Avian zoochory is a probable pathway of European perch colonization in artificial lakes: a reply to Vasemägi et al (2023)

Reply on Vasemägi, A., Huss, M., Gårdmark, A., Ozerov, M., 2023. On ability of perch to colonize new waterbodies - indirect evidence and sticky facts: A comment on: ‘Multiple lines and levels of evidence for avian zoochory promoting fish colonization of artificial lakes’ (2023), by Garcia et al. Biology Letters. 2023;19(3):20220533. (10.1098/rsbl.2022.0533)

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Vasemägi et al. (2023) suggested that the multiple lines and levels of evidence (MLLE) approach presented by Garcia et al. (2023) failed to provide adequate support for avian zoochory promoting the colonization of gravel pit lakes by European perch (*Perca fluviatilis*). We graciously disagree, and welcome the opportunity to further bolster the robustness of our original findings. The utility of the MLLE approach is the manner in which it merges different types (lines) of evidence from diverse fields of study, while also considering the strength (level) of the evidence to infer causal relationships that cannot be studied or assessed directly. This indirect method is inspired by epidemiological studies where direct demonstration of ‘causation’ is difficult or impossible due to the difficulty, and at times the ethics, of conducting empirical and experimental studies (3). Given the infeasibility of empirical demonstration of avian zoochory occurrence, we provide MLLE indicating that avian zoochory “*is a highly probable primary colonization pathway for pioneer fish species like European perch in newly-created artificial lakes*” (2) and then laid the foundation for future studies where certain sources of evidence could be tested. Vasemägi et al. (2023) have regrettably overlooked the core philosophy of the MLLE approach by attempting to critique individual pieces of evidence rather than considering the totality of evidence we presented. However, in doing so, they have now offered us the opportunity to present additional evidence that further supports of original conclusion.

First, our data clearly demonstrates a high likelihood of temporal overlap between waterfowl presence and European perch spawning in the study area. We agree that co-occurrence between fish and waterfowl, taken alone, falls short in proving avian-mediated zoochory, but that was not the intention. Rather, the mere instance of co-occurrence is simply the first line of evidence required to invite the possibility of avian-mediated zoochory. We agree that perch spawning was not directly quantified during 2012-2019, but it is well acknowledged that the onset of European perch spawning is largely determined by the post-winter rise in water temperature, initiating between 8°C and 10°C (4). While temperature loggers were not installed in each of the 37 lakes sampled, high-frequency measurements were made in 17 gravel pit lakes (45.9 %) distributed across the full extent of the study area (**Fig 1**). The study extent is small (64 km²) with limited thermal variability between lakes, notably during the winter (**Fig 2**). Therefore, this does not affect our estimates of perch spawning period. Vasemägi et al. (2023) also take issue that our quantification of waterfowl abundance was from a single lake located “*in the center of the study area, and more than a decade earlier, in 1996-1998*”. They fail to recognize that monthly waterfowl abundance data are rare and, in this case, more than sufficient to identify wintering period. Furthermore, they overlooked that weekly censuses of waterfowl community were conducted during the winter in 9 lakes (censuses on neighboring SOA/SOB/SOC/SOD lakes merged in (2)) over a 14-year period (2005-2018), including 6 of our sampled lakes (**Fig 1**). These waterfowl censuses were used to assess waterfowl community structure, but also confirmed high waterfowl densities in the study area during the wintering period, for years that overlap with fish community surveys (2012-2019; (2)).

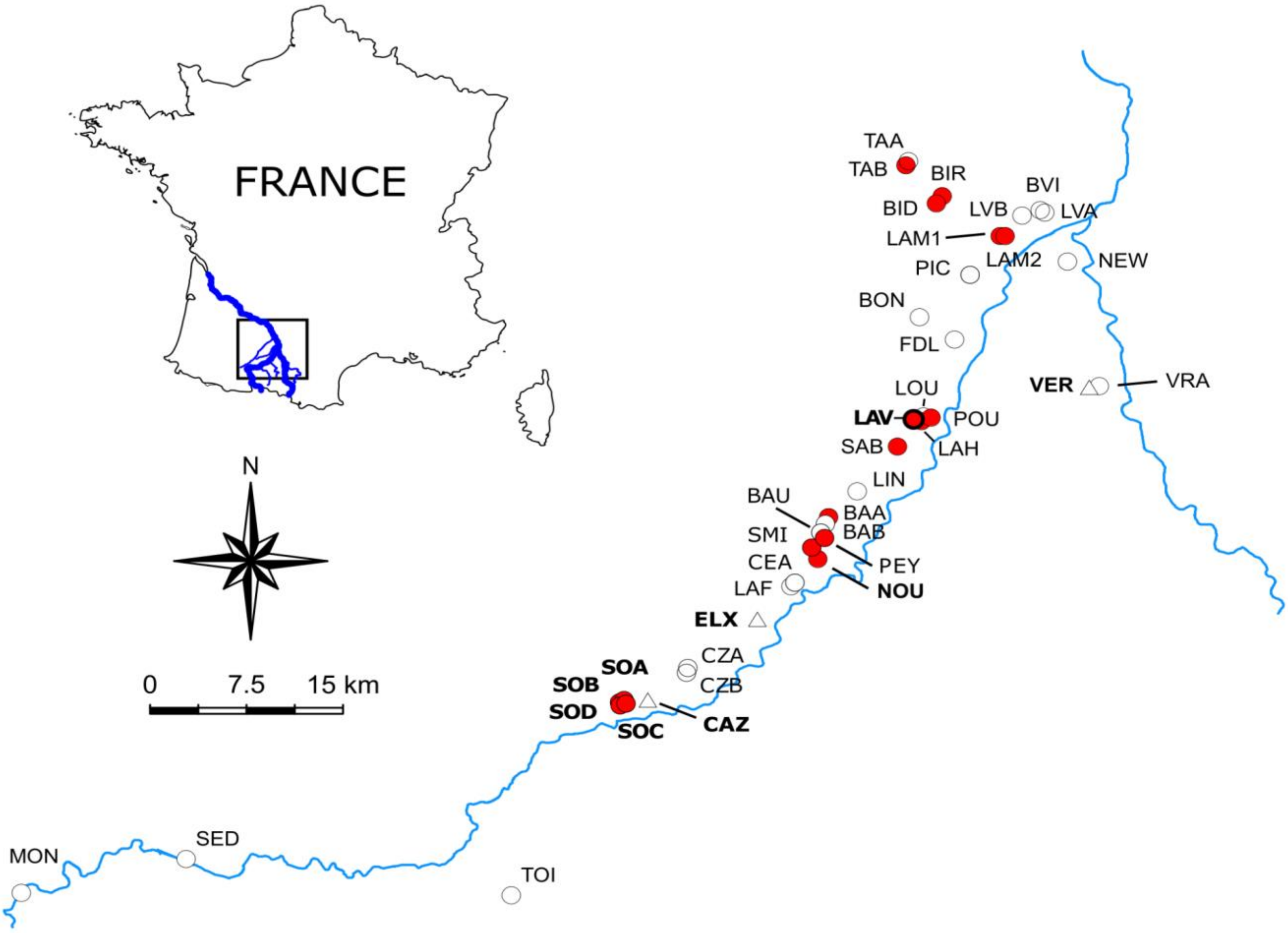

Figure 1. Map of the study area (2). Gravel pit lakes subject to fish community surveys and targeted surveys for European perch (white circles), temperature monitoring (red circles), weekly waterfowl censuses from 1996 to 1998 (bolded circle), and annual censuses from 2005 to 2018 (lake names in bold, and triangles for unsampled lakes) are delineated.

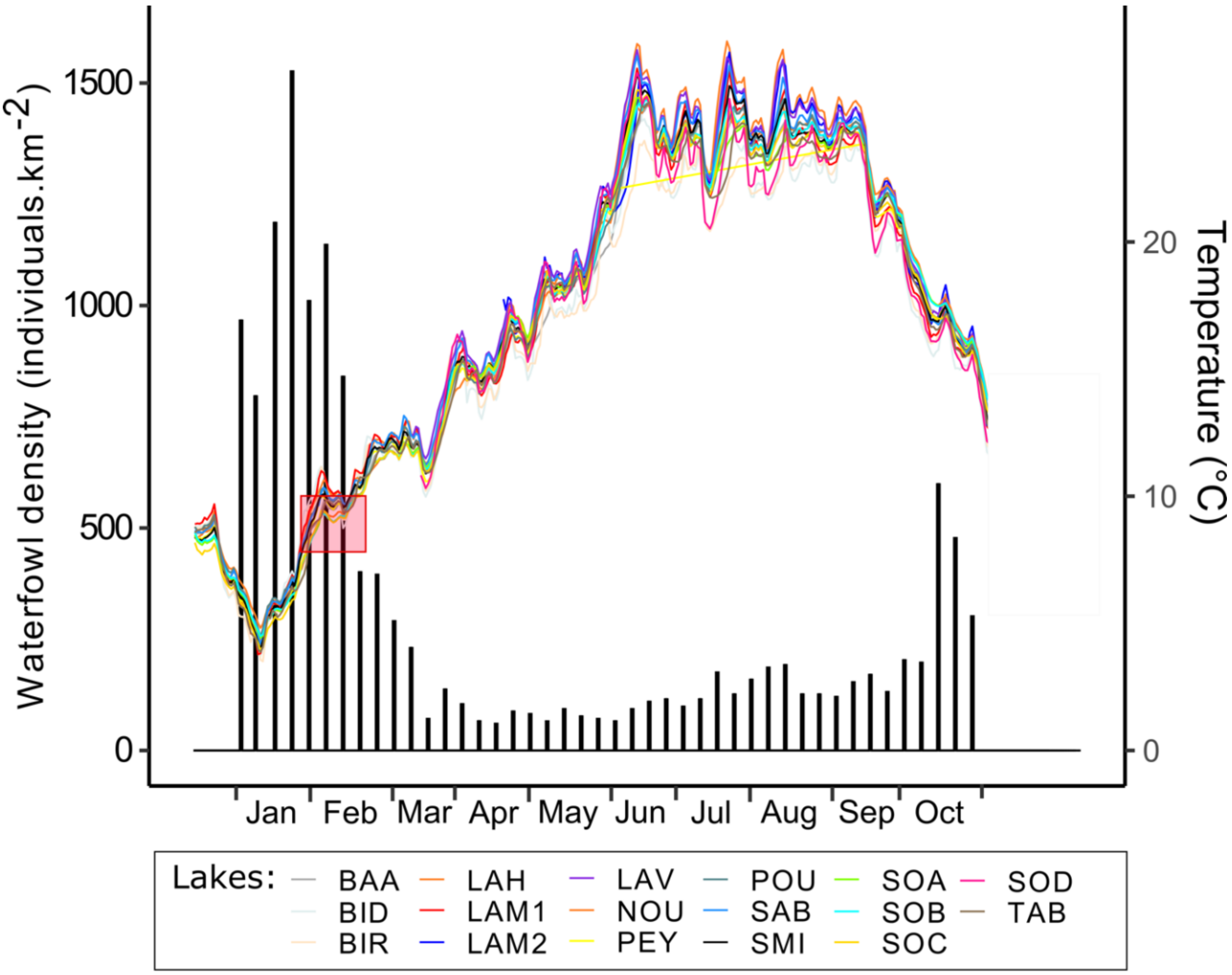

Figure 2. Weekly waterfowl density measured in lake LAV between 1996 and 1998 (ind.km^{-2}; (5)) and daily water temperature (°C) measured in 2020–2021 in 17 lakes. The red area represents the onset of the European perch spawning period (8–10°C) which is very similar across lakes.

Second, Vasemägi et al. (2023) argue that the transport of European perch eggs by waterfowl is not plausible because eggs are not sticky, thus limiting the likelihood of ectozoochory. However, the fact remains that perch deposit long, gelatinous ribbons of eggs that measure up to several meters, in submerged aquatic vegetation, roots, and dead tree branches (4,6,7) where mallards and coots frequently dabble and forage. They point out that egg ribbons of perch might deter a variety of teleost and invertebrate predators, because of unpalatability or due to the presence of potentially noxious components in perch egg shells and matrix (8,9), suggesting that avian endozoochory was unlikely. We disagree with this viewpoint. The studies cited by Vasemägi et al. (2023) do not demonstrate that perch eggs with their gelatinous matrix are not consumed by some invertebrates and fish; only a preference towards the consumption of eggs without this matrix is shown (8,9). It is also worth stating that the dietary preferences and food item toxicity effects in invertebrates and fishes are vast (10) and can be far different from those of birds (e.g., 11); making such extrapolation tenuous. Nonetheless, mallards and coots actively feed on fish eggs (12,13), suggesting that perch eggs might represent a potential food resource for waterfowl when other resources are scarce. European perch is the dominant species in many gravel pit lakes (14), spawning much earlier than other common fish species such as *Rutilus rutilus*, *Lepomis gibbosus*, *Scardinius erythrophthalmus*, *Tinca tinca*, *Cyprinus carpio* or *Micropterus salmoides* (4), leading to the production of extreme egg biomass when waterfowl are highly abundant.

Third, despite Vasemägi et al. (2023) claims, we reiterate that human-mediated introduction of European perch is very unlikely. Indeed, humans strongly influence the introduction of some freshwater species in the study area (15,16). Yet, our survey revealed that only one single recreational angler (i.e. 1% of the anglers questioned, n=99) released European perch, suggesting that human-mediated introduction of perch cannot solely explain their distribution in our study area. This is notably because this species is the main target of only 8.5% of anglers questioned, which is much lower compared to targeted species (e.g., Common carp *Cyprinus carpio*=40.4%, Largemouth bass *Micropterus salmoides*=23.4%). To further support our assertion, we compared European perch to a species known to be widely introduced by humans in the study area like Largemouth bass (17). They are significantly more present in public-access lakes compared to the perch, and are stocked both by managers and anglers; demonstrating that angler surveys can detect illegal stocking (**Table 1**).

Table 1. Comparisons of pattern in occurrence, genetic and reproduction characteristics between Largemouth bass (*Micropterus salmoides*) and European perch (*Perca fluviatilis*).

	Largemouth bass (*Micropterus salmoides*)	**European perch (*Perca fluviatilis*)**
Occurrence (prohibited-access vs public-access lakes)	Higher in public-access lakes (glmm: $\chi2$ = 8.350; p = 0.004; n=1/12 vs n=17/25)	Similar in both lake types (glmm: $\chi2$ = 0.022; p = 0.883; n=1/12 vs n=17/25)
Legal stocking by managers	0.178 kg/ha/year	0 kg/ha/year
Illegal stocking by anglers	9.1 % of anglers report introducing during their lifetime (n = 9/99)	1.0 % of anglers report introducing during their lifetime (n = 1/99)

Preferred species for anglers	23.4%	8.5%
Isolation by Distance (IBD)	Non significant (r = 0.155 ; p = 0.23)	Significant (r = 0.230; p = 0.04)

Fourth, Vasemägi et al. (2023) challenge our interpretation that a significant isolation by distance (IBD) is supportive of bird zoochory because significant IBD can be also generated by human-mediated dispersal. We understand their rationale and would agree if our interpretation of the significant IBD pattern was not made in the light of all other lines of evidence (2). In addition, when performing a similar analysis (sequence-based microsatellite genotyping workflow (18), see methodological details in Supporting Information of (2)) for Largemouth bass using 21 microsatellite markers on 333 individuals from 16 lakes (17), we failed to detect a significant IBD (**Table 1**) despite the fact that the dispersal of this species is mostly human-mediated. Vasemägi et al. (2023) also suggest that the eight first-generation migrants we detected might represent misassignments. They claimed that separating misassignments from real immigrants is challenging and that the accurate detection of migrants requires a thorough characterization of type I and type II errors. We fully agree with their statement but regret that they did not acknowledge the sensitivity analyses in our original article (Supporting Information in (2)). Further, Vasemägi et al. (2023) performed additional simulations to highlight that misassignments can occur and stated that, despite an overall very high assignment accuracy (99.49%), some populations displayed a lower accuracy (i.e. 94.2, 96.5 and 98.4%). These populations correspond to three neighboring lakes of the Soulance complex (SOB, SOC and SOD, Supporting Information in (1)) that we already identified as being more prone to misassignments due to their closeness (Supporting Information in (2)). We then conducted the same simulations as Vasemägi et al. (2023), but by considering all lakes of the Soulance complex as a single population. The overall assignment accuracy was 99.88%, and the lowest accuracy measured at the lake level was 99.4% (**Table SI.1**). Although misassignments are inevitable, the accuracies reported in our study are extremely high, demonstrating the robustness of our approach and genetic dataset.

In conclusion, Garcia et al. (2023) and the additional results reported here confirm that avian zoochory is a highly probable pathway for European perch colonization of artificial lakes. We also agree with Vasemägi et al. (2023) that more empirical and experimental studies investigating the role of avian-mediated zoochory of fish are needed. Such investigations should aim to provide direct evidence of i) the consumption of European perch eggs by waterfowl (experimentation *as per* (19)), ii) the transport of European perch by waterfowl (eDNA on bird feces and feathers) and iii) the arrival of European perch and other fish species by monitoring the long-term dynamics of community assembly in newly created freshwater ecosystems.

Data accessibility. Supplementary material, including the results of genetic assignment simulations performed using ONCOR software (20), is provided after the references of the main text (Table SI.1).

Authors' contributions. F.G.: analysis, writing original draft, revision and editing; I.P.-V.: genetic analysis, revision and editing; J.D.O.: revision and editing; J.C.: revision and editing. All authors gave final approval for preprinting and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare having no competing interests.

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Supplementary Material

Table SI.1. Genetic assignment accuracy of P. fluviatilis populations genotyped at 21 microsatellite markers (2) assessed using the same approach as in Vasemägi et al (1) based on simulated mixture samples composed of a single population only (100% simulations; 1000 mixtures of 200 individuals generated for each population) performed in ONCOR (20). The only difference with Vasemägi et al's analysis is that we pooled together individuals from the four neighboring lakes of the Soulance complex (SOA, SOB, SOC and SOD in Garcia et al's and Vasemägi et al's 2023 papers). The resulting pool of individuals is named SO in the table, and we assumed it corresponds to a single population.

Population ID	**Average**	**95% Confidence Interval**	**Standard deviation**
BIR	0.9988	(0.9916, 1.0000)	0.0023
BID	0.9997	(0.9955, 1.0000)	0.0011
LVB	0.9999	(0.9966, 1.0000)	0.0008
BVI	1.0000	(1.0000, 1.0000)	0.0001
LAM1	1.0000	(1.0000, 1.0000)	0.0004
LAM2	0.9999	(1.0000, 1.0000)	0.0004
NEW	0.9998	(0.9968, 1.0000)	0.0008
PIC	0.9995	(0.9950, 1.0000)	0.0014
FDL	0.9969	(0.9857, 1.0000)	0.0045
LOU	0.9945	(0.9798, 1.0000)	0.0058
LAV	0.9948	(0.9832, 1.0000)	0.0051
LAH	1.0000	(1.0000, 1.0000)	0.0002
POU	0.9971	(0.9872, 1.0000)	0.0037
LIN	0.9999	(0.9998, 1.0000)	0.0004
BAB	1.0000	(1.0000, 1.0000)	0.0003
BAA	0.9999	(0.9973, 1.0000)	0.0007
PEY	0.994	(0.9789, 1.0000)	0.0061
BAU	0.9999	(0.9990, 1.0000)	0.0006
SMI	0.9989	(0.9921, 1.0000)	0.0023
LAF	1.0000	(1.0000, 1.0000)	0.0002
SO	0.9998	(0.9967, 1.0000)	0.0008
SED	0.999	(0.9918, 1.0000)	0.0023
MON	0.9999	(0.9966, 1.0000)	0.0007
TOI	1	(1.0000, 1.0000)	0