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SUMMARY OF A WORKSHOP : OTOLITH MICROCONSTITUENT ANALYSIS OF ATLANTIC BLUEFIN TUNA

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SUMMARY

Otolith micro-constituent analysis has been recommended as a method for measuring homing fidelity in Atlantic bluefin tuna. The premise of the approach is that certain elements are incorporated into otoliths in direct proportion to their availability in ambient water or food. Thus, larvae or juvenile bluefin exposed to either western Atlantic coastal waters or Mediterranean waters might incorporate different mixtures of elements into their otoliths. A workshop was convened in March, 1997, to evaluate the feasibility of using otolith chemistry to distinguish bluefin tuna spawned in the Mediterranean Sea from those spawned in the Gulf of Mexico and the Florida Straits.

Technology: Inductively coupled plasma mass spectrometry is the most promising technology in analyzing Atlantic bluefin tuna otoliths. Although this technology permits the quantification of elements <ppb levels, elements which can be reliably measured are useful for discrimination in otoliths have typically numbered less than ten. Successful application of otolith chemistry to discriminate stocks assumes that all life stages can be identified according to nursery of origin. Therefore, the region of the otolith formed as larvae and juveniles are exposed to nursery waters will need to be isolated from otoliths of juvenile and adult bluefin tunas. Further research is needed to standardize probe-based techniques and develop clean coring techniques.

Methodology: Elements such as Mg, Ca, Sr, and Ba, are incorporated and retained in the otolith's inorganic lattice structure. Other elements are more easily lost from the otolith (e.g. Na, S. K, Cl) because they are associated with organic material or interstitial spaces. These elements could be lost from otoliths due to chemical degradation in the fish due to stress or death. After removal, otolith handling and cleaning could result in additional leaching of certain elements. Analytical protocols should be carefully examined for possible bias due to contamination or leaching.

Biology: The core region represents the tuna's first year of life in either the Mediterranean Sea or inshore coastal western Atlantic Ocean nurseries. Annual variations in climate or oceanography could obscure an elemental pattern associated with the two nurseries. Heterogenous distribution of trace element concentrations within nurseries could also contribute variation in elemental signatures. Early research should examine otolith composition of multiple year-classes collected over multiple sites for both nurseries.

RÉSUMÉ

L'analyse des micro-éléments des otolithes a été recommandée comme méthode pour mesurer le degré de fidélité de retour au lieu d'origine du thon rouge de l'Atlantique. Les prémisses de l'approche sont que certains éléments sont compris dans les otolithes de façon directement proportionnelle à l'espace vital et à l'alimentation dont ils disposent. Par conséquent, les larves ou les juvéniles de thon rouge dans les eaux côtières de l'Atlantique occidental ou de la Méditerranée pourraient comprendre des mélanges différents d'éléments dans leurs otolithes. Des Journées d'étude ont été convoquées en mars 1997 afin d'évaluer la faisabilité de l'usage de la chimie des otolithes pour distinguer les thons rouges nés dans la Méditerranée de ceux nés dans le Golfe du Mexique et dans les Détroits de Floride.

Technologie-- La spectrométrie de la masse du plasma assemblée par induction est la technique la plus prometteuse en matière d'analyse des otolithes de thons rouges de l'Atlantique. Bien que cette technologie permette de quantifier les éléments < aux niveaux ppb, on a dénombré typiquement moins de dix éléments qui peuvent être mesurés de manière fiable et qui sont utiles à la distinction dans les otolithes. L'application réussie de la chimie des otolithes pour distinguer les stocks suppose que tous les cycles vitaux puissent être identifiés d'après la nourricerie d'origine. Par conséquent, la région de l'otolithe qui se forme alors que les larves et les juvéniles se trouvent dans les eaux de nourricerie, devra être isolée des otolithes de thons rouges juvéniles et adultes. Il est nécessaire de poursuivre la recherche pour standardiser des techniques fondées sur les investigations et élaborer des techniques essentielles appropriées.

Méthodologie-- Des éléments tels que le Mg, le Ca, le Sr, et le Ba, sont compris et conservés dans la structure inorganique en treillis des otolithes. On perd plus facilement d'autres éléments des otolithes (par exemple, Na, S, K, Cl) parce qu'ils sont associés à des matières organiques ou à des espaces interstitiels. Ces éléments pourraient disparaître des otolithes en raison d'une dégradation chimique du poisson due à la tension ou à la mort. Après leur déplacement, la manipulation et le nettoyage des otolithes pourrait mener à une filtration supplémentaire de certains éléments. Les protocoles analytiques devraient être examinés avec soin en raison des éventuels biais dûs à la contamination ou la lixiviation.

Biologie-- La zone centrale représente la première année de la vie du thonidé dans la Mer Méditerranée ou dans des nourriceries près des côtes de l'Atlantique Ouest. Des variations annuelles du climat ou de l'océanographie pourraient dissimuler un mode élémentaire associé aux deux nourriceries. Des distributions hétérogènes de concentrations d'éléments à l'état de traces dans les nourriceries pourraient également contribuer à une variation de la signature élémentaire. La recherche devrait examiner sans tarder la composition des otolithes de diverses classes d'années collectés sur différents sites pour les deux nourriceries.

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RESUMEN

Se ha recomendado el análisis de microelementos de otolitos como método para medir la fidelidad de la costumbre de regresar al lugar de nacimiento en el atún rojo atlántico. La premisa del enfoque es que se incorporan a los otolitos ciertos elementos en proporción directa a su disponibilidad de comida en aguas circundantes. En consecuencia, las larvas o juveniles de atún rojo en aguas costeras del Atlántico oeste o del Mediterráneo podrían incorporar diferentes mezclas de elementos en sus otolitos. Se convocaron unas jornadas de trabajo en marzo de 1997 para evaluar la viabilidad de utilizar la química en los otolitos, para distinguir el atún rojo desovado en el mar Mediterráneo del atún rojo desovado en el Golfo de México y estrecho de Florida.

Tecnología - La espectrometría de masa del plasma acoplada de modo inductivo es la tecnología más promisoria para analizar los otolitos de atún rojo del Atlántico. Si bien esta tecnología permite la cuantificación de niveles de elementos <ppb, los elementos que pueden ser medidos de forma fiable y que son útiles para la discriminación en los otolitos típicamente son inferiores a diez. La aplicación con éxito de la química en otolitos para diferenciar los stocks asume que todas las etapas vitales pueden identificarse según el lugar de origen de la crianza. Por tanto, la región del otolito que se forma según las larvas y juveniles se encuentran en aguas del criadero, necesitará aislarse de los otolitos de juveniles y adultos de atún rojo. Se precisa mayor investigación para estandarizar las técnicas basadas en investigación y desarrollar técnicas esenciales apropiadas.

Metodología - Los elementos tales como Mg, Ca, Sr, y Ba se incorporan y quedan retenidos en la estructura inorgánica del latis del otolito. Otros elementos del otolito se pierden con mayor facilidad (por ejemplo, Na, S, K, Cl) dado que se encuentran asociados a material orgánica o espacios intersticiales. Estos elementos podrían desaparecer de los otolitos debido a la degradación química del pez por estrés o muerte. Tras recolectarlos, el manejo y limpieza de los otolitos podría traducirse en una lixiviación de ciertos elementos. Los protocolos analíticos deberían ser cuidadosamente examinados para determinar la existencia de un posible sesgo debido a contaminación o lixiviación.

Biología - La región central representa el primer año de vida del atún en el mar Mediterráneo o en zonas costeras de cría del océano Atlántico oeste. Las variaciones anuales climáticas u oceanográficas podrían ocultar un esquema elemental asociado a las dos zonas de cría. Las distribuciones heterogéneas de concentraciones de los elementos traza dentro de las zonas de cría también podría contribuir a variaciones en las señales de identificación elementales. Debería investigarse sin tardanza la composición de los otolitos de clases anuales múltiples recolectadas en múltiples lugares para ambos criaderos.

Introduction

High international demand for the fatty flesh of giant bluefin tuna has resulted in declining catches of bluefin tuna in coastal waters adjacent to North America (NMFS 1995). Migratory behaviors of Atlantic bluefin tuna allow them to be harvested in jurisdictional waters of many countries, as well as interjurisdictional regions. The high worldwide demand for giant Atlantic bluefin tuna and their migratory behaviors dictates a multi-national management strategy.

Despite pan-oceanic distribution, known spawning grounds are restricted to the Gulf of Mexico (GOM) and the eastern Mediterranean Sea. In addition, tagging studies have indicated low rates of transoceanic migrations by juveniles and adults. Based upon limited tagging data, geographically separate fishing grounds, and two geographically disparate spawning grounds for Atlantic bluefin, the International Commission for the Conservation of Atlantic Tunas (ICCAT) implemented a two stock premise for management in 1982. However, data is sparse and stock assessments based upon the two stock assumption have been called into question by various interests (ICCAT 1992, 1994, 1995, 1996, 1997; Magnuson et al. 1995). Initiation of research to examine stock structure, homing fidelity, or mixing rates has been identified as critical for improved assessment and management (Dean and Woodley 1994; NMFS 1995; Wagner 1996).

Goal

A workshop on bluefin tuna was convened to evaluate the feasibility of using otolith chemistry to identify Atlantic bluefin tuna spawned in each of the known spawning areas, the GOM and Mediterranean. The purpose of the workshop was to review the merits of otolith chemistry as a technique to evaluate spawning site fidelity of bluefin tuna and estimate mixing between spawning contingents. Otolith chemistry has been applied to problems of stock structure (Edmonds et al. 1898; Campana et al. 1995; Proctor et al. 1995) and migration rates (Secor and Piccoli 1996). The goal of the workshop was to:

Evaluate otolith chemistry as a method to differentiate bluefin tuna spawned in the Mediterranean or Gulf of Mexico.

Seventeen scientists with expertise in fisheries stock assessment and ecology, population genetics, analytical chemistry, ecotoxicology, and chemical oceanography attended the workshop, held in Charleston, SC from 30 April to 2 May 1997. Agenda issues included:

1. What are the bluefin tuna stock assessment assumptions with regard to homing fidelity and mixing rates?
2. What is the status of genetic markers and tagging studies in the evaluation of assessment assumptions?
3. How can otolith composition be used to address stock assessment assumptions and data needs?

4. Are otoliths environmental CD ROMS?
5. What technologies are most appropriate for analysis of otolith chemistry?
6. What are expectations for otolith chemistry in the resolution of stock assessment and management issues for Atlantic bluefin tuna?

Background

Stock Assessment Data Needs

A lack of a complete understanding of the biology of Atlantic bluefin tuna (ABFT) clouds stock assessment and management issues for this highly migratory multi-jurisdictional species. Based upon research on the GOM and Mediterranean spawning aggregations, the western stock of ABFT is believed to be sexually mature at 200 cm (about 8 years), but the eastern stock may mature earlier at 130-140 cm (about 5 years). Spawning takes place in the western stock from April-June while in the eastern stock spawning peaks later in the summer. It is unclear whether these apparent regional differences between the stocks are the result of genetic, environment or other influences (i.e. fishing). Additionally, estimated age differences of spawning stocks of the east and west stock of ABFT are based upon different methods of estimating size at age (mark-recapture for the west and anal spines for the east), hence size and age at maturity are still uncertain parameters. Nevertheless, differences in spawning seasons and two distinct spawning areas for ABFT provide rationale for current management which is based upon eastern and western Atlantic management units with arbitrary zones delimiting fishing areas for each unit.

Tagging efforts in the 1960's and 70's established trans-Atlantic migrations of some ABFT. For example, large fish have moved from the Bahamas to Norway in just a few months. Relatively small rates of mixing between east and west stocks have been estimated based upon tagging data. Different assumptions about the behavior of bluefin which cross the Atlantic have very different impacts on the interpretation and implications from tagging data. To date all analyses using tagging data have assumed that a fish which crossed has no greater chance of returning to its original side of the ocean than a resident (no memory assumption). However, inaccurate reporting of recaptures could pose a problem for these data and models. If a small percentage of the eastern stock mix with western stock then the outcome of the models significantly changes because 1) the eastern stock is larger and 2) rate processes are cumulative. Age composition of mixing fish can further complicate the problem because of age structure differences between east and west stocks. A major question to be answered is "Does a mature individual always return to it natal spawning ground?"

Approaches to Separate Stocks

Molecular markers which may be useful for ABFT stock structure analysis and are currently being researched include Mitochondrial DNA (MtDNA), Restriction Fragment Length Polymorphisms (Charleston Laboratory, NMFS), introns of the actin gene and anonymous loci from nuclear genes (Virginia Institute of Marine Science), micro-satellites (Texas A&M University), introns of LDH genes and DNA sequencing of mitochondrial and nuclear DNA (University of South Carolina). Results to date have discovered a minimum of 12 loci from nuclear and mitochondrial genes that have the potential of yielding data suitable for addressing ABFT stock questions. However to date, no single genetic marker has been discovered that will unambiguously separate a fish spawned in the GOM from one spawned in the Mediterranean. Nevertheless, researchers believe that continued genetics research and the aggregate of data from the various markers will eventually resolve questions about AFBT stock structure.

Another advancement that may improve the basis for bluefin management is a new generation of sophisticated fish tags. Archival tags have excited great interest due to the detailed information they can provide on daily, seasonal, and size-dependent migrations. The cost of the tag (ca. $1500) and the need to retrieve archival tags from fishers currently limit the application although over 150 archival tags were implanted in bluefin tuna captured off North Carolina by Drs. B. Block, E. Prince, and their colleagues in winter and spring 1997 in a project supported by NMFS and private sources. "Pop-up" tags (c.a. $2500 each) are tags which can be programmed to surface at specified time intervals after tagging and transmit their location and limited archived data to satellite. Archival and Pop-up tags are expected to improve the quality and recovery rates of information pertinent to bluefin migrations. However, their expense, the lack of information on the fishes origin prior to tagging and possible low return rates probably limits their potential to definitively answer questions related to spawning stock fidelity with a high degree of confidence and on a reasonable time scale.

Otolith Chemistry as a Tool for Stock Identification

I. Definitions

Otolith: Calcium carbonate concretions which occur in the membranous labyrinth of fishes. Typically otolith refers to the *sagitta*, one of three pairs of otoliths. Sagittae are located along the medial plane in the neurocranium. Otoliths contain annuli (Latin, rings), defined by their bipartite optical characteristics; under transmitted light, *annuli* are composed of *translucent and opaque zones*. Annuli in otolith sections of ABFT have been used to assign yearly ages.

Core Region: Region in ABFT sagittae circumscribed within the first opaque zone. It is assumed that this represents the period of time from hatching to the end of the first year of life. During this larval and early juvenile growth phase, ABFT are believed to reside in west Atlantic or Mediterranean Sea/east Atlantic nursery regions.

Microconstituents: Trace elements occurring in otoliths. The prefix *micro-* refers to the distribution of elements within the microstructure of otoliths. Another term, *microchemistry* also refers to the elemental composition of the microstructure of otoliths.

ABFT Nursery Regions: Nursery regions are defined according to the analytical constraints in isolating the otolith's core region (i.e. the first year of life). The Mediterranean nursery region encompasses the entire Mediterranean Basin and adjacent waters of the eastern Atlantic. The Western Atlantic nursery comprises the Gulf of Mexico, and inshore coastal regions of the South

and Mid-Atlantic Bight. These two regions represent areas in which young-of-the-year and yearling bluefin are expected to disperse.

Elemental Pattern or "Fingerprint": Utilization of otolith chemistry as a tool to discriminate stocks is dependent upon the identification of a multi-elemental pattern which is unique to a specific stock or geographic location. This pattern is sometimes referred to as an elemental "fingerprint." Multivariate statistics are used to characterize distinguishing elemental patterns among groups of fish.

WDS (Wavelength Dispersive Spectrometry): Probe-based technique for analyzing microchemistry of otoliths based upon electron induced x-ray emissions. Routinely used to quantify strontium and calcium concentrations in otoliths, WDS has superb spatial resolution (c.a. 10 μm) but limited sensitivity (~100 ppm). Time- and cost-intensive method for analyzing elemental fingerprints.

PIXE (Proton Induce X-ray Emission Spectrometry): Probe-based technique for analyzing microchemistry of otoliths based upon proton induced x-ray emissions. Application of PIXE otolith analysis is still in its developmental phase but recent improvements in standardization and sensitivity could result in combination of high spatial resolution and sensitivity. Time- and cost-intensive method for analyzing elemental patterns.

ICPMS (Inductively Coupled Plasma Mass Spectrometry): ICPMS has the capability to simultaneously assay multiple elements at very high sensitivity (sub ppb detection limits). *Solution-based ICPMS* analyses require that otolith material be introduced in solution after dissolving them in acid. Core regions can be analyzed through otolith coring or laser ablation techniques (see below).

Isotope Dilution ICPMS: A version of *solution-based ICPMS* where a known amount of a specific isotope of the target element is introduced into the unknown solution to be analyzed. By analyzing the unknown solution with and without the isotope addition, the isotopic spectra are more precisely defined and spectral interference can be corrected, resulting in more accurate and precise quantification of the target element.

Otolith Coring: Portions of an otolith can be sampled and isolated from the earliest deposited portion of the otolith (e.g. the *core region*). One method is to prepare a section (c.a. 0.1 to 1 mm thick) of an otolith which contains the core region (see Secor et al. 1991). Under a dissecting microscope, material peripheral to the first annulus can be removed with a dental drill, dremil, or hollow bit drill-press. The remaining material can be acid rinsed to remove some contamination resulting from the coring drilling procedure and then digested for ICPMS analysis. Also titanium wafering blades and drill bits might reduce contamination of key target elements. However, some contamination can occur, including elements that are candidates for analysis.

Laser Ablation: Otoliths are sectioned to expose the core region (see above). The section can be decontaminated with a laser raster, prior to ICPMS analysis (Campana et al 1994). Energy from a laser blast ablates and vaporizes material from the surface of a solid sample forming a crater in the sample (Denoyer et al 1991; Huang al 1993). The excited and vaporized material is conveyed to the detector via a carrier gas. During its transit, sample may precipitate along the conveying tube's inner walls. This problem has made standardization of laser ablation important. Thorrold et al. (1997) standardized elemental concentrations to Ca concentration, assuming that elements were ablated and conveyed in proportion to calcium.

II. Case Studies

Dr. Campana and his colleagues have embarked on a successful research program to distinguish stocks of Atlantic cod *Gadus morhua* on the eastern coast of Canada (Campana et al. 1995; Campana et al. in press). Isotope dilution ICPMS improved precision of elemental measures in whole otolith, solution-based analyses (Campana et al. 1995) and supported discrimination of several cod stocks. Examination of Atlantic cod stock structure was also accomplished using LA-ICPMS which permitted elemental ratios to be estimated in portions of otoliths associated with the first year of life. Previous genetic-based studies provided equivocal results on Atlantic cod stock structure. Recent investigations by Campana and his colleagues used elemental "fingerprints" as natural tags to track local migrations of Atlantic cod across the Scotian Shelf. Otolith chemistry has also been used to characterize stock structure for *Chrysophrys auratus* (Sparidae), *Hoplostethus atlanticus* (orange roughy), and *Aldrichetti forsteri* (Mugilidae) (Edmonds et al. 1989, 1991, 1992).

Over the past ten years, Dr. Thresher and his group have been investigating the use of probe-based techniques (WDS and PIXE) to examine stock structure and migration patterns in several pelagic species. Discrimination of stock structure was observed for morwong (Cheilodactylidae)(Thresher et al. 1994). Microchemical analysis of southern bluefin tuna juveniles captured across large distances in the Pacific and Indian Oceans, showed no discriminating elemental patterns for the elements surveyed (Proctor et al. 1995). Panmictic spawning behavior and high rates of dispersal of young, or lack of sufficient difference in ambient water chemistry associated with nursery regions could have contributed to the lack of discrimination. Alternatively, assayed elements may not have included all those most appropriate for stock discrimination.

III. Are Otoliths Environmental CD ROMS?

The premise of otolith chemistry is that certain elements are incorporated into otoliths in proportion to their concentrations in the surrounding water or in the fish's food. Thus, larvae or young-of-the-year juvenile bluefin tuna exposed to either Gulf of Mexico/west Atlantic or Mediterranean/east Atlantic waters might be expected to incorporate different mixtures of elements into their otoliths if significant differences exist in the chemistry of the two areas. Relatively few laboratory experiments have been conducted to verify the assumption that otoliths can record environmental histories. Laboratory studies indicate that most calcium and strontium deposited onto the otolith originates through gill uptake of these elements (Farrell and Campana

1996). Several rearing experiments have shown that otolith incorporation of strontium is related to its ambient concentration (Limburg 1996; Secor et al. 1996; Thorrold et al. 1996). However, physiological factors, temperature, and genetics may also affect uptake of specific elements into otoliths (Kalish 1989, Thresher et al. 1994; unpubl. data).

Dr. Hansen (NMFS) reported on an unpublished study in which he used solution-based ICPMS to study trace element signals in Atlantic croaker *Micropogonias undulatus* otoliths from Galveston Bay as part of the U.S. National Status and Trends Program. A series of stations was sampled along a salinity/pollution gradient. Hansen found a strong trace metal signal along the gradient and that Ca, Co, and Mg explained 90% of the variation in the elemental signal. He also found a higher weight percent of calcium in the upstream samples and a change in molar ratio along the salinity gradient. Hansen found some analytes particularly difficult to detect in otoliths. Zn was low but detectable in croaker otoliths. Also Hg, Pb and Sn were very low. Hansen suggested pollution might change the concentration of some metals in otoliths, possibly due to effects of pollution on physiology of the fish.

Dr. Thresher (CSIRO) presented a summary of his research on otolith chemistry for a variety of fishes, including research on southern bluefin tuna. Asked to address the question: Are otoliths environmental CD ROM's? he responded, "not entirely". Drawing on experience with southern bluefin tuna (SBT) as an example he elaborated. Thresher's laboratory found tuna otoliths to be relatively "clean" (i.e. low in trace contaminants) compared to other otoliths. They also detected significant ontogenetic variability in some elements, such as Sr, while other elements, such as Cl, were uniform in their distribution.

Thresher discussed problems they had encountered with sample contamination. In samples of morwong otoliths they encountered unusually high concentrations of Fe, Ni, and Zn in their analyses. They also found a Mo signal in SBT otoliths they deemed unusual. The unusual elemental patterns (contaminants) were found to be due to brief contact of the otolith surface with a stainless steel holder during polishing. They also had a problem with Zn contamination at the margins of otoliths that they associated with an organic source. In other analyses with morwong, *Cheilodactylus sp.*, they noted differences between the right and left otoliths pairs from the same fish. Sr concentrations matched exactly while other elements such as K, Na, Cl, S were different. They later determined that one otolith had been soaked in freshwater for 2 minutes while the other had not. After this experience they began experimenting with handling procedures to look for potential effects on the elemental signal. Thresher suggested for their spectrum of elements (Ca, Na, Sr, K, S, Cl) that freezing may be worst while fresh or ethanol preservation may be the best choice. He also noted that they detected little effect of their procedures on the trace elements. Thresher concluded that it may be best to remove otoliths from the live fish when possible and be careful with ultrasonic cleaning or soaking if one wants to work with Na, S, K, and Cl.. Further, it is important to treat all specimens the same way. Discussants at the workshop indicated that these elements may be easily lost from the otolith because they are not incorporated into the calcium carbonate portion of otoliths.

Technological Constraints

I. Elemental Patterns

Because otoliths show low rates of natural contamination in their calcium carbonate matrix, relatively few elements have contributed in characterizing elemental fingerprints in some of the most recent and technologically up to date studies. Campana et al. (1995) found that Ba, Li, Mg, Zn, Sr, and Pb explained most of the variation among groups of Atlantic cod, while in Thorrold et al. (1997) reported that Sr, Ba, Mg, and Zn were best for Atlantic croaker. Elements most useful to Edmonds et al.'s studies were Na, Mg, Si, P, S, K, Fe, and Sr. In preliminary ICPMS analysis on Atlantic and Pacific bluefin tuna otoliths Na, Mg, Ca, Mn, Ni, Zn, Sr and Ba showed measurable variations (Secor and Zdanowicz, unpubl. data). WDS and PIXE analyses of southern bluefin tuna (Proctor et al. 1995) found detectable levels of Na, K, S, Cl, Ca, and Sr.

In a presentation at the workshop, Tim Shaw (Univ. South Carolina, Chemistry Department) suggested that oceanographic differences between the Western Atlantic Ocean and Mediterranean Sea may result in substantial differences in ambient water chemistry. The Mediterranean is an evaporative basin which receives downwelling water from the Atlantic Ocean, and outflow of surface water results in a large influence of oceanic (metal depauperate - with the exception of Sr) water on the Mediterranean Sea. The Gulf of Mexico and coastal regions of the Western Atlantic are more heavily influenced by riverine and estuarine inputs of terrestrial metals. Barium in particular is expected to occur at higher levels in the Western Atlantic than in the Mediterranean Sea. Other transitional metals: Fe, Cu, Co, Mn, Cd, and Ni may also be enriched due to shelf diagenesis in western Atlantic waters. Anthropogenic sources of metals may also contribute to differences between water chemistry between the two principal nurseries. Another possibility is higher Pb in the Mediterranean Sea because Pb continues to be used as an additive in European gasoline. If differences in chemical oceanography between the nursery regions is reflected by otolith composition, candidate elements to discriminate stocks include the transitional metals - Fe, Cu, Co, Mn, Cd, and Ni - and Sr, Ba, and Pb.

II Otolith Handling and Cleaning Protocols

Surface contamination of otoliths can occur due to dissection, handling, storage, or cleaning procedures. Currently there is no agreed upon set of protocols to guarantee consistent interpretations among otolith microconstituent studies. Typically distilled water (Edmonds et al. 1989) or highly purified water (e.g. super Q water) have been used to brush, rinse, and sonificate otoliths to remove or reduce surface contamination and organic residues. Studies by Dr. Thresher's group (unpubl. data) have indicated that rinsing in water or acid can preferentially leach certain elements from otoliths. Dr. Campana reported that his group found no effect of water or acid rinses on the concentrations of Li, Mg, Ba, or Sr. The degree to which handling and cleaning procedures either contaminate or leach elements needs to be assessed for each application. Protocols can be tested on pairs of otoliths from the same fish, leaving one otolith from each pair untreated (e.g. uncleaned). In cleaning steps, water used to clean the otolith should be collected and analyzed along with the digested otolith to determine if elements were leached disproportionately to their occurrence in the otolith. Also procedural blanks should be run to detect and correct for sources of contamination other than the cleaning procedure itself. Probe-based techniques are especially vulnerable to contamination and leaching artifacts due to the increased amount of sample preparation required (sectioning and polishing) and by exposure of an internal plane to contamination and leaching.

III. Standardization

ICPMS has several advantages over other methods of trace element analysis including low detection limits and easy detection of ICP mass spectra. One of the major problems that can be encountered by ICP is formation of oxides and presence of polyatomic moieties which can make interpretation of certain mass spectra difficult (Olesik 1991). Operational skill and knowledge of the chemistry of various elements is essential to avoiding and correcting these potential pitfalls. In some cases, isotope dilution ICPMS can circumvent some of these problems; however, several potentially important elements lack the multiple isotopes necessary to apply this technique to all cases. While more costly and time consuming, isotope dilution ICPMS can also increase the precision of elemental analyses. This may prove essential for elements contaminating otoliths near the detection level of the instrument.

Another potential problem with ICPMS analysis is limitations associated with external standards used for otolith analyses. NIST traceable calcium carbonate (SRM 915a) can be used for otolith analyses. But while the NIST standard is certified, it does not reflect the spectrum of elements and calcium matrix of otoliths, which is significantly different than the NIST standard. Also the elements of particular concern, the trace elements, are not certified. This is a significant problem for otolith chemistry of bluefin tuna because the results from different laboratories must be comparable based on documented QA protocols and must be reproducible to avoid controversy in any data generated to aid in stock assessments. This potential problem could be resolved by producing an appropriate reference material to standardize analyses between laboratories and within a given lab.

Characteristics required for either a CRM (Certified Reference Material) are that the material be 1) stable in composition 2) have a reasonable shelf life and 3) have a chemical composition that can be accurately determined. Producing a CRM for otoliths could be extremely costly, time consuming and difficult. A CRM requires that a material similar in composition to otoliths be produced and then analyzed by multiple laboratories and multiple analytical methods. Once certified, the CRM is then typically sold to laboratories to recoup production cost.

Uncertified reference material (RM) is a common and less expensive alternative to standardization. Campana produced an RM for his cod research by removing otoliths from cod collected at a single site and then grinding and mixing them into a relatively homogeneous powder. Otolith RM can be made widely available if enough material exists and allow multiple laboratories to compare methods and data. Ultimately, a set of "consensus" values emerges that are useful and in many cases as accurate as certified values.

Hypotheses Development

Application of otolith chemistry depends upon a series of operational hypotheses.

1. A sufficient array of elements can be accurately and precisely measured in ABFT otoliths with currently available technology.

   Although current technology permits the quantification of elements at sub-ppb levels, elements which can be reliably measured and are useful for discrimination (i.e. those which provide discrimination among groups) typically number less than ten. Advancement of ABFT otolith chemistry must carefully consider all sources of bias in the measurement process and expect to develop discriminant functions on the basis of ten or fewer elements. Furthermore, differences in elemental patterns may be subtle and will require maximum possible precision.

2. Environments encountered early in the life history of ABFT are reflected in the chemistry within the core region of their otoliths.

   Research on relatively few elements indicate that otolith composition may reflect ambient water chemistry and temperature. However, physiological control in otolith deposition has been strongly implicated for certain elements (e.g. Ca, Cl, Na, K, and S). Thus the operational hypothesis is probably valid only for certain elements.

3. Once the calcium carbonate matrix of an otolith is formed, its elemental composition does not change.

   Research has shown that once incorporated into the otolith, calcium and strontium are not resorbed or remodeled. Similar studies remain to be performed for other elements. Certain elements, e.g. Mg, Sr, and Ba, are incorporated and retained in the $CaCO_3$ lattice structure. Others elements may be more easily lost from the otolith (e.g. Na, S, K, Cl) because they are associated with organic material or interstitial spaces. These interstitial spaces could make otoliths porous to chemical changes in the endolymph due to stress or death, or handling and decontaminating procedures associated with their chemical analysis. Studies of ABFT otolith chemistry should carefully consider possible leaching or contamination of elements due to handling and cleaning procedures.

4. Probe-based, or coring technologies can isolate portions of otoliths corresponding to nursery habitat.

   Successful application of otolith chemistry to stock identification assumes that all life stages can be identified according to nursery of origin. Therefore, the core region will need to be isolated from the otoliths of juvenile and adult ABFT. Further research is needed to standardize probe-based techniques and develop clean coring techniques.

5. The effect of annual variations in nursery conditions on the otolith are unimportant or can be precisely characterized.

   The core region represents the tuna's first year of life in either the Mediterranean Sea/east Atlantic or inshore coastal Western Atlantic Ocean nurseries. Annual variations in climate or oceanography could obscure an elemental pattern associated with either of the two nurseries. Early research should examine otolith composition of multiple year-classes from the two nurseries. If annual variation is important, then stock-specific fingerprints will need to be characterized for each year-class.

6. The effect of spatial variation within nursery regions on otolith composition is unimportant or can be statistically isolated.

Heterogenous distributions of ambient metal concentrations within nurseries could contribute variation in elemental patterns. Early research should examine otolith composition from several sites within each nursery. If spatial variation within nurseries is less than between nursery variation, then the application will be supported.

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