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Layman's Report





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Genetic structure of pike (*Esox lucius*) reveals a complex and previously unrecognized colonization history of Ireland

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About

This research was carried out as part of a wider PhD research project conceived by Inland Fisheries Ireland and University College Dublin. The overall aim of the project is to update and inform managers as to the biology and ecology of pike (*Esox lucius*) in Ireland, a historically understudied species in the Irish context. This project has three main aims, one of which dealing with the genetics of pike in Ireland is the subject of this report. The main aim of this project was to investigate the genetic variation of Irish pike populations and their relationship with European groups, in an attempt to identify where they came from, as it is largely assumed to have been introduced by humans over the past few hundred years. Following on from this will be reports on the dietary habits of pike in lake, river and canal habitats, along with a report comparing life history and morphology (the study of form and structure in relation to function) between these habitat types. The field work and sampling has been carried out opportunistically in collaboration with Inland Fisheries Ireland, and with the help of many individual anglers, as well as angling clubs, particularly those associated with the Irish Federation of Pike Angling Clubs. This study has been generously funded by Inland Fisheries Ireland, with contributions from the Irish Federation of Pike Angling Clubs.



The Irish Fauna

Ireland, an island that was isolated from the rest of Europe swiftly after the last ice-age, is limited in its flora and fauna. This is because the wildlife that occurs on islands depends on a number of inter-related factors such as the area, latitude and the distance that needs to be crossed in order to colonise it, as well as those relating to the species in question, such as its life-history, migration capabilities and its ability to adapt to new environments once it arrives. As such, there is much debate surrounding how Irish animals, particularly mammals, managed to colonise the island at all, with many thinking that land-bridges between Ireland and Britain or mainland Europe must have existed at some time in our past to allow them to get here (Davenport *et al.*, 2008). However, this once popular hypothesis has recently been debunked as research has shown sea levels to have risen much faster than previously thought (Brooks *et al.*, 2007; Edwards & Brooks, 2008). As more research is carried out, the story becomes increasingly complex, with common patterns failing to emerge, and even multiple colonization events being revealed (e.g. red deer; Carden *et al.*, 2012; pygmy shrew; McDevitt *et al.*, 2011). Overall, little thought has been given to considering how freshwater fish species arrived in Ireland, despite the fact that marine waters represent a barrier to their dispersal, similar to that of land animals.

The isolation of Ireland since the last ice age created a somewhat depauperate freshwater fauna (Griffiths, 1997; Maitland, 2004; King *et al.*, 2011), which consisted of only a subset of our the species in Britain and mainland Europe. Irish freshwater fish are almost exclusively made up of **diadromous** species such as the brown/sea trout (*Salmo*

Diadromous: fish that migrate between fresh and salt water. There are two main types; **Anadromous** fish migrate from the sea into freshwater to spawn; and **Catadromous:** fish that migrate from freshwater to the sea to spawn

trutta Linnaeus, 1758), salmon (*Salmo salar*, Linnaeus, 1758), European eel (*Anguilla anguilla*, Linnaeus, 1758) and lampreys (Petromyzonidae), all of which would have been able to naturally recolonize Ireland at the end of the last glaciation due to their tolerance of saline water (Wheeler,

Stenohaline fish are unable to withstand a wide variation in salinity of surrounding water; whereas **Euryhaline** organisms are able to adapt to a wide range of salinities.

1977; Maitland, 2004; King *et al.*, 2011). **Stenohaline** species are all assumed to have been introduced by man during the last few hundred years (Fitzmaurice, 1984; Griffiths, Igoe, 2004; King *et al.*, 2011). It is well established that man made introductions

of fish and other organisms have occurred throughout history, and continue to create a wide range of environmental problems. Globalization has resulted in the opening of barriers and trade routes across the entire planet, which has contributed to a huge rise in the introduction rate of alien species from one part of the world to another (Cambray, 2003; Minchin, 2007; Gozlan *et al.*, 2010). In Ireland today the few freshwater natives (11 species, Table 1) are outnumbered by non-natives (13 species). These non-native species create increasing pressures, mostly linked to competition for resources, such as food and habitat (Stokes *et al.*, 2004; King *et al.*, 2011). However, almost half of the introduced fish species present in Ireland have actually been here for hundreds of years (Fitzmaurice, 1984), and many have no known date nor source of introduction (King *et al.*, 2011).

Table 1: Checklist of freshwater fish species of Ireland (scientific and common names) taken from King *et al.* (2011). Those with no data have often been presumed to have been introduced in the 12th century by the Normans.

Common Name	Scientific Name	Status
River lamprey	<i>Lampetra fluviatilis</i>	Native
Brook lamprey	<i>Lampetra planeri</i>	Native
Sea lamprey	<i>Petromyzon marinus</i>	Native
Killarney shad	<i>Allos fallx killarensis</i>	Native
Atlantic Salmon	<i>Salmo salar</i>	Native
Brown / Sea trout	<i>Salmo trutta</i>	Native
Rainbow trout	<i>Oncorhynchus mykiss</i>	1899-1901 - considered domesticated
Arctic charr	<i>Salvelinus alpinus</i>	Native
Pollan	<i>Coregonus autumnalis pollan</i>	Native
Pike	<i>Esox lucius</i>	16th Century
Common carp	<i>Cyprinus carpio</i>	17th Century
Gudgeon	<i>Gobio gobio</i>	No Data (presumed 12th Century)
Tench	<i>Tinca tinca</i>	17th Century
Common bream	<i>Abramis abramis</i>	Pre-18th century
Minnow	<i>Phoxinus phoxinus</i>	No Data (presumed 12th Century)
Rudd	<i>Scardinius erythrophthalmus</i>	Widespread and naturalised by 1900
Roach	<i>Rutilus rutilus</i>	1889
Dace	<i>Leuciscus leuciscus</i>	1889
Chub	<i>Leuciscus cephalus</i>	2001
Stoneloach	<i>Barbatula barbatula</i>	No Data (presumed 12th Century)
European eel	<i>Anguilla anguilla</i>	Native
Three-spined stickleback	<i>Gasterosteus aculeatus</i>	Native
Ten-spined stickleback	<i>Pungitius pungitius</i>	Native
Perch	<i>Perca fluviatilis</i>	No Data (presumed 12th Century)

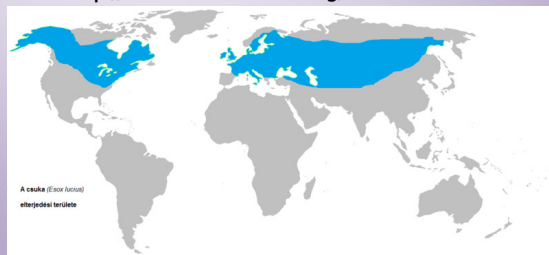
Northern Pike Origins

Northern pike (*Esox lucius* Linnaeus, 1758) is a member of the family Esocidae, a group of fish thought to have emerged during the **Cretaceous** period when Eurasia and North America were still joined (Wilson *et al.* 1992; Craig 2008). This group consists of two subgenera: *Esox*, representing the pikes (3 species), and *Kenoza*, representing the pickerels (2 species, one with 2 subspecies) (Grande *et al.* 2004; Lopez *et al.* 2004). The esocids have a rich fossil record ranging from Late Cretaceous to the present (Grande 1999). Fossil representatives of the *Esox* genus have been found throughout the northern hemisphere, with its oldest known member collected in Canada, dating to the Late Cretaceous (Grande *et al.* 2004). Northern pike have a **circumpolar** distribution in the Northern Hemisphere (Maes *et al.*, 2003; Aguilar *et al.*, 2005), giving it the largest

geographic distribution of all 5 of the extant Esocidae species by a sizeable margin (Senanan & Kapuscinski 2000; Grande *et al.* 2004). It is also the only esocid still present in Europe (Lucentini *et al.* 2009). Throughout its range, pike is of particular interest owing to its socio-economic value through

The **circumpolar** distribution of pike (*Esox lucius*)

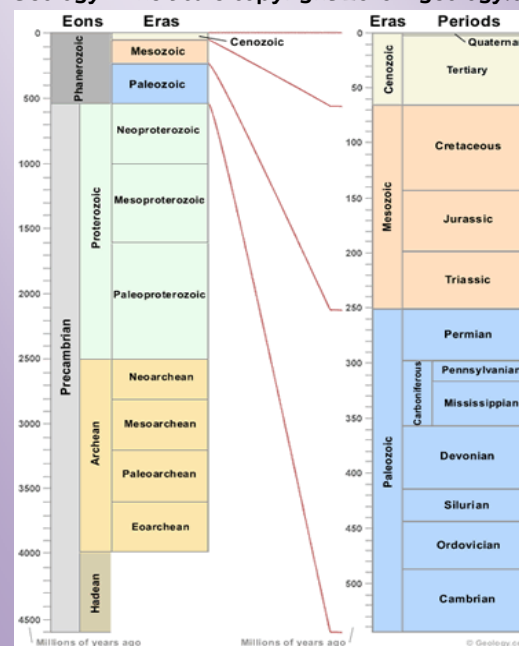
source: <http://commons.wikimedia.org/>



Ireland. Went concluded that there were no references to pike prior to the 16th century, and that

Cretaceous is a geological time period that spans around 79 million years from about 145 to 66 million years ago. The Cretaceous ended with a large mass extinction, in which many groups, including non-avian dinosaurs, pterosaurs and large marine reptiles, died out.

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recreational and commercial fishing (Laikre *et al.*, 2005; Launey *et al.*, 2006; Lucentini *et al.*, 2009). Pike occur throughout Ireland, however, they have long been thought to be non-native (Kennedy, 1969; Fitzmaurice, 1984; O'Grady & Delanty, 2008), based almost exclusively on the seminal paper by Went (1957) in which he attempted to trace the earliest evidence of pike in

where references did exist they pertained to its absence, leading many to interpret Went's paper as a suggested introduction date of the 16th century. This has led to contentious debate on the status of pike in Ireland within many stakeholder (primarily angling) groups (e.g. Barbe & Garrett, 2013). Controversial policies, such as culling and transfer of pike during predator control operations aimed at protecting the native brown trout (Fitzmaurice, 1984; O'Grady & Delanty, 2008), have been common in the management of this species during recent decades, potentially compromising the integrity of genetic stocks [Inland Fisheries Trust (IFT) annual reports e.g. IFT (1966–67, 1979–80); Minchin, 2007].

This lack of scientifically-based colonisation information and other information on the biology of the species led to the development of this project. The genetics components set out to investigate genetic variability in Irish pike populations to see if this could inform us about their colonization history, and to establish whether there are different stocks (as seen in brown trout; Massa-Gallucci *et al.* 2010), all of which could help in the future management of this species.

Pike Genetics

Surprisingly little is known about the genetics and population structure of northern pike throughout its range because little genetic variation has been uncovered to date (Miller & Senanan 2003; Hansen *et al.* 1999; Jacobsen *et al.* 2005; Bruzan *et al.* 1998; Maes *et al.* 2003; Healy & Mulcahy 1980). In fact, northern pike is noted for its pronounced lack of genetic variability when compared to other freshwater fish (Seeb *et al.* 1987; Senanan & Kapuscinski 2000) and even other non-fish species noted for their lack of **polymorphism** (e.g. honeybee (Estoup *et al.* 1995). Even usually highly variable genetic markers (e.g. microsatellites) often show much less variation than expected in pike, which makes detecting genetic patterns difficult (Hansen *et al.* 1999; Senanan & Kapuscinski 2000; Miller *et al.* 2001; Laikre *et al.* 2005). It has been suggested that severe bottlenecks caused by expansion from very few refuge (unglaciated) sites after the last ice-age has led to such reduced genetic variability (Maes *et al.*, 2003; Jacobsen *et al.*, 2005; Launey *et al.*, 2006). In short, if there were only a few areas not under ice, then there would be very few pike that would have been able to survive. Fish that have been isolated from other populations for a long time would have interbred, leading to them become

genetically similar. This strongly suggests that the low variability observed in pike is due at least partly to ancient population history (Jacobsen *et al.* 2005). At first this theory seems sensible; however, the same patterns are not observed in other freshwater fish species that must have been similarly isolated (Miller & Senanan, 2003). It is possible that because pike is a top predator, it was subject to more dramatic population bottlenecks than other fish species because predator populations are restricted in how large they can grow by how much prey fish are available for them to feed upon.

Prior to the present study, there has only been one study that has examined nuclear genetic variation in pike in Ireland (Jacobsen *et al.*, 2005). They found monomorphism (i.e. no differences) between the individuals examined. Other

Mitochondria are structures within cells that convert the energy from food into a form that cells can use. Each cell contains hundreds to thousands of mitochondria. **Mitochondrial DNA** (mtDNA) is the DNA located in the mitochondria and is thought to have evolved from the circular genomes of bacteria that were engulfed by the early ancestors of today's modern complex cells

studies that used **mitrochondrial DNA** (mtDNA) also showed extremely low variability so were unable to help in deciphering how pike colonised Ireland (Maes *et al.*, 2003; Nicod *et al.*, 2004). To date, studies using mtDNA and microsatellites have suggested that all northern European populations recolonized from a single glacial refuge, and that there is at least one (Balkan; Maes *et al.* 2003;

DNA (deoxyribonucleic acid) is the hereditary material of organisms. Most DNA is located in the nucleus of the cell (nuclear DNA). The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T) The order, or sequence, of these bases acts a code for the set of genetic instructions



mage source: <http://www.astrochem.org>

Jacobsen *et al.* 2005), but possibly multiple, separate southern European races (e.g. southwestern France; Launey *et al.* 2006, Lucentini *et al.* 2011).

Before this project, the number, extent and source of introductions of pike into Irish waterways was unknown.

MATERIALS AND METHODS

Sampling

Pike were sampled from 15 locations around Ireland using a combination of electrofishing, gill-netting and angling, between August 2010 and November 2011. Gill-netting and electrofishing were carried out opportunistically in collaboration with Inland Fisheries Ireland during their routine surveys (Table 2). Samples were also obtained while attending angling competitions (Table 2), and occasionally through organized trips to sample areas of particular interest (River Lee & Lough Bane). Sampling locations were chosen to give as broad a representation and coverage of Irish pike populations as possible (Fig. 1). Tissue or scale samples were also obtained from England, France, Sweden, Germany and Romania through collaborations with other universities and researchers (Table 2). European samples were selected to cover hypothesized European lineages i.e. the previously identified genetically distinct northern European and Balkan (Danubian) populations, and to include the most likely sources of introduction/natural colonization (Britain, north-western France).

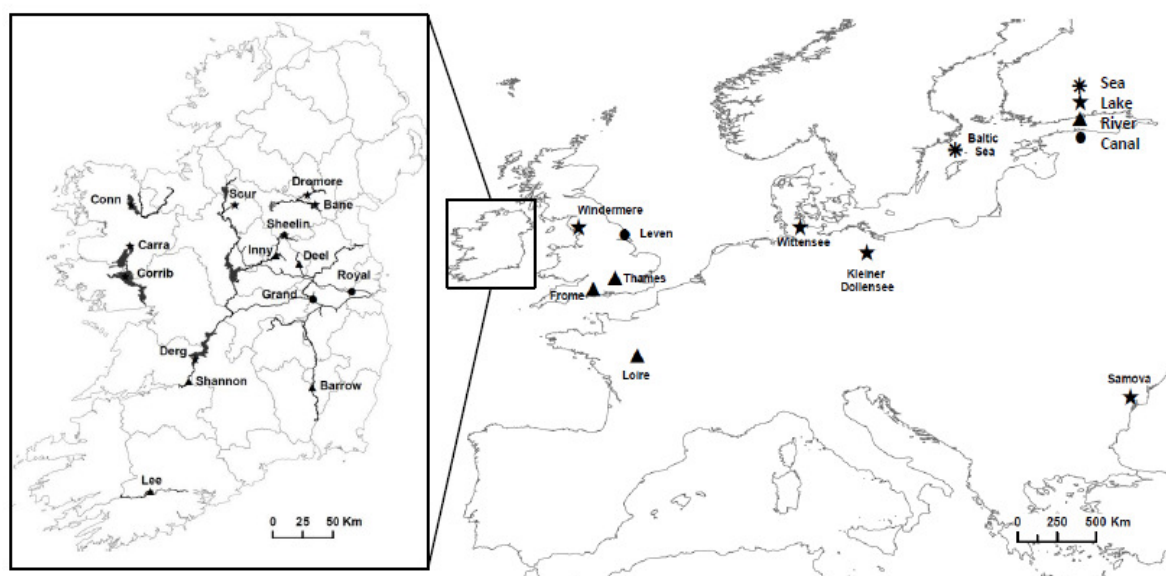


Figure 1. Locations of sampling sites in Ireland and Europe. The Shannon, Derg, Inny, Sheelin and Scur are all directly part of the Shannon system. Dromore River and Lough Bane are a part of the Erne system, which connects to the Shannon via the Shannon-Erne waterway (16 locks) at Upper Lough Erne in County Fermanagh. The Grand Canal connects the River Liffey in Dublin to the River Shannon at Shannon Harbour, Co. Offaly, via 44 locks, and connects with the River Barrow via the Barrowline Canal (9 locks). The Royal Canal also connects Dublin's River Liffey with the Shannon, at the more northerly Abbeyshrule in County Longford, meeting the River Deel along its way.

Table 2 Pike samples from Ireland and Europe. Study sites, indicating site name, region, sample size (n), method employed (gill = gill nets, elec = electrofishing, ang = angling) and average number of alleles per locus (A). German and Swedish samples were shipped as tissue by colleagues. Irish samples were collected between August 2010 to November 2011, European and British samples were collected between March 2002 and September 2012.

Site	Habitat	Method	n	A	Site	Habitat	Method	n	A
Europe					Ireland				
Somova	Lake	Gill	10	8.83	Bane	Lake	Ang	12	1.33
Baltic	Sea		20	9.00	Barrow	River	Elec	48	2.67
Wittensee	Lake		39	8.17	Carra	Lake	Gill	20	1.67
Dollnsee	Lake		44	5.50	Conn	Lake	Gill / Ang	30	1.67
Loire	River	Elec	24	7.50	Corrib	Lake	Gill	39	2.17
Britain					Deel	River	Elec	35	2.00
Frome	River	Elec	32	3.17	Derg	Lake	Ang	40	2.33
Thames	River	Elec	30	4.83	Dromore	River	Ang	25	2.00
Winderemere	Lake	Gill	29	4.00	Grand	Canal	Elec	44	3.17
Leven	Canal	Elec	30	4.17	Inny	River	Elec	34	4.00
					Lee	Various	Ang	52	2.50
					Royal	Canal	Elec	50	2.50
					Scur	Lake	Gill	27	2.17
					Shannon	River	Ang	8	2.00
					Sheelin	Lake	Gill	40	2.83

Methods

DNA was isolated from tissue samples. Previous studies had suggested that many **loci** would be required to identify genetic differences between pike populations

In genetics, a **locus** (plural **loci**) is the specific location of a gene or DNA sequence or position on a chromosome.

Microsatellites, are repeated sequences consisting of two, three or four nucleotides (the basic building blocks of DNA), which can be repeated 3 to 100 times. These repeating regions mutate faster than many other regions due to slippage events that add or remove extra repeats. This leads to a high level of **polymorphism** (i.e. multiple forms), which is helpful for telling individuals and populations apart.



because of the low levels of variability even at a global scale in this species (see above). As such, we targeted 30 different **microsatellites** from the literature to test. We picked those that had showed the most variation previously, and that had been tested in a range of geographical areas. However, through the initial screening process, only six of these turned out to be variable. We then use these six microsatellites to analyse a total of 752 individuals from throughout Ireland.

RESULTS

Genetic analysis

The selected microsatellites worked well, and did show genetic variation - something not previously detected in Ireland. Some of the microsatellites were more informative than others (Table 2), but all contributed to improving our understanding. A pattern of decreasing genetic diversity was observed between pike from Europe (more diverse) and those from Ireland (less diverse). Our testing found genetic differentiation among all the samples examined.

The microsatellite data revealed clear genetic structuring of Irish pike populations, and allowed for inferences of relationships at large spatial scales. Relatively high levels of genetic diversity were found, contrasting with the published findings for this species which showed a distinct lack of variation. Findings from the European populations were generally consistent with other studies on pike in those areas.

An *allele* is one of a number of alternative forms of the same gene or same genetic locus. In the case of microsatellites it refers to versions with different numbers of repeats.

Private alleles (i.e. alleles unique to one location) were rare in Ireland (only 10%), and 22% of the Irish pike alleles were shared with European pike but were not found in Britain. Mainland European samples shared a lot of alleles with each other that were not observed in either Britain or Ireland, and 25% of British pike alleles were shared with those from Europe, but not with Ireland.

Population Structure

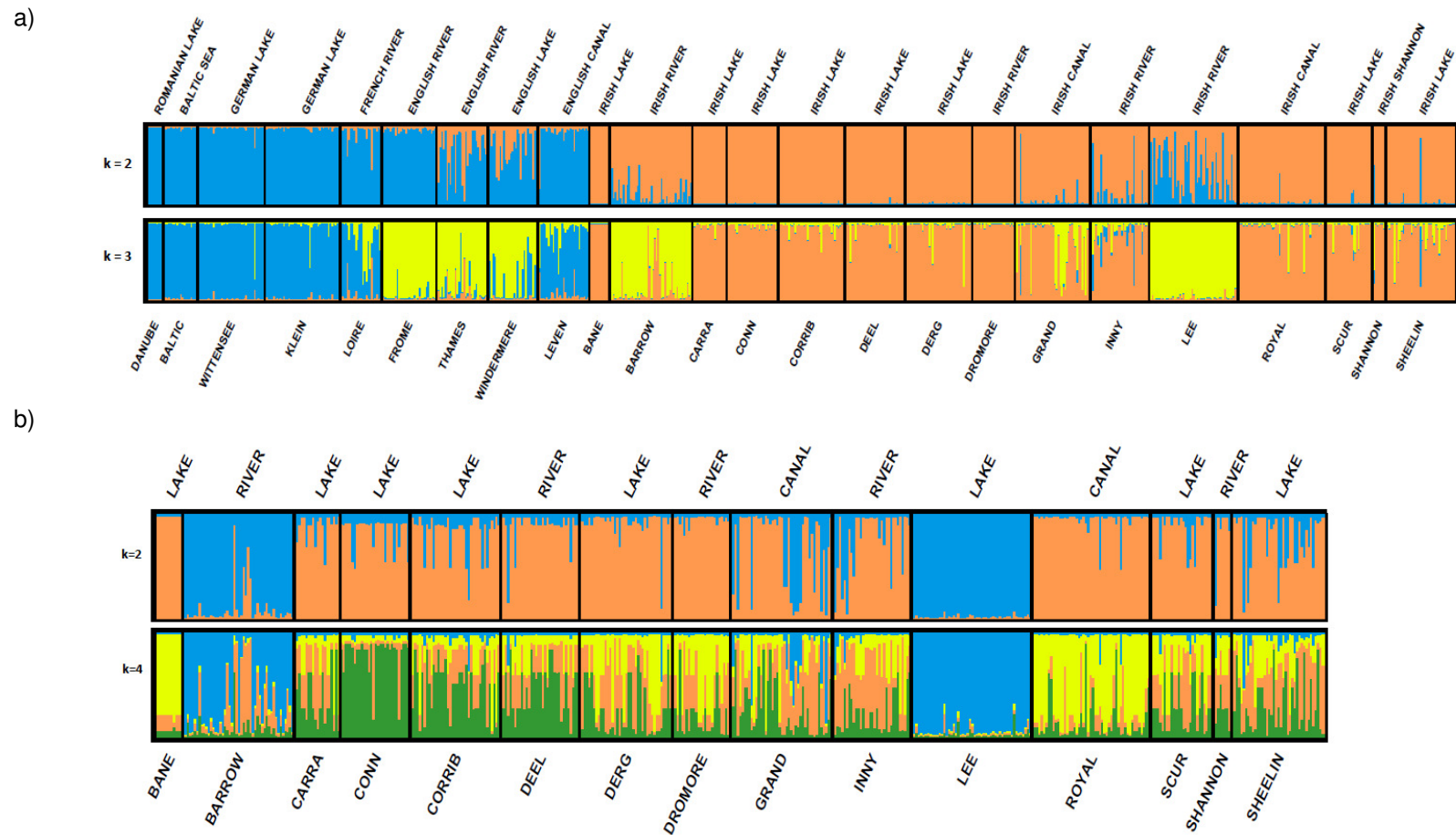
Population substructure was assessed using a program called STRUCTURE 2.3.4 (Pritchard *et al.*, 2000), which helps to identify the most likely number of populations in a sample, based on their genetic differences. We carried out this analysis firstly with all sample populations, and then again separately for only the Irish populations.

This analysis indicated that with all of our samples included, that we were likely dealing with either 2 or 3 main groups. These groupings can be seen in Figure 2a, highlighted in different colours (each population is labelled and separated by black bars). The 2 group scenario highlights how different the Irish group (coloured orange) is in relation to both mainland European and Britain (blue). The 3 group scenario shows the separation of pike from Britain (yellow). Interestingly, two of the Irish samples (Lee and Barrow) are now seen to strongly group with Britain, while all the other Irish samples remain in the orange group. Also of note is the Leven Canal (East Yorkshire, along the east coast of England) sample which is more similar to northern European populations than to the other British ones.

When we looked at just the Irish samples in order to examine the finer scale structure within Ireland, we found most support for 2 or 4 groups. The two group scenario completely supports the previous findings of two highly divergent strains within Ireland (the blue group, related to the British samples, and the remaining distinct 'Irish' group, shown in orange Figure 2b). Examination of the 4 group graph demonstrates that divergent groups exist within Ireland, such as Lough Conn (now green), and the Royal Canal (now yellow). The individuals with multiple colours are ones that do not strongly group with any of the divergent populations. These individuals are from samples collected from rivers and lakes connected to the River Shannon, our largest river system.

The Lee–Barrow–Windermere (blue group) signal can be seen to a lesser degree in the Grand Canal, which can be explained by the connection existing between the River Barrow and the Grand Canal in the form of the Barrowline Canal.

Figure 2 Results from STRUCTURE analysis indicating population clustering of a) 752 individuals from all 24 locations sampled (Ireland, Britain and Europe; Table 1), and b) 504 individuals from 15 locations around Ireland. The population locations are listed below the figure, with the site type above. Each segment of the graph separated by black bars represents a population that we sampled. Within these sections, each narrow vertical coloured bar represents a single individual, and the group to which they are assigned based on genetic similarity (colours). Some segments are wider than others as we had larger sample sizes from these locations (see Table 1). For 'Ireland Only' (part b), the distinction of the Lee & Barrow populations is evident at 2 groups and maintained at when 4 groups are examined. The distinction of Lough Conn (large amount of green), Lough Bane & the Royal Canal (mostly yellow) can only be seen in the four group scenario.



Visualising Relationships

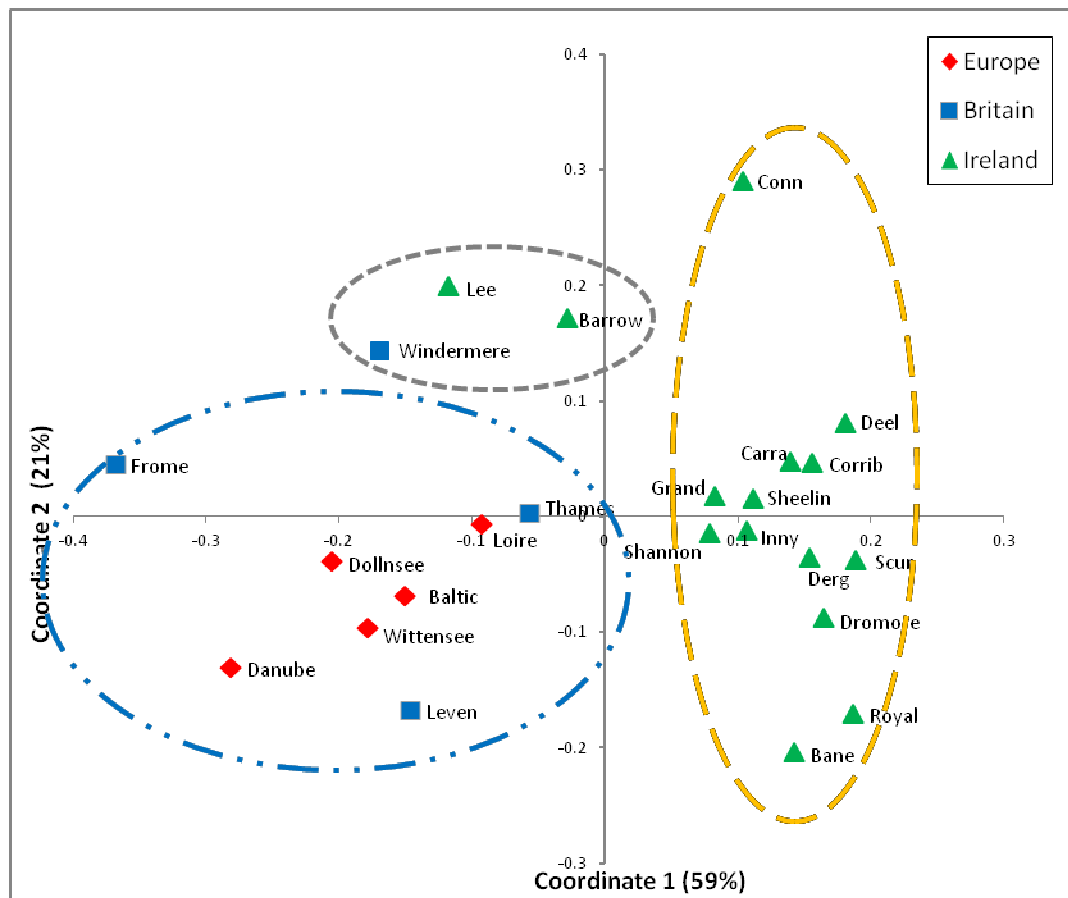


Figure 3 Multi-dimensional scaling (MDS) plot of population pairwise genetic distances enabling visualization of evolutionary relationships between populations. The x-axis accounts for 59% of the variation observed, and the y-axis accounts for 21%, meaning that overall, the placement of the populations as above, explains 80% of the differences in the genetic variation we examined. The orange oval reflects the orange Irish grouping from Figure 2. The blue oval indicates how the majority of British (blue squares) and European (red diamonds) samples group together as in Figure 2a. The Grey circle highlights how the River Lee and the River Barrow are more closely related to the Lake Windermere population from Britain, than they are to the other Irish populations.

The above plot illustrates the pairwise genetic distances between each population, i.e. the closer a population is to another one, the more genetically similar they are. An interesting clustering of the Irish Lee, Barrow and British Windermere samples in the top left quadrant can be seen (grey oval), which supports the findings of Figure 2. Within the 'Shannon' group (the multicoloured sections of Figure 2b), the samples that are not directly connected with the main river system appear on the outskirts of the group (Bane, Carra, Corrib, Deel and the Royal Canal). Lough Conn appears highly distinct, occurring on its own, much further out from the other Irish groups.

The pike from the River Thames appear very close to the River Loire from north-western France. This may actually reflect a historical connection, as these two waterways may have been connected prior to inundation of Doggerland which removed any remaining connections between Britain and Europe

Doggerland is the former landmass in the southern North Sea that connected Great Britain to mainland Europe during and after the last Ice Age, surviving until around 8,000 years ago before becoming flooded by rising sea levels.

around 7000–8000 years ago (Wheeler, 1977; Weninger *et al.*, 2008), thus isolating populations in mainland Europe from those in the British Isles.

Another method called hierarchical analysis of molecular variance (AMOVA) was also performed in order to test the most likely groupings, essentially as a cross-check. Tests were carried out based upon geographical location (e.g. Europe, Britain & Ireland), and subsequently tests were informed by the above STRUCTURE and MDS plots. The results fully supported the previous analysis and so are not further reported here.

Modelling History

A complex method of statistical modelling called approximate Bayesian computation (using the program DIYABC v.1.0.4; Cornuet *et al.*, 2008) was used to simulate the likelihood of alternative scenarios for the initial introduction of pike into Ireland. Essentially, we provide the program with a number of known or estimated parameters, and design a number of potential colonisation scenarios that we wish to test. The program then simulates data and enables one to test which scenario has the most support (i.e. is the most likely). As we were interested in natural colonisation routes the Baltic Sea and Danubian samples were excluded as they were unlikely to be the direct source for Irish pike populations, and the English Leven site was not included in the 'Britain' group, as we have shown that it groups more with European than British samples. In total we tested 17 different scenarios.

Despite the wide range of scenarios tested with DIYABC, one in particular stood out for its best fit to the observed data, repeatedly producing the highest support values (Fig. 4). The first split in this scenario suggests colonization of Ireland and Britain from Europe around 8000 years ago. The second split appears to indicate a split between the Irish and British populations some 4000 years ago. The third and final split illustrates a more recent introduction from Britain into Ireland around 1000 years ago.

DIYABC analysis rejects the seemingly more obvious, 'simple' explanation according to which Ireland would be colonized by pike from Britain and any more population subdivision would have resulted from more recent processes within the island. Instead, analyses indicate that around 3500–4000 years ago, Irish and British pike populations became isolated; this may have corresponded to the Irish Sea becoming fully marine as it is now, and as such becoming an impassable barrier for a freshwater fish. Subsequently, a second pike contingent appears to have entered the island around 1000 years ago and is currently distributed in the south of the island.

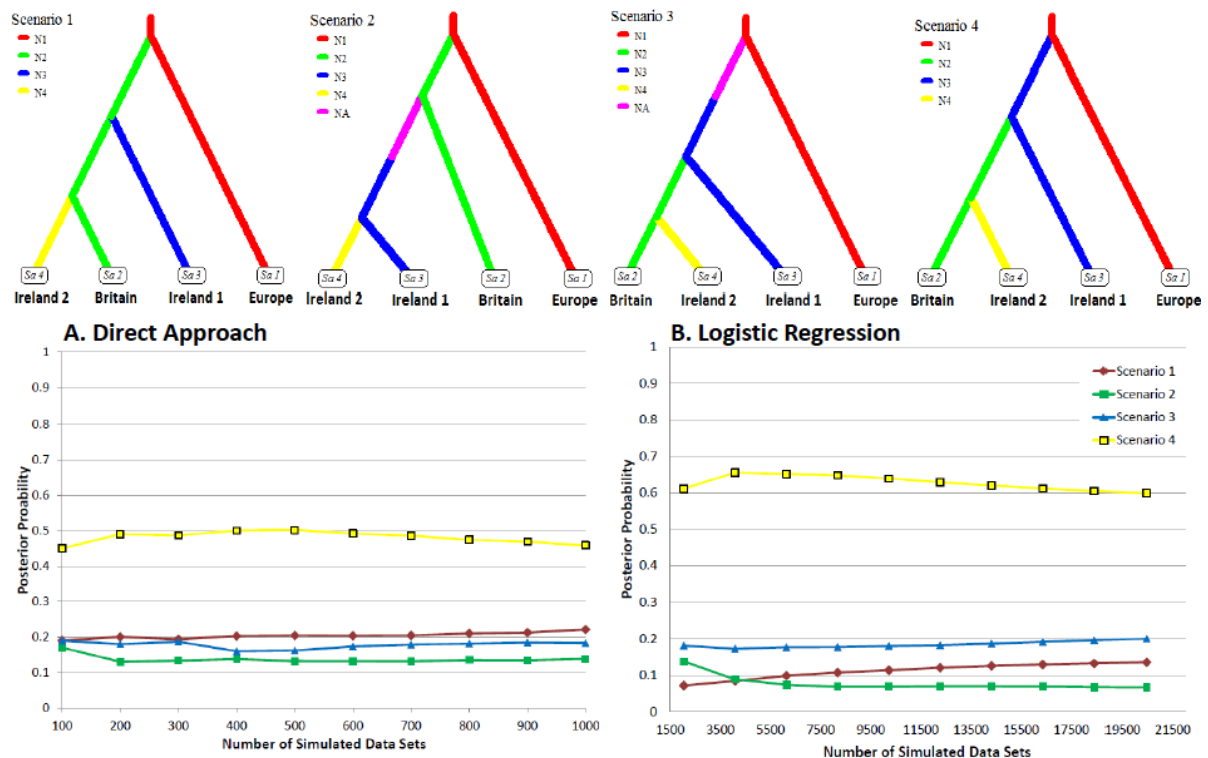


Figure 4 DIYABC analysis was used to estimate the relative likelihood of alternative scenarios for the initial introduction of pike into Ireland. The top graphs illustrate the four best supported scenarios. Ireland 1 (blue) refers to the main group of Irish genotypes, Ireland 2 (yellow) refers to the Barrow and Lee populations, which group with the British Windermere in Fig. 3. For each group the scenario is illustrated (colours indicate different populations), and each split in the graph indicates a divergence event. The bottom graphs indicate the likelihoods of the four best scenarios compared by two different methods. They clearly illustrate that Scenario 4 (yellow) is the scenario with the most support.

It must be noted that this is a statistical modelling approach, and although specific values are reported here for simplicity (further details can be found in the paper Pedreschi *et al.* 2013), there is a wide estimated range around these values. Although the output from the program is given in generations, this was then converted to years by taking a value of 2 years as average age at first spawning, as

reported for Ireland (Healy, 1956, Roche *et al.*, 1999, O'Grady & Delanty, 2008) and Europe (Raaijmakers, 1988, Arlinghaus *et al.*, 2009). Confidence intervals for the time estimations are indeed large, however our estimates fit in well with known historical timelines. Furthermore, it is worth noting that the four best supported scenarios all gave reasonably similar estimates for the time parameters.

Isolation by distance

The association between genetic divergence and geographic distance (km overland) was also investigated. Isolation by distance essentially describes the tendency of populations that are geographically closer to be more similar than populations that are further apart, as one would expect. Within Ireland, significant isolation-by-distance was observed, which was maintained when only the more widespread older strain was examined. When rivers and lakes were examined separately, we found that lakes are responsible for the majority of the isolation effects, which may be expected from rivers which act as corridors in which more movement and hence gene flow (reproductive mixing) occur.



DISCUSSION

Overall, the multiple and varied methods used here, agree with one another and together indicate a strong separation between two distinct Irish units, whose introduction to Ireland may have followed rather different paths. The first more widespread group appears to have reached Ireland and Britain shortly after the retreat of the ice sheets of the last ice-age. The second, which is mainly present in the southern river catchments of the Lee and the Barrow, was likely introduced by humans during the middle ages. This is also further supported by the fact that Ireland and Britain both share alleles with Europe, that they do not share with each other. If all Irish pike had colonized from Britain, Irish pike should display a subset of the British alleles. This is also interesting as it ties in with a similar geographical division that has previously been observed between the north and west (Boreal race) and south-east (Celtic race) in Ireland in relation to the Atlantic salmon, *Salmo salar* (Child *et al.*, 1976).

The greater level of genetic mixture observed from the populations connected to the Shannon is not surprising as the Shannon system has been a major focus of pike management works since the 1960s, involving both culling and transfer of pike among many areas of the system (IFT annual reports 1952–1980). However, further subdivision is detectable within this ‘older’ Irish group. For instance, it seems some divergence may have occurred in very recent times due to human activities – such as the closure of the Royal Canal in 1961, provoking the subsequent isolation and drift of the population for almost 50 years until the canal was reopened in 2010. This is indicated in Figure 2b by the high proportion of the yellow colour in the Royal Canal samples as opposed to other sites, and supported by Figure 3 where the Royal Canal samples can be seen as being quite separate from the main Irish group. Similar processes may have been at work in Lough Bane, which is a very small, somewhat isolated waterbody (approximately 200m × 400m). Other patterns are more difficult to reconstruct; for instance, the lack of divergence of Lough Corrib and Lough Carra from the main ‘Shannon’ group may be linked to recurrent management operations on these waterbodies. The divergence of the Lough Conn population, which lacks unique alleles, probably reflects a relatively recent founding event. Anecdotal evidence suggests that pike have been present in this lake for less than 200 years.



History of pike in Ireland and management implications

Northern pike are thought to have been anthropogenically introduced to Ireland around the 16th century (Went, 1957). However, our results refute this simplistic view. One strain has indeed likely been introduced from Britain, perhaps from populations related to the Windermere pike; however, a much earlier introduction has been found to be incompatible with anthropogenic transfers. This widespread older Irish strain is both lacking in genetic variability and considerably divergent from both the British and European sites examined here. The two main Irish groups also seem to exhibit little geographical overlap (Fig. 1).

The more recent introduction of pike to Ireland may have been facilitated by the Normans in the 12th century, who are responsible for many of the introductions to this island [e.g. hedgehogs (*Erinaceus europaeus*), fallow deer (*Dama dama*), black rat (*Rattus rattus*), rabbit (*Oryctolagus cuniculus*); McCormick, 1999]. In support of this date of introduction is the very rare finding of two pike cleithra (head bones) bones from excavation of the Anglo-Norman castle at Trim, Co Meath, dating to the late 13th–early 14th century (Hamilton-Dyer, 2011). The fact that they are head bones indicates that the pike may have been present here alive, as the usual method of shipping fish at that time was beheaded and dried (Hoffmann, 2009). Furthermore, contrary to the quotes by Went (1957), Longfield (1929) actually states that pike were likely to have been introduced by the 14th century, and that by the 16th century they were thoroughly at home in Ireland. At this time, pike exports from the south of Ireland (Youghal, Dungarvan, Cork and Kinsale) to southern English towns (Longfield, 1929) greatly exceeded those of brown trout. In one year alone, 1507, Dartmouth imported 3850 pike from Ireland. This certainly suggests that they must indeed have been present in decent numbers before this date.

Went (1957) stated that there was no old Irish name for pike, and that the modern name is '*gailliasc*' which literally translates into 'strange or foreign fish', thus suggesting an introduction (Fitzmaurice, 1984). However, Farran's (1946) paper on the local names of Irish fish contains over 10 variations of names for pike, which included *liús*, *lús*, *lusaigh* and *lusc* – all of which are similar to both the old English name for pike (luce) and the Latin '*lucius*' or '*lupus*'. It has since been confirmed by the School of Irish, Celtic Studies, Irish Folklore and Linguistics, at UCD that references to *liús* (pre-1600s) pre-date references to *gailliasc* (1846).

This study has revealed greater population structure than was previously hypothesized to be present based on the expectations of 16th century introduction and a previous account of genotypic variation (or more aptly the lack thereof) in Irish pike (Jacobsen *et al.*, 2005). The evidence from our study indicates that pike population structure within Ireland warrants thoughtful consideration and further examination of its current habitats and populations is recommended. Careful consideration should be given to life history and ecological interactions, particularly between these units, and monitoring should continue using molecular genetic approaches, which may lead to the identification of further divergent populations. Recently developed genomic approaches should be used to monitor and investigate possible adaptive divergence in different environmental contexts, which will also add support to the analysis of evolutionary history and colonization pathway of the species

We recommend that management practices should remain precautionary and avoid breaching population barriers such as through translocations (Miller & Senanan, 2003; Tallmon *et al.*, 2004), especially between the two strains. It seems that this may already be occurring between the Grand Canal and the River Barrow (Fig. 2), due to the Barrowline Canal connection.

Furthermore, as lakes have been shown to maintain isolation-by-distance despite translocation practices, it may indicate that translocated individuals do not adapt well in the new habitat, which would be an important finding to take into consideration to minimize inefficiencies of management strategies.

In conclusion, the present study unveils for the first time the genetic diversity within and among pike populations inhabiting Ireland's water bodies, and clarifies their relationships with populations from European locations. We found evidence for strong geographical structure, and the existence of distinct populations, probably corresponding to multiple colonization dates, which indicates that pike may have first colonized Ireland naturally. This information is significant for the reappraisal of current management strategies in this economically (angling) and ecologically (top-predator) important species, and will contribute new perspectives to the long-standing debate on the mechanisms and timing of colonization dynamics of Britain and Ireland (Lynch, 1996; Griffiths, 1997; Woodman *et al.*, 1997; Carden *et al.*, 2012).

As Irish systems come under increasing pressure, particularly from invasive species [e.g. curly waterweed (*Lagarosiphon major*), zebra mussel (*Dreissena polymorpha*) and the freshwater clam

Corbicula fluminea], particular attention must be paid to these newly discovered Irish pike population units. This research provides the first piece of evidence to help achieve that goal, and highlights the complexity inherent in natural systems, and the need for empirical knowledge as a basis for appropriate biodiversity and fishery resource management.

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