

The inclusion of sub-detection limit LA-ICPMS data, in the analysis of otolith microchemistry, by use of a palindrome sequence analysis (PaSA)

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Abstract

Fish otoliths are a reliable source of historical data regarding individual fish. Optical and chemical data obtained from otoliths are used to reveal the fish's age, natal areas, dispersal trajectories, and more. However, whereas optical methods are well established and widely practiced, methods for obtaining and interpreting chemical data continue to evolve. Despite rapid advances in the past decade, analytical approaches to otolith microchemistry continue to develop as new opportunities and limitations emerge. Otolith chemistry often reflects environmental conditions such as the chemical composition, temperature, and salinity of the ambient water. However, it is not always clear how these interact to produce the chemical signature observed in the otolith. Such gaps and inconsistencies in our knowledge may result partly from valuable information being discarded when measured concentrations fall below customarily defined detection limits (DL). Below we review some of the inaccuracies that may arise when analyzing chemical data obtained from laser-ablation inductively coupled mass spectrometry (LA-ICPMS). We argue that measurements traditionally defined as sub-DL can contain valuable information that would otherwise be discarded. We base our argument on the analysis of sub-DL signals obtained from both sides of otoliths core. Specifically, we show that these signals often form sequences that are symmetrical about the core (palindrome), and that the probability of obtaining even the simplest palindromic sequence by chance falls well below the customary 5% significance level. We conclude by discussing how this microchemical data can be analyzed in a manner that is insensitive to errors in concentration readings.

"Fish otoliths can be thought of as the fish-equivalent of an airplane's black box. They are continuously logging data about the growth and health of the fish and about the water it swims

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in" (Thorrold 2004). Presently, no other biological structure is more important to fishery scientists than otoliths because of the various types of information they contain (Begg et al. 2005). Microscopic daily increments in the otoliths are routinely and extensively used to infer pelagic duration, larval size-at-age, and pre- and post-settlement growth rates (Thorrold and Hare 2002). Moreover, the chemical composition of otoliths is increasingly used to identify natal grounds, migratory routes, and larval dispersal trajectories (Swearer et al. 1999; Arai et al. 2003a; Rooker et al. 2003; Arai and Morita 2005; Patterson et al. 2005; Sandin et al. 2005). The latter use of otoliths has generated considerable excitement and effort, since it potentially opens a window on the mysteries of larval dispersal. As with many new, potentially breakthrough technologies, however, a growing number of challenges have emerged as analyses have become more detailed. Here, we focus on two of these technical issues that currently limit the inferences that may be drawn about larval origins and disper-

sal pathways from micro-chemical analyses of fish otoliths. We then demonstrate how these issues may be circumvented, using data obtained from reef fish collected in the Gulf of Eilat.

Fish otoliths are composed of calcium carbonate, mostly in the form of aragonite. During deposition, a small proportion of the Ca^{++} ions are replaced by other metals. Physiological processes that regulate the composition and osmotic pressure of body fluids cause the concentrations of several common salts (e.g., Na, K, Mg, and Cl) to be much lower than their concentrations in the water. As a result, their concentrations in otoliths will not reflect those in seawater. On the other hand, the concentration of trace elements, whose uptake is more likely to be unregulated (e.g., Sr, Zn, Cu, Pb, Mn, Ba, and Fe) will be expressed in the otolith's chemistry (Campana 1999). More than 30 different elements have been detected in fish otoliths (Campana 1999). Since otoliths are physiologically inert, it is widely accepted that their chemical composition remains unchanged over time, thus providing a permanent record of the historical environmental conditions experienced by the fish (Mugiya et al. 1991; Dove and Kingsford 1998; Campana and Thorrold 2001; Fowler et al. 2005).

Many papers published over the last decade describe studies of otolith microchemistry (Begg et al. 2005; Campana 2000). One of the most intriguing uses of these records is to characterize fish movement. Data from the central core of otoliths have been used to distinguish between the natal areas of fish stocks (Edmonds et al. 1991; Campana et al. 1994; Thorrold et al. 1998a; Thorrold et al. 1998b; Milton and Chenery 2001; Rooker et al. 2001; Rooker et al. 2003) and to identify the source of newly recruited coral reef fishes (Swearer et al. 1999; Thorrold et al. 2001; Jones et al. 1999; Jones et al. 2005). Similarly, changes in chemical composition across the otolith's growth increments (i.e., from the core outwards) have been used to infer migratory routes and larval trajectories (Arai et al. 2003b; Arai and Morita 2005; Patterson et al. 2005; Sandin et al. 2005). The accuracy of larval tracking that such techniques can provide rests on the lower bounds of elemental concentrations that can be resolved along the otolith's radius. Running transects across otoliths increments can be done with several different approaches (e.g., Jones and Chen 2003), however, as argued below, the profiling of otoliths along a longitudinal (e.g., Sandin et al. 2005) or vertical (Brophy et al. 2004) axis can lead to erroneous inferences.

Obtaining chemical profiles requires sophisticated technologies (Campana et al. 1997), such as electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). The latter is the current method of choice (Patterson et al. 1999; Swearer et al. 1999; Zacherl et al. 2003; Brophy et al. 2004; Patterson et al. 2004; Patterson et al. 2005; Ruttenberg et al. 2005; Sandin et al. 2005; Warner et al. 2005). However, these approaches have two inherent sources of error—random noise in the LA-ICPMS signal (from machine errors, sample contamination, etc.) and inaccuracies that arise when water

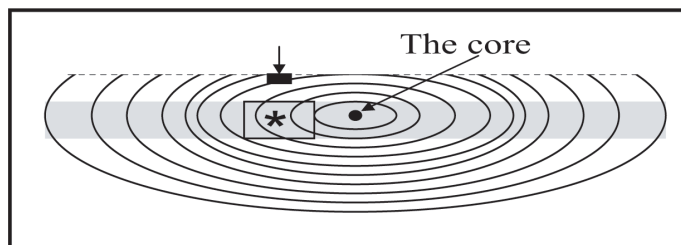


Fig. 1. A schematic side view of a long-section, top-plane polished otolith, showing the otolith increment sections that are normal to the polished plane ("vertical increments"). The scheme demonstrates sampling artifacts, which may occur when the "detection plane" (i.e., the focus depth, in which the daily growth band can be detected and counted; indicated by gray background) does not merge with the polished plane (indicated by broken line). In the figure, the "detection plane" is below the polished plane, and therefore, the pit location (indicated by black rectangle and arrow) does not match the detected "growth band" (indicated by quadrat and asterisk). The sampled band in the scheme is 2 days older than the observed band (indicated by *). In this case, the only way to ensure that the observed band will be the sampled one is by polishing exactly to the core level, and to have a completely flat otolith (as in the example). If the otolith is convex (as in most cases), there will be no plane in which all seen increments will be identical to those placed on the surface at the same spot.

and otolith elemental chemistry are uncorrelated. These points are expanded upon below.

Difficulties in profiling—

Longitudinal profiling. This approach is commonly used, since the otolith's increments are visible and indicate, presumably, the age associated with each micro-chemical sample. Prior to ablation, otoliths are polished along a longitudinal plane. Since many otoliths are convex, no single plane will give direct access to all increments. Even if the otolith is completely flat, it must be polished exactly to the core level to sample the increment seen in a microscope. Moreover, since the layers are concentric, variation in the distance between the polished surface and the core (e.g., Sandin et al. 2005 polished their otoliths down to 5–25 μm above the core) will cause sampling of a different (younger) increment than the one focused upon in the microscope (Fig. 1).

Vertical profiling. This technique is used to increase the chance of finding the core (Brophy et al. 2004; Ruttenberg et al. 2005). Independent of the exact procedure, the precise amount of ablated material is never known. Differences from one ablation to the next, or between otoliths, are due to differences in the focus of the laser and natural heterogeneity in the aragonite (Fig. 2). Ablating deeper into the sample results in progressively shallower pits of smaller radius, unless the focus is adjusted. Since the width of increments around the core are not consistent either within a given otolith or between otoliths of different fish, it is impossible to determine, when transecting through the core, which, and how many, increments were sampled with each ablation. Distance from the core, measured in the number of successive ablations, will not indicate exactly which increment was analyzed.

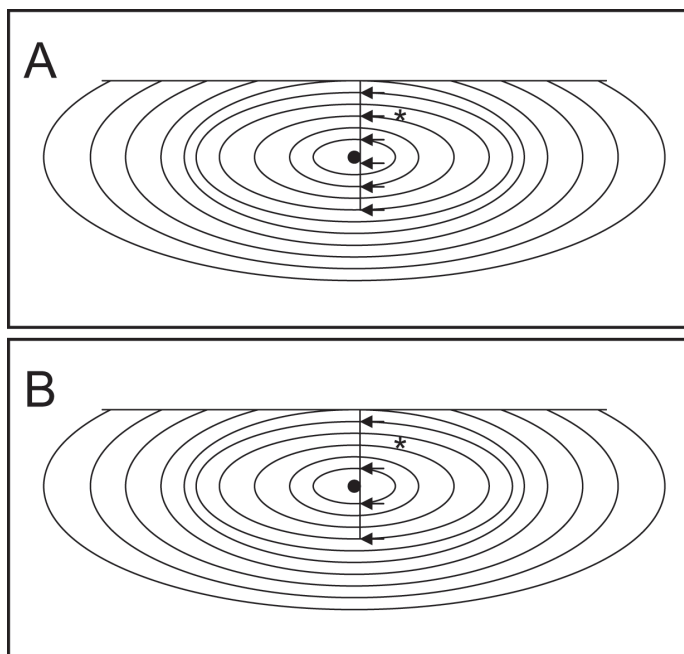


Fig. 2. A schematic side view of a long-section, top-plane polished otolith, showing two distinct hypothetical transects of ablation samples, which may lead to different chemical analysis results. Each of the horizontal arrows represents the final depth (the bottom) of each consecutive ablation in the transect. Differences in the depth of each ablation will create differences in the distances (from the core) and magnitudes of the signals: that is, high concentration of an element that exists only in the fourth increment from the core (marked with *) will be seen at a distance of 2 ablations from the core in the upper transect (A) and 1 ablation from the core in the lower one (B). The ratio of the concentration of this element to Ca concentration will be lower in the lower transect since it will be diluted by a larger mass of aragonite.

Element concentrations—Otolith concentration of elements such as Sr, Ba, or Mg are the most commonly used indicators of the temperature and salinity of the environment experienced by the fish (Friedland et al. 1998; Campana 1999; Bath et al. 2000). As these elements are found throughout the otolith, changes in otolith concentrations are assumed to reflect changes in the water ‘sampled’ by the fish. However, even in the same setting, the concentrations of these elements may vary among species, may depend on interactions between various trace elements (de Vries et al. 2005), and/or may vary for unexplained reasons (Patterson et al. 2004). Hence, even a meticulous analysis of the concentrations of these elements may not readily distinguish among different populations or locations (Kalish 1989; Thorrold et al. 1997). Likewise, elements such as Mn, Mg, and Ba often show high core concentrations regardless of their water concentrations (Brophy et al. 2004; Ruttenberg et al. 2005). These elements are therefore unlikely to provide useful information for distinguishing among natal areas, since core concentrations do not reflect the location where they were generated. Similar difficulties may arise when considering the chemical signatures of anthro-

pogenic contaminants, whose concentration in the water is often spatially variable, but which for reasons not fully understood, do not necessarily correlate with those in the otoliths (Warner et al. 2005).

These unaccountable sources of variation in concentrations contribute to type II errors: i.e., failure to detect true differences among individuals from different natal locations. One way to address these limitations on statistical power is to increase the number of elements being analyzed to garner more information. One of the biggest challenges here is that the elemental concentrations of many elements obtained using LA-ICPMS are quite small. To evaluate whether these minute signals are real or random noise, standard practice is to measure concentrations on blank samples of some “pure” material that are presumably free of metal contaminants. If otolith concentrations fall below the calculated detection limit (DL) of the instrument, they are presumed to be random sample error and are not usable in subsequent analyses. DL is typically defined as 3 times the standard deviation of values obtained from blank samples. The choice of three standard deviations is purely a convention chosen to be conservative and avoid interpreting instrument noise as a sample signal.

Here we show that this ad hoc definition of a detection limit may commonly be overly conservative. As a result, many studies may have inadvertently discarded useful data under the presumption that the observed signal was nothing more than instrument noise or sample contamination. Sinclair (2005) showed that consistent temporal patterns can be found in LA-ICPMS data obtained from corals skeletons that are below the presumed DL. We show here that, in some conditions, chemical signals from otoliths may contain important information even when signals fall below standard definitions of DL. Although DLs defined solely by a statistical convention derived from blank analyses may be a necessity when no other means to assess signal reliability exists, this standard practice may be compromising the true power of otolith microchemistry as a tool.

The hint that there may be true signals in otolith chemical concentrations that fall below DL comes from the recognition that there is information in both the absolute concentrations of elements and in the order/timing that spikes of elevated concentrations occur. Signal sequences are created by the discrete elevations in the concentration of trace elements, which span one or several otolith increments. For example, imagine a vertical profile through the core of an otolith where strontium shows spikes of concentration in the following otolith increments: 7, 12, 14, 23, 24, and 50 d and Mn shows spikes at 5, 19, and 37 d. Suppose each of the spikes was at a concentration that was below the instrument DL. By convention, one would have to conclude that strontium and manganese provided no inference. Yet, as the vertical profile passes through the otolith core, we get to sample these daily increments again. Imagine we see the same order for the sequence of strontium and manganese spikes on the opposite site of the core. The chance of obtaining such consistency in the tempo-

ral ordering of spikes would be exceedingly small if they, indeed, represented only random noise. Suppose further that analyses of the paired otoliths from the same fish showed the same palindromic sequence on either side of its core. There would be little doubt that such repeatability of sequence pattern, even if it associated with spikes of small magnitude, implies significant information. This information is stored in signal sequence, not solely in signal magnitude, and has been, heretofore, largely ignored. Yet, by calculating the probability that observed sequences arise simultaneously by chance, we show that such sub-DL signals can often contain relevant information. We begin by presenting technical details of our micro-chemical analyses and the data they generated. We then provide the statistical rationale for evaluating whether sub-DL signals are indeed devoid of relevant information.

Methods and procedures

Sample collection—Using clove oil and hand nets, we collected 281 new recruits of six species of fishes from the Gulf of Aqaba, Red Sea, on reefs near Eilat, Israel. The six species (*Dascyllus marginatus*, *Dascyllus aruanus*, *Dascyllus trimaculatus*, *Chromis viridis*, *Neupomacentrus miryae*, and *Pseudanthias squamipinnis*) were collected during the recruitment season of 2003 and 2004 (early summer to early winter for *Dascyllus spp.* and *C. viridis* and midwinter for *N. miryae* and *P. squamipinnis*) at several points along the 10km coast of Eilat and preserved in 95% ethanol. Anthropogenic inputs from this densely populated coastal region, such as extensive commercial and military port activity, aquaculture, irrigation runoff and sewage outflow, provide potential sources for significant levels of trace elements to be incorporated into the calcium carbonate matrix of the otoliths of fishes.

Otolith preparation and micro-chemical analysis—Both sagittae were dissected from all 281 fish collected. One sagitta (for some of the fish both) was then cleaned of any organic tissue with dissecting pins, mounted in low viscosity epoxy resin (Epo-Thin epoxy resin, Buehler), and polished to within 15–25 μm above the core. These sagitta were further cleaned of any surface contaminants and organic components by soaking in semiconductor grade 15% H_2O_2 and Suprapur NaOH (0.05N), followed by a series of ultrapure water (N-pure) rinse steps, and air drying in a class-100 flow bench (Ruttenberg et al. 2005). Micro-chemical analyses of the treated samples used a Finnegan Element 2 double focusing sector ICP-MS with the VG-UG Microprobe 266 nm laser ablation system (for methodological details see Ruttenberg et al. 2005, Sandin et al. 2005, Zacherl et al. 2003).

For each sample, we generated a vertical profile (i.e., perpendicular to the longitudinal axis) based on sequential discrete ablations through the core (e.g., Ruttenberg et al. 2005). Profiles consisted of 30 pits (fewer in some of the smaller otoliths that had been polished much thinner than the average) starting from the polished surface of the otolith, above the region visually identified as containing the core. From

each pit we measured counts for ^{24}Mg , ^{48}Ca , ^{52}Cr , ^{55}Mn , ^{87}Sr , ^{138}Ba , and ^{208}Pb in medium resolution mode ($R = 3000$). Molar ratios of analyte to ^{48}Ca were calculated and corrected using analyte isotope counts and the elemental mass bias, calculated using calibration standards of known analyte-to-Ca ratios; standards were analyzed following every third sagittae.

Solid glass standard reference material (NIST 612) was analyzed at the beginning and the end of each working day to evaluate the instrument's analytical accuracy. Estimate precision as %RSD (Relative Standard Deviation = CV) ranged between 12% to 19% for Mg/Ca, 12% to 16% for Mn/Ca, 14% to 26% Sr/Ca, 11% to 17% for Ba/Ca, 11% to 16% for Cr/Ca, and 9% to 19% for Pb/Ca. Blank samples were acquired while only aspirating 1% HNO_3 after every sagitta sample and set of standards. Elemental counts for each sagitta pit were blank-subtracted using the blank sample analyzed before each sagitta. Detectable limits (DL) of sample elemental signal for ICP-MS analysis were characterized using the customary method of calculating $3 \times \text{SD}$ of the blanks for every three sagittae sampled and corresponding standards.

Assessment

We ran two controls in order to validate the performance of our micro-chemical analysis. First, we profiled both sagittae from each of eight fish and compared the paired elemental sequences. These fish were randomly chosen prior to the chemical analyses, and the ablation of each pair member was conducted on a different day to minimize the probability that they would show concordance in pattern due to reasons other than a true concordance in their chemical signal. Second, we compared the elemental profiles obtained from the sagittae of four 40-d old *Sparus aurata* that had been reared (from hatching) in the same holding tank. In both controls, we expected "real" signals to result in profile concordance among replicates: paired sagittae (control 1) or cohort members (control 2).

The validity of sub-DL signals—Micro-chemical analysis of small amounts of material often results in low-level signals. Analyzing the sequence of signals in a vertical transect that spans the core may help differentiate between real signals and random noise. Due to the nature of otolith structure, a vertical profile through the core would sample each increment twice—once before reaching the core and a second time after passing through it. Hence, we would expect the sequence of signals on one side of the core to be mirrored on the other. For each sagitta, we identified the ablation corresponding to the core from high values of Mn/Ca, Mg/Ca, Ba/Ca, and Sr/Ca (Ruttenberg et al. 2005). For each of these elements, we searched for signals whose order of appearance on either side of the core formed a mirror image of each other (Figs. 3 and 4). We allowed only one exception: two different elements could occupy two consecutive ablations on one side, while occupying only one ablation on the other. Such cases, which proved rare, can result from differences in pit width and relative location (Fig. 2).

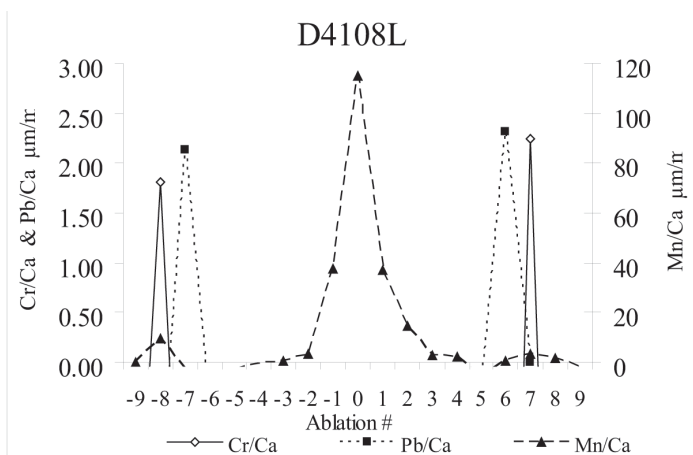


Fig. 3. Palindrome of signals of 3 elements in an otolith of *D. marginatus*. The core was identified by the high signal of Mn. The calculated probability to obtain the signal of Cr and Pb in the obtained mirror order in two otoliths of the same fish is 5% (without taking into account the option to obtain them both at the same ablation, as seen with the signals of Cr and Mn). The full data obtained from this fish are shown in Fig. 4.

The symmetry in signal sequence on both sides of the core gives rise to a palindrome, whose probability of occurrence provides an indication of the biological relevance of the signals. Below we evaluate the probability of such palindromes arising from random noise by focusing on simple sequences for which these probabilities are easily computed. We suggest that if these simple sequences can be shown to be significantly different from random (e.g., with a Type I error rate of < 5%), then (1) more complex palindromes would be more unlikely to arise by chance, and (2) signals of the corresponding magnitude should not be discarded.

Consider a profile that consists of a sequence of N non-overlapping signals, half of which fall on either side of the core. Consider further that the sequence contains two signals of each of $N/2$ elements (see Fig. 3 for an example with $n = 4$ and two elements: Pb and Cr). The total number of possible sequence arrangements is M . The number of sequence arrangements in which each element appears on one side of the core is as follows: $(N/2)! \prod (N-2i)$ where $i = 0, 1, \dots, (N-2)/2$; of these, $1/(N/2)!$ will form palindromes. Hence, the probability

of a random palindromic sequence is $p = \frac{\prod (N-2i)}{M!}$ given the same representation of the $N/2$ elements in two similarly sized sequences (i.e., one for each of the paired sagittae), the probability of two random sequences forming seemingly identical palindromes is $P \times (2^{N/2}/M!)$, where the term in brackets is the product of the probability of a prescribed sequence and the number of permutations that are similar to it. For a sequence of 4 readings and 2 elements, this probability equals 0.055. With $n = 6$ and 3 elements, it equals 7.5×10^{-4} . Notably, the probability of obtaining palindromic sequences (P) would be lower than the value derived above, since the signals of the different elements may overlap (e.g.,

Fig. 3 where the signals of Cr and Mn, signals nr 2, are located at the same pit).

Microchemistry results—We identified the core in 230 of 281 otoliths analyzed (82.9%) by observing high spikes of Mg and Mn. Failure to detect the core in 31 (11%) of the otoliths is most likely explained by our having missed the core during the ablation profile. The remaining otoliths were either cracked ($n = 10$) or with clear contamination ($n = 7$).

In 228 (99.1%) of the otoliths with detectable cores, the signal sequence of Cr, Pb, and Mn formed mirror images across the core. Only 2 otoliths did not have the same signal sequence on both sides of the core. The total number of paired signals, summed across the elements Cr, Pb and Mn, varied from 1 to 12 (average: 7.2 ± 1.75 ; Fig. 4). The majority of the signals were below the calculated detection limit, but all were higher than the average readings of the blank. The number of paired signals whose magnitude fell below the average readings of the blank was minimal (5% for Pb and 2% for Cr and Mn; $n = 60$ fish). Unlike the patterns for Cr, Pb, and Mn, there were no clear paired palindrome signals for Sr, Mg or Ba.

All eight comparisons of paired sagittae (control 1) revealed similar signal sequences across the two otoliths; i.e., the order of the Cr, Pb, and Mn signals on both sides of the core was the same in both otoliths. Signal distances from the core (measured in number of ablations) were not identical (as would be expected given the irregular shape of the otoliths and the variance in laser pit widths and depths per ablation), although the differences were small (average difference is 0.89 and a difference of more than 2 is uncommon). Differences were also found between the magnitudes of the signals on the two sides of the same otolith, and between the two otoliths of the same fish (examples in Fig. 4). The sequence pattern was thus much more consistent than signal magnitude.

The sagittae of all four *S. aurata* (control II) showed the same pattern of elemental signals (Fig. 5). Small differences in signal magnitude and distance to core are consistent with the technical issues discussed above.

Discussion

Our proposed palindrome sequence analysis (PaSA) is designed to overcome two major impediments that are common to the analysis of otolith microchemical data. First, concentrations obtained using LA-ICPMS can vary markedly among individuals for a variety of technical reasons that may obscure useful information (e.g., Fig. 2). This variation inherently detracts power from statistical analyses of concentration data. Second, the empirical studies analyzed here suggest that separating signal from noise using DL standards as commonly defined in otolith research is overly conservative. Nearly all sequences analyzed in this study exhibited strong signals in the otoliths (i.e., they included palindromic sequences of element spikes) even when element concentrations were substantially below the minimum DL. These findings suggest that the common practice for defining DLs is overly conservative and is labeling relevant signals as

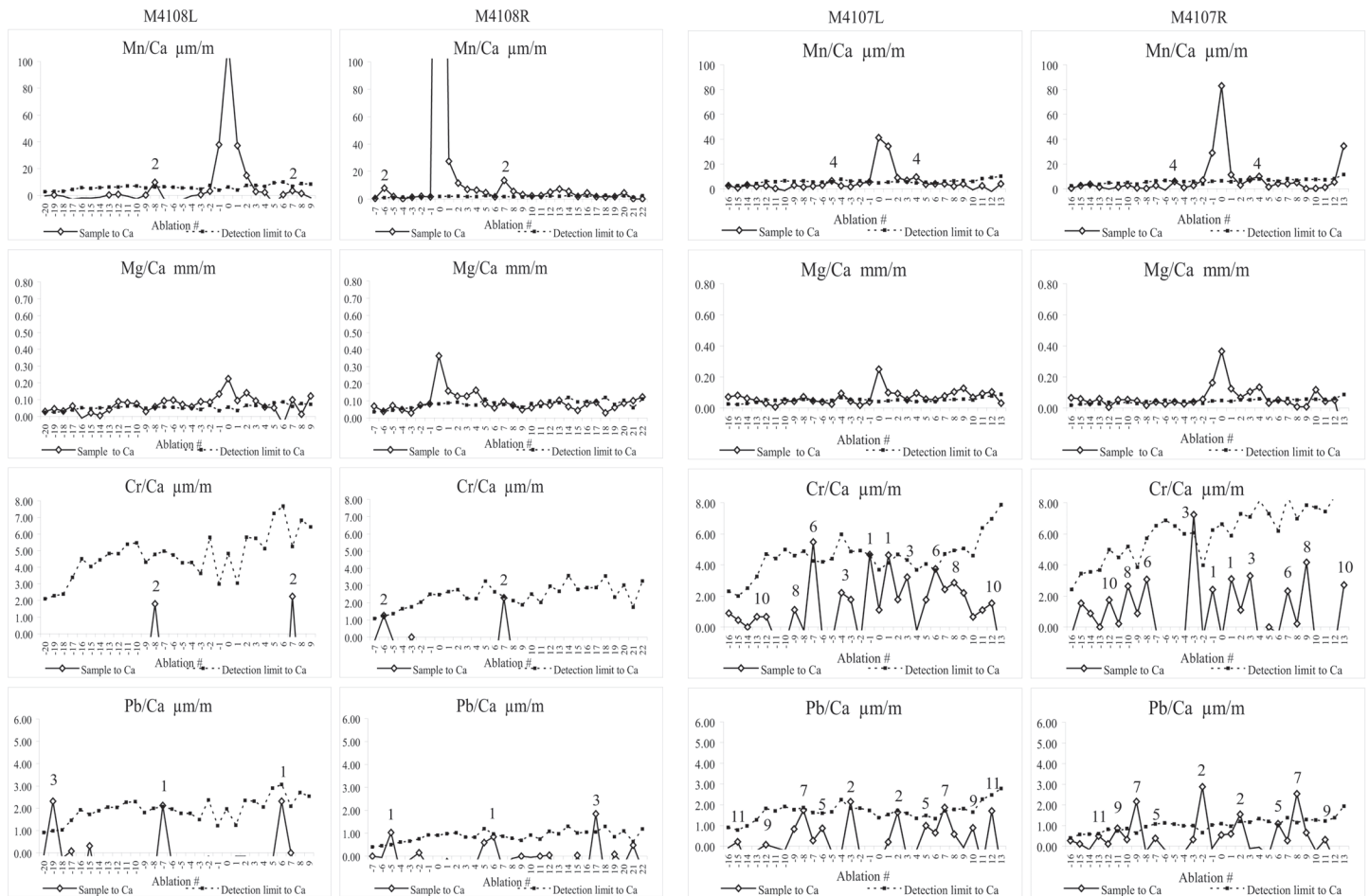


Fig. 4. Comparison of the microchemistry analysis of the two sagittal otoliths of (left) *Dascyllus marginatus* nr 108 with only a few heavy metals signals and (right) *D. marginatus* nr 107 in which plenty of heavy metals signals were found. The ratios of Ca to Mn, Mg, Pb, and Cr are presented for each otolith. The core was identified from the high concentrations of Mn and Mg and the x axis was adjusted to it (Mn/Ca ratio at the core is above the max value of the Y scale at some of the samples). Ablations before the core are presented with negative values and after the core with positive values. The value is the distance in ablations from the core. The ratio of the detection limit (3 standard deviations of the blanks) of each element to Ca is presented for each ablation on all graphs. An identical order of high concentrations of heavy metals is found at both sides of both otoliths of the same fish. The order of the pairs of signals is marked with numerals; 1 for that closest to the core and the highest value for the most distant one. Where high concentrations of two elements are identified in the same ablation they are marked with the same number. The order of the signal from the core to the edges in fish nr 107 is: 1. Pb, 2. Cr and Mn, and 3. Pb. In fish nr 108 the order is: 1. Cr, 2. Pb, 3. Cr, 4. Mn, 5. Pb, 6. Cr, 7. Pb, 8. Cr, 9. Pb, 10. Cr, and 11. Pb. Pairs of Mg signals are not clear and were not counted.

noise. Therefore, there may be far more information in otolith transects than is commonly extracted.

The existence of palindromes in the signal sequence of most otoliths also suggests that we need to move beyond solely comparing elemental concentrations to extract information about the location where otolith material was deposited. The sequences that make up the palindromes and the location of their elements relative to the core are sources of data that may provide valuable information on larval histories. The sequences themselves can be used as powerful multivariate metrics for statistical analyses. For example, in our ongoing study of reef fish in the Gulf of Eilat, we are comparing the number of paired signals of each of the elements that

form palindromes and the relative distance from the core to the closest pair of the same elements. These data can be used to discriminate between fish trajectories (for sample of possible application see the appendix) in ways that avoid the large variation inherent in solely using elemental concentrations.

The data for Sr, Mg, and Ba were not included in the PaSA, because no clear paired palindrome signals were observed. These elements are found in relatively high concentrations throughout the entire otolith (Sr/Ca ~ 3mm/m, Mg/Ca ~ 50 µm/m, and Ba/Ca ~ 5µm/m). It might be that natural fluctuations in their concentration mask the relatively low signals produced by the small concentration changes in the sea (for instance, the problematic Mg signal in Fig. 5).

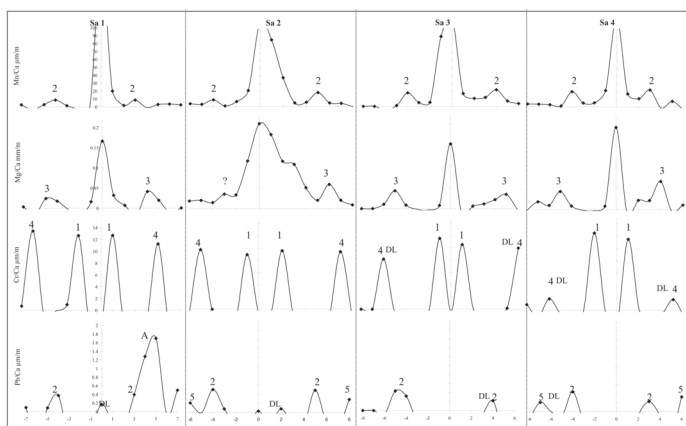


Fig. 5. Results of microchemistry analysis of four sagittal otoliths taken from four juvenile *Sparus aurata* (columns Sa1, Sa2, Sa3, and Sa4) reared together in the same water tank. Ratios of Mn/Ca (top), Mg/Ca (second row), Cr/Ca (third row), and Pb/Ca (bottom) along the transect through each otolith are shown. The core was identified by the high concentrations of Mn and Mg. The Y axis is on the core mark (Mn/Ca ratio at the core is above the max value of the Y scale at all 4 fish). Ablations before the core are presented with negative values and after the core with positive values. The value is the distance in ablations from the core. Mirror order of the high concentrations of heavy metals is found on both sides of each core, and the same order is found in all 4 fish (except for one Mg signal in Sa2, which is marked by "?"). The order of the pairs of signals is marked with numerals; 1 for that closest to the core and the highest value for the most distant one. Where high concentrations of two elements are identified in the same ablation, they are marked with the same number. The order of the signal from the core to both edges in all otoliths is: 1. Cr, 2. Pb and Mn, 3. Mg, and 4. Cr. Signals below detection limit are marked DL. The high signal of Pb/Ca in Sa1 (marked A) was found only there and is probably contamination.

When the concentrations of an element fluctuate around zero (after the deletion of the blank) every small signal can be easily seen, as with Cr, Pb, and Mn in Figs. 4 and 5. Our instrument and method (single collector ICP-MS and tiny samples ablated by the laser) limit the number of the examined elements, but our findings suggest that it is likely that similar information can be obtained from palindrome analyses of other trace and ultra trace elements using alternative instruments and techniques.

Through the use of PaSA, and by comparing data for several elements from two radii of the same otolith, analytical power may be enhanced. Consistency of pattern helps separate true signals from random noise. The natural asymmetry of otoliths and the differences in the increments' width, together with the reduced pit depth along a vertical transect, are all sources of noise in LA-ICPMS transects. Therefore, one would not expect to obtain similar signals at similar distances on both sides of the core, even if the same increments were ablated on each side. Although the specific magnitude of the signals and their actual distance from the core are compromised by many sources of random variation, the order of signal peaks on either side of the core may provide a powerful and consistent

marker of the environmental component of otolith microchemistry. The palindromic sequences obtained from the vast majority of the otoliths confirm that informative trace signals are common, even though many of the signals supposedly fall below the widely-accepted DL.

The variation in the sequences that were found between the otoliths of the collected fishes was high. One possible way to examine if this variation is, in fact, recording true changes in otoliths' chemistry is to examine fish that have experienced exactly the same water throughout their entire lives. The examined otoliths of *S. aurata*, which showed the same sequence of high magnitude signals (except for one Mg signal on one side of a single otolith; Fig. 5) confirm that the observed variation in the wild fish's otoliths is unlikely to be noise. There was variation in the magnitude of the signals between fish, but no signals were missing for any element in any of the examined otoliths. An alternative explanation for the similarity in the *S. aurata* otoliths palindromes is that it arises from variable physiological processes rather than from changes in the water chemistry. There is diverse evidence showing that variation in water chemistry is reflected in otolith chemistry (e.g., Campana et al. 1994; Campana et al. 1995, Campana and Thorrold 2001, Dove and Kingsford 1998). To date, evidence for physiologically-induced changes in trace element concentrations is limited to naturally elevated concentrations of some elements in the core (Ruttenberg et al. 2005). Further support for environmental control of these otolith sequences comes from comparisons of laboratory and field raised fish. The consistency of palindromes across individuals that was found in otoliths of lab reared *S. aurata* was not found between individuals of the same species (e.g., *C. viridis* or *D. marginatus*) that were collected from the same coral and were identical in age, Pelagic Larval Duration (PLD), and recruitment date (shown by otoliths reading). Since the individuals collected in the field could have been at separate locations in the past, their observed differences in otolith chemistry sequences likely reflect changes in the water chemistry that the fish had experienced.

Applying the PaSA method to the study of fish may provide additional clues to their early life history, especially when sequence analyses are combined with data on element concentrations to obtain more reliable and more insightful results. This will be true especially in seas with prolonged anthropogenic contamination.

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