THE REFISH (RESTORING ENDANGERED FISH IN STRESSED HABITATS) PROJECT, 1988-1994

T.R.K. DALZIEL[1], F. KROGLUND[2], L. LIEN[3], and B.O. ROSSELAND[3]

[1]*Power Technology, PowerGen plc, Ratcliffe-on-Soar, Nottingham NG11 OEE,* [2]*NIVA, Televeien 1, N-4890 Grimstad, Norway,* [3]*NIVA, P.O. Box 173, Kjelsås, N-0411, Norway*

Abstract. The REFISH (Restoring Endangered Fish In Stressed Habitats) Project was established in 1988 to assess acid-tolerance among indigenous Norwegian strains of brown trout. The work, comprising both laboratory and field studies, has involved the restocking and subsequent test-fishing of thirteen lakes with five brown trout strains. There was considerable variation in the ability of individual lakes to support adult trout. This did not appear related to ANC (acid neutralising capacity) or any single chemical factor. One strain, Bygland, was found to be relatively acid-tolerant, accounting for more than 60% of all fish recaptured by test-fishing over 1990-1994. This is consistent with better survival of young life-stages of the Bygland strain, compared with that of the other strains, in laboratory experiments employing acidic conditions. Strain-specific differences in calcium metabolism may be the physiological basis for acid tolerance.

1. Introduction

Many lakes in southern Norway are acidic and are unable to support brown trout, *Salmo trutta* L., the dominant fish species. Many other lakes have water qualities which are marginal for trout survival (i.e. they have low pH, low calcium (Ca) concentrations and elevated concentrations of inorganic aluminium (Al_i)).

Biological recovery, or improvement in water quality, of acidified lakes and other surface waters is possible (Wright & Haus, 1991) but is predicted to be a long process (Christophersen *et al.*, 1990, Wright *et al.*, 1991, Warfvinge *et al.*, 1992, Skeffington & Brown, 1992).

In the meantime, liming to reduce acidity is currently the most commonly used method for restoring (or protecting) fish populations in acidified surface waters (Hindar & Rosseland, 1991). This is costly and has to be repeated in order to remain effective. An additional method of fishery restoration might be to restock acidified lakes with relatively acid-tolerant strains of brown trout.

Differences in salmonid acid tolerance are well documented (Jensen & Snekvik, 1972; Grande *et al.*, 1978; Rosseland & Skogheim, 1984). Laboratory work has shown strain-specific acid tolerance in brook trout, *Salvelinus fontinalis* (Robinson *et al.*, 1976) and brown trout (Gjedrem, 1976, 1980; McWilliams, 1980, 1982; Rosseland & Skogheim, 1987; Swarts *et al.*, 1978; Turnpenny *et al.*, 1988). Studies employing both laboratory experiments and long-term field trials have, however, been lacking. The REFISH (Restoring Endangered Fish In Stressed Habitats) Project started in 1988 with the intention of assessing, through laboratory experiments and field work, acid tolerance in indigenous Norwegian strains of brown trout.

Results from the laboratory experiments and field work have been reported in

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internal reports of the sponsoring agencies. For example, Sadler & Lynam (1989a, b), Dalziel & Lynam (1991, 1992, 1993) - laboratory work, and Rosseland *et al.* (1990), Kroglund *et al.* (1992) and Rosseland (1994) - field work. This paper provides an overview of data collected for the Project over 1988-1994.

2. Materials and methods

The five strains of trout chosen for the study were selected from populations inhabiting waters covering the range of chemical conditions encountered in southern Norway. The strains are known as *Tunhovd* (from the high mountain Lake Tunhovd), *Fossbekk* (from Fossbekken Brook), *Bygland* (Lake Byglandsfjorden), *Bustul* (Lake Bustul/Oyevatn) and *Gjedrem*, a product of a selective breeding programme to select acid tolerant trout involving 250 strains from southern Norway (Gjedrem, 1980).

Laboratory experiments using ranges of pH, Ca concentration and Al_i concentration, were conducted in the UK over 1988-1992 using imported eyed eggs and fry of the five strains. The experiments were conducted in a temperature-controlled room and involved the use of flow-through systems with artificial soft water media to simulate as closely as possible field water qualities. In addition to obtaining survival data, sublethal effects on whole body mineral concentrations and extent of skeletal calcification were also assessed (Sadler & Lynam, 1989a, b; Dalziel & Lynam, 1991, 1992, 1993).

For field trials, thirteen lakes in three regions in southern Norway were selected for restocking and subsequent test-fishing. All lakes had lost their native fish populations of perch (*Perca fluviatilis*) and brown trout since the 1970s. The three regions are known as *Birkeland*, *Lyngdal* and *Valle/Njardarheim*.

Restocking of the lakes commenced in September 1988 and was subsequently repeated at the same time each year to 1992. First year (0+) fish (4-5 cm) of the five strains were stocked on each occasion, based on lake size. Fish of each strain were fin-clipped in a specific way to allow strain identification of those caught subsequently by test-fishing.

Test-fishing, using gill nets, commenced in 1989 and continued at the same time in each subsequent year, following the procedure employed in the SNSF Project (Rosseland *et al.*, 1979, 1980) and the Norwegian Monitoring Programme (SFT, 1983). Data were collected on numbers of fish of each strain caught (from fin-clipping), length, weight, sex, stage of sexual maturity, flesh colour and degree of stomach fullness. Samples of scales and otoliths were taken to assess fish ages, and samples of stomach contents for eventual dietary composition analysis.

Water samples from the lakes were collected periodically (biweekly or monthly for most lakes, except in the Valle/Njardarheim region: three or four times per year), and sent immediately to NIVA at Oslo for analysis.

3. Results

The laboratory experiments (Sadler & Lynam, 1989a, b; Dalziel & Lynam, 1991, 1992, 1993) showed the Bygland strain to be consistently more acid-tolerant than the other strains. The extent of skeletal calcification of the developing Bygland strain fry was, however, always less than that of the other strains, irrespective of pH. Conversely, Tunhovd strain fry consistently showed the most advanced calcification of any strain but, under acidic conditions, very poor survival.

A total of 479 fin-clipped brown trout were caught in the thirteen lakes over 1990-1994, comprising 62% Bygland, 20% Gjedrem, 8% Bustul, 5% Fossbekk, and 4% Tunhovd. Table I shows the numbers of fish of each strain caught in each lake. The most successful lake, in terms of recaptures was Mjåvatn (Lyngdal region), which yielded 25% of all fish caught.

TABLE I

Numbers of fish of each strain caught, 1990-1994

(Region) Lake	Bygland	Gjedrem	Fossbek	Bustul	Tunhovd	Σ
(Birkeland)						
Repstadvatn	17	4	0	3	0	**24**
Barkevatn	25	9	4	8	3	**49**
Mørkelivatn	0	0	0	0	0	**0**
(Lyngdal)						
Homsvatn	12	3	0	1	0	**16**
Mjåvatn	85	19	5	8	3	**120**
Skjekelivatn	22	12	2	7	3	**46**
Sandvatn	48	15	4	4	6	**77**
Trollselvvatn	2	2	0	0	0	**4**
(Valle/Njardarheim)						
Rennevatn	0	0	0	0	0	**0**
Hyttetjørni	0	0	0	0	0	**0**
Skammevatn	2	1	0	0	0	**3**
Kringlevatn	33	9	0	3	0	**45**
Smalevatn	52	22	10	6	5	**95**
Totals	**298**	**96**	**25**	**40**	**20**	**479**

All fish caught had been feeding well and were in good condition, in terms of length, weight and flesh consistency and colour. Older fish were sexually mature and some had evidently spawned in the previous autumn.

Mean lake water chemistry data are shown in Table II.

TABLE II

Mean lake water chemistry, September 1988 - December 1994

(Region) Lake	pH	$[Ca^{2+}]$ mg/l	$[Al_i]$ µg/l	[TOC]* mg/l	[ANC]+ µeq/l	n
(Birkeland)						
Repstadvatn	4.8	1.02	170.9	2.49	-34.8	113
Barkevatn	4.7	1.03	159.0	3.69	-29.8	115
Mørkelivatn	4.6	0.68	166.9	4.29	-38.7	117
(Lyngdal)						
Homsvatn	4.8	0.56	97.4	1.93	-28.7	57
Mjåvatn	4.7	0.60	52.7	4.97	-9.5	58
Skjekelivatn	4.7	0.48	63.6	3.69	-22.0	58
Sandvatn	4.7	0.50	63.7	3.99	-19.5	58
Trollselvvatn	4.5	0.35	55.9	4.89	-22.0	61
(Valle/Njardarheim)						
Rennevatn	5.2	0.44	135.8	0.46	-17.5	18
Hyttetjørni	5.1	0.29	80.6	0.50	-18.9	24
Skammevatn	5.3	0.37	67.4	0.38	-10.1	21
Kringlevatn	5.2	0.33	51.1	0.42	-12.6	27
Smalevatn	5.2	0.29	53.2	0.75	-12.3	29

*Total organic carbon concentration
+Acid neutralising capacity

4. Discussion

ANC is used to classify lakes according to their ability to support fish. An ANC of 20 µeq/l is proposed as an acceptable limit for fish in Norwegian freshwaters (Lien *et al.*, 1992). However, the fact that fish survive in most of the thirteen lakes used in the REFISH Project, particularly in Repstadvatn, Barkevatn and Homsvatn (Tables I and II), suggests that ANC may not be a sufficient measure of whether fish can be supported. The REFISH Project data suggest that no single chemical factor is adequate to explain the occurrence or absence of fish. Instead, this seems to be lake-specific and is determined by several factors including pH and concentrations of Ca and/or Al_i.

Differences in strain-specific acid tolerance in brown trout have been reported by Brown & Lynam (1981), Turnpenny *et al.* (1988), Gjedrem (1976, 1980), McWilliams (1980, 1982), Rosseland & Skogheim (1987) and Swarts *et al.* (1978). The REFISH Project results over 1990-1994 demonstrate marked differences in acid tolerance between strains of Norwegian brown trout, with one strain, Bygland, being markedly superior compared with the others.

Sayer *et al.* (1993) discuss possible reasons for strain-specific acid tolerance, suggesting that fertilization and hardening of eggs in hard hatchery water prior to introduction into soft, acid water may lead to greater chance of survival than if fertilization occurred within treatment. The water quality of origin of a particular strain, rather than hatchery water, may also be important. So, too, may be the maternal contribution to the mineral and nutrient stores of each individual. In most years, all the strains used to stock the different lakes were raised from the eyed egg stage to summer-old fry in the same hatchery (OFA Fish Farm). Since most of the pre-stocking history is identical, the significant differences in lake survival most probably are due to genetic differences in tolerance.

The laboratory experiments showed fry development, in terms of skeletal calcification, to be most advanced in the Tunhovd strain. However, Tunhovd also consistently demonstrated poor acid tolerance and provided only 4% of fish caught by test-fishing. Conversely, Bygland fry showed less advanced development with age than fry of the other strains, but greater acid tolerance; Bygland fish accounted for 62% of all fish caught. It might be that acid tolerance in the Bygland strain arises through more body calcium being utilized in gill membrane integrity (rather than in skeletal calcification), thus helping to protect against the effect of acidity increasing the rate of passive ion loss to the external medium. McWilliams (1982) demonstrated that trout from a population inhabiting low pH, low Ca, water (similar to the water of origin of the Bygland strain) had much lower gill permeability to H^+ and Na^+ ions (i.e. increased gill membrane integrity), than that of another population from higher pH, higher Ca concentration water.

In addition to continuing to collect data on adult fish survival, the Project is currently focusing efforts on assessing the reproductive success of the strains. Over the last two winters, freshly fertilised eggs have been planted out in mesh egg boxes in tributary streams. Data from this work should be reported shortly.

5. Conclusions

Lake ANC may not be the most important chemical factor determining adult trout survival. Rather, this appears to be lake-specific and determined by several factors including pH and concentrations of Ca and/or Al_i.

The Bygland strain is significantly more acid-tolerant than the other strains assessed in the REFISH Project. Laboratory survival experiments corroborate this. Strain-specific differences in Ca metabolism may be the physiological basis for acid tolerance.

Restocking with relatively acid-tolerant strains of trout may offer a useful fishery management tool in areas with acidic waters in which fisheries have been lost. Further data are required, however, on survival throughout the complete life-cycle.

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