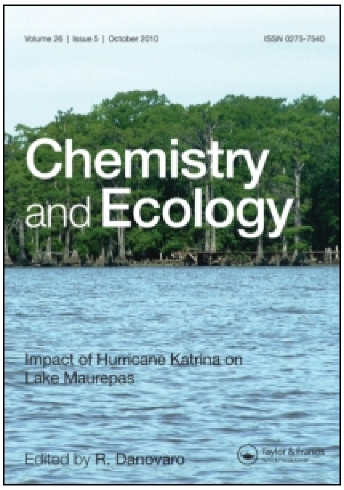

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TRACE METAL VOLTAMMETRIC DETERMINATION OF OTOLITHS OF TENCH *TINCA tinca* (L.): ITS POSSIBLE USES IN MONITORING THE QUALITY OF FRESH WATERS

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New data of heavy metals in otoliths of fresh water teleosts are reported. Differential pulse anodic stripping voltammetry (DPASV) has been used for determining copper, lead, cadmium and zinc in otoliths of tench, *Tinca tinca* (L.). The analytical procedure followed gives data of good repeatability and with low detection limits, in a simple and inexpensive way. Tench of age ranging from 3 to 8 years were examined. Four year old fish had maximum concentrations of heavy metals, and an otolith weight adequate for experimental determinations in bulked samples. Fresh water teleosts are less mobile than marine ones, and could be used for monitoring the quality of waters suspected for pollution, and eventually verifying the influence of the environmental variations on the metal bioaccumulation.

Keywords: Trace metals; otoliths; fresh water teleosts; tench *Tinca tinca* (L.); fresh water quality; DPASV

INTRODUCTION

Grady *et al.* (1989) proposed the metal contents in otoliths as an indicator parameter for age and origin of fish; some heavy metals are

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present only occasionally and can be considered as anthropogenic pollutants.

The otoliths of teleosts perform a statolithic function in the inner ear. They have a complex polycrystalline structure (Carlstrom, 1963). Degens *et al.* (1969) found they mainly constitute calcium carbonate, as aragonite, within an organic proteic matrix. Assuming that the laying down of alternating proteic and aragonitic zones goes on at a constant rate as the fish is growing, it is possible to evaluate the age of the same animal. Beside this function of age-indicators, the otoliths can act also as environmental indicators. Analyses of structure and ionic composition allow evaluation of the temperature range and the degree of pollution of the aquatic environment where the fish has lived (Gauldie *et al.*, 1980; Radtke and Morales-Nin, 1989).

Fish tend to accumulate heavy metals, as do other aquatic organisms. It seems therefore interesting to attempt a monitoring of the heavy metal pollution of waters by analysing the metal contents in the otoliths, as for instance Macpherson and Manriquez (1977), Protasowicki and Kosior (1988) and Papadopoulou *et al.* (1980) have suggested. This is possible since the otoliths are growing quite regularly, enclosed in the labyrinth, during all the fish life (Jones, 1985; Ralston and Williams, 1988). Studies have up to now only considered marine fish. The mobility of the fish in the marine environment, particularly migratory species, hinders verification of the influence of environmental variations on the metal bioaccumulation, and hence evaluation of the quality of sea water (Edmonds *et al.*, 1991; Campana *et al.*, 1995). Fresh water fish, on the contrary, tend to be less mobile. This led us to study the possibility of monitoring the quality of inland waters, monitoring otolith metal contents, in particular in fish inhabiting channels receiving waste from industrial areas and agricultural activities, where heavy metal pollution is suspected.

The very low content of trace metals in otoliths needs a repeatable analytical procedure, with low detection limits: Papadopoulou *et al.* (1980) used neutron activation analysis (NAA), Protasowicki and Kosior (1988) used atomic absorption spectrometry (AAS), Campana *et al.* (1995) used isotope dilution-inductively coupled plasma mass spectroscopy (ID-ICP-MS), Arai *et al.* (1995) used particle induced X-ray emission (PIXE). In the present paper, we set out to assess the suitability of a voltammetric method, *i.e.*, differential pulse anodic stripping voltammetry (DPASV), for determining copper, lead, cadmium and zinc as metals of environmental relevance in otoliths of sampled fish. The DPASV method allows the determination of metals at ng g^{-1} concentration levels, providing satisfactory analytical results without requiring particularly expensive instrumentation. We here attempt to verify if the DPASV method is a good choice for determining trace metal contents in otolith matrices in tench.

In this preliminary study we chose the tench, *Tinca tinca* (L., 1758) (Osteichthyes, Cyprinidae), typical of still and slow-flowing waters, and resistant to sudden changes of temperature and to low dissolved oxygen. It is native in continental Italy, and has medium-large size (maximum length 700 mm, weight up to 8 kg). Gandolfi *et al.* (1991) reported that it spawns in May–June, and is sexually dimorphic. This fish could be considered as a target organism, because it is tolerant of a wide range of environmental conditions.

EXPERIMENTAL

Sample Collection and Treatment

We collected tench of different size in a channel (Roggia Storta) near Torviscosa (Udine, Northern Italy), flowing in an area with many industrial sites and agricultural activities (see Fig. 1); weight and total body length were recorded for each individual. Two otolith types, '*asterisci*' and '*lapilli*', were extracted from fish by manual extraction with steel forceps, then rinsed by MilliQ water. The '*asterisci*' otoliths were used for the age determination, examining the otolith layers by an optical microscope: the age values were validated comparing the results with those obtained by scale examination. The '*lapilli*' otoliths were used as samples for trace metal analysis taking one otolith for each animal: they were oven dried at 70°C for 48 hours, then individually weights and stored in polyethylene vials.

Trace Metal Analysis

The DPASV analysis was performed on bulked samples (about 20–40 mg) of otolith, obtained grouping otoliths of same age fish. Bulked otolith samples were immersed in 0.5 ml of 68% nitric acid superpure

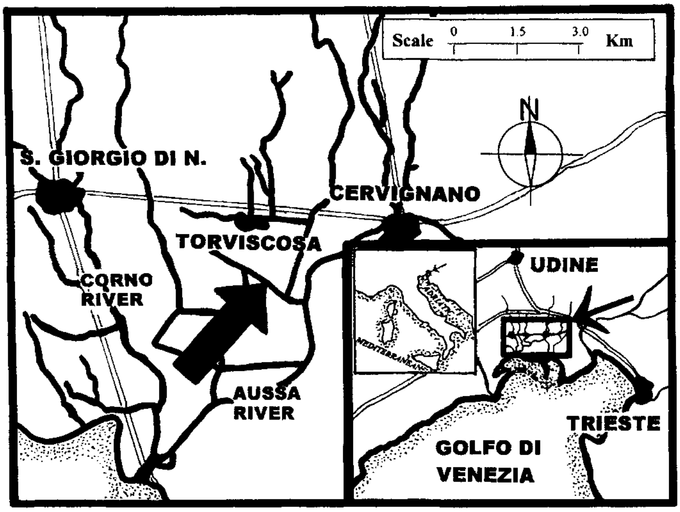

FIGURE 1 Map of the industrial area near Udine (Northern Italy); the arrow indicates the channel (Roggia Storta) where the fresh water teleosts were collected.

(Carlo Erba) directly in the voltammetric cell, and then heated at 80°C to near dryness. This treatment was repeated twice, and the dry residue was finally redissolved and made up to 10 ml with MilliQ water. The voltammetric measurements were made by a polarographic analyser AMEL Model 433 equipped with a three-electrode cell: the working electrode was a hanging mercury drop electrode (HMDE), the counter electrode was a platinum wire, and the reference electrode was silver–silver chloride. All glassware were pretreated with nitric acid 1:4 v/v and then rinsed with MilliQ water. Pre-electrolysis times ranged between 3 and 5 min, dependently on the metal concentration levels. The concentrations were evaluated by means of standard additions.

RESULTS

Fish were partitioned in six age groups of 10–15 individuals ranging from 3 to 8 years. Average fish length (mm), fish weight (g), and otolith weight (mg) for each of the six groups were calculated (see Tab. I).

Otolith weight increases with the age, and therefore with both length and weight of fresh water fish: the experimental trends of these biological parameters are plotted in Figure 2.

TABLE I Average values and standard deviations (SD) of fish length (mm), fish weight (g) and otolith weight (mg) for each of the six age groups

Fish age (*years*)	*No. of fish*	*Fish length* (*mm*)		*Fish weight* (*g*)		*Otolith weight* (*mg*)	
		average	*SD*	*average*	*SD*	*average*	*SD*
3	15	99	6	11	2	1.3	0.6
4	15	123	12	22	7	2.0	0.5
5	15	193	21	108	34	3.3	0.6
6	12	296	17	440	111	7.5	1.2
7	12	349	28	723	186	9.4	0.9
8	10	433	35	1597	375	16.4	2.1

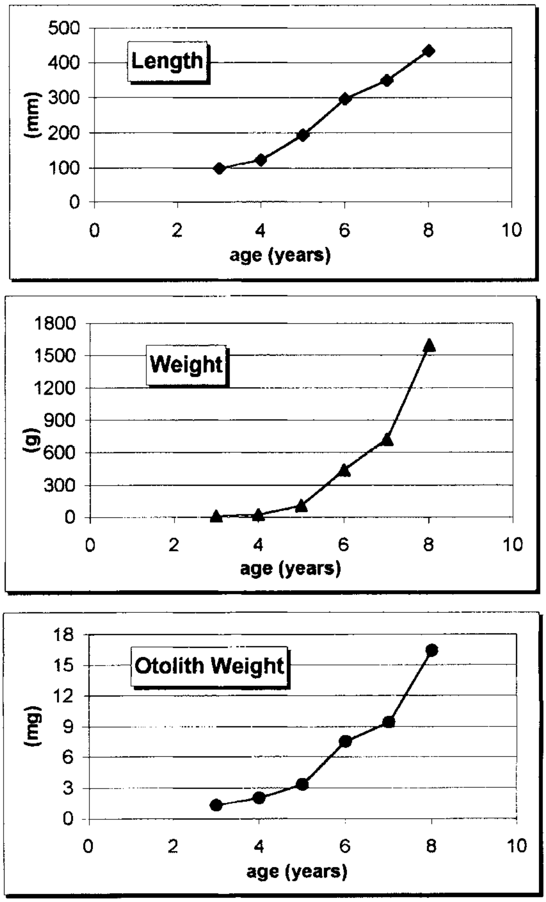

FIGURE 2 Average length, weight and otolith weight of *Tinca tinca* (L.) against fish age.

Overall average metal concentrations (μg g^{-1} otolith dry weight) were determined by DPASV for each age group; on this purpose we used bulked samples (20 – 40 mg), collecting otoliths of same group (see Tab. II).

Typical DPASV voltammograms obtained from an otolith bulked sample are shown in Figure 3 with operational conditions: the four peaks correspond to copper, lead, cadmium and zinc. The calibration curve for determining the zinc concentration by means of three standard additions is also shown. All calibration curves had correlation coefficients higher than 0.999, showing a satisfactory linearity.

The repeatability of our procedure, calculated on 5 replicates, ranged from 10 to 20% (as RSD%, Relative Standard Deviation expressed as percentage) in bulked samples obtained from 7 and 8 year old age groups (see Tab. III). Limits of detection (LOD), ranging from 0.03 to 0.08 μg g^{-1}, calculated following the IUPAC rules (Long and Winefordner, 1983) and evaluated for 30 mg of otolith bulked samples, are also reported in Table III.

No certified reference material (CRM) was available for evaluating the accuracy of the analytical procedure on these particular biological samples.

The obtained results show that the heavy metal concentrations in otoliths have a tendency to decrease as the fish age increases. Both copper and cadmium display a concentration maximum in fish aged 5 years; these data constitute statistical outliers when compared to the measurements for the other samples (as can be seen by means of boxplots, not reported here). It is relevant to note that in all the other samples cadmium was below the limit of detection (see Tab. II), and this prevents the possibility of making further considerations on this parameter, based on numerical values. Lead and zinc show maxima in 4 year old fish, then their concentrations in otoliths decrease regularly

TABLE II Overall average metal concentrations (μg g^{-1} otolith dry weight), as determined in otoliths of each age group

Fish age (yrs)	*Metal conc. (μg g^{-1} otolith dry wt.)*			
	Cu	*Pb*	*Cd*	*Zn*
3	0.48	1.10	< 0.03	9.44
4	0.35	1.44	< 0.03	10.9
5	2.25	0.63	0.16	6.20
6	0.07	0.13	< 0.03	1.48
7	0.07	0.16	< 0.03	1.74
8	0.11	0.13	< 0.03	1.79

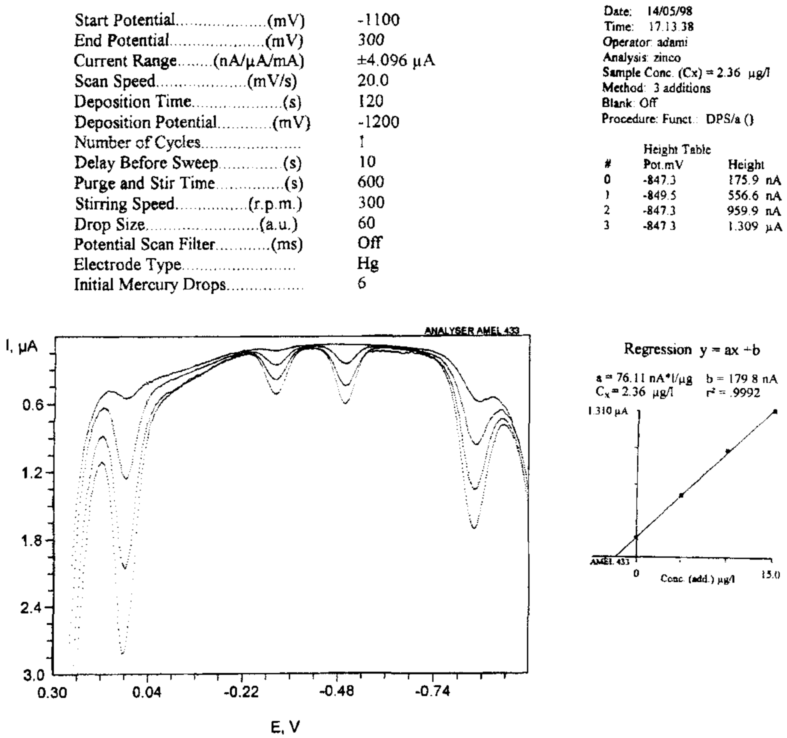

FIGURE 3 DPASV voltammograms obtained from an otolith bulked sample; operational conditions are reported; the four peaks correspond to copper, lead, cadmium and zinc (in that order). A calibration curve based on three standard additions (5, 10 and 15 μg l^{-1}) is plotted for zinc.

TABLE III Limits of detection (LOD) and repeatability (RSD%) for the considered metals; LODs are calculated for an otolith amount of 30 mg; RSD% was calculated by means of 5 replicates on bulked samples of 7 and 8 year old age groups

	Cu	*Pb*	*Cd*	*Zn*
LOD (μg g^{-1} otolith dry wt.)	0.05	0.04	0.03	0.08
RSD% (7 years)	15	16	*	11
RSD% (8 years)	13	20	*	13

* Not evaluated.

towards very low, practically constant values (see Fig. 4a); similar decreasing trend occurs for copper. It is possible to calculate the total metal contents in each otolith, multiplying the overall average metal

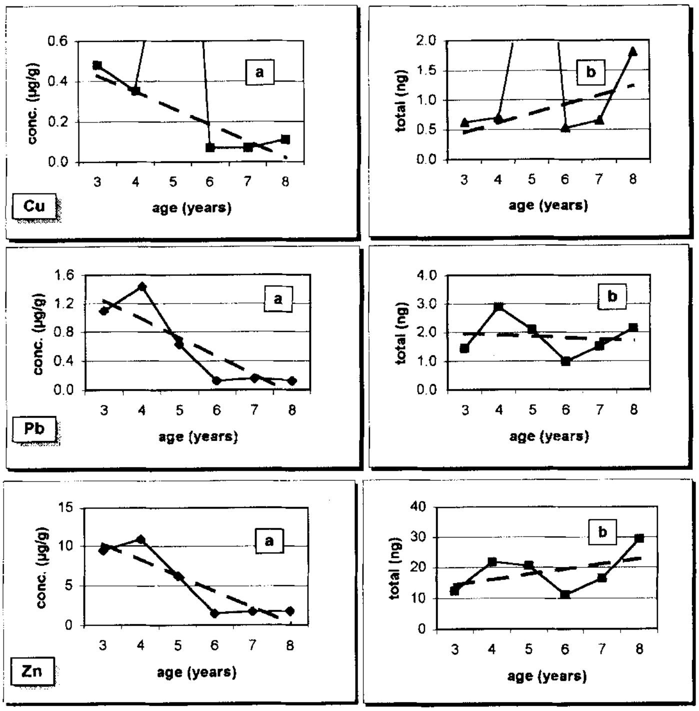

FIGURE 4 (a) Copper, lead and zinc overall average concentrations (µg g^{-1} dry wt.) in otolith against fish age (years); (b) total metal content (ng) in a single otolith against fish age. Dashed lines represent the Ordinary Least Square interpolation of values (for copper, the sample obtained from 5 year old fish was excluded from these computations).

concentration for average weight of the same age group otoliths. Plotting the total metal contents (ng) against the fish age (Fig. 4b) shows that the absolute content values do not decrease, but are quite constant or slightly increasing, depending on the specific metal. The decrease of concentrations with age is therefore probably not due to a reduction of bioaccumulation, but rather to the decrease in the weight ratio metal:aragonite-organic matrix; and it is well known that the otolith weight increases sharply with age (see Fig. 2).

A comparison of our results with other data was made for zinc, as the heavy metal more present in current literature relative to marine teleosts. In particular, we have considered the results obtained from *Scomberomorus cavalla* by Grady *et al.* (1989) and *Scomber japonicus colias* by Papadopoulou *et al.* (1976, 1980), plotted in Figure 5, the

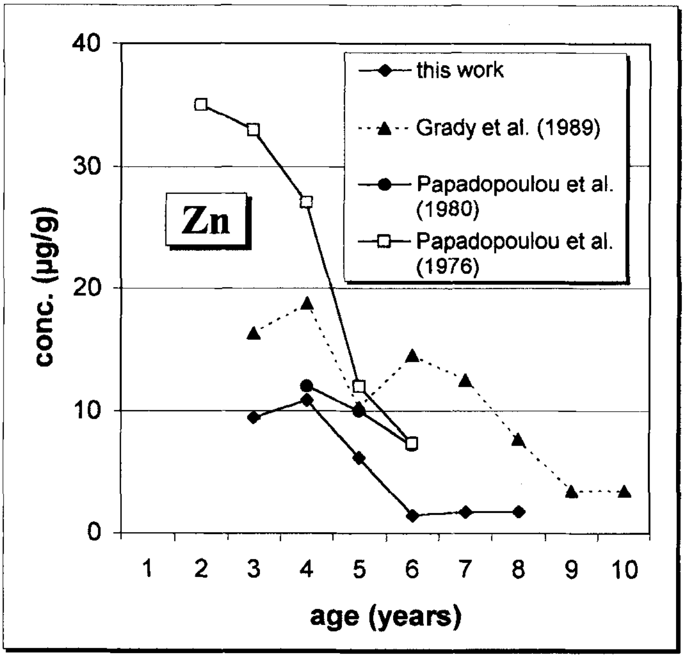

FIGURE 5 Comparison of zinc concentrations (µg g^{-1} dry wt.) in otoliths against fish age, as obtained from *Scomberomorus cavalla* by Grady *et al.* (1989) and *Scomber japonicus colias* by Papadopoulou *et al.* (1980, 1976).

zinc concentrations (µg g^{-1}) in otoliths against fish age. In all cases the concentrations have very similar trends, decreasing towards the minimum values found in older fish (8 – 10 years). We can also observe that, at least for zinc, the otolith metal concentration is lower, and reaches its minimum earlier in the tench considered in the present paper compared with the marine teleosts.

CONCLUSIONS

This work presents data of heavy metals in otoliths of fresh water teleosts, which were lacking in current literature. The analytical procedure here used (DPASV) gives data of good repeatability, and has low LODs. This voltammetric technique is far less expensive than analytical methods used for similar studies, *e.g.*, NAA, ID-ICP-MS, PIXE or AAS. Four year old teleosts had maximum concentrations of zinc and lead in their otolith, and had otolith weights suitable for experimental determinations: this group of fish seem to have useful characteristics as organisms to be used as 'bioindicators' of metal pollution. The DPASV technique could be a valid procedure for monitoring metals in otoliths taken from fresh water fish sampled under different environmental conditions, *i.e.*, polluted *versus* unpolluted channels, considering also different teleosts, such as carp, *Cyprinus carpio* (L.), or pike, *Esox lucius* (L.).

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