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Elemental analysis of Scottish populations of the ectoparasitic copepod *Lepeophtheirus salmonis*

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Abstract

Conventional nebulisation ICPMS (Inductively Coupled Plasma Mass Spectrometry), was used to determine the concentration of a broad range of elements in the salmon louse *Lepeophtheirus salmonis*. Lice samples were collected from Atlantic salmon in seven localities (4 fish farms and 3 wild salmon fisheries) on two separate sampling occasions and prepared for analysis. Sixty six elements were measured, 35 of these were found to be variable and were subjected to univariate and multivariate statistical analysis. The results of the single element comparisons showed that not all individual sites could be discriminated from each other. Sea lice collected from cultured salmonids could be discriminated from those on wild salmonids at the same site using the elements magnesium (<0.05%), vanadium (<0.01%) and uranium (<0.05%). Using discriminant analysis based on 28 elements, the separation of all sampled sea lice localities from each other was clear (100% correct classification) giving each an individual signature. Further analysis examined the effects of sequentially removing elements from the discrimination model in order to determine the minimum number of elements required to obtain satisfactory discrimination of populations. It was found that 16 elements could still provide 100% correct classification, whilst 12 elements still provided 97.30% correct classification. This pilot study has shown elemental analysis to be a potentially successful method for the discrimination of populations of *L. salmonis*, although the biological basis of the elemental signatures derived remains to be established.

Contents

Introduction

The identification and discrimination of different populations of *Lepeophtheirus salmonis* has recently become a subject for considerable research attention. The principal concern has been the question of whether and to what extent lice originating from wild salmonids interact with lice from farmed salmonid hosts. The identification of potential lice population markers is currently being undertaken using a wide variety of approaches including morphometrics, enzyme profiling, gene sequencing and the search for molecular markers. Not all of these approaches have proven useful, largely because it is not known to what extent the measured variable is under environmental influence. Water temperature for instance, has been found to influence morphological size and form making morphometric data inadequate for differentiating populations of sea lice (unpublished data). Recent studies scoring the allelic frequencies of allozymes of sea lice collected over a wide geographic range have shown some discrimination of sea lice from divisions within large waterbodies and between different watersheds/basins (Isdal, Nylund & Nævdal, 1997; present authors – unpublished data). Further, the findings of Todd, Walker, Wolff et al., (1997) suggest that it is possible to discriminate sea lice parasitising farmed salmonids from those on wild salmonids and one farm site from another using

randomly amplified polymorphic DNA (RAPD's) profiles.

The marine environment is not homogeneous, the water of each sea loch possesses its own chemical signature according to its geochemical and geophysical origins. The determination of such elemental signatures is possible by use of conventional nebulisation Inductively Coupled Plasma Mass Spectrometry (ICPMS), a well established and powerful mass spectrometric technique for determining the concentrations of elements in solutions (Hutton, Eaton & Gosland, 1990; Schmit, Youla & Gelinas, 1991). ICPMS has been successfully used in stock identification of fish (Campana, Fowler & Jones, 1994) since the elemental composition of hard tissue (e.g. otoliths and scales) is directly influenced by the fish's environment and diet. Since otoliths and fish scales grow as the fish grows, the spatial distribution of elements in the tissue additionally provides an environmental / dietary history of the individual fish. Due to the large number of potentially mineralised elements available for analysis by ICPMS (60-70), the potential for statistical discrimination is large.

Little is known about the origins of sea lice in the natural environment and speculation regarding the impact of lice from farms on wild salmonid populations of economic importance has fuelled controversy in Scotland and Ireland. This study has been undertaken to investigate whether ICPMS might allow for the discrimination of lice originating from different areas through whole-body elemental analysis of the adult female stage.

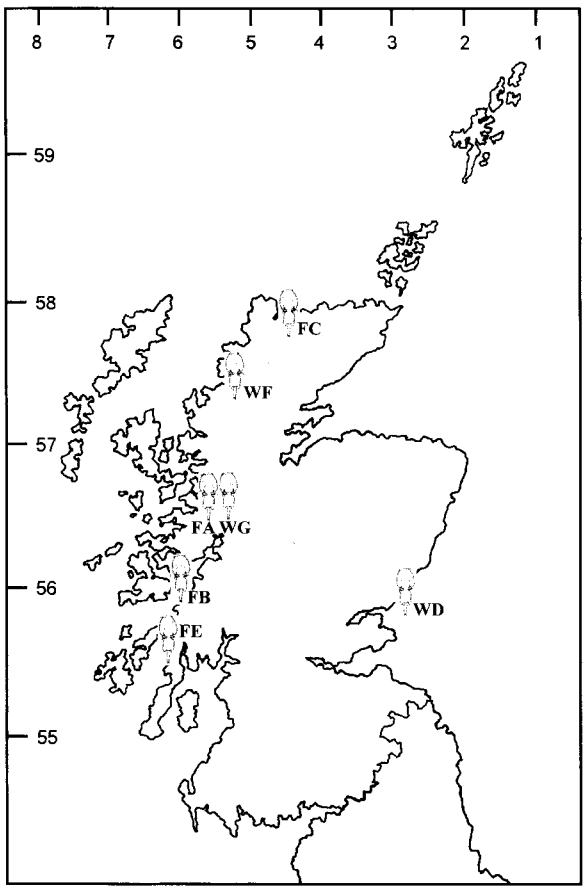

Fig. 1. Map of *Lepeophtheirus salmonis* collection sites in Scotland. (F = farm; W = wild).

Materials and methods

Collection of samples

Samples of adult female *Lepeophtheirus salmonis* were collected from Atlantic salmon, *Salmo salar* L. either at harvest from cultured salmon or from wild salmon captured by rod and line or nets. To protect the identity of the commercial sites from which sea lice were collected, the sites are labelled A-G (F = farm; W = wild) (Fig. 1). Where possible, sea lice sites were sampled on two occasions. Sea lice were transported alive in local fresh sea water to the laboratory and maintained for 24 hours at 10°C to allow for the assimilation and evacuation of gut contents. Lice were then rinsed several times in nanopure distilled water and dried onto filter paper.

Sample preparation and analysis

Dried sea lice samples were weighed into metal-free polypropylene tubes and 100µl of ultrapure nitric acid (Seastar) was added. The tubes were loosely capped, and then placed in a water bath at 95°C for 30 minutes. The resulting digest was made up to a final volume of 5ml with 18Mohm deionised water. The digest solutions were diluted five-fold for running on the ICPMS machine. Indium was added to the tubes at a concentration of 10ppb as

an internal standard, and blanks and calibration standards made up in 2% nitric acid solution. Single point calibration standards were prepared by serial dilution of "Spex" multielement standard solutions. A Perkin Elmer/Sciex ELAN 6000 ICPMS machine was used to perform the elemental composition analysis using standard instrument set-up parameters as outlined in Table 1. All 66 elements were analysed at once, and detection limits were based on 3 × standard deviation of 10 reagent blanks. Blank subtraction involved subtracting the internal standard normalised counts for each element in a reagent blank from those of the samples and standards. Interferences were corrected on-line by the instrument software.

Statistical analysis

Elemental titres (measured using $\mu g g^{-1}$ (ppm)) showing variability between samples, were analysed using univariate (Dunn's and Kruskal-Wallis non-parametric tests) and forward Stepwise Discriminant analyses. The latter was carried out using Statistica 6.0 StatSoft, Inc. 1997.

Results

Univariate statistics

Table 2 shows the summary statistics for the thirty five elements found to be most variable between sites and selected for analysis from a total of 66 elements tested for by ICPMS. Table 3 presents the results from the pairwise comparisons using Dunn's test. These univariate statistics were able to discriminate the majority of sites where sea lice were collected on farmed fish from those sites where lice were collected from wild salmon, but not all. For example, only three out of four farm sites were separated by this method. A significant find was that sea lice collected from farmed and wild salmonids at the same site (FA and WG), could be discriminated using the elements magnesium, vanadium and uranium.

Discriminant analysis

Table 4 presents the model generated by the discriminant analysis which includes 28 of the thirty five elements given in Table 2. A chi-square test applied to successively removed roots illustrates that the first four roots can be confidently used for the discrimination of sea lice specimens (Table 4). The first root accounts for 98% of the total variation between specimens and the first two roots for 99.57%. The principal ten elements in decreasing order of their contribution to specimen separation (as determined by partial lambda) were titanium, magnesium, arsenic, manganese, cobalt, mercury, phosphorus, rubidium, caesium and barium. The classification functions for each element and site of collection are given in Table 5. Using these, a louse of unknown origin could be classified by multiplying the actual concentration of each element (determined by ICPMS) with its respective classification function value given in Table 5, as follows:

$$S_i = C_i + (W_{i1} \times X_1) + (W_{i2} \times X_2) + \ldots\ldots$$

where: C_i = constant for the $_i$th group
W_{ij} = weight for the $_j$th variable
X_i = observed value (in micrograms per gram (ppm))
S_i = classification score for individual

The largest value obtained through application of the formula for each site to the elemental data, determines the most likely origin of the specimen. The separation of sea lice localities for the first four roots of the discriminant analysis based on the determined concentration of 28 elements from each sea louse is shown in Fig. 2. Each site is clearly discriminated and importantly the specimens of sea lice from farmed and wild salmonids within a single locality, FA and WG, are markedly separated from each other.

The discriminant analysis was repeated with elements sequentially removed, to ascertain whether a similar level of discrimination could be achieved based on analysis of a more restricted number of elements. Fig. 3 illustrates the increase in percentage misclassification with the sequential removal

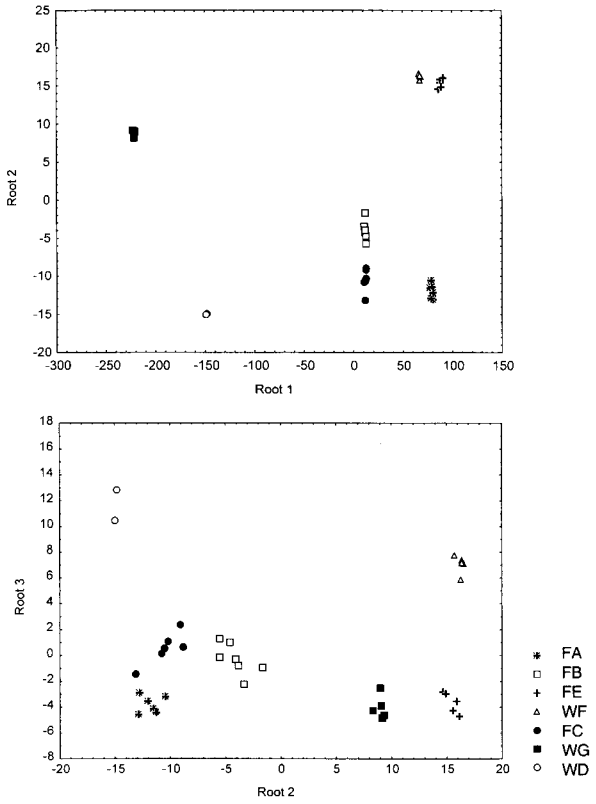

Fig. 2. Map of the thirty seven specimens of *Lepeophtheirus salmonis* in the first three planes of the discriminant analysis based on information from 28 elements. Each specimen is identified by a symbol representing the locality in which the host was sampled.

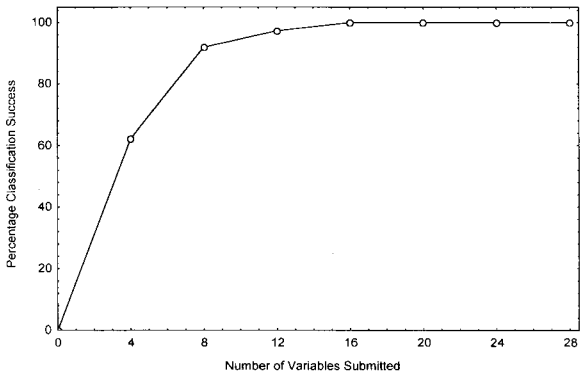

Fig. 3. Graph of the percentage increase in the total number of sea lice correctly classified to their respective site with an increase in the number of elements (variables) used by the discriminant analysis.

of elements (elements with the largest partial lambda values in Table 4 are removed first) from the 28 selected by the first discriminant analysis. While

Table 1. Standard instrument set-up parameters for the Perkin Elmer/Sciex Elan 6000 ICPMS machine.

Parameter	Instrument setting
Nebuliser gas (Ar)	0.9 L/min
Auxilliary gas (Ar)	1.1 L/min
Coolant gas (Ar)	13 L/min
RF power	1000 Watts
Acquisition mode	Peak hopping
Dwell time per peak	20 milliseconds
No. of replicates	2

100% correct classification is obtained using 16 or more elements, less than perfect classification is obtained using 12 or less elements (12 = 97.30%, 10 = 89.19%, 8 = 91.89% and, 4 = 62.16%). Using eight elements, it is still possible to discriminate all lice from the wild salmonids studied from the lice from farmed salmon but using 4 elements, lice from farms are misclassified as belonging to wild sites and vice versa.

Discussion

This paper describes the successful use of elemental analysis in the discrimination of populations of Crustacea for the first time, although this technique has previously been used to identify the origins of fish (Calaprice, Lapi & Carlsen, 1975; Campana et al., 1994; Schroder et al., 1995; Wang et al., 1994). Univariate statistics applied in this study indicated that the separation of certain sites was possible but by no means complete. Discriminant analysis, however, using a selected 28 out of the 35 variable elements, provided a clear separation of sites. These statistical techniques also allowed discrimination of sea lice taken from farmed salmonids from those taken from wild salmonids in the same locality. By sequential reduction of the number of elements submitted to discriminant analysis, it was further found that 16 elements still provided a 100% correct classification of sea lice. This study has therefore demonstrated the potential for ICPMS to allow statistical discrimination of sea lice and this technique may therefore provide a further tool in the elucidation of the epidemiology of sea louse infestations, thereby enabling improved management and control of this parasite. The ability of

Table 2. ICPMS analysis of adult female *Lepeophtheirus salmonis* from several localities. The 35 most variable elements are given in micrograms per gram (ppm) dry weight as the mean and standard deviation followed by the median and range in parentheses.

Element	FA (n = 6)	FB (n = 7)	FC (n = 7)	WD (n = 2)	FE (n = 5)	WF (n = 5)	WG (n = 5)
Lithium	0.322±0.097 (0.29) (0.23-0.44)	0.134±0.059 (0.11) (0.08-0.24)	0.206±0.049 (0.21) (0.13-0.26)	0.575±0.290 (0.575) (0.37-0.78)	0.290±0.142 (0.23) (0.16-0.50	0.266±0.069 (0.27) (0.16-0.34)	0.128±0.048 (0.11) (0.09-0.21)
Boron	9.283±7.253 (10.0) (1.0-19.7)	7.000±6.429 (7.0) (1.0-17.0)	7.600±1.39 (7.2) (6.0-10.0)	15.500±7.778 (15.5) (10.0-21.0)	16.200±7.981 (13) (8-29)	15.400±3.975 (14) (12-22)	11.200±6.301 (9.0) (4.0-20.0)
Magnesium	2015±269.722 (1945) (1770-2540)	1412.857±175.187 (1450) (1130-1630)	1481.429±157.791 (1450) (1220-1720)	3150.000±1470.782 (3150) (2110-4190)	1832.000±493.528 (1500) (1460-2480)	2192.000±430.952 (2170) (1750-2860)	1278±182.264 (11800) (1120-1550)
Aluminium	11.683±5.253 (10.45) (7.1-20.3)	21.157±12.099 (16.8) (11.2-41.5)	12.186±6.424 (8.8) (6.3-23.2)	9.350±4.879 (9.35) (5.9-12.8)	13.800±9.406 (9.5) (7.0-30.2)	15.440±6.512 (14.3) (7.1-24.7)	23.220±11.304 (919.8) (12.5-37.3)
Mercury	0.193±0.117 (0.2) (0.07-0.3)	0.167±0.097 (0.15) (0.08-0.3)	0.163±0.129 (0.07) (0.04-0.3)	0.300±0.000 (0.3) (0.3-0.3)	0.038±0.011 (0.03) (0.03-0.05)	0.058±0.041 (0.03) (0.03-0.12)	0.126±0.029 (0.13) (0.08-0.15)
Phosphorus	9318.333±1270.975 (8975) (7860-10900)	9657.143±834.357 (9440) (8270-11000)	8767.143±981.949 (9420) (7450-9650)	8535.000±1251.579 (8535) (7650-9420)	6260.000±485.798 (5960) (5870-6990)	7024.000±551.072 (7240) (6200-7600)	7518.000±1243.953 (73800) (6180-9000)
Potassium	9741.667±1777.779 (9605) (7320-12200)	9045.714±1411.404 (8610) (7580-11400)	8440.000±1285.535 (8750) (6840-10200)	5425.000±21.213 (5425) (5410-5440)	4692.000±791.688 (4430) (3700-5760)	5114.000±1889.373 (6020) (3030-6840)	5938.000±1937.542 (6320) (2990-8120)
Calcium	925.000±140.677 (885) (760-1170)	572.857±104.842 (550) (470-770)	708.571±94.592 (700) (600-880)	1450.000±707.107 (1450) (950-1950)	968.000±276.080 (790) (750-1270)	852.000±174.557 (780) (700-1110)	914.000±213.495 (840) (720-1210)
Scandium	0.767±0.647 (0.65) (0.2-2.0)	1.029±0.663 (0.8) (0.2-2.0)	0.657±0.431 (1.0) (0.1-1.0)	0.200±0.000 (0.2) (0.2-0.2)	1.520±0.965 (1.0) (0.8-3.0)	1.600±0.548 (2.0) (1.0-2.0)	0.700±0.158 (0.7) (0.5-0.9)
Titanium	0.683±0.194 (0.65) (0.5-0.8)	0.886±0.436 (0.7) (0.4-1.60)	0.571±0.198 (0.5) (0.4-1.0)	0.450±0.071 (0.45) (0.4-0.5)	0.420±0.045 (0.4) (0.4-0.5)	0.480±0.192 (0.4) (0.3-0.8)	1.480±0.760 (1.2) (0.8-2.6)
Vanadium	0.822±0.247 (0.79) (0.54-1.1)	1.083±0.259 (1.06) (0.62-1.4)	1.717±2.074 (1.0) (0.40-6.35)	0.820±0.283 (0.82) (0.62-1.02)	1.080±0.110 (1.0) (1.0-1.2)	1.040±0.134 (1.1) (0.9-1.2)	1.640±0.167 (1.6) (1.5-1.9)
Chromium	2.027±1.209 (1.99) (0.4-3.6)	2.446±1.142 (3.0) (0.51-3.8)	2.029±1.267 (2.9) (0.45-3.1)	2.575±2.284 (2.575) (0.96-4.19)	2.280±0.130 (2.2) (2.2-2.5)	2.640±0.182 (2.6) (2.4-2.9)	3.680±1.026 (3.3) (3.0-5.5)
Manganese	2.993±0.910 (3.03) (1.5-4.12)	3.630±2.848 (2.5) (1.0-10.1)	2.911±0.299 (2.8) (2.6-3.5)	2.720±1.598 (2.72) (1.59-3.85)	1.420±0.476 (1.7) (0.6-1.7)	1.440±0.219 (1.4) (1.2-1.8)	2.520±0.817 (2.4) (1.4-3.5)
Iron	113.333±30.111 (110) (80-170)	72.857±44.949 (60) (30-170)	105.714±24.398 (120) (70-140)	85.000±7.071 (85.0) (80-90)	44.000±8.944 (40) (40-60)	42.000±17.889 (40) (20-60)	116.000±35.071 (120) (60-150)
Cobalt	0.258±0.396 (0.070) (0.05-1.05)	0.089±0.054 (0.050) (0.04-0.2)	0.070±0.047 (0.05) (0.03-1.7)	0.565±0.771 (0.565) (0.02-1.11)	0.082±0.072 (0.05) (0.05-0.21)	1.630±2.956 (0.38) (0.05-6.89)	0.278±0.396 (0.11) (0.05-0.98)
Nickel	0.260±0.296 (0.15) (0.1-0.86)	0.229±0.180 (0.20) (0.1-0.6)	0.189±0.120 (0.1) (0.1-0.4)	0.395±0.460 (0.395) (0.07-0.72)	0.120±0.045 (0.1) (0.1-0.2)	0.140±0.089 (0.1) (0.1-0.3)	0.360±0.434 (0.1) (0.1-1.1)
Copper	11.853±11.909 (7.66) (4.9-36.0)	7.566±6.309 (5.2) (3.9-21.6)	5.186±1.410 (6.00) (3.16-6.7)	8.865±2.737 (8.865) (6.93-10.8)	8.340±2.352 (9.3) (5.9-11.2)	8.060±3.093 (6.6) (5.8-13.3)	9.840±3.620 (9.1) (5.0-14.0)
Zinc	280.5±100.717 (264.5) (159-439)	255.429±151.421 (214) (138-565)	181.429±24.711 (188) (152-217)	754.500±446.184 (754.5) (439-1070)	282.400±133.067 (374) (126-388)	346.000±185.286 (302) (175-631)	605.200±340.851 (507) (355-1180)
Gallium	0.017±0.008 (0.015) (0.01-0.03)	0.016±0.011 (0.01) (0.01-0.04)	0.010±0.00 (0.01) (0.01-0.01)	0.070±0.042 (0.07) (0.04-0.10)	0.014±0.009 (0.01) (0.01-0.03)	0.030±0.019 (0.03) (0.01-0.06)	0.026±0.011 (0.03) (0.01-0.04)
Arsenic	11.692±2.434 (10.85) (9.55-15.4)	7.983±5.856 (5.8) (3.41-20.5)	6.781±2.309 (6.0) (3.57-10.5)	1.460±0.212 (1.46) (1.31-1.61)	6.980±1.221 (6.3) (5.8-8.4)	3.620±0.536 (3.9) (3.0-4.2)	7.740±1.940 (7.2) (5.9-10.3)
Selenium	2.750±1.786 (2.75) (0.8-5.0)	1.157±0.973 (0.7) (0.5-3.0)	1.714±1.380 (1.5) (0.5-4.0)	4.500±0.707 (4.5) (4.0-5.0)	0.980±0.691 (0.6) (0.5-2.1)	0.960±0.559 (0.8) (0.5-1.9)	6.260±1.629 (5.7) (4.3-8.0)
Rubidium	3.668±0.955 (3.43) (2.70-4.91)	3.360±0.437 (3.14) (3.07-4.16)	3.189±0.667 (3.11) (2.44-4.35)	2.405±0.120 (2.405) (2.32-2.49)	1.614±0.382 (1.41) (1.26-2.09)	1.912±0.543 (2.24) (1.17-2.40)	1.876±0.567 (1.90) (1.05-2.54)
Strontium	15.783±3.002 (15.5) (12.8-20.7)	9.691±1.744 (10.1) (7.14-12.5)	11.847±1.556 (11.9) (9.83-14.5)	26.450±13.506 (26.45) (16.9-36.0)	18.060±6.777 (13.5) (12.9-27.4)	15.580±4.129 (13.1) (11.9-21.3)	10.576±2.811 (9.96) (7.71-13.9)
Zirconium	0.077±0.053 (0.06) (0.02-0.17)	0.053±0.042 (0.04) (0.01-0.13)	0.021±0.015 (0.02) (0.01-0.05)	0.025±0.007 (0.025) (0.02-0.03)	0.114±0.043 (0.14) (0.05-0.15)	0.066±0.104 (0.02) (0.01-0.25)	0.208±0.409 (0.02) (0.01-0.94)
Silver	0.023±0.039 (0.007) (0.005-0.102)	0.006±0.0026 (0.005) (0.005-0.012)	0.013±0.009 (0.011) (0.005-0.031)	0.051±0.011 (0.051) (0.043-0.059)	0.039±0.030 (0.030) (0.018-0.092)	0.064±0.075 (0.044) (0.005-0.192)	0.026±0.017 (0.016) (0.015-0.056)
Cadmium	0.050±0.054 (0.03) (0.02-0.16)	0.049±0.072 (0.02) (0.01-0.21)	0.023±0.008 (0.02) (0.01-0.03)	0.040±0.028 (0.04) (0.02-0.06)	0.052±0.066 (0.02) (0.02-0.17)	0.342±0.698 (0.02) (0.02-1.59)	0.066±0.040 (0.06) (0.02-0.12)
Tin	0.048±0.020 (0.055) (0.02-0.08)	0.039±0.027 (0.03) (0.01-0.09)	0.030±0.031 (0.02) (0.01-0.08)	0.055±0.064 (0.055) (0.01-0.10)	0.148±0.076 (0.18) (0.06-0.24)	0.156±0.125 (0.17) (0.03-0.34)	0.054±0.021 (0.05) (0.03-0.08)
Antimony	0.103±0.047 (0.095) (0.05-0.18)	0.046±0.034 (0.03) (0.01-0.09)	0.054±0.040 (0.06) (0.01-0.12)	0.015±0.007 (0.015) (0.01-0.02)	0.036±0.019 (0.03) (0.01-0.06)	0.028±0.018 (0.03) (0.01-0.05)	0.058±0.033 (0.06) (0.02-0.11)
Tellurium	0.118±0.142 (0.075) (0.03-0.40)	0.100±0.040 (0.1) (0.03-0.17)	0.071±0.036 (0.1) (0.03-0.1)	0.100±0.057 (0.1) (0.06-0.14)	0.100±0.000 (0.10) (0.10-0.10)	0.100±0.000 (0.1) (0.1-0.1)	0.100±0.000 (0.1) (0.1-0.1)
Caesium	0.052±0.017 (0.055) (0.02-0.07)	0.177±0.350 (0.04) (0.04-0.97)	0.040±0.014 (0.04) (0.03-0.06)	0.020±0.000 (0.02) (0.02-0.02)	0.016±0.005 (0.02) (0.01-0.02)	0.020±0.007 (0.02) (0.01-0.03)	0.060±0.031 (0.06) (0.03-0.1)
Barium	0.348±0.347 (0.275) (0.06-1.02)	0.337±0.184 (0.38) (0.13-0.63)	0.186±0.090 (0.14) (0.09-0.32)	1.905±1.294 (1.905) (0.99-2.82)	0.170±0.110 (0.15) (0.08-0.36)	1.004±0.413 (0.91) (0.71-1.71)	0.640±0.215 (0.62) (0.34-0.88)
Lanthanum	0.442±0.596 (0.07) (0.02-1.50)	0.167±0.243 (0.1) (0.01-0.71)	0.884±1.435 (0.19) (0.02-3.84)	0.035±0.021 (0.035) (0.02-0.05)	0.338±0.163 (0.41) (0.12-0.52)	0.340±0.332 (0.22) (0.13-0.92)	0.092±0.066 (0.07) (0.05-0.21)
Cerium	0.117±0.080 (0.125) (0.02-0.21)	0.034±0.045 (0.01) (0.01-0.13)	0.346±0.815 (0.05) (0.02-2.21)	0.015±0.007 (0.015) (0.01-0.02)	0.012±0.004 (0.01) (0.01-0.02)	0.014±0.009 (0.01) (0.01-0.03)	0.060±0.031 (0.06) (0.03-0.11)
Lead	0.738±0.507 (0.555) (0.22-1.54)	0.589±0.339 (0.54) (0.09-1.04)	0.360±0.172 (0.29) (0.21-0.64)	0.065±0.078 (0.065) (0.01-0.12)	8.988±11.131 (1.89) (0.31-22.7)	1.008±1.830 (0.25) (0.11-4.28)	1.154±0.853 (1.07) (0.23-2.54)
Uranium	0.028±0.009 (0.027) (0.018-0.043)	0.037±0.025 (0.026) (0.012-0.087)	0.027±0.007 (0.028) (0.017-0.035)	0.049±0.016 (0.049) (0.037-0.060)	0.049±0.012 (0.050) (0.031-0.191)	0.033±0.022 (0.03) (0.005-0.063)	0.096±0.046 (0.093) (0.052-0.172)

Table 3. Pairwise comparisons using Dunn's tests for each element and for each collection of *Lepeophtheirus salmonis*. The test value for the Dunn's test for each appropriate element is given in parentheses.

Site	FA	FB	FC	WD	FE	WF	WG
FA	-						
FB	Li* (3.163) Ca* (3.304) Sr* (3.121)	-					
FC	-	-	-				
WD	As* (3.490)	Ca* (3.051)	Ga* (3.136)	-			
FE	P* (3.270) K** (3.519) Rb** (3.377) Cs* (3.132) Ce* (3.145)	P*** (3.814) K* (3.219) Rb** (3.496) Sr* (3.154) Ag* (3.157) Cs* (3.254)	P* (3.059) Zr* (3.105) Sn* (3.160)	Hg* (3.434)	-		
WF	K* (3.107) As*** (3.988)	-	Ba* (3.353)	-	Ba* (3.157)	-	
WG	Mg* (3.217) V** (3.537) U* (3.226)	Se* (3.459)	Zn* (3.174) U* (3.385)	-	Ti*** (3.695) Se* (3.269) Cs* (3.127)	Mg* (3.316) Ti* (3.205) Se* (3.152)	-

Dunns test: *0.05% = 3.038, **0.01% = 3.494, ***0.005% = 3.675.

this technique to discriminate lice obtained from wild and farmed sources in the same location also suggests that it might be used to determine the origin of lice recovered from wild and farmed salmonids. In order for the true potential of this technique to be evaluated in the future, however, a number of variable parameters need to be investigated such as the seasonality / stability of elemental composition and its relationship to the host's / parasite's environment.

In decapod crustaceans the trace metal content has been suggested to be divisible into three partitions:- that passively adsorbed onto the cuticle, that present in ingested food contained in the gut but not assimilated into the body and that which is absorbed and accessible to physiological processes (principal component) (Rainbow, 1988). In order to interpret data obtained from the elemental analysis of sea lice it is therefore important to determine the contribution of each of these partitions to the overall elemental content and the stability of elemental composition within each partition. The contribution of metal contained within the gut can, however, be ignored in this study as the lice used were starved prior to analysis.

The elemental signatures of sea lice are likely to be heavily dependent upon a combination of the host elemental composition and the physico-chemical properties of the marine environment e.g. the form in which elements are bioavailable and how their state and concentration are affected by factors such as salinity and depth. In terms of the host, differences are likely to rest particularly upon a) dietary components which may differ between farms and between wild and farmed fish b) genetic factors and c) environmental factors which will affect the elemental composition of both host and parasite as elements are not homogeneously bioavailable. Riley & Skirrow (1965) have shown a ~16.5% increase in the concentration of the ions Na^+, Mg^{2+}, Ca^{2+}, K^+ and Sr^{2+} with increased salinity from 30‰ to 35‰ whilst Butler (1998) has demonstrated that although certain elements such as V, show no concentration change with depth, others increase (Sc, Ti, Fe, Ni, Cu, Zn, Si and P) or decrease (Co and Mn). Research on *Palaemon elegans* by Nugegoda (1986, cited in Rainbow, 1988) supports the suggestion that such environmental variability could affect elemental composition since it was shown that decreasing salinity

Table 4. Chi-Square tests with successive roots removed and standardized coefficients for the canonical variables of the top 28 elements selected by the Discriminant Analysis, the eigenvalues and the proportion of the variance explained by the first four roots. The elements with the smallest partial lambda figures contribute the most to overall discrimination of the analysed specimens.

Roots removed	Eigen-value	Chi-Square	df	p-level
0	13013.73	452.90	168	0.00000
1	169.41	277.64	135	0.00000
2	25.15	182.58	104	0.00000
3	19.20	122.20	75	0.00048
4	10.00	66.60	48	0.03909
5	2.33	22.24	23	0.50557

Variable	Root 1	Root 2	Root 3	Root 4	Partial lambda
Antimony	-2.40	-3.75	-1.56	-0.45	0.1106
Arsenic	49.47	3.44	-3.11	1.20	0.0024
Barium	37.85	10.65	1.49	1.67	0.0105
Boron	5.07	3.08	0.85	-1.84	0.1983
Cadmium	-11.27	-0.29	-0.45	-1.32	0.0196
Caesium	-43.53	-4.78	1.83	-1.90	0.0093
Calcium	-44.89	1.19	-3.65	-5.65	0.0188
Cerium	5.84	2.68	0.03	2.05	0.1548
Cobalt	-50.53	-5.04	2.62	0.21	0.0031
Chromium	-37.07	-7.95	1.26	-0.79	0.0199
Gallium	-16.37	-1.74	-1.77	-0.42	0.0343
Lanthanum	-25.05	-5.95	1.89	-0.40	0.0165
Lead	-8.36	1.67	1.45	2.21	0.0707
Lithium	-43.20	-5.11	1.67	6.74	0.0117
Magnesium	75.79	-1.13	2.91	-1.93	0.0022
Manganese	117.92	8.69	-2.77	3.84	0.0029
Mercury	-72.91	-14.64	0.78	-1.27	0.0033
Nickel	-12.68	2.18	0.30	0.31	0.0355
Phosphorus	-73.37	-6.46	-0.82	-3.58	0.0033
Rubidium	57.38	4.65	1.64	-0.42	0.0042
Scandium	-6.30	5.90	1.55	4.84	0.2995
Selenium	-4.30	3.13	1.07	1.91	0.2783
Strontium	7.62	-0.66	-0.33	2.79	0.2970
Tin	22.51	-1.45	-3.31	-5.61	0.0415
Titanium	-49.60	-1.53	0.71	-1.18	0.0020
Vanadium	-7.84	-2.12	-0.23	0.44	0.0331
Zinc	-15.04	-2.41	-0.07	0.97	0.0195
Zirconium	14.73	1.47	-0.55	1.66	0.0139
Eigenvalue	13013.73	169.4061	25.14587	19.19628	
Cum. Prop.	.98	.9957	.99762	.99907	

reduced zinc complexation leading to an increase in its passive uptake.

Seasonal or temporal variation of environmental parameters such as temperature and salinity and of host or parasite life-cycle parameters such as feeding and moulting / reproductive state are also likely to affect elemental composition. The developmental stage of the parasite chosen for analysis is also important since without knowledge of the stability of elemental composition within and between different developmental stages, comparisons between samples from different sources may not be justified. Losses or gains during moulting between stages may be particularly important although many of the cuticular components might be expected to be conserved since previous research has shown that cuticular metal can be reabsorbed at moult in decapods (Rainbow, 1988). Parasite genetic factors might also play a part in elemental composition through differential uptake, bioassimilation and excretion, but their contribution cannot as yet be quantified.

The range of parameters that could affect the elemental composition of free-living copepods led Båmstedt (1986) to note that in order for hypotheses concerning differences related to geolocality to be valid, it is essential to use data or specimens collected not only within the same season but having the same developmental stage and trophic position. The present study satisfies such requirements in that it employed only adult female lice, with all the samples being taken within a narrow time frame. It is clear, however, that any further studies must determine whether the adult female is the optimum stage to choose. The environmental, seasonal and host parameters affecting the elemental composition of the parasite and the stability of elemental composition over time and under variable conditions must also be established.

This study has clearly demonstrated the potential for this methodology to allow discrimination of sea louse populations. The preliminary nature of the work, however, involving as it has, low sample sizes, a relatively small number of populations and the analysis of a very large number of elements, leaves a number of critical aspects of the method to be established. This study has indicated that a smaller subset of elements should provide enough variability to discriminate samples, however, any such elemental subset needs to be constructed using larger numbers of individuals and populations to ensure that the discriminatory powers of the model will be widely applicable. In addition, the high classification success demonstrated in the present study results from testing only the modelling data. Testing of new data with the same model would be likely to give a lower classification accuracy and might therefore require more elements to provide similar discriminatory efficiency. The lack of detailed knowledge concern-

Table 5. Classification functions for the discrimination of sea lice. The identification of a sea louse or its discrimination from sea lice at other sites given by the systematic use of the values in the equation Constant + (Antimony × value for first site) + (Arsenic × value for first site) + (Barium × value at first site) + ... etc. For example for FA = -31039.4 + (ICPMS value for Antimony × 25073.2) + (ICPMS value for Arsenic × -3871.1) + ... etc.

Variable	FA p=0.16216	FB p=0.18919	FC p=0.13514	WD p=0.13514	FE p=0.18919	WF p=0.13514	WG p=0.05405
ANTIMONY	25073.2	28708.1	21152.0	22266.2	29246.3	43905.5	40535.7
ARSENIC	-3871.1	-4933.7	-3685.2	-4030.7	-4937.5	-8628.9	-7506.5
BARIUM	-28962.7	-36205.5	-26996.0	-29299.1	-36356.3	-61855.6	-54346.3
BORON	-225.6	-277.5	-205.7	-217.8	-281.1	-470.6	-422.4
CADMIUM	10630.7	13558.7	10155.9	11085.1	13521.6	23661.6	20450.1
CAESIUM	68358.2	86679.4	64691.3	70743.5	86757.3	150857.6	131449.4
CALCIUM	51.3	65.7	49.3	53.8	65.5	115.3	99.1
CERIUM	-4517.2	-5557.9	-4134.4	-4509.8	-5571.4	-9151.5	-8111.0
CHROM	8406.2	10561.9	7869.5	8588.7	10603.7	18220.3	15974.6
COBALT	11094.0	14106.3	10529.7	11501.2	14135.2	24655.3	21483.8
Constant	-31039.4	-48916.5	-27291.5	-32414.5	-49043.9	-146365.1	-110978.1
GALLIUM	308224.3	390838.0	292466.1	317021.9	390650.3	680690.4	590709.6
LANTHANU	8989.2	11289.1	8404.2	9178.8	11343.7	19427.1	17079.5
LEAD	432.7	569.3	427.4	468.6	569.9	1046.9	900.6
LITHIUM	107375.8	136649.6	101992.2	110845.1	137336.5	240862.9	210364.9
MAGNES	-45.1	-57.9	-43.4	-47.2	-57.8	-102.4	-88.4
MANGAN	-19142.8	-24363.0	-18206.5	-19884.7	-24369.8	-42572.0	-36998.2
MERCURY	207512.7	261365.4	194895.2	212191.6	262166.7	451180.5	395139.3
NICKEL	11849.4	15476.0	11613.3	12713.0	15420.4	27927.7	23889.9
PHOSPHOR	18.3	23.3	17.5	19.0	23.3	40.6	35.3
RUBID	-22641.2	-28838.2	-21584.6	-23444.6	-28850.3	-50400.3	-43793.3
SCAND	1427.2	2157.6	1638.9	1811.9	2139.4	4698.2	3837.8
SELEN	574.7	806.7	607.9	684.3	800.9	1646.7	1363.0
STRONTIU	-425.1	-543.1	-402.0	-451.6	-540.7	-964.2	-822.3
TIN	-85209.1	-110655.6	-82673.7	-90459.8	-111061.3	-200681.3	-173908.8
TITAN	31848.7	40691.7	30441.6	33244.5	40673.8	71464.9	61928.4
VANAD	2101.5	2643.8	1971.8	2129.0	2658.3	4544.1	3996.9
ZINC	19.7	24.9	18.6	20.2	25.0	43.4	37.9
ZIRCON	-23191.8	-29514.2	-22051.0	-24067.9	-29488.1	-51066.4	-44461.8

ing the origin and stability of the elemental compositions recorded in this preliminary study means that to attempt to draw detailed conclusions from the tables presented and to account for the observed differences in elemental concentrations between populations of lice would be inappropriate. Nevertheless we believe that this study has successfully demonstrated a promising technique for the future discrimination of samples of sea lice and indeed of other parasitic and free-living aquatic arthropods from different sources and we hope that the necessary further research will validate this conclusion.

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