

# Mass mortality among colony-breeding seabirds in the German Wadden Sea in 2022 due to distinct genotypes of HPAIV H5N1 clade 2.3.4.4b

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## Abstract

Mass mortality was observed among colony-breeding seabirds in the German Wadden Sea area of the North Sea during the summer months of 2022. Several species' colonies were affected, most notably sandwich terns (*Thalasseus sandvicensis*), common terns (*Sterna hirundo*) and Germany's only northern gannet (*Morus bassanus*) colony on the island of Heligoland. Mortality in some tern colonies reached 40%, while other colonies were almost spared. In all cases, infections with the high-pathogenicity avian influenza virus (HPAIV) subtype H5N1 of clade 2.3.4.4b were identified to have caused the epidemic. Phylogenetic analysis of whole-genome sequences revealed that the outbreaks were dominated by two genotypes, Ger-10–21 N1.2 and Ger-10–21 N1.5, previously identified in Germany. Spatiotemporal analyses of phylogenetic data suggested that these viruses could have entered the continental North Sea coastal region via the British Isles. A close linkage of viruses from tern colonies in the German Wadden Sea was evident with further connections to breeding colonies in Belgium and the Netherlands, and further spread to Denmark and Poland. Several of the affected species are endangered, such that negative effects of epizootic HPAIV infections on populations are feared, with uncertain long-term consequences.

## INTRODUCTION

Infections by high-pathogenicity avian influenza viruses (HPAIVs) of clade 2.3.4.4b of the H5 goose/Guangdong (gs/GD) lineage have emerged repeatedly in Germany since 2016 [1]. Regional and temporal accumulations of HPAIV-infected wild birds were detected at the German coasts of the Baltic and North Sea, with the worst affected species varying between seasons [1]. In 2016/17, for example, the majority of cases were observed in diving duck species, such as tufted ducks (*Aythya fuligula*) and common pochards (*Aythya ferina*), whereas geese species such as barnacle geese (*Branta leucopsis*), at the North Sea coast, and to a lesser degree also graylag geese (*Anser anser*), have dominated the epizootic since 2020 [2]. The 2020/21 and 2021/22 HPAI winter periods exceeded all previously recorded HPAI epizootics in Germany in terms of the number of wild bird cases recorded, the genetic diversity of the viruses and the duration of virus activity [3]. Moreover, a geographical shift of wild bird cases towards the Wadden Sea coast was observed [4].

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**Keywords:** seabirds; highly pathogenic avian influenza; mass mortality; H5N1; transmission; species conservation.

**Abbreviations:** BA, Bayesian factor; HA, hemagglutinin protein; HPAI, highly pathogenic avian influenza; HPAIV, highly pathogenic avian influenza virus; MCC, maximum clade credibility; NA, neuraminidase protein; Q, quartal of a year.

Sequence accession numbers are listed in Table S1. Data associated with spatiotemporal spread are accessible at <https://zenodo.org/> under DOI: 10.5281/zenodo.6901960.

All supporting data, code and protocols have been provided within the article or through supplementary data files. Three supplementary figures and two supplementary tables are available with the online version of this article.

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This trend culminated in HPAI-associated mass mortality of a large flock of wintering red knots (*Calidris canutus islandica*) from the Greenland/Canada population in December 2020 (Globig *et al.*, in preparation). In addition, an increased incidence was observed in raptor and gull species [5].

From May 2022, a new and unprecedented situation was observed in Europe when several colony-breeding water- and seabird species, such as the sandwich tern (*Thalasseus sandvicensis*), the common tern (*Sterna hirundo*), the northern gannet (*Morus bassanus*), the black-headed gull (*Chroicocephalus ridibundus*), and – mainly at the Baltic Sea coast – the cormorant (*Phalacrocorax carbo*) started showing mass mortality induced by HPAIVs [6]. Other European colony breeding species affected by HPAI in the breeding season 2022 are the Eurasian spoonbill (*Platalea leucorodia*) in the Netherlands and, in other parts of northwest Europe, common guillemots (*Uria aalge*). This development turned out to be embedded in a larger scale expansion of HPAIVs of the gs/GD lineage of Southeast Asian origin into the Northern Atlantic and onward to North America [7, 8]. In early May 2022, AI was detected in a sandwich tern colony on the German Baltic coast (Langenwerder). In late May 2022, high mortality was observed in breeding colonies of sandwich terns in Calais, France, and in the Dutch islands of Zeeland and Texel, and HPAIV of the H5N1 subtype was detected. At Shetland and the Orkney Islands, UK, outbreaks of HPAIV infections at northern gannet and great skua (*Stercorarius skua*) breeding spots were observed in 2021 and from April 2022 onwards. In early June 2022, HPAIVs of subtype H5N1 were detected for the first time in colony-breeding seabirds at the German Wadden Sea coast (sandwich terns, common terns).

Here we assemble data documenting the impact of HPAIV H5N1 on colony-breeding seabirds in Germany and analyse phylogenetic and phylogeographical aspects of virus incursion and circulation within and between the breeding colonies.

## METHODS

### Ornithological data collection

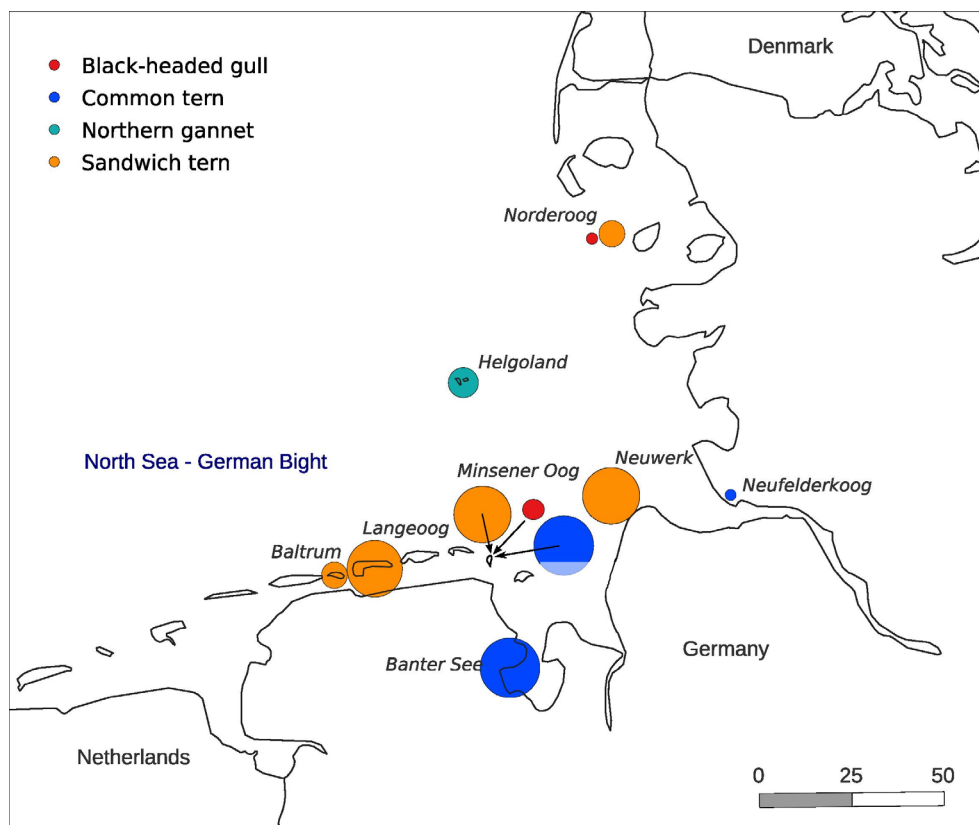
Since 1991, the three countries bordering the Wadden Sea coastline of the North Sea, Denmark, the Netherlands and Germany, have committed themselves to jointly monitor birds in the Wadden Sea in a co-ordinated scheme that has its roots in the survey for oiled birds (e.g. [9]). The surveillance programme is coordinated by the Common Wadden Sea Secretariat. The methods of recording dead birds differ depending on the species and have been detailed by Hälterlein *et al.* [10]. Many breeding colonies in the German Federal States of Schleswig-Holstein and Lower Saxony have been recorded in recent years using drones and aerial photographs. Breeding pairs of gannets on Heligoland are recorded according to Südbeck *et al.* [11] and bird flu monitoring was carried out at Heligoland daily on the main island and the dune. Carcasses in the gannet colony were collected on three dates during the outbreak. Drift line checks take place at regular intervals at designated coastline sections. All dead animals are recorded and documented by various institutions such as the national park administration or nature conservation associations [12].

Three German Federal States share the Wadden Sea coastline of Germany: Schleswig-Holstein, Hamburg and Lower Saxony (Fig. 1). In both Schleswig-Holstein and Lower Saxony, no colonies were entered during the breeding phase to avoid disturbing the birds. It was feared that the birds could adopt other habitats in the wake of the disturbance and that this could contribute to further spread of HPAI viruses. At Minsener Oog, Lower Saxony, however, a transect of ~1.3 km was established near the colony and the dead birds were recorded during the breeding season on a daily basis. The number of dead birds over time was used to monitor the progression of infection within the colony. The colonies on the island of Norderoog were checked after the end of the breeding season on 23/24 July. All carcasses were registered with GPS co-ordinates. Time of death and, in the case of chicks, age, were estimated. Affected parts of the colony that were not recorded (~40–50% of the area) were extrapolated. At the common tern colony at the Banter See, Lower Saxony, the colony area and the lake were searched twice a day and all dead terns were collected. On the island of Neuwerk, Hamburg, the carcasses of birds were collected and disposed of but not recorded, such that an assessment of the number of dead birds is currently not available. Future breeding pair counts may, however, allow us to retrospectively estimate the impact of HPAI 2022 on sandwich terns at this island, as well as at other locations, since even for the other locations mortality estimates are currently based on the sightings of dead birds, and may be underestimated.

General uncertainty of exact mortality rates exists because (i) some dead birds will not have been found, such that the number of dead birds will be underestimated to an unknown extent; (ii) retrospective calculations on the basis of next season's counts may suffer from unknown levels of (additional HPAI-induced) mortality in the wintering areas; (iii) the number of breeding pairs is not always easy to estimate, due to variable numbers of replacement and second clutches, with perhaps more pronounced difficulties in the disrupted breeding season of 2022; (iv) many populations contain prospectors and/or non-breeders that may have been found dead, but do not appear in estimates of the number of breeding pairs; and (v) the breeding site fidelity of some species is not very pronounced and relocations frequently take place.

### Molecular virus detection and phylogenetic analyses

Clinical material, mainly oropharyngeal and cloacal swabs, was collected from wild bird carcasses obtained from affected colonies or washed ashore. RNA was extracted with the Qiagen Mini Viral kit (Qiagen, Germany). We used real-time RT-PCR (RT-qPCR) to test for the presence of AIV-specific RNA, as well as for sub- and pathotyping [13].



**Fig. 1.** Map of affected seabird breeding colonies along the North Sea coast of Germany. The colour of the dots represents species, as shown in the legend, and the size of the dots depicts the level of mortality, as detailed in Table 1. Scale, km.

Full-genome sequencing of selected HPAIV-positive samples was executed by a previously described nanopore-based real-time sequencing method with prior full genome amplification [14]. Samples were selected on the basis of their viral load, the species origin and the location.

HPAIV genome amplification was conducted by universal AIV-End-RT-PCR using Superscript III One-Step and Platinum *Taq* (Thermo Fisher Scientific, USA) using a single primer pair (Pan-IVA-1F: TCCAGTCACGACGTCGTAGCGAAAGCAGG; Pan-IVA-1R: GGAAACAGCTATGACCATGAGTAGAAACAAGG), which binds to the conserved ends of the viral genome segments.

After purification of the PCR products with AMPure XP Magnetic Beads (Beckman-Coulter, USA), full-genome sequencing on a MinION platform (Oxford Nanopore Technologies, ONT, UK) using the Rapid Barcoding kit (SQK-RBK004, ONT) for transposon-based library preparation and multiplexing was performed. Sequencing was directed according to the manufacturer's instructions with a R9.4.1 flow cell on Mk1C device with MinKNOW Software Core (v4.3.11).

Live basecalling of the raw data with Guppy (v5.0.13, ONT) was followed by a demultiplexing, quality check and trimming step to remove low-quality, primer and short (<50 nt) sequences.

After sequencing, full-genome consensus sequences were generated using a map-to-reference approach utilizing MiniMap2 [15]. Reference genomes are a curated collection of all haemagglutinin (HA) and neuraminidase (NA) subtypes alongside an assortment of internal gene sequences chosen to cover all potentially circulating viral strains.

Polishing of the final genome sequences was done manually after consensus production according to the highest quality (60%) in Geneious Prime (v2021.0.1, Biomatters, New Zealand).

Segment-specific and concatenated whole-genome multiple alignments were generated using MAFFT (v7.450) [16] and subsequent maximum-likelihood (ML) trees were calculated with RAxML (v8.2.11) [17] utilizing model GTR GAMMA with rapid bootstrapping and searching for the best scoring ML tree supported with 1000 bootstrap replicates or alternatively with FastTree (v2.1.11) [18]. Time-scaled trees of concatenated sequences of the different genotypes were calculated with the BEAST (v1.10.4) software package [19] using a GTR GAMMA substitution model, an uncorrelated relaxed clock with a lognormal distribution and coalescent constant population tree models.

**Table 1.** Mortality of seabirds in breeding colonies and along the drift line of the German Wadden Sea coast, June–August 2022

Species	Location*	Colony size†	Carcasses		Adult mortality‡	Carcasses§ (drift line)
			Adult	Juvenile		
Sandwich tern	Minsener Oog	4765	2967	2807	31.1%	
	Baltrum	649	14	NA	1.1%	
	Langeoog	165	112	38	33.9%	1904
	Neuwerk	>660	>850	NA	>60%	
	Norderoog	6442	650	2900	5.0%	
Common tern	Banter See	690	510	1350	37.0%	
	Minsener Oog	190	176	74	46.3%	105
	Neufelderkoog	1070	7	94	0.3%	
Black-headed gull	Minsener Oog	2852	121	151	2.1%	350
	Norderoog	1670	29	101	0.9%	
Northern gannet	Heligoland	1485	259	689	8.7%	697

\*Depicted in Fig. 1.

†No. of breeding pairs.

‡Calculated by dividing the no. of adult carcasses by twice the no. of breeding pairs.

§Total no. counted along the German North Sea shore line.

NA, not available.

Spatiotemporal analysis was modelled on discrete sampling locations (countries) using a symmetric model, applying Bayesian stochastic search variable selection (BSSVS) procedures. Bayes factors (BFs) were calculated and the potential geographical pattern of dissemination visualized considering BFs >3 and posterior probabilities >0.7 as significant. For inferring the detailed spread of cases, their latitude and longitude location coordinates were derived via adaptive optical means. A continuous relaxed random walk model with lognormal distribution was applied. Chain lengths were set to 20–50 million iterations, depending on the data set, and their convergence checked via Tracer (v1.7.1). Time-scaled summary maximum-clade credibility (MCC) trees with 10% post-burn-in posterior were created using TreeAnnotator (v1.10.4) and visualized with FigTree (V1.4.4). The robustness of the MCC trees was evaluated using 95% highest posterior density (HPD) confidence intervals at each node and posterior confidence values as branch support. Spatiotemporal spread was inferred on MCC trees using SPREAD (v1.0.7) [20] and visualized with QGIS (V3.24.3, QGIS.org). Associated data and underlying source data are available in the Zenodo repository <https://zenodo.org/> under DOI 10.5281/zenodo.6901960.

## RESULTS

### High adult mortality at breeding colonies of seabirds along the German Wadden Sea coast was found during summer 2022

Breeding colonies of terns in Germany are mainly scattered across small flat sandy islands along the Wadden Sea coast (Fig. 1). The largest sandwich tern colonies in Germany at Minsener Oog, Lower Saxony, and Noorderoog, Schleswig-Holstein, were affected differently by HPAI (Table 1). On Minsener Oog, adult mortality amounted to 31.1%, while on Noorderoog it remained at 5.0%.

In the beginning of June, HPAIV was only detected sporadically in dead adult and young birds on Noorderoog. In late June the mortality of adult sandwich terns increased exponentially after the late settlement of birds that had presumably already made unsuccessful breeding attempts in other colonies during this breeding season (among these a high proportion of birds ringed in the Netherlands), and most of the juveniles died from the infection. The largest common tern colony in Germany at Neufelderkoog, Schleswig-Holstein, is very elongated, with large distances between the nests, and was almost unaffected by HPAI (mortality rate 0.3%). This very low impact also applies to Noorderoog, where common terns breed in the vicinity of the sandwich tern colony. The colonies at the Banter See and on Minsener Oog, both Lower Saxony, in contrast, showed the highest mortality rates of >37% of adult birds.

In the black-headed gull (*Chroicocephalus ridibundus*) colony on Minsener Oog, Lower Saxony, a mortality rate of 2.1% was recorded, similar to what was seen at Noorderoog island.

For the northern gannets on Heligoland, Schleswig-Holstein, a mortality rate of 8.7% was calculated, based strictly on dead birds counted within the colony. Since more than 400 individuals were additionally recorded dead during drift line inspections, the actual mortality is likely higher, up to 22.5% if all of the washed up birds were Heligoland breeders.

## Mortality was due to HPAIV H5N1 infection

All samples ( $n=63$ ) examined from species listed in Table 1 tested positive for HPAIV of the H5N1 subtype. Viral loads varied considerably but were in general high for fresh carcasses retrieved from breeding colonies (cycle thresholds 14–21). Considerably lower virus loads were detected in carcasses found along the coastal drift lines, especially when in a state of enhanced decomposition. This mainly affected findings of northern gannets (cycle thresholds  $>34$ ).

A total of 111 full-genome sequences were obtained from German samples, of which 23 originated from colony breeders (4 gannets, 13 terns, 2 cormorants, 4 gulls, (Supplementary Material), whereas the other sequences originated from other wild birds, predators and poultry holdings. For each case, the RT-qPCR-based sub- (H5N1) and patho- (HP) typing was confirmed. All haemagglutinin sequences clustered with the gs/GD clade 2.3.4.4b. Further genetic analyses were used to map the relationship of HPAIV H5N1 from colony-breeding seabirds and to trace trajectories of introduction and spread within and among the breeding colonies. This showed that the outbreak in Germany since May 2022 was dominated by two known genotypes that have been found to circulate in Germany since October 2021: Ger-10–21 N1.2 and the reassortant Ger-10–21 N1.5 [7]. These two genotypes also prevailed in previous outbreak events in wild birds as well as in poultry from early 2022 and comprise ~75% of all sequences obtained across various regions of Germany.

## Detection of three genetic clusters of HPAIV H5N1 cycling within and between breeding colonies around the North Sea

The German sequence data were supplemented with publicly available sequences, resulting in curated data sets for each reassortant of 191 sequences for analysing tern and cormorant colonies (genotype Ger-10–21 N1.5) and 109 sequences for analysing gannet colonies (genotype Ger-10–21 N1.2). Data sources and acknowledgments are listed in Table S1 (available in the online version of this article). Bayesian stochastic search variable selection (BSSVS) with calculation of BFs is summarized in Table S2.

The only German breeding colony of northern gannets is located on the North Sea island of Heligoland. Although this colony was affected by HPAIV (see above), no samples from that location yielded valid sequences that could be included in the analyses. Instead, four northern gannet samples collected during drift line inspections in Germany in the summer of 2022 confirmed positive for HPAI H5N1 viruses and yielded sequence information identifying them as belonging to the Ger-10–21 N1 genotype. A single gannet sample collected in February 2022, however, clustered with the HPAIV H5N1 genotype Ger-10–21 N5. Viruses of this genotype were detected in the German North Sea and Baltic Sea coastal regions in Q4 2021 in other wild birds (<https://doi.org/10.5281/zenodo.6838094>) and travelled westward in Q1 2022. These viruses were consistently detected in samples from Germany until 1 March 2022.

Phylogenetic analysis via maximum likelihood (Figs S1 and S3) shows that these viruses are closely related to contemporary viruses collected in the UK and are related to viruses from the Netherlands. An incursion of these viruses into the continental North Sea coastal region via the British Isles could be confirmed by spatiotemporal analysis via time-scaled MCC phylogeny of subsets of clustered sequences and inferring their spread (cluster 1, Figs 2a, S2a and 3a). The potential incursion via the British Isles could, however, not be corroborated by ornithological findings: Although ringing data of gannets washed up on the North Sea island of Sylt revealed that they hatched in British breeding colonies, this merely reflects that they were likely ringed as chicks in the UK, but they could still have been regular breeding birds of the Heligoland colony for years. At the Heligoland breeding site, gannets from very different hatching colonies can be found.

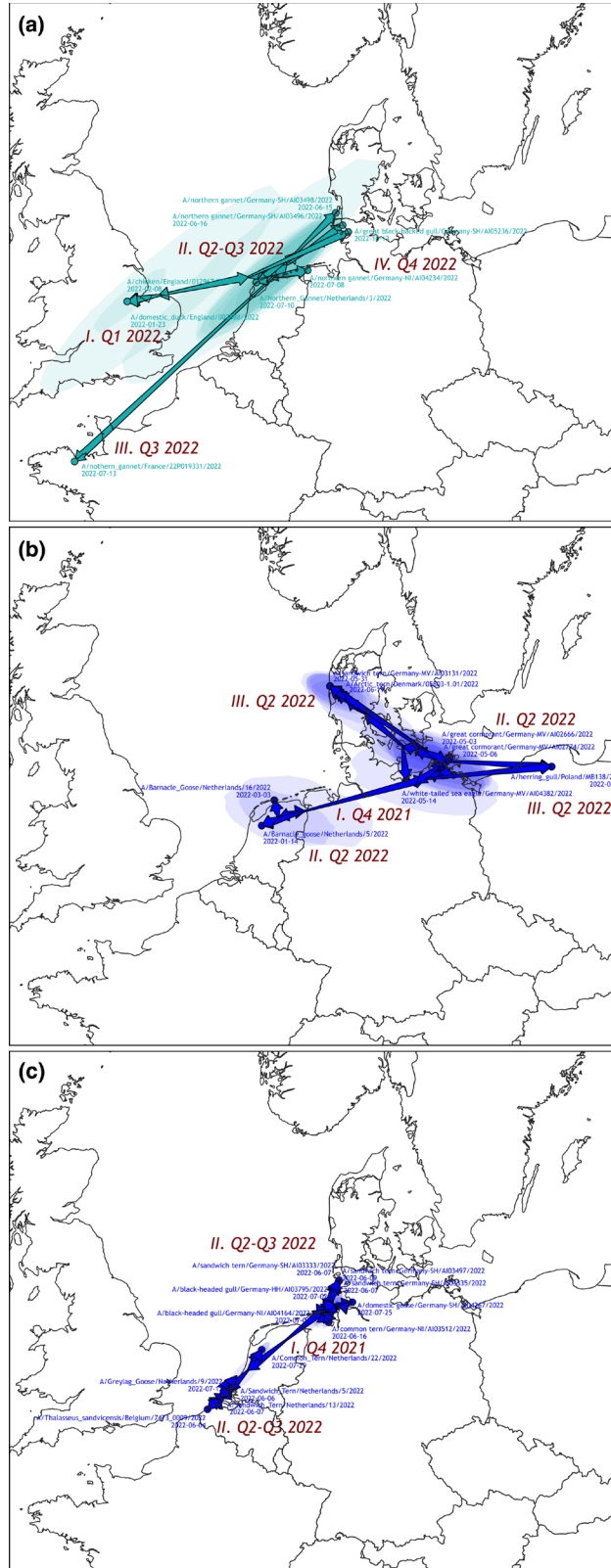
The tern cases (common and sandwich terns) are caused by a different reassorted virus: Ger-10–21 N1.5 (Fig. S1). This genotype has been present in Germany since at least October 2021. The tern cases in Germany can be assigned to two different clusters within this genotype (Figs 2b, c, S2b, c and S3b): cluster 2 could mainly be traced back to infections of geese in Q1 2022 in the Netherlands. These viruses moved further eastward, affecting wild birds and colony-breeding great cormorants in Mecklenburg-Vorpommern on the Island of Rügen. Subsequently, white-tailed eagles (*Haliaeetus albicilla*) on the island of Rügen and sandwich tern colonies were infected by this virus, causing widespread fatality (Figs 2b, S2b and S3b). Spatiotemporal analysis revealed the further spread of this genotype from Germany to Poland and to the Danish North Sea coast.

Cluster 3 reflects a close linkage of tern colonies in the German Wadden Sea. The virus was detected in common terns at the North Sea coast in Wilhelmshaven and in Sandwich terns on the island of Minsener Oog in Lower Saxony, part of the Wadden Sea, in June 2022. Similar viruses caused infection in sandwich terns in the Netherlands and Belgium. The virus was subsequently also detected further north in Germany in sandwich terns found dead on Hallig Hooge nearby the Island of Norderoog in Schleswig-Holstein (Figs 2c and S2c).

## DISCUSSION

The mass mortality among seabirds in the area of the North Sea – triggered by HPAIV H5N1 in the summer of 2022 – is unprecedented. During the breeding season, seabird colonies have high population densities, and the close physical contact of the birds to one another is likely to increase HPAIV transmission rates greatly. According to observations made in a number of tern





**Fig. 2.** Cluster areas (polygons) with their directed spread (arrows) of HPAIV genomes (points) inferred by spatio-temporal phylogeography with noted key steps of virus dissemination. (a) Cluster 1 viruses from northern gannets Q1–Q2 2022. (b) Cluster 2 viruses from common and sandwich terns Q1–Q2 2022. (c) Cluster 3 viruses from common and sandwich terns Q2–Q3 2022.

colonies in Germany, the spread of HPAIV within a colony seemed to require some time to develop from an easily overlooked latent phase with few individual infections and deaths to a fulminant epizootic. As populations shrink due to rising mortality, dispersal of affected birds or the regular end of breeding activities, infection chains are disrupted. However, the risk of epizootic infections did not appear to be distributed evenly, as significant differences in mortality rates were observed between different HPAIV-affected colonies of the same species. Differences in the time of HPAIV incursion into the colony could be one explanation. Additionally, however, it needs to be considered that the presented mortality rates are based on rough calculations made using numbers of dead birds found and the estimated number of breeding pairs per population, whereas the exact population size of most colonies is unknown and probabilities of finding dead birds may differ among populations as well.

The current summer cases among colony breeding birds may have particularly grave consequences for the continuation of at least some of the heavily hit colonies. In recent years, an increase of cormorant populations in the Wadden Sea has been observed. The population of sandwich terns was stable, while the populations of black-headed gulls, common terns and common eiders decreased [9]. All of the aforementioned species require advanced ages for sexual development (e.g. gannets >5 years), and the average breeding success per year is quite low, e.g. in common terns 0.12–1.57 fledglings/brood [21], and increases with age [22], such that the loss of older, more experienced breeders is especially detrimental [23]. As such, it can be assumed that the loss of a large number of adult birds will have a negative long-term impact on population development. The complete impact of the HPAI epizootic in 2022 on long-term population patterns will not be known until breeding pair counts in the future. If colonies are similarly affected by HPAI in upcoming years, it may be damaging to population trends in the Wadden Sea in the event of an enzootic entrenchment of HPAIV.

There are currently no preventive measures in place to protect seabird colonies from future HPAIV reincursion events, and the intervention options are few. Most importantly, the early collection and safe disposal of all carcasses at the beginning of an outbreak found in breeding areas by appropriately trained personnel aids in the reduction of virus loads in the environment [24]. Not only do the carcasses themselves remain infectious (especially muscles and feathers, depending on temperature, 3–4 weeks at 20 °C [25]), but carcasses floating in water will flush out infectious viruses. Virus-contaminated surface water has been shown to act as a highly potent transmission medium [13]. AI virus persistence in surface water is strongest at a low temperature (<17 °C), a neutral-to-basic pH (7.0 to 8.5), low salinity (<0.5 ppt) and a low dissolved ammonia concentration (<0.5 mg l<sup>-1</sup>). Small shallow fresh water pools in the vicinity of breeding colonies in Lower Saxony are often visited by terns for bathing and grooming and might have contributed to virus spread via contaminated surface water. However, even in sea water with salinity >3 p.p.t. it was estimated to take 3 days to achieve a 1 log reduction of virus titre [26].

From an epizootiological perspective, seabird colonies amplified HPAIV H5N1 in the North Sea and other North Atlantic regions in the summer of 2022. This undoubtedly aided in maintaining year-round virus replication, establishing enzootic HPAIV circulation in Northern Europe, and increasing virus presence in the environment. The consequences of persistently high HPAIV infection pressure are severe for both avian wildlife and poultry rearing, particularly in free-range poultry operations. In addition, the presence of HPAIV H5N1 in wild bird populations increases the risk of infection in mammalian species such as foxes, mustelids and seals [27–30]. Overall, the risk of emerging HPAIV variants with zoonotic propensity increases.

Controlling HPAI in poultry globally is fundamental to lowering the risk of virus spillover from poultry to wild birds [31], while also lowering the risk of human exposure. Strict biosecurity measures for poultry holdings, combined with a test-and-cull strategy, have been shown to be effective in areas where outbreaks occur infrequently. Risks of spill-out events of HPAI virus from infected poultry premises to natural habitats of wild birds, e.g. during culling operations or removal of manure and waste, must be minimized. The same strict hygiene principles should also be employed when entering wild bird habitats, visiting wild bird breeding colonies or handling wild birds for other purposes (e.g. ringing). However, vaccination of poultry may be required as an additional layer of protection when incursion pressure remains consistently high. HPAI vaccination options in wild birds have yet to be explored. Baited vaccination of foxes and other carnivores against rabies or wild boar populations against European swine fever has a long history of success in Europe [32]. Discussions must be initiated to determine whether similar vaccine options can be envisioned for use in some of the much more diverse, mobile, volatile and endangered wild bird populations. Access will be shaped by foraging habits, with gulls being much less fastidious compared to terns, which strictly feed on small live fish.

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Author Dr Lina Stacker was not available to confirm co-authorship, but the corresponding author Dr Harder affirms that author Dr Stacker contributed to the paper and vouches for author Dr Stacker's co-authorship status.

## Author contributions

A.P., T.H. and M.B. conceived the study; O.S., F.P., B.H., V.H., E.B. and S.B. provided samples and ornithological population data; J.K., L.S., A.G., Ch.H., A.G., Y.L. and C.H. carried out the experiments and generated sequences; AP and JK analysed sequence data; A.P., T.H., O.S. and S.B. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript, and agreed with the final version.

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