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Use of fish otoliths in habitat fingerprinting

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Abstract

Utility of Otolith chemistry in fisheries management has not been comprehensively examined and thus this review of articles is been done to compile these facts that indicates its applicability in habitat conservation by analysing its microchemistry. Otolith chemistry publications span a wide variety of topics starting from becoming an important technique for identifying and delineating fish stock units, their habitats, migration pattern of the highly migratory stocks. The capacity to differentiate between distinct groups due to differences in otolith chemical composition has made it a successful natural tag in stock discrimination experiments. Otoliths are continuously deposited components in fish that contain vital information about the environment. The history and development of otolith chemistry in general, as well as their application in the field of fish stocks and their management are briefly summarised here.

Keywords: ear bone, fish stocks, microchemistry, natural marker

Introduction

Health of Aquatic environment is always crucial to fish biodiversity. Many fisheries biologists are concerned about the aquatic environment in order to understand the relationship between fish and habitat so that they can focus on management and mitigation practices to improve habitat conditions so that it would be more conducive to live in. Some Studies shows that, habitat destruction is one of the major factors that leads to stock depletion and abrupt the migration pattern of many catadromous and anadromous species. This is a major issue in stock discrimination, fish distribution, habitat and migration pattern. Under the assumption that stocks loosely represent populations, tagging (Wise l963), morphometrics (Thorpe, 1976), meristics (Blouw *et al*. 1988), parasite loads (Scott and Matin 1957), ichthyo-plankton surveys (O'Boyle *et al*. 1984), immunological characteristics (Schill and Borazio, 1998) and other approaches have all been used to confirm the presence of multiple stocks, but none has provided a reliable indicator of stock identity.

Many biologists suggest that microchemistry and stable isotopic analyses of fish otoliths can be a major tool that can be used to find out the environment of fish where it grew (Zeigler and Whiteledge 2010) [65]. The trace elements in fish otoliths can act as natural geographic marker to discriminate fish stocks in Marine environment (Campana *et al*. 2000) [8].

The inner ears of all teleost fishes contain three calcified structures (Sagitta, Lapilus and Astriscus), which acts as balancing and hearing organs (Popper and Zhongmin, 2000; Popper *et al*., 2005). The structure is composed of calcium carbonate (approximately 96%) and trace elements present in a protein matrix (Campana *et al*., 1997) [9]. Also, sagitta otolith has been used as a taxonomical tool for identifying fishes due to their large size and degree of interspecific differences (Lagler *et al*. 1977; Harvey *et al*., 2000, Battaglia *et al*., 2010).

Otoliths remain unaffected by short-term changes in fish and therefore, it may be considered as a tool for species discrimination, stock analysis and even in testing the function and ecological significance (Cardinale *et al*., 2004). Therefore, it may be considered to serve as the permanent record of the life history of an individual fish (Clausen *et al*., 2007). Also, they hold a wealth of information on daily age, size, growth and ontogeny of fishes (Gerard & and Malca, 2011). Hence, they serve as one of the most reliable tools for identification of growth rates, structure of age in a certain population and for fisheries management. Furthermore, the analysis of microstructure and elemental microchemistry of otolith have greatly been developed for stock identification, feeding ecology of predators and the determination of migration direction in fish species (Campana & and Thorrold, 2001; McFarlane *et al*., 2010). Highly regional species and Non-migratory species may contain unique chemical composition in otoliths conforming of the location where they inhabit (Kingsford and Gillanders, 2000; Lo-Yat *et al*., 2005). Moreover, oxygen and carbon stable isotope ratios have also been used successfully as natural tags for fish population structure studies (Edmonds and Fletcher, 1997; Gao *et al*., 2004; Correia *et al*., 2011) [17, 21, 14].

Elements (isotopic) and microchemistry

Elemental microchemistry is an important tool to determine natal origin of fish stocks, population structure and migration patterns to address a wide range of ecological issues such as the extent of stock purity and habitats used by the stock (Campana, 1999; Elsdon *et al*., 2008; Pracheil *et al*., 2014; Ruttenberg *et al*., 2005) [6, 19, 49]. Otoliths are having three important key properties.

1. They continuously grow throughout daily deposition of calcium carbonate (CaCO3) aragonitic crystals on protein layers (Campana and Neilson, 1985) [10].
2. These are acellular and metabolically inert, so they neither reworked nor resorbed, even during times of starvation (Campana and Neilson, 1985) [10]. So they keep the information by the time frame permanently which is reported as natural tag (Panfili, 2002) [44].
3. The chemical elements in the otoliths is somehow influenced by the ambient water (Elsdon and Gillanders, 2003; Sturrock *et al*., 2012) [18, 54]. Nursery area Identification is a very significant tool which may ensure the regulations of sustainable management of fisheries (Beck *et al*., 2001; Colloca *et al*., 2009). A well-managed nursery area promote maintenance of fishery resources thereby improving the socio-economic status of the fishermen community.

There are so many elements (some are trace) present in otoliths that exert great influence in discriminating the fish stocks which may be habitat and/or species-specific variation. For example, Ba:Ca, K:Ca, Li:Ca, Mg:Ca, Na:Ca and Sr:Ca showed significant spatial variability in the Gangetic population of *S. aor* (Nazir and Khan, 2017) . A number of freshwater fish species, including *Oncorhyncus clarki lewisi* from the Coeur d'Alene River, have had similar reports on the Ba:Ca, Mg:Ca, and Sr:Ca ratios (Wells *et al*., 2003) [60]. The Ba:Ca, Sr:Ca and Zn:Ca were found to be of great influence in delineating the natal origin/ stocks in fish species like *Cichla temensis* and *Prochilodus lineatus* inhabiting Negro River basin of Uruguay and Parana rivers of the Plata basin respectively, (Avigliano *et al*., 2017; Garcez, Humston, Harbor, & Freitas, 2015) [1, 22].

Otolith chemistry could be utilised to distinguish *Heteropneustes fossilis and Channa punctata* fish stocks obtained from the Ganga and its tributaries, the Yamuna and Gomti rivers, with nearly full success (>96 percent). (Khan *et al*., 2012 [54] and Khan *et al*., 2014). Nazir and Khan (2018) found that Otolith chemistry appears to be more sensitive to detect even small variations in the environmental conditions that may not be producing variations at genetic and morphometric levels. This could be the reason for dramatic changes in trace element concentrations at spatial levels, effectively dividing the population into distinct stocks based on their location.

Impact of environmental changes on the otoliths

Environmental changes (salinity, temperature, water chemistry) and otolith chemistry are closely associated with stock discrimination or connectivity studies (Campana, 1999; Elsdon *et al*., 2008; Thorrold *et al*., 1998) [6, 19], the reconstruction of environmental histories of fish and description of fish movements and differential habitat use (Elsdon *et al*., 2008; Martin *et al*., 2013) [19]. For assessing spatio-temporal variations between otolith and ambient water chemistry can reveal the consistency of natural chemical markers between cohorts and years (Walther & Thorrold, 2009). Environmental factors primarily influenced the otoliths shape, rather than genetic factors (Campana and Casselman, 1993) [7]. Some elements (e.g., Sr, Ba) appear to be directly reflect ambient water concentrations, whereas some other elements like Cu, Zn have effects on the physiology and less likely to reflect adjacent environmental health (Campana, 1999; Sturrock *et al*., 2012; Izzo *et al*., 2018) [6, 54, 30]. Furthermore, some elements like Cu, Pb influences fish health and metabolic rates, resulting in differences in otolith composition. (Geffen *et al*., 1998; Hamer and Jenkins, 2007) [23, 24].

Because of the salinity gradients, freshwater, marine water and estuaries are radically distinct from one another. The concentration of Sr in fish otoliths varies depending on the chemical composition of the water and, the temperature of the environment (Campana, 1999; Wells *et al*., 2003) [6, 60]. It was found to be positively correlated with water salinity (Kraus and Secor, 2004), while the otolith Sr:Ca ratio was shown to be positively and negatively correlated with water temperature above and below 10 °C, respectively (Campana, 1999) [6].

Water concentrations of the microelements strontium (Sr) and barium (Ba) in coastal systems are inversely connected, with lower Sr and higher Ba in freshwater zones, despite their usage in environmental and migratory histories (Macdonald and Crook, 2010; Walther and Limburg, 2012). Estuaries are also known as a nutrient trap of the ecosystem due to the mixing of marine and freshwater inputs. Fishes are move in to these water bodies having different water chemistry, salinity pattern, temperature and trace elements.so there is a link occurs between the habitat use pattern and the life history of fish and otoliths chemistry. Estuaries may provide potential sources of Ba because this element is closely bound to fluvial sediments (Li and Chan, 1979; Coffey *et al*., 1997) [35, 12]. Another source of Ba may be from upwelling of oceanic waters (Lea *et al*., 1989) [34]. Sr:Ca was used as a habitat tracer because it reflected changes in water constituent composition between different type of aquatic environment. (Elsdon *et al*., 2008; Sturrock *et al*., 2012; Walther and Limburg, 2012) [19, 54]. Salinity and other parameters such as osmoregulation, on the other hand, have little effect on the link between aqueous and otolith strontium (Kraus and Secor, 2004). According to Gaetani and Cohen (2006), Sr incorporation in abiotic aragonite must be independent of salinity. Sr is a trace element whose concentration is modified by its surroundings (Bath *et al*., 2000; Elsdon and Gillan) [3].

There is a positive correlation between otolith Sr/Ca and water salinity (Bath *et al*., 2000; Secor and Rooker, 2000; Elsdon and Gillanders, 2002) [3, 53]. On the other side, otolith Ba concentration is negatively correlated with water salinity (Miller, 2011). The Sr/Ca and Ba/Ca ratios of otoliths were used to study the movement of catadromous and diadromous species from a fluvio-marine habitat (e.g. Tabouret *et al*., 2010; Feutry *et al*., 2012). Feutry *et al*. (2012) and Tabouret *et al*. (2010) look at the migratory patterns of three Kuhlia species and European eels between varying salinities of water bodies. There is a link between the environment and the otolith. Ba has been paired with its natural variation patterns, allowing it to be used as a tracer to reconstruct fish life histories as they occur (Elsdon and Gillanders, 2005a; Hamer *et al*., 2006). Bouchoucha *et al*. (2018) [5] found that Ba was the most discriminating element in the brackishwater habitat and its concentration was higher in the otoliths of Diplodus species. Endogenous factors (e.g., fish condition - Izzo *et al*., 2015) [29] can influence Ba concentrations in otoliths, although they mainly reflect ambient environmental concentrations (Campana, 1999; Elsdon *et al*., 2008) [6, 19]. Sarkar *et al*. (2015) discovered that greater Ba and Sr concentrations in the ganga basin's mid and downstream suggested the O. bimaculatus' preferred habitat. Strontium isotopes were employed for a vulnerable estuary fish species to detect nursery locations. (Hobbs *et al*., 2010).

Reis-Santos (2013) [47] discovered a positive association between temperature and Sr:Ca in otoliths, although there have also been reports of negative and non-significant correlations between otolith Sr:Ca and temperature (Campana, 1999; Elsdon *et al*., 2008; Sturrock *et al*., 2012) [6, 19, 54]. Higher Sr:Ca concentrations are linked to greater salinity water, while higher Ba:Ca ratios are linked to freshwater (Campana, 1999; Elsdon and Gillanders, 2003a; Elsdon *et al*., 2008) [6 18, 19]. Temperature, salinity, and biological parameters like development rate, however, can all affect the Sr:Ca and Ba:Ca ratios (Campana, 1999) [6].

Further, elements such as Li, Mg, Mn, Zn and Cu incorporated within otoliths are also affected by environmental features such as water chemistry, temperature and diet of fishes (Bouchard, Thorrold, & Fortier, 2015; Ranaldi & Gagnon, 2008) [4, 46]. There is no clear association between the content of Mn and Mg in otoliths and the concentration of Mn and Mg in surrounding water, and change appears to be connected to unknown endogenous and exogenous processes (Martin and Wuenschel, 2006; Hamer and Jenkins, 2007; Woodcock *et al*., 2012) [39, 24]. Mn and Mg are essential for a number of cellular functions, implying that modulation of such elements can produce significant physiological reaction (Hamer and Jenkins, 2007; Barnes and Gillanders, 2013) [24, 2]. Manganese is persist in considerable amount from the beginning of the otolith via maternal transmission, and has thus can serve as a reference point in the environmental alterations in various species (e.g. Ruttenberg *et al*., 2005; Warner *et al*., 2009; Clarke *et al*., 2011) [49, 59, 11]._Li presents a high success rate for distinguishing fish reared at different salinities, as the ratio Li/Ca increases with salinity under controlled conditions (Hicks *et al*., 2010) [26].

Table 1: It contains the components as well as their impacts in that specific environment.

Elements	Types of water resources	Analyse	Fish species	Effects	References
Al, Bi, Cd, Co, Cu, Ga, Mn, Ni, Pb, V, and Zn	Hudson River Estuary	Habitat Use	American Eels	AI, Bi, Cd, Mn, Ni, and V contributed most to the discriminant function.	Zikri Arslan and David H. Secor, 2005
Sr/Ca and Ba/Ca ratios	freshwater–estuarine environment	Habitat	silverside (*Odontesthes bonariensis*)	Increase the Sr/Ca ratio with increasing water onductivity	Avigliano *et al*., 2014
Sr:Ca and Ba:Ca	Saline (high, medium, low)	Effect of env. parameters	European sea bass *Dicentrarchus labrax*	Sr:Ca and Ba:Ca increased with increasing at all salinities concentraction.	Reis-Santos *et al*., 2013 [47]
Ba:Ca	Different saline water	Effect of env. parameters	Chinook salmon *Oncoryhynchus tshawytscha*	three-way interaction between temperature, salinity and water Ba:Ca ratios	Miller, 2011 [36]
Sr, Ba, and Mg, Al, B, Cu,Li,Pb, Zn, Ca, Na, Mg, Mn	Lake Superior	coastal wetland habitats and nursery area	yellow perch, *Perca flavescens*	Large fish having high concentration of Sr	Brazner *et al*., 2004
18O and 87Sr	Great Lakes	macrohabitat use	barramundi, *Lates calcarifer*	Stable isotope analysis	Pender and Griffin 1996
18O and 87Sr	Great Lakes	macrohabitat	striped bass, *Morone saxatilis*	Stable isotope analysis	Secor and Piccoli 1996, Secor *et al*. 2001, Zlokovitz *et al*. 2003
18O and 87Sr	Great Lakes	macrohabitat	Atlantic croaker *Micropognias undulatus*	Stable isotope analysis	Thorrold *et al*. 1997 [56]
87Sr/86Sr isotope ratios	Great Lakes	inter-habitat movements	Atlantic salmon (*Salmo salar*)	Sr isotopes should be investigated as a habitat discrimination tool	Kennedy *et al*. 2002
24Mg, 51V, 55Mn, 63Cu, 66Zn, 88Sr,118Sn, 138Ba and 208Pb	Brackishwater body	Nursery habitat	Diplodus. vulgaris *D. sargus*	Ba discriminate the brackish water body and the conc. Is higher	Bouchoucha *et al*., 2018 [5]
23Na,24Mg, 88Sr, 115In, 138Ba, 43Ca	Marine water in southern Australia	stock discrimination	mulloway (*Argyrosomus japonicus*)	Ba:Ca and Sr:Ca conc was highest	Ferguson *et al*., 2011 [20]
44Ca, 88Sr, 137Ba,26Mg, 55Mn, 7Li, 60Ni, 54Fe, 208Pb and 66Zn	shallow coastal waters in south Brazil	population and connectivity studies	coral reef fish *Stegastes fuscus*	To assess the connectivity among fish groups	Daros *et al*., 2016 [15]
Li, Mg, Mn, Sr, Ba, Zn	Marine environment	Stock identification;	adult cod (*Gadus morhua*)	suited as biological tracers of groups of fish	Campana *et al*., 2000 [8]
Sr/Ca, Ba/Ca, Mg/Ca,	Marine water NE	Population structure	blue jack mackerel	Increases the Sr, Ba decreases	Moreira *et al*., 2017

Pb/Ca, Li/Ca, Fe/Ca and Mn/Ca	Atlantic		(*Trachurus picturatus*)	Li, mg, Mn	
Ba, Sr, Zn, Mn	Ganges Basin, India	Potential habitat	Ompok (Siluridae)	Ba and Sr are the main determinants for habitat preferences	Sarkar *et al*., 2015
Li, Mg,Ca, Mn, Zn, Ba, K, Na, Sr	Ganga basin	Stock discrimination	Sperata aor	Ba and Sr used to discriminate the stock, others are ontogenic	NAZIR and KHAN, 2018

Role of oxygen and carbon isotopes

δ^{18}O in otoliths and water δ^{18}O is deposited in equilibrium, although temperature and salinity alter it. Temperature affects the fractionation factor of δ^{18}O in otoliths (Thorrold *et al*., 1997) [56] and a negative linear relationship between water temperature and δ18O in otoliths has been shown for various species (Høie *et al*., 2003). Salinity is also widely employed as a proxy for water ^{18}O values, particularly in shallow coastal areas, where the fractional quantity of runoff-sourced freshwater is present, and the δ^{18}O profiles of otoliths can reflect seasonal freshwater inflow (Matta *et al*., 2013). It is also known that ontogenetic changes in trophic levels that comprise the fish diet and metabolism can affect δ^{13}C values measured in fish otoliths (Schwarcz *et al*., 1998; Gao and Beamish, 2003; Gao *et al*., 2004) [21], but may also reflect geographic variation in the δ^{13}C of the dissolved inorganic carbon (DIC) of the ambient water (Thorrold *et al*., 1997; Patterson, 1999; Solomon *et al*., 2006) [56]. The coastal upwelling not only boosts food availability, but it also allows larvae and juveniles to remain in the area, highlighting the increased δ13C values detected in the otoliths (Roy *et al*., 1989; Santos *et al*., 2001; Sala *et al*., 2013) [51].

Aside from these, numerous factors like food and feeding habits of fish also tend to affect the chemistry of otoliths (Walther and Thorrold, 2006; Webb *et al*., 2012) [58]. Because of changes in metabolism and otolith generation, trace element accumulation in otoliths varies by species (Geffen *et al*., 1998; Hamer and Jenkins, 2007; Vasconcelos *et al*., 2007) [23, 24, 57]. The buildup of trace elements in fish otoliths with the environment and their bioavailability are influenced by the fish's physiological condition (exchange rate between the external and internal environments) (Bouchoucha *et al*., 2018) [5].

Advantages

- Whole otoliths remain stable in any kind of the environment over a long period of time.
- It expands in size throughout their lives.
- It's a stock identity indicator.
- It serves for tracking stock migrations

Campana *et al*. (2000) [8] investigated that otoliths element used as a natural tags having three central assumptions:

1. Each group has distinct and repeatable identifiers,
2. The group mixing has been characterised by all conceivable groups.
3. The marker remains consistent during the time between characterization and mixing

Assumption-1. Each group has distinct and repeatable identifiers.

When elements (such as Na, K, S, P, and Cl) are subjected to strict physiological regulation, there is little diversity among fish populations (Thresher *et al*., 1994; Proctor *et al*., 1995), and are hence unlikely to be used in elemental fingerprinting. Even physiologically controlled elements (Li, Mn, Ba, and Sr), on the other hand, should be suitable for use as a group identifier if they vary among groups. But the rate of Mg incorporation, which is strongly physiologically regulated, is probably impacted mainly by otolith crystallisation rate through the proxy of water temperature (Mitsuguchi *et al*., 1996). (Mitsuguchi *et al*., 1996). All the elements are not chemically stable and reproducible group-specific indicators.

Assumption 2: The group mixing has been characterised by all conceivable groups.

The uncharacterized fishes contained in the mixture, which could be considered one or more of the reference groups in this situation (Wood *et al*., 1987, 1989; Wirgin *et al*., 1997). It can be reduced if the samples are taken at a period when the groups are known to be quiet (e.g., on the spawning or nursery grounds). Reference samples acquired at a less aggregated time may be used depending on the hypothesis being investigated.

Assumption 3: The marker remains consistent during the time between characterization and mixing

The elemental composition of otoliths, as well as the stability of an environmentally induced marker, cannot alter significantly over time. Because the group-specific marker must stay constant during the interval between characterisation (e.g., spawning group) and mixing, such short-term consistency is a crucial requirement of the biological tracer technique.

Current challenges

Due to the climate change and habitat alteration the aquatic environment faces so many stress. So that the fish stock delineate day by day. Diet variations associated with life cycle phases may have a confusing influence on group specific tags, particularly for stock delineation studies, because fish occupying the same habitat but feeding on different food may have different otolith chemical compositions. Furthermore, dietary adjustments toward higher trophic levels will result in larger otolith concentrations of bioaccumulating elements (e.g., Gray,2002). Regarding ontogeny, Though the exact processes are unknown, there is evidence that physiological changes associated with age and growth affect elemental uptake and incorporation (Sturrock *et al*., 2014). According to Darnaude *et al*. (2014) [16] used oxygen isotope ratios in fish otoliths to differentiate marine stocks and recreate previous temperatures. They observed that physiological reactions to temperature differed between sub-stocks, and that, despite an overall i18Otrend of otolith mostly mirroring environmental temperature i18O and salinity, the mechanisms underlying otolith incorporation were complex.

Genetic effects on otolith elemental incorporation can have large confounding effects on otolithchemical data interpretation, which has consequences for stock delineation. The intricate combination of internal and extrinsic factors that leads in otolith elemental incorporation was highlighted. In the most extreme situation, genetic variations in otolith chemical composition attributable to environmental variation may exceed or hide differences in otolith chemical composition caused by environmental variation (Clarke *et al*.,

2011) [11] and Barnes *et al.* (2013) [2]. One of the limiting constraints for its application in the marine environment has been pointed out as the elements (e.g., Mg, Ca, Mn, Sr, andBa) relatively homogenous distribution in otolith chemical analysis (Sturrock *et al.*, 2012; Chang and Geffen, 2013) [54]. Isotopic ratios of O and C have been found to be the greatest option for improving spatial resolution when used alone or in combination with element Ca. Ecogeochemical techniques and produced isoscapes (spatial maps of expected isotopic variation) have recently revealed significantly larger regional variability than previously thought, allowing them to be used as the foundation for migration patterns and stock delineation investigations (Darnaude *et al.*, 2014) [16]. Furthermore, Manganese can be used to track some habitat-specific environmental variables like hypoxia, which could be important in stock assessments (Limburg *et al.*, 2011, 2015) [36, 37].

Overall, otolith chemistry has the potential to increase our understanding of population structure, as well as spawning components, natal origin, and migration patterns. Using otolith chemistry, we are able to overcome the aforementioned obstacles and close information gaps in population structure and connection research.

Conclusion

The Indian fishery is multispecies, multifleet, and open access means most of the stocks are a mix of several species. Anthropogenic stress on the aquatic environment is on the rise as the human population grows. Pollution (especially plastic pollution) and habitat deterioration in many aquatic environments are increasing as urbanisation progresses. In order to conserve any species, it is necessary to identify its habitat it resides. Maximum likelihood stock mixture analyses using genetic or morphometric markers have now proven themselves as a reliable method for estimating group proportions in a stock mixture (Wood *et al.*, 1989; Millar, 1990; Wirgin *et al.*, 1997), Because both genetic and otolith markers serve as multivariate tags that can be utilised to estimate mixture proportions rather than individual identifications, stock mixture analyses based on otolith elemental fingerprints work in parallel. These techniques can provide stronger evidence for population structure, as well as insight into the mechanisms that maintain population structure. The application of structural chemistry can be particularly significant in circumstances with delicate population structures that may not be recognised by genetic applications alone. Continued otolith chemistry research and development in stock delineation studies can help to promote interconnection between management actions and biological processes, as well as the design of effective fishery management units.

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