What can otolith examination tell us about the level of perturbations of Salmonid fish from the Kerguelen Islands?

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Abstract – Otoliths preserve a continuous record of the life cycle from the natal through the adult stage. For that reason, the morphological and chemical characteristics of otoliths of two nonnative Salmonids, brown trout (*Salmo trutta*) and brook charr (*Salvelinus fontinalis*) from populations on the Kerguelen Islands were compared. Several approaches were used to study the relationships between otolith morphometry, crystal morph and chemical elemental composition. These salmonids sampled in Kerguelen are well differentiated in terms of species through their otolith shape. The results indicate that ecotypes and river populations can be reasonably well differentiated on the basis of otolith shape. The crystallisation study has revealed the presence of a particular form: the vaterite, present at a high rate: 45% of *S. fontinalis* and 18% from *Salmo trutta fario*. Moreover, vaterite and aragonite otoliths presented differences in chemical composition.

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Key words: Salmonidae; Kerguelen Islands; otolith shape; chemistry; crystallography

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Introduction

The Kerguelen Islands, located in South Indian Ocean (49°S, 70°E), are subjected to cold sub-Antarctic oceanic climate. The hydrographic system is complex (rivers, fjords and lakes) and no endemic freshwater ichthyofauna is present. Many fish species have been introduced between 1951 and 1991 (Duchêne 1989; Duhamel et al. 2005), exclusively Salmonidae (*Salvelinus fontinalis, Salmo trutta* and *Salmo salar*…). The majority of these species quickly acclimated and then colonised new rivers. The Kerguelen Islands present a favourable environment for studying these species because of the absence of freshwater endemic ichthyofauna, the homogeneity of sites and the simplicity of freshwater communities.

Environmental contaminants (heavy metals, organic pollutants…) may be responsible for physico-pathological troubles (highest disease sensibilities, sexual variations…) in wild fish populations in many world regions. However, the pressure of human activities on fish populations disturbs the determination of the real responsibility of environmental contaminants in physico-pathological perturbations observed. In the Kerguelen Islands, the living Salmonid populations are potentially protected from anthropogenic perturbations. However, the recent studies carried out in two Kerguelen rivers, Château River and Ferme River have shown differences between the two sites in the

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immune competence characteristics [number of circulating leucocytes, splenosomatic index and phagocyte oxidative activity (Betoulle et al. 2005) of *S. trutta* and *S. fontinalis*]. These different immune parameters between the two salmonids populations of Château River and Ferme River could be due to differences in exogenous (food availability and quality, particular physico-chemical characteristics…) and in endogenous biological factors (age, reproductive and epidemiological status of organisms).

Otoliths are an indirect means to study the relationships between environment and organisms, through their shape, crystal morph and their chemical composition. Minor and trace elements incorporated into otoliths during growth may record environmental conditions experienced by fishes. They are considered as the 'black box' of the fish (Lecomte-Finiger 1999). Chemical elements were used because (i) environmental physico-chemical conditions influence incorporation, (ii) there is no turnover (Campana 2005). Otolith size and shape were species specific but interindividual variations might exist in functions of geographic areas. With environmental disturbances or stress, asymmetry between right and left otoliths can be observed (Campana 2005). Otoliths are composed of $CaCO_3$ that normally precipitates as aragonite, which is metabolically inert (Campana 1999). In few otoliths, the $CaCO_3$ occurs as calcite or vaterite. These three polymorphs of $CaCO_3$ differ in the geometry of the crystal: calcite is trigonal, vaterite is hexagonal and aragonite is orthorhombic. Otoliths may be completely or partially composed of vaterite. Vaterite otoliths have been reported in a number of marine and freshwater fishes from different environments (Gauldie 1986; Oliveira & Farina 1996; Tomas & Geffen 2003). Calcite and vaterite could be linked to physiological disturbances or to environmental stress (Morales-Nin 1987).

Salmonids (brown trout *S. trutta* and brook trout *S. fontinalis*) from various Kerguelen aquatic systems were compared through their otolithometric characteristics. In this paper, we tested the hypotheses (i) that otolith shape can be used to differentiate Salmonids species and/or populations, (ii) that otoliths shape, crystallisation and chemistry can be used to evaluate environmental stress among the Salmonids. To answer these questions the role of external factors including stress, food quality and quantity, freshwater quality is discussed.

Materials and methods

Studied areas

Between January and August 2005, brown and brook trout were sampled in six different aquatic systems of Kerguelen: Studer Lakes, Château, Sud and Ferme Rivers, and Sablière and Décharge Ponds (Fig. 1). The first three sites were relatively far from the scientific base of Port-aux-Français, whereas the other three sites were very close to the base (Fig. 1). It is important to note that studied sites were represented by lentic (lakes and ponds: Sablière and Décharge) and lotic systems (rivers: Ferme, Studer, Sud and Château). Each month between January and August 2005, water physico-chemical characteristics of the six different sites were recorded from samples taken in the middle of each month. They included temperature, pH, conductivity, and levels of dissolved oxygen, nitrogen–ammonia, nitrates, nitrites, orthophosphates, potassium and sulphates, according to Standard Methods of the American Public Health Association (American Public Health Association 1992). Each analysis (one for each month between January to August 2005) was done in triplicate (Table 1).

Salmonids

A total of 199 fish were captured by electrofishing. Weight and length were measured (Table 2) and *sagittae* (310) were extracted directly in the field. These fishes belonged to three Salmonids species: *Salmo trutta fario, Salmo trutta trutta* and *S. fontinalis*. As other Salmonids these species are sometimes able to realise marine migration and returned to their native river by homing.

Otolith morphometry

To test whether otoliths shape could be used for differentiate Salmonids species and population, otoliths were cleaned and weighted (accuracy 0.001 mg; Semi-microbalance Sartorius Genius, S.A. Sartorius Mechatronics Belgium N.V. Leuvensesteenweg, 248/B, 1800 Vilvoorde ME 235P). Each otolith, systematically placed with the *sulcus acusticus* oriented towards the observer, was examined under a stereomicroscope fitted with (Sony XC-77CE CCD industrial Camera, Sony, France) linked to a computer. Digital images were then acquired with the software VISILOG 6.3 (Noesis, Orsay, France) which also calculated the surface area of the otolith (*A*o), its perimeter (*P*o), its length (maximum measure, *L*o) and its width (maximum measure, *l*o) to the nearest 10^{-2} mm. These measures allowed the calculation of five shape indices (Table 3) which are independent from differences in otolith size (Tuset et al. 2003). Moreover, the shape of each otolith was assessed with a Fourier series. Among several possibilities for analysing shape with Fourier series, the elliptic Fourier analysis (EFA) descriptors are often considered more powerful than those derived from fast Fourier transformation (FFT) for shape analysis, and was therefore used in this work, although

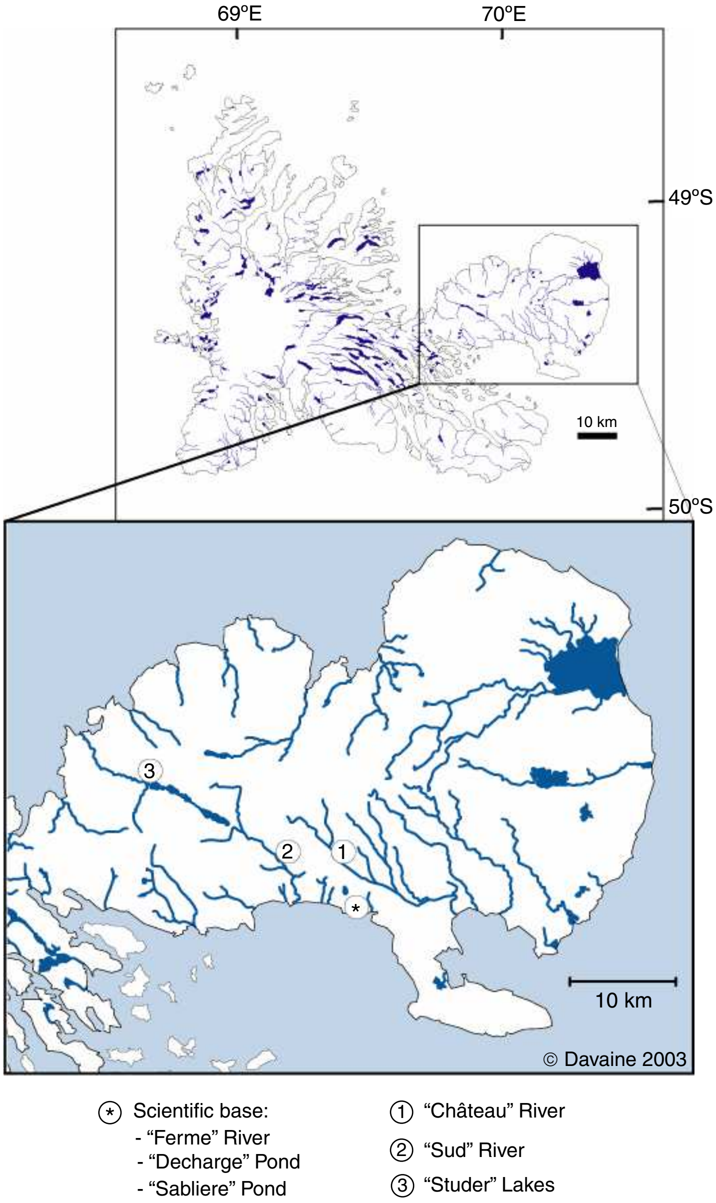

Fig. 1. Map of the study area.

Table 1. Environmental factors: temperature, pH, dissolved oxygen, conductivity, chlorine, sulphate, nitrate, phosphate, potassium, iron, DCO, phenolic compounds and total hydrocarbures.

	Château River	Sud River	Studer Lakes	Ferme River	Décharge Pond	Sablière Pond
Temperature (°C)	4.6 ± 1.7	6.3 ± 1.9	5.8 ± 1.1	5.9 ± 2.5	5.2 ± 1.8	5.4 ± 1.5
pH	7.5 ± 0.2	7.6 ± 0.1	7.5 ± 0.1	7.6 ± 0.1	7.9 ± 0.2	7.7 ± 0.05
Dissolved oxygen ($mg \cdot l^{-1}$)	12.2 ± 0.3	10.8 ± 0.5	11.5 ± 0.3	11.7 ± 0.6	11.4 ± 0.2	12.3 ± 0.3
Conductivity ($\mu S \cdot cm^{-1}$)	67.8 ± 1.6	64 ± 1.6	74.8 ± 1.7	150.4 ± 5.6	169.4 ± 3.8	146.8 ± 4.5
Cl^- ($mg \cdot l^{-1}$)	11.9 ± 0.9	8.8 ± 0.4	10.7 ± 0.2	23.9 ± 0.5	18.4 ± 2.4	23.9 ± 0.5
SO_4^{2-} ($mg \cdot l^{-1}$)	0.9 ± 0.5	1.5 ± 0.4	0.75 ± 0.2	2.4 ± 0.5	1.75 ± 0.4	0.75 ± 0.2
NO_3^- ($mg \cdot l^{-1}$)	0.022 ± 0.006	0.018 ± 0.004	0.016 ± 0.003	0.021 ± 0.009	0.012 ± 0.002	0.01 ± 0.002
NO_2^- ($mg \cdot l^{-1}$)	0.005 ± 0.0005	0.004 ± 0.0006	0.004 ± 0.0005	0.005 ± 0.0007	0.003 ± 0.0003	0.005 ± 0.0008
PO_4^{3-} ($mg \cdot l^{-1}$)	0.88 ± 0.04	0.84 ± 0.07	0.97 ± 0.07	0.64 ± 0.08	0.71 ± 0.07	0.76 ± 0.05
NH_3-N ($mg \cdot l^{-1}$)	0.035 ± 0.014	0.035 ± 0.013	0.033 ± 0.007	0.153 ± 0.01	0.123 ± 0.01	0.167 ± 0.02
K^+ ($mg \cdot l^{-1}$)	0.3 ± 0.02	0.39 ± 0.14	0.27 ± 0.02	0.62 ± 0.04	0.7 ± 0.05	0.55 ± 0.05
Total iron ($mg \cdot l^{-1}$)	0.12 ± 0.05	0.14 ± 0.03	0.18 ± 0.06	0.38 ± 0.04	0.41 ± 0.09	0.24 ± 0.04
DCO	43.25 ± 15.9	52.2 ± 17.2	31 ± 13.3	52 ± 21.5	37.6 ± 14.9	41.75 ± 13.6
Phenolic compounds ($mg \cdot l^{-1}$)	0.013 ± 0.009	0.021 ± 0.009	0.004 ± 0.001	0.05 ± 0.015	0.06 ± 0.02	0.04 ± 0.01
Total hydrocarbures ($mg \cdot l^{-1}$)	0.0005 ± 0.0003	0.004 ± 0.002	0.002 ± 0.001	0.001 ± 0.0003	0.001 ± 0.0004	0.0006 ± 0.0003

DCO, Dissolved Carbon Organic.

Table 2. Salmonids characteristics.

Name	Number of individuals	Size (cm), minimum/ maximum	Weight (g), minimum/ maximum
Salmo trutta fario	88	12.7–51.4	30–1370
Salmo trutta trutta	4	26.5–56.2	195–2250
Salvelinus fontinalis	103	14.1–48.5	20–1171
Unknown	4	16.5–45.6	60–1010

Unknown because of bad weather during sampling.

Table 3. Shape indices establish from morphometric measures.

Shape indice	Formula
Form factor	$(4\pi Ao)/Po^2$
Roundness	$(4Ao)/(\pi Lo^2)$
Circularity	Po^2/Ao
Rectangularity	$Ao/(Lo \times lo)$
Ellipticity	$(Lo–lo)/(Lo + lo)$

EFA is in fact less used than FFT (Kuhl & Giardina 1982; L'Abée-Lund 1988; Pothin et al. 2006; Gonzalez-Salas & Lenfant 2007; Mérigot et al. 2007). In this technique, an object (the otolith) can be described as a periodic function $x(t)$ and $y(t)$ formed by a sum of trigonometric series of sinus and cosines. These series are characterised by several components named harmonic or ellipse. Each harmonic is characterised by four coefficients resulting from the projection of each point of the outline on two normal axes (x) and (y). The higher the number of harmonics, the greater is the accuracy of the outline description (Kuhl & Giardina 1982). For each digital image, the software SHAPE 1.2 (free software) H. Iwata © 2001, see http://cse.naro.affrc.go.jp/iwatah/shape (Iwata & Ukai 2002) calculated the Fourier coefficients to make them independents from otolith size and orientation (and position) regarding the start point of the outline, which is arbitrarily defined. In addition, the Fourier power (FP) spectrum was calculated to determine the sufficient and necessary number of harmonics for the best reconstruction of the otolith outline (Crampton 1995). The FP of a harmonic is proportional to its amplitude and provides a measure of the amount of 'shape information' described by this harmonic.

For the nth harmonic, FP (PF_n) is given by the expression:

$$PF_n = (A_n^2 + B_n^2 + C_n^2 + D_n^2)/2$$

where A_n, B_n, C_n and D_n were the Fourier coefficients of the nth harmonic.

Then, we can calculate the cumulated power percentage (PF_c) defined by:

$$PF_c = {}_1^n PF_n$$

For such a purpose, we randomly choose subsamples of 20 otoliths, from the six sites, and calculated both the Fourier series and the FP. The increase in number otoliths until 30 does not change the FP. The threshold of 99.99% of the mean cumulated FP was chosen to define the adequate number of harmonics to be considered in the analyses. The FP indicates that 17 harmonics described 99.99% of the outline of otoliths for the two Salmonidae. However, the coefficients derived from the first harmonic were not taken into account, because the outline reconstructed with these coefficients is a simple ellipse resulting in a maximum FP. These coefficients would then mask the information derived from the other harmonics. Thus, 16 harmonics with four coefficients (64 variables) were used for discrimination.

Crystallography

To evaluate the impact of environmental stress among the Salmonids, X-ray diffraction was used to identify the polymorph composition of $CaCO_3$ in normal and aberrant otoliths. Samples were crushed and introduced in X-ray diffractometer (PW1729 Philips X-ray Generator with a goniometer 1731). The excitation was achieved with a copper lamp and a scan 2θ angle between 2° and 60° (0.02° s^{-1}).

Otoliths were observed with an environmental Scanning Electron Microscope (Philips XL, 30 ESEM) to obtain photographs of crystals.

Chemical elemental composition

The chemical elemental composition of otoliths has been used to characterise differences between morph of crystallisation, and was analysed with inductively coupled plasma mass spectrometry (Ultra mass 700 ICP-MS, Varian, Inc. Corporate Headquarters, Palo Alto, CA, USA) and ICP atomic emission spectroscopy (Vista-PRO, Varian, Inc. Corporate Headquarters, Palo Alto, CA, USA). Otoliths from the two species from six areas and a sample of vaterite and aragonite otoliths were analysed ($N = 60$). Each otolith was dissolved in 2 ml of suprapur nitric acid (65%). Solutions were mineralised 2 h at 110–120 °C and solubilised in 5 ml of suprapur nitric acid (5%); 50 μl of intern standard (indium 0.1 mg·l^{-1}) were added. A standard range was realised with a multi-element solution (ASTASOL MIX MO101, Analytika, Prague, Czech republic). The quality control was realised with three standards (bonemeal, TORT (Labster hepatopancreas) 2 and otoliths crushing).

Data analysis

The different shape indices might present redundancy between them because some are built with the same parameters, i.e., surface area, perimeter, etc. So, because of possible links between these indices, Pearson correlations were first calculated to ovoid redundancies in the use of these indices. Although a correlation exists between the coefficient of form and circularity ($r = -0.990$, $P < 0.01$) and between roundness and rectangularity ($r = 0.590$, $P < 0.01$), all shape indices were separately used in the following analyses in a Student *t*-test for paired sampling (SPSS Version 10, SPSS Inc. Headquarters, Chicago, Illnois, USA).

Reclassification of undetermined fish and determination of differences between the six geographical sites for each fish species was evaluated by canonic discriminant analysis (CDA). Each of 16 harmonics was defined by four coefficients. Therefore one otolith was described by 64 variables. The CDA was performed on these variables to investigate the validity of predefined groups. The performance of the discriminant analyses was assessed with the Wilk's λ. This statistic is the ratio between the intragroups variance and the total variance, and provides an objective means of calculating the chance-corrected percentage of agreement between real and predicted groups membership. The values of Wilk's λ range from zero to one, the closer the Wilk's λ is to zero, the better is the discriminating power of the CDA. To validate the performance of the CDA, a Cohen's kappa statistic test was used. This statistic analysis provides an objective means of calculating the chance-corrected percentage of agreement between actual and predicted group membership; kappa values range from zero to one, with zero indicating the CDA yields no improvement over chance, and one indicating perfect agreement (Titus et al. 1984).

A principal components analysis was performed for the two species to analyse the relationships between abiotic conditions in sampling sites and vaterite presence in otoliths (SPAD 4, Coheris. SPAD, Courbevoie, France).

Different ratios of trace elements Sr/Ca, Mn/Ca, Mg/Ca and Ba/Ca were calculated for sampling areas and for vaterite and aragonite otoliths. Differences in ratios were analysed by Mann–Whitney and Kruskal–Wallis test (SPSS).

Results

Population differentiation

Shape indice mean were not significantly different between right and left otoliths for the two Salmonids species (*Salmo trutta fario* and *S. fontinalis*) (Table 4). No dimorphism between otoliths was observed. Right and left otoliths were then used indifferently for discriminant analyses. In the first step, discriminant analysis was realised to classify all Salmonids in their respective species group. The discrimination between the two species has a Wilk λ of 0.18 ($P < 0.05$) and Cohen-kappa test revealed that 89% of fish were correctly classified. This analysis has allowed the determination of 12 misdetermined individuals.

Similar analyses have been realised with otoliths from fishes of the six rivers to search inter-sites differences. Classification groups were Studer, Château, Ferme, Sud and Base (Décharge and Sablière). The first discriminant function (Fig. 2a) was significant for *Salvelnus fontinalis* with a Wilk λ to 0.062 ($P = 0.002$). For *Salmo trutta fario* (Fig. 2b) the first two discriminant functions were significant Wilk λ [0.002 ($P = 4 \times 10^{-6}$) and 0.034 ($P = 0.015$)]. Individuals were correctly classified respectively at 97% and 100%. For the two species, this analysis has revealed differences between groups and so between sampling sites.

In fact, *S. fontinalis* samples were subdivided into three groups. The first discriminant axis separated individuals from Sud to individuals from Studer and Base aquatic systems. *Salmo trutta fario* samples were grouped in the four sites: Château, Ferme, Studer rivers and Base. First discriminant function distinguished Studer River from areas near the scientific Base of Port-aux-Français namely Château, Ferme Rivers and Base. The last two areas were only

Table 4. Comparison by *t*-test for shape indice means between right and left otoliths of the two species.

			Mean ± standard error				
Shape indice	Species	*N*	Right	Left	*t*	d.f.	*P*-value
Form factor	*Salvelinus fontinalis*	46	0.4255 ± 0.031	0.4277 ± 0.036	−0.525	45	0.602
	Salmo trutta fario	63	0.4202 ± 0.048	0.4191 ± 0.047	0.199	62	0.843
Roundness	*Salvelinus fontinalis*	46	0.4786 ± 0.046	0.4746 ± 0.049	0.770	45	0.445
	Salmo trutta fario	63	0.5479 ± 0.067	0.55 ± 0.064	−0.416	62	0.679
Circularity	*Salvelinus fontinalis*	46	29.6994 ± 2.3	29.5939 ± 2.6	0.322	45	0.749
	Salmo trutta fario	63	30.3219 ± 3.8	30.3873 ± 3.6	−0.141	62	0.888
Rectangularity	*Salvelinus fontinalis*	46	0.6491 ± 0.21	0.6443 ± 0.24	1.353	45	0.183
	Salmo trutta fario	63	0.6612 ± 0.03	0.66 ± 0.028	0.359	62	0.721
Ellipticity	*Salvelinus fontinalis*	46	0.2679 ± 0.36	0.2686 ± 0.39	−0.175	45	0.862
	Salmo trutta fario	63	0.2141 ± 0.051	0.2112 ± 0.046	0.852	62	0.397
Shape	*Salvelinus fontinalis*	46	1.4897 ± 0.12	1.4817 ± 0.14	0.486	45	0.629
	Salmo trutta fario	63	1.5208 ± 0.18	1.5209 ± 0.18	0.006	62	0.995

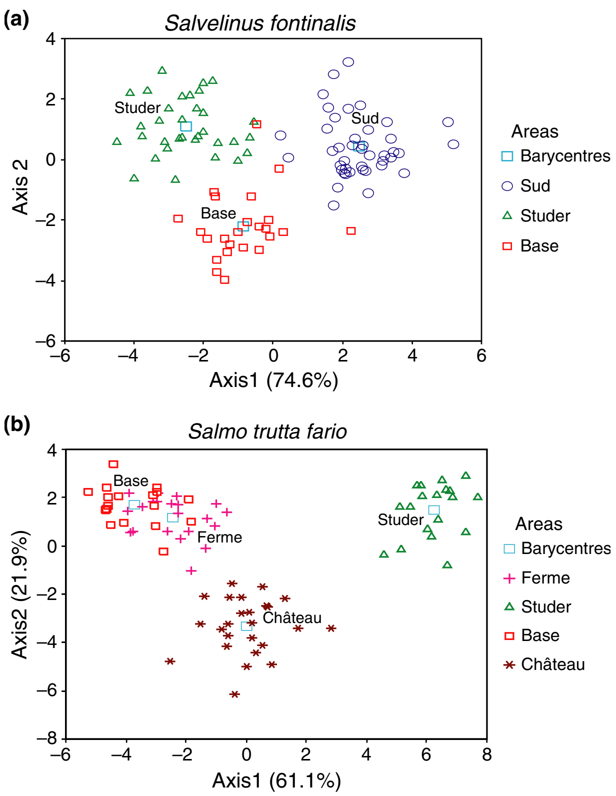

Fig. 2. Canonical discriminant analysis achieved with elliptic Fourier coefficients for *Salvelinus fontinalis* (a) and *Salmo trutta fario* (b), discrimination in function of sampling areas.

discriminated from Château River by second discriminant function. Individuals of this species were also separated in three groups: Studer, Château Rivers and a group represented by Ferme River and Base. *Salmo trutta fario* populations are distributed along a gradient going from rivers far the Base to rivers near the Base. These two analyses showed that morphometry and particularly Fourier analysis could be used for population differentiation.

Crystallography

Environmental stress could affect the crystallisation morph of calcium carbonate of otoliths. Preliminary observations revealed the presence of transparent and opaque otoliths (Fig. 3a and e). To test, whether these differences are the result of a switch in crystallisation morphs (aragonite, vaterite or calcite) caused by an environmental stress, the morph of crystallisation have characterised by X-ray diffraction. It revealed that the 'aberrant' transparent otoliths are composed of vaterite (Fig. 3h) and the 'normal' opaque otolith (Fig. 3d) of aragonite. The two morphs present variations in their diffraction peaks because of variations in inter-atomic distance (Fig. 3). Moreover, their structures are different: aragonite crystallises in needle whereas vaterite in smooth balls. Aragonite crystals are orthorhombic (Fig. 3c) and vaterite hexagonal (Fig. 3g). The proportion of vaterite otoliths varied with species: 46% for *S. fontinalis* and 18% for *Salmo trutta fario*.

Chemical elemental composition

The concentration of Ca, Sr, Mg, Mn, Ba, Cu, Zn and Al were measured using MS (ICP-MS) and then compared between vaterite and aragonite otoliths. Ratio to calcium were computed for strontium, manganese, magnesium and barium, for both vaterite and aragonite otoliths. Differences in concentration were observed for Sr/Ca ($U = 101$, $P < 0.05$), Mn/Ca ($U = 37$, $P < 0.05$) and Mg/Ca ratios ($U = 12$, $P < 0.05$) but no for Ba/Ca ratio (Fig 5a–d). Moreover, heavy metal concentration e.g., zinc and aluminium were different between the two crystallisation morphs (Fig. 5e, $U = 20$; Fig. 5f, $U = 12$, $P < 0.05$) but not for Cu concentration (Fig. 5g). Elemental concentration comparisons between crystallisation morphs showed an increase of Mg, Mn, Zn and Al and a decrease of Sr in vaterite otoliths while Ba and Cu concentrations were the same for the two morphs. Moreover, analyses of these elements for all sampling rivers also showed concentration variations. But, no site effect was directly observed.

Relation between otoliths and abiotic conditions

To test whether the two crystallisation morphs characterised on otoliths are in relation with environmental stress, a principal component analysis has been realised. It revealed relationships between some abiotic factors (Table 1) of the aquatic systems and vaterite otoliths (Fig. 4). This table presents mean of each abiotic factor measured during the 6 months of sampling. However, some of them can have many variations during the day as temperature which can vary to 10 °C. For *S. fontinalis* two groups of rivers are separated on the four axes (respectively 27.76%, 17.1%, 11.25% and 8.8%): Studer, Sud and Château in one hand, Décharge and Sabliére on the other hand. These two last rivers are characterised by a high pH, their conductivity and are linked to the presence of phenolic compounds and vaterite otoliths. For *Salmo trutta fario* the opposition between Décharge, Sabliére, Ferme and Château, Studer and Sud was only present on the first axis (34.68% of variability). A relation between phenols, hydrocarbons and vateritic otoliths is present on three axes (respectively 15.17%, 9.22% and 8.54% of variability). The rivers near the scientific Base are characterised by waters with high conductivity and presence of phenols and fish with vaterite otoliths (Fig. 4).

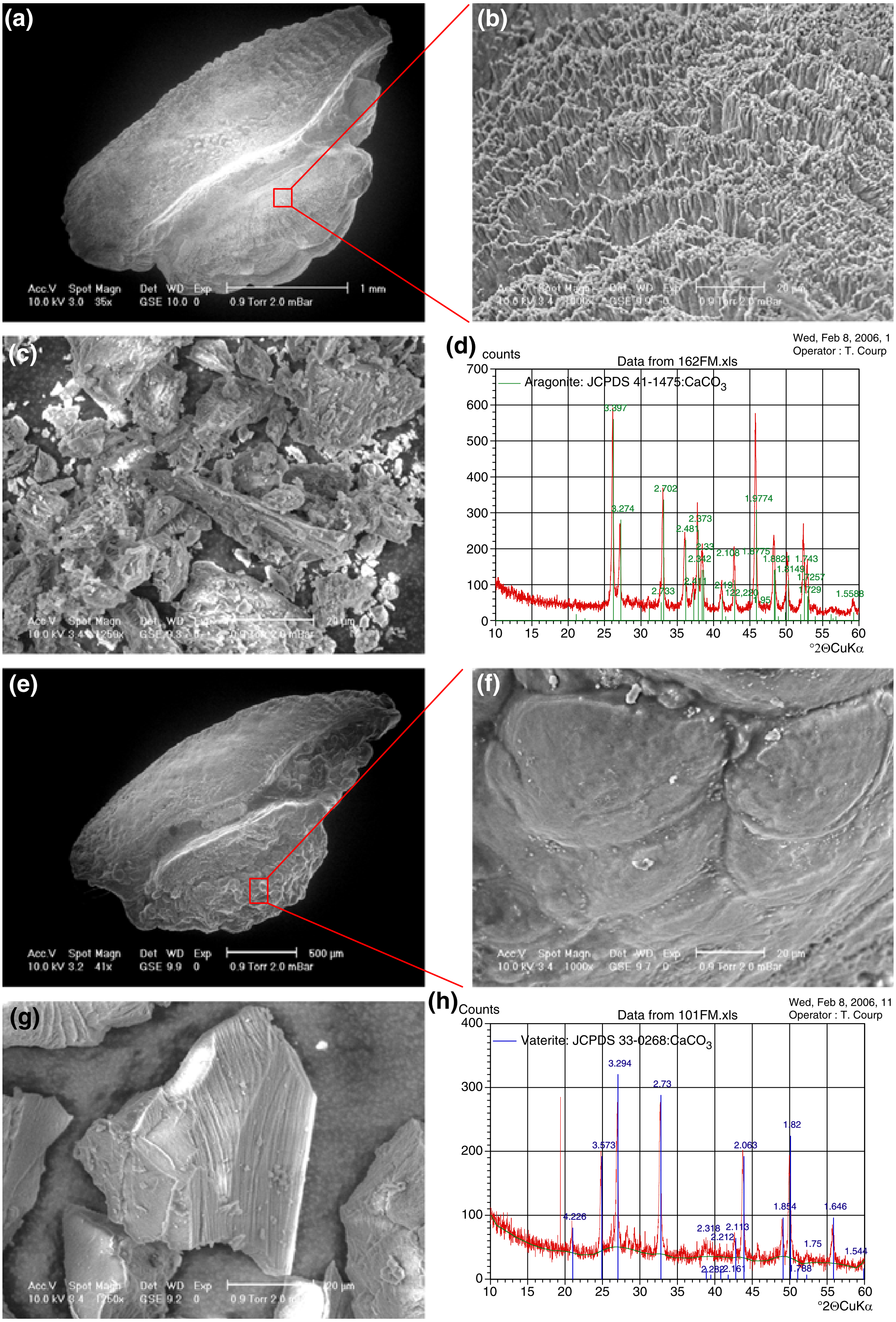

Fig. 3. Aragonite (35×) (a) and vaterite (41×) (b) *sagittae* photograph (environmental SEM); details of surface structure: aragonite (b) and vaterite (f) (1000×); aragonite (c) and vaterite (g) crystals (1250×). X-ray diffractogram of aragonite (d) and vaterite (h).

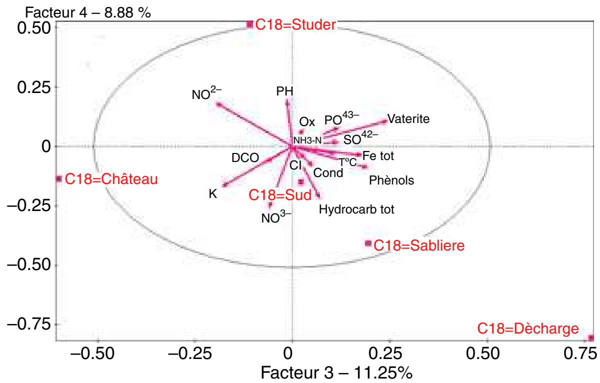

Fig. 4. Principal component analysis achieved with all abiotic factors and vaterite. Areas were just illustrative variable and were not used in analysis.

Discussion

Can otolith shape be used as a tool for differentiating among salmonids species and salmonids 'populations'?

The salmonids sampled in Kerguelen were well differentiated in term of species. Otolith shapes have been used in many biological studies. Juveniles of two salmonids species (*S. salar* and *S. trutta*) have been distinguished by otoliths shape descriptors (L'Abée-Lund 1988). In our study, otolith shape analyses were used for the discrimination of the species of salmonids (*Salmo trutta fario* and *S. fontinalis*) and have allowed the determination of species for misdetermined individuals (these fish were not determined in the field because of bad conditions during the sampling).

The results of our study indicate that ecotypes and river populations can be reasonably well differentiated on the basis of otolith shape. Recent studies have used Fourier harmonics for the description of otoliths shape. This technique associated with statistical analysis allowed a discrimination between fishes of the same species living in various geographic areas (Pothin et al. 2006; Mérigot et al. 2007). Otoliths from one river were well differentiated from other river (97% in their river of origin for *S. fontinalis* and 100% for *Salmo trutta fario).* These high replacement levels were better than levels observed for *S. salar* (87%) by Friendland & Reddin (1994). This specificity for their river can be the result of homing that characterises salmonids. Migrant individuals were able to return to their native river. The number of mistake would be

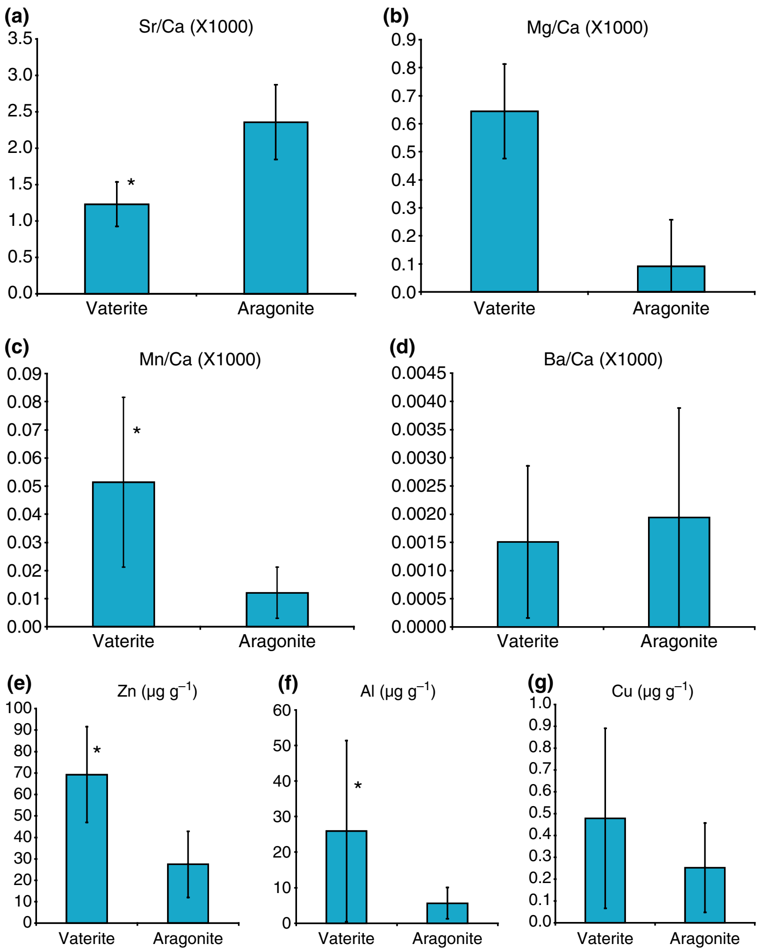

Fig. 5. Sr, Mg, Mn, Ba versus Ca ratio (a–d) in function of crystallisation (vaterite, aragonite). Composition in Zn, Al and Cu (e–g) in function of crystallisation. *Significant difference ($P = 0.05\%$), Mann–Whitney test.

low and the result of a broad geographic region with close estuaries (Beall & Davaine 1981). This high intraspecific similarity observed for individuals from a same river could be a result of this homing. The similarity for sites near Port-aux-Français (Base and Ferme) might be the result of mistake or to similar environmental conditions in the various rivers.

Moreover, this analysis has confirmed the presence of two ecotypes for each species: one 'lake ecotype' (Studer) and one 'river ecotype' (Château and Sud). These two ecotypes have been described earlier in literature and linked to a behavioural variability and to an evolution of genetic variability from a same 'French' genetic pool (Bruslé & Quignard 2001). A 'third ecotype' appeared near Port-aux-Français. This ecotype, never described in literature corresponds to an adaptative population to this particular ecosystem composed of ponds and connecting short rivers near the Base.

Can otolith shape be used as a tool for evaluating stress among the Salmonids?

Fluctuating asymmetry is assumed to reflect the developmental instability caused by environmental or genetic stress (Sahyoun et al. 2007). Fish otoliths represent a good tool to evaluate the consequence of different effects on asymmetry. In our study, the differences between right and left otoliths were estimated by image processing. Asymmetry between right and left otoliths was not significant. Nonasymmetric otoliths were found in individuals of the two Salmonids species revealing that potential stress observed in Salmonids living near Port-aux-Français was not so strong as to create an asymmetry in otoliths. The shape of the otoliths was unaltered by the presence of vaterite.

Can otolith crystallisation be used as a tool for evaluating stress among the Salmonids?

X-ray diffraction and SEM analysis revealed that crystalline otoliths were formed by a particular crystallisation morph of $CaCO_3$: the vaterite. This morph was described in earlier studies (Gauldie 1993; Tomas & Geffen 2003), but was considered as unusual. Vaterite was an ancestral form found in otoliths of primitive fishes as sturgeon, lamprey and hagfish (Carlström 1963) whereas the normal morph was aragonite. Vaterite otoliths have been observed when fishes were stressed in particular in fish farming. However, this morph was found in wild population of *Salvelinus namaycush* in North American Great Lakes (Bowen et al. 1999). Salmonids seemed to present more vaterite otoliths than other fish family (Gauldie 1986). In our study 45% of otoliths from *S. fontinalis* and 18% from *Salmo trutta fario* were composed of vaterite. These rates were higher than those observed in other Salmonids species: 22% for *S. namaycush* and 26% for *Oncorhynchus tshawytscha* (Bowen et al. 1999). Those differences might be explained by protein matrix modifications, and then by mutations and switch of amino acids (Gauldie 1993). *In vitro* experiment have shown that vaterite crystallisation could be induced by the presence of acidic amino acids: glycine and alanine ($6.5 < pH < 7.5$) (Shivkurama et al. 2006). Most external factor including stress, food quality, viral infections and/or temperature might induce an expression of mutated genes and the switch from aragonite to vaterite.

Water temperature was a primordial factor that could induce the shift between aragonite and vaterite. A small variation of water temperature (8.30–8.85 °C) could decrease significatively the rate of aragonite in otoliths of *O. tshawytscha* (Gauldie 1986).

The high rate of aberrant otoliths in Salmonids populations in Kerguelen Island could confirm stress conditions because of environmental factors especially temperature fluctuations. Water temperature in the Kerguelen Rivers presents a large range of variations up to 10 °C during a day and between seasons (Delarue & Massé 1981). These changes could induce a thermal stress and a switch of crystallisation in the otoliths. Another important factor that could induce a change of crystallisation is linked to food accessibility. In Kerguelen Salmonids (juveniles and adults) have to feed on small invertebrates. This type of prey is more adapted to juveniles than to adults that are generally fish eaters (Beall & Davaine 1981) because no native freshwater ichthyofauna is found in Kerguelen Rivers. Moreover, the low density and the low diversity of preys induces a high level of intraspecific competition (Wojtenka & Steenberghe 1981). *S. fontinalis* and *S. trutta* are, moreover, sympatric species in Kerguelen Rivers (Bruslé & Quignard 2001).

In the present study vaterite and aragonite otoliths show differences in elemental concentrations. Indeed, in vaterite Sr decreased and Mn, Mg Zn and Al increased. Variations in Sr, Na and K have been observed in other species such as *Stenodus leucichthys* (Brown & Severin 1999). For Tomas & Geffen (2003) crystalline geometry was so different between vaterite and aragonite that this privileged incorporation of chemical elements with small ionic radius as Zn, Mg, and Mn (0.6–0.9 Å: Curti 1999). Moreover, the low Sr rate in vaterite otoliths might be the result of temperature variations in the rivers. Indeed for *O. tshawytscha* decrease in Sr has been correlated to temperature variations (Gauldie 1996). Mn presents a high solubility for acidic pH. An increase of this concentration could be the reflection of variation of the pH of the endolymph (Tomas & Geffen 2003). However, it is difficult to dissociate the two parameters: water temperature in the rivers and endolymph pH. Indeed abiotic factors (temperature, salinity, pH and food quality) interact with endogenous factors (stress, reproduction, pathology…) across barriers (gills, intestine) and interact indirectly with hormonal systems that control the endolymphatic fluid composition (across saccular epithelium).

The principal component analysis has revealed that vaterite otoliths were more frequent in the aquatic system near the Base and linked on the one hand to particular physico-chemical characteristics. Water conductivity in aquatic system near the Base (Sablière, Décharge and Ferme) was higher than in the other sites because of proximity with the sea side, which lead to an enrichment in chlorine ions of the waters by wind and rain. On the other hand, these salmonid populations are submitted to high fishing pressure and to anthropogenic pollutants, because of the human presence at the Base.

In conclusion, the results from this otolith study provide support for the view that environment quality represents an important factor for the 'quality' of the Kerguelen Salmonids. Otoliths shape presents some disturbances. Moreover, vaterite in high proportion was observed. Many factors can produce this abnormality, such as temperature fluctuations, dietary deficiencies and contaminations.

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