

Chapter 1: Introduction to the Project

1.1 Overview of chapter

Chapter 1 provides the background, aims and objectives to the project and the requirements for monitoring freshwater fish in Ireland under the Water Framework Directive. The advantages of using fish as indicators of ecological quality are outlined and there is a brief review of fish species in Ireland.

1.2 Background to the project

This project explores the relationship between fish communities and environmental influences that control them. The primary aim of the project is “*to develop a predictive model for the composition, abundance and age structure of the fish fauna based on Q-values, faunal and floral communities, physical and hydrological environment plus environmental variables such as nutrient concentrations*”. To achieve this main aim the project team sampled fish populations at 374 river sites in Ireland gathering extensive physical/hydromorphological, biological and chemical data. A further 145 sites were extracted from the Central Fisheries Board (CFB) archival database and included in the project database. This report outlines the analysis of this dataset and the resultant relationships between the fish populations and the hydromorphological environment initially, and then taking water quality issues into account using the Environmental Protection Agency’s (EPA) Quality Rating System (Q-Values) and water chemistry (Chapters 2 and 3). As an integral part of a fish assessment system it is necessary to consider spatial and temporal variability of fish populations. Chapter 4 examined this aspect and recommends sampling methodologies for rivers and some preliminary results for Irish lakes. Additional studies were undertaken (Chapters 5 and 6) to answer questions raised in the project specification regarding specific mechanisms of water quality impacts on fish – specifically, diurnal oxygen variation as it affects fish movements and micro distribution and the impact of eutrophication and siltation on salmonid egg survival.

Water quality in Irish rivers has been assessed, by the EPA, since 1971 primarily on the basis of macroinvertebrate communities in riffle areas, i.e. the Quality Rating System (Q-value). The system is based principally on macroinvertebrates but also takes into account aquatic macrophytes, phytobenthos and hydromorphology (Flanagan and Toner, 1972; Clabby *et al.*, 1993; McGarrigle *et al.*, 2002). The Quality Rating System has been shown to be a robust and sensitive measure of riverine water quality and has been linked with both chemical status and land use pressures in catchments (Clabby *et al.*, 1992; McGarrigle, 1998; Donohue *et al.*,

2006). The system facilitates rapid and effective bankside assessment of the water quality of rivers and streams. There are nine possible scores, ranging from one to five (intermediate scores such as Q4-5 are also possible). High ecological quality is indicated by Q5, Q4-5 and Q1 indicates bad quality (Table 1.1) (see Appendix 1 for a summary of the derivation of Q-value classes). Whilst some impairment is evident at Q4, primarily as a consequence of eutrophication, the ecological conditions at such locations are considered to be acceptable for salmonids. This is an assumed relationship. This project examined this association and developed a statistical relationship between the existing Q rating system and fish community structure / composition.

Table 1.1: General characteristics of the various biological Quality Classes of the Quality Rating System (after Clabby *et al.*, 2001) (note: intermediate scores, e.g. Q4-5 are also possible)

Quality classes Q ratings	Class A		Class B	Class C	Class D	
	Q5	Q4	Q3-4	Q3	Q2	Q1
Pollution status	Pristine, unpolluted	Unpolluted	Slight pollution	Moderate pollution	Heavy pollution	Gross pollution
Fishery potential	Game fisheries	Good game fisheries	Game fish at risk	Coarse fisheries	Fish usually absent	Fish absent

In response to the decline in water quality throughout Europe, the European Parliament and Council passed into law EC Directive 2000/60/EC establishing a framework for community action in the field of water policy, commonly known as the Water Framework Directive (WFD). This was transposed into Irish law by the European Communities (Water Policy) Regulations - S.I. No. 722 of 2003. The WFD requires that the quality of surface waterbodies should be assessed relative to defined type-specific reference conditions with a key defining issue that quality will largely be based around the concept of ecological quality. Article 8 (1) requires each Member State to establish monitoring programmes in accordance with Annex V and to conduct a comprehensive overview of water status in each River Basin District (RBD). Water bodies will be classified into five status classes consistent with the five normative definitions of *high, good, moderate, poor and bad* (ECOSTAT, 2003). The principal aim of the WFD is that waters currently exhibiting High and Good ecological status should be maintained as such and waters which are currently otherwise should be restored to good status by 2015. The WFD requires the inclusion of fish as a biological element in the assessment of ecological quality.

1.3 Requirements in relation to fish in the Water Framework Directive

The Water Framework Directive lists fish amongst the biological elements (Annex V) which should be used for the classification of ecological status of surface waters (rivers, lakes and transitional waters (estuaries). “Ecological status” (Article 2 (21)) is an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters, classified in accordance with Annex V (Wallin *et al.*, 2003). Member States are required to establish methods/tools for assessing ecological status and guidance on the approach to classification is provided (ECOSTAT, 2003). The variables to be used in any fish index are composition, abundance and age class structure. In high quality or reference sites, species composition and abundance must correspond totally or nearly totally to undisturbed conditions; all type specific disturbance sensitive species are present; the age structure of the fish communities show little sign of anthropogenic disturbance and are not indicative of a failure in the reproduction or development of any particular species. Other quality classes (good, moderate, poor and bad) will show a gradual decrease in these variables (Table 1.2).

Table 1.2: Normative definitions of ecological status class for fish as per EC Directive 2000/60/EC (After CEC, 2000).

Staus	Fish fauna
High	Species composition and abundance correspond totally or nearly totally to undisturbed conditions All the type specific-sensitive species are present The age structures of the fish communities show little sign of anthropogenic disturbance and are not indicative of a failure in the reproduction or development of any particular species
Good	There are slight changes in species composition and abundance from the type-specific communities attributable to anthropogenic impacts on physico-chemical and hydromorphological quality elements. The age structure of the fish communities show signs of disturbance attributable to anthropogenic impacts on physico-chemical or hydromorphological quality elements and in a few instances are indicative of a failure in the reproduction and development of a particular species, to the extent that some age classes may be missing.
Moderate	The composition and abundance of fish species differ moderately from the type-specific communities attributable to anthropogenic impacts on physico-chemical or hydromorphological quality elements. The age structure of the fish communities show major signs of anthropogenic disturbance, to the extent that a moderate proportion of the type specific species are absent or of very low abundance.
Poor	Waters showing evidence of major alterations to the values of the biological quality elements for the surface water body type and in which the relevant fish communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions, shall be classified as poor.
Bad	Waters showing evidence of severe alterations to the values of the biological quality elements for the surface water body type and in which large portions of the fish community normally associated with the surface water body type under undisturbed conditions are absent shall be classified as bad.

To facilitate comparability of ecological classification systems across Europe, Member States monitoring results shall be expressed as Ecological Quality Ratios (EQR). “These ratios shall represent the relationship between the values of the biological parameters observed for a given body of surface water and the values for these parameters in the reference conditions applicable to that body”, i.e. the observed biological value divided by the reference biological value (Annex V: 1.4 ii). To calculate EQRs it is fundamental, therefore, to consider the origin, composition and distribution of fish species in Irish waters in order to differentiate reference from disturbed communities.

1.4 Freshwater Fish Communities in Ireland

Went and Kennedy (1969, 1976) compiled a “List of Irish Fishes”. Maitland and Campbell (1992) also provide valuable commentary on species distribution, origin, lifecycle and biology of the freshwater fishes of Britain and Ireland. The latter authors estimate that *circa* 215 freshwater fish species occur in Europe generally of which about 80 species exist in the north-western part; they identify 55 species in Britain of which only 31 occur in Ireland. Twenty-nine species of fish known to occur in Irish freshwaters are listed in Table 1.3 with observations on their origin and current status. Three species included in the freshwater list by Maitland and Campbell (1992) have been omitted as they are primarily species of Irish coastal and estuarine waters, these are: bass (*Dicentrarchus labrax* Linnaeus 1758), thick-lipped mullet (*Chelon labrosus* Risso 1827) and thin-lipped mullet (*Liza ramada* Risso 1810).

Of the other species included here in Table 1.3, the allis shad (*Alosa alosa* Linnaeus 1758) has only been recorded occasionally from some estuaries and rarely in freshwater (J. King, *pers. comm.*). The sturgeon (*Acipenser sturio* Linnaeus 1758) is “really only a vagrant to freshwaters in the British Isles since it never breeds here” and “only very occasionally does one venture into freshwater here” (Maitland and Campbell, 1992). The twaite shad (*Alosa fallax* Lacepede 1803) and the smelt (*Osmerus eperlanus* Linnaeus 1758) enter the lower reaches of a small number of Irish rivers annually to spawn. The flounder (*Platichthys flesus* Linnaeus 1758) is known to penetrate freshwater and in some systems occurs a considerable distance upstream of the tidal influence. Like the bass and the mullets, these five species are perhaps more accurately described as fishes of transitional (estuaries) and coastal waters.

The origin and diversity of freshwater fishes in Ireland is discussed by Went 1945, 1950, 1957 and 1979; Fitzmaurice 1984; and Moriarty and Fitzmaurice, 2000. Perhaps the most comprehensive account of species distribution is provided in a series of publications which

deal respectively with bream (*Abramis brama* Linnaeus 1758) (Kennedy and Fitzmaurice, 1968), tench (*Tinca tinca* Linnaeus 1758) (Kennedy and Fitzmaurice, 1970), trout (*Salmo trutta* Linnaeus 1758) (Kennedy and Fitzmaurice, 1971), gudgeon (*Gobio gobio* Linnaeus 1758) (Kennedy and Fitzmaurice, 1972), cyprinid hybrids (rudd x bream, rudd x roach and roach x bream) (Kennedy and Fitzmaurice, 1973) and rudd (*Scardinius erythrophthalmus* Linnaeus 1758) (Kennedy and Fitzmaurice, 1974). Fitzmaurice (1983) provides an account of carp (*Cyprinus carpio* Linnaeus 1758) in Ireland and lists waters to which the species was stocked between 1950 and 1978. In their discussion on the status of the rudd, Kennedy and Fitzmaurice (1974) grouped the fish fauna as follows:

- (a) Species native to Ireland: allis and twaite shad, salmon, trout, char, pollan, smelt, eel, 3-spined and 10-spined sticklebacks.
- (b) Species known to have been introduced: rainbow trout, pike, carp, tench, roach, dace.
- (c) Species whose range in Ireland is more extensive than the past: bream and minnow
- (d) Species found in Ireland but for which there is no historical documentary data: rudd, gudgeon, stoneloach, perch.

Table 1.3: List of freshwater fish species of Ireland (scientific and common names)

Common Name	Scientific Name	Status	
Species which spend their entire life or the major part thereof in freshwater			
River Lamprey	<i>Lampetra fluviatilis</i> (Linnaeus 1758)	W	A
Brook Lamprey	<i>Lampetra planeri</i> (Bloch 1784)	L	C/R
Sea Lamprey	<i>Petromyzon marinus</i> (Linnaeus 1758)	L	C/R
Killarney Shad	<i>Alosa fallax killarnensis</i> (Regan)	L	R
Atlantic Salmon	<i>Salmo salar</i> Linnaeus 1758	W	A
Brown Trout/Sea trout	<i>Salmo trutta</i> Linnaeus 1758	W	A
Rainbow Trout	<i>Oncorhynchus mykiss</i> (Walbaum 1792)		
Arctic Char	<i>Salvelinus alpinus</i> (Linnaeus 1758)	L	R
		L	R
Pollan	<i>Coregonus autumnalis</i> (Pallas 1776)	L	R
Pike	<i>Esox lucius</i> Linnaeus 1758	W	A
Common Carp	<i>Cyprinus carpio</i> Linnaeus 1758	L	C
Gudgeon	<i>Gobio gobio</i> (Linnaeus 1758)	W	A
Tench	<i>Tinca tinca</i> (Linnaeus 1758)	L	C
Common Bream	<i>Abramis brama</i> (Linnaeus 1758)	W	A
Minnow	<i>Phoxinus phoxinus</i> (Linnaeus 1758)	W	A
Rudd	<i>Scardinius erythrophthalmus</i> (Linnaeus 1758)	W	C
Roach	<i>Rutilus rutilus</i> (Linnaeus 1758)	W	C
Dace	<i>Leuciscus leuciscus</i> (Linnaeus 1758)	L	R
Chub	<i>Leuciscus cephalus</i> (Linnaeus 1758)	L	R
Stoneloach	<i>Barbatula barbatula</i> (Linnaeus 1758)	W	A
European Eel	<i>Anguilla anguilla</i> (Linnaeus 1758)	W	A
Three-Spined Stickleback	<i>Gasterosteus aculeatus</i> Linnaeus 1758	W	A
Ten-Spined Stickleback	<i>Pungitius pungitius</i> (Linnaeus 1758)	L	C
Perch	<i>Perca fluviatilis</i> Linnaeus 1758	W	A

Species which enter freshwater to spawn near the upstream limit of tidal influence			
Twaite Shad	<i>Alosa fallax</i> (Lacepede 1803)	L	R
Smelt	<i>Osmerus eperlanus</i> (Linnaeus 1758)	L	R
Species which may enter freshwater for variable periods but principally occur in marine or estuarine waters			
Allis shad	<i>Alosa alosa</i> (Linnaeus 1758)	L	R
Sturgeon	<i>Acipenser sturio</i> (Linnaeus 1758)	L	R
Flounder	<i>Platichthys flesus</i> (Linnaeus 1758)	W	C

Native species in Bold type

L – Local; W – Widespread; R – Rare; C – Common; A – Abundant.

Kennedy and Fitzmaurice (1974) express the view that bream and minnow are almost certainly introduced. The stone loach, which is erratically distributed throughout Ireland, they suggest might be native; “the gudgeon has a patchy and imperfectly known” distribution, and the perch which is “absent from several catchments where conditions are not unsuitable for it” cannot be assessed. They regard the distribution of the rudd as that of an introduced rather than an indigenous species. Kennedy and Fitzmaurice (1974) concluded “that most if not all, of the purely freshwater fishes found in Ireland to-day were introduced during the period from the Norman invasion to the late nineteenth century”. Maitland and Campbell (1992) suggest that stone loach, gudgeon and minnow (all three species “originally indigenous to south-east England”), were used as bait (live and dead) and this is a likely mechanism for their distribution. This may explain the somewhat random distribution of these three species in Ireland. The perch is also indigenous to south-eastern England but has been widely redistributed “for their food value and ease of catch” (Maitland and Campbell, 1992). Moriarty and Fitzmaurice (2000) concluded the freshwater fishes of Ireland belong to two groups: diadromous native species which became widely distributed in lakes and rivers and purely freshwater species introduced since the 16th century.

Salmon occur in every waterbody in Ireland to which they can gain access (McGinnity, *et al.*, 2003). Brown trout occur in almost every rivulet, brook, stream and river in Ireland (Kennedy and Fitzmaurice, 1971) and eels are thought to exhibit a similar distribution (Moriarty and Dekker, 1997). The Killarney shad, char and pollan are confined to lakes, as are rainbow trout. Carp, tench, bream and rudd are primarily species of standing waters but also occur in very slow flowing deep water in some rivers (habitats not surveyed in this project).

Roach, introduced to Ireland in 1889 (Went, 1950), have been distributed to many waters, mostly by anglers (Fitzmaurice, 1981) and now occur in almost all the rivers draining Ireland’s central limestone plain. Roach x rudd hybrids were positively identified, for the first time in Lough Conn in 2002 (O’Grady, *pers. comm.*). Dace, introduced with roach to the Munster Blackwater in 1889 (Went, 1950) have developed populations since 1975 in the

River Nore, Co. Kilkenny and the Bunratty River, Co. Clare, a tributary of the Shannon (Moriarty and Fitzmaurice, 2000). This species has recently also been identified in the Shannon at Castleconnell and its tributary the Mulcaire River, occurring upstream and downstream of the weir at Annacotty. Dace now also occur in the River Barrow (J. Caffrey, *pers. comm.*) and the Doon Lakes in east Co. Clare.

1.4.1 “Exotic” species

Occasional occurrences of ‘exotic’ non-native species have been recorded from Irish freshwaters; e.g. catfish (*Octalurus melas*) Fitzmaurice (1984) and huchen (*Hucho hucho*) (Moriarty and Fitzmaurice, 2000) but there is no evidence to suggest that either species became established. In 2000 live crucian carp (*Carassius carrassius*) were confiscated from a group of French anglers fishing the River Fergus at Clarecastle (M. Fitzsimons, *pers. comm.*). These tourist anglers brought the fish to Ireland as ‘live bait’. In 2000 a single chub (*Leuciscus cephalus*), allegedly captured on the River Inny at Ballinafad, was positively identified. Live specimens were again positively identified from the River Inny in 2004 and 2006 (J. Caffrey, *pers. comm.*). The species is thought not to be present elsewhere in Ireland.

1.5 Fish Stock Assessment in Ireland

There is no tradition of systematic monitoring of fish stocks in Irish freshwaters and no national monitoring programme exists. Qualitative information on fish stocks was compiled by the Inland Fisheries Trust from 1950 to 1979 and thereafter by the Central and Regional Fisheries Boards, to assess the angling potential of rivers and lakes. In 1975 a quantitative technique was developed to assess the trout populations in lakes managed as game fisheries (O’Grady 1981). At that time trout stocks in selected rivers were also beginning to be quantitatively assessed (Champ, 1983). These operations provide partial information only on the fish community composition for the waters surveyed.

Since 2000, predator control has been suspended in most lakes and knowledge of fish communities in these waters is less reliable. Likewise electric fishing surveys, also conducted intermittently, provide information on riverine fish communities and frequently have been designed to deliver quantitative information only on salmonids. Following a period of collaborative work between the Central Fisheries Board (CFB) and the Office of Public Works (OPW) the Central Fisheries Board adopted a policy of carrying out standard baseline fish habitat surveys in all catchments where in-stream works, drainage maintenance etc. are carried out (O’Grady *et al.*, 1991).

Investigative surveys, of waters with angling potential for coarse fish, were conducted throughout the 1960s and 1970s but this practice was discontinued in the 1980s. Selected

coarse fish waters (circa 250 lakes) were resurveyed between 1994 and 1997 by the CFB. Reports of anglers catches, returns to the Irish Specimen Fish Committee (ISFC), information compiled in occasional surveys of selected trout lake fisheries, intermittent localised surveys of riverine salmonid populations, fish kill incidents and surveys conducted by the Irish Char Group, provide the only updates regarding the current distribution of freshwater fish in Ireland.

However, no national monitoring programme exists for fish in Irish lakes or rivers. In England and Wales monitoring of fish in rivers has been carried out for a number of years over an extensive network of sites by the Environment Agency and a standard methodology has been adopted by the UK Environment Agency (Alan Starkie, *pers. comm.*).

1.6 The use of fish as indicators for the ecological quality of running waters

The use of fish communities as indicators for the ecological quality of running water is becoming more common worldwide (Karr, 1981; Scott and Hall, 1997; Kestemont *et al.*, 1998; Appelberg *et al.*, 2000; Belpaire *et al.*, 2000; Kesminas and Virbickas, 2000; Schmutz *et al.*, 2000; McCormick *et al.*, 2001; Joy and Death, 2002; Mebane *et al.*, 2003; FAME CONSORTIUM, 2004; Pont *et al.*, 2006). Fish have been overlooked in the past due to a number of disadvantages, i.e. fish mobility in time and space, manpower needs for sampling are higher than for other taxa, therefore field sampling is more costly (Karr, 1981; Berkman and Rabeni, 1986). However, fish provide powerful tools for assessing aquatic environments and have proved their suitability as indicators of human disturbances for many reasons and provide a dramatic impact when mortality occurs. Fish provide a highly visible and dramatic manifestation of environmental degradation when fish mortality occurs. Fish have a number of advantages as indicator organisms for biological monitoring programmes (Karr, 1981; Harris, 1995; FAME CONSORTIUM, 2004).

1. Fish are present in most surface waters, even in the smallest streams, and occupy a variety of habitats.
2. Fish are relatively easy to identify (i.e. training periods for fish identification are relatively short in comparison to other taxa such as macroinvertebrates), most samples can be identified in the field with the release of the sample after processing.
3. Information on the ecological requirements and life histories of most species of fish are well documented
4. The sensitivity to disturbances is well documented for many species and their responses to environmental stressors are often known.

5. Fishes have evolved complex migration patterns making them sensitive to continuum interruptions.
6. The longevity of many fish species enables them to be sensitive to disturbance over relatively wide temporal and spatial ranges.
7. Fish communities comprise a range of trophic levels, e.g. omnivores, herbivores, insectivores, planktivores and piscivores.
8. Depressed growth and recruitment are easily assessed and reflect stress.
9. Fishes are valuable economic resources and the public can relate to them. Using fishes as indicators confers an easy and intuitive understanding of cause effect relationships to stakeholders beyond the scientific community.

Standardised fish-based methods to assess ecological integrity of running waters were developed in the USA in the 1980s (Karr, 1981; Angermeier and Karr, 1986; Karr *et al.*, 1986; Karr *et al.*, 1987). Karr (1981) predicted that regular use of fish can improve the resolution of water quality monitoring and assessment programmes. In Europe, the development of fish-based methods are increasingly important now that fish are one of the four biotic elements listed in the EU Water Framework Directive (CEC, 2000) on which waterbody status will be assessed and a number of studies have been undertaken (Appelberg *et al.*, 2000; Belpaire *et al.*, 2000; Kesminas and Virbickas, 2000; Schmutz *et al.*, 2000; Pont *et al.*, 2006). A multimetric fish-based index (i.e. European Fish Index (EFI)) was developed by the FAME project (Pont *et al.*, 2006), a consortium of researchers from 12 countries in Europe (Austria, Belgium, France, Germany, Lithuania, Poland, Portugal, Spain, Sweden, the Netherlands and the United Kingdom) based on the concept of the Index of Biotic Integrity (Karr, 1981).

The Irish freshwater fish fauna broadly consists of two distinct groups, the salmonids, which require low temperatures and high oxygen and the cyprinids which prefer higher temperatures and display a range of tolerance to low oxygen. High species richness is an advantage in the development of a biological quality index (e.g. IBI, Karr, 1981), however, Joy and Death (2000) successfully correlated a limited fish species composition with habitat quality in New Zealand rivers. Therefore, it is considered possible that the different tolerances displayed by the various elements of the Irish ichthyofauna, to physio-chemical and environmental pressures, could facilitate the development of an index despite the restricted species diversity.

1.7 Aims and objectives of the project

The aims and objectives of the project are:

1. To assess the impact of degraded water quality, as described by the EPA's Quality Rating System, on riverine fish stocks by establishing a relationship between fish stocks in rivers and the EPA's Q-value system.
2. To develop a model, with known accuracy and precision, to predict the composition of fish in rivers (based on the integration of both hydromorphological and the biotic elements of the aquatic ecosystem).
3. To provide recommendations for fish stock assessment in rivers (and to investigate methods for lakes).
4. To develop an increased understanding of the impacts of eutrophication on fish populations and communities.

A number of tasks were identified in order to achieve the main aims and objectives (Table 1.4).

Table 1.4: Details of tasks that were undertaken by the project in order to achieve the four main objectives

Task No.	Overall activity	Main components
1	Project planning	Project management and logistics
2	Literature review	
3	Standard methodology	Q-value workshop, standard survey sheet and standard protocol produced
4	Compilation of database and transfer into GIS	Develop a database for the project
5	Archival data	Retrieve data on fish, macroinvertebrates and abiotic variables from CFB archival database and transfer into project database
6	Work Package 1	Establish relationship between Q-value and fish stock composition and abundance
7	Work Package 2	Develop an appropriate sampling protocol for assessment of the status of fish stocks in rivers
8	Work Package 3 (a)	Detailed evaluation of fish stocks in selected sites on base/core rivers (Rye Water, Oona, Dunkellin and Robe)
9	Work Package 3(b)	Microdistribution in relation to diurnal oxygen variation-telemetry
10	Work Package 3 (c)	Survival of salmonid eggs in relation to silt and blanket weed

Chapters 2 and 3 deal with relationships between the fish communities and the physical environment, layering water quality aspects on top of hydromorphological factors using a range of statistical and modelling approaches. With there currently being no national monitoring programme for fish in Ireland, the project evaluated stock survey methods and developed a standard fish stock sampling protocol for rivers (Chapter 4), potential sampling protocols for the assessment of fish populations in lakes were also investigated. Chapters 5 and 6 attempt to answer questions posed in the original project specification on the impact of

diurnal oxygen variation (Chapter 5) and silt and blanket weed on spawning (egg survival) (Chapter 6).

1.8 Project Management, role of participants and structure of report

The project management structure and the deliverables of the project are outlined in Appendix 2. The project was coordinated by the Central Fisheries Board.

In addition to the main deliverables a number of supplementary reports and publications have arisen from the project. There were also some student project theses written in part fulfilment of a Ph.D. etc. (also in Appendix 2).