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APPLIED ISSUES

Zebra mussels (*Dreissena polymorpha*) in Ireland, AFLP-fingerprinting and boat traffic both indicate an origin from Britain

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SUMMARY

1. The zebra mussel (*Dreissena polymorpha*) is an aquatic nuisance species that invaded Ireland around 1994. We studied the invasion of the zebra mussel combining field surveys and genetic studies, to determine the origin of invasion and the vector of introduction.
2. Field surveys showed that live zebra mussels, attached to the hulls of pleasure boats, were transported from Britain to Ireland. These boats were lifted from British waters onto trailers, transported to Ireland by ferry and lifted into Irish waters within a day. Length-frequency distributions of dead and living mussels on one vessel imported 3 months earlier revealed a traumatic occurrence caused by the overland, air-exposed transportation. Results show that a large number of individuals survived after re-immersion in Irish waters and continued to grow.
3. Zebra mussels from populations in Ireland, Great Britain, the Netherlands, France and North America, were analysed using amplified fragment length polymorphisms (AFLP)-fingerprinting to determine the origin of the Irish invasion. Phylogenetic analysis revealed that Irish and British mussels clustered closely together, suggesting an introduction from Britain.
4. Ireland remained un-invaded by the zebra mussel for more than 150 year. The introduction of the zebra mussel to Ireland occurred following the abolition of value added tax in January 1993 on imported second-hand boats from the European Union (UK and continental Europe). This, together with a favourable monetary exchange rate at that time, may have increased the risk of invasion of the zebra mussel.

Keywords: AFLP, *Dreissena polymorpha*, invasions, Ireland, trade

Introduction

Biological invasions continue to have many unpredictable ecological and economic consequences (Pimentel *et al*., 2000). The rapid expansion of the zebra mussel [*Dreissena polymorpha* (Pallas, 1771)] throughout Europe and North America has caused severe detrimental impacts to native fauna, and resulted in high mainten-

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ance costs for industries by the clogging of pipelines used for water abstraction (Ludyanski, McDonald & MacNeill, 1993; Van der Velde *et al*., 1994; Schloesser, Nalepa & Mackie, 1996; Strayer *et al*., 1999).

Over the last 200 years, zebra mussels spread to Western Europe from their native Ponto–Caspian region where they were found in lakes and delta areas of large rivers draining into the Black and Caspian seas. Human activities enabled the expansion of the zebra mussel and its further spread was made possible by a combination of natural and human-mediated dispersal mechanisms (Carlton, 1993; Johnson & Padilla, 1996; Johnson & Carlton, 1996; Minchin, Lucy & Sullivan, 2002). This dispersal began at the end of the 18th century, at a time when shipping trade became increasingly important and zebra mussels were transported (upstream), attached by means of their byssus threads, to the hulls of ships. Canals were built, linking different navigable river systems and thereby allowing downstream dispersal of its planktonic larvae. Zebra mussels, in these ways, became established in the Curonian Lagoon in the Baltic Sea, via the Dnieper–Neman waterway, which opened in 1803 (Orlova, Vladislav & Komendantov, 2000). They most probably spread from the Baltic with seagoing ships carrying exports of damp timber to Britain (by 1824), the Netherlands (*c*. 1826), Germany (*c*. 1830) and France (*c*. 1840) (Kerney & Morton, 1970; Kinzelbach, 1992). Further canal interconnections led to its dispersal through much of central Europe – via a central corridor covering the Dnieper, Vistula, Oder, Elbe and Rhine rivers- to the north-western, continental part of Europe (Bij de Vaate *et al*., 2002). In 1992, a route was opened with the construction of the Main-Danube Canal, connecting the Rhine and Danube Rivers (Ricciardi & MacIsaac, 2000; Bij de Vaate *et al*., 2002). Although the zebra mussel became widely spread in lowland lakes, rivers and waterways in Europe, mountain ranges like the Alps and Pyrenees remained barriers for the species. However, with the increasing popularity of recreational watersports, craft on trailers have more frequently been transported to and between high alpine lakes. As a result, the species became established in these lakes and spread to northern Italy (Giusti & Oppi, 1972) and north-eastern Spain (Anonymous, 2001). Since the 1980s, zebra mussels have been found in the brackish waters of the south-eastern Gulf of Finland, and have progressed further northwards along the Finnish coast (Valovirta & Porkka, 1996; Orlova *et al*., 2000). The zebra mussel invaded the Great Lakes in North America by 1986, most likely introduced as larvae with the ballast water of transoceanic ships coming from the Black Sea (Hebert, Muncaster & Mackie, 1989; Carlton, 1993; Stepien, Taylor & Dabrowska, 2002).

The zebra mussel arrived in Ireland in 1994 or earlier (Minchin & Moriarty, 1998a,b) and has since spread to most interconnected waterways with recreational boating (Fig. 1). Ireland remained uninvaded for almost 150 years after its establishment in Britain and north-western Europe and the reason for its sudden invasion, the vector of introduction and the source region remained unknown. A number of recent studies have demonstrated the usefulness of genetic markers as a tool in elucidating invasion source regions. Genetic analyses can be used to determine phylogeographic relationships, expressed in a hierarchical descendence, which may reflect invasion corridors and can help retrace source populations (Hebert & Cristescu, 2002) and such methods have successfully been applied to a number of different organisms such as plants (Novak & Mack, 2001), freshwater cladocerans (Cristescu *et al*., 2001; Hebert & Cristescu, 2002), bivalves (Stepien *et al*., 2002), brittlestars (Roy & Sponer, 2002) and fishes (Waters, Shirley & Closs, 2002). Here we present evidence of an anthropogenic introduction of the zebra mussel from Britain to Ireland, based on field surveys and genetic studies using AFLP-fingerprinting (Vos *et al*., 1995). We also ascribe a vector of introduction and suggest why it became established around 1994.

Methods

Field survey and length-frequency analysis

Over the period 1997–2001, managers of companies who transport and import boats (transportation of boats using wide load trailers is a specialised service) were interviewed. In addition, locations in Britain, the Netherlands and Germany, where boats had been lifted from the water onto trailers and directly imported and re-immersed in Ireland, were recorded.

Contacts were then made with boat marinas in Ireland likely to receive used imported craft, and over the period 1998–2001 four imported boats were selected and examined for the presence of zebra mussels; three boats were examined upon arrival in Ireland and

one boat was examined 3 months after re-immersion in Irish waters. Date, origin and destination of importation were noted. Zebra mussels were sampled by scraping a 2 × 2 m area of the hull surface with a 15-cm wide steel blade. Shell lengths of living mussels obtained from the first three boats were measured and mussel densities were estimated according to a logarithmic scale. The shell lengths of dead and living zebra mussels attached to the hull of the fourth boat, examined after 3 months, were measured to estimate mortality related to overland transportation and post-invasion growth of surviving mussels.

Sample collection and conservation

Zebra mussels for the genetic analyses were collected in Ireland, England, France, the Netherlands and the U.S.A. (Table 1). The locations in England, the Netherlands and France were chosen because these were likely sources for the introduction of the zebra mussel to Ireland. Where possible, sample locations were chosen in known source areas (based on the field survey) for boat export to Ireland.

Zebra mussels were collected by taking living individuals from submerged substrates and immediately placing them in 50 mL conical polypropylene tubes (Greiner Bio-One, Alphen aan den Ryn, the Netherlands) filled to the rim with a saturated aqueous solution of Cetyltrimethylammonium-bromide (CTAB; Merck, Amsterdam, The Netherlands) containing 20% Dimethylsulfoxide (DMSO; Merck) and stored at room temperature.

DNA extraction

After addition of 150 μL (95 °C) CTAB-buffer [1.4 M NaCl, 0.2% CTAB, 0.1 M Tris, 0.02 M ethylenediaminetetraacetic acid (EDTA), 0.2% β-mercapto-ethanol), a small piece of tissue from an individual mussel was homogenised with sterile potters. The homogenate was incubated in 10% sodium dodecyl sulphate (SDS) and digested with 8 μL Proteinase-K (10 g mL^{-1}; MBI Fermentas, St Leon-Rot, Germany) at 65 °C overnight. After 1 h of incubation, another 4 μL Proteinase-K was added. Prior to the DNA extraction, RNAse treatment was performed for 0.5 h at 37 °C (6 μL of a stock solution with a concentration of 10 mg mL^{-1}). DNA was extracted by treatment with phenol (pH 8.0) and chloroform/isoamyl alcohol (24 : 1, v/v) (Sambrook, Fritsch & Maniatis, 1989) and precipitated by adding 2.5 volumes 96% ethanol and 0.1 vol. 3 M Na-acetate at −20 °C overnight. The precipitate was washed with 70% (v/v) ethanol, dried and dissolved in 50 μL sterile, deionised water. The quality of the genomic DNA was checked by electrophoresis in 0.7% agarose gels, and the DNA concentration (μg μL^{-1}) was determined using a smartSpec™3000 Spectrophotometer (Bio-Rad, Veenendaal, The Netherlands).

AFLP procedures

AFLP techniques were applied as described by Vos *et al*. (1995) with several modifications. The double digestion of the DNA by *Eco*R1 and *Mse*I was performed at 37 °C for 1.5 h, in a total volume of 20 μL. The reaction mixture consisted of 0.5 μL *Eco*RI (12 U μL^{-1}, Takara, Wokingham, UK), 1.0 μL *Mse*I (4 U μL^{-1}, New England Biolabs, Frankfurt am Main, Germany), 4 μL 5xRL-buffer [50 mM Tris-HAc pH 7.5, 50 mM MgAc, 250 mM NaAc, 25 mM dithiotheritol (DTT), 250 ng μL^{-1} bovine serum albumine (BSA)] and 12.5 μL sterile deionised water. The ligation of the adapters was performed by adding 5 μL of a solution containing 2 μL sterile deionised water, 1 μL 5xRL-buffer, 0.5 μL 5 mM ATP, 0.5 μL 50 pmol μL^{-1} *Eco*RI-adapter (5′-CTCGTAGACTGCGTACC, CTGACGCATGGTTAA-5′), 0.5 μL 50 pmol μL^{-1} *Mse*I-adapter (5′-GACGATGAGTCCTGAG, TACTCAGGACTCAT-5′) and 0.5 μL T4-ligase (MBI Fermentas) for 3 h at 37 °C.

The PCR amplifications were performed in two steps. In the first polymerase chain reaction (PCR), tenfold diluted template DNA was amplified using a *Eco*RI (5′-GACTGCGTACCAATTC-3′) and *Mse*I (5′-ATGAGTCCTGAGTAA-3′) primer combination. The PCR product was used as a template for a second (selective) amplification using *Eco*RI/+CA (5′-GACTGCGTACCAATTCCA-3′) and *Mse*I/+CAT primers (5′-ATGAGTCCTGAGTAACAT-3′). The amplified fragments were separated on 9% denaturing polyacrylamide gels using the mini-PROTEAN® 3 (Bio-Rad). The AFLP fingerprints were visualised by silver staining (Bassem, Caetano-Anollés & Gresshoff, 1991; Chalhoub *et al*., 1997). The gels were wrapped in cellophane-foil and dried on a glass plate in a dry and dark place at room temperature.

Data analysis

The polyacrylamide gels were scanned on a GS-700 Imaging Densitometer (Bio-Rad) and optimised using the Molecular Analyst® software version 1.4.1 (Bio-Rad). A three-step procedure was applied to facilitate reliable comparisons between individuals: First, the gel band patterns were converted into graphical peak patterns using the Profile Analysis menu from the Molecular Analyst® Software in which the peak intensity of the graph represented the staining intensity on the gel. Next, the values on the *x*-axis, corresponding to the identified peaks, were transformed into relative distances to compensate for small differences in electrophoresis procedures (e.g. different running time for the gels), using two prominent bands (X1 and X2), which were observed in every individual, as an internal standard. The distance between X1 and X2 (i.e. Internal Standard Distance, ISD) was set at the arbitrarily chosen number of 100, where the relative distance for band X1 ($R_{D,X1}$) was set at 0 and the relative distance for band X2 ($R_{D,X2}$) at 100. All *x*-values were then converted into relative distances using the following formula:

$$R_{DY-X1} = (|Y - X1|/I.S.D) \times 100$$

where $R_{D\ Y-X1}$ is the relative distance from band Y to band X1, $|Y-X1|$ is the absolute value of the subtraction of the *x*-value of peak Y minus the *x*-value of peak X1 and I.S.D. is the subtraction of the *x*-value of peak X2 minus the *x*-value of peak X1. Finally, the relative distances were used to detect the presence or absence of bands. The obtained information was then used to compose a binary 1/0 character data matrix, signifying the presence and absence of bands, respectively.

Fig. 1 (a) Map of Ireland showing various water bodies, canals and sampling sites. (b) Spread of the zebra mussel in Ireland in the period 1995–2001.

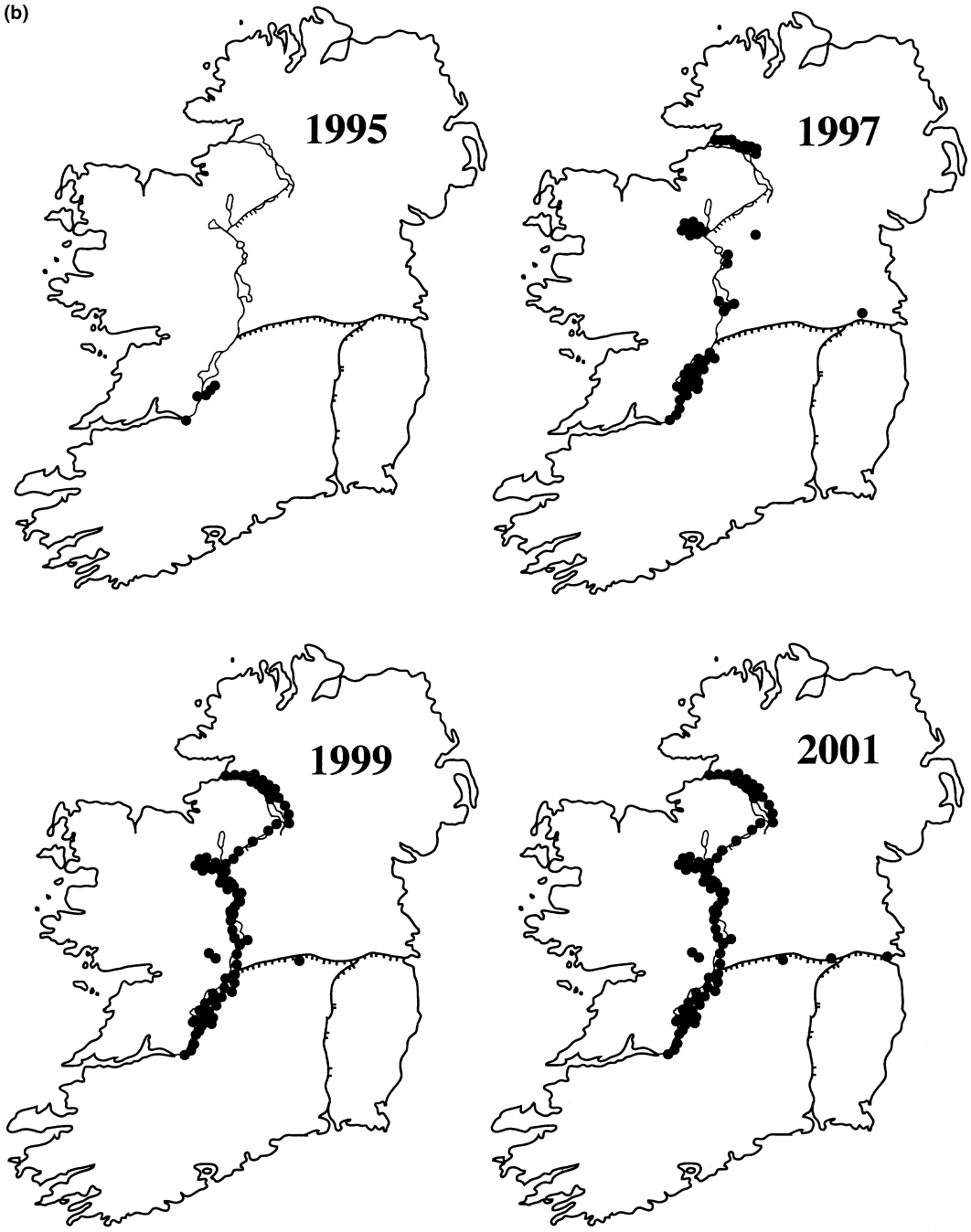

Fig. 1 (*Continued*).

Phylogeographic analysis

The binary data matrix was used for the phylogeographic analyses. A principal component analysis (PCA), a non-hierarchical grouping technique, was performed using the GeneMaths (2000) program version 1.50 (Applied Maths™, St-Maartens-Latem, Belgium), to detect relatedness between individual samples. The data were normalised by subtracting the mean and dividing by the root mean square. In the two-dimensional PCA-ordination diagram samples were spread according to their relatedness, with samples on opposite sides of the *x*- or *y*-axis being negatively correlated (Jongman, ter Braak & van

Table 1 Location, date of collection and number of individuals used for AFLP analysis

Country	Location	Collection date	Number of individuals
Ireland	Lough Derg	02-12-1999	3
	Lough Key	02-12-1999	3
	Lower Lough Erne	02-12-1999	2
Great Britain	Brayford Pool (near Lincoln)	25-07-2000	2
	Thames River (near Chertsey)	02-01-2000	6
The Netherlands	Lake Groene Heuvels	29-03-2000	2
	Lake Ressen	28-06-2000	3
	Waal River (near Weurt, Vuren)	16-06-2000	7
France	Petit Rhône River (near St Gilles)	02-05-2000	4
North America	Mississippi River (near Baton Rouge)	15-05-2000	2

Tongeren, 1987). Phylogenetic relationships were inferred by the neighbour joining (Saitou & Nei, 1987) and the maximum parsimony methods using PAUP* 4.0 (Swofford, 2001). The samples from the Mississippi (Baton Rouge, LA, U.S.A.) were regarded as an outgroup population. For the neighbour-joining analysis, the genetic distances were computed using the Nei-Li distance estimation (Nei & Li, 1979). Unweighted maximum parsimony analysis was performed using the tree-bisection-reconnection branch swapping and closest taxon addition in a heuristic search. The overall consistency index of the most parsimonious trees (length 50) was 0.54. The 50% majority-rule consensus was computed for the most parsimonious trees (not shown). The percent frequencies of grouping of clades in the consensus tree are presented in Fig. 4.

Results

Field surveys

Based on interviews, we identified the origins of boat imports from the Netherlands and Britain, in which boats were lifted from the water onto trailers, transported by ferry to Ireland and re-immersed in freshwater (Table 2). Four boats, imported from Britain to Ireland were examined for the presence of zebra mussels. Origin, destination and date of importation of the examined vessels are given in Table 3. Living zebra mussels were found on all four boats, providing evidence that live zebra mussels were introduced to Ireland via this mechanism. The boats from near Bristol, Bridgewater and Norfolk Broads, each had >2000 living individuals on each hull. The vessel from Hampton Court had <500 individuals on its hull. Shell length-frequency distributions are given in Fig. 2 (a–d).

Post-invasion survival

Three boats were examined before re-immersion in Irish waters, the fourth boat was examined 3 months after importation to Ireland. It had been lifted from British waters near Bristol, transported to Ireland, lifted into the Grand Canal at Sallins (Ireland) on 6 August 1997, then navigated to nearby Lowtown, an area without an established zebra mussel population, where it remained. On 7 November 1997, thousands of zebra mussels were found on its hull. These were made up of mature individuals, ranging 9–23 mm in shell length (Fig. 2d). There is no possibility that these zebra mussels could have attained adult sizes from settlement within the 3 months following their arrival. In Lough Derg (Ireland), an area of favourable conditions, zebra mussels attained a maximum shell length of 7–10 mm after 1 year (Minchin & Moriarty, 1998b). Furthermore, on live mussels a distinct shock ring was visible on 17 mm shell length individuals (the modal size), corresponding to the modal shell length of dead specimens (14 mm) still attached to the hull (Fig. 2d). This suggests that a recent sudden traumatic occurrence, such as an overland, air-exposed transportation of zebra mussels attached to the hulls of boats, resulted in the death of a number of individuals, whereas the surviving mussels continued to grow after re-immersion in Irish waters.

Phylogeographic analysis

AFLP fingerprints allowed discrimination of up to 32 bands, of which 22 were parsimony informative. Both

Table 2 Information on location of origin in Great Britain or the Netherlands, boat type, year of importation and location of destination of boats imported to Ireland (information based on interviews)

Boat origin	Canal, river or catchment	Boat destination	Boat class	Year	Source
Britain					
Beccles	Waveney River	Dromineer	Cruiser	1997	T. Knight
Brundall	Yare River	Athlone	Cruiser	1993	D. Briscoe
		Killaloe	Cruiser	1997	D. Briscoe
		Banagher	Cruiser	1995	D. Briscoe
Chertsey	Thames River	Athlone	Steel barge	1997	D. Briscoe
Colchester	Colne River	Carrick-on-Shannon	Cruiser	1994	D. Briscoe
Dartford	Cray Catchment	Carrick-on -Shannon	Canal boat	1995	D. Briscoe
Doncaster	Don River	Banagher	Cruiser	1994	D. Briscoe
Ely	Ouse River	New Ross	Cruiser	1996	D. Briscoe
Evesham	Avon River	Banagher	Canal boat	1994	D. Briscoe
Goole	Aire and Calder Navigation	Banagher	Canal boat	1994	D. Briscoe
Horning	Bure River	Banagher	Cruiser	1996	D. Briscoe
			Crusier	1997	D. Briscoe
Inverness	Ness Canal	Killaloe	Crusier	1993	D. Briscoe
Kingston	Thames River	Dublin, Grand Canal	Cruiser	1994	D. Briscoe
Leighton Buzzard	Union Canal	Lowtown	Canal boat	1995	R. Few
Napton	Napton Canal	Lowtown	Canal boat	1998	R. Few
Near Exeter	Exeter River	Carrick-on-Shannon	Cruiser	1993	D. Briscoe
Norwich	Wensum River	Banagher	Cruiser	1994	D. Briscoe
Rochester	Medway River	Athlone	Cruiser	1994	A. Chatterton
Snodland,	Medway River	Athlone	Cruiser	1994	D. Briscoe
Spalding	Welland Canal	Lowtown	Canal boat	1994	R. Few
Thorne	Don River	Lough Corrib	Cruiser	1997	D. Briscoe
Wargrave	Thames River	Banagher	Cruiser	1995	D. Briscoe
Weybridge	Thames River	Banagher	Cruiser	1994	D. Briscoe
Windsor	Thames River	Athlone	Cruiser	1996	D. Briscoe
Windsor	Thames River	Killaloe	Cruiser	1996	B. Carroll
The Netherlands					
Alkmaar	N-Hollandsch Canal.	Carrick-on-Shannon	Steel barge	1997	D. Briscoe
Lemmer	IJsselmeer	Killaloe	Steel barge	1995	D. Briscoe
Lochem	Twente Canal	Athlone	Steel barge	1997	D. Briscoe
Loosdrecht	Vecht River	Banagher	Steel barge	2000	D. Briscoe

the two-dimensional PCA-ordination diagram (Fig. 3) and the phylogenetic tree (Fig. 4) show cohesion among samples from the same sampling site and differentiation between samples from different sites. In the PCA-ordination plot (Fig. 3), clusters from Ireland and Britain lie close together, indicating similarity between Irish and British individuals. Clusters lying on opposite sides of the *y*-axis are negatively correlated, suggesting a distinctiveness between Irish-British clusters lying on the left side and Dutch-French clusters lying on the right side of the *y*-axis. The cluster from the Mississippi River (U.S.A.) lies clearly

Table 3 Location of origin in Britain, location of destination in Ireland, estimated time of transportation and date of arrival of the four boats examined for the presence of zebra mussels

Boat origin	Boat destination	Estimated time of transportation (in days)	Date of arrival	Estimated density (ind. m^{-2})
Brundall, Yare River	Killaloe	1	21 April 2000	10–100
Bridgewater Canal	Grand Canal	1	25 April 2000	10–100
Hampton Court	Lowtown	1	5 September 2001	1–10
Sharpness Canal	Lowtown	1	6 September 1997	10–100

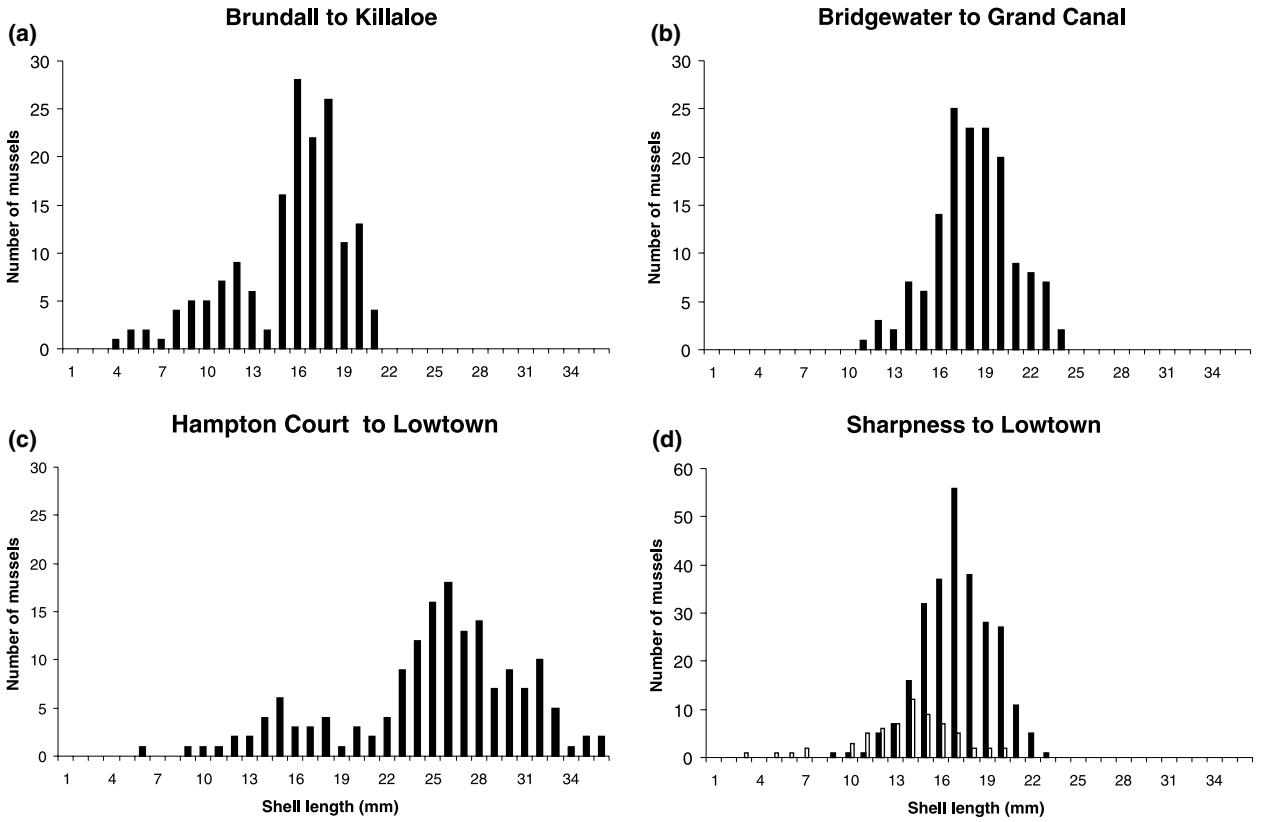

Fig. 2 (a–c) Length-frequency distributions of zebra mussels obtained from three boats examined upon arrival in Ireland between 1997 and 2001 (location of origin and destination of boats are given in each graph). (d) Length-frequency distributions of both living ($n = 266$, black bars) and dead ($n = 65$, white bars) zebra mussels, recovered 3 months after arrival in Ireland from a boat imported from Sharpness Canal to Lowtown in 1997.

separate from all other clusters. The phylogenetic tree (Fig. 4) shows that the examined individuals from the Irish populations are genetically more similar to individuals from the British populations than to individuals from the Dutch, French or North American populations. The results of both the PCA analysis and the phylogenetic analysis suggest that Britain is the source region of invasion.

Discussion

This study strongly suggests that the zebra mussel was introduced to Ireland attached to the hulls of pleasure craft, carried on trailers and imported by ferry from Britain. Hauliers recall zebra mussels attached to the hulls of several leisure craft imported to Ireland (Table 2). The field surveys provide evidence that mature zebra mussels, transported either during April or September, arrived alive from Britain to Ireland (Table 3). Moreover, length-frequency distributions showed that the zebra mussel survived the introduction and continued to grow after arrival in Irish waters (Fig. 2d).

Normally boats imported from Britain are lifted and returned to water within a day. As zebra mussels were found to survive several days out of water under damp conditions, such a mode of introduction is likely (Ricciardi, Serrouya & Whoriskey, 1995; Paukstis *et al.*, 1999). In Ireland zebra mussels exposed to air have been found to survive under cool damp conditions in March for at least 18 days (Minchin, personal observation), although this period is likely to be much shorter during summer (McMahon, 1996; Ussery, Miller & Payne, 1998; McMahon, 2002). In North America, trailered boating traffic has already been recognised as one of the most important vectors for the transport of zebra mussels among unconnected bodies of water and is considered responsible for the non-riverine spread through North America (Padilla, Chotkowski & Buchan, 1996; Johnson & Padilla, 1996;

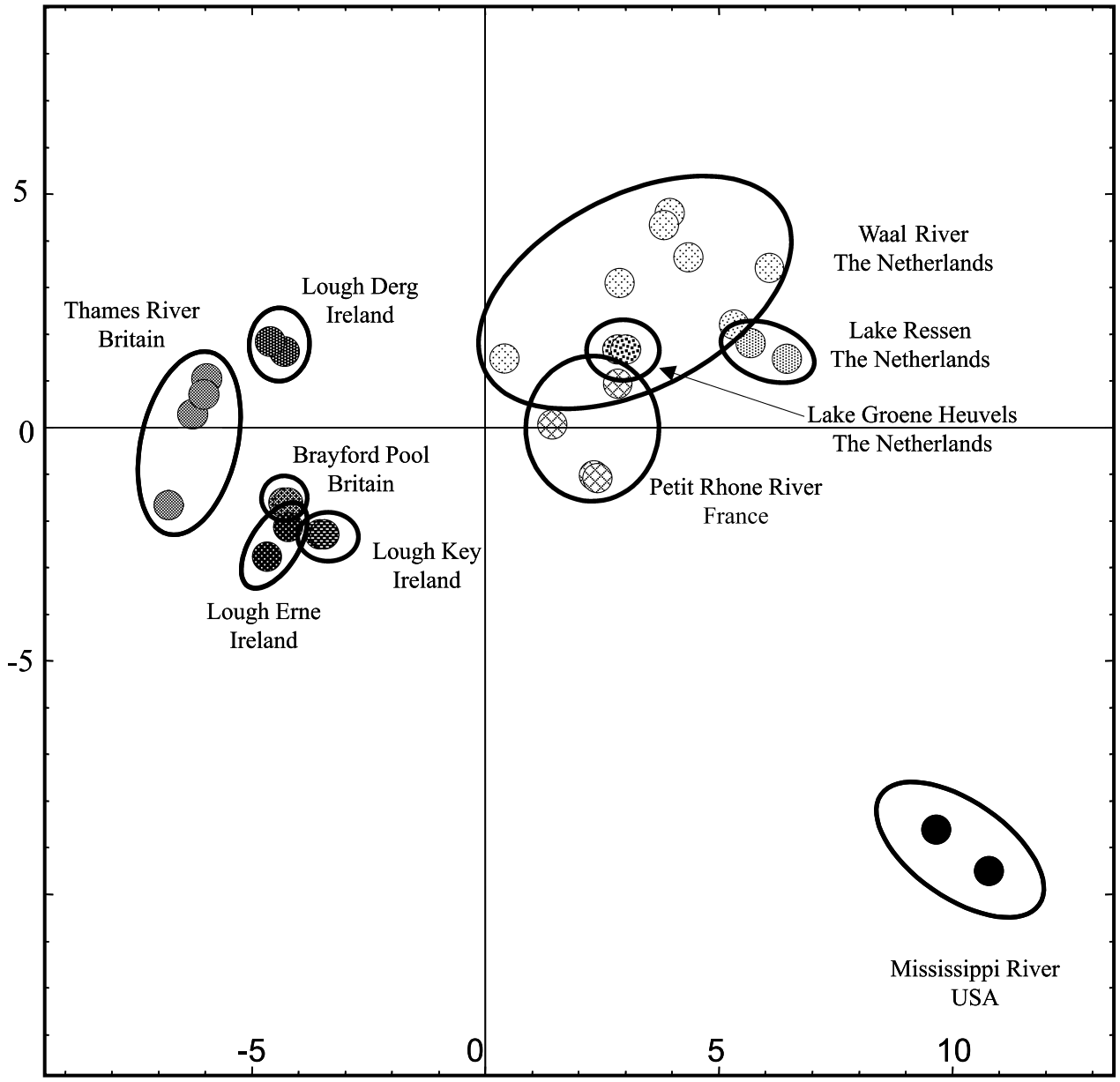

Fig. 3 Two-dimensional PCA-ordination diagram, inferred from AFLP characters of the various populations of the zebra mussel, showing relatedness between individuals. Each individual is presented as a small circle. Individuals belonging to the same population were given a similar pattern and were encircled.

Johnson & Carlton, 1996; Buchan & Padilla, 1999; Johnson, Ricciardi & Carlton, 2001). In this study we show that trailered boating traffic need not be restricted to transport of zebra mussels among unconnected bodies of water on a mainland, but may also be responsible for transport of zebra mussels overseas.

Ireland imports boats from Britain and the Netherlands. It is possible that zebra mussels imported from the Netherlands may also survive such a journey. However, the phylogeographic analysis suggests that the Irish populations are genetically more similar to British populations than to the Dutch, French, and North American populations. This again suggests that British mussels created the founder population in Ireland. We are unable to state whether there was more than one inoculation event from Britain. However, the similarity of the Derg, Key and Erne populations in Ireland is consistent with their pro-

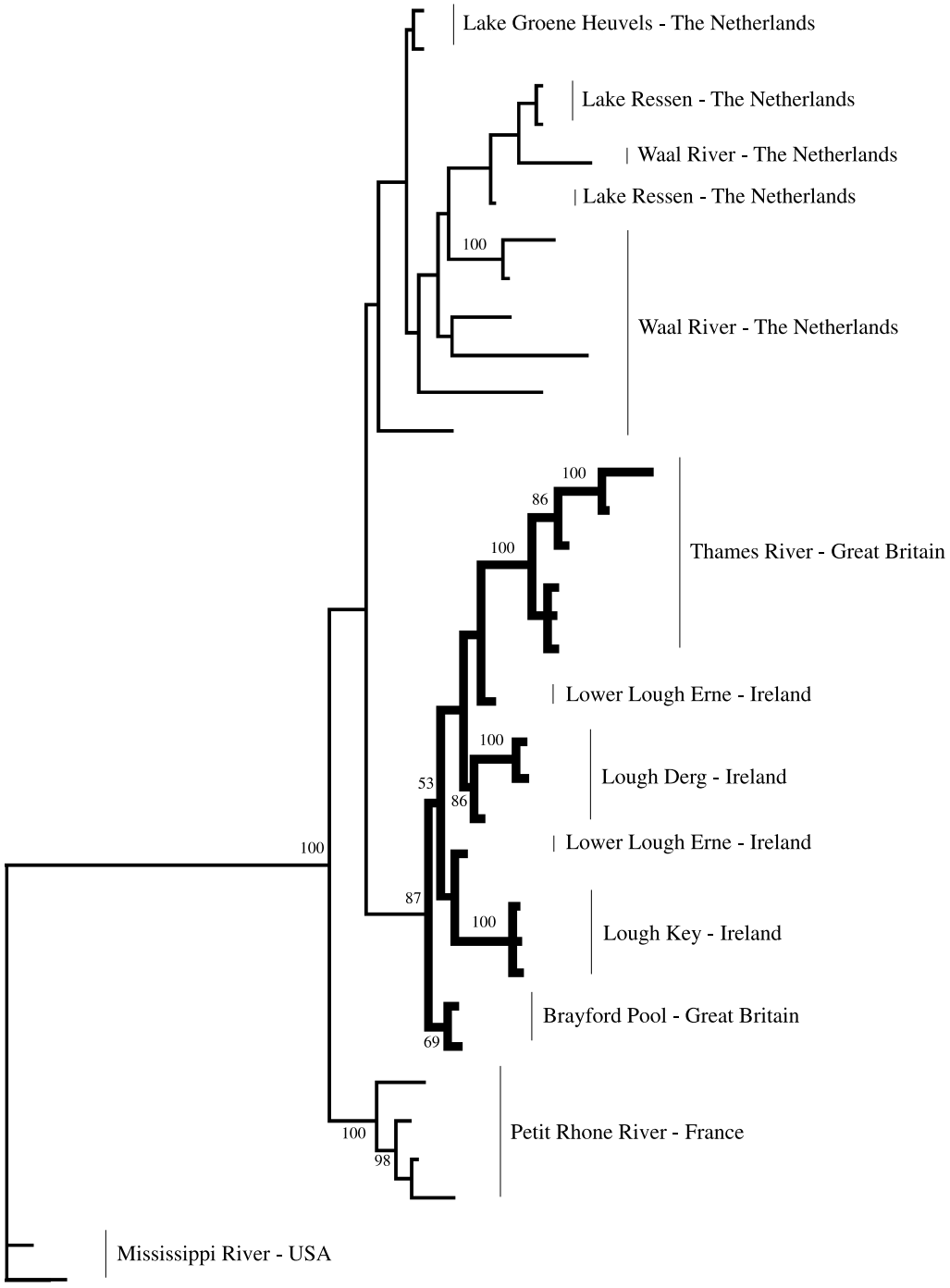

Fig. 4 Neighbour-joining tree inferred from the AFLP characters of the various populations of the zebra mussel. The clustering of the Irish and British populations (indicated by the thick lines) was supported in the maximum parsimony analysis as suggested by the 50% majority-rule consensus tree for the most parsimonious trees (number at the nodes are the percent frequencies of grouping of the clades).

posed spread from the lower Shannon region on the hulls of infested watercraft (Minchin, 2000; Minchin, Maguire & Rosell, 2003), thus suggesting a single source region (Fig. 1).

Although zebra mussels were first recognised in Ireland in 1997 (McCarthy, Fitzgerald & O'Connor, 1997), it appears that they became established in 1994 or earlier. Considering their capability to be dis-

persed, it is remarkable that Ireland had not become invaded at an earlier time. Notably, the estimated date of introduction in 1993 or 1994 coincides with the abolition of the value added tax (VAT) in January 1993 on used boats purchased within the European Union. This abolition of VAT resulted in an increase of boat imports (predominantly from Britain and the Netherlands), almost certainly enhanced by a favourable exchange rate between the Irish pound and the British pound at that time. These circumstances may have contributed to their arrival at this time.

Although we have no evidence against the arrival of zebra mussels in ballast water to the docks of Limerick port on the Shannon River, where salinity are low, circumstantial evidence argues against this. Usage of ballast water by ships' has taken place since about 1880, and since this time there have almost certainly been opportunities for zebra mussels to arrive with ships coming from the Baltic, and from elsewhere in Northern Europe. Even if they would have arrived with ballast water from Britain and become established in Limerick Dock in the Shannon Estuary, it is very unlikely that the species would have gained access upstream on the Shannon River to Lough Derg, as small craft seldom berth in Limerick Docks, and should they do so, it is only for a few days. Moreover, the ascent upriver by craft is subject to navigation restrictions and a passage to a higher level of 30 m at the lock within the Ardnacrusha hydroelectric dam between Limerick and Lough Derg, and records show there were <50 upward movements of craft in 1993 and 1994.

Introduction by attachment to imported timber has been considered responsible for the spread of the zebra mussel to Britain, Germany and the Netherlands in the 19th century (Kerney & Morton, 1970; Kinzelbach, 1992). However, Ireland at this time was not importing timber from Europe. Moreover, it is common practice nowadays for imported timber to be bark-stripped, sawn and exported dry, consequently it is unlikely that this vector still operates. A further possibility for their establishment could be a deliberate release of zebra mussels, perhaps to change the water quality in the Shannon system or a release from an aquarium. No evidence for such vectors are known and the pattern of spread (Fig. 1) argues against this as the introduction appeared to have happened in a boating region.

It is difficult to reconstruct invasion corridors and source regions. However, the severe adverse economic and ecological consequences of many invasive species summons the need of a better understanding of the mechanisms underlying invasions, in order to prevent their further spread. Here we report a new vector of introduction – overseas ferry transportation of pleasure craft fouled with zebra mussels. We also implicate an unexpected barrier of invasion, the VAT, showing that changes in the value of craft with removal of taxes may be a contributing factor in enhancing the movements of invasive species.

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