Chapter 6: Work Package 3 (c): Survival of Salmonid Eggs Along a Water Quality/Nutrient Gradient

6.1 Overview of chapter

This chapter deals with an investigation to examine the survival of eyed ova and alevin incubated in hatching boxes in river sites representing a water quality gradient. Twenty six sites (16 sites on the east coast and 10 sites on the west coast) were included in the study ranging from Q2-3 to Q5.

6.2 Introduction

Over the last three decades, Irish rivers have been subject to increased nutrient inputs from agricultural and urban sources leading to eutrophication (McGarrigle, 2001). In Irish rivers, a median annual threshold value of 30µg/l (or a mean value of 47µg/l) molybdate reactive phosphate can give rise to eutrophic conditions (Clabby *et al.*, 1992, Mc Garrigle 2001). Phosphate promotes prolific growth of aquatic macrophytes and increased algal biomass, which can impede flow and cause the accumulation of silt around the plants (Ladle and Casey, 1971). This ultimately leads to siltation and compaction of spawning gravels. Other biological changes arising from eutrophication in freshwater environments include severe diurnal fluctuations in dissolved oxygen levels and a reduction in biodiversity (Mc Garrigle, 2001). These conditions threaten the long-term viability of salmonid populations, which are considered to be sensitive to low oxygen levels and increased siltation (Hendry *et al.*, 2003).

Salmonids preferentially select clean, well-oxygenated gravels when spawning, as fines may affect egg survival. Fines are usually defined as particles <1mm, although some authors refer to fines as any particle <2mm (Ottaway *et al.*, 1981, Soulsby *et al.*, 2001). The fine particles (<1mm) may become entrapped in small gravel thus reducing essential inter-gravel flow as it supplies dissolved oxygen to the eggs and removes metabolic wastes from the developing alevins (Hausle & Coble, 1976). Fine sediments can also adhere to and abrade the chorion of salmonid ova (Adams and Beschta, 1980). Furthermore, accumulation of fines material may trap the alevin and reduce their living space and escape cover (Dill and Northcote, 1970). Many authors have used laboratory and field experiments to document the detrimental effects of siltation on egg survival and alevin emergence (Adams and Beschta, 1980; Peterson & Metcalfe, 1981; Witzel and MacCrimmon, 1983; Chapman, 1988; Kondolf and Wolman, 1993; O'Connor, 1998). However, Crisp (1993) found that alevins of brown trout and Atlantic salmon could successfully penetrate a sand layer up to 8cm in thickness.

Oxygen is essential for embryo survival and is obtained as water moves through the redd. Dissolved oxygen concentration is determined by the flow through the gravel, which in turn is affected by gravel bed porosity (Crisp, 1993). Hatching success and alevin size are strongly dependent on gravel permeability and concentrations of dissolved oxygen (Turnpenny and Williams, 1980). Low oxygen concentration can also result in a reduction in fry size (Kondolf and Wolman, 1993). Other factors affecting salmonid egg survival and alevin size at emergence include gravel size, depth of egg burial and egg density (Dill and Northcote, 1970). Temperature is also an important factor for egg development as freezing conditions may kill the eggs and higher than normal temperatures speed up hatching (Embody, 1981).

Although there has been extensive research carried out on brown trout egg and alevin survival rates in relation to fines and dissolved oxygen concentration throughout Europe, little work has been carried out to date on linking these factors to eutrophication and examining survival along a pollution gradient. Less work has been carried out in the Republic of Ireland with the exception of work carried out on the survival rate under acidic conditions in the Wicklow mountains (Kelly-Quinn *et al.*, 1993).

6.2.1. Aims and objectives

The main aims of this work package was to examine the survival of eyed ova and alevin, incubated in hatching boxes in river sites representing a water quality gradient on the east and west coast of Ireland and to examine which biological, chemical or physical factors were affecting survival.

6.3 Materials and Methods

The egg survival study was carried out over six weeks from January through to March in 2002 and 2003. It has been established that a strong relationship exists between the EPA Q-value and mean levels of molybdate reactive phosphate (McGarrigle, 2001), therefore, published EPA Q-values were used to select potential sites which represented a nutrient and water quality gradient. A two-minute kick sample was taken to establish macroinvertebrate communities and confirm the Q-value.

6.3.1 Site selection/ study area

A pilot study was carried out in 2002 on nine sites ranging in quality from Q2-3 (moderately polluted) to Q4 (unpolluted). The number of sites and the range in quality were increased in 2003 to include 26 sites (16 sites on the east coast and 10 sites on the west coast), ranging from Q2-3 to Q5. A detailed description of each site is provided in Table 6.1.

The sites chosen in the east of Ireland were located on the Liffey, Slaney, Broadmeadow, Ward, Tolka and Dodder catchments and those in the west flowed through the Robe and Aille catchments. The River Liffey rises in a large expanse of high-level blanket bog and travels 138km, draining a catchment area of 1616km^2, before entering the sea in Dublin. Once an excellent angling river, diffuse and point source pollution have degraded sections of the system. The River Slaney flows southwards from the Wicklow Mountains across blanket bog (Reynolds, 1998) and rough grazing for 80km draining a catchment area of 1943.5km^2 to enter the sea at Wexford. Low-level agriculture in the upland stretches has ensured its high water quality status. The Dodder rises at 630m and flows northwards through the valley of Glenasmole which has two reservoirs for a length of 24km before entering Dublin Bay at Ringsend. Although it has good water quality and is a good trout stream, it is prone to severe flooding. The Broadmeadow, Ward and Tolka are small catchments situated in Co. Dublin. The Broadmeadow rises at an altitude of 110m near Dunshaughlin in Co. Meath and flows eastwards for approximately 22km where it joins the Ward river south of Ashboune. The Ward river rises near Kilbride at an altitude of 80m in Co. Meath where it winds its way (21km) through a number of small towns. The Broadmeadow river joins it after it has flowed though Swords and the river enters the sea just north of Swords. The Tolka rivers rises at an altitude of 80m north west of the small village of Batterstown and is slightly larger than the previous two catchments flowing for 30km through an extremely built up area of the city before entering Dublin harbour at Fairview park. The Broadmeadow, Ward and Tolka rivers flow across glacial till and boulder clay occupying wide flat valleys before entering the sea. Their path through the increasing conurbation of Dublin city ensures their status as moderately polluted systems.

The River Robe rises west of Ballyhaunis and drains a 320km^2 area of lowland of south Mayo before flowing into Lough Mask downstream of Ballinrobe. The Robe is the largest of the Lough Mask tributaries (63.6 km in length). It is an important trout spawning and nursery river, however, water quality in the catchment has been deteriorating over the last number of years due to eutrophication. The Aille River (30 km in length) rises in the Partry mountains northwest of Lough Mask. The river runs underground for approximately 3 kilometres (17 km from its source). The geology of each site is coded in Table 6.1.

Table 6.1. Site Descriptions

Catchment	River	Code	Year	Q value	Catchment area (km^2)	Stream order	Altitude	Geology
Broadmeadow	Broadmeadow	**UCD20**	2003	3	39.72	3	60	16.1
Ward	Ward	**UCD21**	2003	2-3	16.76	4	66	16.2
Tolka	Tolka	**UCD22**	2003	2-3	21.07	4	67	16.2
Liffey	Gollymochy stream	**UCD11**	2002	3	11.31	2	67	16.1
Liffey	Canal supply	**UCD012**	2002	3	4.09	1	75.	16.2
Liffey	Liffey	**UCD13**	2002	3	1616	4	120	
Liffey	Rye Water	**UCD14** **UCD23**	2002 2003	3 3	193.54	4	39	16.2
Liffey	Morell stream	**UCD15** **UCD24**	2002 2003	4 3-4	13.62	2	95	16.1
Liffey	Painestown	**UCD16** **UCD25**	2002 2003	3 3	13.08	3	81	16.1
Liffey	Painestown	**UCD17** **UCD26**	2002 2003	4-5 4	20.73	4	81	16.1
Liffey	Brittas	**UCD27**	2003	4	8.94	4	247	10
Liffey	Hartwell	**UCD28**	2003	4	13.77	3	105	16.1
Liffey	Killcullen	**UCD18** **UCD29**	2002 2003	4 4	38.96	3	101	10
Liffey	Griffin stream	**UCD19** **UCD35**	2002 2003	2-3 2-3	28.9	4	69	16.2
Dodder	Owendoher	**UCD30**	2003	4	3.58	3	173	1
Slaney	Derreen	**UCD31**	2003	4	35.12	4	160	1
Slaney	Knickeen	**UCD32**	2003	4-5	12.09	3	216	1
Slaney	Little Slaney	**UCD33**	2003	4-5	17.58	3	165	1
Slaney	Little Slaney	**UCD34**	2003	5	4.34	2	195	1
Robe	Vincent Walsh	**CFB01**	2003	3	9.67	3	48	16.1
Robe	Ballindine	**CFB02**	2003	3	4.88	2	52	16.1
Robe	Mayfield	**CFB03**	2003	3	12.28	3	54	16.1
Robe	Claremorris	**CFB04**	2003	3	0.273	2	54	16.1
Robe	Ballygowan	**CFB05**	2003	3-4	13.63	2	63	16.1
Robe	Kilnock	**CFB06**	2003	4	1.67	1	64	16.1
Robe	Bricken stream	**CFB07**	2003	4	11.46	3	62	15
Robe	Scardaun	**CFB08**	2003	3	5.65	2	47	16.1
Aille	Aille	**CFB09**	2003	4-5	78.67	4	90	2
Aille	Camoge	**CFB10**	2003	4-5	6.08	3	98	10

CA= Catchment area **HA**=Hydrometric area

Code	Description of geology
1	**Granite, Felsite & other intrusive rocks rich in silica**
2	**Diorite, Gabbro, dolerite & other intrusive rocks poor in silica**
10	**Ordovician**
15	**Lower avonian shales & sandstones, carboniferous slate series & calciferous sandstone series**
16.2	**Middle carboniferous limestone**
16.1	**Lower carboniferous limestone**

6.3.2 Fieldwork

Brown trout eggs were planted in the above river sites in January 2002 and 2003 and mortality was monitored over a six-week period. Farm bred brood stock females were stripped and fertilised on the 08/11/01 at Cullion fish farm in Mullingar. They were held at the fish farm for 61 days in circulating water with an average temperature of 6.9°C. They were collected from the fish farm and planted at nine sites on the east coast on the 08/01/02. Eggs were also stripped on the 22/11/02 and held for 67 days at an average temperature of 6.3°C (Charlie Nolan, *pers. comm.*). These eggs were collected and transported to the east and west coast sites on the 28/01/03.

Vibert boxes were constructed to house the eggs (Harris, 1973). The boxes (10.6cm x 6.5cm) were lined with mesh (size 2mm) and contained only one compartment. The mesh allowed the movement of water through the box. The boxes were slightly modified to include a secure screw-cap which facilitated faster handling during loading and later examination of the eggs. A total of 50-eyed ova were placed in each box and carefully interspersed with gravel which had been washed and sieved to remove excess fines. Although the size of available spawning gravel may vary substantially from site to site depending on climate, land use, drainage area and basin typology (Kondolf & Wolman, 1993), the same gravel was used in all boxes, ranging in size from 4mm-70mm. Three groups of six vibert boxes were buried at each site to a depth of approximately 10cm below the gravel surface.

Egg boxes were lifted at weekly intervals over a six-week period. Each week, one box was lifted from each of the three groups at all sites. Sites were visited in the same order over a two day period. Egg boxes were lifted from the east coast sites between 10.00 and 16.00 and were placed in a plastic bag which was filled with river water. Egg boxes were returned immediately to the laboratory and observations on the egg/alevins and estimation of mortality rate took place between 18.00 and 23.00.

Weekly water samples were collected and returned to the laboratory for chemical analysis. On site measurements were made for temperature, oxygen saturation, conductivity and pH.

6.3.3 Laboratory Analysis

The contents of the Vibert boxes were poured into a white tray and the number of live eggs/alevins counted. Percentage egg mortality was determined by the number of dead eggs/alevins divided by the total number of eggs originally placed in the artificial redd (See Appendix 10). At a number of sites, length measurements were made on a random sample of alevins.

Median mortality was calculated for each site from the data shown in Appendix 10. Average mortality was then calculated for each Q-value band. Mortality was determined for four different life stages; egg, hatching, emergent and swim-up stage, however there may be some overlap between development stages at each site. Survival at the egg stage was determined by a count of bright orange-eyed ova. White eggs were considered dead. The hatching developmental stage was determined when the majority of the eggs in each box had hatched into alevins. The emergent stage was reached when the alevins were still using their yolk sac for food, they were not recorded as being at the 'swim up' stage until their yolk sac had diminished. Average egg/alevin percentage mortality was calculated for each Q-value (Figs 6.1 and 6.2).

The gravel and other substrate materials from each box were placed in a warm dry environment where they were left to dry out for a number of weeks. Once dry, the sediment was passed through a series of mechanical sieves (aperture 2mm to 0.032mm). The contents of each sieve were weighed and the percentage of fines (substrate < 1mm and <2mm) was determined. The relationship between sieve aperture (mm) and the commonly used descriptive scale A.G.U.D.S. (American geophysical union descriptive scale) for substrate analysis is shown in the Table 6.2.

Table 6.2. Relationship between sieve aperture (mm) and A.G.U.D.S.

Sieve aperture (mm)	**American geophysical Union Descriptive scale**
32 to 64	Very coarse gravel
16 to 32	Coarse gravel
8 to 16	Medium gravel
4 to 8	Fine gravel
2 to 4	Very fine gravel
1 to 2	Very coarse sand
0.5 to 1	Coarse sand
0.25 to 0.5	Medium sand
0.125 to 0.25	Fine sand
0.063 to 0.125	Very fine sand
0.032 to 0.063	Coarse silt

The water samples were analysed for total suspended solids (TSS), molybdate reactive phosphate (MRP), total oxidised nitrogen, ammonia and alkalinity. Other parameters including altitude, stream order, slope, catchment area and geology were derived from the EPA Digital Terrain Model.

6.3.4 Statistical Analysis

Statistical analysis was carried out using SPSS and SAS. Spearman rank-order correlation was carried out to determine what parameters were associated. A Friedman test, a non-parametric analogue of a two-way ANOVA, was used out to examine if the mortality varied between Q-values.

A repeated measure analysis using Proc Mixed in SAS was used to eliminate the background error due to site and weekly changes. Once background noise was eliminated, predictions were back transformed to produce a graph of predicted mortality at each site. Finally logistic multiple regression using a stepwise progression was carried out to show how much of the variation in mortality was accounted for by using the relationship between the 'variables' and the 'effect' i.e. mortality. Analysis was carried out on the logit scale.

6.4 Results

Mortality was calculated for each 'vibert' hatching box at all sites (see Appendix 10). In general, as expected mortality in both years increased over time but the pattern differed between sites and development stage.

6.4.1 Egg mortality along a water quality gradient

Data were only available for the nine east-coast sites in 2002 (Fig. 6.1). Data from east and west coast rivers were combined in 2003 for analysis (Fig. 6.2.). Eyed ova were planted earlier in 2002 than in 2003 therefore the first two weeks of data were combined to determine egg survival in 2002. Heavy rain and flooding occurred after hatching so it was not possible to lift any of the boxes on the 05/02/02.

In 2002, mortality of eggs in weeks one and two was extremely low (<11% at all sites). However, mortality increased substantially at the hatching stage in week 3 at sites rated Q2-3 and Q4 but remained low at Q4-5. On returning to the sites on the 12/02/02 after substantial flooding, mortality had further increased. Sites UCD19 (Q2-3) and UCD11 (Q3) had 100% mortality following the flood. Mortality rates at Q3 and Q4 sites were similar after the hatching stage and only slightly lower mortality at the emergent and swim-up stage was found at Q4-5 (UCD17). The Friedman test detected a statistically significant difference between mortality at the various Q-values ($P<0.05$) and between different life stages ($P<0.05$). The same test was repeated with data from 2003 but there was no statistical difference in average mortality between each Q-value. However, there was a difference between mortality at each of the life stages ($P<0.05$). The variation in mortality between Q-values in 2003 (Fig. 6.2.) was not as significant as appeared in 2002 (Fig. 6.1).

In 2003 the egg stage only included one week of data because egg planting took place quite late. Hatching had taken place at all sites by the 11th February 2003. The alevins survived on the content of their yolk sacs for three weeks and finally reached swim up stage by the 11th March 2003.

Egg mortality was low across all Q-values. Mortality reached its highest level in the Q4-5 band largely due to two sites, CFB09 in the west and UCD32 in the east. These low values were attributed to hydrochemical conditions (see Section 6.4). Hatching was more successful in 2003 with little variation across sites. Mortality during the emergent stage was highest at the Q3-4 sites. One of the sites in this band had extremely high mortality (median 96%), which lowered the overall band average. However, at the other sites survival was good and mortality values remained below 60%. At the end of the study period, mortality at swim-up stage was similar between sites rated Q3, Q4 and Q4-5. High mortality was found again at Q3-4 and at Q5. Only one site was available in the category Q5 (clean water) and the low value may be site specific and a factor of inadequate replication. Surprisingly, the lowest mortality was at the most polluted sites, which were in Q2-3 quality band. These sites had the highest survival throughout the survey.

6.4.2 Predicted mortality at each site.

A repeated measures analysis was carried out to remove some of the error produced by weekly sampling. The estimates calculated by the Least Square Means were back transformed to produce the following proportions. These proportions allow comparisons of mortality between sites and between years to be made.

In 2002, the lowest mortalities were found at UCD17 (Q4-5 site) and site UCD13 (Q3) on the main Liffey, one of the largest rivers in the survey. Mortality was highest at UCD11, UCD16 and UCD19 (Fig. 6.3). These sites had 100% mortality after the flooding incident.

Predicted mortalities in the east in 2003 showed large variation across Q-value bands (Fig. 6.4.). Some of the lowest mortalities were found at sites with low water quality such as UCD21 and UCD22. In contrast, UCD32, a Q4-5 site, had extremely high mortality. Six sites examined on the east coast in 2002 were surveyed again in 2003, they included; UCD24, UCD23, UCD29, UCD25, UCD26 and UCD35. These sites displayed considerable variability in survival across years. Sites UCD24, UCD26 and UCD29 had increased mortality in 2003 whereas sites UCD23, UCD25 and UCD35 showed a decrease in mortality. A Mann-Whitney U test was used to compare mortality at these sites between years. Statistically, the only site with significant differences between 2002 and 2003 was UCD26 (P=0.021).

The highest mortality recorded on the west coast (See Fig. 6.5) was at CFB09, a Q4-5 site and the cleanest site in this area. Temperature at this site was consistently lower than at all the other sites. Site CFB06 (a Q4 site) has the lowest mortality and highest temperature readings throughout the survey.

Details of actual mortality estimates at each site in 2002 and 2003 have been included in Appendix 10.

6.4.3 Relationships between biological, chemical and physical variable measured at each site.

Spearman's correlation was used to produce a correlation matrix for each data set (see Appendix 11). The data from the sites in the west of Ireland were quite different from the east coast rivers so analyses were carried out separately. There were a number of relationships present in the data but only the important correlations are discussed below. Data on percentage fines were only available for sites in the east. Fines correlated positively with altitude and temperature but negatively with stream order. In 2002, fines were associated with increased alkalinity and total oxidised nitrogen. However, in 2003 they were negatively correlated with conductivity, pH, nitrite, MRP (molybdate reactive phosphate), geology and alkalinity. Q-value, as expected, was negatively correlated with MRP, ammonia and geology but positively correlated with altitude. Conductivity was positively associated with alkalinity, MRP and nitrite in the east but not the west in 2003. Nitrite data were collected on the east coast and they were positively correlated with TON (total oxidised nitrogen) as would be expected but they were also correlated with MRP, ammonia, TSS (total suspended sediment) and alkalinity. Increases in MRP were associated with increases in ammonia in all catchments and ammonia was positively correlated with total suspended sediment. As expected, oxygen was negatively correlated with temperature.

6.4.4 Factors affecting egg/alevin mortality

A logistic regression was carried out using a stepwise procedure to examine which factors were having an effect on mortality. In 2002 a larger number of factors were isolated including fines <2mm, altitude, stream order, alkalinity, pH, nitrite, TON, MRP and geology (Table 6.3).

Table 6.3: Model of factors affecting egg mortality for west coast data in 2002 (0.15 = significant level for entry into the model).

	Parameter estimator	Standard error	Type II SS	F value	Pr>F
Intercept	664.97	149.13	39.02	19.88	0.0002
Fines <2mm	0.25	0.05	53.28	27.14	<0.0001
Altitude	-0.30	0.04	98.59	50.23	<0.0001
Stream order	-2.74	0.58	43.34	22.08	<0.0001
Alkalinity	-0.10	0.02	76.80	39.13	<0.0001
pH	-1.77	0.89	7.74	3.94	0.0587
Nitrite	40.51	22.89	6.15	3.13	0.0895
TON	0.23	0.07	20.30	10.34	0.0037
MRP	-36.11	7.93	40.71	20.74	0.0001
Geology	-36.83	8.75	34.76	17.71	0.0003

TON= Total oxidised nitrogen

MRP= Molybdate Reactive Phosphate

In 2003 all data from sites in the east and west were combined to produce a model (Table 6.4). A smaller number of parameters, fines<2mm, pH, MRP, temperature and ammonia, met the 0.15 significant level for entry into the model and therefore in combination were having an effect on egg /alevin mortality.

Table 6.4: Model of factors affecting egg mortality for all data in 2003 combined. (0.15 = significant level for entry into the model).

	Parameter estimator	Standard error	Type II SS	F value	Pr>F
Intercept	-7.63	2.64	30.80	8.33	0.0049
Fines 2mm	0.10	0.03	38.12	10.30	0.0018
pH	0.68	0.34	14.29	Variable	0.0525
MRP	-14.89	4.61	38.63	10.44	0.0017
Temp.	0.36	0.10	44.55	12.04	0.0008
Ammonia	2.70	1.09	22.70	6.14	0.0151

Temp. = Temperature

MRP=Molybdate reactive Phosphorus

East and west site data were then modelled separately to establish any spatial effects. Temperature was the only factor having an effect on egg/alevin mortality on the west coast (Table 6.5). However, for sites in the east in 2003, altitude, conductivity, nitrite, MRP, temperature and catchment area all had an effect on mortality (Table 6.6).

Table 6.5: Model of factors affecting egg mortality for west coast data in 2003

	Parameter estimator	Standard error	Type II SS	F value	Pr>F
Intercept	-3.71	0.70	33.20	27.77	<0.0001
Temperature	0.42	0.11	19.04	15.93	0.0003

Table 6.6: Model of factors affecting egg mortality for east coast data, 2003

	Parameter estimator	Standard error	Type II SS	F value	Pr>F
Intercept	-7.86	1.27	21.93	38.63	<0.0001
Altitude	0.03	0.01	11.69	20.58	<0.0001
Conductivity	0.01	0.00	16.29	28.69	<0.001
Nitrite	-42.58	16.22	3.91	6.89	0.0112
MRP	-9.33	3.78	3.45	6.08	0.0168
Temperature	0.29	0.05	18.54	32.65	<0.0001
Catchment area	8.10E-9	2.37E-9	6.64	11.70	0.0012

MRP= Molydate reactive Phosphate

6.4.5 Alevin growth in relation to temperature.

The size of the alevins after hatching was related to the temperature of the water (Fig. 6.6). A degree-day, which is used in Fig. 6.6, is equal to 1C above 0^0C for 1 day. Lower temperatures were experienced at UCD22, UCD21 and UCD35 in week 1 and the alevins hatching from these sites were smaller than alevins at other sites. The highest water temperatures were experienced at UCD24 and UCD29 throughout the period and the alevin at these sites were larger than at other sites. The eggs stripped on the 8/11/01 2001 had all hatched by the 29/01/02 and those stripped on 22/11/02 had hatched by the 11/02/03.

6.5. Discussion

Brown trout spawning may occur in Irish rivers between November and December. The eggs are incubated in gravel over the winter months and hatching takes place between February and April. The developing embryo subsists on protein, fats and carbohydrates stored in the yolk sac. During this period of incubation, the eggs are extremely vulnerable and they require a constant supply of oxygen especially just before hatching (Sedgwick, 1982). Infiltration of fine sediment into the redd can be critical for egg/alevin survival (Soulsby *et al.*, 2001). Fry emerge from the gravel 6-8 weeks after hatching when the yolk sac has been absorbed.

In the present study egg/alevin survival in 2002 was significantly different between sites with varying water quality ratings. However, the pattern generally deviated from the hypothesis of increasing mortality/decreasing survival with declines in water quality from Q4-5 to Q2-3. Egg survival was high being >89% at all sites. This appears to be within the expected range for brown trout. LeCren (1961) also recorded a survival value of 94% for brown trout eggs in the wild. Survival of eyed ova planted in acidic streams in the Wicklow Mountains in 1992 ranged from 61% to 86% (Kelly-Quinn *et al.*, 1993). In the present study survival during the hatching period decreased substantially at all but the Q4-5 sites, the lowest values occurred at sites within the Q2-3 and Q4 bands. The reason for the poorer survival at hatching at Q4 sites than the Q3 sites is not clear from the data. It is generally accepted that the hatching stage can be more vulnerable to low oxygen levels (Sedgwick, 1982). The oxygen saturation values in waters at these sites were satisfactory, however no measurement of oxygen levels within the hatching boxes was undertaken. Fines were within acceptable range for both sites in the Q4 band (UCD18, UCD15). A similar experiment was carried out by Harshbarger and Porter (1982) in North Carolina. They placed brown trout eyed ova in whitlock-vibert boxes and recorded survival rates of 16% from eyed to hatching and 31% survival through direct plants of egg into the gravel. Survival at Q2-3 and Q4 were similar to these results but survival at Q3 and Q4-5 were substantially higher than their findings at 49% and 92% respectively. An earlier study by Harshbarger and Porter (1979) found 0% survival at the hatching stage due to fungus in the boxes. This was not found in the current study. McKenzie and Moring (1988) also recorded survival rates as high as 74% for salmon ova from planting to hatching stage.

Flooding had a detrimental effect on survival to the emergent stage at all sites in 2002 but particularly within the band Q2-3 and Q3 where there was 0% survival. In the egg boxes with 0% survival, only one had substantial levels of fines (UCD11 had 41% fines). Lisle and Lewis (1992) also found that high flow events caused temporal variation in survival and cross channel variations in bed load transport. It is possible that high levels of other pollutants washed into the stream during the flood contributing to mortality. High rainfall events may also result in elevated levels of nitrate, phosphate and ammonia run-off from agricultural land and roads. These may be detrimental to the developing embryos as discussed later. There was little change in survival from the emergent to the swim-up stage; the highest survival values were at the Q4-5 site. Harshberger and Porter (1979) recorded survival rates of 8% in boxes. This was similar to our findings at Q3 (13%) and Q4 (8%) however the current study recorded survival rates of 22% at Q 4-5, which was similar to the survival recorded for direct egg plants of Harshbarger & Porter (1979).

In the following year, 2003, there was no statistical difference between survival rates in the various water quality bands. In fact, survival was consistently higher at the low Q-value sites at each stage of development. As in 2002 egg survival was > 90% at all sites with the exception of two Q4-5 sites; CFB09 had high levels of unionised ammonia during sampling; site UCD32, a high altitude site in the Wicklow Mountains, which may have been subjected to acid pulses (Kelly-Quinn, *pers. comm.*). Egg survival of 91% was also found by other authors using similar methodology (Harshbarger and Porter, 1982). The median egg survival at site UCD32 was 68% and although this value was low in comparison to other sites in the survey, it was similar to the values found by Kelly-Quinn *et al*. (1993) in Wicklow mountain streams.

Survival at hatching in 2003 was a lot higher (>43%) than in 2002 at all sites. This value was similar to survival rates of brook trout in a laboratory experiment which reported 55.3% survival in the control trough and 40.6% when fines <0.85 were greater than 25% (Argent and Flebbe, 1999). In 2003, the highest hatching survival was found at the Q4-5 sites (85%), which is similar to hatching success of brown trout in a control stretch in South Wales (Turnpenny and Williams, 1980). Survival to emergence dropped slightly as expected but was lowest at Q3-4 sites. This band included the site UCD24 that had consistently poor survival throughout the survey period. Finally, survival to the swim-up stage yielded the most unexpected results. The highest survival rates were at the Q2-3 sites. Intermediate survival rates were recorded at the Q3, Q4 and Q4-5 sites. The lowest survival rates were recorded at Q5 and Q3-4 sites. Survival at Q3, Q4 and Q4-5 was similar to that recorded in previous studies for direct planting of brown trout eggs. The survival recorded at Q3-4 was identical to that recorded for eggs planted in Whitlock vibert boxes as previously discussed (Harshbarger and Porter, 1982). However, the survival at Q2-3 sites was substantially higher than survival figures reported in the literature. It was similar to the finding of Hausle and Coble (1976) who placed brook trout in a laboratory trough in Wisconsin. However, those alevins would not have been exposed to predation or flooding effects like those in the field.

Once survival across the Q-value bands had been established for both years it was then necessary to determine which sites had the highest predicted mortality and what had caused it. The repeated measures analysis carried out on data in 2002 showed that the highest mortalities were experienced on the three sites most affected by flooding during the survey (UCD11, UCD16 and UCD13). The cleanest site (UCD17) had the lowest predicted mortality possibly because this site had a constant supply of oxygenated water. Low mortality was also found at UCD13, which was situated on the main Liffey, and therefore its size may have had a dilution affect on pollutants. In 2003, along the east coast, survival was extremely variable, many sites with better water quality had the highest predicted mortality whereas the sites in the low water quality band had the lowest predicted mortality. The main causes of mortality will be discussed in the next section. In the 2003 west coast data set, CFB09 had the highest predicted mortality and this may be related to high levels of unionised ammonia, which will be discussed later.

The logistic regression model identified five main factors which affected egg/alevin mortality in 2003. Those included molybdate reactive phosphorus (MRP), fines <2mm, pH, ammonia and temperature. Data from sites in the east were quite different to those in the west so separate models were produced for each data set separately which will be discussed later.

The first factor selected by the model was MRP. Although it does not have a direct effect on biota, high concentrations indicate anthropogenic inputs and levels >30ug/l are indicative of eutrophic conditions (McGarrigle, 2001). As previously mentioned phosphorus is generally the limiting nutrient in freshwater and it can control algal growth and therefore primary production within the river (McGarrigle, 2001). MRP values were correlated with Q-values and therefore lower mortality occurred in higher quality classes. This was the case in 2002 but that pattern was not evident in 2003.

Although fines <1mm had an effect on mortality, they were removed in the stepwise procedure as fines <2mm were more significant. Regression analysis carried out by Adams and Beschta (1980) indicated that watershed slope, area, relief and land use influenced the amount of fine sediment in the bed. Correlation analysis in the present study also found a negative relationship between fines <2mm, stream order and slope with catchment area negatively correlated in 2002 but not in 2003. In 2003, percentage fines <2mm was positively correlated to sites with high altitude and negatively correlated to low pH and low conductivity. This would indicate that higher fines were found at higher altitude, low order streams with low pH. In a study carried out by Harshbarger and Porter (1982), they found that sediment deposition was 100% greater in first and second order streams as opposed to third order streams.

Although fines emerged as a significant factor in the overall 2003 model, it did not come out as one of the factors affecting mortality in the east coast sites. Despite the fact that three east coast sites had levels of fines, that according to the literature, should cause high mortality, they had some of the lowest predicted mortality. Hausle and Coble (1976) showed that 20% sand (<2mm) slowed emergence and reduced the number of fry emerging. Crouse *et al.* (1981) found that when fines <2mm constituted 26% and 31% by volume, fish production decreased. However, in three sites (UCD27, UCD33 and UCD31) levels of sand (<2mm) higher than 20% were recorded but 100% mortality was only found in one out of 16 such boxes, which would indicate that fines alone, was not sufficient to cause mortality at these sites.

Fines < 2mm were established as one of the factors affecting egg/alevin mortality in 2002. They did not appear to be a problem in the first couple of weeks but after flooding a large number of boxes contained >20% fines and mortality in those boxes was high. O' Connor (1998) carried out an experiment to determine the percentage of sand that had an effect on mortality. When incubated with 25% fines (0.063-1mm) none of the alevin survived. They concluded that oxygen deprivation due to clogging of the interstitial pockets caused the mortality and that infiltration of >10% could have a negative effect on alevin survival. Therefore, even if high fines content did not cause total mortality, it may still have a negative impact on trout production in a stream. Mortality in redds following spawning on the Newmills Burn, a highly canalised tributary of the River Don in Aberdeenshire, was as high as 86%, this may have been related to fine sediment infiltration, reduced permeability and reduced oxygen supply to ova. The sediment loads in this catchment were mobilised from intensively managed land (Soulsby *et al.*, 2001).

River pH was the third factor, which had an effect on egg/alevin mortality in 2003. The relationship between pH and egg mortality is well established (Weatherley *et al.*, 1990; Kelly-Quinn *et al.*, 1993; Donaghy and Verspoor, 1997). There were a number of slightly acidic sites in this survey, UCD32, UCD34 and UCD33 in the Wicklow Mountains and CFB09 on the west coast. In Week 4, the lowest pH value of 5.4 was recorded at UCD33. Site UCD34 had a pH of 6 at week 4. Site UCD32 experienced its lowest pH (6.07) during hatching which may have resulted in particularly high mortality rates at these sites. It is also possible that these sites experienced pulses of lower pH waters, which were not detected by the present sampling. High gradient mountain streams draining poorly weathered geology are prone to episodic acidity when pH values can fall to 4.2 (Kelly-Quinn *et al.,* 1993). Other research carried out in Wicklow Mountain streams found that eggs became brittle at sites exposed to acid pulses especially when pH fell below 4.5 (Kelly-Quinn *et al*., 1993). Low pH values reduce the activity of the hatching enzyme chorionase, which dissolves the inner wall of the egg, and so the time before and during hatching is particularly vulnerable (Weatherley *et al*., 1990; Mason, 1996). The pH range suitable for fisheries is considered to be between 5.0 and 9.0 however between 6.5-8.5 is preferred (Flanagan, 1990). pH also has an affect on the speciation of other important parameters including ammonia and metals such as aluminium.

Ammonia was another factor isolated by the model. It arises from the breakdown of nitrogenous organic and inorganic material in water and soil, excretion by aquatic biota, industrial processes, and gas exchange reduction of nitrogen gas by micro-organisms (Chapman, 1996). Total ammonia in surface waters is usually less than 0.2mg/l N but may reach 2-3mg/l, however, these levels are indicative of organic pollution. Levels > 0.2 mg/l N were only exceeded at one site (UCD19-weeks 1, 2, 4 and 5) in 2002 and two sites in 2003 (UCD21-weeks 3, 4 and 6 and CFB02-week 4). The relative concentration of ionised and unionised ammonia in water is a function of pH and temperature. As pH increases there is a shift toward the unionised form. It is this unionised form that is more toxic to fish and other aquatic biota. In 2002, UCD19 had levels of unionised ammonia >0.02 mg/l, this occurred in week four and 100% mortality was found in each egg box. In 2003, high levels were found at CFB09 in week 5 but mortality was only 48%. In week 4 at Site UCD21 total unionised ammonia was estimated to be 0.017mg/l N, here mortality values of 58%, 38% and 28% were recorded. Other sites with high levels of unionised ammonia, although not exceeding the critical limit, included sites CFB10 and CFB02, both on the west coast.

Nitrite ($N0_2$) was highlighted as an important factor affecting egg mortality in 2003 in the east coast only but it didn't emerge as a factor in the joint 2003 model. It normally occurs in small amounts as it is the intermediate stage between oxidisation of ammonia to nitrate and nitrogen is more likely to occur as ammonia or nitrate. Clean water has levels <0.01 $mg.l^{-1}$ NO_2 and higher values may indicate sewage pollution (Flanagan, 1990). The EC salmonid water regulation (S.I. No. 293, 1988) for nitrite recommends an upper limit less than 0.05$mg.l^{-1}$ NO_2 (Flanagan, 1990). This limit was only exceeded in 2002 at UCD19. In week one, values reached 0.068$mg.l^{-1}$ NO_2 but egg mortality was still low suggesting that this value had less of an effect on egg mortality. In week 4 when values reached 0.109$mg.l^{-1}$ NO_2, eggs were already dead at this site. Sites UCD18 and UCD17 had levels >0.01 $mg.l^{-1}$ NO_2 which would indicate some level of sewage pollution. Although the critical limit was not reached in 2003, a number of sites had levels >0.01mg.l^{-1} $N0_2$. Two sites regarded as high quality UCD29 and UCD28 had levels of nitrite >0.01 which would indicate some level of sewage pollution.

Water temperature was the final important parameter highlighted in the 2003 logistic model. Temperature throughout the study was within the optimum range 0-13^{o}C for brown trout egg as outlined by Elliott (1981). Stream temperature can control the rate of development and therefore determine the incubation period of fish eggs (Embody, 1981)**.** For trout on the east coast in 2003, it took between 437-480 degree days for 50% of brown trout to hatch. Eggs laid in November or December will usually hatch after 444-degree days (Elliott, 1994) and so the present results were within the expected range. Eggs from the same batch from the same female may hatch at different time from 3-4 days at high temperature to as much as 15-20 days at low temperatures. Variations in hatching times are also found between females of the same species (Embody, 1981).

Eggs were taken from the same batch and yet length of alevin varied between site. Sites UCD15 and UCD18 had consistently higher temperature and alevins hatched first at these sites and were larger than at other sites. In 2002 alevins hatching at Site UCD15 were 15mm in length and at site UCD18 they reached 14mm but at UCD19 the alevins were between 10-13mm in length due to the lower temperatures. This pattern was repeated in 2003 when higher temperature at UCD24, UCD29 and UCD28 produced alevin lengths of 14.9mm to 15.7mm whereas sites with cooler temperatures at UCD22 UCD21 and UCD25 produced smaller alevins (13.5mm to 14mm). Larger alevins have a longer time to find food and therefore have a natural advantage over their smaller rivals (Elliott, 1994). However in this study the sites with large alevin often had the highest predicted mortality. Predicted mortality at sites UCD24 and UCD29 in 2003 was extremely high whereas at site where hatching alevins were particularly small predicted mortality was low. Perhaps when alevins are larger there is more competition for space and oxygen within the vibert boxes so mortality is higher.

Increased temperature is negatively correlated with dissolved oxygen (Flanagan, 1990). Only surface oxygen was measured in this survey but many authors have found that oxygen in the interstitial gravels may be substantially lower than surface values. Research carried out in the UK found oxygen levels in the interstitial areas after 28 days were 6-8mg/l lower than the surface waters. They also found that 50% mortality occurred when interstitial oxygen was at 6.5mg/l (Shumway *et al*., 1964). Adequate oxygen is particularly important during the incubation of eggs. The metabolism of the developing embryo demands oxygen and low dissolved oxygen may slow growth or in extreme cases the embryo may suffocate (Sedgwick, 1982). Although the need for oxygen continues during the alevin stage, the most critical period is just before hatching. Surface dissolved oxygen levels were not critical in this study, it is possible that dissolved oxygen levels fell within the gravels at certain times at some locations. Experiments carried out by Shumway *et al.* (1964) found that salmonid embryos reared at reduced dissolved oxygen or velocity resulted in a reduction in size of newly hatched fry and it increased the length of the incubation period. Long incubation periods were not experienced at sites with poorer water quality but smaller alevins were recorded at some sites. Witzel and MacCrimmon (1983) also recorded that shorter emergence periods may have been a response to stress such as hypoxia and compaction.

During the final week in 2003, most alevins were > 24mm with the exception of two sites UCD21 (22.9mm), UCD34 (23.1mm). Low temperatures throughout the study time may explain the reduced length of alevins at Site UCD34. However, alevin size at UCD21 may be attributed to low oxygen and velocity in the gravels (Shumway *et al.,* 1964). Undersized fry are less likely to survive in these conditions for a prolonged period (Silver *et al.,* 1963). An earlier electric fishing survey in the summer of 2002 found no fry at Site UCD21.

This chapter has highlighted the tremendous variability in egg and alevin survival rates. Several possible factors responsible for egg mortality in 2002 and 2003 were highlighted which may be of use to fishery managers involved in restocking programmes.

6.5.1 Other problems encountered during the project

There were some problems experienced during the survey, the first of which was flooding which has already been discussed. High flows at UCD14 in 2002 prevented egg boxes from being lifted for two weeks. In 2003, high flows at several sites dislodged egg boxes and sometimes it was only possible to lift one box out of a possible three to calculate mortality. Although 50 eggs were placed in each box, it was often difficult to find 50 individuals back at the laboratory. Alevins may have escaped from boxes. Argent and Flebbe (1999) also reported that fry were able to escape from ‘whitlock vibert’ boxes. Eggs disintegrate quite quickly in acidic conditions so remnants are often difficult to find. Brown trout expertly locate and cut redds in clean silt free gravel. Placing vibert boxes in fast flowing water in a suitable spawning location is subjective.

6.6 Conclusions

Survival was highly variable along a water quality/nutrient gradient. This has also been reported in other studies. Expected survival can vary from year to year (Lisle & Lewis, 1992) and from egg box to egg box (Harshbarger & Porter, 1982). Sites must be individually examined to assess the potential factors that may affect mortality. Some of the “high” altitude sites with high Q-value may be prone to acid episodes and therefore may not be suitable nursery areas. Flooding during the developmental period was shown to be detrimental to survival of brown trout. Alevin length was related to water temperature. High survival rates were found at some low quality sites. However, this is no indication of whether the site could sustain a brown trout population throughout the entire life cycle. A myriad of factors were responsible for egg mortality and there was some evidence to suggest this was linked to water quality in 2002. However, the relationship was not evident in the 2003 study. There is no simple predictor of survival/mortality. Clearly egg survival at all stages is determined by a complex array of interacting environmental factors and it was not possible in this study to isolate mortalities associated solely with eutrophication effects.