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REVIEW ARTICLE

Fish Otolith Analysis in Southeast Asia: Expanding its Taxonomic Roots to More Ecological Targets

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ABSTRACT

Otoliths, crucial tools for understanding fish biology and ecology, offer valuable insights into fisheries science in Southeast Asia. Traditionally used for age identification through shape analysis and growth ring counting, otolith studies have evolved to encompass broader ecological investigations. This review synthesizes literature on fish otoliths in Southeast Asia to identify research gaps and suggest future directions. Utilizing targeted keywords, we mined data from scientific search engines, focusing on peer-reviewed articles. Our analysis spans 1988 to 2021 and collated 129 publications, which were trimmed to 91 reviewed scientific articles. Otolith microstructure emerged as a primary research area of interest, with a focus on catadromous fishes from the Anguillidae family. Geographically, publications are concentrated in marine fish populations across archipelagic nations such as Indonesia, the Philippines, Malaysia, and the Vietnam Peninsula. Practical gaps, including validation precautions and cost-benefit analyses, potentially underscore the importance of methodological rigor and innovation in otolith research, but advances in mass-based age determination techniques and radiometric analysis present promising avenues for addressing these challenges. As global otolith research expands beyond aging studies, we urge fisheries scientists to explore innovative research areas outlined here to enhance their expertise in otolith microstructure and morphometric techniques. By integrating innovative approaches and multiple validation methods, researchers can enhance the reliability and accuracy of otolith-based age determination, ultimately advancing our understanding of fish population dynamics and supporting sustainable fisheries management practices in the Southeast Asian region.

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1. Introduction

Fish otoliths are not only anatomical structures but also invaluable tools for understanding fish ecology and informing fisheries management practices. To understand the significance of otoliths in fishery science, it is important to study their anatomy, physiological function, and utility in fish studies. Otoliths, tiny structures embedded within the inner ear cavity of all teleost fishes, play an important role in assessing fish populations across various aquatic environments (Stevenson and Campana 1992). Composed primarily of calcium carbonate (CaCO3), specifically in the form of aragonite, otoliths resemble bones in their fully calcified state (Fortaleza and Nanola 2017; Luceño et al. 2018). Functionally, these structures aid fishes in orienting themselves and maintaining balance during locomotion (Figure 1). The vestibular labyrinth of the fish's inner ear contains three pairs of otoliths: lapillus, asteriscus, and sagitta, each comprising left and right structures (Tuset et al. 2003). Sagittal otoliths are predominantly utilized in research and assessment (Nolf 1995). Through analyses of their morphological, microstructural, and microchemical features, otoliths serve as repositories of crucial data pertaining to fish growth and habitat at a temporal scale (Kalish 1989; Stevenson and Campana 1992).

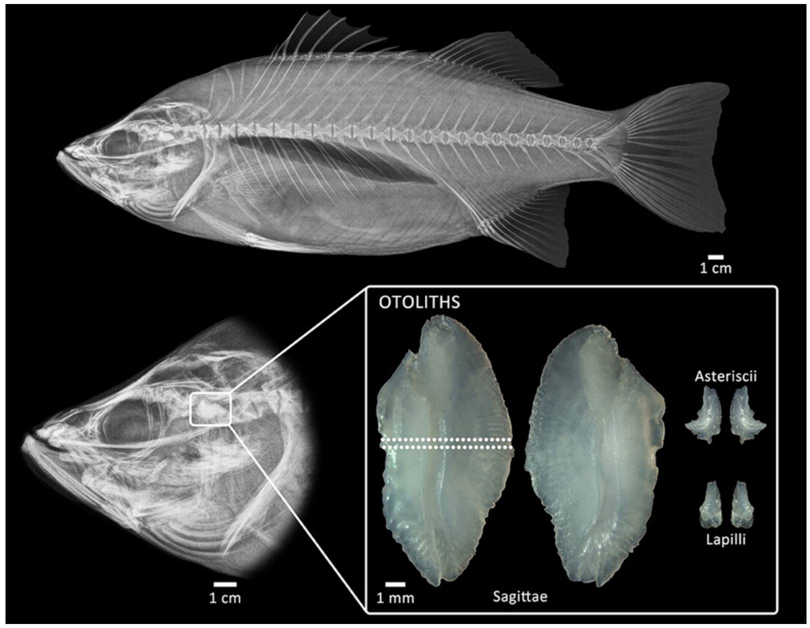

Figure 1. Details of teleost fish x-ray, showing the location of otoliths. Fish have three pairs of otoliths: sagittae, asteriscii, and lapilli. Dotted lines in the sagitta represent areas where the otolith is sub-sectioned to reveal the nucleus and growth rings. Image was adapted from Morais et al. (2018), with permission for use from Dr. Kathryn L. Phillips (University of California, Davis).

The morphological structure of fish otoliths provides vital information about fishes and their life history (Popper and Tavolga 1981; Rodriguez-Mendoza 2006). Analyses of the increments, as well as the shape of the otoliths, have been utilized for population analysis and growth (Gust et al. 2002; Megalofonou 2006; Molony and Choat 1990; Neuman et al. 2001). Technological advances in recent years have also allowed the microchemical analysis of these structures, enabling the examination of approximately 50 major and trace elements in the otolith and providing insights into fish environmental histories and physiological information. Such technological developments have propelled this research field to prominence in the 21st century, with new methods and discoveries continually emerging on a global scale (Campana 2005). Otolith studies are a well-established research field in fisheries science worldwide. However, similar topics of interest in otolith research are less explored in tropical Southeast Asia compared to our temperate counterparts (Blackman et al. 2021) despite the high fish biodiversity found in tropical freshwater and marine ecosystems in the region. Tropical Southeast Asia, comprising Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar (Burma), the Philippines, Singapore, East Timor, and Vietnam, is known to host nine biodiversity hotspots exhibiting high occurrences of endemism due to the region's archipelagic nature (Myers et al. 2000; Hughes 2017; Kano et al. 2013).

Despite the significant fish diversity in the region, there is a paucity of systematic reviews on otolith studies in Southeast Asia. Such a synthesis is crucial now, given the various opportunities for innovative methods in otolith research and the numerous threats to fishery stocks, ranging from climate change to the increasingly unsustainable demand for fish products in the international market (Nong 2019). In this paper, we compiled and analyzed published fish otolith research in Southeast Asia to underscore its utility as a tool for environmental, biological, and conservation studies, identify prevailing research trends in the region, and recommend areas for further research based on the identified gaps in the literature review.

To compile a comprehensive review of literature, a systematic search was conducted across various academic databases, digital libraries, and online search engines such as Google Scholar, ScienceDirect, the National Center for Biotechnology Information (NCBI), and Journal Storage (JSTOR), with a focus on otolith research studies published in the Southeast Asian region from September 1, 1988, up to July 31, 2021. Key terms, including [OTOLITH or FISH OTOLITH] combined with [NAME OF SOUTHEAST ASIAN COUNTRY], were utilized to ensure a structured approach to data collection. Efforts were made to access full-text articles from all journals, although in some cases, only abstracts of older material were available. Subsequently, a digital database was established, with each article tagged with metadata such as title, authorship, study location, primary research area, sub-research area, publication year, journal name, and complete citation. To facilitate organization, papers were initially categorized into five main areas: morphometry, microstructure, microchemistry, or mixed topics, and further subdivided into relevant sub-research areas such as age determination, migration patterns, and stock assessment. We acknowledge that despite these efforts, some articles may have been inadvertently omitted due to limitations in search terms. To address this potential gap, thorough reviews of article references were conducted to ensure comprehensive coverage of relevant literature.

2. Southeast Asian otolith research throughout the years

A total of 129 published data entries were retrieved and trimmed to 91 primary reviewed scientific articles for this review. Increasing interest in otolith research, spanning from 1998 to 2021, is evident across different countries and regions within Southeast Asia (Figure 2). Over the past decade, there has been a three-fold increase in the number of published papers, with a corresponding rise in the number of publications per major research area. The utilization of analytical chemistry in the "big data era" has facilitated interdisciplinary collaborative topics (Valcarcel 2012), including otolith microchemistry, which involves elemental analysis of hard accretionary tissues in aquatic animals (Gibson-Reinemer et al. 2009). From 2010 to 2021, otolith morphometric studies emerged as a popular topic, despite the global establishment of otolith microchemistry research since the 1990s. This trend is likely attributed to equipment limitations and local expertise. Studying the structure, form, and size of both fish and their otoliths is more replicable, requiring only general laboratory materials for observation and measurement, in contrast to highly technical topics such as isotope analysis, which demand precision equipment and costly laboratory supplies.

3. Geographic distribution of otolith-related publications in Southeast Asia

Among the literature, several published papers were studies that encompassed a regional setting, where the samples were analyzed across multiple countries. In these, we listed the paper as a representative count for each country. Across all countries, no studies were focused on fish from Singapore, Brunei, and East Timor.

Indonesia yielded the greatest number of published studies, with 36 (24%) studies in total. Published otolith research in the country is well distributed among the three major research areas, with microstructural studies leading with n = 14 papers. Popular sub-research areas in the country include age determination, migration, and early life history. Indonesia is known as the largest archipelagic country in the world, and its unique geography allows numerous fish species to flourish, therefore becoming one of the biggest biodiversity hotspots in the world, only second to Brazil. The archipelagic nature of the country allows the formation of three distinct biodiversity hotspots and one of the world's largest centers of endemism: the Sundaland, Wallacea, and Sahulland, all of which have high species diversity. There were also instances where habitat differentiation of fish species was analyzed through otoliths, such as that for *Anguilla celebesensis* from the Indonesian and Philippine coasts.

Vietnam had the second most otolith studies conducted, with 31 (20%) published papers. Here, morphometry and microchemical studies (n = 11) are more represented than microstructural studies. Many of these were done in the Mekong River Basin (MRB), looking into migration routes and patterns of fish species along this large river system. A myriad of studies concerning interspecific variation, as well as the discovery of new fish species, was also observed. MRB is known for its high fish diversity and is considered the second most biodiverse river in the world, next to the Amazon River. The river is also known for its high levels of migratory and cryptic fish populations; thus, assessment of genetic structure and diversity in the river system is a common trend observed in Vietnam, as well as the countries included in the Greater Mekong Sub-region.

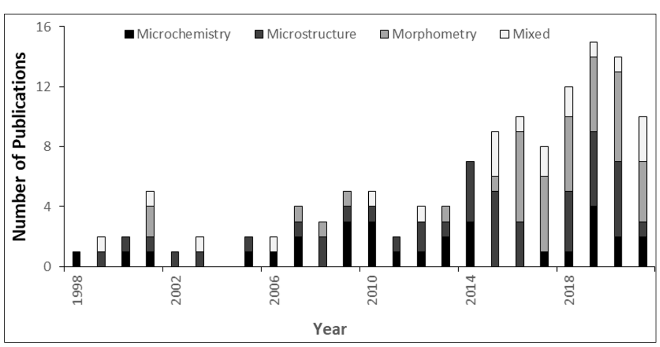

Figure 2. Timeline of otolith-related publications in Southeast Asia from 1998 to 2021 subdivided into four major research areas.

Other countries that encompass the Greater Mekong Sub-region also have a differing focus on fish otolith research. In Thailand (n = 14, 9%) and Laos (n = 13, 8%), most otolith research focused on fish growth and dimension, habitat, stock assessment, and migration. In Myanmar (n = 6, 4%), stock assessment and fish aging studies are the most common. Lastly, otolith research in Cambodia (n = 5, 3%) was commonly microchemistry and migration studies, but these are not distinctly unique to the country, as all of the otolith research in Cambodia were part of a larger-scale research funded by other countries. It is important to note that most research on the otolith of freshwater fish is focused on those countries surrounding the Mekong River.

A total of 25 (16%) otolith studies have been conducted in the Philippines, and the majority of these studies have focused on otolith microstructure. Trends of otolith research in the country are inclined toward age determination and somatic growth of fish through otolith microstructure examination, most of which focused on tropical eels (*Anguilla* spp.) The Philippines is also considered a biodiversity hotspot for freshwater fishes and home to numerous bodies of water, from rivers to lakes. Despite this, freshwater studies in the country are still lacking and highly fragmented. Similarly, Malaysia also recorded 23 (15%) otolith studies, with the majority of those focusing on otolith microstructure and microchemistry. Similar to the Philippines, trends are highly inclined toward fish age determination and somatic growth. Many habitat and migration studies are also common in Malaysia, which is known for its high fish diversity.

It is important to note that among these published works, otolith research among freshwater fish is highly skewed to MRB, with little to no other studies among lakes and rivers across tropical Southeast Asia. Transboundary water systems are common in the mainland due to the physical connectivity of some countries. MRB runs through multiple countries in the region, and it is the longest river in Southeast Asia, running through China, Myanmar, Laos, Thailand, Cambodia, and Vietnam, comprising the Greater Mekong Sub-region. The river serves as a breeding ground and migratory corridor for numerous fish species. While gathering data, studies in the Mekong River were abundant, leading to instances wherein a study might encompass two or more countries, and these were most common in otolith studies focusing on migratory patterns and life history. This was evident in the study of the life history of *Pangasius krempfi* along the Mekong Delta, where sampling was done in multiple sites throughout the river, encompassing multiple countries. This is an important gap to explore, as both marine and freshwater biodiversity are threatened in the region because of habitat degradation, over-harvesting, and other anthropogenic activities.

Predictably, the countries with the greatest number of publications also had access to open waters, such as the West Philippine Sea and the Indian Ocean. This includes Indonesia, Vietnam, the Philippines, and Malaysia, and thus the number of publications can also be associated with the country's geographical features. Countries such as Laos and Cambodia had limited to no access to open waters, as both countries are mostly landlocked. However, there were also instances wherein even countries surrounded by waters, such as Singapore, Brunei, and East Timor, had no publications produced. Not to mention, most of these countries, despite not having access to open waters, have a relatively good number of freshwater ecosystems. It is important to note that interest in otolith research must also be a limiting factor, as publication- and funding-rich countries like Singapore seem not to have otolith-related publications. We can only infer that the noticeable scarcity of fish otolith studies in Southeast Asia might be related to the limited number of fisheries experts interested in working on fish otoliths within the region.

4. Research topics and fish families of interest

Microstructure studies are still the most evident topic across the years within Southeast Asia, comprising a total of 42 studies (33%). This is followed by morphometry, microchemistry, and mixed studies, with 38 (29%), 30 (23%), and 19 papers (15%), respectively (Figure 3c). Microstructure studies analyze increments present in the cross-section of fish otoliths as well as on their polished and unpolished otolith surface. These increments can be utilized for population analysis, specifically estimating age and growth rates, timing of life history events, determining somatic response to habitat change, or shift among fish populations (Gust et al. 2002). Furthermore, fish ecology in response to different environmental variables can also be inferred. Perhaps the reason behind the abundance of studies focusing on this approach is its importance in determining the age structures of stocks for fishery management. Morphometry studies, on the other hand, correlate the allometric relationship existing between otolith size and somatic growth rate of fish, as well as the various possible factors that might have impacted this correlation (Megalofonou 2006; Molony and

Choat 1990; Neuman et al. 2001). Identification of fish species and determination of variations between species have also employed this method. Habitat zones of fish species in relation to depth can also be known to influence the size of otoliths directly (Paxton 2000; Lombarte and Lleonart, 1993). Moreover, changes in temperature can also cause an accumulation and formation of translucent zones in the otolith microstructure, which can indicate seasonal changes as well as migratory patterns through analysis of otolith microchemistry (Pilling et al. 2007; Zlokovitz et al. 2003). Both microstructure and morphometry are considered traditional approaches in otolith science and are expected to comprise the bulk of most otolith research in the region.

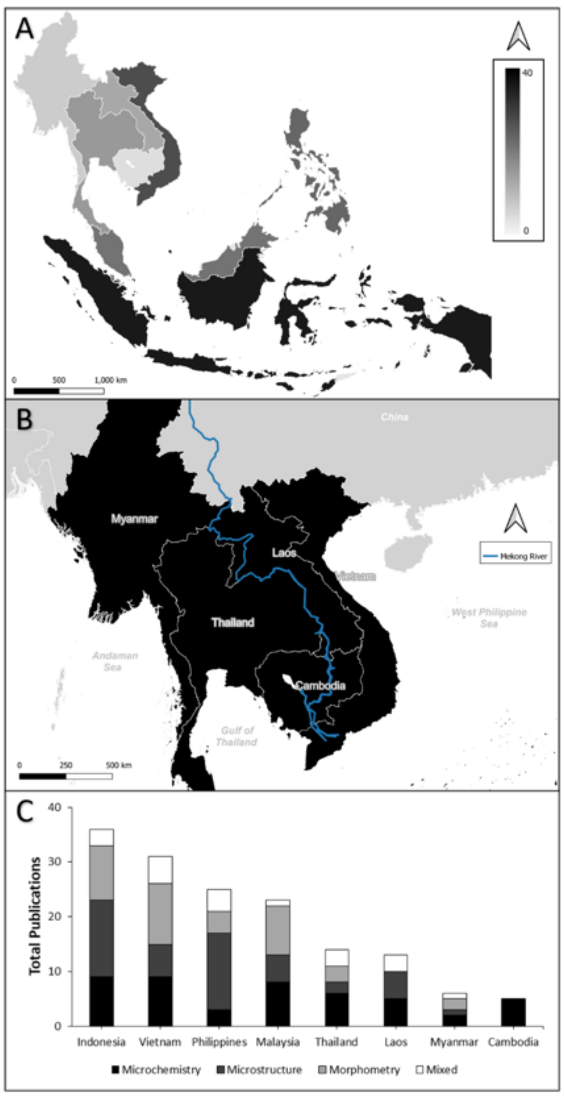

Figure 3. Geographic distribution of otolith-related publications in Southeast Asia. (A) Geographic map visualizing the number of publications per country. (B) The Greater Mekong Subregion comprising of China, Myanmar, Laos, Thailand, Cambodia, and Vietnam. (C) Bar graph showing the distribution of publications per country according to the four major research areas.

Overall, otolith microchemistry has the least number of studies per research area. Chemical analysis of otoliths can deduce physiological information in response to environmental stress. Aragonite in otoliths is usually contaminated by a variety of ions from its environment (Rodriguez-Mendoza 2006; Radtke and Shafer 1992). These calcified structures are decoded in otolith microchemistry and are analyzed to determine fish environmental histories. Examination of strontium (Sr) and barium (Ba) concentrations reveals differences in temperature and salinity of the water environment for fish species, providing information about the migratory history of diadromous species (Tzeng et al. 1997; Elsdon and Gillanders 2004). Variation in elemental concentrations has also been quantified in numerous studies relative to different environmental conditions, including occurrences of environmental disasters such as oil spills, pollution, as well as volcanic activities (Peebles and Hollande 2020; Campana 1999). Compared to the other methods, microchemical analysis arguably needs a more technical approach.

The number of otolith research publications in Southeast Asia encompasses a variety of sub-research areas. A total of 15 different topics have been identified out of the 129 studies analyzed (Figure 4a). As expected, there were overlaps with some of the topics in the majority of the studies conducted; thus, these were all counted separately. It is noticeable that despite the diversity of research areas that the different papers explored, there is still a clear and huge inclination towards otolith morphology and microchemistry studies. Age determination has been a very popular research area in the region ever since the early 2000s, with 46 (26%) publications. Age determination is made possible due to the calcified structures in the otoliths that are proven to be a vital tool in assessing anything related to the fish itself, as the data on age can be used in understanding important characteristics of a species and its population (e.g., age at recruitment and sexual maturity, reproduction periods, lifespan, migration patterns, mortality, etc.) (Panfili et al. 2002). Thus, age determination is the backbone of otolith research, as most otolith studies are dependent on observable increments (Palla et al. 2016; Bobiles et al. 2015; Morioka et al. 2016).

Otolith dimensions are the second most studied research topic in the region, with 45 (25%) papers. The dimensions of fish and their otoliths are usually compared because of the allometric relationship between the two (Stevenson and Campana 1992).

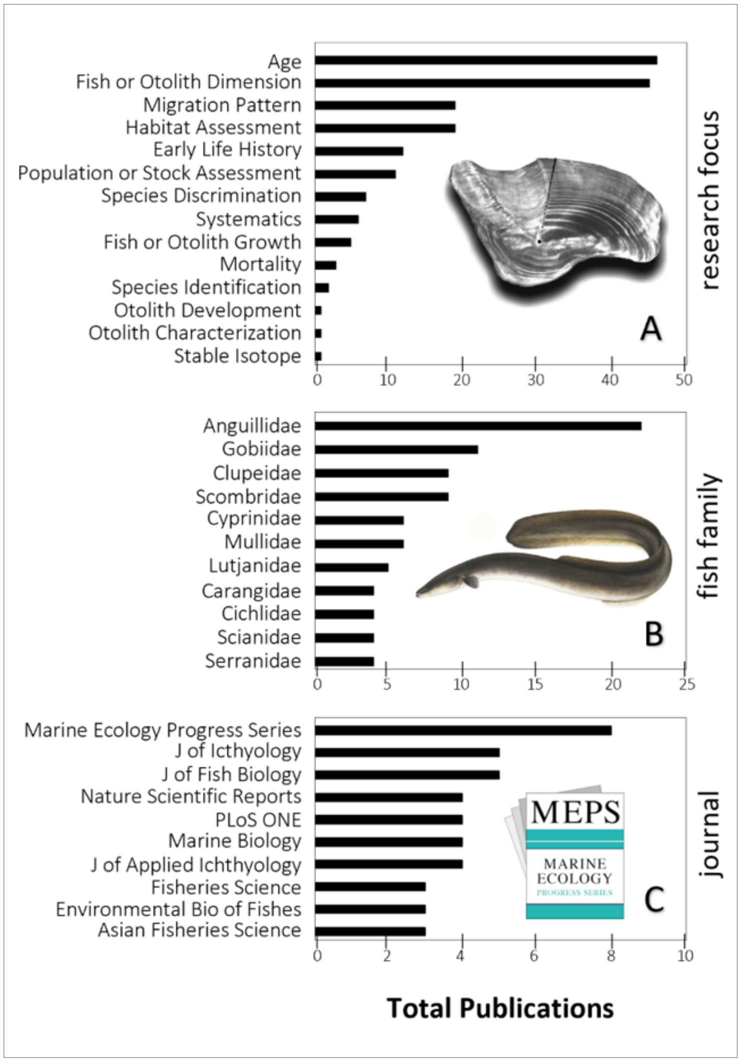

Figure 4. Summary of publication contributions to otolith studies in Southeast Asia. (A) Distribution of research topics otolith-related publications; otolith ageing photo courtesy of Steve Vanderkooy. (B) Studied fish families for otolith research; anguillid drawing courtesy of Wildlife Journal Junior. (C) Top 10 journals that published otolith studies in Southeast Asia; Marine Ecology Progress Series icon lifted from website.

Otolith growth is caused by incremental deposition in the structure, and this corresponds with the somatic growth of the fish. Thus, this information can be used for the estimation of age, growth rates, and even the assessment of populations and stock (Jutagate et al. 2013; Kwangkhwang 2016). Otolith dimensions are also used for species identification and habitat discrimination (Utayopas 2001; See et al. 2016). The asymmetry of otoliths requires proper analysis of their dimensions and geometry, which is partnered with proper standardization to ensure the data being extracted from these structures are accurate and reliable. Other than genetic factors, the shape of the otolith can also be affected by the fish's environment, and differences in water properties, such as salinity, pH, as well as differences in the water's chemistry, can be determined via its structure (Clark et al. 2021; Tanner et al. 2013; Ferguson et al. 2011).

Habitat assessment and migration patterns are the next most studied topics, with 19 papers (11%) each, respectively. These two research areas largely employ microchemical analyses in relation to differences in environmental conditions. Sr/Ca ratios and temperature are primarily the topics of microchemical studies as they can be used to reconstruct environmental and migratory histories (Radtke and Shafer 1992). Otolith research on early life history (n = 12 papers, 6.7%) and population stock assessment (n = 11 papers, 6.2%) are the next most popular otolith research topics in Southeast Asia. Early life history mostly includes physiological change and habitat shift studies. It is also one of the earliest research topics that have been published in Southeast Asia (Arai et al. 1999). Population stock assessment, on the other hand, primarily focuses on the strategic management and sustainability of fish stocks in a specific body. Shortage of fish has been a huge problem in Southeast Asia due to the overexploitation of resources, and studies focusing on fish populations are beginning to become more and more relevant (Mogea et al. 2019; Koolkalya et al. 2020; Jamal and Susanto 2020).

The other four research areas all have fewer than 10 publications each, namely species discrimination (n = 7, 3.9%), fish systematics (6, 3.4%), fish/otolith growth (5, 2.8%), and fish mortality (3, 1.7%). Sagittal otoliths are widely used as identification tools for fish species as each otolith's shape differs for every species (Thuy et al. 2015; Tuset et al. 2006; Luceño et al. 2018; Palla et al. 2016; Salimi et al. 2016). Publications on systematics consisted of papers dealing with taxonomy, phylogenetics, and the discovery of new species (Dolar et al. 2003; Pavlov 2021; Nielsen and Prokofiev et al. 2010). Fish/otolith growth focused more on the growth of the otolith and the fish itself throughout their lifespan, while fish mortality studies estimated the mortality rates of a species by confirming its life history correlated with age and life history studies. The remaining topics all have either 1 or 2 representative papers and are somewhat related to the other major groupings but are highly specific in their methodology. Papers on species identification focus solely on individual species based on otoliths found in various conditions, while the other topics are related to increment deposition and embryonic development, focusing on the process of ring formation in otoliths (Pavlov et al. 2012).

A total of 59 families across all research publications have been studied in Southeast Asia. To confirm this, we validated fish taxonomic families via the International Taxonomic Information System to obtain the most updated designation per species. Herein, we discuss the research effort across various fish families.

Anguillidae was the most studied fish family in Southeast Asia, with 22 (14%) studies (Figure 4B). Anguillids comprise catadromous freshwater eels that are widely distributed throughout the globe (Watanabe et al. 2009; Arnold 2009). Many of the species listed under this family are facing threats from multiple pressures such as habitat loss, barriers to migration, pollution, and parasitism, and thus many species were listed as "Threatened" and "Near Threatened" by the International Union for Conservation of Nature (Jacoby et al. 2015). Knowing this, the concern for members of the Anguillidae family is growing, and such concern is reflected in otolith research in the region. Authors such as T. Arai., K. Tsukamoto, M. Kuroki, and N. Chino are some of those who have consistently published otolith studies of different anguillid species. Studies done on the family are also diverse: ranging from microstructural, morphological, and microchemical topics. Along with gobiids, they are the only otolith-focused studies in Southeast Asia that have more than 10 published papers.

The second most studied fish family in the region is Gobiidae (7.5%), which constitute gobies. It is considered one of the largest acanthomorph families in the world, with more than 1000 species described (Thacker and Roje 2011). They are mostly small marine fishes commonly found in benthic areas, but some can inhabit freshwater and estuarine habitats (Thacker 2011). Being widely distributed, many biologists in Southeast Asia have also taken interest in this family, including Q. Dinh, J. Pattuinan, and R. Aini, encompassing a huge array of research areas as well, similar to the anguillids. Some studies have also cited the importance of gobies in economic growth as an alternative fishery resource, consequently making it prone to overexploitation (Vedra et al. 2014, 2013; Furukawa et al. 2019; Dinh et al. 2015; Dinh 2017).

The third most studied fish families are Clupeidae and Scombridae, which both represent 9 papers (5.8%) each. Clupeids consist of sardines, herrings, and shads (Rajan 2018) and are predominantly marine, with some interestingly becoming successfully adapted to freshwater habitats. This includes the only freshwater *Sardinella* to exist, the *S. tawilis* (Mamaril 2001; Willette et al. 2014) from Lake Taal in the Philippines. Mostly residing in tropical countries, it is also one of the most diverse and most studied fish families globally. On the other hand, the scombrid family consists of tunas and mackerels (Collette et al. 2001). Globally distributed throughout tropical and temperate seas, many otolith studies have also utilized this family for their studies (Ida et al. 1993). Considering these, studying other fish families in Southeast Asia is an important gap to fill in otolith research, given the wealth of information and applications that we can derive from these.

5. Top journal and author contributors

Scientific publications on fish otoliths play a crucial role in disseminating knowledge, providing data, and publishing innovative studies from various authors we reviewed. The reputation, performance, and productivity of authors can significantly depend on the journals they choose to submit their papers (Rallison 2015). Among the collected published papers reviewed, academic journals were ranked in the top ten to determine which ones contributed more to otolith studies in Southeast Asia (Figure 4c).

The top contributor is the Marine Ecology Progress Series (MEPS), with 8 otolith research papers published. MEPS focuses on marine, coastal, and estuarine ecology, covering a wide range of biological organizations, species, and habitats in both fundamental and applied research. The reviewed papers from MEPS all address sub-research topics related to the early life history, natal origin, and migration of various fish species, aligning with the journal's specialty (Arai et al. 1999; Newman et al. 2000; Marui et al. 2001; Sugeha et al. 2001; Arai et al.1999; Shiao et al. 2010; Wells et al. 2012).

Following MEPS, the Journal of Fish Biology and Journal of Ichthyology rank second and third, respectively, with each having five publications. The Journal of Fish Biology covers various aspects of fish biology, exploitation, and their significance to human society. Most of its published papers focus on the migration of fish species, such as the tropical Asian catfish *P. krempfi* (Hogan et al. 2007), catadromous *A. bicolor* (Arai and Chino 2019), and anadromous *Tenualosa ilisha* (Arai et al. 2019), which are important for human consumption. The Journal of Ichthyology, in collaboration with the Russian Academy of Sciences, focuses on original studies of fish taxonomy, ecology, genetics, and protection. Its contribution to otolith studies primarily centers on morphology, morphometrics, aging, interspecific variations, and taxonomy, with Pavlov D.A being a notable contributor. From the fourth to the tenth rank, the number of papers contributed decreases, with each journal having less than seven papers. This indicates a decreasing trend in contributions as the ranks go down.

Considering the 79 journals datamined in this review out of 129 papers, it is expected that the number of articles per journal is not high, with an average of 1.6 papers published per research journal. Moreover, international academic journals are more likely to be chosen by authors to submit their otolith research. While national, regional, or international journals are equally important, authors may prefer international journals to disseminate their findings more easily across different countries, especially considering that English is the lingua franca in scientific communication.

Among the collated 91 papers, a total of 324 authors contributed to otolith research. The year 2019 yielded the highest number of authors (n = 75), reflecting a collaborative effort among authors. Notably, Dr. Takaomi Arai leads in publication counts with 16 papers. Arai's expertise in fish biology, marine and fisheries sciences, and tropical ecology, particularly in the ecology of anguillid eels, has significantly contributed to otolith research in Southeast Asia. Dr. Katsumi Tsukamoto follows with nine publications, focusing on the biology, ecology, and conservation of freshwater Japanese eels using otoliths. Dr. Naoko Chino, with seven publications, also made significant contributions to the study of *Anguilla* species and their habitat transitions.

Despite the rise in popularity of otolith studies in the 20th century, the research has a long history dating back to the 19th century. Dr. Johannes Reibisch's publication in 1899 demonstrated the utility of otoliths in determining a fish's age (Reibisch 1899; Dahl 1909; Jackson 2007). In Asia, the first fish otolith study was published in Japan in 1932, comparing the otolith and somatic growth of yellowfin and bluefin tuna (Kimura 1932). While otolith studies have been relevant for over a century, Southeast Asia still lags behind, highlighting a need for further exploration in this area (Kano et al. 2013; Carpenter and Paxton 1997).

6. Challenges and ecommendations

In this paper, we argued how progress in the utilization of fish otoliths as vital tools can further our understanding of fisheries science in Southeast Asia. However, there are also challenges that need to be addressed in order to move forward. Here, we list how practical gaps can be addressed in the use of otoliths by looking into precautions in validation, cost and benefits, and novel methods in aging. These challenges stem from the inherent complexity and variability of otolith structures, as well as limitations associated with age-determination methods. With this in mind, we would like to stress how the selection of appropriate techniques for otolith extraction and analysis is crucial to mitigate errors and biases inherent in known methods for fish otolith analysis (Kimura and Kastelle 1995).

The common issue encountered in studying fish otoliths is post-mortem alterations to otolith composition, which present significant challenges to data interpretation. Improvements in otolith extraction techniques will contribute to cost savings and efficiency (Chalupnicki and Dittman 2016). However, haphazard procedures in otolith collection and preparation can variably affect measured elemental concentrations (Proctor and Thresher 1998). These alterations underscore the importance of careful sample handling and preparation to minimize artifacts and ensure accurate interpretations of otolith data. However, a poor understanding of otolith biomineralization mechanisms can lead to misinterpretations and erroneous conclusions (Fablet et al. 2011). For this, future researchers must abide by developed models of otolith biomineralization to improve the accuracy of otolith interpretations and their applications in fisheries science and environmental management.

Otolith-increment age estimates have long provided valuable insights into fish age and growth patterns. However, this method is time-consuming and requires capacity-building for skills in counting and validation (Stewart et al. 1995). There are now advancements in otolith mass-based age determination techniques that offer faster, less labor-intensive alternatives to traditional aging methods, providing cost-effective options for fisheries management and conservation efforts (see Lepak et al. 2012). Efforts to address the challenges associated with otolith analysis have led to the development of innovative and cost-effective methods for processing large quantities of otoliths (Green et al. 2002). Previously, the use of otolith thermal marking techniques offered a noninvasive means of identifying hatchery-produced fingerling, providing valuable insights into fish origin and migration patterns (Volk et al. 1999). However, this protocol has only been applied to a limited number of fish representatives. Additionally, advancements in computer vision technology offer promising solutions to reduce labor and costs associated with otolith analysis (Rypel 2008). At present, the most widely used method for fish otolith characterization is radiometric analysis which utilizes isotopes (see Stewart et al. 1995). Radiometric aging estimates have equally valid accuracy and reliability as compared to traditional increment counting but are less time-consuming. This, however, can still be affected by within-sample heterogeneity in otolith age and mass-growth rate, leading to some uncertainties in age estimates (Francis 2003).

To address challenges in radiometric analyses of fish otoliths, various approaches have been proposed to enhance the accuracy and precision of age estimates (Siskey et al. 2016). For instance, extracting otolith cores has been identified as a valid method for radiometric age determination, circumventing issues encountered with modeling otolith mass growth and improving reliability (Kimura and Kastelle 1995). Additionally, advancements in radiometric analysis techniques, such as the use of short-lived natural isotopes, offer promising avenues for refining age validation methods and enhancing the accuracy of age estimates in fish otoliths (Fenton and Short 1992). By leveraging innovative approaches and integrating multiple validation methods, researchers can overcome inherent limitations and biases in otolith-based age determination, ultimately enhancing our understanding of fish population dynamics and supporting sustainable fisheries management practices.

Radiometric analysis of fish otoliths can also be used in fisheries science to understand long-term environmental conditions, yet we need to note that their reliability can be influenced by various factors. For example, environmental variables such as temperature and precipitation rates can introduce significant discrepancies in otolith composition (Campana et al. 1997). Recent studies demonstrated that the novel use of stable isotopes (SI) as fish stock descriptors can be complicated by factors like variable fish growth and otolith precipitation rates co-varying with temperature (Carbon SI) and water conditions (Oxygen SI), which pose as additional challenges for their use as environmental indicators (Thomas et al. 2017). In this way, certain elements may reflect changes in the fish's surroundings, while others indicate internal physiological processes. This distinction complicates interpretation, as elements in different fractions of otoliths may have different environmental significance. While elements exclusively in the calciferous fraction are indicative of environmental conditions, those in both fractions can blur the lines between internal and external influences. Understanding these nuances is crucial for accurately interpreting otolith chemistry and its implications for fisheries science and environmental monitoring.

7. Conclusions

Numerous areas in ichthyological research remain unexplored and open for investigation in Southeast Asia. Given the utility of otolith research in providing information about all aspects of fish biology and its implications in fisheries management and conservation studies, we highly encourage more researchers to take an interest in this branch of fisheries science. Southeast Asia boasts one of the most biologically diverse waters globally but faces significant economic and environmental threats due to anthropogenic causes (Pomeroy et al. 2007).

Acknowledging the importance of leading research topics in fish and otolith studies, which yield diverse information about their composition and biology, should not deter scientists from exploring alternative approaches. Microchemistry analysis, for instance, continues to grow, representing a novel method for otoliths. The research, technology, and the economy contribute to ongoing advancements in modern biodiversity pathways in Southeast Asia (von Rintelen et al. 2017). Additionally, ongoing debates persist regarding the use of otolith chemistry to determine movement and habitat usage in complex ecosystems like estuaries (Walther 2019). Various factors, including environmental elements such as water temperature, salinity, and ambient concentrations, alongside intrinsic factors like genetic background, fish diet, and physiological processes, may contribute to variations in otolith elemental composition (Izzo et al. 2018). Furthermore, differences in otolith elemental composition appear to be species-specific (Chang et al. 2012).

Despite potential challenges, microchemical analyses offer opportunities for interdisciplinary collaborations. For example, geochemistry represents a viable method in paleoenvironments of fossil fish otoliths, aiding in environmental reconstructions through isotopic assays with the development of microsampling instrumentation and laser-based techniques (Campana 2005).

While the diversity of fish families studied for otolith research in Southeast Asia is considerable, the distribution of these studies remains highly imbalanced. A significant portion of research focuses on the family Anguillidae, and partially the family Gobiidae. Conversely, for other families, only a small percentage of studies have been conducted, with more than half (56%) having only one publication representing their family. Given Southeast Asia's high fish diversity and endemism, the lack of otolith research on these fishes is concerning, especially for freshwater species facing significant anthropogenic pressures such as overexploitation, pollution, and habitat modifications (Dudgeon 1992; Ng and Tan 1997; Sodhi et al. 2004; Rashid et al. 2015; Friedman et al. 2018).

Furthermore, it seems that only families with high commercial value receive attention, while those less economically significant are overlooked. Many government-led conservation policies heavily rely on economic valuation, potentially biasing the allocation of funds for biodiversity and conservation studies (Martin-Lopez et al. 2008; Demir 2013). However, while greater attention must be given to threatened taxa, other species should not be neglected. Otolith studies offer valuable insights with implications for fish biodiversity conservation, underscoring the importance of researchers in Southeast Asia engaging in otolith research to understand fish biology and life history better. Policymakers and practitioners should prioritize research on fish species in Southeast Asia, recognizing that human activity is a leading cause of biodiversity loss globally and must actively participate in conservation efforts.

With these considerations in mind, we hope that this review stimulates interest in the scientific community to address the gaps in fish otolith research in Southeast Asia and fosters collaboration within the region.

AUTHOR CONTRIBUTIONS

Calizo JDA: data gathering, database compilation, data analysis, wrote first manuscript draft. **Valenzuela RB Jr.:** data gathering, database compilation, data analysis, wrote first manuscript draft. **Martinez VV:** contributed to revisions of the initial manuscript draft. **Briones JCA:** conceptualization, database compilation, data analysis, prepared the manuscript's final version.

CONFLICTS OF INTEREST

We declare that we have no involvement or affiliation with any organization that may have any financial or non-financial conflict of interest in the subject matter or materials discussed in this paper.

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