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Population ecology

Multiple lines and levels of evidence for avian zoochory promoting fish colonization of artificial lakes

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Understanding how obligate freshwater organisms colonize seemingly isolated ecosystems has long fascinated ecologists. While recent investigations reveal that fish eggs can survive the digestive tract of birds and successfully hatch once deposited, evidence for avian zoochory *in natura* is still lacking. Here, we used a 'multiple lines and levels of evidence' approach to demonstrate possible bird-mediated colonization of lakes by the European perch (*Perca fluviatilis*). We studied a set of newly-formed and isolated artificial lakes that the public is either prohibited to access because of gravel extraction or allowed to access (mainly for angling). The motivating observation is that a large proportion of prohibited-access lakes (greater than 80%) were colonized by European perch even though stocking by anglers and managers never occurred. Three supplementary lines of evidence supported avian zoochory. First, European perch spawning occurs when waterfowl abundance is very high. Second, European perch lays sticky eggs at shallow depths where they can be eaten by waterfowls or attached to their bodies. Third, genetic analyses suggested that European perch actually migrate among lakes, and that distances moved match with daily flight range of foraging waterfowl. Together, multiple lines of evidence point to avian zoochory as a probable pathway for fish colonizing remote or newly-formed freshwater ecosystems.

1. Introduction

Fascinated with the biogeography of freshwater life, Charles Darwin [1] notoriously suspended the feet of a duck in an aquarium where the ova of mollusks were hatching and observed that many larvae firmly attached themselves to the duck's feet. He remarked that 'These just hatched molluscs, though aquatic in their nature, survived on the duck's feet, in damp air, from 12 to 20 h; and in this length of time a duck or heron might fly at least six or seven hundred miles, and would be sure to alight on a pool or rivulet, if blown across the sea to an oceanic island or to any other distant point'. Fast forward over a century and a half, and scientists remain puzzled by the presence of putatively strict waterborne dispersal organisms in isolated lakes and ponds. Take freshwater fish as an example. Past connections between water bodies or human-mediated introductions can explain fish presence in many lakes [2,3]. Yet, fish also occur in lakes that have never been connected to other water bodies such as those separated by vast stretches of dry land or open ocean

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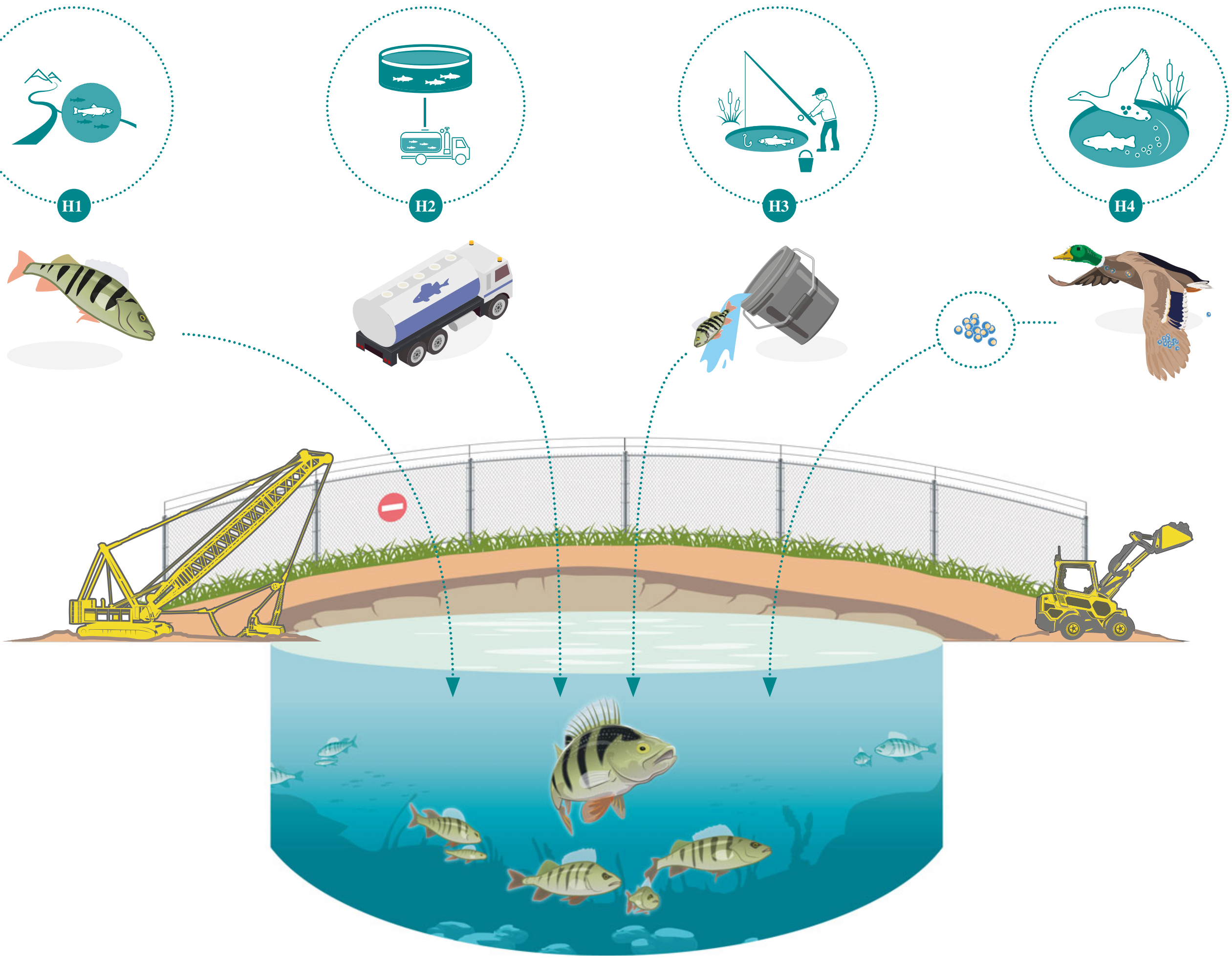

Figure 1. Hypothesized colonization pathways of European perch, *Perca fluviatilis*, in artificial gravel pit lakes: (H1) active waterborne colonization; (H2) legal stocking by lake managers; (H3) illegal 'bucket release' by anglers; (H4) colonization through avian zoochory.

crater and desert lakes [4]; or even lakes shielded from human stocking such as newly-created artificial lakes which are now teeming with fishes [5].

Growing evidence now points to the importance of bird-mediated colonization, i.e. *avian zoochory*, in shaping the distribution of aquatic plants, zooplankton and macroinvertebrates [6–9]. Avian zoochory has also long been suggested to serve as a potential colonization vector for freshwater fish, mostly through *ectozoochory* [10,11] (i.e. dispersal of propagules externally on birds' bodies). However, recent experimental studies suggest that fish dispersal through avian *endozoochory* (i.e. a mechanism widely supported for other organisms such as mollusks and plants; [12,13]) is mechanically possible for fish eggs, which can survive through the digestive tract of waterfowl and successfully hatch [14,15]. Yet, empirical evidence of bird-mediated fish dispersal in the wild is still lacking.

Direct characterization of bird-mediated fish colonization of a water body is challenging, as it would require demonstrating that (i) fish were truly absent from the recipient water body prior to any bird-mediated colonization event, (ii) bird(s) carrying fish eggs move from a fish source population to the formerly fishless water body, (iii) birds 'release' fish eggs in the recipient water body, and that (iv) these eggs successfully hatch and ultimately establish a self-sustaining population. However, indirect characterization in support of bird-mediated fish colonization is probable by providing empirical evidence where other possible colonization pathways are excluded and the aforementioned conditions for avian zoochory are deemed possible [11].

Here, we deploy a 'multiple lines and levels of evidence' approach to support bird-mediated fish colonization of artificial gravel pit lakes by the European perch, *Perca fluviatilis* (figure 1). Originally developed in epidemiology, multiple lines and levels of evidence approaches are powerful when experimental data are unavailable, and challenges to inferring causality exist. First, we used a set of multidisciplinary approaches (interviews, questionnaires, field surveys, population genetic analyses and the literature) to exclude active aquatic colonization by fish (H1) and human-mediated colonization either through legal stocking by lake managers (H2) or illegal release by anglers (H3). Second, we indirectly demonstrated that avian zoochory was a likely colonization pathway for fish (H4) by showing that there is (*i*) a synchrony between the onset of fish spawning and the waterfowl wintering period, (*ii*) an overlap between egg laying locations and waterfowl foraging areas, and (*iii*) egg transport and survival between lakes as supported by the literature.

2. Material and methods

The distribution of European perch was examined in 37 artificial gravel pit lakes (aged from 9 to 56 years following the start of gravel extraction) located within a 70 × 75 km area of the

Garonne River floodplain, France ([5]; electronic supplementary material, figure SI(1)). Lakes are disconnected from the hydrographic network, and no flood events have connected lakes among them or with the hydrographic network because lakes are relatively far from the main rivers. 'Prohibited-access lakes' (12/37 lakes) are where gravel extraction is performed by private companies (electronic supplementary material, figure SI(1)). Legally permitted for industrial use, these young lakes have restricted access regulations and are most often fenced with signs indicating that public access and leisure (angling, navigating and bathing) are prohibited, limiting access to the sites. They are usually geographically clustered because a given company will excavate several lakes, and restricted access is enforced until the last lake is filled by precipitation and groundwater infiltration; a process that usually takes a couple of decades. After that period, the lakes become generally 'public-access lakes' (25/37 lakes; electronic supplementary material, figure SI(1)) and support recreational activities such as angling, shoreline walking and watersports. These older lakes are managed under different regulations and are predominantly accessible to the general public, with no specific regulations in terms of access.

Fish communities were surveyed in each lake at least once during the period 2012–2019, using an integrative approach combining gillnets and electrofishing [5]. In total, 13 814 fish belonging to 29 species were sampled, including 4360 European perch individuals (mean = 92.8 ± 152.6 individuals · lake^{-1}). European perch was the dominant species (present in 78% of the studied lakes). A sub-sample of 531 individuals from 27 populations were genotyped using 23 sequence-based microsatellite loci to quantify genetic diversity (AR, i.e. allelic richness), determine the genetic structure (assignment of individuals to clusters, i.e. groups of genetically homogeneous individuals) within the study area and identify potential first-generation migrants (i.e. individuals in a sampled population that are significantly assigned to another population on the basis of multilocus genotype data) (electronic supplementary material). The occurrence of an isolation-by-distance (IBD) pattern was tested to explore the relationship between geographical and genetic distances among European perch populations (electronic supplementary material). Interviews with lake managers ($n = 31$) were performed to assess the species and quantity of stocked fish between 2011 and 2018, resulting in responses for 86% of lakes containing European perch. Questionnaires to local anglers ($n = 94$) were used to assess illegal 'bucket release' practices in gravel pit lakes. If anglers performed illegal 'bucket release', information about the species released and body size at release were collected.

We assessed temporal variability in waterfowl abundance (individuals · km^{-2}) according to weekly censuses conducted over a 3-year period (1996–1998) in one lake located at the centre of the study area [16]. In addition, bird counts performed annually in February over a 14-year period (2005–2018) on six lakes were used to assess waterfowl community composition. The spawning phenology of European perch was determined by combining information from the literature on spawning temperature and temperature measured in 17 lakes using Hobo sensors.

3. Results and discussion

(a) Rejected colonization pathways

Active aquatic colonization by European perch (H1) was impossible as the study lakes demonstrate seepage hydrology and, are disconnected from other water bodies. The occurrence of European perch was high and did not statistically differ between prohibited-access lakes (83%, $n = 10/12$) and public-access lakes (76%, $n = 19/25$) (glmm: $\chi^2 = 0.022$; $p = 0.883$). This pattern could not be explained by legal stocking (H2) because all interviewed managers of prohibited-access lakes indicated that fish stocking had never occurred in the lakes they managed. Our survey of anglers revealed that illegal introduction through bucket releases (H3) was also unlikely to explain this pattern because (i) interest for European perch angling was relatively low (i.e. primary species of interest for only 8.5% of anglers) and (ii) illegal bucket release of European perch was extremely rare. In fact, only 20% ($n = 19$ of 94) of anglers admitted illegal bucket releases of predominantly largemouth bass (*Micropterus salmoides*, with 39.1%) or common carp (*Cyprinus carpio*, with 17.1%). Importantly, only one angler among 94 admitted performing bucket release of European perch. Furthermore, these rare instances of illegal releases are more likely to occur among public-access lakes. Despite the lack of interest of anglers by European perch, this species could have been accidentally introduced as a contaminant while releasing other species (e.g. species misidentification). Although this may have occurred if very small juveniles of other species were released by anglers, our survey of anglers revealed that large individuals (ranging from 100 to 730 mm; 335.26 ± 204.38 mm) were exclusively released in the rare instances of illegal stocking. Importantly, this evidence for lack of legal stocking by fisheries agencies (H2) and negligible illegal bucket releases from anglers (H3) were further supported by genetic analyses. If humans were involved in European perch colonization, the genetic diversity and structure of European perch populations would be higher in public-access lakes, where stocking occurs [17]. Nevertheless, we found no differences between prohibited-access lakes (AR: 2.260 ± 0.296; mean number of represented genetic clusters: 2 ± 1) and public-access lakes (AR: 2.303 ± 0.217; genetic clusters: 1.722 ± 0.895) (lmm: $\chi^2 = 1.170$; $p = 0.279$ and glmm: $\chi2 = 0.089$; $p < 0.765$, respectively).

Genetic analyses were also used to identify potential European perch first-generation migrants, revealing that (i) recent migration is occurring in our study area (i.e. 8/531 genotyped individuals were identified as first-generation migrants with a high accuracy, see details in electronic supplementary material) and (ii) 25% of these migrations occurred between prohibited-access lakes. Taken together, because of the impossibility of European perch active colonization, the limited presence of humans, and the absence of legal and illegal stocking practices in prohibited-access lakes, we posit that European perch colonization by avian zoochory is a viable hypothesis worth further consideration.

(b) Avian zoochory as a probable pathway

European perch spawning occurs when water temperatures are between 8 and 10°C [18]. Based on temperature monitoring of gravel pit lakes, this condition occurs from end of January (8°C reached on 30 January) to early February (8°C ≤ T ≤ 10°C until 18 February). This spawning period aligns with the end of the waterfowl wintering period (figure 2*a*) when bird abundance remains very high (i.e. 1137 individuals · km^{-2} in the first week of February). During this period, the waterfowl community comprises 35 species, numerically dominated by mallard (*Anas platyrhynchos*, Anatidae) and Eurasian coot (*Fulica atra*, Rallidae) (figure 2*b*). Furthermore, European perch eggs are held

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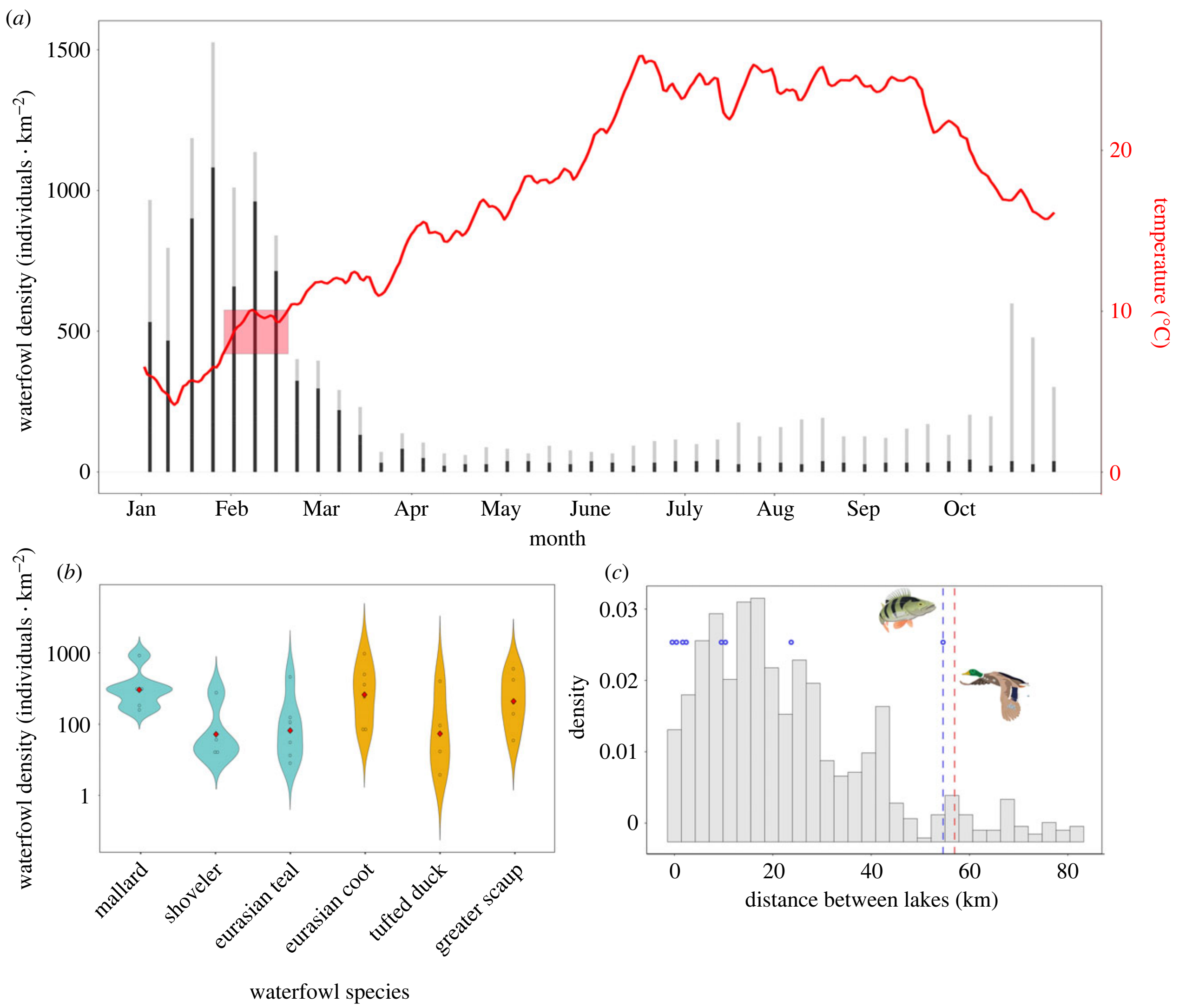

Figure 2. (*a*) Waterfowl density (individuals · km^{-2}; left *y*-axis) between 1996 and 1998 [16] and daily water temperature measured in 2020–2021 (in degrees Celsius; right *y*-axis). Red square indicates the onset of European perch spawning period (8–10°C). (*b*) Waterfowl density (individuals · km^{-2}, log-transformed) of common waterfowl species observed in February, with species with dabbling tendencies displayed in blue and species with diving tendencies displayed in yellow. (*c*) Histogram of straight-line pairwise distances between the study lakes (bars) with migration distances inferred for potential first-generation migrants (blue dots) and the maximum distance putatively travelled by genetic-based potential first-generation migrants of European perch (blue-dotted lines), and the maximum reported foraging distance for mallard ducks (red-dotted line [19]).

together in a long gelatinous ribbon, ranging in length from 0.1 m to 1.5 m, and containing up to 200 000 eggs per kilogram of female [18]. Eggs are laid in sticky strings becoming fixed to aquatic plants and rocks at shallow depths in the littoral zone of lakes [18], where they can easily come into contact with the webbed feet and feathered-bodies of waterfowl. This may promote ectozoochory by gelatinous European perch eggs becoming attached to waterfowl, and survival of eggs is enhanced, to some extent, by desiccation tolerance [10]. Furthermore, both diving and dabbling waterfowls, especially mallards and coots, consume fish eggs [20,21], and recent studies have shown that fish eggs can survive through waterfowl guts and then hatch, suggesting that avian endozoochory is likely [14,15]. Piscivorous birds (e.g. herons) might occasionally contribute to single fish dispersal events among lakes by picking up an individual and dropping it unintentionally in another lake. However, this type of avian zoochory is likely very rare in our study area compared to waterfowl-driven zoochory due to a relatively low number of piscivorous birds compared to waterfowl and a very low propagule pressure per dispersal event (a single individual versus multiple eggs).

The occurrence of bird zoochory was corroborated by results from genetic analyses. First, a significant IBD pattern was observed (Mantel $r = 0.230$; $p = 0.04$; i.e. geographically closer lakes were genetically similar; electronic supplementary material). Significant IBD patterns can be associated with natural stepping-stone colonization which, in absence of active aquatic colonization (cf. 'Rejected colonization pathways') and human-mediated dispersal, may be mediated by birds (with decreasing probability of dispersal with increasing distance among lakes). Second, 50% ($n = 4/8$) of the potential European perch recent migration events identified were less than 2 km away from the original lake, whereas the other 50% ranged between 9.4 and 54.6 km (figure 2*c*). The distribution of these potential migration distances is in accordance with the detected IBD pattern (electronic supplementary material) and is very similar to foraging flight distances of mallards (less than 2 km for most of them, with a maximum of 56.9 km; [19,22]). Moreover, these waterfowl foraging distances cover 92% of all pairwise distances among the study lakes (figure 2*c*).

Multiple lines and levels of evidence based on interviews, questionnaires and multidisciplinary field investigations revealed that avian zoochory is a highly probable primary colonization pathway for pioneer fish species like European perch in newly-created artificial lakes. The only piece of evidence missing here is demonstrating that perch eggs can survive bird digestion or attach to the body of waterfowl. While avian zoochory may still be relatively infrequent compared to natural and human-mediated pathways for colonization, we highlight its likely importance in newly-formed lake ecosystems with implications for community assembly.

Ethics. Fish sampling and sample collection were performed under permits granted from French authorities (Arrêtés Préfectoraux 20160706, 20170309, 20180717 and 20190814).

Data accessibility. All data and R code are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.xd2547dmw [23].

Electronic supplementary material is provided in Figshare [24].

Authors' contributions. F.G.: conceptualization, data curation, formal analysis, investigation, writing—original draft, writing—review and editing; I.P.-V.: formal analysis, writing—review and editing; A.G.: data curation, writing—review and editing; J.D.O.: conceptualization, funding acquisition, writing—review and editing; J.C.: conceptualization, funding acquisition, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare no competing interests.

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