Habitat use and diet of terns in north western Sweden

Potential effects of Northern Pike

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Abstract

In this study I examine the habitat, breeding and diet preferences of two tern species (*Sterna hirundo* and *Sterna paradisaea*) in inland lakes in Sweden. Previous work has shown that Northern Pike *(Esox lucius)* can induce a dwarf ecotype of European Whitefish *(Coregonus lavaretus)*, a potentially valuable food resource for piscivorous birds. Here I investigate if pike presence or absence in north western Swedish lakes has is linked to habitat use and diet of terns. Through visual surveys of 54 lakes, the capture and measurement of tern chicks, and the collection of faeces for metabarcoding analysis in summer 2024, I was able to demonstrate statistically significant preference of terns for lakes with pike. Metabarcoding analysis of the faecal samples revealed European Perch *(Perca fluviatilis)* with a fraction of 65 % as most important and whitefish (25 %) as second most important component of fish-diet in terns. Different proportions of whitefish in relation to pike absence or presence could not be identified. This also applies for tern chick condition, which I could not thoroughly assess due to data limitation. In conclusion, I was able to identify links between pike presence and tern habitat use. However, further studies are needed to better understand this ecological relationship.

Keywords: Sterna hirundo, Sterna paradisaea, diet, lakes, breeding success

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Abbreviations

Abbreviation	Description
EMBL	European Molecular Biology Laboratory
GAM	Generalised additive model
MOTU	Molecular Operational Taxonomic Units
NCBI	National Center for Biotechnology Information
SLU	Sveriges lantbruksuniversitet

1. Introduction

The use of bioindicators is a well-established approach for monitoring ecosystem health (Burger 2006). Identifying such indicators can provide information about food web processes that helps us detect and explain changes at the ecosystem level, thereby providing policy makers with invaluable information (United Nations Economic Commission for Europe 2023). Hence, finding suitable bioindicators is of crucial importance in light of present and future shifts in climate, land use, and other anthropologically driven changes (Sala et al. 2000).

One approach to using bioindicators is to harness the connectivity between trophic levels, using predators as indicators of prey abundance and population structure. In aquatic systems, fish are often used as indicators for pollution (Chovanec et al. 2003) or prey availability (Montevecchi 1993). However, birds are also highly interesting candidate indicators as they hold the potential to link aquatic and terrestrial ecosystems. A promising connection exists between land-dwelling piscivorous birds and their prey with the added advantage that birds are often relatively easy to study (Sutherland 2006), while providing insights into less easily accessible, aquatic systems. Seabirds are commonly discussed and used as bioindicators for marine ecosystems. Nesting along the shore, and foraging at sea, they can reflect the status of prey fish populations (e. g. Frederiksen et al. 2007; Piatt et al. 2007). However, less closely examined is the trophic ecology of piscivorous birds in inland lakes.

Lake-dwelling piscivorous birds such as divers, mergansers and terns, highly specialised for fish hunting and adapted in both morphology and behaviour, are commonly recognised as being sensitive to prey availability. In particular, they often depend on availability of sufficiently small prey fish (Pearson 1968; Cramp 1989). Environmental factors that affect availability of such prey may therefore be linked to richness of piscivorous birds. In large northern Swedish lakes, presence of Northern Pike *(Esox lucius)* may constitute one such important factor as it has been shown to induce a dwarf life history strategy in populations of European Whitefish *(Coregonus lavaretus)* (Öhlund et al. 2020). Consequently, bird surveys on these lakes have shown a higher density of these birds in lakes with pike were small whitefish are plentiful (Öhlund et al. 2024).

Divers are known to preferentially exploit the pike-induced whitefish resource when available and mergansers and terns also appear to profit (Söderlund 2020; Öhlund et al. 2024) In this study, I aim to investigate closely whether we can find positive effects of pike on terns.

The two tern species with established broods in our study area are Common Tern *(Sterna hirundo)* and Arctic Tern *(Sterna paradisaea)* (see Ottosson 2012). Hereafter, when both species are referred to together, they will be collectively called “terns”. These birds are opportunistic feeders, consuming a variety fish and

insect species depending on availability (see Cramp 1989). Fish as principal prey appears to play a crucial role during the breeding season. Terns are known to choose nesting sites in proximity to constant food supply (Palmer 1941; Hawksley 1957; Cramp 1989) and to be sensitive to reduced food availability (Newton 2003; Mallory et al. 2017). However, most studies so far have focused on populations residing at sea or in very large lakes (e.g. Becker 1995; Wails et al. 2014; Mallory et al. 2017; Scopel & Diamond 2018).

I hypothesise that pike presence in northern Swedish whitefish lakes is associated with 1) higher tern abundance, 2) tern chicks that are in better condition, and 3) a greater proportion of whitefish in tern diet. To test these hypotheses, I will collect tern abundance data through visual surveys, identify dietary components using faecal samples from roosting birds and captured chicks through metabarcoding analysis, and assess chick condition by capturing and measuring/weighing chicks from broods in the respective lakes.

2. Methods

2.1 Occurrence of terns in the sampling area

2.1.1 Sampling Area

Data collection was carried out in 54 lakes in north western Sweden (Fig. 1). These lakes have surface areas ranging from 1 to 10 square kilometres, and are situated in the counties of Västerbotten or Jämtland. While the fish communities in the lakes vary, they all have populations of European Whitefish.

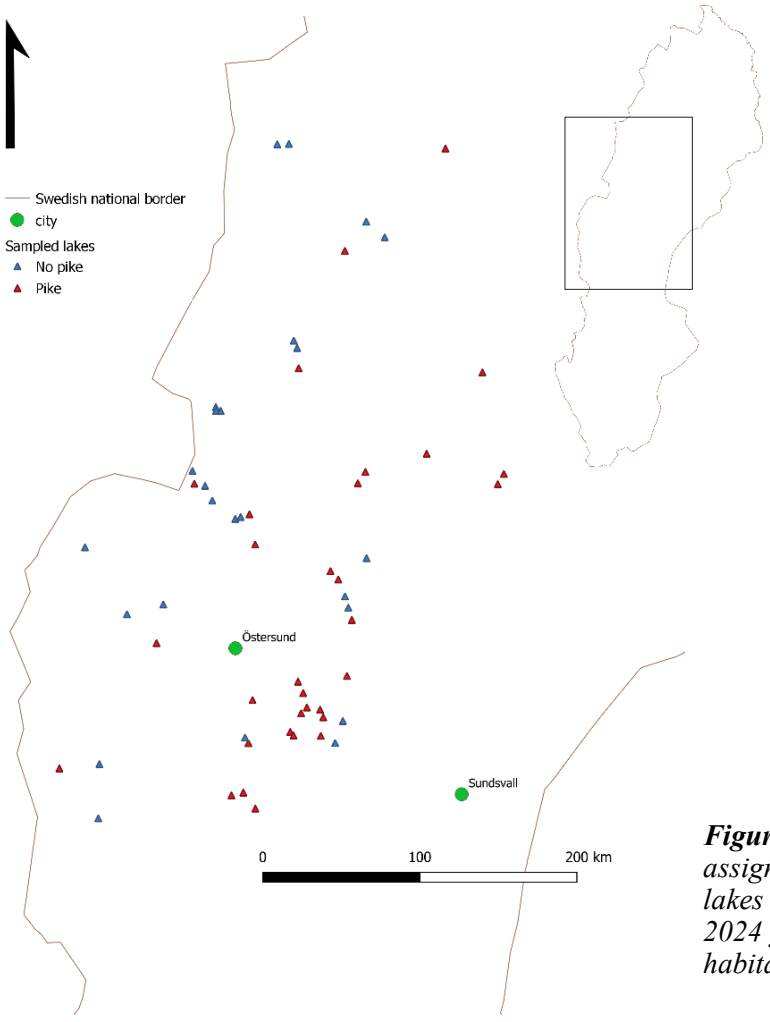

Figure 1 *Location and assigned category of the 54 lakes sampled in summer 2024 for this study on tern habitat and diet.*

All of these lakes were classified in two categories: “Pike” and “No Pike”, the category “Pike” inholding the whitefish dwarf form which the category “No Pike” lacks.

2.1.2 Data collection: Visual survey

Visual surveys were conducted from vantage points at the lake shore by scanning the water surface from there. The aim was to cover the entire lake surface and only adult birds were counted (Bibby et al. 1992). Recordings were paused during strong winds (> 5 bft, estimated in the field) or during precipitation.

A 30x77 spotting scope was used to scan distant areas and 7x50 binoculars for close ranges, to survey the lake as far as possible. Considering tern behaviour, the lake shores and fields up to 20 meters above the water were treated equally important as the water surface. The scan was repeated after 15 minutes. At each scan I recorded tern sightings, estimated weather characteristics, position of the vantage point marked in a GPS device (Garmin eTrex 10) and the field of view outlined on a paper map. Lake areas were considered surveyed if terns could be identified at genus level. At absence of terns, other birds or objects served as reference for visibility. Special attention was given to broods or indirect signs of nesting terns. To ensure presence and activity of the birds we decided to perform the visual survey in June and July between 05:00 and 22:00.

2.1.3 Analysis of the visual survey data

The actual area surveyed in each lake and the total lake area were calculated in QGis 3.34 from the field of view and the total lake outline, as planned in the survey design. I downloaded geospatial data from Open Street Map (OpenStreetMap contributors 2024) using the query *key = natural, value = water* for the spatial extent of the venture points.

The number of terns detected per lake is the sum of birds in all scanned areas, using the highest count of each the two scans. When it could be determined that an individual bird had already been counted in another scanning area, this bird was excluded from the scan.

To test if terns show a preference for lakes with pike, I analysed tern presence as binary data with respect to lake category using a Fisher's exact test.

2.2 Tern chick capture

2.2.1 Sampled lakes and time for chick capture

We sampled 29 lakes in total: 27 lakes that were part of the visual surveys and two additional lakes with previously recorded tern nesting sites (Öhlund et al. 2024). This subset was generated to cover the complete sampling area as evenly as possible and to keep the paired structure of "Pike" and "No Pike" lakes.

2.2.2 Data collection: Capturing and measuring tern chicks

Capturing of tern chicks was carried out from end of June till end of July, in all-weather unless it made boating dangerous. To locate nesting sites, we navigated the lakes with a motorboat, mostly along the shoreline, scanning the surroundings with binoculars. If active breeding sites were already known we headed there, but continued searching for other nests along the way. Upon reaching a nesting site I jumped ashore to capture the chicks by hand, while my assistant stayed in the boat, circling around the nesting site, to spot birds hiding out of my view, or catch any that tried to escape swimming using a hand net. I placed the caught chicks in paper bags. For morphometric measurements, I took each chick out of its bag, held it in the "ringers grip" and measured hand length using a ruler and head to bill tip using callipers. After that the chick was placed back in, and weighted with the bag. I read the measurements to full millimetres for the ruler and callipers and to 0.5-gram for the scale. After measuring, we waited for the chicks to defecate into the bags. If we obtained at least one dropping per nest we released the chicks at once and left. Faeces were sampled later directly from the inside of the bags by swabbing with a cotton tip applicator. The tip was inserted in a 2 ml screw cap tube filled with ethanol (95%) and swirled to dissolve material and then removed. A field control was generated for each sample, with a new applicator by repeating the procedure immediately after sampling, but without allowing the tip to contact any faeces.

2.2.3 Analysis of tern chick capture data

To evaluate chick condition, I used a condition index based on weight and hand length. I obtained comparative values by fitting a general growth curve to the individual chick measures from this study. I ran a GAM regression in R Studio (version 4.4.2) using the *gam* function with a smooth factor to account for nonlinear relationships, from the mgcv package (Wood 2017). Hand length was used as predictor and weight as response. The resulting growth curve and measured chick weights are presented in Fig. 4. To assess differences between categories, I

compared the residuals from hand to weight condition, averaged over all chicks per lake (not shown in Fig. 4), to the model's predictions based on our own data using a Welch's t-test.

2.3 Diet analysis

2.3.1 Data collection: faeces sampling in the field

The analysed samples originate partly from tern chick capturing, the remaining were collected at the lakes whenever we saw a tern sitting somewhere and discovered it had defecated. The same procedure was applied as for the other samples: swabbing with a cotton tip applicator, generating a field control and dissolving in ethanol.

All samples were stored in the tubes as they were sampled, within a cooling box, and upon my return from fieldwork, were frozen in a freezer until further processing.

2.3.2 Processing of the samples - DNA extraction, PCR, metabarcoding

At the SLU laboratories DNA from the faecal samples was extracted using the QIAGEN DNeasy® Blood & Tissue Kit. I followed the producer's protocol except for the initial lysis. Adapting to preserved faecal samples I discarded superfluous ethanol from the tubes, and then incubated the samples in a solution of buffer ATL, proteinase K and 1 M DTT (Dithiothreitol) (17:2:1) for 12 hours at 56° C. After extraction the samples were cleaned with the OneStep PCR Inhibitor Removal Kit from ZYMO RESEARCH following the protocol. I estimated DNA content in the cleaned samples with a Nano Drop measurement. All field samples and one environmental control per sampled lake were extracted.

The cleaned DNA was amplified in a PCR using Teleo02 primers targeting the mitochondrial 12S rRNA gene of fish (Teleostei) (Taberlet 2018) in a 96 well PCR plate. A detailed description of the procedure is provided in the appendix. PCR results were checked by running a 1% agarose gel.

Concluding, 5 µL from each well were pooled and purified with the QIAquick PCR purification kit (Qiagen) and sent to Novogene Europe (Cambridge, United Kingdom) for library preparation and NovaSeq paired-end sequencing (Novogene strategy: PE250).

2.3.3 Analysis of diet data

The metabarcoding output suggested a considerable amount of fish DNA in the controls (see appendix 4), presumably due to cross-contamination during PCR and tag-jumping in both PCR and metabarcoding, and was filtered prior to further processing; details are provided in the appendix. The reassessed sequencing data were used for taxonomic assignment with *obitag* against the full EMBL database (version 143, April 2025) annotated with full NCBI taxonomy, resulting in the identification of the fish components in tern diet in the sampled lakes.

As next step, I tested for patterns in tern diet by tern species, age, time of the year and lake category. Owing to the small sample size and the non-normal distribution of the data, I used a Wilcoxon rank-sum test as most appropriate analytical method. Shannon diversity index was applied to assess diet diversity.

3. Results

3.1 Visual surveys

I surveyed 54 lakes between 14 June and 20 July 2024. On average 84 % of each lake´s total surface was surveyed. Three lakes with less than 65 % surface covered were excluded from further analysis.

Table 1 *Total detections of the study species (Sterna hirundo, Sterna paradisaea, Sterna sp.) in the 51 representatively surveyed lakes in 2024.*

Species	**Number of detections**
Sterna hirundo	13
Sterna paradisaea	3
Sterna sp.	49
Total number of observed terns	65

Most tern detections in the visual survey were not at species level, therefore we remained at genus level for the analysis. In the visual survey 65 terns were counted. Detections in 13 out of the 51 sampled lakes resulting from 11 lakes with pike and 2 without pike. This corresponds to an overall zero detection rate of 76% (39/51) with 62 % in lakes with pike (18/29) and 91 % in lakes without pike (20/22). Of these 13 “tern lakes”, 9 hosted tern nesting sites. All but one nesting site were located in lakes with pike.

Using binary data on tern presence, a Fisher’s Exact Test showed that there is a significantly higher likelihood of finding terns in lakes with pike (N = 51, odds ratio = 5.912, p = 0.025) compared to lakes without pike.

Despite a higher observed proportion of nesting sites in the “Pike” category across all lakes, the association missed statistical significance (N = 51; Fisher’s Exact Test, p = 0.117 , odds ratio = 6.477).

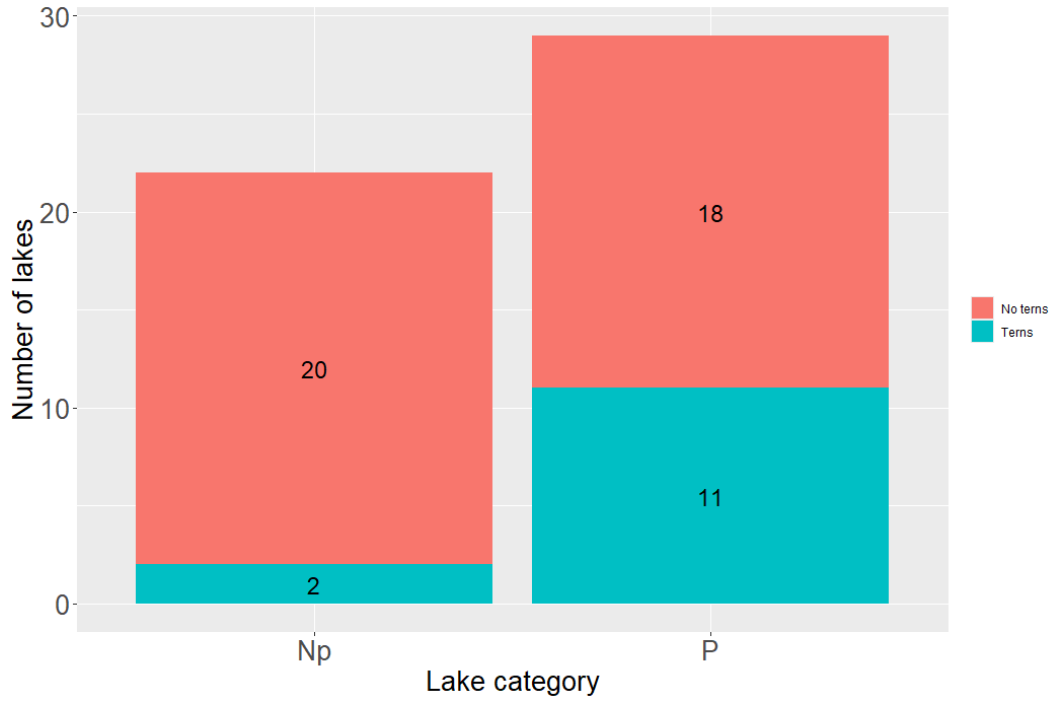

Figure 2 *Tern presence in the sampled lakes during the visual survey in summer 2024 in the two different categories "No Pike" (Np) and "Pike" (P).*

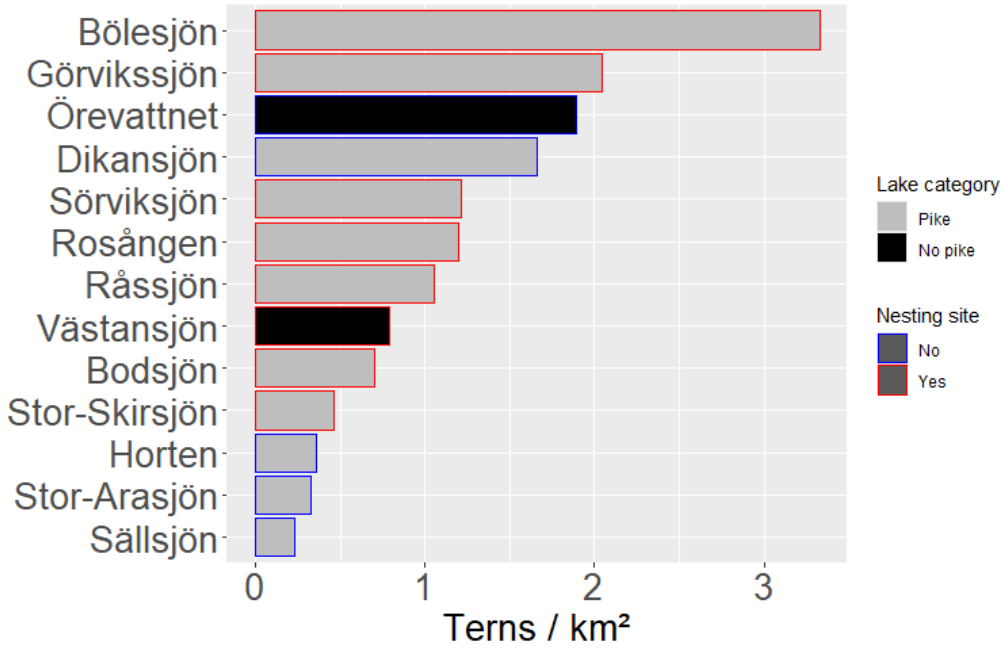

Figure 3 *Tern detections per km^2 lake surface in the visual surveys 2024, lakes with tern nesting sites framed in blue, without, in red. Omitted are 38 lakes with no detections.*

3.2 Tern chick capturing

We captured and measured 28 tern chicks: 16 Common and 12 Arctic Terns. Brood size was 2.4 chicks per nest in average. Chick weight ranged from 17.5 g to 122 g, with a mean of 79.0 g and a standard deviation of 36.8 g. Hand length ranged from 15 mm to 167 mm with a mean of 85 mm and a standard deviation of 46.7 mm.

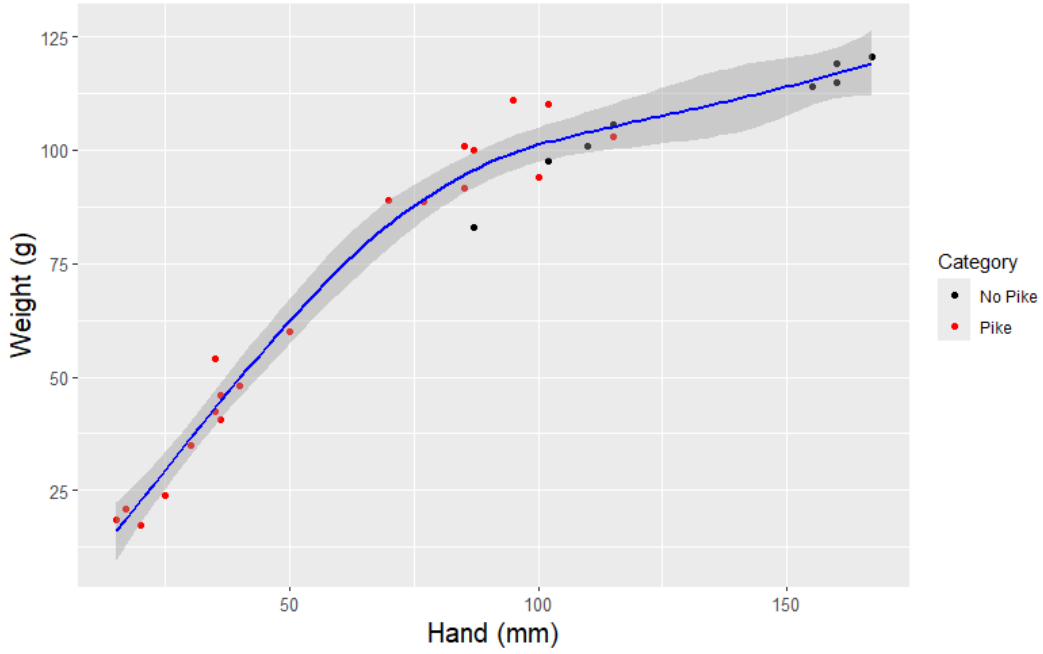

Figure 4 *Dots represent the weights of 28 tern chicks from the capture in summer 2024 in relation to their hand length and the lake categories in which they were caught. The line shows the trajectory, the shade the 95 % confidence intervals, of a growth model fitted using a GAM bases on the observed study data (the dots).*

3.3 Diet analysis

We collected 25 faecal samples from terns, 15 from Common and 10 from Arctic Tern. Five samples were from adult birds (4 Common, 1 Artic Tern) and 20 from chicks (11 Common, 9 Arctic Tern). Of these, 20 samples from 10 lakes yielded reliable results in the metabarcoding analysis. We identified the following five prey fish species: European Perch *(Perca fluviatilis)*, European Whitefish, Common Minnow *(Phoxinus phoxinus)*, Nine spine Stickleback *(Pungitius pungitius)* and Northern Pike

In the total sample, across bird species, lake categories and age, averaged across lakes the most common tern prey was perch (65.1%), followed by whitefish (25.3%), minnow (6.5%), stickleback (2.4%) and pike (0.7%).

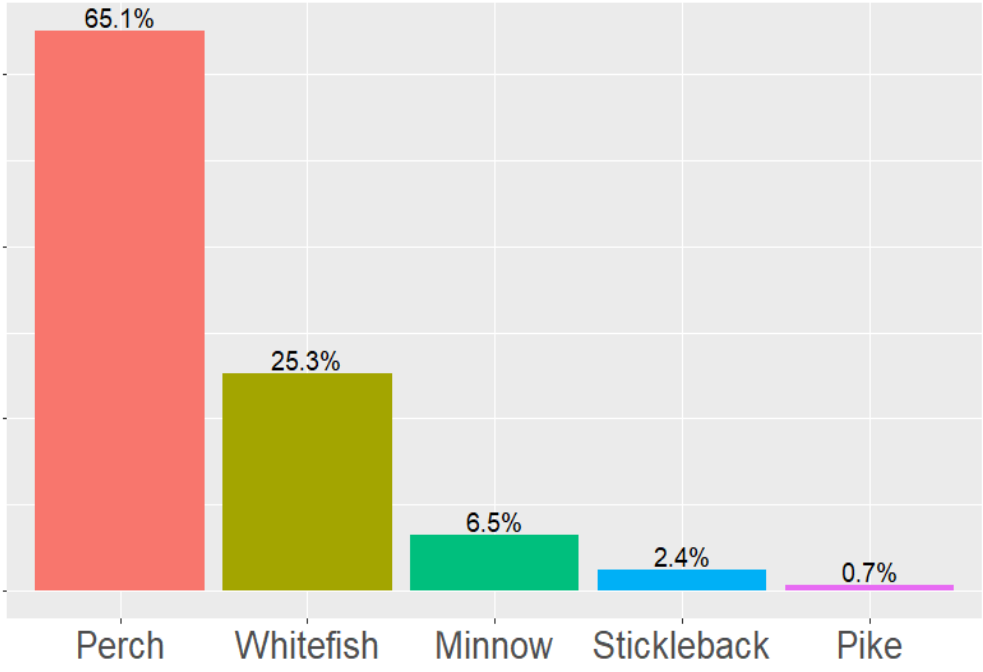

Figure 5 *Distribution of prey fish found in the metabarcoding analysis of all 20 tern samples, averaged across the 10 different lakes from the field recordings in 2024.*

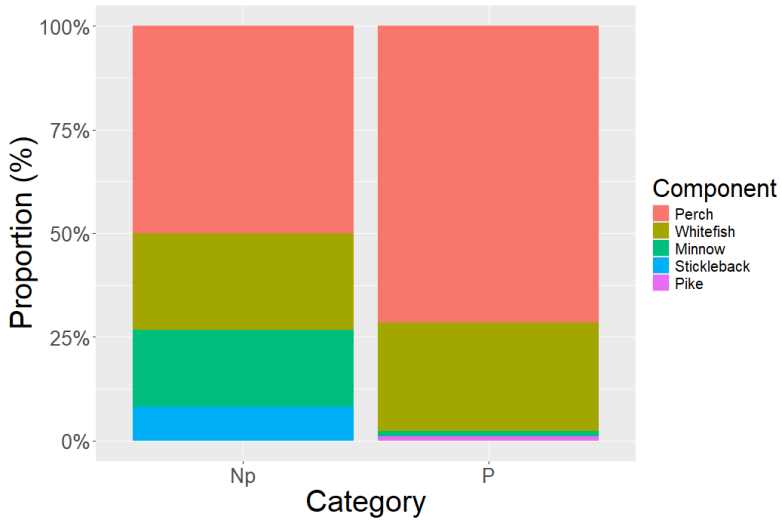

Figure 6
Proportions of fish species in the diet from 20 tern samples, averaged across lake, in the two different categories "No Pike" (Np) and "Pike" (P).

Averaged by lake, samples in the "No Pike" category exhibited higher Shannon diversity in diet composition compared to those in the "Pike" category. The mean Shannon diversity was 0.74 (N = 3) in pike absent lakes and 0.18 (N = 7) in pike presence lakes. Statistically significant differences in diet diversity between the two categories were narrowly missed (N = 10, Wilcoxon rank sum test with continuity correction, W = 19, p = 0.067).

Comparing between lake average proportions of whitefish in the diet (23.2 % in "No Pike", 26.2 % in "Pike"), I found no significant difference between pike presence lakes and pike absence lakes (N = 10, Wilcoxon rank sum test with continuity correction, W = 15, p = 0.347).

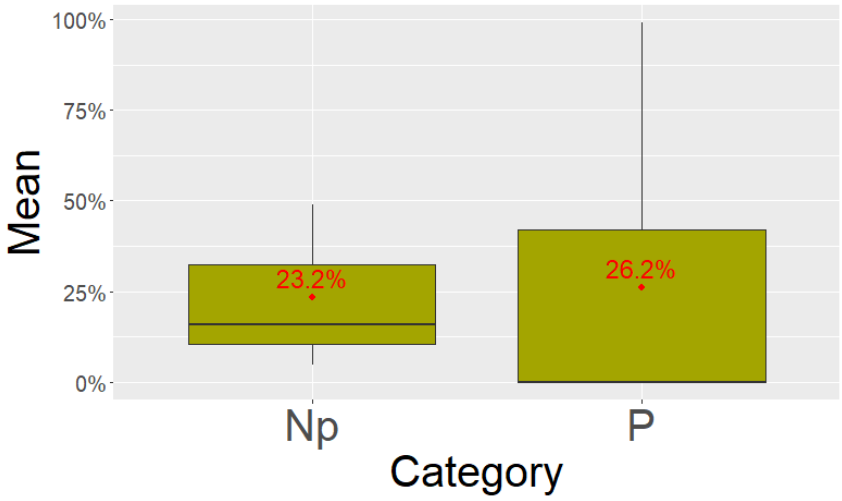

Figure 7
Boxplot of whitefish proportion in the two different categories "No Pike" (Np) and "Pike" (P), the median (black line) and then mean (red dot).

The proportions of perch in the diet showed lager, but not significant differences. In the "Pike" category it was 71.5 %, and in "No Pike" 50.1 % (N = 10, Wilcoxon rank sum test with continuity correction, W = 5, p = 0.253)

4. Discussion

In this study I was able to show that Arctic and Common Tern in inland Swedish lakes are more frequently found in lakes with pike than in lakes without pike. This supports findings from Öhlund et al. (2024), who reported generally higher abundance of piscivorous birds in these lakes, likely due to the higher biomass of small prey fish. It also aligns with general habitat and breeding preferences of Common and Arctic Terns, which favour high quality foraging conditions (Palmer 1941; Hawksley 1957). Our sampling design, based on two lake categories, and the hypothesis assuming more terns in pike lakes, justify applying simple presence - absence data derived from the visual survey. Complexity will increase if more and finely gridded data is available: Tern species, the type of detection (e.g. breeding / non-breeding), presence of further species, as well as their interaction, can be considered as response variables. Actual amount of prey in the lakes, geospatial attributes and limnologic characteristics can be included as predictors.

The analysis of tern diet revealed no substantial differences between the categories in relation to the proportion of whitefish; it was nearly equal in both lake categories. The most evident limitation arises from a lack of data, in particular only three data points were available from lakes without pike.

A higher, though not statistically significant, proportion of perch in the diet in lakes with pike might reflect underlying differences in fish community composition between the two lake categories. Compared to Öhlund et al. (2024), the unexpectedly high fraction of perch in tern diet could result from seasonal or interannual related peaks in availability of perch as prey fish. Diet diversity in ”No Pike” lakes was higher, although the difference narrowly missed statistical significance. If this trend is interpreted as an indication of less favourable foraging conditions, it could suggest that terns are driven to rely on a wider range of potentially less preferred prey. Especially as it includes higher proportions of minnow and even stickleback, species we assume to be rather unattractive prey for terns. However, this conclusion must be approached with caution as in the ”No Pike” category, only 6 individual samples from 3 lakes imply that a single sample substantially affects the outcome. Furthermore, 5 of the 6 samples came from Arctic Terns which are believed to generally select a more diverse diet than Common Terns (see Cramp 1989). The samples from ”Pike” lakes are mostly from Common Terns (12/14). Potential differences in diet diversity may therefore be due to species-specific foraging preferences rather than prey availability.

Terns, particularly Arctic Terns, are known to feed also on insects. Insects can be considered energetically as valuable as fish, but may require higher foraging efforts (Gilbert & Servello 2005). Therefore, they might be less favoured, particularly as chick provision and may serve as substitute prey indicating fish scarcity. Unfortunately, we could only test for fish in the diet and might have missed

important patterns in feeding ecology. Future research should aim for a broader set of primers in the amplification step of the extracted DNA from the faecal samples to reveal the full spectrum of diet components. This also brings the potential of an increased sample size, as all discovered bird faeces could be sampled and later excluded if the metabarcoding reveals they do not come from terns. Digital droplet PCR and a greater sequencing depth from the metabarcoding service could improve the detection of patterns, especially if a finer-scale analysis is desired.

I could not clearly answer the hypothesis of tern chicks being better conditioned in "Pike" lakes, mostly because of a lack of sufficient observations. Given the small sample size of 28 samples from 8 lakes, I nonetheless made efforts to evaluate chick condition using the data we collected. I fitted a GAM and analysed the residuals averaged by lake. The uncertainties resulting from averaging were appraised less severe than those from using pseudo replicates, as I would have done using the individual samples and chicks from the same lake appeared to be in similar growth phases. Here no significant differences between the category became apparent. However, this is problematic, since the model acts as a reference for the very data it was derived from, limiting the validity of the comparison and underscoring the need for independent data. Our data only provide measurements from both categories in a relatively short section at a medium hand length (see Fig. 4). Assessing the influence of pike absence or presence will therefore only be meaningful if the reference data comes from both categories. This suggested to redo the analysis with a subset, but too few data were left for meaningful statistical testing.

During the visual survey, identification of terns at genus level was feasible. They can be distinguished from other white birds such as gulls by their flight style and silhouette. However, I only relied on identification at species level, when terns were closer than one kilometre and clearly visible. Consequently, I performed the analysis of the visual survey data at genus level, unfortunately losing the ability to detect species specific habitat preferences.

At the lakes, terns were mostly detected when flying near the shore, searching prey, returning to nests or mating partners on stones, or resting on stones. I scanned not only the open water surface, but also the shoreline and moving objects in the air. Since birds might temporally disappear from the observer's view, the sampling procedure included two scans at 15-minute intervals to increase detection probability. I strived to survey in comparable weather conditions; Strong winds were avoided as it likely would reduce terns` foraging activities due to declining prey yield or drive them into sheltered waters (Dunn 1972; Taylor 1983). Precipitation made surveys impossible because after a few minutes the lenses of the spotting scope and binoculars were fogged. Sun glare, reflections, heat haze and mist reduced the detection distance, landscape features as islands, headlands or bays

could obscure parts of the lake. In any of these cases I increased the number of observation points to assure even coverage of the sampled lakes. Recording were performed during the main breeding season in June and July (Cramp 1989), to account for daytime dependant differences in the detection probability of terns we limited the survey to the main part of the day. The Observer bias is and remains inherit to this method (Bibby et al. 1992), it will not affect comparisons between lakes in this study, as all recordings are carried out by the same person, but might do so when comparing the results to other studies.

To keep the sampling effort manageable for a single person, we included only lakes with a surface area below 10 km². This may lead to an underestimation of tern occurrence, if extrapolated to the entire region, as they are likely to show preferences for larger lakes.

In conclusion, I am confident that I have reliably analysed and mapped tern distribution in the sampled lakes. However, it is important to carefully account for eco-geographical differences if these results are to be used for another purpose.

The faecal samples from the chick capturing yielded reliable results in the metabarcoding as they were fresh and clearly from our study species. But results may be biased, especially in regard to the, as prey fish, relatively large whitefish dwarf ecotype: A chick will not be able to swallow fish as large as an adult (Boecker 1967), and therefore more small fish will be represented in samples from chicks. More samples from adult birds are needed to control for possible age-dependant diet shifts. Especially Common Tern, reported to feed on fish up to 15 cm as adult (del Hoyo et al. 1996) is promising in the context of the whitefish dwarf ecotype as additional prey. But faecal samples, unless obtained from captured birds, are rarely found with the bird present and therefore not reliable if not genetically tested for species.

The method of tern chick capturing proved suitable for our purpose and field recordings were generally successful. However, it is important to acknowledge that multiple environmental factors influencing breeding success were not included in our analysis. For example, incorporating clutch size can be valuable for assessing prey availability when combined with chick condition, as larger clutches require more provisioning by the parents. Our focus on foraging quality did not account for other potential influences on breeding success, such as weather conditions or predation (Becker 1995; Becker et al. 1997; Scopel & Diamond 2018). We did not find any broods in lakes where none had been detected during the visual inventories. Future capturing should therefore be focused on lakes with confirmed, or expected nesting sites to increase the sampling efficiency. Generally terns are considered to be relatively tolerant to disturbance as common practice of visiting tern colonies suggests (e.g. Gochfeld 1981; Becker et al. 1997; Sanchez-Guzman & Viejo 1998; Paillisson et al. 2008; Wails et al. 2014). After sampling we could observe the adult birds returning to the nests quickly when we left. Nonetheless we were eager to

keep the handling time as short as possible and refrained from obtaining faecal samples from all chicks, as long as at least one sample per nest was retrieved. We sampled once in moist, cool conditions, but the chicks were fully feathered and less prone to hypothermia than younger, less developed chicks would have been. Handling chicks as we did it, requires close attention to ensure that each chick is handled with its corresponding bag to maintain the link between chick measurements and faeces. Even more important, because it impacts not only data quality but also animal welfare, is to prevent bags containing chicks from being blown away or accidently leave chicks in bags when concluding the recording. As the adult birds used to stay in the air above the nest, occasionally even attacking us, we were able to identify them at species level. Because of the small sample size, I conducted the analysis at genus level but took species level distinctions into account when interpreting the results.

Large amounts of data, ideally from time series are needed to construct representative growth models (e.g. LeCroy & LeCroy 1974; Klaassen et al. 1986) and evaluate chick condition.

However as previously reported (e.g. Palmer 1941; Hawksley 1957) and the distribution of nesting sites in this study suggest, terns might not establish broods under conditions of unsatisfying food supply by choice, or even because insufficient courtship feeding prevented egg laying (Nisbet 1977). For future studies, an alternative way of relating tern fitness to local environmental conditions may be to document fledging success (e.g. Green 2004).

This study supports an association between abundance of terns with pike presence in northern Swedish lakes. Although limitations in the dataset preluded definitive conclusions regarding breeding performance and diet composition, we could identify evidence for ecological effects. The successful data collection demonstrates the feasibility of our approach and provides valuable foundations for further recordings, ideally on a larger scale. Our work might prove valuable when combined with additional observations and supports the necessity and attraction of holistic approaches to combining aquatic and terrestrial ecology.

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Why do Sweden's terns eat and breed like, and were they do?

Terns are a very diverse and widely distributed bird group with approximately 50 known species. Scientists from Sweden closely looked at two of them: The Arctic and the Common Tern. In Sweden these two species are mainly found at the Baltic Sea, but also spread into the inland regions where they stay at lakes. Both tern species only come north for breeding. Common Terns stay during our winter along the southern coasts of Africa and Arctic Terns even fly into the Antarctic, which makes them the farthest migrating species on Earth! But besides this, terns are also fascinating in ecology, as terrestrial animals that eat fish we can see diversity patterns in the water reflected on land. Investigations have shown that in Swedish lakes a large fish predator, the Northern Pike, has a surprising effect on one of its prey fish: Whitefish can diversify into two types to reduce the risk of being eaten by pike. Either they grow very large and stay in the same water zones as pike, or they avoid pike and move to the open waters where they do not need to invest much in growth and stay quite small. But other predators have noticed! Fish eating birds like mergansers, terns and especially divers seem to adapt and preferably hunt for these small whitefish. Now a team of scientists from Sveriges lantbruksuniversitet in Umeå took a close look at terns in particular. An experienced data collector has been out to over 50 lakes and visually searched for terns. And the result is clear: Terns are nearly six times more likely found in lakes where pike is also present. But not enough, the scientists also wanted to find out if chicks from terns that breed in lakes with pike were doing better and if terns there would eat more whitefish. To investigate they captured chicks, measured them and took samples from the birds´ droppings. But this is where nature showed them who is in charge: They only found very few terns nesting in lakes without pike and could not gather enough data to compare chicks' condition. It is likely that terns just do not want to breed if there is not enough food for their family. The droppings were genetically analysed and showed that these terns mostly fed on perch, the second largest fraction was whitefish, proving its importance. Still no differences in tern diet in the two lake categories were found. But the tern-team from Umeå is not ready to throw in the towel so quickly! With the new field season, plans are already made for more data collections with additional species and more lakes and maybe this year they will find some more pieces in this fascinating bird-fish jigsaw puzzle.

Further readings: Detailed, scientific descriptions of Terns are given in (del Hoyo et al. 1996), the article about pike-whitefish interaction is (Öhlund et al. 2020) and (Taberlet 2018) give full, but demanding descriptions of environmental sampling and processing of DNA.

Appendix

Detailed description of the PCR procedure

The 25 tern samples were processed with another 30 samples from different bird species. I used a 96 well plate for the samples and a single 8 wells column for the extraction controls. The PCR reaction volume per sample was 25 µL which was a mix of 12.5 µL GoTaq Green Mastermix (Promega), 7.5 µL nuclease free water, 1 µL forward – and 1 µL reverse primer, and 3 µL of faecal DNA extract. Every approximately 10 samples one well was filled with the normal reaction mix but no DNA was added to control for contamination. Further 1 well per column was filled with exclusively distilled water to account for tag-jumping. All samples and controls obtained an individual combination of forward and reverse primer to allow pooling of all samples for metabarcoding. The array of 55 faecal samples, 23 field controls, 5 extraction controls, 13 water-filled wells, 9 wells with DNA free reaction mix and the primer combinations is shown in appendix 1. The filled plate and columns placed in a thermal cycler starting a PCR protocol of an initial denaturation phase at 95°C for 10 minutes, followed by 35 cycles at 95°C for 30s, 57°C for 30s, and 72°C for 1 minute, concluding with a final elongation at 72°C for 10 minutes and permanent hold at 8°C.

	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F13**
R1	H2O	B1	B3	F3	X	H7	I9	A9	E5	C2	E10	H6	Ex1	
R2	X	H2O	B5	F7	H1	H9	J1	C3	E7	C4	F6	I2	Ex3	
R3	J8	B9	H2O	G1	H3	X	J6	C5	F9	D4	F8	I4	H20	Ex5
R4	K2	C1	B7	H2O	D5	I1	A3	C7	J3	D8	X	H2O		X
R5	K4	X	E1	G3	H2O	I3	X	C9	A2	E2	H2O	I8		Ex6
R6	K6	D1	E9	G5	D7	H2O	A5	E3	A4	H2O	F10	J4		Ex7
R7	K8	D3	X	G7	F5	I5	H2O	X	H2O	E4	G6	J7		
R8	A1	D9	F1	G9	H5	I7	A7	H2O	B4	E6	G8	K1		

Appendix 1 *Array of the samples* ***and*** *controls for the PCR. Left the 96 well plate and right the 8 well column. Letter + number represents samples and controls (field controls from A2 onwards, extraction controls in the 8 well column), X is PRC mix without DNA and H2O pure water. The individual primer combination in each well, save the H2O wells that contain none, can be drawn from the forward primer in the first row and the reverse primer in the first column.*

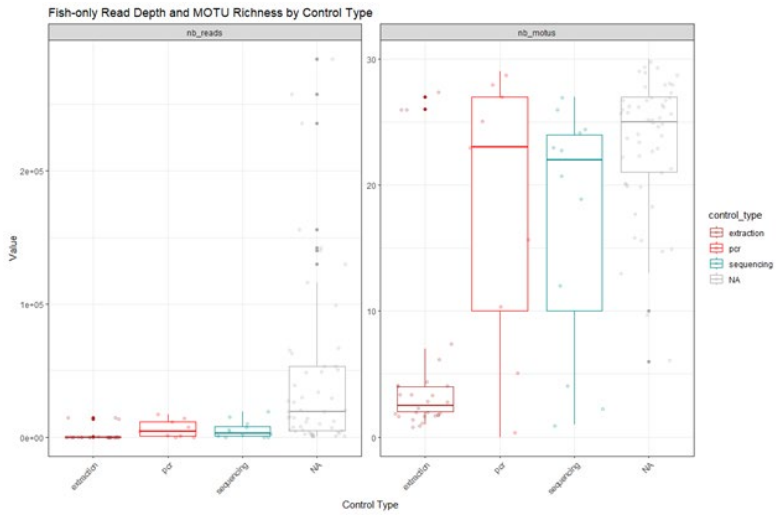
Details of the used bioinformatics

The pooled samples resulted in a total number of 17288958 raw reads. Data cleaning was performed to remove artefacts caused by contamination or replication errors.

Raw Illumina paired-end reads were processed using OBITools4. Forward and reverse reads were assembled using *obipairing* with *--min-identity=0.8* and *--min-overlap=10* to retain only well-aligned pairs. Unpaired reads were excluded using *obigrep -p 'annotations.mode != "join"'*. Reads were demultiplexed using *obimultiplex*, which used strict tag matching (@param,matching,strict*)*, allowed up to two primer mismatches *(@param,primer_mismatches,2)*, and excluded indels *(@param,indels,false)*. Unassigned reads were stored separately. The dataset was dereplicated by collapsing identical sequences with *obiuniq -m* sample, which tracked the abundance of each unique sequence across samples. Only the count and merged_sample attributes were retained using *obiannotate -k*.

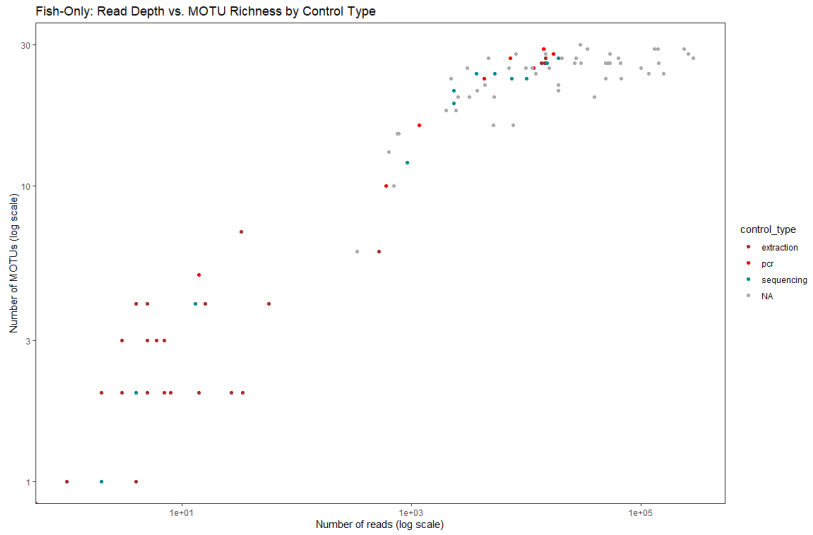
To identify true biological variants, PCR and sequencing errors were filtered with *obiclean -s sample -r 0.05 --detect-chimera -H*, retaining only "head" sequences considered genuine. Singletons, sequences with abundance < 1% of total reads and sequences shorter than 100 bp were excluded, as they are unlikely to originate from fish DNA in our samples.

Taxonomic assignment was carried out using *obitag* against the full EMBL database (version 143. April 2025) annotated with full NCBI taxonomy). Reads assigned at minimum to the order ray finned fish (Actinopterygii) were selected for further filtering. Fish reads were explored and filtered using the R package *metabaR* (Zinger et al. 2021). The number of reads and MOTUs per PCR was computed and visualized across control types using boxplots and log-log scatterplots to identify potential anomalies (appendix 2).



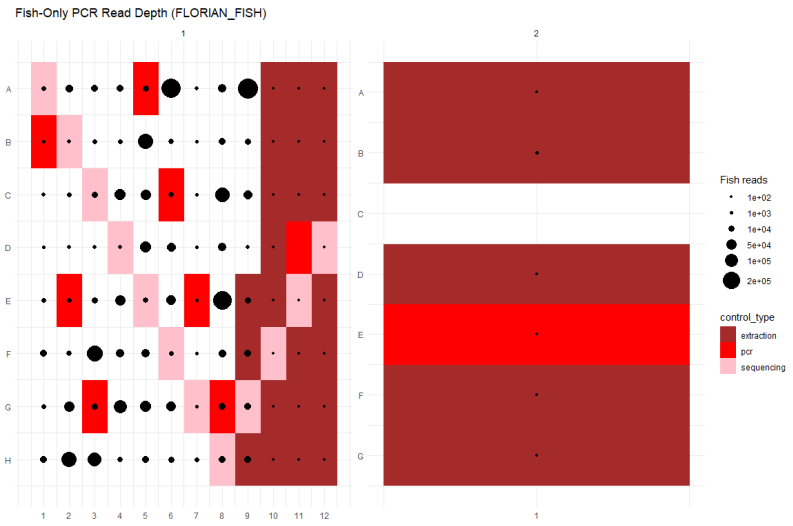
Appendix 2 *Boxplots showing read depth (nb_reads) and MOTU richness (nb_motus) by PCR control type in the fish-only dataset resulting from the metabarcoding of the processed faecal samples . N/A indicate biological samples, the field controls are included in extraction.*

Sample PCRs (NA in control_type, grey) generally showed higher read counts and more MOTUs than negative controls. Sequencing controls showed low read counts but can contain numerous MOTUs, indicative of tag-jump events. Sample PCRs (NA, grey) show high read counts and MOTU numbers. Negative controls, especially extraction and PCR (red shades), exhibit lower sequencing depth and diversity. Sequencing controls (cyan) cluster at low read depth but show a broad range of MOTUs, suggesting tag-jump events. (appendix 3).



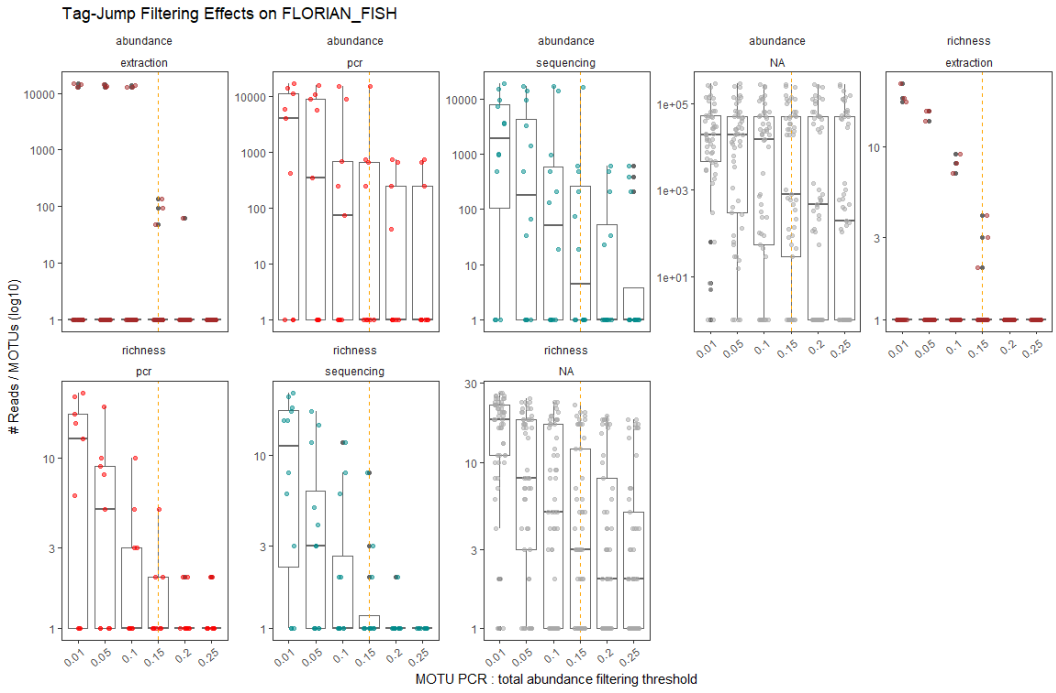
Appendix 3 *scatterplot showing read depth (nb_reads) and MOTU richness (nb_motus) by PCR control type in the fish-only dataset. N/A indicate biological samples.*

The spatial layout of the PCR setup was examined. Extraction and PCR controls (red) generally show low read counts. PCR and sequencing negative controls (dark red and pink) showed sporadic read presence, consistent with low-level tag-jumps. (see appendix 4, for sample IDs see appendix 1).



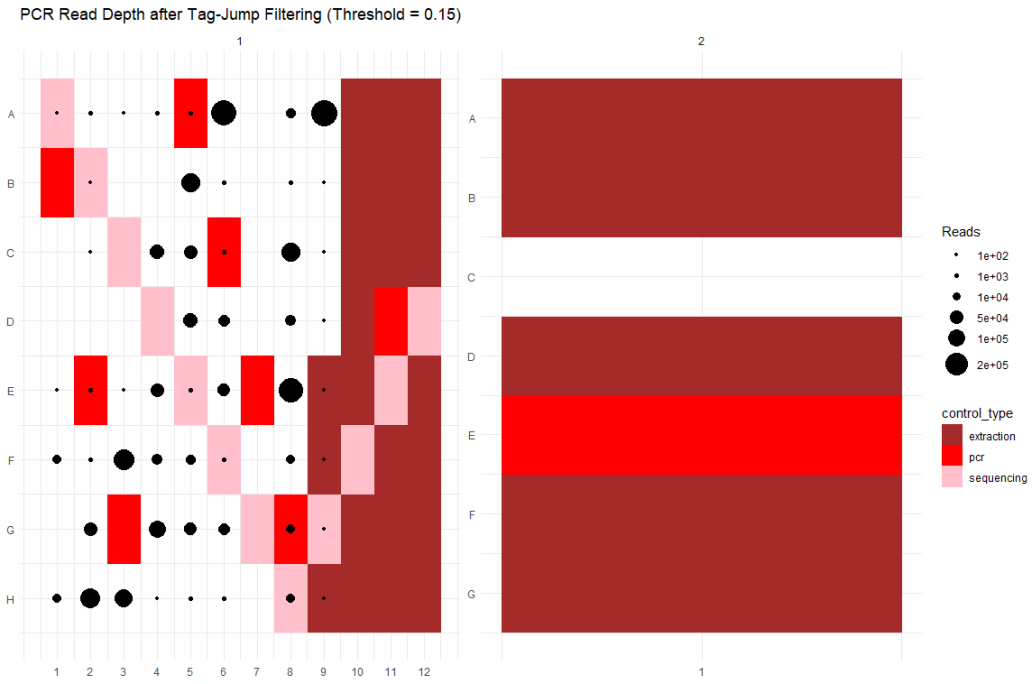
Appendix 4 *Heatmap and dot plot showing fish-only read depth across PCR plates by well position and control type. Dot size reflects total fish read counts per PCR; background colour indicates the type of control. White background indicates biological samples.*

Tag jumping occurs when primer combinations assigned to DNA fragments from one sample, shift place and bind to those of other samples (Schnell et al. 2015). If not removed, this leads reads being assigned to the wrong sample or false reads in appearing in controls. Tag-jumps were filtered using "tagjumpslayer" by applying a relative abundance threshold with visual validation across control types. Consistent with tag-jump removal, abundance thresholds reduced both read and MOTU counts in controls, especially in sequencing negatives. A threshold around 0.15 (orange dashed line) balances the reduction of artefacts in controls while preserving richness in biological samples (NA) (appendix 5).



Appendix 5 *Effects of tag-jump filtering thresholds on fish-only read abundance and MOTU richness across control types and samples. Each panel shows how increasing the MOTU PCR relative abundance threshold (x-axis) affects the number of reads and MOTUs per PCR (log scale on y-axis), with separate panels for each control_type and samples (extraction, PCR, sequencing, and NA for samples).*

After filtering, read counts in sequencing negative controls drop markedly (appendix 6). Tag jump filtering unfortunately removed all reads from 13 of 55 biological samples, resulting in the loss of 5 out of 25 tern samples.



Appendix 6 *Fish-only PCR read depth across the plate after tag-jump filtering (threshold = 0.15). Each circle represents a PCR reaction, with its size indicating the number of reads retained after filtering. PCRs are arranged by plate layout, and shaded by control type (extraction, PCR, sequencing). White background indicates biological samples.*

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Publishing and archiving

☒ YES, I, Florian Feind, have read and agree to the agreement for publication and the personal data processing that takes place in connection with this.