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Epidemiology of *Renibacterium salmoninarum* in wild Arctic charr and brown trout in Iceland

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Renibacterium salmoninarum (Rs) is common in wild Arctic charr *Salvelinus alpinus* and brown trout *Salmo trutta* in Iceland. Of 22 charr and nine trout populations none were free of Rs antigens. In two charr populations only one fish exceeded the Rs antigen detection limit and in one of these cases the ELISA value was within uncertainty limits of the infection criterion. Mean prevalence of infection was 46% for Arctic charr (range: 3–100%) and 35% for brown trout (range: 6–81%). No infected fish showed gross pathological signs of bacterial kidney disease (BKD). The ubiquity and high prevalences of infection indicated that the bacterium has been endemic for a long time, and is probably a normal, low density resident in the fish. A lack of correlation in mean intensity of Rs antigen and prevalence of infection between sympatric populations of Arctic charr and brown trout suggests that the dynamics of infection and internal proliferation of bacteria can be quite independent in the two species even if they live in the same lake. Rs intensity and its coefficient of variation decreased with age in older fish, suggesting a connection between Rs intensity and host mortality. However, this can be caused by other ecological factors that decrease survival, especially low food availability, which simultaneously increase the susceptibility to Rs infection and internal proliferation.

Key words: bacterial kidney disease; epidemiology; Salmonidae; life history; ecology.

INTRODUCTION

Research on bacterial and viral diseases has been confined mainly to aquaculture environment, and less has been done to assess their epidemiology in natural environment (Austin & Austin, 1993). Bacteria and viruses are integral components of the natural environment and may be expected to be influenced by ecological processes within lake ecosystems in a similar way as larger parasites are (Curtis, 1982; Frandsen *et al.*, 1989; Dorucu *et al.*, 1995). Systematic studies of the epidemiology of infective micro-organisms in nature have a bearing on policy making in aquaculture, and in stocking and conservation of freshwater fish.

Bacterial kidney disease (BKD), caused by the Gram-positive bacterium *Renibacterium salmoninarum* (Sanders & Fryer, 1980), Rs, is one of the most serious diseases in salmonid farming in the Atlantic and Pacific region (Margolis & Evelyn, 1987; Fryer & Lannan, 1993; Olea *et al.*, 1993). It can be acute to chronic and infections can occur over a wide range of temperature (Sanders

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et al., 1978; Mitchum *et al.*, 1979). Rs survives intracellularly and is transmitted both horizontally and vertically (Wood & Wallis, 1955; Bullock *et al.*, 1978; Evelyn *et al.*, 1986).

Infections by Rs have been recorded in wild fish populations (Smith, 1964; Pippy, 1969; Evelyn *et al.*, 1973; Wood, 1974; Ellis *et al.*, 1978; Mitchum *et al.*, 1979; Paterson *et al.*, 1981; Banner *et al.*, 1986; Souter *et al.*, 1987; Sanders *et al.*, 1992; Meyers *et al.*, 1993), and in some cases clinical signs of BKD have been observed (Smith, 1964; Pippy, 1969; Evelyn *et al.*, 1973; Mitchum *et al.*, 1979; Banner *et al.*, 1986; Souter *et al.*, 1987). The transmission of BKD between wild and farmed fish has been demonstrated (Mitchum & Sherman, 1981).

In Iceland there are three native species of salmonids, Arctic charr *Salvelinus alpinus* (L.), brown trout *Salmo trutta* L. and Atlantic salmon *S. salar* L., and one imported species, rainbow trout *Oncorhynchus mykiss* (Walbaum). Rs has been detected sporadically in wild salmonids, but outbreaks of BKD have been documented only in intensive salmonid culture. That Rs has been detected in wild fish in Iceland is taken into account in salmonid stocking schemes and aquaculture (Gudmundsdóttir *et al.*, 1993; Jónsdóttir *et al.*, unpubl.). The present study examined the association of prevalence of infection and intensities of Rs antigens with age, length, condition factor, sex and maturity stage of fish in wild populations of Arctic charr and brown trout from 23 lakes. Infection patterns of Rs in the fish populations were also analysed in relation to ecological, limnological and geological features of the lakes.

MATERIALS AND METHODS

SAMPLING OF MATERIAL

Arctic charr (691 from 22 populations), brown trout (261 from nine populations), benthic invertebrates and zooplankton were collected and 12 abiotic variables were measured in 23 lakes across the country in August 1993, 1994 and 1995 (Fig. 1, Table I). Sampling procedures were as described for the Ecological Survey of Icelandic Lakes (Malmquist *et al.*, unpublished).

To screen for Rs, sterile kidney samples were excised from individual charr and trout and kept on ice for up to 6 h, and frozen at − 20° C until processed for an enzyme linked immunoabsorbant assay (ELISA) test.

ELISA

The sample process and the ELISA test included a built in correction for non-specific binding to the catching antibody (Gudmundsdóttir *et al.*, 1993).

To establish a negative/positive threshold, ELISA values (E values) from Rs negative charr stocks from eight fish farms where Rs has never been detected were used as a reference. The threshold was defined at the upper 99% confidence limit (CL) of the frequency distributions of E values from the negative fish ($n = 349$). A fish was therefore judged infected if it had an E value $>0{\cdot}050$ (Fig. 2).

In the preparation of negative controls for the ELISA test, pools of samples from Arctic charr, brown trout and salmon were used. Repeated measurements of the negative test pool in 1994 were used to assess the accuracy of the ELISA. The mean of 33 measurements repeated in 11 different tests had a 95% CL of $\pm 0{\cdot}0024$, and the 95% CL of measurements were $\pm 0{\cdot}0139$.

In preparation of the positive control, bacterial growth was collected from an agar plate and suspended in 10 ml of phosphate buffered saline (PBS), then 250 µl of HemoDe (terpene and butylated hydroxyanisole, Fisher Scientific, Pittsburg, PA, U.S.A.) were

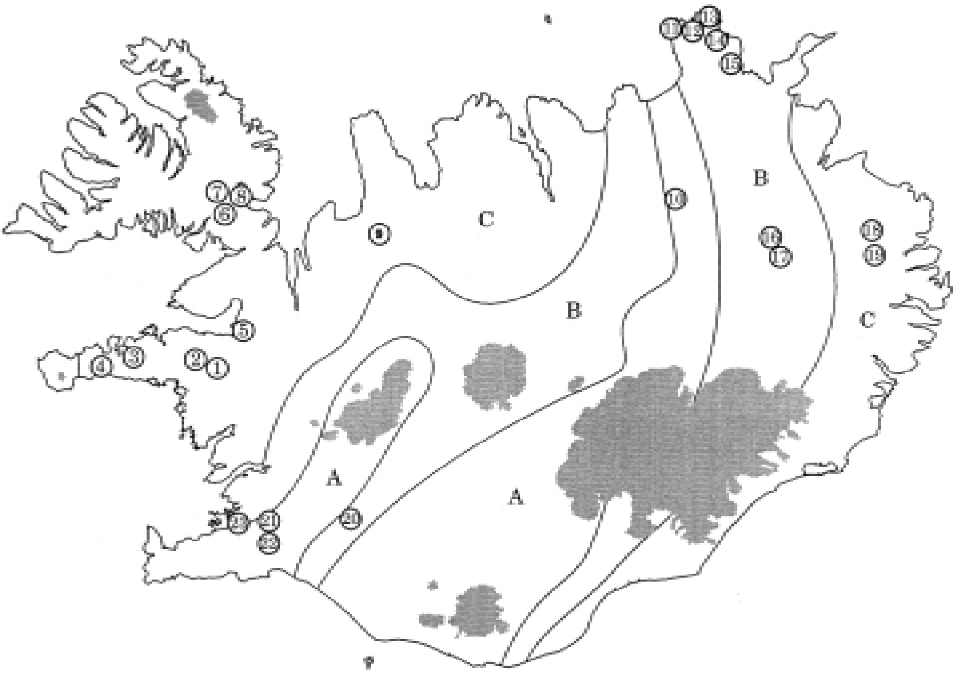

FIG. 1. Map of Iceland showing major geological zones and sites of lakes analysed (numbers in circles refer to names of lakes, see Table I). A, Areas dominated by post-Glacial (<0·01 million years) basalt lavas; B, areas dominated by Pleistocene (0·01–3·1 M years) basalt formations; C, areas dominated by Upper Tertiary (>3·1 million years) basalt formations (modified from Jóhannesson and Sæmundsson, 1989).

added, and the suspension heated at 100° C for 15 min. After centrifugation, the supernatant was collected and tested in an ELISA in twofold dilution steps between 1 : 32 and 1 : 1024. Each sample was tested twice. The relationship between E values and the twofold dilution steps of the bacterial sample test was examined by regression analysis. A highly significant, positive linear correlation was observed ($r^2 = 0{\cdot}983$, $P < 0{\cdot}001$), hence the more bacteria present in a sample, the higher the E value.

DATA HANDLING AND EXAMINATION

Prevalence of infection (%, of fish with E value >0·050) and intensity of Rs antigens (E values) were analysed with respect to the following variables of fish: species; age (year); fork length (cm); wet weight (g); Fulton's condition factor; sex and maturity stage (fish with maturity stage >2 are prospective spawners). Fulton's condition factor was calculated from: $K = 100\ W L_F^{-3}$, where W is wet weight (g) and L_F is fork length (cm). For ecological variables, prevalence and E values of fish were analysed with respect to: density of benthic macroinvertebrates (animals retained in a 250-µm sieve) in the rocky surf zone (0·2–0·5 m depth); density of benthic macroinvertebrates in the soft sediment zone (>0·5 m depth); density (no. ind $10\ \text{l}^{-1}$) of zooplankton in the pelagic zone; acidity (pH); electrolytic conductivity (indicator of the amount of dissolved nutrient salts); temperature; height above sea level; mean depth; volume; catch of fish per unit effort (indicator of fish density); number of sympatric salmonids; access from sea; stocking of fish (a possible way of transmission of Rs between populations); geographical location; and type of bedrock in catchment area.

In statistical analyses of E values, all comparions were performed on ln- or arcsine-transformed data, which conformed to normality.

TABLE I. Lakes analysed (no. in parentheses refer to site of lake, cf. Fig. 1) and biotic and abiotic variables of the lakes

Lake	Year	Loc	Height	Sea	Bdrc	Cond	pH	T	Md	Vol	cpue	Sd	Stock
Hítarv. (1)	1994	1	147	1	1	56	6·4	12·4	8·8	67	1·1	2	3 (3)
Oddastadav. (2)	1994	1	65	0	1	71	7·1	14·2	5·4	16	1·6	2	1
Baulárvallav. (3)	1994	1	193	0	2	53	7·0	12·8	17·7	28	0·6	1	1
Vatnsholtsv. (4)	1994	1	10	1	1	109	7·1	15·2	1·0	0,3	1·2	3	3 (3)
Haukadalsv. (5)	1994	1	37	1	1	56	7·3	14·8	23·4	78	0·9	2	3 (3)
Ónefnt v. (6)	1995	2	470	0	1	40	7·6	8·5	2·5	1	0·1	1	1
Högnav. (7)	1995	2	410	0	1	40	7·9	7·4	2·0	1	0·7	1	1
Thidriksvallav. (8)	1995	2	73	0	1	84	8·0	9·3	30·0	45	0·7	2	1
Svínav. (9)	1993	2	123	1	1	99	7·6	10·7	12·5	147	2·6	3	2 (3)
Mýv. (10)	1993	3	277	0	3	186	9·3	11·5	2·4	90		2	3 (1,2)
Kötluv. (11)	1993	3	0	0	2	4950	7·6	8·4	3·0	4	0·9	1	1
Sigurdarstadav. (12)	1993	3	0	0	2	313	8·4	8·0	1·3	2	0·4	1	1
Hraunhafnarv. (13)	1993	3	0	0	2	112	7·3	9·2	2·0	9	1·5	2	1
Y.-Deildarv. (14)	1993	3	38	1	2	92	7·9	10·6	1·5	1	1·2	3	2 (3)
St.-Vidarv. (15)	1993	3	151	0	2	85	8·1	7·9	15·0	6	1·4	1	1
Sænautav. (16)	1994	3	524	0	2	86	9·4	9·8	7·8	18	0·9	1	0
Ánav. (17)	1994	3	521	0	2	62	11·1	11·2	6·0	29	0·6	1	0
Eidav. (18)	1994	3	32	0	1	64	6·7	13·8	4·4	5	1·6	2	0
Urridav. (19)	1994	3	38	0	1	98	9·8	14·6	4·4	5	0·1	1	0
Apav. (20)	1993	4	59	1	2	83		11·2	1·5	20	0·6	2	3 (1)
Thingvallav. (21)	1994	4	101	0	3	79	8·8	9·0	31·1	2855		2	3 (2)
Úlfljótsv. (22)	1993	4	79	0	3	80	8·8	9·0	4·7	17	3·6	2	3 (2)
Ellidav. (23)	1993	1	73	0	3	91	8·8	10·9	1·0	2	2·0	3	3 (2,3)

Year, Year of sampling; Loc, geographical location of lake (1, west Iceland; 2, Westfjords and north-west Iceland; 3, north and north-east Iceland; 4, south Iceland); Height, height (m) of lake above sea level; Sea (0, no access from sea; 1, access from sea: Bdrck, Bedrock type [1, Upper Tertiary basalt rocks (>3·1 million years); 2, Pleistocene basalt formations (0·01–3·1 million years); 3, post-glacial lavas (<0·01 million years)]; Cond, electrolytic conductivity of lake (µS cm^{-1}); T, lake temperature (° C); Md, mean depth (m) of lake; Vol, volume (Gl) of lake; cpue, catch per unit effort; Sd, salmon diversity (no. of sympatric salmonid species: 1, single species; 2, two species; 3, three species); Stock, stocking of fish into lake (0, no stocking; 1, unlikely; 2, likely; 3, Yes. Numbers in parentheses refer to stocked species; 1, Arctic charr; 2, brown trout; 3, Atlantic salmon). Height, Md, and Vol based on Adalsteinsson (1990) and own measurements. Bdrc is modified from Jóhannesson and Sæmundsson (1989).

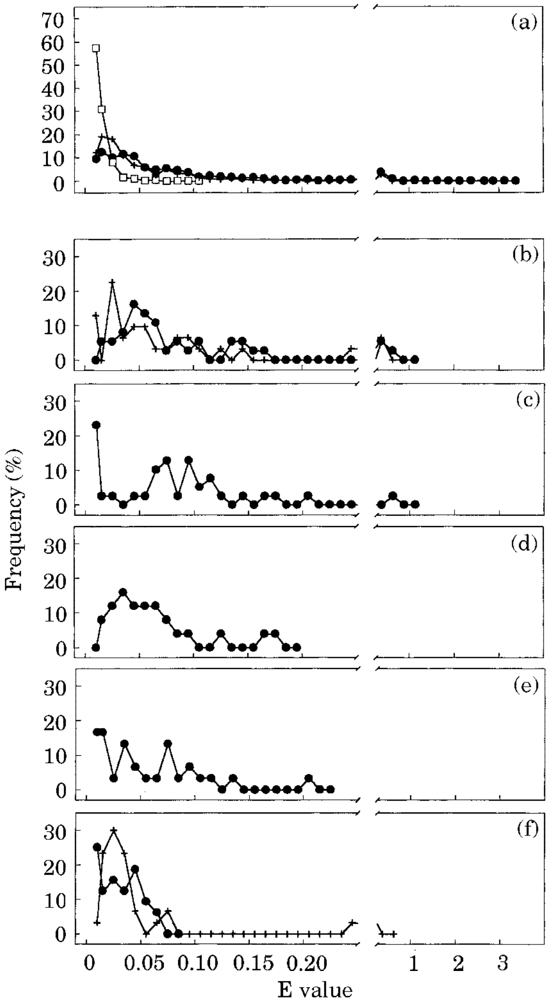

FIG. 2. Frequency distributions of E values (intensity of Rs antigens) in (a) all lakes pooled and [(b)–(f)] selected lakes with different infection profiles (●, Arctic charr; +, brown trout). Infected fish have E values >0·050. A reference sample of farmed Arctic charr stocks free of Rs antigens (□) is also displayed with the pooled data (a). Note the change in scale in E values above 0·25.

RESULTS

Antigens of Rs were detected in kidneys of Arctic charr and brown trout from all 23 lakes sampled (Fig. 2, Table II). No gross pathological changes of BKD were observed.

Prevalence of infected charr (E value >0·05) ranged between 3% and 100%, and between 6% and 81% for brown trout, and mean E values ranged between 0·013 and 0·228, and between 0·018 and 0·082 for charr and brown trout, respectively (Table II, Fig. 2). There was a significant difference among lakes in E values for both Arctic charr (overall analysis of variance, ANOVA, $F_{21,669}=16{\cdot}055$, $P<0{\cdot}001$) and brown trout (overall ANOVA, $F_{9,251}=5{\cdot}476$,

TABLE II. Prevalence (P, %) of infected fish (E value >0·05) and mean of E values (ME) of all fish

Lake	Arctic charr						Brown trout					
	P	ME	L1	L2	Max	*n*	P	ME	L1	L2	Max	*n*
Ánav.	3	0·013	0·009	0·019	0·551	29						
Apav.	3	0·014	0·011	0·018	0·060	33						
Mýv.*	4	0·019	0·014	0·025	0·098	26						
Y.-Deildarv.	14	0·021	0·014	0·032	0·156	29	27	0·030	0·024	0·038	0·102	30
Ellidav.	16	0·025	0·018	0·035	0·070	32	13	0·029	0·023	0·037	0·245	30
Hraunhafnarv.	10	0·028	0·022	0·036	0·091	30						
St.-Vidarv.	17	0·033	0·026	0·042	0·086	29						
Úlfljótsv.	30	0·034	0·020	0·056	0·480	20						
Thidriksvallav.†	49	0·042	0·027	0·066	0·340	33						
Eidav.	40	0·043	0·033	0·056	0·086	20	17	0·026	0·018	0·037	0·409	30
Urridav.	53	0·046	0·032	0·065	0·340	30						
Haukadalsv.	56	0·046	0·027	0·075	0·351	32						
Sigurdarstadav.	52	0·050	0·038	0·066	0·176	25						
Sænautav.	58	0·058	0·047	0·072	0·155	26						
Oddastadav.	57	0·059	0·046	0·075	0·373	37	81	0·082	0·056	0·119	2·603	30
Thingvallav.	53	0·062	0·049	0·078	3·044	97	58	0·047	0·023	0·094	0·256	12
Hítarv.	65	0·069	0·053	0·091	0·664	37	48	0·044	0·029	0·067	0·265	31
Kötluv.	76	0·088	0·070	0·111	0·233	21						
Svínav.	94	0·104	0·082	0·132	0·302	18	33	0·038	0·027	0·053	0·492	27
Högnav.	90	0·106	0·083	0·135	1·091	39						
Vatnsholtsv.	77	0·110	0·079	0·155	1·747	30	6	0·018	0·014	0·023	0·073	33
Ónefnt v.	100	0·228	0·139	0·374	2·353	15						
Baulárvallav.							28	0·030	0·018	0·049	1·901	35
Mean	46	0·047	0·043	0·082		22	35	0·036	0·026	0·057		9

The lakes are ranked in ascending order of ME of Arctic charr. L1 and L2 are lower and upper 95% CL of ME. Max, maximum E value in fish. *n* is number of fish analysed.

*Brown trout is also present in Lake Mývatn but was not sampled.

†One uninfected brown trout from lake Thidriksvallavatn, the only brown trout caught there, is not included in the table.

$P<0{\cdot}001$). For Arctic charr, maximum E values in individual fish within lakes ranged between 0·060 and 3·044, and between 0·073 and 2·603 for brown trout (Table II).

E values and prevalence were correlated positively for infected charr [Pearsons's $r=0{\cdot}725$, d.f. = 19, $P<0{\cdot}01$, one outlier (fish) from Ánavatn excluded] but not for trout, ($r=0{\cdot}250$, d.f. = 6, $P>0{\cdot}05$).

Overall Arctic charr had higher E values ($t=3{\cdot}975$, d.f. = 950, $P<0{\cdot}001$) and prevalence of infection ($G=13{\cdot}551$, d.f. = 1, $P<0{\cdot}001$) than did trout (see Table II). In the eight lakes pooled where the species co-occur (Mývatn charr not included because trout was not sampled there), the difference was even more marked (mean E value: charr = 0·055, trout = 0·035, $t=5{\cdot}134$, d.f. = 525, $P<0{\cdot}001$, prevalence: charr = 53%, trout = 33%, $G=21{\cdot}976$, d.f. = 1, $P<0{\cdot}001$). However, we found no significant association in prevalence of infection or mean E values between charr and trout in these eight lakes (prevalence: $r=0{\cdot}225$, d.f. = 6, $P>0{\cdot}05$; mean E value: $r=0{\cdot}044$, d.f. = 6, $P>0{\cdot}05$), but such association would be expected if interspecific infections were frequent.

INFECTION AND LIFE HISTORY OF FISH

A second-order regression accounted significantly for variation of log-transformed E values with age in charr ($F_{2,685}=18{\cdot}713$, $P<0{\cdot}001$), but not in trout ($F_{2,208}=2{\cdot}881$, $P=0{\cdot}058$) (Fig. 3). Negative, linear relationships were found between E values and length in both charr (regression coefficient $=-0{\cdot}051$, $t=-10{\cdot}078$, $P<0{\cdot}001$) and trout (regression coefficient $=-0{\cdot}042$, $t=-5{\cdot}606$, $P<0{\cdot}001$). The strength and independence of the relationship between E values and length in Arctic charr was investigated further by regressing residuals of the quadratic regression of E values on age against length. The effect of fish length was still highly significant after removing the effect of age ($F_{1,686}=118{\cdot}860$, $P<0{\cdot}001$) (Fig. 4).

Highly significant differences were found in age, length and condition factor of charr among lakes (ANOVAs, age: $F_{21,666}=26{\cdot}304$, $P<0{\cdot}001$; length: $F_{21,669}=15{\cdot}486$, $P<0{\cdot}001$; condition: $F_{21,669}=9{\cdot}960$, $P<0{\cdot}001$). A closer inspection on a lake basis (Table III) showed that E values were correlated significantly with age in nine lakes (41%), length in 13 lakes (59%) and condition factor in three lakes (14%). However, the sign of the correlation coefficients differed among lakes (Table III). In general, in lakes where the fish population was composed of relatively young individuals, E values and age of fish increased together, but in lakes with a relatively high mean age, E values decreased with increasing age of fish. Furthermore, dispersion of Rs antigen levels, measured as variance to mean ratio of Rs antigen intensity (E value), declined with increasing age of charr (Fig. 5).

Infected charr were significantly shorter than uninfected fish in five lakes; significantly younger than uninfected fish in two lakes, but significantly older in two lakes (Table IV). No difference was found in mean age, length or condition factor between infected and uninfected brown trout.

With respect to sex neither charr nor trout differed in prevalence of infection (all G-statistics with $P>0{\cdot}05$) or in E values (all lakes combined) (charr: $t=1{\cdot}858$, d.f. = 680, $P=0{\cdot}064$; trout: $t=1{\cdot}541$, d.f. = 218, $P=0{\cdot}125$). There was a significant difference in E values, between prospective spawners (maturity stage >2, mean E

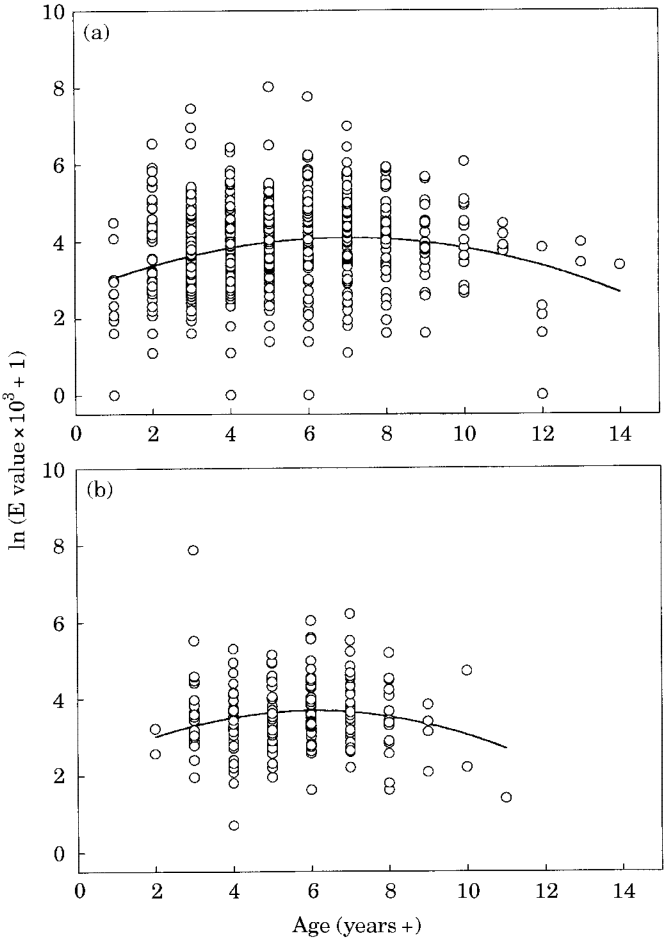

FIG. 3. A plot of ln-transformed E values (the intensity of Rs antigens) *v*. the age of the host. The curves give the least squares second-order fit to the data. (a) Pooled data for Arctic charr in 22 lakes (quadratic coefficient of regression = − 0·029, $P<0·001$). (b) Pooled data for brown trout in seven lakes (quadratic coefficient of regression = − 0·041, $P<0·001$). For brown trout, age was not available from Lake Baulárvallavatn.

value = 0·063) and non-spawners (mean E value = 0·034) among charr ($t = 7·547$, d.f. = 647, $P<0·001$), but not among trout (mean E value of prospective spawners = 0·029, for non-spawners = 0·036, $t = 1·274$, d.f. = 196, $P = 0·204$). Similarly, prevalence of infection was higher in prospective spawners among charr ($G = 30·326$, $P<0·001$) but not among trout ($G = 1·177$, $P>0·05$).

INFECTION AND LAKE ECOLOGY

For charr, mean E values were associated with density of macroinvertebrates in the surf and sediment zones of lakes (Fig. 6). Prevalence of infected charr also correlated inversely with density of macroinvertebrates in the surf zone ($s = −0·617$, d.f. = 18, $P<0·01$) and the deeper sediment zone ($s = −0·667$, d.f. = 18, $P<0·01$). For trout, mean E values correlated negatively with density of benthic invertebrates in the surf zone, but not in the sediment zone (Fig. 6).

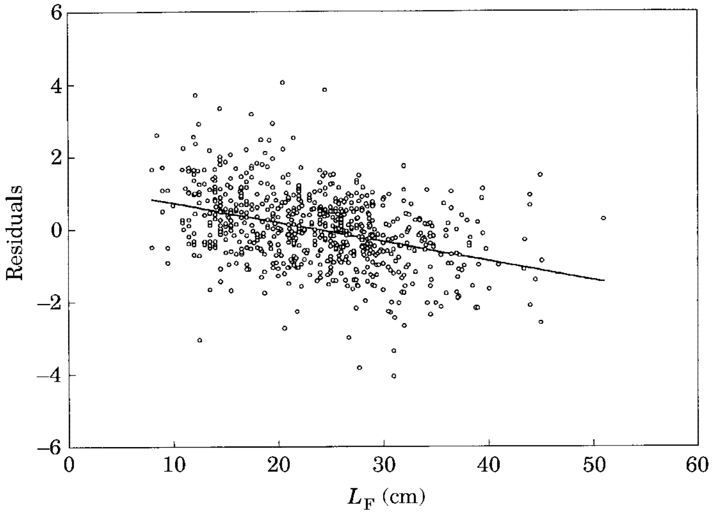

FIG. 4. Regression of residuals of the quadratic regression of E values on age against fork-length, L_F (cm) in Arctic charr. Regression equation: Residual=1·262–0·053 × length. $F_{1,686}=118·86$, $P<0·001$.

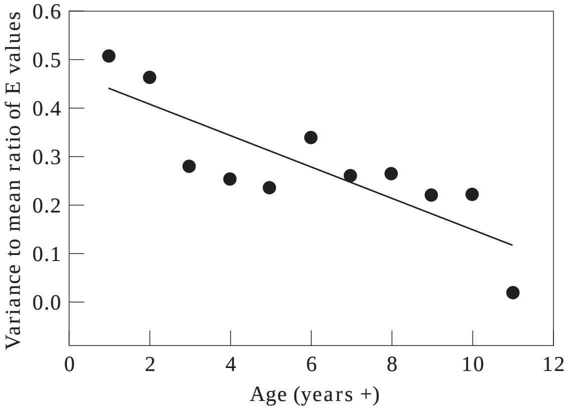

FIG. 5. Dispersion (variance to mean ratio, $s^2/\bar{X}$) of E values (intensity of Rs antigen) in relation to age of Arctic charr (fish from all lakes pooled). ANOVA, $F_{1,9}=19·568$, $r^2=0·685$, $P=0·002$; linear coefficient= − 0·032, $P=0·002$.

Similarly, prevalence of infected brown trout correlated inversely with density of benthic invertebrates in the surf zone ($s=-0·833$, d.f.=6, $P<0·05$), but not in the sediment zone ($s=-0·286$, d.f.=6, $P>0·05$). Significant positive correlations were observed between density of benthic macroinvertebrates in the surf zone and the condition factor of charr ($r=0·629$, d.f.=18, $P<0·01$) and trout ($r=0·784$, d.f.=5, $P<0·05$).

Mean E values of Arctic charr were associated negatively with lake acidity ($s=-0·459$, d.f=19, $P<0·05$) and mean E values of brown trout correlated positively with mean depth ($s=0·664$, d.f.=9, $P<0·05$) and volume of lakes ($s=0·720$, d.f.=9, $P<0·05$). Correlations with other variables, i.e. density of

TABLE III. Correlation (Pearson's *r*) between intensity of E values and age, length and condition factor of Arctic charr; the lakes are ranked in ascending order of mean age

Lake	P	Mean E value	Mean age	Pearson's *r*						
				Age		Length		Condition		*n*
Apav.	3	0·014	2·7	0·565	**	0·649	**	0·343	*	33
Mývv.	4	0·019	3·0	0·181		0·224		0·073		26
Vatnsholtsv.	77	0·110	3·1	−0·372	*	−0·449	*	−0·171		30
Kötluv.	76	0·088	3·2	0·224		0·369		0·255		21
Ellidav.	16	0·025	3·5	0·588	**	0·618	**	0·393	*	32
Urridav.	53	0·046	3·8	−0·017		−0·113		−0·177		30
Hraunhafnarv.	10	0·028	3·9	0·517	**	0·544	**	−0·055		30
Y.-Deildarv.	14	0·021	4·1	−0·183		−0·380	*	−0·205		29
Oddastadav.	57	0·059	4·5	−0·100		−0·212		0·165		37
Haukadalsv.	56	0·046	4·6	0·508	**	−0·813	**	−0·421	*	32
Svínav.	94	0·104	4·8	0·395		−0·195		−0·223		18
Sigurdarstadav.	52	0·050	4·9	−0·564	**	−0·689	**	−0·361		25
Ánav.	3	0·013	5·4	−0·383	*	−0·427	*	−0·250		29
St.-Vidarv.	17	0·033	5·9	0·025		−0·496	**	−0·068		29
Thingvallav.	53	0·062	6·3	−0·091		−0·315	**	−0·098		97
Hítarv.	65	0·069	6·5	0·372	*	0·245		0·073		37
Ónefnt v.	100	0·228	6·5	−0·101		0·115		−0·241		15
Högnav.	90	0·106	6·6	−0·101		−0·550	**	−0·072		39
Eidav.	40	0·043	6·8	−0·162		−0·233		−0·298		20
Sænautavy.	58	0·058	7·3	−0·168		−0·144		0·336		26
Úlfljótsv.	30	0·034	7·4	−0·128		−0·467	*	0·063		20
Thidriksvallav.	0		7·8	−0·505	**	−0·697	**	−0·178		33

P, prevalence (%) of infection. *n*, Sample size. **P*<0·05; ***P*<0·01.

TABLE IV. Mean age, mean length and mean condition factor of infected and uninfected Arctic charr

Lake	Age (+ years)			Length (cm)			Condition			*n*
	Uninf.	Infect.	*P*	Uninf.	Infect.	*P*	Uninf.	Infect.	*P*	
Stóra-Vidarv.	6·1	5·2	NS	24	16	**	1·007	0·984	NS	27
Úlfljótsv.	7·5	7·2	NS	29	20	**	1·142	1·118	NS	18
Thidriksvallav.	8·9	6·6	*	29	16	***	1·114	1·026	*	30
Haukadalsv.	3·8	5·2	*	28	18	***	1·085	0·986	*	30
Sigurdarstadav.	5·7	4·2	**	41	28	***	1·106	1·026	NS	23
Hítarv.	5·8	6·9	*	25	28	*	1·048	1·055	NS	35

Only lakes with significant differences in intensities of E values between infection classes are shown. Based upon Kruskall–Wallis test (not tested if *n*<5).

n, Sample size; NS, not significant. *P*>0·05; **P*<0·05; ***P*<0·01; ****P*<0·001.

zooplankton in the pelagic zone, electrolytic conductivity, lake temperature, height above sea level, or catch of fish per unit effort, were not significant (*P*>0·05) for either charr or trout.

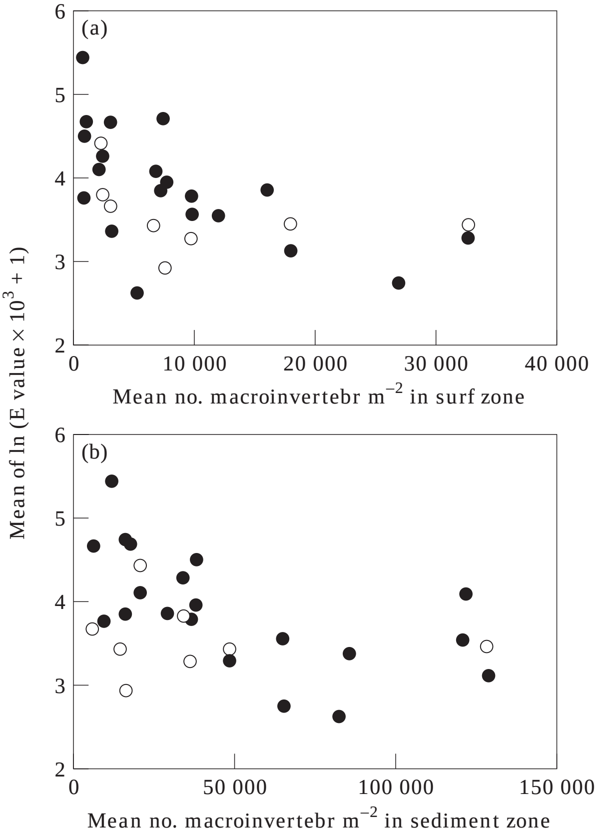

FIG. 6. A plot of mean ln-transformed E values (intensity of Rs antigens) in Arctic charr (●), ($n=20$) and brown trout (○), ($n=8$) *v.* (a) density of benthic macroinvertebrates in the surf zone of lakes and (b) density of benthic macroinvertebrates in the sediment zone of lakes. For Arctic charr, Spearman's $s=-0{\cdot}639$ ($P<0{\cdot}01$) in the surf zone, and $-0{\cdot}659$ ($P<0{\cdot}01$) in the sediment zone. For brown trout, Spearman's $s=-0{\cdot}714$ ($P<0{\cdot}05$) in the surf zone, and $-0{\cdot}286$ ($P>0{\cdot}05$) in the sediment zone. Data on invertebrates was not available for Lake Mývatn and Lake Thingvallavatn.

Mean prevalences (mean of mean prevalences) and mean E value (mean of mean E values) for charr in lakes situated on basic bedrocks from Upper Tertiary was significantly higher than in lakes of areas dominated by Pleistocene basalt formations (probabilities from Tukey's multiple comparisons, prevalence: $P=0{\cdot}019$; mean E values: $P=0{\cdot}021$) and in areas dominated by postglacial lavas (prevalence: $P=0{\cdot}048$; mean E values: $P=0{\cdot}041$) (Table V). Furthermore, in lakes on Upper Tertiary bedrock, the density of benthic macroinvertebrates in the surf zone (mean=5·268 animals m^{-2}) was significantly lower than in surf zones of lakes on Pleistocene bedrock (mean=9·861 animals m^{-2}) or on Post-Glacial lavas (mean=21·344 animals m^{-2}) ($F_{2,18}=3{\cdot}980$, $P=0{\cdot}037$). Similar differences were observed for densities of benthic invertebrates in the sediment zone.

Infection in charr also differed geographically (Table V). Prevalence was significantly higher in north-west Iceland than in north and north-east

TABLE V. Mean prevalence (MP, %) of infected fish (E value >0·05) and mean of E values (ME, mean of means) of all fish among lakes, grouped by ecologcial variables

Ecological variable	Arctic charr			Brown trout		
	MP	ME	*n*	MP	ME	*n*
Bedrock type						
Upper Tertiary basalt	66	0·074	10	37	0·037	5
Pleistocene basalt	29	0·034	8	28	0·031	2
Post-glacial lavas	26	0·033	4	36	0·038	2
F	5·739**	5·369**	22	0·019 NS	0·121 NS	9
Geographical location						
West Iceland	54	0·057	5	35	0·037	5
Westfjords and north-west Iceland	83	0·103	4	33	0·038	1
North and north-east Iceland	30	0·036	10	22	0·029	2
South Iceland	29	0·032	3	58	0·047	1
F	5·565**	3·579*	22	0·520 NS	0·246 NS	9
Access from sea						
No access	43	0·047	16	39	0·040	5
Access	52	0·049	6	29	0·032	4
t	0·424 NS	0·101 NS	22	− 0·394 NS	0·749 NS	9
No. of sympatric salmonids						
One	56	0·059	8	28	0·031	1
Two	34	0·039	10	41	0·047	4
Three	50	0·051	4	20	0·029	4
F	1·252 NS	0·853 NS	22	1·038 NS	1·547 NS	9
Stocking of fish						
None and or unlikely	48	0·053	12	32	0·041	3
Yes and or likely	41	0·041	10	31	0·034	6
t	− 0·610 NS	− 0·811 NS	22	− 0·286 NS	− 0·606 NS	9

n, Number of lakes analysed. *F* and *t* refer, respectively, to ANOVA and *t*-tests. NS, not significant. $P>0·05$; $*P<0·05$; $**P<0·025$. MP are tested on arcsine-transformed data.

Iceland ($P=0{\cdot}007$) and south Iceland ($P=0{\cdot}032$), and average E value was also significantly higher ($P=0{\cdot}035$).

Access from sea, number of sympatric salmonids, or stocking history did not influence infection pattern (Table V).

DISCUSSION

A high proportion of the wild fish were infected but have low levels of Rs antigens in the kidney (E values in the range 0·050–0·100). A few fish had high E values. Comparable results have been observed for wild salmonids carrying Rs antigens by Paterson *et al.* (1979) using IFAT and Meyers *et al.* (1993) using ELISA.

The low (but frequent) occurrence of Rs antigens could be explained by at least three mechanisms, possibly acting concurrently. First, some fish may just have become infected. Second, fish recovering from an infection might harbour Rs antigens in immune complexes deposited in the kidney (Kaattari *et al.*, 1989; Sami *et al.*, 1992). In that case, the bacterium might be present or absent and it might reside in some other organ. Third, the bacterium might be a low density resident in wild fish forming ‘ a synergetic or controlled parasitic relationship with the host ’ (Austin & Austin, 1993). Rs has co-evolved with the salmonids for a long time and its survival strategies, e.g. its intracellular location (Gutenberger *et al.*, 1997) appear to be highly successful. Occasional homeostatic disturbances within infected fish may induce its occasional proliferation and high levels of Rs antigens. Our findings are in accordance with this.

The absence of external and internal clinical signs of BKD in the present study, even in fish that had high E values, conforms to findings by Paterson *et al.* (1979) and Meyers *et al.* (1993) for heavily infected wild salmonids. Clinical signs in diseased fish result from complex host–pathogen interactions (Bruno, 1986). So lack of clinical signs, despite a heavy infection, may be connected with weak or absent host responses. Brown *et al.* (1996) studied the development of immunity in salmon hatched from eggs exposed at an early stage to p57, the main extracellular antigen and putative virulence factor of the bacterium. p57 had a partially immunosuppressive effect on the development of the specific immune system. Fish derived from eggs containing the protein showed an increased susceptibility to active challenge from Rs, production of antibodies against p57 decreased and respiratory burst activity of phagocytic cells as well. These authors postulated that ‘ the intra-ovum presence of Rs and its soluble antigens may contribute to the inability of many salmon to mount an effective immune response to Rs ’. Turaga *et al.* (1987) showed that soluble antigens produced by Rs are capable of suppressing antibody response *in vitro*. Information on immune reactions towards specific antigens of Rs are limited but Wood & Kaattari (1996) demonstrated that the removal of p57 enhanced immunogenicity of the bacterium. These findings suggest that symptom free, although heavily infected fish, might arise from eggs exposed to p57.

Although E values pooled over lakes and overall prevalence were higher in charr than in trout we found no significant association in these factors between the two species in lakes where they co-occur. This suggests that the dynamics of infection and internal proliferation of bacteria can be quite independent in the two species even if they live in the same lake. Both species congregate at spawning sites and this may be the the time and place where intraspecific, horizontal infections are most likely. Since the species very rarely show temporal and spatial overlap in spawning activities horizontal infections might be far less likely between the species than between conspecifics.

INFECTION AND FISH LIFE HISTORY

Higher E values (intensity of Rs antigens) and prevalence of infection among prospective charr spawners (fish were sampled *c.* 1·5 months prior to spawning) suggests increased probability of activation of Rs during maturation. This along with the results of Lee & Evelyn (1989) that only low levels of the bacterium are needed for vertical transmission of Rs and the result of Brown *et al.* (1996) that exposure to p57 during the egg stage can cause immunosuppression in subsequent fry, shows that the bacterium is well adapted for vertical transmission.

The second-order relationship of intensity of Rs antigens with age of fish with maximum intensities in intermediary ages suggests that Rs infection may be a significant cause of host mortality. The data fit well with outcomes of Monte-Carlo simulation experiments by Anderson & Gordon (1982) of a single parasite–host model, assuming a constant mean infection rate through time and a linear probability function of host mortality resulting from parasite burdens. According to Anderson and Gordon (1982) such a convex age–intensity relationship, concomitant with a decline in the degree of dispersion (variance to mean ratio) of intensity in older age classes, may be evidence of the induction of host mortality by parasite infection. However, as stressed by Anderson & Gordon (1982), peaked, or curved age–intensity curves may also result from age related changes in the average rate of infection, with infection of older hosts ceasing, e.g. due to changes in foraging behaviour or to acquisition of acquired immunity. These factors, will not normally create a concomitant decline in the degree of dispersion in older hosts. The dispersion of Rs antigens levels in the charr decline with increasing age of the fish (Fig. 5) thus suggesting a connection with host mortality. However, since most fish harboured relatively few bacteria while a few fish harboured the major proportion of the bacterial population, it is unlikely that Rs was a frequent and direct cause of death. The negative relationship between age and intensity of Rs antigens in older fish more likely stems from other age-dependent mortality factors that, through differential mortality, result in both lowered intensity and dispersion of Rs antigens in the older part of the population. Factors likely to work in this manner are food shortage, sexual maturation and other growth limiting factors. Starved fish in poor condition are more likely, to harbour flourishing Rs colonies and to die. Older fish are those that have been more successful in acquiring food and maintaining good condition which concurrently makes them less likely to experience infection and proliferation of Rs. After the effect of age had been removed, there was still a highly significant, negative effect of fish length on Rs intensity. Factors such as food shortage, that are likely to have an adverse effect on survival, also have a negative effect on length at age. The above scenario does not imply adverse effects of Rs proliferation. Such effects, whether acting directly or via interaction with other mortality inducing factors, may be important in natural population.

Anderson & Gordon's (1982) model applied primarily to parasites that have a long life span relative to that of the host, e.g. larval helminth parasites. Given a fairly constant infection probability, and making assumptions as to how ecological factors may increase the risk of death and probability of Rs proliferation simultaneously, it may be argued that the Rs antigens–age relationship mimics the helminth parasite system.

The intensity–age relationship discussed above is a composite picture, based on data drawn from separate lake populations that differ considerably in age and length distribution of fish. A comprehensive, long-term study of a single fish population within a lake might provide valuable information on the infection rate of Rs.

INFECTION AND LAKE ECOLOGY

The density of benthic macroinvertebrates correlated negatively with Rs infection, notably in charr, and infection patterns differed according to bedrock and geographical location of lakes. These results conform with the expectation that ecological features of lakes can influence the dynamics of host–parasite interaction (e.g. Curtis, 1982; Frandsen *et al.*, 1989; Dorucu *et al.*, 1995).

The density of macroinvertebrates in the surf zone correlated positively with condition factor of both charr and trout in Icelandic lakes. If density indicates accessibility to fish, then the lower the food availability the more fish suffer infection and proliferation of Rs. More specifically, higher prevalence of infection and E values in fish of nutrient-poorer lakes may be the result of insufficient energy acquisition, a stress that upsets the balance in the synergetic host–parasite relationship.

Differences in infection patterns in relation to the type of bedrock in catchment areas of lakes probably result from ecological differences induced by specific hydrogeological features of each bedrock type. According to Gardarsson (1979), lakes in areas of permeable post-Glacial lavas, which receive water primarily through underground springs, may be biologically richer than lakes situated in areas of the older, less permeable Pleistocene and especially Upper Tertiary bedrocks, where water enters lakes primarily as direct run-off from the surface. The patterns of macroinvertebrate density in lakes, with respect to the different bedrock types, are in good agreement with ecological expectations according to Gardarsson (1979). The geographical difference in infection pattern also appears to be better explained in terms of ecological difference, induced by hydrogeological features, rather than by location *per se*, since geographical location is an integrated part of bedrock type, hence of hydrogeological features.

The few studies that have addressed ecological associations with patterns of Rs in wild salmonids seem to point in the same direction. Mitchum *et al.* (1979) identified a combination of instability in water flow and water temperature and small water volume, as important causes of BKD in three salmonid species. Moreover, Paterson *et al.* (1981), who studied fish under controlled circumstances, identified inadequate food as important in determining BKD infection patterns.

The high prevalence of Rs antigens in fish from a number of lakes implies that Rs has been endemic for a long time. This is substantiated by the observation that among charr populations with the highest levels of Rs antigen and highest prevalances of infection are those in Ónefnt vatn (no. 6, Fig. 1) and Högnavatn (no. 7, Fig. 1), which most likely have never been exposed to stocked fish. Both lakes are small, shallow, and situated in high altitudes quite far from human settlement, and with impassable waterways.

In summary, the frequent but low levels of Rs antigens in fish indicate that the bacterium is a normal, low density resident in wild charr and trout. It is unlikely that stocking has affected the infection pattern of wild charr or trout. We infer that the epidemiology of Rs infection in wild, Icelandic lake populations of Arctic charr is influenced primarily by ecological features of the lake ecosystem, in particular, favourable food conditions result in a decreased susceptibility to Rs. A causal framework of this kind also conforms with the relationship observed between the intensity of Rs antigens and the age and size of the host. On a geographical scale the important ecological features of lakes that influence the epidemiology of to Rs are clearly linked to hydrogeological features of catchment areas which in turn reflect the age of geological formation.

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