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Tilapia health: *quo vadis?*

Guest Editors

Devin Bartley
Michael Philips
Kevin Fitzsimmons
Richard Arthur
Melba G. Bondad-Reantaso

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Aims and Scope: The primary aim of *Reviews in Aquaculture* is to provide a forum of reviews on developments in aquaculture techniques, policies and planning. The Journal will publish fully peer-reviewed review articles, invited or otherwise, on major aspects pertaining to aquaculture, including: global, regional and/or national production and market trends; aquaculture practices and technological developments; aquaculture–environment interactions; indigenous and alien species in aquaculture; the biology and culture of aquaculturally important and emerging species; utilization of primary and secondary resources in aquaculture; developments in artificial propagation of individual species and or groups; developments in feeds and feeding; genetics and aquaculture; health management in aquaculture; policy developments pertaining to aquaculture; aquaculture product quality and traceability; and socio-economics of aquaculture and impacts.

The cover image is based on the Review *Cultivation of fish ciliate parasites: Progress and prospects* by Ming Li et al., https://doi.org/10.1111/raq.12708.

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REVIEWS IN Aquaculture

LETTER

Tilapia health: *quo vadis*?

Species and farmed types of tilapia have become one of the world's most popular aquaculture products and provide nutrition and livelihood to rural and urban communities around the world.* This popularity has been due to technological advances in fish health, farming systems, breeding and genetics, engineering, marketing as well as wide consumer acceptance and the special biological characteristics of tilapia that enable them to survive and grow in a variety of environments and farming systems. As the farming of tilapia has become more widespread, so have the concerns around fish health and disease prevention and cure.

To review the status of tilapia health, disease prevention and cure, the Food and Agriculture Organization of the United Nations (FAO) and INFOFISH convened a virtual International Technical Seminar, Tilapia health: *quo vadis*,† that brought together 1700 participants from over 100 countries. A call for expression of interest was released after the event, to develop the presentations into full articles for publication as a Special Issue at Reviews in Aquaculture. Eight groups of authors responded to the call and the eight papers now constitute this Special Issue. The FAO Lead Technical Officer (Melba G. Bondad-Reantaso), guest editors (J.R. Arthur, Devin M. Bartley, Kevin Fitzsimmons, and Michael J. Phillips), authors and entities that funded the papers as Open Access (indicated in individual papers) are gratefully acknowledged. The virtual event was supported by two FAO projects, namely: GCP/RAF/510/MUL Enhancing capacity/risk reduction of emerging Tilapia Lake Virus (TiLV) to African tilapia aquaculture and TCP/INT/3707 Strengthening biosecurity (policy and farm-level) governance to deal with Tilapia lake virus.

The special issue builds on the recent review of tilapia published as a special virtual issue in RAQ 15:1 (2023); with articles that focus on fish health issues along with other articles that set the scene for tilapia farming globally. The paper by El-Sayed and Fitzsimmons, *From Africa to the World- the Journey of Nile tilapia*, documents how Nile tilapia, first farmed by ancient Egyptians 4000 years ago, has been moved from Africa to farming systems around the world to become one of the most important farmed species. The review further reveals that although Nile tilapia often supports inland capture fisheries as well as aquaculture production, tilapia introductions have in some cases adversely impacted local cichlid species, and reduced abundance of other fishery resources.

Farmed tilapia producers and processors have been leading the seafood industry in several aspects of processing, value adding, and packaging. The dependable supply and pricing of farmed tilapia and its value in several sectors, for example, food service, fast casual dining, frozen meals, as well as fresh seafood counters, have allowed processors and marketers to invest in novel value-added processing and packaging. This has only been possible because the tilapia industry has invested in advanced production systems and fish health.

Much of the tilapia sold in international trade is processed in the producing country. Often fillets are marketed leaving about 70 percent of the fish unused. The paper, *How value addition by utilization of tilapia processing by-products can improve human nutrition and livelihood*, by Peñarubia and co-authors, reveals a wide range of other products that are or could be utilized in the supply chain. So-called 'by-products' of processed tilapia that may be discarded or used for animal feed, can provide additional income to workers in the tilapia supply chain. In addition to nutritious products such as fish cakes and sausage, tilapia skins are processed to produce leather and gelatin, heads and bones can be turned into flour or supplements, and collagen from fish scales and bones have potential in the cosmetic and pharmaceutical fields. The authors point out that further research and development, along with marketing are needed to fully utilize the wide range of products available from tilapia.

The paper by Zimmerman and co-authors, *The future of intensive tilapia production and the circular bioeconomy without effluents: biofloc technology, recirculation aquaculture systems, Bio-RAS, partitioned aquaculture systems, and integrated multitrophic aquaculture*, describes several of the most innovative fish farming systems allowing farmers to grow more fish in smaller areas with fewer inputs. Recirculating systems, aquaponics, bioflocs, in-pond raceways are just some of the more advanced, or intensive, aquaculture methods that were mostly pioneered using tilapia and are now being tested with other species. The paper provides a nice overview of these systems and their particular pro's and con's.

In, *Strategies to enhance tilapia immunity to improve their health in aquaculture*, Wang and co-authors provide an overview of the benefits of enhancing the immune response to improve tilapia health, in turn reducing the levels of pathogens within tilapia farming systems. The authors review the immune system of tilapia and the importance of the gut microbiome. They then summarize the strategies used to reduce the impact of disease in tilapia culture through enhancement of the immune system, including the feeding of probiotic and prebiotic supplements to modulate the gut microbiota, the use of herbal

medicines and immunostimulants to enhance immunity to disease, and the existing and potential use of vaccines to prevent infections. They note that emerging and re-emerging diseases such as streptococcosis, tilapia lake virus disease, and infectious spleen and kidney necrosis virus disease have resulted in high levels of morbidity and mortality, production losses and trade restrictions. In the absence of effective husbandry management, disease prevention strategies and appropriate biosecurity measures, these and other infectious diseases will continue to challenge the sustainability of global tilapia aquaculture. They then look at the economics of applying immune enhancement strategies in tilapia culture, noting that the decision to use these products depends on multiple factors including farming practice, farmer perception, and the overall cost-benefits. They note that effective husbandry management through maintaining high water quality, adequate nutrition, and good biosecurity will create a less stressful environment for fish. Rapid response to epidemic events and the availability of rapid and accurate diagnostic methods are important to limit the damage caused by disease. Vaccination as a means of controlling infectious diseases is one of the most significant and successful health practices within the aquaculture industry.

The review, *Improving tilapia biosecurity through a value chain approach*, by MacKinnon and co-authors explores the value chain perspective to assess and manage risks of disease threats and losses in tilapia aquaculture. The paper outlines the tilapia value chain as a starting point, then assesses the important infectious agents of tilapia that may affect different parts in the value chain. The paper then describes how risk analysis can be applied to identify critical control points in the value chain and potential risk mitigation measures that may be implemented at those points. It emphasizes, as many of the other papers illustrate, that the control of diseases of tilapia requires a multi-faceted approach across the whole 'aquaculture system', with control measures chosen based on their feasibility, effectiveness and sustainability.

In, *A global review of problematic and pathogenic parasites of farmed tilapia*, Shinn and co-authors provide an extensive and detailed global accounting of the protistan and metazoan parasites of tilapias, with emphasis on those species having demonstrated or potential impact to tilapia aquaculture. The authors summarize more than 2500 host–parasite records from 73 countries and more that 820 recorded tilapia introductions. For each major parasite taxonomic group, they highlight those parasites that have been translocated along with their tilapine hosts or have been acquired from the new environments into which tilapia have been introduced, together with remarks on their taxonomy, reported geographic distribution (including translocations), pathology, status, and future directions of research, and approaches to treatment and control. They note that while Africa has enormous potential for aquaculture development, substantial knowledge gaps about tilapia parasites remain for many African states, which creates associated production and biosecurity risks. Globally, tilapias host a rich fauna of parasites, with new species still being encountered. This review and its associated supplementary tables will be of high value to fish parasitologists in general, to diagnosticians, to tilapia farmers encountering parasite problems in their facilities, and to government scientists conducting import risk analyses for proposals to introduce tilapias to new geographic areas.

Tilapia has often been described as extremely hardy fish with very few disease problems. While this is generally true, rearing more and more fish intensively can induce stressful conditions that allow pathogens to take hold and allow a disease to spread. Likewise, fish selected for fast growth, colour morphs and/or body conformation may have allowed some of the innate hardiness to have been degraded. The paper by Haenen and co-authors, *Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance* provides a thorough overview of bacterial diseases and some associated pathologies that have and are affecting commercial farms rearing tilapia. While the losses endured by the tilapia industry have been less than salmon or shrimp farming, no farmer wants to lose fish and see income decline. Treatments and vaccines are described for the various bacteria which have been associated with significant mortalities.

In their review, *From the basics to emerging diagnostic technologies: What is on the horizon for tilapia disease diagnostics?*, Ha Dong and co-authors stress that the intensification of tilapia farming has exacerbated losses due infectious diseases and that the disease diagnostics play a crucial role in aquaculture biosecurity and health management. However, the recent proliferation of cutting-edge molecular methods in aquaculture has shifted the focus of researchers and users away from basic approaches and towards molecular diagnostics, despite the fact that many diseases can be rapidly diagnosed using inexpensive, simple microscopic examination. This review highlights the importance of the three levels of diagnostics for diseases of tilapia to promote the integration of both basic and advanced methods to achieve accurate and meaningful diagnostic results. The authors thus emphasize the need for frequently overlooked but basic procedures such as case history records, gross pathology, presumptive diagnostic methods, and histopathology. They also provide an in-depth review of current and emerging molecular diagnostic technologies for tilapia pathogens, including polymerase chain reaction methods, isothermal amplification methods, CRISPR-based detection, and lateral flow immunoassays. They also discuss the future of tilapia disease diagnostics, including next generation sequencing, artificial intelligence, environmental DNA/RNA and point-of-care testing, and a future vision for transferring these technologies to farmers and stakeholders for a sustainable aquatic food system transformation.

Undoubtedly, tilapia, and especially Nile tilapia, will continue to be one of the most important groups of farmed aquatic species. The material reviewed in this special edition provides valuable information to resource managers, fish farmers, processors, marketers, and vendors to help ensure that tilapia aquaculture develops responsibly and provides beneficial outcomes to communities and the environment.

KEYWORDS

Aquaculture, biosecurity, fish health, non-native, Tilapia, value adding

AUTHOR CONTRIBUTIONS

Devin M. Bartley: Conceptualization; project administration; writing – original draft; writing – review and editing. **J. Richard Arthur:** Conceptualization; writing – original draft; writing – review and editing. **Kevin**

Fitzsimmons: Conceptualization; writing – original draft; writing – review and editing. **Michael J. Phillips:** Conceptualization; writing – original draft; writing – review and editing. **Melba G. Bondad-Reantaso:** Conceptualization; writing – original draft; writing – review and editing.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Devin M. Bartley[1]
J. Richard Arthur[2]
Kevin Fitzsimmons[3]
Michael J. Phillips[4]
Melba G. Bondad-Reantaso[5]

[1]*Michigan State University, East Lansing, Michigan, USA*
[2]*Barriere, British Columbia, Canada*
[3]*University of Arizona, Tucson, Arizona, USA*
[4]*FUTUREFISH Company Limited, London, UK*
[5]*Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy*

Correspondence
Devin M. Bartley, Michigan State University, East Lansing, MI, USA.
Email: devinmichaelbartley@gmail.com

ORCID

Devin M. Bartley https://orcid.org/0000-0001-7877-4186
Kevin Fitzsimmons https://orcid.org/0000-0003-3759-8168
Melba G. Bondad-Reantaso https://orcid.org/0000-0002-2380-3549

ENDNOTES

* Food and Agriculture Organization of the United Nations. *The State of World Fisheries and Aquaculture 2020. Sustainability in action*. FAO, Rome; 2020. https://doi.org/10.4060/ca9229en

† http://infofish.org/tilapia/

‡ https://onlinelibrary.wiley.com/doi/toc/10.1111/(ISSN)1753-5131.Tilapia

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REVIEW

REVIEWS IN Aquaculture

From Africa to the world—The journey of Nile tilapia

Abdel-Fattah M. El-Sayed[1] | **Kevin Fitzsimmons**[2]

[1]Oceanography Department, Faculty of Science, Alexandria University, Alexandria, Egypt

[2]Department of Environmental Science, College of Agriculture and Life Sciences, University of Arizona, Tucson, Arizona, USA

Correspondence
Abdel-Fattah M. El-Sayed, Oceanography Department, Faculty of Science, Alexandria University, Alexandria, Egypt.
Email: abdelfatah.youssif@alexu.edu.eg

Abstract

Despite the fact that Nile tilapia (*Oreochromis niloticus*) (Linnaeus, 1758) are African freshwater fish, they have been introduced into many countries inside and outside Africa. The journey of Nile tilapia started during the second half of the 20th Century, especially in Southeast Asia and the Americas, mainly for aquaculture and fisheries enhancement. Most of these introductions became well established in aquaculture. As a result, aquaculture of Nile tilapia has been steadily expanding in many countries. These fish play an important role in the livelihoods of local societies on different continents. This review summarizes the current knowledge on global Nile tilapia introductions and sheds light on their social and economic contributions to the countries into which they have been introduced, as well as their share in the global fish market. The ecological impacts of tilapia introductions have also been addressed. The success stories of the major Nile tilapia producers were highlighted so that the lessons learned from their experiences could be transferred to other countries.

KEYWORDS
Africa, Asia, impacts, introductions, Nile tilapia, The Americas

1 | INTRODUCTION

Tilapia are African freshwater fish belonging to the family Cichlidae. They are distributed all over Africa, except the northern Atlas Mountains and Southwest Africa.[1] About 700 Cichlid species have been reported in the African Nile basin.[2] Of those, Philippart and Ruwet[3] reported 76 tilapia species across Africa. Fishbase[4] suggested that the family Cichlidae includes 52 tilapia species (32 *Oreochromis*, 13 *Sarotherodon*, and seven *Tilapia* species). More recently, the names of *Tilapia dageti* (Thys van den Audenaerde, 1971), *Tilapia guineensis* (Günther, 1862), *Tilapia zillii* (Gervais, 1848) and *Tilapia mariae* (Boulenger, 1899) have been reclassified as *Coptodon dageti* (Thys van den Audenaerde, 1971), *Coptodon guineensis* (Günther, 1862), *Coptodon zillii* (Gervais, 1848), and *Pelmatolapia mariae* (Boulenger, 1899), respectively. Past publications may refer to the older invalid names, but the updated names are used in this document. This means that there is still confusion among researchers in the taxonomic classification of tilapia.[5]

Eleven tilapia species are currently farmed in Africa,[6] compared to only three species in 1980. However, Nile tilapia (*Oreochromis niloticus*) (Linnaeus, 1758) is, by far, the most widely cultured tilapia species,[7] due to its economic value as one of the most important farmed fish species in the world. The culture of other tilapia species is also practiced, together with "not elsewhere included" or 'nei' 'tilapia'. These species include blue tilapia (*Oreochromis aureus*) (Steindachner, 1864), longfin tilapia (*Oreochromis macrochir*) (Boulenger, 1912), three spotted tilapia (*Oreochromis andersonii*) (Casteinau, 1861), Mozambique tilapia (*Oreochromis mossambicus*) (Peters, 1852), Shire River tilapia (*Oreochromis shiranus*) (Boulenger, 1897), Tanganyikan tilapia (*Oreochromis tanganicae*) (Günther, 1894), mango tilapia (*Sarotherodon galilaeus*) (Linnaeus, 1758), blackchin tilapia (*Sarotherodon melanotheron*) (Rüppell, 1852), redbelly tilapia (*C. zillii*) (Gervais, 1848) and redbreast tilapia (*Coptodon rendalli*) (Boulenger, 1897).

However, the production of this group is very limited, being only 62,393 metric tons (mt) (4.6%) of total farmed tilapia production in Africa in 2019.[7]

Nile tilapia are naturally distributed in the Nilo-Sudanian region, Ethiopian Rift Valley, the western Rift Lakes (Lake Albert, Lake Edward, Lake George, Lake Kivu and Lake Tanganyika) and Lake Turkana in the eastern Rift Valley. Nile tilapia is also naturally established in Central and Western Africa (Senegal, Gambia, Volta, Niger, Benue and Chad river basins) (Figure 1).[1,3] Nile tilapia are highly adapted to tropical, subtropical and temperate environments. They are characterized by their fast growth rates, tolerance to extreme environmental conditions (such as temperature, salinity, pH, and low dissolved oxygen), high resistance to stress and diseases, trophic plasticity and feeding on low trophic levels, and their ability to reproduce in captivity.[6,8,9] These attributes made them an ideal candidate for aquaculture all over the globe. It is no surprise, therefore, that 114 Nile tilapia introductions have been recorded worldwide (Figure 2), mainly for aquaculture and fisheries enhancement.[10] As a result, tilapia culture has been developing at a high rate since the 1990's. Production of farmed tilapia increased from only 1 million mt in 2000 to over 6 million mt in 2019, with Nile tilapia contributing 74% to this production[7] (Figure 3).

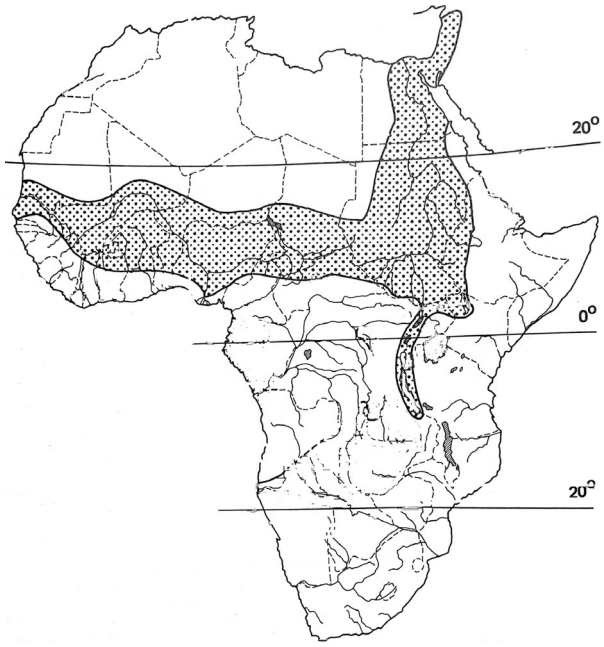

FIGURE 1 Natural distribution of Nile tilapia in Africa. Modified from: Philippart and Ruwet.[3]

Nile tilapia, often described as the 'aquatic chicken' can be a low-priced fish compared with other farmed fish, which feed on higher trophic levels.[11] Therefore, it is sometimes considered a food for the poor, or the fish for the masses.[11] In many locations tilapia has played a significant role in rural development, poverty alleviation, hunger eradication and human health improvement in the developing and least developed countries, thereby directly contributing to the achievement of the United Nations Sustainable Development Goals (e.g., SDGs 1, 2 and 3). (https://www.undp.org/sustainable-development-goals). This has been achieved through fish supply for domestic consumption and export, generating more foreign currency, raising producers' income and creating employment opportunities.

We review global Nile tilapia introductions, and the economic and social roles they play in the regions and countries into which they have been introduced, and the success stories of the major Nile tilapia producers. It also discusses the adverse ecological impacts which may

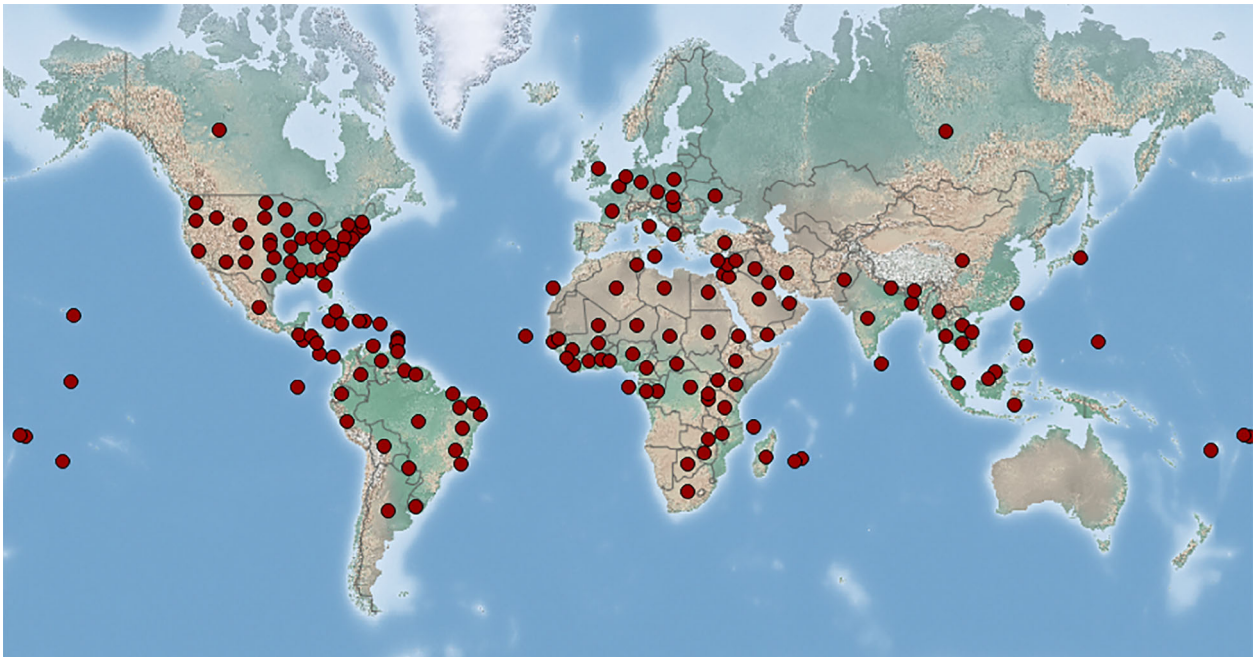

FIGURE 2 Global Nile tilapia introductions. Source: CABI Invasive Species Compendium (https://www.cabi.org/isc/datasheet/72086).

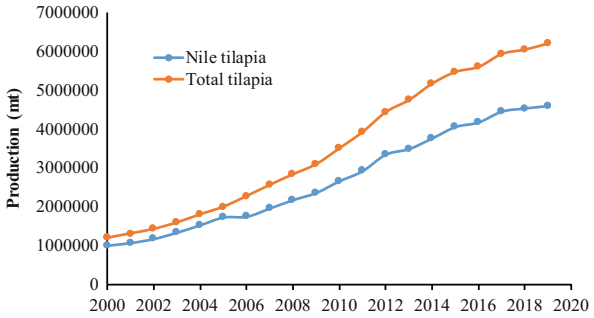

FIGURE 3 Global farmed tilapia production 2000–2019.[7]

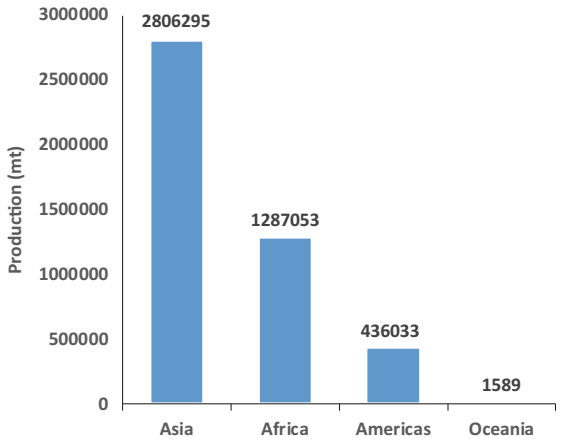

FIGURE 4 Production of farmed Nile tilapia by continent in 2019.[7]

result from tilapia introductions and transfers, so that the necessary precautions can be taken to avoid, or at least minimize, these adverse impacts.

2 | THE JOURNEY OF NILE TILAPIA IN AFRICA

The story of Nile tilapia culture in Africa started about 4000 years ago. As indicated from the biblical references and illustrations from ancient Egyptians tombs, tilapia farming is, arguably, rooted in Egypt, at about 4000 BC, about 1000 years before cyprinid carps culture was practiced and published in the first aquaculture text by Fan Li in China.[12,13] Although a recent article[14] hypothesizes carps may have been reared as far back as 8000 years ago. In recent history, the first tilapia culture trial dates back to 1924 in Kenya, when water ponds were stocked with different species of tilapias (especially *Oreochromis niger*) (Günther, 1894) followed by African catfish (*Clarias gariepinus*) (Burchell, 1822) and common carp (*Cyprinus carpio*) (Linnaeus, 1758).[15] Ten years later, the first scientific fish farming trials took place in Egypt.[16] Many other tilapia farming trials were conducted during the 1950s and 1960s throughout the continent, where thousands of small ponds were constructed for tilapia farming, mainly for subsistence, using extensive farming techniques.[15]

The real journey of Nile tilapia in Africa started in the 1990s, when tilapia was introduced, transferred or translocated in many African countries, mainly for commercial aquaculture and fisheries enhancement.[17–19] By 2020 Nile tilapia culture was practiced in 30 countries throughout Africa,[7] and their production increased from only 27,000 mt in 1990 to 1,287,053 mt in 2019, representing 95.4% of farmed tilapia production in Africa, and 68% of African inland water aquaculture outputs,[7] and contributing 28% of global Nile tilapia output (Figure 4). However, Nile tilapia farming in Africa is dominated by a single country, Egypt, which contributed 84% (1,081,202 mt) to farmed Nile tilapia output in Africa in 2019. Egypt is also ranked third among the top global Nile tilapia producers in the world, after China and Indonesia (Figure 5a). Tilapia culture is also expanding in Uganda, Ghana, Kenya, Mali, Sudan, Tanzania, and Zambia (Figure 5b).

3 | EGYPT'S EXPERIENCE AND LESSONS LEARNED

Tilapia farming in Egypt is practiced mainly by small-scale farmers, using simple farming technologies and outputs. Extensive, semi-intensive (SI), intensive and integrated farming are applied; however, SI remains the most popular system. Over 80% of aquaculture output is produced under SI systems in earthen ponds, with mono-sex Nile tilapia being the primary species.[20] Farmed tilapia (and other fish) are fed with high quality extruded feeds (both sinking and floating pellets).[21,22]

Nile tilapia, despite being freshwater fish, are able to tolerate a wide range of water salinity.[23] They can grow in brackish water (BW), at salinity of up to 15‰. They can also reproduce at water salinity ranging from 13‰ to 29‰.[23] Due to the scarcity of fresh water and abundance of BW in Egypt, Nile tilapia production is practiced mainly in BW environments, especially in the northern delta lakes areas along the Mediterranean coast. In 2019, 89% of farmed Nile tilapia in Egypt were produced from BW, whereas global Nile tilapia production in BW environment represented 21% of total Nile tilapia outputs.[7]

Nile tilapia culture has a major contribution to animal protein supply and food security for millions of Egyptians, especially among the poor and middle-class communities in rural areas.[24] Farmed Nile tilapia contributed 66% to national aquaculture production, and 43% to total fish consumption in Egypt in 2019.[20] Thus, out of 25.38 kg of fish consumed per capita per year, 11 kg come from farmed Nile tilapia. This confirms the significant contribution of farmed Nile tilapia to food security for the Egyptian population, especially among the poor and middle-class communities.[24]

Almost all Egyptian tilapia production is directed to the domestic market; only about 10,000 mt are exported (GAFRD, personal communication, 2022),[20] mainly to the Gulf Cooperation Council region.[24] Farmed tilapia play a significant role in poverty and malnutrition eradication of rural Egyptian households, especially for nutritionally vulnerable groups, through providing good quality animal protein at low prices.[24,25] In addition, Nile tilapia farming improves the livelihoods of tens of thousands of households, mainly by generating employment opportunities through the whole aquaculture value chain. Taking into account that 14 full-time jobs

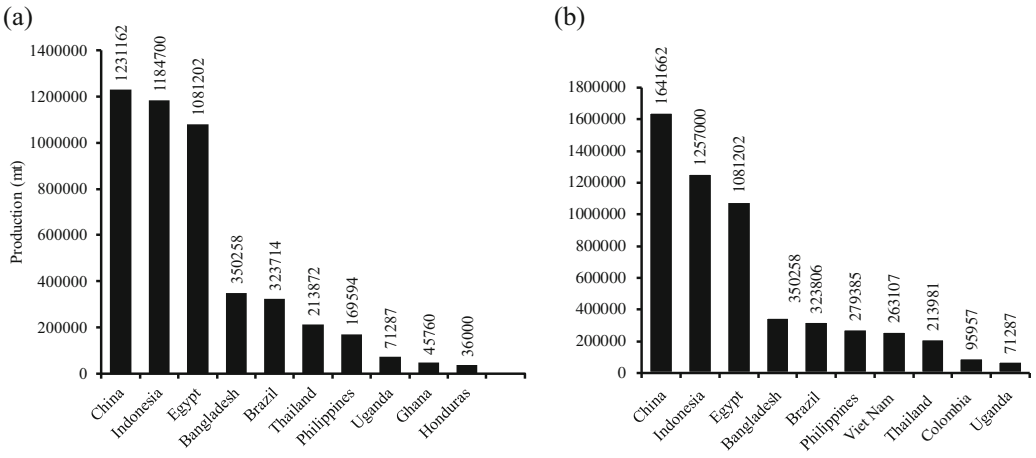

FIGURE 5 (a) Top producers of farmed Nile tilapia in 2019.[7] (b) Top producers of total farmed tilapia in 2019.[7]

are required to produce 100 mt of fish,[26] it has been estimated that full-time employment in the tilapia value chain is currently about 151,000 persons. Many part-time jobs are also created throughout the production cycle (e.g., harvesting, transportation, and pond preparation).

3.1 | Other African countries

Tilapia farming plays a significant role in food security in several other sub-Saharan African regions. Countries such as Benin, Burundi, Cameroon, Congo, Cote d'Ivoire, Mozambique, and Rwanda are expanding Nile tilapia farming.[27] Different types of incentives are offered to encourage local and foreign investors to invest in tilapia culture which has been proven profitable. For example, Nile tilapia in Rwanda are farmed as a cash crop, with the cash generated from their sale used for buying necessary commodities.[28] In Tanzania, households of small-scale farmers in Mvomero and Mbarali districts consume substantial amounts of Nile tilapia they produce, while the remaining portions were sold mostly fresh to their neighbours.[29] In Zambia, tilapia contributes directly to food security and indirectly to income generation through fish retail and job creation. About 40% of fish farming households use all of the harvest for family consumption, while about 57% sell their harvest in local markets.[30–32] Similarly, farmed Nile tilapia has become a major livelihood source for smallholder households in Ghana,[33–35] Kenya,[36] Nigeria,[37] and Uganda,[38] through improving rural income, food and nutrition security and poverty alleviation.

4 | TILAPIA FARMING SYSTEMS IN AFRICA

Despite the huge potential of Nile tilapia farming in Africa, it has not kept pace with the changes in aquaculture technologies and lags far behind other producing regions. Tilapia farmers in most African countries, except a few countries (e.g., Uganda, Ghana and Kenya) adopt non-commercial extensive systems in earthen ponds, mainly for subsistence. Farm ponds are generally very small (<100 m^2–1000 m^2).[27,39,40] In Cote d'Ivoire, the Acadja-enclos system is still used in ponds with tree branches or bamboo poles placed in ponds to encourage periphyton to grow where the tilapia can easily graze. The branches and/or poles are then removed to simplify harvest.[41] Family labour is generally used on African farms, while the use of hired labour is very rare because many small-scale farmers do not have the cash to hire external labour. Small fish ponds have been reported to improve food security, poverty reduction and health status and economic standard of rural households in Zambia,[31,32] Uganda[38,42] and Tanzania.[29]

In commercial tilapia culture systems, both monoculture of Nile tilapia (*O. niloticus*) and polyculture of tilapia with North African catfish (*C. gariepinus*) and common carp (*C. carpio*) are commonly practiced, especially in sub-Saharan Africa (SSA).[43] The use of mixed-sex tilapia is common, although sex-reversed (all male) tilapia culture is spreading in a number of SSA countries.[6] This system is primarily based on fertilization using a compost crib built inside the pond, where farmed tilapia depends on the enhanced natural food production. Stocking densities of tilapia fingerlings in this system are generally low, ranging from 1 to 4 fish/m^3.[26] The more advanced farming systems, namely SI and intensive farming technologies are also practiced, especially in Egypt, Uganda, Ghana and Kenya. SI tilapia farming in earthen ponds is popular; where tilapia monoculture and polyculture systems are commonly used.[6,39]

Intensive tilapia culture is practiced in a few African countries (Egypt, Ghana, Kenya, Nigeria, Malawi, Zambia, Zimbabwe, Cote d'Ivoire and Uganda), mainly in cages and, to a lesser extent, in tanks, raceways and recirculating systems.[44,45] The aquaculture of Nile tilapia in floating cages is widely practiced in Egypt, with a production amounting to 119,291 mt in 2019, representing 11% of total farmed tilapia production and 7.3% of total aquaculture output.[20] While tilapia cage culture is developing in other African countries, it is still a relatively low contributor to total tilapia production.[27,45] Small (48 m^3) to medium (108 m^3) cages, made of locally available materials, are

generally used. Commercial, large-scale cages (800–1200 m^3) are also used in Ghana and Zimbabwe.[39,46] In 2021 and 2022, multi-million dollar international investments have been placed for the cage culture of Nile tilapia in Ghana, Zimbabwe, and Lake Victoria (Uganda and Kenya) (El-Sayed, 2022, personal survey).

Intensive tank tilapia culture in Africa, especially in Egypt, is also growing. Most tank tilapia farmers in Egypt use concrete tanks for raising all-male Nile tilapia, at densities ranging between 25 and 100 fish m^{-3}, depending on the initial stocking size. Tank-raised tilapia are generally fed with extruded feeds (sinking or floating pellets-25%–30% crude protein), to grow to about 200–300 g over a 5–7-month period. Tilapia culture in concrete tanks and raceways in the rest of Africa is very limited and is practiced in only a few countries, such as Kenya and Zambia.[39] The size and shape of tilapia culture tanks vary depending on the culture objectives. A few other large-scale commercial tilapia enterprises have been established in SSA (e.g., Republic of South Africa and Congo) (A.-F.M. El-Sayed, 2018, personal survey). Fish are fed either farm-made pellets or commercial feeds (mostly imported).

5 | THE JOURNEY OF NILE TILAPIA OUTSIDE AFRICA

During the second half of the 20th Century, Nile tilapia was introduced into many countries outside Africa, especially into Southeast Asia and the Americas, mainly for aquaculture and fisheries enhancement.[4,47] Most introductions were successful; domesticated broodstocks and self-sustaining Nile tilapia populations are now established in many countries.[10,48] Consequently, Nile tilapia became an important component of inland fisheries in many tropical and subtropical countries.[49–51] Tilapia aquaculture was successful in many countries and has played a considerable role in the livelihoods, health and economies of rural societies in these countries.[11,49] The following sections will briefly describe Nile tilapia culture in Asia and the Americas, with emphasis on major producers.

6 | THE JOURNEY OF NILE TILAPIA TO ASIA

Mozambique tilapia (*O. mossambicus*) was introduced in Indonesia in 1939, and to many other Asian countries during the 1950s and 1960s; but it was not widely accepted by the consumers.[52] Alternatively, Nile tilapia was introduced from Africa to some Asian countries in the 1960s and early 1970s, for aquaculture and fisheries enhancement.[3,49,52] During the following years, it was widely distributed throughout the continent. For example, Nile tilapia was introduced to Japan from Egypt in 1962, and was then introduced to Thailand and Taiwan in mid-1960s.[49] In the 1970s through 1990s, this species was introduced from Thailand to several Asian countries, including Bangladesh, India, Lao PDR, Malaysia, Myanmar, Nepal, Philippines, and Vietnam.[49] Several Nile tilapia introductions from unknown origins have also been reported.[4]

As a result, Nile tilapia culture has become a popular, well-established, and profitable activity in Asian countries, such as Bangladesh, China, India, Indonesia, Myanmar, Pakistan, Philippines, Sri Lanka, Thailand, and Vietnam.[6] Nile tilapia culture is currently playing an important role in food security of local rural poor, due to the provision of cheap, high-quality food both to farmers' households and local markets and generation of cash incomes.[11,53]

Nile tilapia has been a subject of continuous genetic improvement for many years in many countries. For example, the WorldFish Centre (formerly known as the International Centre for Living Aquatic Resources Management [ICLARM]) initiated a selective breeding research programme in the Philippines, for genetic improvement of Nile tilapia more than 30 years ago, which resulted in the production of the genetically improved farmed tilapia (GIFT) strain.[54] After nine generations, the GIFT strains showed a 64% cumulative increase in growth over the original base population. As a result, GIFT has been disseminated to other Asian countries, including Bangladesh, Indonesia, Malaysia, and Sri Lanka.[11]

7 | ROLE OF TILAPIA IN FOOD SECURITY AND RURAL DEVELOPMENT IN ASIA

Despite the fact that tilapia is not native to Asia, their introductions, especially in Southeast Asia, have resulted in significant benefits, including: a) establishment of capture fisheries in certain countries[55]; b) an important aquaculture fish in most countries in the region, appropriate for a wide range of aquaculture operations[49,56]; c) a source of affordable animal protein in many countries, improving nutritional and economic status of local households[51]; and d) providing employment and increasing income, and playing a significant role in rural development and welfare.[49] For example, introduced Nile tilapia is currently among the most important farmed freshwater fish in Southern China, especially in Guangdong and Hainan Provinces, mainly due to favourable geographic location and weather conditions.[55,57] However, production is seasonal, and there are distinct periods of low growth. The fact that the commercial supply is stable is a testimony to the success in developing an export-oriented sector, with careful control of inventories.

As a result, Asia is currently the largest producer of farmed tilapia in the world, with a production of 4,141,976 mt in 2019, contributing 69% to global tilapia production.[7] Nile tilapia is the dominant species, contributing 68% (2,806,295 mt) to total Asian tilapia production and 45% to global tilapia output (Figure 4). Most of this production comes from small-scale, rural farms.[58,59] These family-owned, often contract farmers, in much of Asia are becoming more competitive to large-scale, commercially-managed tilapia farms.[58,60] Nile tilapia is cultured mainly in polyculture systems or in integrated systems with other plant/animal species and separate information and statistical data on

tilapia production, value, etc., is generally not available in many countries.[43]

Most of cultured Nile tilapia in Asia is produced in China, Indonesia, Bangladesh, Philippines, Thailand, and Vietnam, which are among the most heavily populated countries in Asia. These six countries produced over 65% of total farmed tilapia in Asia in 2019.[7] Tilapia production is directed mostly to domestic consumption; however, significant amounts are exported to foreign countries, in different forms.[61–63] The following section summarizes the role of tilapia production in general, and Nile tilapia in particular, on the livelihood and rural economies in these countries.

8 | BANGLADESH

Nile tilapia (*O. niloticus*) (Chitralada strain) was introduced into Bangladesh from Thailand in 1974, to replace another introduced species (*O. mossambicus*), which was not accepted by the farmers.[64] Nile tilapia was quickly accepted by the public, and has become the primary farmed tilapia species, due to their low prices, and low-cost operation with good financial returns.[11,65] The culture of the GIFT strain has overtaken the Chitralada strain across most of Bangladesh as a means of increasing tilapia production and economic return, due to their better performance. Currently, there are more than 400 fish hatcheries in Bangladesh, using the GIFT strains, with a production of over four billion tilapia fry every year.[66] As a result, tilapia production in Bangladesh has witnessed a sharp increase in recent years. Until 2008, no official tilapia production from Bangladesh was reported in Food and Agriculture Organization (FAO) records; reported production started in 2009, with only 16,237 mt. Since then, Nile tilapia production has grown to reach 350,258 mt in 2019, over only 11 years.[7]

Nile tilapia culture plays a vital role in rural livelihood in Bangladesh since the fish are produced exclusively in freshwater environments in rural areas, through production, direct consumption, distribution and marketing.[53] Tilapia is now the third most important fish species in Bangladesh, after pangas (*Pangasius* sp.) and rohu (*Labeo rohita*) (Hamilton, 1822).[65] Tilapia are used mainly for subsistence and can be an economical source of food fish and additional income. Small-scale Nile tilapia culture in rural regions is basic, requiring very low inputs and labour, and can be undertaken by women and even children.[11,67] Tilapia farmers made significant profit by selling tilapia seeds to other tilapia growers.[68,69] In addition, landless farmers can benefit from tilapia farming by culturing these fish in common property roadside ditches.[70] The integration of Nile tilapia farming with existing farming systems has also significantly improved the livelihoods and reduced poverty of households in rural and peri-urban areas in Bangladesh.[67]

9 | CHINA

China is the world's leader in tilapia farming, with a production of 1,641,662 mt in 2019, contributing 20% to total tilapia outputs (Figure 5b).[7] Also, China contributed 27% (1,231,162 mt) to total global Nile tilapia production. Guangdong, Hainan, Guangxi, Fujian and Yunnan are the major tilapia producing provinces in China; contributing over 95% to national total tilapia production [60]. Small-scale and household-based tilapia farming is widely practiced in these provinces, making an important contribution to sustainable rural development, including food security, employment, income generation, diversifying livelihoods, utilizing family labour, and empowering women.[60]

Tilapia production has been expanding at a steady rate, along with domestic and international markets. Huge governmental support has been provided to tilapia producers, processors, and traders.[71] An example of this support is the development of tilapia culture in Guangdong Province, especially around the city of Maoming. Governmental support comprises services and supervision for all tilapia industry stakeholders, including improving farming technologies, well-trained researchers, strict quality supervision systems, disease

TABLE 1 The contribution of tilapia to total freshwater (FW) fish production in major tilapia producing countries in 2019.[7]

	Africa	Americas		Asia					
Production system	**Egypt**	**Brazil**	**Mexico**	**Indonesia**	**Philippines**	**Bangladesh**	**China**	**Thailand**	**Vietnam**
Tilapia fisheries (mt)	140,702	22,770	91,143	70,450	41,802	NA	NA	19,300	NA
Total FW fisheries output (mt)	229,479	218,932	151,638	480,935	90,421	929,872	1,383,929	129,300	128,120
% tilapia in total FW fisheries output	61.31	10.40	60.11	14.69	46.23	NA	NA	14.92	NA
Aquaculture of Nile tilapia output (mt)	1,081,202	323,714	NA[a]	1,130,000	174,212	350,258	1,231,162	213,872	NA
Total tilapia aquaculture output (mt)	1,081,202	323,806	56,945	1,166,800	279,386	350,258	1,641,662	213,981	263,107
% Nile tilapia in total tilapia output	100	99.97	-	96.84	62.40	100	75.00	99.94	-
Total FW aquaculture output (mt)	1,306,335	527,591	63,017	3,714,500	298,132	2,209,839	25,068,485	383,309	2,950,200
% tilapia in total FW aquaculture output	82.77	61.37	90.36	31.41	93.7	15.85	6.55	55.82	8.92
% tilapia in total FW fish output	70.40	46.42	68.99	29.49	71.9	11.16	6.21	45.51	8.55

[a]Not Available.

prevention and treatment, establishing breeding technology, and low interest financing for contract farming, processing plants and marketing support.[72] As a result, tilapia culture was rapidly developed in the Maoming district, so that it has become China's largest tilapia producer, and also the main centre for tilapia export processing[11,55] (CICCE, China International Cold Chain Equipment and Fresh Logistics Exhibition) (http://www.coldchain-china.com/index.php?m=content&c=index&a=show&catid=66&id=68).

In addition to supplying the domestic market with tilapia as a source of high quality, low-cost animal protein source, huge amounts of tilapia are exported to international markets.[63] The local tilapia fish market is dominated by farmed Nile tilapia, while the contribution of tilapia from captured fisheries is limited (Table 1). China has been the largest tilapia exporter in the world for many years, with a huge competitive advantage over other exporters, due to the low cost of Chinese tilapia. A total amount of 445,844 mt of tilapia (mainly Nile tilapia) was exported in 2018, contributing 87.4% and 79.2% to Asian and global tilapia exports, respectively,[7] with USA and Africa being the main markets for Chinese tilapia.

China realized the importance of tilapia as an export commodity to foreign markets and took strong measures to promote tilapia exports. The Chinese government established an early warning system on tilapia trade for assessing and monitoring the status and trends of tilapia culture industry.[71] The system provides the necessary strategies that facilitate sustainable tilapia development in China, using economics, management science and information technology to analyse the status of the tilapia industry, identify the major warning signals and, in turn, forecast the future trend of the industry.

However, between 2015 and 2020, the China Aquatic Products Processing and Marketing Association reported that cost of production has been increasing 5%–7% per year as government hatcheries have been replaced by private hatcheries, marketing support has decreased, and labour and regulatory costs have increased.[73] Some low-cost loan programs have also been removed. Farmers have had to absorb most of these costs as processors have not offered much higher prices. Many farmers are switching to high-value fish (in marketing terms) or trying to increase production per hectare with intensification and automatic feeders to reduce labour. The COVID-19 crisis also reduced tilapia harvests and domestic and international demand for tilapia products in 2020 compared to 2019.[74] In 2021, increasing demand and exports were resumed, but still not to the level of 2019.[74] Early in 2022, opinion seems to be that Chinese production of tilapia will grow only slightly with much of the demand coming from value added forms in domestic markets, with minimal new demand from international markets at the higher prices demanded by farmers and processors and higher transport costs affecting global trade.

10 | INDONESIA

In Indonesia, Nile tilapia gradually overtook carps and Mozambique tilapia to become the dominant freshwater aquaculture species. Indonesia is currently the second largest tilapia producer in the world (after China). Farmed tilapia output represents 31.4% of total freshwater aquaculture production in 2019; with Nile tilapia contributing 96.8% to total tilapia production.

Tilapia culture is practiced mainly in earthen ponds, by small farmers and households. Raising tilapia in floating cages and rice paddies is also widely practiced.[75] Commercial-scale cage farming of Nile tilapia in lakes and reservoirs for export has also been growing in Indonesia (e.g., Regal Springs). Therefore, in addition to the role of farmed Nile tilapia in local Indonesian markets, exporting tilapia with value adding to international markets has emerged in recent years. In 2019, Indonesia exported 11,107 mt of tilapia, mainly to the US market, in the form of frozen fillet.[7] Tilapia seed production and trade has also become a major source of employment and profit in many rural regions in Indonesia.[76] Tilapia farming is expected to become a major employer by 2030.[77]

11 | PHILIPPINES

Tilapia culture (especially GIFT strain) in the Philippines has witnessed rapid development during the last decades, leading the country to occupy the sixth position among the top tilapia producers in the world.[7] Nile tilapia is currently one of the most important animal protein sources in the Philippines, especially in rural areas.[78,79] In fact, tilapia prices are included in the market basket of goods that determine the cost-of-living government statistics.[79] These fish provide local communities with affordable, high-quality protein, and improve the nutrition and health status of rural households. Nile tilapia farming is also considered a cash crop; generating income and employment opportunities.[78]

The seeds of the GIFT strain of Nile tilapia are currently used throughout the country, leading to a sharp increase in tilapia production from only 13,214 mt in 1980 to 279,386 mt in 2019, representing 94% of freshwater aquaculture production of the country.[7] The use of improved seeds significantly improved the livelihood and profit margins of hatchery operators and fish farmers, created more employment in rural societies, and empowered women.[80]

In continuation with the development of the GIFT technology, a "Strategy for Sustainable Aquaculture for Poverty Reduction in Philippines" has been adopted and implemented by Philippine Department of Agriculture-Bureau of Fisheries and Aquatic Resources (DA-BFAR), with support of the WorldFish Center.[11] The main targets of this strategy were to increase farm incomes through the use of viable tilapia production approaches, promote the investment in post-harvest facilities, and improve the capability of local government units to foster production and marketing partnerships with BFAR and the private sector. The role of the Southeast Asian Fisheries Development Center (SEAFDEC) in tilapia culture development and promotion in the Philippines, and many other Asian countries is outstanding. The centre has carried out a number of research projects on the contribution of small-scale aquaculture to sustainable aquaculture and rural livelihood development. It also provides training and capacity building, extension services and technical supports to stakeholders along the

whole tilapia culture value chain. GET EXCEL tilapia is another new breed developed by the government of the Philippines by combining an improved breed of Nile tilapia using within-family selection and a rotational mating scheme. The Bureau of Fisheries and Aquatic Resources-National Freshwater Fisheries Technology Centre (BFAR-NFFTC) has sustained the development of a fast-growing fish known as GET EXCEL 2002 through the use of genetically improved tilapia.

12 | THAILAND

Thailand is currently the eighth largest world tilapia producer and sixth largest tilapia producer from aquaculture in Asia, after China, Indonesia, Bangladesh, the Philippines, and Vietnam, with a production of 213,981 mt in 2019.[7] Tilapia production comes mostly from freshwater aquaculture; which represented 56% of total freshwater aquaculture production in 2019,[7] with Nile tilapia and the hybrid red tilapia representing 99.9% of total farmed tilapia production.[7,81] The hybrid red tilapia (*O. niloticus* x *O. mossambicus*) strain developed by Charoen Phokpand (locally known as TabTim or Tub-Tim) is a popular variety that is grown in fresh water and brackish water as it has a higher salinity tolerance.[82,83] In some locations it is grown in polyculture systems with shrimp where it is reported to reduce occurrences and severity of shrimp diseases and provide a firmer texture for the tilapia flesh.[84,85] Tilapia farming is practiced primarily in private/family-owned, small to moderate-size ponds (1–3 ha).

Intensive and SI tilapia culture are commonly practiced in Thailand. Polyculture systems with carps and silver barb are some of most popular.[58,86] Integrated agriculture-aquaculture systems (IAAS) (fish-chicken/duck-pig, fish-cum-rice) are also very common in Thailand. Integrated tilapia culture currently plays a significant role in rural areas, by providing high-quality, cheap protein source, generating additional income and rehabilitating the soil through better on-farm nutrient recycling.[58,71,86] In Northeast Thailand, tilapia/carp polyculture is profitable, because pond inputs such as rice bran, crop by-products, broken rice, cattle, and buffalo manure are available, mostly on-farm, at low cost. Cage culture of tilapia in the extensive canal systems across Thailand has grown enormously in recent years, providing economic opportunities for landless farmers.[81,87] However, cage-based culture in rivers faces several challenges including water quality and disease. More research is needed on farm practices and risks, river and water management, and the complete commodity value chain.[87]

13 | VIETNAM

Nile tilapia culture in Vietnam grew at a high rate during the last decade (2010–2019), to become an important component in the food security of local rural poor.[88,89] Vietnam was ranked seventh among the top tilapia producers in the world in 2019.[7] Tilapia is among the most important fish species stocked in integrated systems in the Mekong Delta, second only to carps.[90] Nile tilapia is also integrated with silver barb and common carp in rice fields.[91] In addition, the culture of red hybrid (*O. mossambicus* x *O. niloticus*) in floating cages is widely practiced in the Mekong River, often in cages abandoned by *Pangasius* farmers who moved into intensive pond systems.[92,93]

Most tilapia produced in Vietnam is directed to local consumption. However, Vietnamese tilapia exports have gained significant attention in recent years. The exports increased from 0 mt in 2010 to 11,969 mt in 2019,[7] mainly in the form of frozen whole fish and frozen fillet. Major importers include the United States, Colombia, the Netherlands, Turkey, Italy, Belgium, Spain, Germany, South Korea and Saudi Arabia.[61]

14 | OTHER ASIAN COUNTRIES

In addition to the above-mentioned Asian countries, Nile tilapia (especially GIFT) farming is practiced in 16 other Asian countries at varying intensities. In addition, 14 countries farm other tilapia species, namely, Mozambique tilapia (*O. mossambicus*), blue tilapia (*O. aureus*) Sabaki tilapia (*O. spilurus*) or tilapia hybrids. Large amounts of tilapia are also recorded in the FAO aquaculture statistics under "not elsewhere included (nei)" tilapia' (*Oreochromis* [= *Tilapia* = *Coptodon*] spp). Significant amounts of Nile tilapia are produced in Taiwan, Cambodia, Myanmar, Sri Lanka, Lao PDR, Saudi Arabia, Malaysia, and Nepal.[94–96] Semi-intensive tilapia culture in earthen ponds, intensive systems in cages and tanks and integrated aquaculture/agriculture systems are all adopted at varying levels. Nile tilapia culture is gaining increasing attention in some other Asian countries such as India, Pakistan, Iran, and the Arabian Gulf Cooperation Council (GCC) countries. Extensive research is currently underway in these countries to develop sustainable tilapia culture industries.

15 | THE JOURNEY OF NILE TILAPIA TO THE AMERICAS

In the Americas, Mozambique tilapia (*O. mossambicus*) was first introduced from Malaysia into the Caribbean Island of St. Lucia in 1949.[97] These fish were then introduced from St. Lucia to many American countries during the 1950s and 1960s, mainly for aquaculture, fisheries, mosquito and weed control and fee fishing.[98,99] However, Mozambique tilapia was poorly accepted by the consumers as a food fish. Nile tilapia (*O. niloticus*) was then introduced, during the 1960s and 1970s, as a major candidate for aquaculture and fisheries. According to Fishbase,[4] Nile tilapia was introduced from Cote d'Ivoire to Brazil in 1971 and then to many Latin American countries in the 1970s, including Bolivia, Colombia, Panama, and Peru.[4] In North America and the Caribbean, Nile tilapia was introduced from Africa to Mexico in 1964, and Puerto Rico and the United States in the 1970s.[100] As a result, Nile tilapia has become a major component of freshwater aquaculture industry in some countries in the Americas.

Seventeen countries, especially in Latin America and the Caribbean, now practice Nile tilapia culture, with a production of 436,033 mt in 2019, representing 75% of total farmed tilapia production (584,202 mt), and 42% of total FW aquaculture output (1,031,434

mt).[7] As shown in the following section, Nile tilapia represents 61% of total freshwater aquaculture in Brazil.[7] Moreover, Nile tilapia represents 92% of total freshwater aquaculture in Costa Rica, and the only freshwater fish species with data reported to FAO from Honduras.[7] Red tilapia hybrids are also farmed in Honduras for local consumption and export to the US market,[101] but data are not available on the production of this hybrid. This demonstrates the role Nile tilapia plays in food security in these countries. Most tilapia production in the Americas is directed to domestic markets, with significant value-added tilapia exports to the US.[24,61,100,102]

16 | THE ROLE OF NILE TILAPIA CULTURE IN BRAZIL

Brazil is the most important Nile tilapia producer in the Americas, with a production of 323,714 mt in 2019.[7] Currently Brazil is ranked 5th among the top Nile tilapia producers in the world. Several additional large-scale vertically integrated tilapia projects have been announced in 2019 and 2021.[103,104] If these and other project expansions are all realized, the production would exceed 500,000 mt by 2025. Different farming systems are deployed, including SI in earthen ponds, intensive and integrated practices. Cage culture in lakes, reservoirs and just below hydroelectric power dams has also gained more popularity in recent years[105,106] contributing over 40% of total aquaculture production in Brazil.[106]

Nile tilapia culture in floating cages in reservoirs in semi-arid Brazilian regions is supported by state and federal governments as a means of food security and poverty alleviation.[107,108] Reservoirs that were created by governments, initially for drinking water and irrigation, now support cage culture which has become an important livelihood for resource-poor rural households, which generally live under harsh environmental conditions and low social development.[107,108] For example, it has been demonstrated that Nile tilapia aquaculture and fisheries activities contribute significantly to local economy in two semi-arid Brazilian reservoirs, Santa Cruz and Umari.[108] These activities provide over 44 mt of tilapia per year, for local consumption, in addition to various goods and services, with aquaculture generating higher revenues than fisheries. It has also been reported that cage culture of Nile tilapia in Santa Cruz reservoir is sustainable.[109] The benefit–cost ratio indicated that total revenue was US$ 1.34 for each US$ 1.00 invested (i.e., 34% profit).

17 | OTHER AMERICAN COUNTRIES

As a result of the continuous national and international efforts, small-scale and vertically integrated large-scale production of Nile tilapia in other American countries including Mexico, Colombia, Honduras, Ecuador, Costa Rica, El Salvador and Paraguay have recorded significant increases.[7,102,110,111] These countries produced 105,355 mt of Nile tilapia in 2019, representing 69% of their total farmed tilapia production, and contributed 41% to total tilapia production in the Americas, excluding Brazil.[7]

In Mexico, net pens, constructed out of local materials, are commonly used for raising Nile tilapia by low-income groups or individuals [103]. More sophisticated, large-scale production of Nile tilapia in floating cages are also in use, mainly in irrigation reservoirs. Floating or slow-sinking pelleted feeds are commonly used for feeding caged tilapia. In Colombia, Nile tilapia are also produced, mainly in cages, for export, while red tilapia are produced for the domestic market. Cage culture is practiced in large hydroelectric power reservoirs, using all-male Nile tilapia. The caged fish are generally fed with commercial extruded feed (24%–34% cp). Significant amounts of other tilapia species (e.g., *O. aureus*) and hybrids are produced in other American countries such as Peru and the United States. Over 90% of tilapia production in the Americas is directed to domestic consumption.

However, some of these Latin American countries produce high quality tilapia, which is exported to the United States in the form of fresh fillets. While China remains the leading Nile tilapia exporter to the US market, exports from other American countries are increasing. The rising of production costs in China and the change in government policy with regard to product subsidies will make Chinese producers less competitive over time, leaving more room for imports from neighbouring Central and South American countries. As a result, 13 countries from these regions are currently exporting tilapia and tilapia products to the US market; with Honduras, Colombia, Costa Rica, Mexico and Ecuador, respectively, being the largest exporters, contributing 95% of total tilapia export from these regions to US market.[61] The fresh tilapia market in the United States is dependent almost exclusively on these regions, since they contributed over 99.5% of total fresh fillet imported to the US market.[61] A total of 19,141 mt of tilapia fillet were exported from Colombia, Costa Rica, Honduras, Ecuador and Mexico to the US market in 2019.[7] Domestic production of tilapia in the United States has been fairly stable during the last two decades (2000–2019), with an annual production ranging from 8000 to 10,000 mt.[7] This production meets only 8% of the increasing demand for tilapia in the US market, leading to significant tilapia imports, as explored in the next section.

Tilapia culture development programs are implemented by local and international organizations in some countries in Latin America and the Caribbean, leading to significant economic and social development in rural societies. For example, a rural development program has been implemented by FAO in El Salvador to promote polyculture of tilapia and white-leg shrimp (*Penaeus vannamei*) (Boone, 1931) as a means of income generation and food security.[112] This practice increased the average farmers' income by up to 28%. A similar program has been carried out in Guatemala, also with FAO assistance, for making efficient use of water in Thismuntique village.[112] Nile tilapia farming resulted in a significant improvement in the livelihoods of many households. Rural households consumed 39.5% of fish production, while the remaining 60.5% were distributed among the local schools, leading to improving nutrition among Guatemalan school children. A benefit/cost analysis showed that profit margin for producers was 26%.

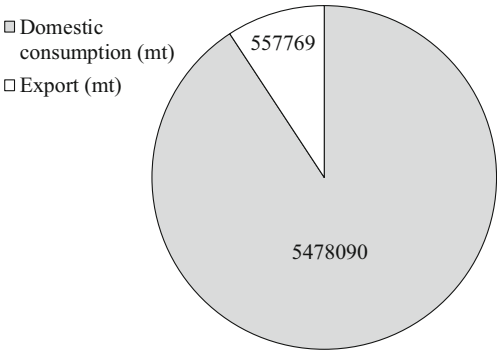

FIGURE 6 Global consumption and export of farmed tilapia in 2018.[62]

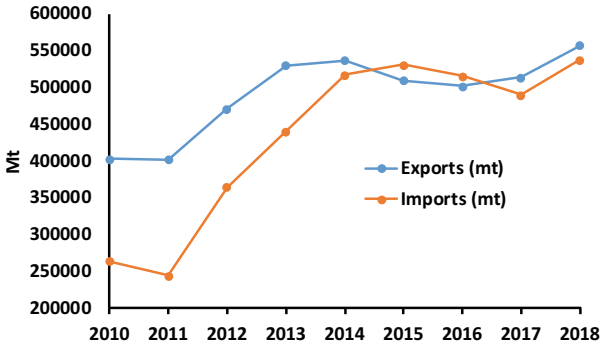

FIGURE 7 Global tilapia imports and exports (mt) during 2010–2018.[62]

18 | CONTRIBUTION OF INTRODUCED NILE TILAPIA TO FRESHWATER FISHERIES ENHANCEMENT

In addition to the socioeconomic benefits of farmed Nile tilapia, tilapia has made a significant contribution to the wild fisheries in countries to which these fish have been introduced and established. For example, Nile tilapia have been introduced in many rivers in south China and become the most dominant exotic fish species in Guangdong, Hainan, Guangxi, Fujian and Yunnan provinces.[57,113,114] Also, when Nile tilapia were introduced into East African Lakes (such as lakes Victoria, Kyoga, and Nabugabo), they became the most important commercial Tilapiine fish in many of these water bodies.[17] Similarly, Nile tilapia was introduced into Lake Kutubu, Papua New Guinea[115] and in just a few years became the most dominant component of the lake fishery, representing over 50% of total fish yield.[116,117] These fish have also become an important source of animal protein and cash crop for local communities; exceeding the benefits provided by native fish.[117] In Mexico, many new reservoirs have been stocked with Nile tilapia fingerlings from government hatcheries to provide a new fishery resource for those fish displaced by the filling of the reservoir.[111] Virtually all the wild fisheries catch of tilapia in Mexico come from these reservoirs and at times have been included in Mexico's aquaculture statistics.

19 | NILE TILAPIA- A FISH FOR THE POOR, AND THE RICH

It is clear from the above review that farmed Nile tilapia is a fish for both the poor and the rich in Asia, Africa, and Latin America. These fish can help feed the world, due to their simple farming techniques, good quality and affordable prices. The demand for Nile tilapia is increasing worldwide, especially in developing countries.[61,118] Consequently, most tilapia are consumed domestically (91% of total tilapia production in 2018)[7] (Figure 6). It should be emphasized, however, that tilapia trade data and statistics did not separate tilapia imports and exports into species; but reported them as 'total tilapia'. Since

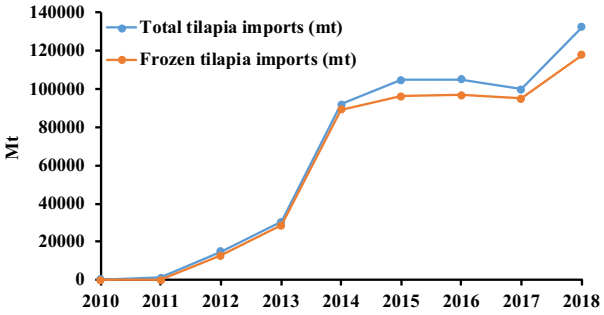

FIGURE 8 Tilapia imports to sub-Saharan Africa during 2010–2018.[62]

Nile tilapia represents about 75% of total farmed tilapia production, it is fair to suggest that Nile tilapia represent a major source of locally consumed and traded tilapia. Tilapia is also in high demand among the middle-income and high-income communities in many countries.[118] Therefore, the global trade of tilapia products has flourished in the last two decades and is expected to continue.[62]

The demand for tilapia is also growing in traditionally non-tilapia producing countries, especially those countries which rely on whitefish species (e.g., Atlantic cod [*Gadus morhua* Linnaeus, 1758], whiting [*Merluccius bilinearis* Mitchill, 1814], haddock [*Melanogrammus aeglefinus* Linnaeus, 1758], hake [*Urophycis* sp.], Asian catfish [Basa] [*Pangasius* sp.] and pollock [*Pollachius* sp.]),[119,120] primarily Europe and the United States. Tilapia is currently imported by over 135 countries worldwide. As a result, total tilapia imports doubled during the last 10 years to reach 537,914 mt in 2018 (Figure 7).[62] The USA is the largest tilapia importer in the world, with imports of 189,565 mt in 2018, representing 35% of total tilapia imports, while China is the largest tilapia exporter; contributing 444,851 mt (80%) to global tilapia exports.[62]

In other markets, especially Europe, Africa and the Middle East, the demand for tilapia is high. For example, over 35 sub-Saharan African (SSA) countries import tilapia, mostly frozen fish, mainly from China.[61] Tilapia imports in SSA increased dramatically from a couple 100 mt in 2010 to 132,475 mt, representing 25% of global tilapia imports in 2018 [63] in just 9 years (2010–2018) (Figure 8).

TABLE 2 Major tilapia importers in Europe, and amounts imported (mt) in 2010 and 2015–2019.[62]

	2010	2015	2016	2017	2018	2019
Belgium	1758	3965	3556	2633	3180	3896
France	769	4649	3839	4418	3555	4800
Germany	2638	3191	2695	2822	2702	2809
Italy	688	2646	2261	2065	2303	2565
Netherlands	2219	6469	5475	5910	6271	7314
Poland	6853	3836	3162	4706	3674	4274
Russian Federation	-	8127	6630	9944	8996	6702
Spain	3424	5498	4933	4856	3919	4408
United Kingdom	477	3622	3488	3693	2791	3852
Other countries	1703	4938	4976	5054	5177	4837
Total	18,826	42,003	36,039	41,047	37,391	40,620
Total Europe	20,529	46,941	41,015	46,101	42,568	45,457

Tilapia imports to 36 European countries, from different global exporters, have also increased during the last decade from 20,520 mt in 2010 to 45,457 mt in 2019.[62] This increase may appear limited on the global scale; however, in a continent where tilapia had no market until a few decades ago, consuming over 45,000 mt a year is impressive. This suggests European tilapia demand will expand further. The largest importers of tilapia in Europe are Belgium, France, Germany, Italy, the Netherlands, Poland, Russian Federation, Spain, and the United Kingdom (Table 2). They accounted for about 90% of the total tilapia imports during 2015–2019.

20 | ADVERSE IMPACTS OF NILE TILAPIA INTRODUCTIONS

Despite the above-mentioned benefits of Nile tilapia introductions, tilapia may pose adverse ecological and socioeconomic impacts in ecosystems into which they are introduced.[48,98,121,122] These impacts include: habitat degradation and loss, disruption of native biota, reduction or eradication of native species, reduction in capture fisheries yield, competition for food and breeding sites with native species, hybridization with native species of tilapia, and spread of aquatic diseases. For example, the introduction of Nile tilapia to the Pearl River in China led to a significant reduction in the relative densities and the body sizes of the native fish; thereby impacting the overall ecosystem function.[122] These changes suggest that Nile tilapia compete with native species for food resources and space,[48,98] and can seriously alter the trophic position and food web of native species.[123,124] Similarly, when Nile tilapia were introduced into Tangxi Reservoir (South China), they became dominant over time, leading to a reduction in the catch of other introduced fish, especially bighead carp.[125] The introduction of Nile tilapia in the Halali Reservoir (India) has also significantly reduced the catch per unit effort (CPUE) of native fish species.[126] Introduced Nile tilapia into Igarapé Fortaleza hydrographic basin (Amazonas River) have also caused significant impacts on native cichlids,[99] presumably due to the competition for food, preying on the eggs and larvae of native cichlids, and occupation of most spawning cites, leaving little room for the spawning of native species.[98,127] Introduced Nile tilapia also have the potential to transmit diseases into recipient aquatic environments, as demonstrated by McCrary, Murphy, Stauffer and Hendrix[128] in Lake Nicaragua (Central America). These authors reported that several native cichlid species were affected by an outbreak of trematodes, which was linked to the dominance of both Nile tilapia and Mozambique tilapia in the lake system.

The capability of introduced tilapia to interbreed with natural populations of their closely related native species is extremely high, which may cause genetic impacts and reduce the population size of these native populations.[48] The introgression between Nile tilapia and native *Oreochromis* spp. in Lakes Victoria and Kyoga resulted in morphological changes and disappearance of native species.[129] Similar effects have been recorded in Limpopo River (South Africa),[48] and the Mindu Reservoir and Kidatu Rufiji river system (central Tanzania),[130,131] where introduced Nile tilapia led to genetic and environmental impacts, including extinction risk of the indigenous species through hybridization, competition exclusion and loss of genetic integrity.

The introduction of tilapia into new freshwater ecosystems may also adversely affect the income of the resource users and other local communities.[10] For example, the increase in Nile tilapia abundance in rivers of Guangdong Province (China) reduced the incomes of local fishers[55,57,113]; as the contribution of tilapia increased, the CPUE of native species decreased.[112] The market prices of tilapia are also lower than that of native species, which are sold at much higher prices, because of their preferences by local consumers.[57,113] Consequently, fishers' income decreased with increasing the proportion of Nile tilapia in the catch.[113] The spread of tilapia can also adversely affect the value of other species, as they compete for food, which in turn leads to growth retardation and low production; thereby contributing to further decreases in fishers' income.[55,57]

Similar impacts have also been reported in Brazil,[98,99] South Africa,[18] and Zambia,[132] where the spread of Nile tilapia seriously impacted the value of other species, due to the competition for food and reduction in growth rates of other fish. Hybridization between introduced Nile tilapia and native tilapias (three spot tilapia *O. andersonii* and green head tilapia *O. macrochir*) in Kafue River (Zambia) resulted in a significant increase in the CPUE of Nile tilapia, whereas the CPUE of native tilapias showed a continuous decrease.[132] This situation poses potential threats to native fish production, which provides local communities with self-sufficiency in food, employment generation, and economic profitability.

Although the potential for deleterious effects caused by tilapia introductions must be recognized, tilapia are hardy and able to thrive in some disturbed and polluted environments in which native fish are ill adapted. Many reports of tilapia invasions fail to describe the alterations in water quality, climate change, and placement of dams changing water temperatures and flow rates, or co-introduction of more predatory non-native species that accompanied the introduction of tilapia into a particular watershed.[10,103,104,133,134] There are also reports that in relatively pristine ecosystems, Nile tilapia introductions did not lead to deleterious effects and that the fish did not even hybridize with indigenous cichlids.[134]

21 | MISMANAGEMENT OF TILAPIA INTRODUCTIONS

Tilapia introductions for insect and weed control, aquaculture and fisheries have occurred in many countries. It is almost impossible to guarantee safe confinement of these fish; their escape from irrigation and aquaculture facilities to natural aquatic ecosystems is inevitable. Therefore, appropriate management measures should be adopted to control tilapia introductions and mitigate any adverse ecological and socioeconomic impacts. In this regard, management strategies vary from one country to another depending on the objectives of tilapia introductions. Some countries adopt protective measures to control tilapia introductions to protect and sustain their native aquatic habitats.[107] For example, the United States has legalized unlimited catch of non-native fish species, as a means of controlling their spread and reducing their stocks.[135] Obligatory best management practices in aquaculture were implemented in some states (e.g., Florida) to control the stocks of these non-native species through preventing their escape.[136,137]

On the contrary, some other countries encourage tilapia introductions for aquaculture and natural fisheries enhancement, regardless of the negative effects they may cause.[107,138] They believe that feeding the public and maintaining better livelihoods of local communities are more important than the conservation of aquatic biodiversity. Current Brazilian federal legislation, for instance, encourages naturalization of non-native fish species, including tilapia, and allows the aquaculture, transportation and trade of these non-native species.[139,140] Aquaculture in China also depends increasingly on introduced species, including tilapia. Although such introductions can be considered assaults on already damaged and compromised aquatic ecosystems, government policy supports such introductions, and substantial domestic market and export fish market sectors have been built around these introduced species.[71,72]

22 | CONCLUSION

This review indicated that while Nile tilapia are endemic to Africa, they have been introduced into many countries worldwide, mainly for aquaculture and fisheries purposes. As a result, aquaculture of Nile tilapia is currently practiced in more than 80 countries and is now ranked third among the top farmed fish species, behind grass carp and silver carp. They make a significant contribution to the livelihoods and economies of rural societies in these countries. In some Asian countries, such as China, India, Bangladesh, Vietnam, and Myanmar, introduced Mozambique tilapia (*O. mossambicus*) has generally not been accepted by consumers. In contrast, the interest in Nile tilapia culture (especially monosex Nile tilapia and the GIFT strain) in those countries is increasing. Nile tilapia are also commercially important in Central and South American countries, especially Mexico, Costa Rica, Honduras, Guatemala, Ecuador, Brazil, and Colombia. The Nile tilapia journey will likely continue to further destinations. The adaptability of Nile tilapia to a wide range of environmental conditions, and their suitability for different farming systems will likely accelerate their spread and distribution worldwide.

Global tilapia markets and trade are expanding, and are expected to continue much further. It is hoped that the success stories of the major Nile tilapia producers will motivate Nile tilapia culture in other countries that intend to introduce this fish for aquaculture purposes. However, countries which introduced, or intend to introduce, Nile tilapia should adopt the necessary policies that support the conservation of native ecosystems and local biodiversity and at the same time, promote tilapia culture and improve the livelihoods of aquatic resource users.

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Abdel-Fattah M. El-Sayed: Conceptualization, data collection and curation, data tabulation and analysis, writing original draft, validation, review and editing, and corresponding author. **Kevin M. Fitzsimmons:** Contributions to original draft and review and editing.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

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ORCID

Abdel-Fattah M. El-Sayed https://orcid.org/0000-0003-3378-1205
Kevin Fitzsimmons https://orcid.org/0000-0003-3759-8168

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REVIEW

REVIEWS IN Aquaculture

The future of intensive tilapia production and the circular bioeconomy without effluents: Biofloc technology, recirculation aquaculture systems, bio-RAS, partitioned aquaculture systems and integrated multitrophic aquaculture

Sergio Zimmermann [1] | Anders Kiessling [2] | Jiasong Zhang [3]

[1]R&D, Zimmermann Aqua Solutions, Sunndalsøra, Norway

[2]Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden

[3]South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, China

Correspondence
Anders Kiessling, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, P.O. Box 7024, Uppsala 750 07, Sweden.
Email: anders.kiessling@slu.se

Funding information
Swedish University of Agricultural Sciences

Abstract

Modern tilapia farming with low use of water aims, as in circular bioeconomy, to reduce inputs and fully reuse waste and effluents, closing flows or links of economic and ecological resources and decentralizing production systems (local production and local consumption). Concerns over diseases, market demand for a clean, sustainable and ecologically correct aquaculture, with greater and more efficient controls, increased predictability and repeatability of activities, are leading to a series of structural changes in the reuse of water and effluents through various closed recirculation systems with the reuse of waste as nutrients. In recent decades, one of the most important innovations and trends of tilapia culture is towards circular bioeconomy, characterized in this review by several recirculation systems, such as biofloc technology (BFT), recirculation aquaculture systems (RASs), bio-RAS, partitioned aquaculture systems (PASs with split ponds, SPs; and in pond recirculation system, IPRS) and integrated multitrophic aquaculture (IMTA). The future of tilapia culture meshes with urban agriculture and waste fermentation, where low-demand water recirculation systems will be the protagonists in the disruption of industries in five main sectors (materials, energy, information, transport and food/health), that still today focus on extraction, into a more sustainable local model.

KEYWORDS
bioflocs, bio-RAS, circular bioeconomy, culture systems, recirculation, tilapia, zero effluents

1 | INTRODUCTION

Resource flows in a circular economy can help reduce the use of increasingly scarce resources, reduce waste production and limit energy consumption. In a world with a growing demand for clean water and healthy food, the economy in a linear model is no longer adequate, since modern societies cannot build a future under a 'take-do-discard' model. The movement towards environmentally sustainable systems is necessary through circular and life cycle thinking to preserve our finite natural resources.[1–4] Water, in particular as a valuable resource, must be treated with respect and managed with methods to reuse and conserve it, putting into action the concepts of circular bioeconomy.

2 | CIRCULAR BIOECONOMY WITHOUT EFFLUENTS

The circular economy can be defined as a production strategy that aims to reduce inputs, as well as waste production, closing economic and ecological resource flows or links, decentralizing production systems (local production and consumption) and questioning tools for measuring economic performance and the role of money and finance in building natural and social economic capital.[3] The analysis of physical resource flows is of two main types: (1) linear, where biological wastes (nutrients) are expected to be reintroduced into the biosphere and (2) circular, with biological wastes (nutrients) being recirculated and used again in the production system, not returning to the biosphere. Traditional aquaculture generates wastes deposited directly into nature, providing high levels of nitrogen and phosphorus to the natural environment. These represent a threat to human health, the welfare of fish and shrimp and the overall environment.[1] The frequent diseases that occur in aquaculture and the growing demand of the population for clean, sustainable aquafarming that is environmentally friendly are leading to the development of alternative production models with greater and more efficient controls, increase in predictability and repeatability of activities. These include a series of structural changes in aquaculture activity that consider the treatment of water and waste through closed land-based recirculation aquaculture system (RASs) and the reuse of wastes as nutrients. The partial or total reuse of water from aquatic crops has generated a series of land-based RAS, undoubtedly the most important innovation in aquaculture in recent decades when integrated with complementary systems forming a donor and receiver system.

Recirculation is based on the water movement through various compartments, tanks or ponds of different sizes. The water passes from one compartment to another and is partially or totally reused, depending on the intensity of the culture, ranging from more extensive/semi-intensive ponds to intensive/super-intensive tanks. The more intensive systems make use of sophisticated biofilters, compartments with biofilters, mechanical filters, geo-membranes/liners and various treatment methods, using any species grown in conventional aquaculture such as fish, crustaceans, molluscs, algae, and so on. Recirculation technology is widely used today in tropical fish farms, primarily for biosecurity reasons. RAS is showing enormous growth in marine shrimp, bivalve and seaweed farming, especially in the initial phases (hatchery and nursery). There is also enormous investment in recirculating water in salmon farming, but at low temperatures filter microorganisms are not very efficient, which greatly increases the costs of biofilters and additional structures.

Low water demand systems, either in isolated or recirculating compartments with intense aeration and high load of omnivorous tilapia or shrimp (more than 8 units/m^3 for fish and 100 units/m^3 for shrimp) end up spontaneously generating bioflocs.[5] In a single compartment, for example, a pond or tank, bioflocs are known as BFT (from biofloc technology). By recirculating the water in more compartments, this system can be called bio-RAS, a combination of the BFTs with the RAS, a term originally coined by Prof. Anders Kiessling back in 2015.[6] The primary objective is to improve the biosecurity of crops in places where water is scarce and/or land is expensive since the minimum exchange of water reduces the incidence of diseases.[7] The recirculation and reuse of water is the most classic application of the circular economy in aquaculture. These techniques are deployed in several aquaculture systems with possibilities of 'zero effluents' (Figure 1), whose focus is to maintain stable water quality and levels, suitable through the recycling of nitrogenous and carbon components, carried out mainly by specific bacteria, which are stimulated by the balance/ratio of carbon and nitrogen (C:N) in the water. The structure of this review is based on a publication prepared by the first author and collaborators for EMBRAPA/Brazil.[8]

3 | RECIRCULATION AQUACULTURE SYSTEMS

Recirculation aquaculture system technology has been developed over the last five decades, and it is becoming more popular and accessible as infrastructure and equipment are proportionally decreasing in price, while fish, labour and especially feed are increasingly expensive. Apart from that, RAS are being well applied in grow-out systems that are extensive in nature (in order to save water, increase yield and lower production costs) and intensive systems (on high-cost property, closer to urban markets and where water is expensive).[8] The main objectives of a more extensive RAS in ponds are to conserve water and generate less effluent that could damage the surrounding environment. To achieve this, an increased technology level is needed, by default increasing productivity. Despite the productive and environmental advantages, the reuse and maintenance of water quality, especially in more intensive RAS, will depend on a series of structures and equipment that are still relatively expensive, such as: settlers, mechanical filters, biological filters, ultraviolet lamps (disinfection), water pumps, air blowers, power generator, emergency aeration, ozone generation, and so on. (Figure 2). In addition to the high investments in building structures and equipment, there are high operating costs such as electricity, maintenance and depreciation. This is in part compensated for by the flexibility to locate production facilities near large markets, complete and convenient harvesting, quick and efficient disease control.[8] RAS have been widely used for hatcheries and nurseries for both freshwater and marine aquaculture. In recent years, large scale production with RAS for grow-out to harvest size have come into commercial success. Unfortunately, there were a significant number of failures of RAS commercial operations before the more recent successes.

4 | BIOFLOC SYSTEMS

Bioflocs are usually formed in isolated compartments (tanks or ponds),[8] but, unlike high technology water purification used in RAS, water recycling occurs directly in the fish production unit, reducing the size and the cost of mechanical and biological pipes, pumps and filtration systems. The process is somewhat similar to an activated

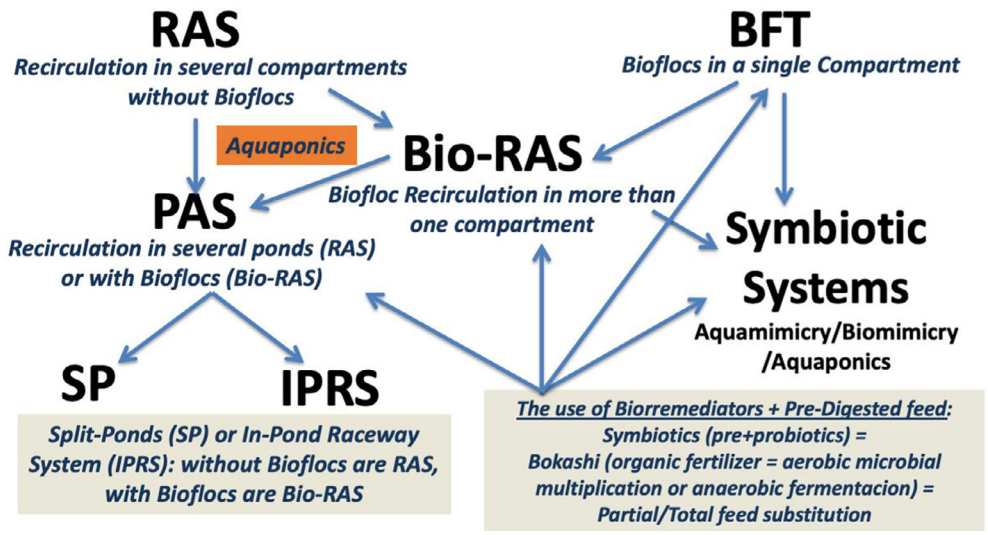

FIGURE 1 Characterization of the main aquaculture systems with low demand for recirculating water without effluents and their various derivations.

FIGURE 2 High technology and clear water recirculation aquaculture system for Tilapia (www.globalfish.pl).

sludge system used for wastewater treatment. The bioflocs are composed of assemblages of heterotrophic, nitrifying and cyano-bacteria as well as various algae and fungi. Therefore, compared with the more intensive RAS, it does not require filtration structures and can simply consist of tanks and aerators/pumps (Figure 3). The BFT can be inserted into a recirculation system (optional), with settler (optional) to control excess solids, drainage system (optional), blower and/or water pump and power generators. The structural and operational advantages of a BFT allow cultivation with high loads of suspended solids in the water, characteristics that affect different species produced in the RAS, but do not impact omnivorous filter-feeding species such as tilapia and marine shrimp, two of the most used species in BFTs around the world.[8] The ability to work with a relatively high solids load makes the BFT less dependent on mechanical filters.[6] It also abolishes the need for either partial water exchange or a secondary denitrification system typical of a highly intensive RAS. Some microorganisms that grow in the bioflocs of the culture water, such as nitrifying bacteria, transform toxic nitrogenous compounds (mainly ammonia and nitrite) to nitrate, also eliminating the need for an external biofilter, mandatory in recirculation systems (RAS). In essence, toxic ammonium is assimilated to organic N by heterotrophic bacteria and algal biomass when carbohydrate is added into culture water,[9] and thereby also function as an additional feed source for the farmed fish/shrimp. Such systems require constant and reliable aeration and physical water movement equipment in order to keep sediments suspended, plenty of available dissolved oxygen and avoid anaerobic sludge accumulation. In addition, careful monitoring and manipulation of dissolved oxygen, alkalinity, pH and C:N ratio is required.[5]

FIGURE 3 BFT (system without water exchange) in a greenhouse, with constant temperature throughout the year, in the sub-tropics of Brazil. *Photo*: Rafael Jung (2002).

FIGURE 4 Bio-RAS with six greenhouse fattening reactors, recirculation tank (bottom) and denitrifying tank or sludge concentration/reuse tank (top left). *Photo*: Sergio Zimmermann (2003).

5 | BIO-RAS

Bio-RAS is the combination of RAS with BFT (a recirculation system with bioflocs in more than one compartment).[8] The advantages of BFT over classical RAS became apparent three decades ago, when different systems based on bioflocs were developed. Currently, there is a trend to merge these two low water demand systems to optimize crops with a reduction in production costs (especially food and electricity). The bio-RAS strategy uses the best and most efficient of each of the previous technologies, with cost reduction combined with the maximization of technological, zootechnical and animal welfare efficiency with the sustainability of the crops.[6] Bio-RAS has been used in the last decade in a number of low-cost aquaculture projects (Figure 4). In bio-RAS, bioflocs can form in part of one or more compartments, or of the entire circulating water (in this case, it requires some adaptations in the filtration system or its exclusion).[8] In most

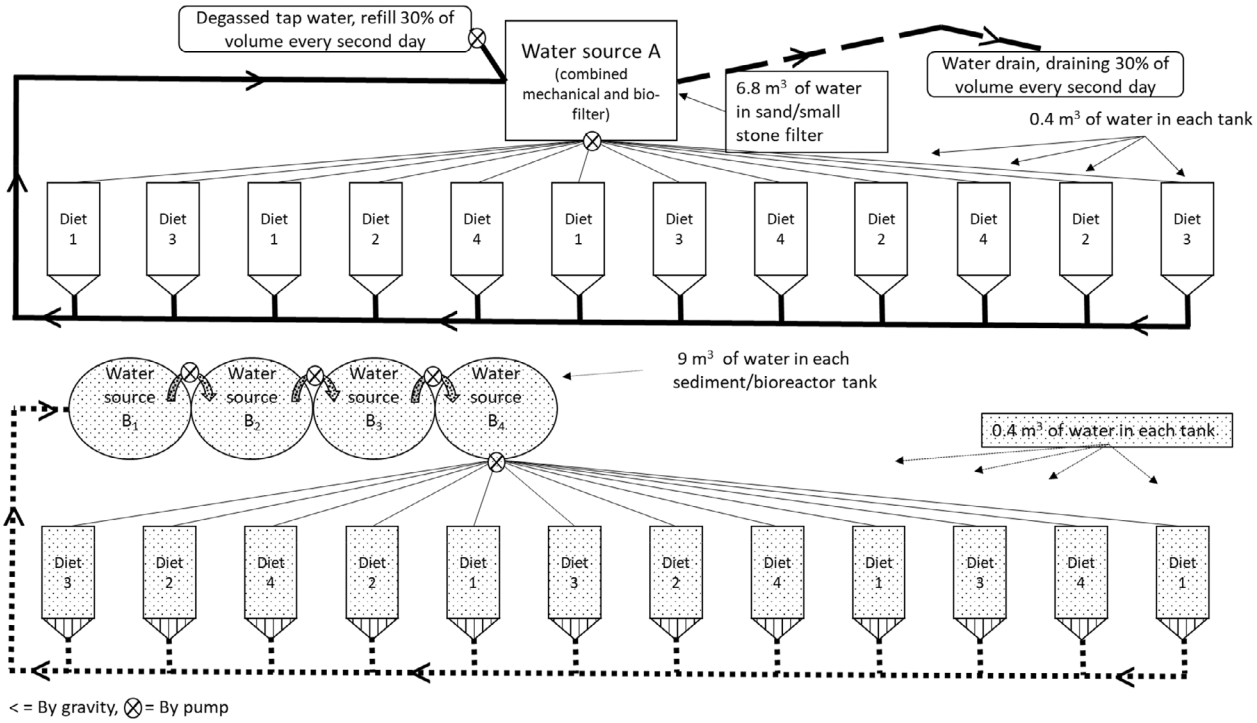

FIGURE 5 A simplified drawing of the CW- and bio-RAS systems used by Kiessling and co-workers[6] at AnGiang University in Vietnam. Extracted from Reference 10.

cases, it is part of the sludge reuse system of a simplified RAS, without effluents (Figure 5 shows a simplified drawing of the CW- and bio-RAS systems used by Kiessling and co-workers at AnGiang University in Vietnam).[6]

6 | PARTITIONED AQUACULTURE SYSTEMS

The partitioned aquaculture system (PAS) was developed in the 1990s in the southern United States to cultivate American channel catfish with recirculation of wastewater. The objective is producing zero effluents,[11] where fish are confined in high densities in concrete tanks (raceways) or smaller channels/ponds, around 5% of the total area for the tank and 95% of the pond or lake for recirculation and reuse of water. The fish residues from its catabolism circulate and recycle through the water body where there are high concentrations of algae (fertilized by these residues), similar to a domestic wastewater treatment, which increases or even doubles the support capacity of the system. By doubling the rate of photosynthesis of algae in these generally isolated baffles and ponds, the rate of removal of nitrogenous, phosphorous and other waste products doubles, thus doubling the potential maximum feeding rate and the consequent carrying capacity to sustain the system and the fish and shrimp production.

PAS represents a high degree of intensification for previously extensive ponds and reservoirs where phytoplankton predominate.[11] In its various forms, productions in the range of 10–50 tons of tilapia per hectare of surface or 10,000 m^3 of total volume are obtainable. Its two main variations are increasingly common around the world: (a) *IPRS* for in pond raceway system with a pond/reservoir/lake holding cages, raceways or containers (Figure 6a–f) and (b) *SPs* for split ponds (Figure 7).

IPRS confine omnivorous fish at high densities in cages or raceways (channels with high water flow) installed along the inside periphery of an existing lake or pond. The water recirculates through the large bodies of water that assimilate the waste from the small, cultivated areas, facilitating the feeding, sampling, protection and harvest of the fish.[11] Although IPRS was originally designed for channel catfish aquaculture in the southern United States, its use expanded and became more popular in the farming of carp, tilapia and other omnivorous fish in China, India, Brazil, Colombia, Thailand and several other countries.[8]

The SP's also originated in the southern United States, taking advantage of the huge dams with reservoirs available as a starting point for the construction of the system. SPs are built by dividing a fish pond into two unequal sections by building a central partition or dike, with water circulating between the two sections with high-volume, low-head pumps. Compared with the IPRS, SPs usually have a relatively smaller recirculation basin (around 80%–85% of the total area) and a larger fish retention basin (15%–20% compared with 5% of the IPRS). In both systems, farmers are increasingly using pumps connected to solar collectors to reduce electricity and electrical installation costs.[11]

Some PAS adopt techniques derived from bio-RAS, with early research and scientific publications using bioflocs as biological water treatment in large-scale commercial systems for intensive fish and

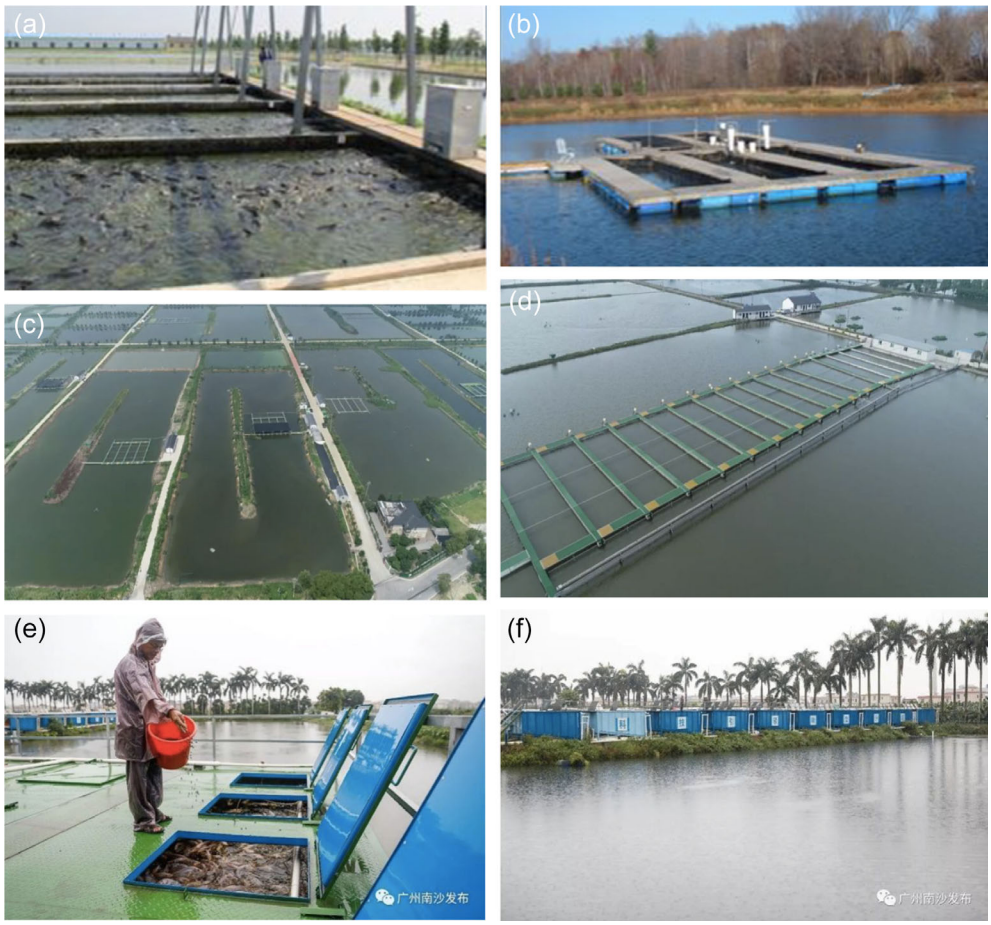

FIGURE 6 Raceways on the banks of a pond or lake (IPRS), floating cages inside a reservoir, IPRS in a typical RAS pond farm and Tilapia being cultured in containers on the banks of an earthen pond used as a purification system. *Photos*: Sergio Zimmermann and Chinese Medias (2021).

shrimp production. At higher densities, PAS change rapidly to a predominance of bioflocs and require more and more aeration, water movement and the addition of symbiotic supplements (pre +- probiotics) as summarized in Figure 1: Bokashi (organic fertilizer/bioremediator), fermentative Premix (FermentAqua®), EM (Enhanced Microorganisms) or mixotrophic products (BlueAqua's Mixotrophic System®), and so on. These have been developed into a series of systems, among which are aquamimicry heterotrophic, autotrophic, photo-autotrophic and the active suspension system, various techniques which use concepts generated in the RAS and BFT systems mixed with bio-RAS.[5,6,8]

7 | INTEGRATED MULTITROPHIC AQUACULTURE (AQUAPONICS AND FERTI-IRRIGATION)

In integrated multitrophic aquaculture system (IMTA), two or more complementary species with different trophic levels or niches are farmed. For example, tilapia with shrimp and seaweeds in brackish water. Another example would be tilapia, silver carp and water lotus in freshwater. In some cases, fish and terrestrial animals and/or hydroponics (vegetables) could be in the same production system in recirculation with single or multiple loops. The integration between aquatic and terrestrial species (such as plants, pigs, poultry, among others) is maintained with multiple relationships between resources (such as space, water, food or nutrients). Generally, these are shared between different species, thus offering greater potential in terms of technical and economic efficiency and redundancy.[12] In the past, the production of more than one aquatic species in the same culture unit, either in earthen ponds or in cages, was called polyculture, while aquatic and terrestrial organisms that were produced together, was called integrated aquaculture (IA). In IA, the waste output from one subsystem generally becomes an input for another subsystem, resulting in greater efficiency in the production of aquatic organisms.

IMTA combines the cultivation of fed species (e.g., tilapia + shrimp) with extractive (species, grazers and filter feeders) feeding on organic matter (echinoderms, molluscs, especially bivalves, micro-crustaceans and worms, other herbivorous fish) and inorganic extracting species (such as phytoplankton and marine macroalgae or hydroponic vegetables). The goal is to match in the right proportions to create balanced systems that generate environmental and economic sustainability and social acceptability. The feeding costs of the IMTA systems are thus distributed between two or

FIGURE 7 Aerial view of split-ponds or split ponds/weirs (partitioned) from the Google Earth program (accessed in August 2020). *Photo*: Sergio Zimmermann.

FIGURE 8 Uncoupled aquaponics system, tilapia juveniles integrated to the production of mini-tomatoes in ferti-irrigation and vegetables in hydroponic profiles. Collection and storage of rainwater (left outside), in the extreme south of Brazil (sub-tropics). *Photos*: Fagner Tafarel Campos de Sá (2021).

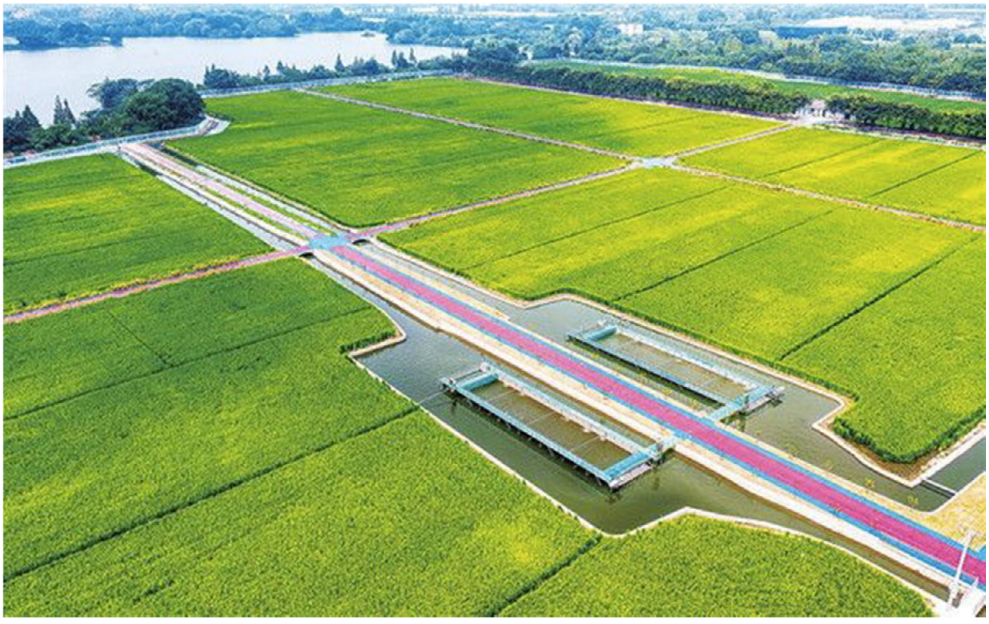

FIGURE 9 Integrating IPRS with rice production in China (2020). *Photo*: Chinese Medias (2021).

FIGURE 10 Tilapia and rice culture in China (2021). *Photo*: Chinese Medias (2021).

more commercial crops where more nutrients can be captured and sequestered, avoiding the loss of valuable inputs. Therefore, IMTA can produce more than one type of edible crop from feed ingredients more efficiently than other conventional production systems. For example, in an integrated system of tilapia with shrimp and hydroponics (aquaponics) with fertigation, the metabolites produced by aquatic organisms serve as nutrients for each other and for plants (Figure 6c–e). In addition, the sludge from RAS and bio-RAS systems can be reused as pre-digested ingredients (highly digestible) in rations for aquatic and terrestrial animals (Figure 8a–c).

Aquaponics is one of the classic examples of IMTA, an interaction between hydroponics and aquaculture, where one crop benefits from the by-products of another, making the respective ecological 'bottlenecks' of both systems become strengths, considerably reducing the need for inputs, nutrients and effluent production, unlike when the same systems are run individually.[13] Aquaponics systems can be important tools to enable economic temperature control, disease prevention, predator control and the full use of the most expensive inputs (rations) and should also be encouraged for their sustainability and biosecurity characteristics (Figures 9 and 10).[14]

8 | COMPARING THE SYSTEMS

In 2021, the Brazilian Ministry of Agriculture published a booklet with the main characteristics and production costs of several tilapia intensive rearing systems,[15] such as BFT, bio-RAS, ponds and cages from the States of São Paulo and Paraná (subtropical climate). The authors stressed that the comparison between technologies should go beyond observation of the production costs per ton of tilapia. It is very important to evaluate the Capital Expenditure/Operational Expenditure (CAPEX/OPEX), and especially the increasing land costs (not considered in the study, thus favouring the more extensive systems such as IPRS and SP), as well as water volumes and the annual production potential of each technology. This last feature altered the financial

TABLE 1 Characterization of the main tilapia aquaculture intensive recirculation systems with low demand of water and effluent production

Culture system	Grow-out volume (%)	Recirculation volume (%)	CAPEX (USD/kg/year)	OPEX (USD/kg/year)	Productivity (kg/m^3/year)	Prod. cycle (days)	Average FCR	Required area (ha) per 100 Mton/year	Profitability index (%)	Levelling point (in kg)	References
BFT	100%	–	2–8	1–2	20–30	120–140	0.9–1.25	0.4	11.22	55,680	5,8,10,15–17
RAS	80%–90%	10%–20%	6–20	3–8	30–50	120–150	0.95–1.30	0.25	4.35	–	5,8,10,15–17
Bio-RAS	50%–80%	30%–50%	3–8	1–1.5	30–40	100–120	0.75–1.20	0.3	24.81	55,895	5,8,10,15–17
SP	20%	80%	1–3	0.5–1.5	5–10	180–210	1.10–1.60	0.6	46.05	53,295	5,8,16–18
IPRS	1%–10%	90%–99%	3–5	1–3	4–6	180–210	1.25–1.75	1	6.05	227.452	8,16–18

Note: Typical grow out/recirculation rates, CAPEX/OPEX per kg annually produced, productivity, FCR, required area, profitability index and levelling point.

result and may favour one or another technology. This is the case of BFT and bio-RAS, which could perform 2.6–3 cycles per year, with the financial differential of low water requirement production technologies, where management efficiency should reach high levels to the systems to be viable, as well as the appeal to be environmentally sound. This study was later published in more detail with a short economic analysis,[16] and it is summarized together with other references characterizing the systems in Table 1.

It is very challenging to summarize quantitative data on nutrient flows or balances when describing most extensive systems such as RAS in ponds or PAS (SP or IPRS).[18] Table 1 summarizes information on how the dimensions of the grow-out and 'purifying/receiving' (recirculation) water bodies (tank, pond or lake) are applied worldwide in order to generate a better understanding of how each of these main five systems are dimensioned, functioning and yielding. The ranges presented were collected in commercial structures in Brazil, Peru, Ecuador, Colombia, Mexico, USA, Thailand, Vietnam and China.[17,19]

9 | CONCLUSIONS

Tilapia culture will evolve along with the trends of food production that are increasingly urban, 'on the roofs of supermarkets' and in urban industry facilities,[20] where aquaponics and water saving/recirculation systems will be the producers in these new forms of Circular Economy.[8,20] During the COVID-19 years and more recently the war in Ukraine, it is clear the increasing disruptions of the centralized extractive industries that today sustain the global economy in the five main sectors (materials, energy, information, transport and food/health), are evolving into a more local model. It is suggested that production and process costs could decrease by an order of magnitude of 10 times by 2030, that is, we will use 90% less natural resources and produce 10 times less waste.[20]

Modern tilapia culture systems with resources flowing in a circular economy will reduce the use of increasingly scarce resources such as water, energy, labour and especially feed ingredients, minimizing waste production. Novel pre-digested dough-like feed (FermentAqua®), produced from inexpensive by-products or diet ingredients are replacing traditional diets with low cost and improvements in productivity.[19] The convergence of precision fermentation and water circularity is enabling rapidly falling costs.[20] The recirculation systems characterized in this review include: BFT, RAS, bio-RAS, PAS with SPs and IPRS and IMTA. Each system has different characteristics in term of production costs, carrying capacities, FCR, cycles per year, CAPEX/OPEX, financial characteristics, water requirements, production technologies and can be chosen based on specific situations such as land prices, market demands/distance, water availability and several other parameters.

AUTHOR CONTRIBUTIONS

Sergio Zimmermann: Conceptualization; investigation; methodology; supervision; validation; writing – original draft; writing – review and editing. **Anders Kiessling:** Conceptualization; formal analysis; funding acquisition; investigation; supervision; writing – review and editing. **Jiasong Zhang:** Visualization; writing – review and editing.

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DATA AVAILABILITY STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article. The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Sergio Zimmermann https://orcid.org/0000-0002-0393-1922
Anders Kiessling https://orcid.org/0000-0002-5325-3779
Jiasong Zhang https://orcid.org/0000-0001-9490-119X

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REVIEW

REVIEWS IN Aquaculture

How value addition by utilization of tilapia processing by-products can improve human nutrition and livelihood

Omar Peñarubia | Jogeir Toppe | Molly Ahern | Ansen Ward | Michael Griffin

Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

Correspondence
Omar Peñarubia, Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Carcalla 00153, Rome, Italy.
Email: omarriego.penarubia@fao.org

Abstract

Aquatic foods, particularly fish, are recognized as a unique source of essential fatty acids, micronutrients and protein many diets lack, especially in poor and vulnerable communities. Tilapia (*Oreochromis* sp.) fillets typically represent 30%–33% of the fish, leaving around 70% of the fish unused for human consumption. These nutrient-rich by-products can be converted into food and other products with medical, pharmaceutical and packaging applications. Heads and backbones of processed tilapias, and undersized tilapia can be used in the development or fortification of food products such as fish cakes, sausages and bread. Tilapia skin can be processed into leather for clothing and leather artefacts. Gelatin from fish skin can be developed into edible films and coating while collagen from fish scales and bones has good application in the cosmetic and pharmaceutical fields. The viscera can be converted into biodiesel or silage and hydrolysates, which are good sources of peptides and enzymes. To ensure 100% utilization, any remaining parts of the fish not used for food, can be transformed into products for animal consumption or for fertilizer. Thus, the conversion of by-products from tilapia processing into value-added products can contribute to improve human nutrition and better livelihood opportunities. However, adopting new technologies in value addition will require additional operational costs for acquiring new equipment and skills, a proven market demand for the products and an enabling policy environment.

KEYWORDS
by-products, livelihood, nutrition, processing, tilapia, value-addition

1 | INTRODUCTION

Human population growth, rising incomes and shifts to healthy diets will increase global demand for nutritious foods in the next decades.[1] Global food demand is rising, and the effects of limited opportunity to expand land-based production have posed negative impacts on the environment and human health. Fish and other aquatic foods are among the world's most traded food items, with a total export value of USD 164 billion globally in 2018.[2] Globally, more than 1 billion people rely on fish for consumption and livelihoods. Aquatic foods are considered nutritionally diverse and have been recognized as particularly nutritious, contributing essential fatty acids, micronutrients, such as iron and zinc, calcium and vitamin A, as well as protein.

Tilapia (*Oreochromis* sp.) production has great potential to contribute to income generation, poverty alleviation, enhanced trade, economic benefits and the supply of protein and essential nutrients. In

2020, global tilapia production was estimated at almost 7 million tonnes.[3] Tilapia is mostly sourced from aquaculture, which accounts for 6.2 million tonnes worth USD 12 billion annually. The vast majority of tilapia production is destined for domestic consumption, with only 507,000 tonnes traded internationally. This trade has an annual value of USD 1.392 billion.[4]

In terms of the consumer market, the consumption of tilapia in the United States, the second most important consumer market for tilapia, remained relatively stable throughout the pandemic.[4] Tilapia, the fourth most popular fish species among US consumers, has increased in retail sales as house-bound consumers sought out easy-to-prepare, versatile aquatic food options. Tilapia imports in 2020 increased 10% in terms of volume and 2.3% in value compared with 2019, where frozen tilapia fillets made up most of these imports, accounting for 61% of value.[5] While the United States remains by far the most import market, its imports of tilapia have fallen significantly since their peak in 2014. Prior to the pandemic, tilapia imports fell by 12% in value terms between 2018 and 2019, 7% in volume. US tilapia imports account for 48% of the value of global trade, followed by Mexico (26%, USD 185 million), Israel (11%, USD 58 million), Côte d'Ivoire (11%, 52 million) and Canada (10%, USD 40 million).

Processing tilapia for international markets is a common form of value addition, with fillets making up 75% of the value of tilapia trade (55% of volume). Fish processing includes bleeding, gutting, beheading, filleting, skinning and trimming before fillets are bought by consumers. The fillet yield in industrial processing is species-dependent and leads to a significant removal of parts of the fish, such as heads, bones, guts or by-products.[6,7] The tilapia fillet industry produces a large amount of processing by-products estimated at 60%–70% of the total weight comprising head, carcass, viscera, fins, skin and scales.[8–10]

By-products of fish were traditionally considered to be of low value and as a waste product contributing to environmental problems. The ever-increasing production of these processing by-products without utilization was resulting in environmental pollution.[11–14] Inappropriate waste management causes environmental pollution leading to breeding grounds for insects and vermin, thus, posing significant public health risks. Consequently, waste management is coming under strict regulations due to environmental issues and has become an increased cost burden for the seafood industry.[12] However, by-products of fish processing provide a good source of macro- and micronutrients and can be converted into a variety of products including fishmeal and oil, fish hydrolysates, fish collagen, fish sauce, fish biodiesel and fish leather, but also nutritious food products. The utilization of by-products has several environmental and economic benefits as well as the possibilities to produce more food from limited resources.[6,13]

Large-scale processing companies may process fish by-products and convert them into value-added products such as fish oil and fishmeal. However, challenges such as the unavailability of waste disposal facilities and programmes for small-scale fish processors and fishing communities often result in missed opportunities to utilize these by-products for potential economic gain to local communities. The transformation of by-products into commercial products must be market-driven or must have a realistic possibility of being sold with an economic gain within a reasonable time period.[6,13]

This review summarizes the importance of fish, and particularly tilapia, as sources of important micro- and macronutrients. Importantly, it will highlight existing studies and practices on how to fully utilize the whole fish by transforming tilapia processing by-products into nutritious fish products for human consumption. Furthermore, it will provide information on other non-food products that can be developed from tilapia and promoted as an additional source of livelihood for small-scale producers and processors.

1.1 | Fish and human nutrition

Aquatic foods are rich in numerous vitamins, minerals, essential fatty acids and micronutrients essential to cognitive development and human health, particularly in the first 1000 days of a child's life.[15,16] However, there is evidence that the crucial first 1000 days extend for an additional 7000 days throughout adolescence, linking two crucial time periods—the first 1000 days of life and women of reproductive age, particularly important for adolescent girls.[17–23]

Aquatic foods can improve human health through at least three pathways: by reducing micronutrient deficiencies that can lead to subsequent disease; by providing a unique source of the omega-3 long-chain polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) which may reduce the risk of heart disease and promote brain and eye health; and by displacing the consumption of less-healthy red and processed meats that can cause adverse health outcomes.[24,25] Many fish, and other aquatic animals, naturally obtain health promoting long chain omaega-3 fatty acids from consumption of, or bio-accumulation from, marine microalgae. These omega-3 fatty acids (particularly EPA and DHA) are exceedingly rare in land-based plant crops, but have many essential roles, including being precursors of eicosanoids, a large component of the central nervous system, a structural element of every cell of the body, and a regulator of cardiac rhythm, thus it can reduce risks for cardiovascular disease.[26,27]

Micronutrient deficiencies account for an estimated 1 million premature deaths annually.[24,28] Nutrient-rich aquatic foods could provide food-based approaches to reducing nutrient deficiencies, with increasing access and consumption offering many advantages over nutrient supplementation.[29] Calcium, iron, zinc and vitamin A from fish could provide a considerable proportion of the recommended dietary allowance (RDA) for adults and children under 5-years old.[28]

Aquatic foods particularly fish is an animal-source food, which provides 17% of animal protein and 7% of total protein consumed globally.[2,15,26] However, in 31 countries—16 of which are LIFDCs and five are small island developing states (SIDS)—where fish and other aquatic foods serve as the backbone to a healthy diet, fish accounts for more than 30% of total animal protein supply.[17]

Tilapia has high crude protein content, minerals such as calcium, phosphorus, magnesium, sodium and iron[30] and fatty acids. Tilapia is also a good source of vitamins A, C, D, E, K, B6 and B12[11] Furthermore, tilapia carcasses contain 23 fatty acids, including the very important n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[30]

1.2 | Value addition

Aquatic foods particularly fish are highly perishable food products due to their chemical composition including neutral pH, and weak connective tissues.[31] Therefore, it is necessary for storage or transport to preserve and process aquatic foods to maintain durability and to retain their nutritional value. In addition to increasing the shelf life, fish processing and the sale of by-products also aims to increase its economic value by developing new fishery products to attract public interest.[17,31–33]

The development of new ingredients or new products in various forms by using tilapia processing by-products has been an important option to increase the added-value of products, avoid economic loss, reduce environmental impact, and supply consumers with a nutritious, low-cost, more stable shelf-life and convenient food.[34,35]

Fish processing by-products deteriorate very rapidly, especially when containing viscera, therefore it is important to preserve them as soon as possible after being produced. Some of the by-products such as heads and trimmings, may in certain cases be used for human consumption while the majority has traditionally been regarded to be of low value or as a problem and used as feed for farmed animals or as fertilizers.[36]

1.2.1 | Food products

Tilapia production is expected to rise and already has one of the most diversified supply chains in aquaculture, increasing the amount of by-products generated after processing. Value addition through production of fish fillets has been an effective strategy to increase consumer acceptability and commercial value, and ensure better utilization of tilapia. With the changes in consumer consumption expectations as well as the development of reliable cold-chains, fresh preprocessed fishery products such as fillets are more popular with consumers and producers because of their convenience for processing and cooking.[31] However, the filleting process results in a significant amount of by-products that can be converted to nutritious low-cost fish products. By-products containing heads, frames and belly flaps, and parts of the viscera like liver and roe are good sources of high-quality proteins, lipids with long-chain omega-3 fatty acids, micronutrients like vitamins A, D, riboflavin (B2) and niacin (B3) as well as minerals such as iron, zinc, selenium and iodine[6,37,38] (Table 1).

Several studies were conducted to convert undersized tilapia and tilapia processing by-products into new food products with good nutrition and various applications. Small-sized tilapia can be converted into flour and used as a flour substitute in brownies to add nutrients such as protein and calcium.[40] Furthermore, it can be converted into spring roll with high acceptability from consumers.[39]

Mechanically separated flesh or the minced fish obtained from tilapia backbones offered good quality and organoleptic characteristics and therefore, have great potential to develop value-added products. From the microbiological perspective, the frozen mince was determined to be safe and had a stable physicochemical and sensorial quality for 6 months of storage.[79]

TABLE 1 Utilization of undersized tilapia and tilapia processing by-products in foods

Fish-based product	Part used
Spring rolls[39]	Undersized tilapia
Flour (brownies)[40]	
Surimi[32,41–45]	Minced meat/mechanically separated meat
Surimi powder[46]	
Burger patties[47]	
Nuggets[9,48]	
Mortadella[10,49]	
Sausage[50–56]	
Flour[11,34]	Head and bone
Flour[30,35,57–60]	Minced fish/mechanically separated meat
Flour[59–62,80,81,109]	Bone
Protein concentrate/flour[63–65]	By-products
Gelatin[66–70]	Skin
Gelatin[71]	Bone
Gelatin[72]	Scales
Gelatin[73]	Muscle
Edible film[74–76]	Skin
Edible film[77,78]	Bones
Edible coating[62]	Skin

Tilapia mince can be converted into surimi[32,41–45] or surimi powder.[46] The proximate composition and quality indices of surimi powder made from minced tilapia suggest that it can be prepared easily, has low distribution cost, requires minimum storage space, is useful in dry mixtures to produce homogenous blends and is easy to standardize protein content.[46] Furthermore, tilapia mince can be directly used as an ingredient in the production of burger patties,[47] nuggets,[9,48] mortadella[10,49] and sausage.[50–56] The mortadella made from minced tilapia had a good acceptance index, higher mineral content, especially calcium, greater softness, less luminosity and greater colour intensity compared with pork and chicken mortadella.[10] A maximum level of 60% minced fish in sausage has been recommended to obtain good nutritional quality and acceptable sensory attributes.[55] Sausages manufactured with mechanically separated tilapia meat represent a sustainable use of this waste, with high consumer acceptance.[50]

The head and minced fish of tilapia can be converted into flour[11] with various food applications. Instant soup and flours from tilapia wastes can be used in the food industry either for development and introduction of new food products on the market or for the replacement in current food products made from conventional flour sources, producing a healthy alternative particularly to gluten-intolerant consumers.[34] Tilapia flour can be used as an ingredient in various food products such as croquettes,[35,57] bread,[58,63] pasta,[59,64] extruded snacks[30,60] and in local soup and vegetable dishes.[16] In addition, tilapia bones can be converted into bone flour and used for noodles and cookies, replacing wheat flour. The tensile strength,

TABLE 2 Utilization of tilapia processing by-products into products with biotechnological or pharmaceutical applications

	Part used
Fish-based products	
Fish oil[82]	Whole fish
Fish oil[83]	Viscera
Fish oil[84]	Head, viscera and skin
Collagen[35,36,85–89,110,111,116]	Skin
Collagen[37,90,91]	Scales
Collagen[85,90]	Bones
Gelatin[92,93]	Scales
Hydrolysates[37,94–97]	Head, tail and frame
Hydrolysates[37,38,98,99]	Viscera
Hydrolysates[88,100,101]	Muscle
Hydrolysates[102]	Scale
Other products	
Fish meal[103,104]	Whole fish and by-products
Silage[36,105]	By-products
Biodiesel[106,112–115]	By-products
Leather[106–108]	Skin

colour and water absorption of noodles significantly decreases as the level of tilapia bone flour is increased from 0% to 15%,[80] while the addition of 20% tilapia flour in cookies resulted in acceptable physicochemical and organoleptic qualities while further increasing the calcium content.[61] Tilapia powder can also be used to improve the textural properties of fish sausage.[81] In addition, snack bars can be prepared with the addition of tilapia dry powder and tilapia hydrolysate powder as alternative options for adding nutraceutical values to food products.[65]

Gelatin can be extracted from tilapia skin,[66–70] bones,[71] scales[72] and muscle.[73] Gelatin made from tilapia skin displayed better thermostability and could form stronger gelatin networks than other reported fish gelatins, which is of commercial value for replacing mammal-based gelatin[66,69] as food additives in various food products.[68] Therefore, gelatin from tilapia can meet the demand of some groups of people who do not consume meat and meat products.[67] The obtained tilapia gelatin can be used in yogurt,[70] panna cotta[71] or for manufacturing of imitation seafood products.[73]

Gelatin made from tilapia can be further processed into edible films and edible coatings for food applications. However, the value of the water vapour transmission rate through the gelatin is high due to the hydrophilic property. To improve the thickness, tensile strength, elongation, solubility and water vapour transmission rate of the edible film, polysaccharide hydrocolloids such as carageenan,[74,77] chitosan,[78] alginate[75] can be added or chemical crosslinking using electrolytes can be used.[76] Gelatin-based edible coating developed using tilapia skin could extend the shelf life of fish meatballs to 14 days in cold storage as compared with fish meatballs without the coating.[62]

1.2.2 | Biotechnological or pharmaceutical applications

The use of fish by-products is increasingly gaining attention, as they offer a significant and sustainable source of high-value biocompounds (Table 2),[109] due to their high content of collagen, peptides, chitin, PUFAs, enzymes and minerals, suitable for biotechnological or pharmaceutical applications since they are considered safe, nutritionally healthy, low cost and with therapeutic benefit.[37,98]

Fish oil contains mainly triglycerides of fatty acids. The lipid composition in fish is quite different from land animal lipids and vegetable oils due to the large quantity long-chain PUFAs, which cannot be synthesized by human body and provide a wide range of critical functions for human health.[37,82] Tilapia oil extracted from viscera can compete with other commercial oil in terms of nutrient content.[83]

Fish collagen is considered to be an alternative to collagen from bovines and pigs and is recently recognized as a promising biomaterial with great potential in pharmaceutical and biomedical applications.[85] Fish collagen can be extracted from tilapia skin, scales and bones. Collagen gels made from tilapia skin have excellent mechanical properties and thermal stability.[86] Collagen/bioactive glass nanofibers prepared with collagen from tilapia skin, can be used for burn treatment as it promotes wound healing and skin regeneration in human, donkeys and bears.[37,87,110,111] A specific extracted collagen peptide from tilapia collagen hydrolysate has the function of preventing ultraviolet (UVB)-induced damage to cells and inhibiting UVB-mediated photoaging of skin.[90] In addition, tilapia scale gelatin which can be extracted from collagen has great potential to be developed as an emulsifier in body creams.[92] Furthermore, tilapia scale gelatin can be used in producing stomach-soluble capsules.[93]

By-products from tilapia processing can be converted into hydrolysate with beneficial functional properties and have potential for applications in food, healthcare and pharmaceutical products.[94,95,100] Protein hydrolysates helped prevent and manage obesity-related comorbidities such as diabetes as they effect intestinal hormones secretion and dipeptidyl peptidase IV (DPP-IV) inhibitory activity.[96] The alkaline-aided protein hydrolysates can be used as a valuable and low-cost source for a natural antioxidant for several food and pharmaceutical applications.[97,102] Furthermore, the amino acids of tilapia hydrolysates have a significant impact on numerous human biological and physiological activities as they have various functional and bioactive properties.[88]

1.2.3 | Other products

More sophisticated products often only utilize certain parts of the fish. The conversion of by-products into fishmeal seems to be the best way of using these residues, since it generates less quantities of wastes.[103] Previously used as a protein and energy source, fishmeal is currently recognized for its high palatability and source of vitamins, essential fatty acids, minerals and oligoelements for animal feeds.[103,104] The fishmeal produced using tilapia processing by-products displayed variations

in its nutritional contents. However, no differences were observed in its biological values or the proportion of protein retained in the body for growth and/or maintenance, that might compromise its use in diets offered for tilapia, or more likely other non-fish species.[103]

Tilapia silage contains high amounts of all essential amino acids, except for tryptophan, making it a potential protein source in the manufacturing of animal feeds particularly for livestocks.[105] The acid silage or the oil and protein hydrolysate obtained from the silage, are useful nutrients when included in moderate amounts in feed for farmed animals and fish. Furthermore, the presence of free amino acids and short-chain peptides in the protein hydrolysate may also function as a feed additive promoting growth performance, not only as a source of amino acids.[36]

Biodiesel can be produced using viscera of tilapia.[112,113] The biodiesel made from tilapia wastes had high levels of acidity for biofuel use and with physicochemical parameters that could meet the specifications for biodiesel such as low-ash content, flashpoint and density.[114,115]

Due to its excellent biocompatibility and biodegradability, fish skin has been widely applied not only in food and pharmaceutical applications but also in the production of leather.[116] Tilapia leather may be used for clothing and leather products in general.[107] Due to its thickness and tensile strength, tilapia leather is stronger than some other leather types.[106]

1.3 | Profit from value addition

Value addition and product diversification could convert more of the fish into food or other valuable products, and provide additional sources of income for processors and venders. Tilapia processing can lead to as little as 30% fillet leaving almost 70% as a by-product usually not used for human consumption. Most tilapia processing plants of a certain size do add value to their by-products by for example converting it into fishmeal, or selling the skins for further processing. Smaller processing plants are less likely to have the capital to invest in equipment for further processing.

The potential for adding value is huge, in terms of financial gain, food security and nutrition. The potential in using more of the tilapia for human food purposes has not gained much attention, although in many parts of the world more than the fillet are eaten when fish is prepared at home. From a food security point of view, utilizing 60% instead of 30% of the fish as food would double the amount of fish for consumption without any increase in fish production. Based on knowledge of other species, using non-fillet parts such as heads, tails and frames, for consumption would also significantly increase the nutritional value in terms of micronutrients.[117] However, there is a need for further studies to generate knowledge on nutritional composition of non-fillet parts. Proving the nutritional value of a product does not necessarily mean the product will be consumed. However, there are a few studies showing that products based on non-fillet parts of tilapia and other species had a high acceptability among school children and could be done in simple way at low cost.[117]

When handling aquatic by-products, the interest has often been on end products with a higher value, such as isolating bioactive compounds and using them as food ingredients, supplements or nutraceuticals. Other bioactive compounds such as collagen and gelatine, chitin and chitosan, enzymes and specific proteins, bioactive peptides and pigments which have food, packaging and pharmaceutical applications can be obtained from processing by-products. However, these processes may require high initial investment and intensive use of technology which is not practical and beneficial for small-scale processors. Upfront investment for innovations can be high particularly for buying new equipment or sophisticated machineries as well as use of reagents for laboratory extraction of isolates. Also, investment must be made for human capital, for example, developing new skills and capacity building.

Many of the promising products developed from the by-products would require investments and technologies not available in many cases. However these are good opportunities for companies that can invest in technologies and in opening new markets. Converting by-products into food or feed products can often be done with simple technologies, at artisanal level and make use of most of the by-product. These might be product of relative low value, but with higher volumes. The high-tech products are of much higher value but at much smaller volumes. Both options are good and should be developed in parallel.

Prior to adoption of new technologies and value addition, studies of market must be conducted to ensure the demand for the product. It is important to know if there are potential buyers or consumers and their buying behaviour. In the end it is the consumer who decides what to buy. Also, having an enabling policy environment that can support the uptake of new technologies and products by providing incentives can play an important role.

2 | CONCLUSION

Fisheries and aquaculture production has seen a massive increase driven by the growth of global population and the subsequent recognition of aquatic foods as a key component in a balanced diet and a healthy lifestyle. With the increase in market demand for convenient fish products, different ways to process and preserve fish and fish products are introduced to meet consumer demands. Accordingly, a remarkable increase in the amount of by-products from fish processing and waste are being generated around the world. This is leading to a potential economic loss and environmental pollution, but could also be an opportunity to improve the economy and the environmental impact of the sector.

Even though more of the tilapia could be used to produce tasty, healthy and low cost foods, most of the by-products will end up as other non-food product. Fishmeal production could be a good alternative for big processing plants, but investment cost will make it difficult for smaller plants to use this technology. Use of simple low-cost technologies such as fish silage production could be a good alternative to handle processing by-products.

Instead of using sophisticated technology, low cost yet efficient techniques can be utilized to convert processing by-products into value-added products with high nutrient contents. Some of these innovations include production of fish powder, fish silage and fish meal, which can be conducted with household equipment and do not require high upfront investment.

Tilapia can be fully utilized by converting by-products from processing into new food, animal feed products, used in packaging or in the manufacture of biotechnological and pharmaceutical products. By doing this, we can reduce environmental pollution, improve human nutrition and provide additional sources of income and livelihood.

AUTHOR CONTRIBUTIONS

Omar Penarubia: Writing – original draft. **Jogeir Toppe:** Conceptualization; formal analysis; supervision; validation; writing – original draft; writing – review and editing. **Molly Ahern:** Conceptualization; formal analysis; resources; validation; writing – original draft; writing – review and editing. **Ansen Ward:** Formal analysis; writing – original draft; writing – review and editing. **Michael Griffin:** Data curation; formal analysis; validation; writing – review and editing.

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CONFLICT OF INTEREST

The views expressed in this publication are those of the author(s) and do not necessarily reflect the views or policies of the Food and Agriculture Organization of the United Nations.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created in this review.

ORCID

Omar Peñarubia https://orcid.org/0000-0001-8395-8887

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REVIEW

REVIEWS IN Aquaculture

Strategies to enhance tilapia immunity to improve their health in aquaculture

Bei Wang[1] | Kim D. Thompson[2] | Eakapol Wangkahart[3] | Jidapa Yamkasem[4] | Melba G. Bondad-Reantaso[5] | Puntanat Tattiyapong[4] | Jichan Jian[1] | Win Surachetpong[4]

[1]Guangdong Provincial Key Laboratory of Aquatic Animal Disease Control and Healthy culture, College of Fisheries, Guangdong Ocean University, Zhanjiang City, Guangdong, China

[2]Aquaculture Research Group, Moredun Research Institute, Edinburgh, UK

[3]Laboratory of Fish Immunology and Nutrigenomics, Applied Animal and Aquatic Sciences Research Unit, Faculty of Technology, Mahasarakham University, Kantarawichai, Mahasarakham, Thailand

[4]Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

[5]Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

Correspondence
Win Surachetpong, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, 10900, Thailand.
Email: fvetwsp@ku.ac.th

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Abstract

Various emerging and re-emerging diseases, such as streptococcosis, tilapia lake virus disease and infectious spleen and kidney necrosis virus disease, have had a significant economic impact on the tilapia industry over the last decade. These diseases have resulted in high levels of morbidity and mortality, production losses and trade restrictions. In the absence of effective husbandry management, disease prevention strategies and appropriate biosecurity measures, infectious diseases will continue to challenge the sustainability of global tilapia aquaculture. Strategies used to reduce the impact of disease include feeding probiotic and prebiotic supplements to enhance tilapia health and to modulate the fish's gut microbiota to improve gut health. Herbal medicines and immunostimulants are also used to enhance immunity to disease, while vaccines are available, or are under development, for a number of economically important pathogens affecting tilapia culture. This review provides an overview of the benefits of enhancing the immune response of tilapia to improve their health, and in turn reduce the levels of pathogens within tilapia farming systems.

KEYWORDS
emerging pathogen, immune response, immunostimulation, vaccine

1 | INTRODUCTION

Tilapia is the second most important fish species group farmed globally, with an annual production of over 4.5 million tonnes.[1] They are hardy, fast-growing fish, able to tolerate a wide range of environmental conditions, including the high stocking densities normally used during their culture.[1] The most commonly farmed tilapia species is the Nile tilapia (*Oreochromis niloticus*), which is now cultured in more than 120 countries around the world[2] and is third globally in terms of production volume, with a production of 4.6 million tonnes produced in 2019.[1] However, various emerging and re-emerging diseases, such as streptococcosis,[3,4] tilapia lake virus (TiLV) disease[5] and infectious spleen and necrosis virus (ISKNV) infections[6] have caused mass mortalities in tilapia farms, which have had a damaging effect on the global tilapia industry, especially over the past decade. Although various measures have been used to control outbreaks of these diseases, including the use of antibiotics and other therapies, improving farm management and biosecurity practices, limiting animal movements

and identifying and eliminating infected fish populations, the negative impacts caused by these infectious diseases on the tilapia industry continue to grow.[7,8] Improving tilapia health through modulation of their immune system is considered an attractive alternative to limit disease outbreaks and reduce the disease-related losses experienced by tilapia farmers. Combining existing methods of disease control with alternative strategies aimed at enhancing the fish's immune response may be a suitable way to improve the fish's ability to resist disease, and in turn, improve the sustainability and profitability of the tilapia aquaculture industry by reducing disease outbreaks. In this review, we summarize strategies and molecules that are being used to improve tilapia health and enhance their immunity against diseases.

2 | IMPORTANCE OF EMERGING AND RE-EMERGING DISEASES IN TILAPIA

According to the statistics of the Food and Agriculture Organization of the United Nations (FAO), the world tilapia aquaculture production grew 10.4% a year, from around 380,000 tonnes in 1990 to 6 million tonnes in 2018.[9] High stocking densities are stressful for the fish, making them more susceptible to infection, and they also promote the rapid spread of emerging pathogens by fish-to-fish contact.[10,11] The movement of live fish carrying a pathogen or contaminated fish products can also transmit pathogens to a new geographical region, contributing to the emergence of new disease epidemics.[11] Another factor contributing to the spread of pathogens is infected migratory fish and resident populations of wild fish transmitting pathogens to farmed fish within their new environment.[8]

Stress is a major factor that affects the well-being of an animal, including fish. It creates a physiological situation that is beyond the normal level of tolerance, thus compromising the natural defences of fish and increasing their susceptibility to pathogens. Cultured fish may experience many sources of stress, for example, poor water quality, high stocking density, poor nutrition, weather events or changes in temperature, handling, transportation and disease treatment. Stress is defined as the physiological response to a threatening situation, initiated and controlled by hormonal systems that regulate secondary stress response factors.[12,13] Acute and intense stress responses can alter the physiological balance of the fish, which may cause behavioural or physical responses such as skin colour change. When animals are exposed to chronic stress, they are less able to elicit an adaptive response to the stress, resulting in the response becoming dysfunctional, leading to growth inhibition, reproductive failure and a negative effect on their immunocompetence to resist pathogens.

It was initially thought that tilapia were relatively tolerant to disease and could easily adapt to a wide range of rearing environments, including those with poor environmental conditions. However, bacterial and viral diseases have recently become a major threat to the tilapia farming industry.[14,15]

Streptococcosis is one of the most frequently reported bacterial diseases in tilapia aquaculture, and is mainly observed in temperate and tropical tilapia-culture areas. The mortality rate can reach 50%–70% in relatively warm seasons, especially during summer. The typical clinical signs of Streptococcal infections in tilapia include abnormal behaviour, exophthalmos and meningitis.[3,4] Hai et al. demonstrated that red tilapia fry are highly susceptible to *Flavobacterium columnare*, with up to 100% cumulative mortality occurring within 24 h of infection.[16] The pathological changes associated with this disease are usually restricted to external lesions such as skin damage, gill necrosis and fin erosion.[17] Recently *Francisella* species have been reported to cause mortality in cultured finfish species.[18] The most information to date relates to disease in tilapia due to infection by *F. noatunensis* subsp. *orientalis*, causing granulomatous inflammatory reactions. Mortalities in the species can be high, and the disease can likely be transferred via live fish movements.[19] Aeromonads are also a common cause of disease in tilapia culture, with Nile tilapia among the wide range of fish species affected.[20] Haemorrhages, slow swimming, pop-eye and reddening skin are the prominent clinical signs seen in most affected tilapia farms.[21]

In addition to bacterial diseases, viral diseases caused by TiLV,[22–29] ISKNV[6,30] and *Tilapia parvovirus* (TiPV)[31,32] cause high mortalities and negative impacts on the global tilapia industry.[31,32] TiLV or *Tilapia tilapinevirus*[33] has been extensively studied over the past 5 years. It has now been reported in 16 countries over four continents, where it affects both wild and farmed tilapia.[5,34] TiLV infection is known to be part of "tilapia 1-month mortality syndrome," causing up to 90% cumulative mortality in fry and juveniles within 1 month of their transfer to grow-out ponds.[25] Moreover, several studies have reported high levels of TiLV-associated mortality in different tilapia species.[22–27,35,36] Another emerging virus, TiPV, has been isolated and characterized from adult Nile tilapia.[31] Concurrent TiLV and TiPV infections have subsequently been reported during disease investigations in farmed red hybrid tilapia,[32] and there are several reports of multiple infections leading to severe pathology in farmed tilapia.[37–39] Despite being the focus of recent, extensive research, no effective methods have been developed to manage these emerging diseases, including the development of effective vaccines.[40] Thus, these pathogens continue to devastate global tilapia culture, with associated socio-economic problems and food security issues related to lost production.[41]

The misuse of antimicrobials in agriculture and aquaculture has led to concerns relating to human and animal health issues, potential environmental and ecological impacts due to the presence of antimicrobial residues in products and the development of antimicrobial resistance (AMR) in bacteria. In 2015, during the 68th World Health Assembly, a Global Plan of Action (GAP) on AMR was established. Members of the World Health Organization have developed and implemented a National Action Plan on AMR based on a "One Health" approach. Commitments to support the GAP were obtained from members attending the 83rd General Assembly of the World Organisation for Animal Health (WOAH) and the 39th Conference of the FAO.[42]

Options for controlling emerging disease problems in tilapia aquaculture include the use of antibiotics and vaccines. Recently, the use of antibiotics in tilapia production has increased due to the rise in infectious disease problems resulting from the sector's rapid

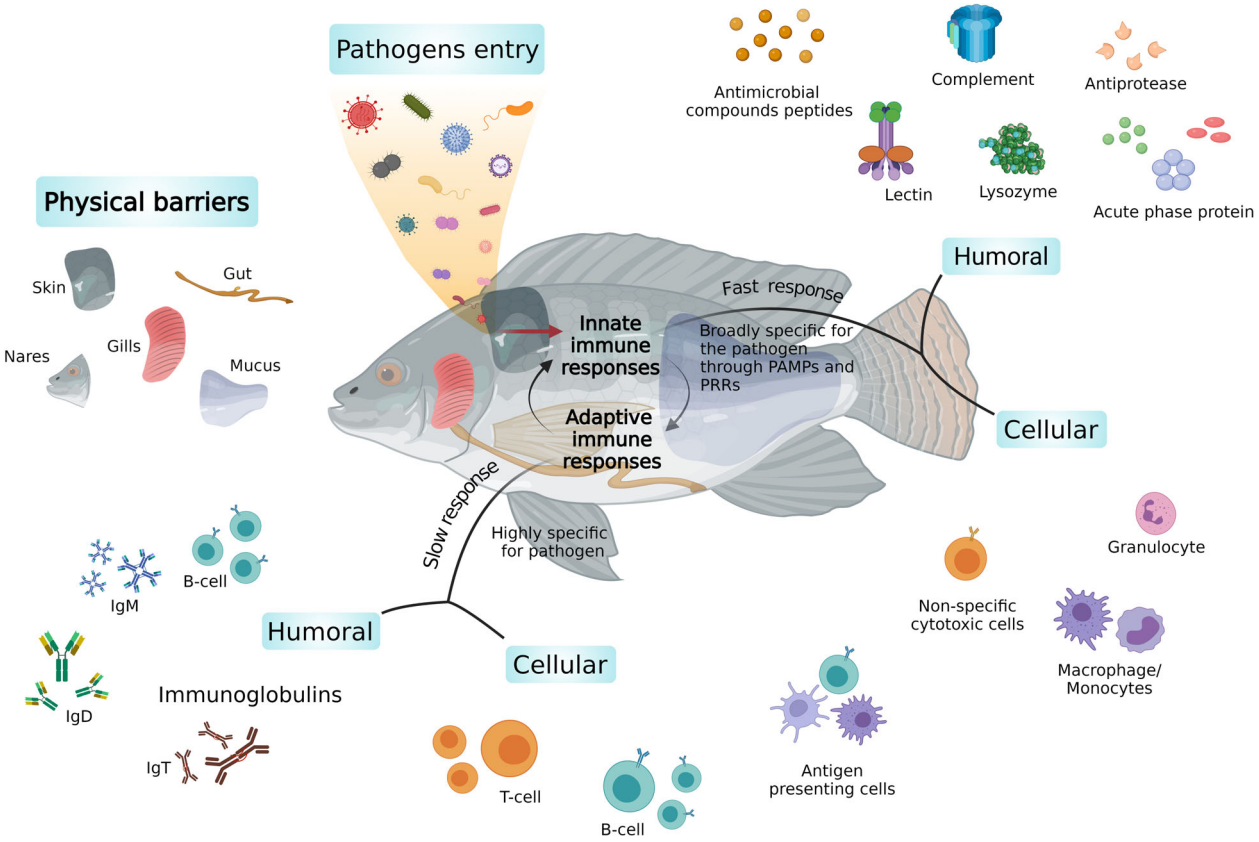

FIGURE 1 A simple overview of the immune response of tilapia to invading pathogens. PAMPs, pathogen-associated molecular patterns; PRRs, pattern recognition receptors.

expansion. Using antibiotics appropriately and correctly would reduce the development of drug-resistant bacteria and increase the effectiveness of treatment.[7,43] Since there is no specific treatments for viral diseases in tilapia aquaculture, rapid screening for viral pathogens and the development of effective vaccines are necessary to prevent and control future outbreaks of viral disease.

3 | IMMUNE SYSTEM IN TILAPIA

Routine husbandry and water quality issues can lead to stress-related immunosuppression that makes fish more susceptible to infections. Understanding the impact that these factors can have on immune function and disease resistance can allow immunosuppressive events to be predicted and appropriate actions to be taken by the fish farmer to alleviate immunosuppression. Knowing how fish respond to pathogens immunologically and being able to manipulate these responses offers an opportunity to enhance the fish's ability to combat disease.[44] Feeds containing probiotics, prebiotics, medicinal herbs and other immuno-stimulatory products have been reported to enhance the fish's immune system at times of immunosuppression or before the fish becomes fully immunocompetent.[45,46] Vaccines, on the other hand, provide protection against subsequent infections through adaptive immunity.[47]

Tilapia have a very effective immune system consisting of both innate and adaptive immune responses[48] that can protect them from invading pathogens. Innate immunity provides the first line of defence against infection,[49,50] while the adaptive immune response, occurring after the innate immune response has been initiated, responds to specific pathogens, producing an immunological memory that can respond to a subsequent re-encounter with the pathogen.[51] A simple overview of the tilapia's immune response to infection is presented in Figure 1.

Innate immune defences of tilapia include physical barriers and various cellular and humoral components.[51–54] Skin, scales and the epithelial layers of gills and the gastrointestinal tract act as physical barriers, helping to prevent the entry of pathogens into the fish. The mucus covering these surfaces also acts as a physical barrier by trapping pathogens and contains various antimicrobial substances such as lectins, lysozymes, complement proteins and antimicrobial peptides (AMPs) that can neutralize and kill the pathogen.[51,53–55]

If the pathogen is able to breach the physical barriers and enter the host, humoral and cellular components of the innate immune system respond in an attempt to prevent the infection from progressing.[44,56] The humoral response uses a diverse range of antimicrobial components (e.g., AMPs, lysozyme, complement proteins and acute phase proteins) to directly destroy the pathogen, or to promote inflammation and phagocytosis by cellular components of the innate

immune response.[51,53–55,57,58] Several studies report on the functional characterization and activity of complement in tilapia.[59–64] Measurement of lysozyme activity is often used as an indicator of the innate immune response in tilapia to various stimuli.[54,65–68] Over 90 AMPs have been identified in teleost fish, including molecules like β-defensins, cathelicidins, hepcidins, histone-derived peptides and fish-specific piscidins.[51] Nile tilapia β-defensin has been shown to have an inhibitory effect on the growth of *Escherichia coli* and *Streptococcus agalactiae*.[69] The therapeutic potential of piscidins,[70] including tilapia piscidins, has recently been reviewed by Hazam and Chen[71] and Raju et al.[72]

The cellular components of the tilapia's innate immune response consist of monocytes/macrophages and granulocytes, that is, neutrophils and eosinophilic granule cells. Initiation of the cellular response is mediated through the binding of pattern recognition receptors (PRRs) on these cells to pathogen-associated molecular patterns (PAMPs) located on different microbial pathogens, including viruses, bacteria, fungi and parasites, or to danger-associated molecular patterns (DAMP) found on proteins or other molecules released from stressed or injured cells.[51] Toll-like receptors (TLRs) are a group of PPRs responsible for monitoring the extracellular environment for pathogens. TLRs detected in tilapia include TLR3, 7, 21 and 22, with high expression of TLR3 found in tilapia liver, brain and spleen.[73,74] Retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs) on the other hand, detect viral RNA. Tilapia appear to lack RLR genes RIG-I and LGP2, but possess MDA5 and RLR adaptor IPS-1 genes.[73] The nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) that have been detected in tilapia include NOD1, NOD2 and NRL3.[75] These molecules are responsible for detecting host cell membrane damage and intracellular pathogens, and the stimulation of inflammatory cytokine expression, such as interleukin-1β (IL-1β), leading to the formation of inflammasomes.[76]

The process of inflammation is the host's response to infection, and is aimed at eliminating the pathogen and initiating tissue healing. Various inflammatory cytokines have been identified in tilapia, and their expression profiles investigated in response to various pathogens, for example, upregulation of IL-1β and tumour necrosis factor alpha (TNFα) during infection by *Lactobacillus rhamnosus*[77] or *S. agalactiae*[78,79] showed that IL-10 was up-regulated, and TNFα and IL-6 down-regulated during an infection by *S. agalactiae*, indicating antagonistic pro- and anti-inflammatory cytokines responses.

The main phagocytes in fish are monocytes/macrophages and neutrophils.[80] Monocyte/macrophages, neutrophils and eosinophils have been shown to have phagocytic activity in fish,[81] with many reports available on the phagocytic activity of tilapia macrophages, for example, phagocytosis of *Aeromonas hydrophila* in vivo.[82] During phagocytosis, the pathogen is taken up by the phagocyte and is enclosed into a phagosome, which then combines with a lysosome to form a phagolysosome, where the pathogen is killed by antimicrobial substances such as reactive oxygen species (ROS) released during respiratory burst and nitric oxide (NO).[54,83–85]

If a pathogen persists within the host, the adaptive immune system becomes activated. Adaptive immunity in tilapia, like other teleosts, is divided into cell-mediated and humoral immune responses,[86,87] with B and T lymphocyte cells responsible for delivering cellular immunity. Antigen presenting cells (APCs) from the innate response, including dendritic cells, monocyte/macrophages and B cells present processed phagocytosed materials to the T cells of the adaptive immune system in a process known as antigen presentation,[51] linking the innate and adaptive immune responses. Genes associated with pathogen recognition, antigen presentation and activation of the adaptive response have been detected in tilapia through transcriptomic analysis.[88] Presentation occurs in association with major histocompatibility complex (MHC) antigens on the surface of the APC and the T cell receptor (TCR) to stimulate T cell differentiation. Types of T cells include helper ($CD4^+$) T cells, cytotoxic ($CD8^+$) T cells and regulatory T cells (Treg).[51,86,89] The protein structure of the TCR β chain has been characterized in Nile tilapia.[90] CD4 is the T-cell co-receptor found on APCs, and is associated with the recognition of processed antigens presented by MHC-II. CD8 is the T-cell co-receptor associated with binding to antigens presented via the MHC-I molecule.[51] MHC class Iα gene,[91] and two MHC-II molecules, MHC-IIa and MHC-IIb have been described for Nile tilapia.[92] Both CD4 and CD8 genes have also been identified in tilapia.[93,94] The $CD8^+$ T cells (also referred to as cytotoxic T lymphocytes or CTLs) are an important immune defence against intracellular pathogens (viruses and bacteria) or tumour cells. The $CD4^+$ T cells, also referred to as T helper cells (Th cells), play a major role in initiating and regulating adaptive immune responses.[51,95]

The humoral adaptive immune response is delivered by B cells, which produce high-affinity immunoglobulins (Ig) specific for their target antigen. Three classes of Ig have been identified in teleosts, IgM, IgD and IgT, all of which have been detected in tilapia.[96–98] IgM is the major Ig found in serum and the systemic response of tilapia, and is found in all immune tissues. Although its function remains unclear, IgT plays a major role in mucosal immunity and is found predominantly in the mucus on mucosal surfaces of gills, skin and intestines.[99] IgM is frequently used as a measure of systemic antibody responses, while IgT is often used as a measure of a mucosal antibody response, including in tilapia.[94,96,100]

The mucosal-associated lymphoid tissues (MALTs) are important in preventing pathogen invasion into the fish during the early stages of infection and are associated with skin (skin-associated lymphoid tissue [SALT]), gut (gut-associated lymphoid tissue [GALT]), gills (gill associated lymphoid tissues [GIALT]) and nares (nasopharynx-associated lymphoid tissue [NALT]).[55,101] While the contribution of MALTs in tilapia immunity has not yet been fully elucidated, mucosal vaccination of tilapia with a nanoparticle vaccine against *F. columnare*, delivered by immersion, was shown to trigger upregulation of IgT, IgM, TNF α, IL1-β and MHC-1 in the gills (mucosal response)[102] and of IgT, IgM, TNF α and IL1-β in the blood (systemic response)[103] of vaccinated fish.

Since the products discussed in this review tend to be delivered orally to fish, we need a better understanding of how they influence the GALT as well as other MALT responses. For example, alternations to the intestinal microbial community of the host after feeding

probiotics are known to influence growth, digestion, immunity and disease resistance of the fish and development and function of the fish's immune response.[45,46]

Understanding how these products influence different components of the tilapia's immune response may provide insight into how they can be used against specific types of pathogens, for example, bacteria, viruses or parasites. Several reviews are available describing the effects of dietary immunostimulants on various immune responses of fish.[45,46,104–106] However, these responses depend on the type and dose of immunostimulant used and how it is administered (route and duration). The overuse of immunostimulants can actually lead to immunosuppression.[106] These aspects need further investigation to optimize the use of immunostimulants in tilapia.

4 | MODULATION OF GUT HEALTH TO IMPROVE TILAPIA IMMUNITY AGAINST DISEASES

The uncontrolled use of antibiotics for treating diseases can lead to an imbalance in the natural dynamics of microorganisms present in fish cultivation.[107] Researchers are keen to find lasting, environmentally friendly solutions for disease control in tilapia farming systems. In this section, strategies to manage the digestive health of tilapia including understanding and modulation of gut microbiota, and supplementation of probiotic and herbal extract are discussed.

4.1 | Gut microbiome

Intestinal bacteria play an important role in promoting fish health and are involved in the development of lymphoid tissues in the intestine.[108] Although the role of gut microbiota in the development of lymphoid organs and GALT have been investigated in other fish species, this remains to be examined in tilapia. Data generated from other fish species may help to inform on the role of the microbiome in promoting gut immunity in tilapia. The proposed roles of gut microbiota on the intestinal immune system and pathogen regulation in tilapia are shown in Figure 2.

In other fish species such as zebrafish (*Danio rerio*) and ayu (*Plecoglossus altivelis*), the gut microbiota are involved in the recruitment and development of immune cells and the formation of lymphoid tissue in the fish's intestine.[108–110] It has been shown that the absence of the gut microbiota and lack of intestinal alkaline phosphatase in zebrafish limits the differentiation of the gut epithelium and results in the depletion of goblet cells and enteroendocrine cells.[111,112]

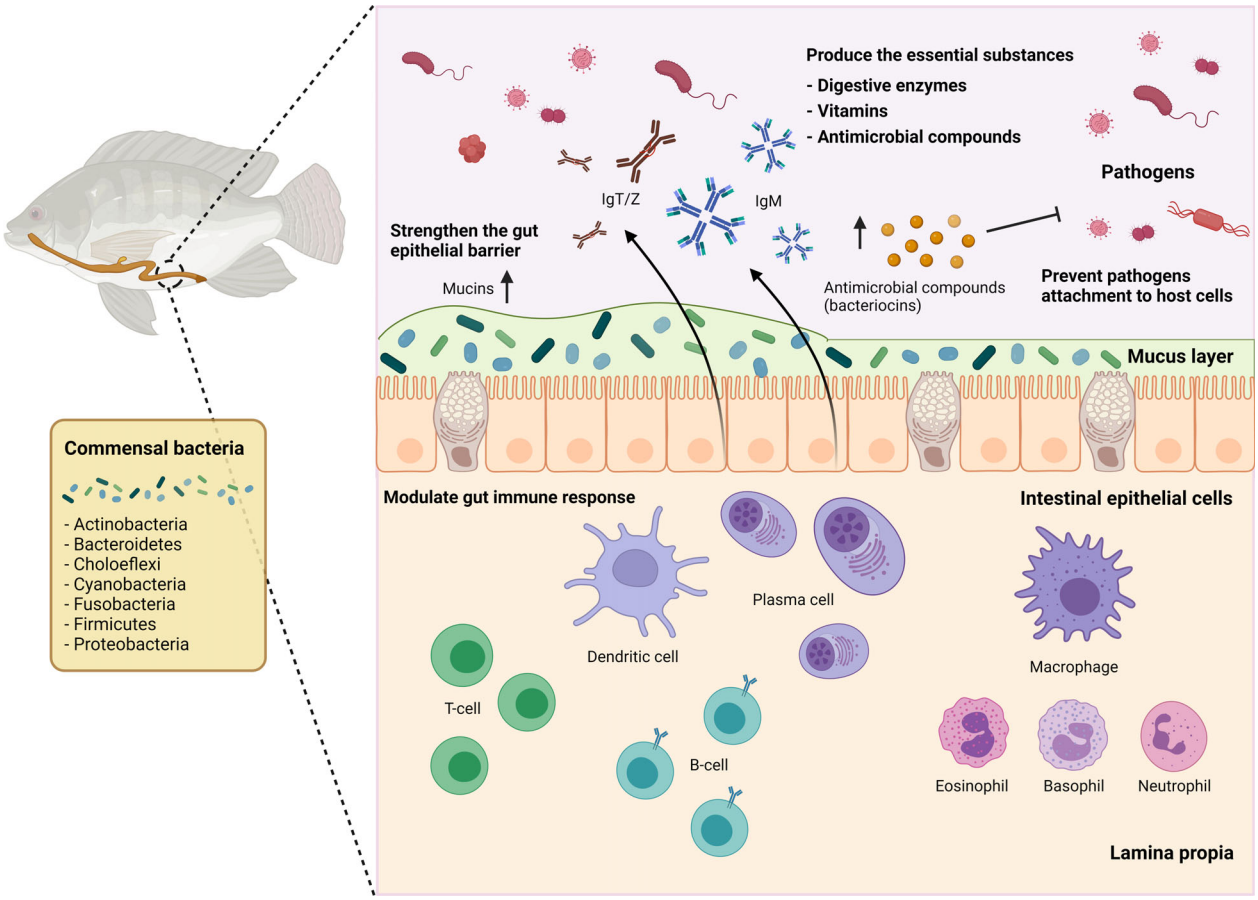

FIGURE 2 The proposed roles of gut microbiota on the intestinal immune system and pathogen regulation in tilapia.

Furthermore, the gut microbiota influences the development of GALT by providing essential signals, such as cytokines to recruit macrophages into the intestine and the GALT.[113,114] In zebrafish, the gut microbiota can modulate the expression of genes involved in maintaining homeostasis of the intestinal environment and gut epithelial regeneration.[109] As such, disruption of the gut microbiota and gut homeostasis may result in excessive inflammation and depletion of lymphoid tissue.[110–112]

Lessons learnt from other species may help define future studies in tilapia. As well as its role in GALT development, the gut microbiota produces important enzymes that are required for nutrient metabolism and to stimulate the uptake of vitamins and minerals in fish.[109,111,115,116] For instance, Ray et al.,[115] showed that gut microbiota produce enzymes such as amylase, protease, xylanase and cellulose, which are able to break down indigestible substances, including complex protein, cellulose and hemicellulose, and help with the absorption of these nutrients in the fish's intestine. Hence, better nutrient absorption may lead to improved feed conversion ratios, and improved growth and feed efficiency in tilapia.[117,118] Lastly, many beneficial bacteria can protect the intestinal epithelium by competing with pathogenic bacteria to prevent their attachment and multiplication on the intestinal surface.[110,119] It is important to study the composition and role of gut microbiota in promoting and maintaining gut health, and its interaction with the intestinal immune system of fish.[113] This information is important to develop appropriate strategies to maintain an optimal gut microbiome in tilapia.[120]

Several molecular techniques are used to study the 16S ribosomal RNA (16S rRNA) of bacteria conserved between different groups of bacteria. Such methods include denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), fluorescence in situ hybridization (FISH) and DNA microarrays.[121] These techniques allow the identification of bacterial communities and changes in the quantities and compositions of these populations after exposure to specific environmental changes, such as infection, therapeutic treatment or feed supplementation. In tilapia, the use of 16S rRNA sequencing has revealed that the most common bacterial species in the intestine belong to the phyla Fusobacteria, Firmicutes, Proteobacteria,[122] Actinobacteria, Bacteroidetes, Chloroflexi and Cyanobacteria.[122,123]

Many different factors can affect the diversity of the intestinal bacteria found in tilapia, including seasonal influences, farming location, culture system and feed ingredients. For example, recent evidence has suggested that the number and type of bacterial communities within the same fish species is affected by seasonal change.[122,124] In general, five bacterial phyla make up the main bacterial populations within the tilapia's intestine, that is, Bacteroidetes, Cyanobacteria, Firmicutes, Fusobacteria and Proteobacteria. During the pre-rainy and rainy season, the Firmicutes dominate the intestinal tract more than other bacterial groups, while the Fusobacteria are primarily found during the end of the rainy and dry season.[122] Similarly, high and low water temperature during summer and winter, respectively, can lower the number and diversity of mesophilic bacteria in the tilapia's intestine compared with the autumn months.[124] However, the composition and number of gut microbiota during different seasons await further investigation.

Besides the effect of seasonal change, farming locations and different rearing systems also affect the diversity of gut microbiota.[122,125,126] Bereded et al.[122] reported different populations of gut microbiota in fish raised in the natural environment in Lake Tana compared with indoor facilities in Ethiopia. During the same season, the abundances of Proteobacteria, Chloroflexi and Cyanobacteria were higher in tilapia collected from the indoor aquaculture facility, while Firmicutes and Fusobacteria dominated in the intestine of fish collected from their natural environment. In addition, tilapia reared in recirculating systems had a rich and diverse bacterial community in their gut, especially *Cetobacterium* spp. in tilapia larvae, which was believed to have resulted in a higher survival rate than fish in a flow-through aquaculture system.[126] The reason for this increased survival might be a result of *Cetobacterium* inhibiting the growth of invading pathogens, as shown for other freshwater fish species.[127,128] Although the protective mechanisms of *Cetobacterium* in tilapia gut health have not been fully investigated, a decrease in *Cetobacterium* in the gut microbiota of zebrafish as a result of Olaquindox supplementation appears to make the fish more susceptible to an infection by *A. hydrophila*, leading to bacterial septicemia and high levels of mortality.[129]

Antibiotics have been widely used by the aquaculture industry to treat bacterial infections within their stock. However, the misuse of antibiotics can alter the quantity and diversity of intestinal bacteria in fish and, in turn, increase the host's susceptibility to pathogen infection.[7,129] To avoid such problems, alternative strategies have been used to make the fish more resistant to disease. For example, dietary supplementation with a combination of organic salt, potassium diformate and phytogenic compounds derived from plant extracts was fed to tilapia to make them more resistant against an infection by *Francisella* spp.[130] Interestingly, it was shown in this study that a combination of 0.5% phytogenic compounds and 0.2% organic salt could maintain the diversity of gut microbiota in tilapia during an infection by *Francisella* spp. Notably, tilapia that received a combination of phytogenic compounds and organic salt had fewer opportunistic bacterial pathogen species present in their microbiome, including *Plesiomonas shigeloides* and *Vibrionacea* spp., than groups of fish receiving only one of these dietary supplements.[130] Moreover, microbial diversity and fish survival can be enhanced using probiotic and prebiotic supplementation in fish feed.[126,131,132] For instance, supplementation with fermentation products from *Saccharomyces cerevisiae* yeast can inhibit infection by pathogenic *E. coli* and *Pseudomonas fluorescens* in a dose-dependent manner and improves survival of tilapia fed with these diets.[133] Although various products and factors that affect the composition of gut microbiota of tilapia have been extensively studied, an in-depth analysis of the mechanisms and interaction between pathogenic bacteria and the gut microbiota of tilapia should be conducted.

4.2 | Probiotics, prebiotics and immunostimulants

Feeding probiotics to tilapia is an effective and attractive way to modulate the intestinal microbial composition and to maintain and promote host health.[134,135] Probiotics are defined as live microbial adjuncts that

have a beneficial effect on their host. The primary mechanisms of action of probiotics include improved epithelial barrier function, their adhesion to intestinal cells and pathogen inhibition by occupying adhesion sites, production of antibacterial substances and activation of humoral and cellular immunity.[136–138] Probiotics also have the ability to improve the quality of the host's living environment, by inhibiting the growth and reproduction of harmful bacteria by decomposing organic matter in the water.[108] The major mechanisms for biological control resulting from the application of probiotics include induction of systemic resistance in the fish, competition for nutrients and space and the production of biologically active compounds.[139–141] Various bacterial species displaying these properties have been used as probiotics in aquaculture over the years to enhance fish growth and immunity, including *Arthrobacter*, *Bacillus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Micrococcus*, *Pediococcus*, *Aeromonas*, *Burkholderia*, *Enterobacter*, *Vibrio*, *Pseudomonas*, *Rhodopseudomonas*, *Roseobacter* and *Shewanella*.[133,142]

Specifically, the benefits of feeding probiotics to enhance tilapia innate immune responses have been described. Abdel-Tawwab et al.[143] found that Nile tilapia fed spirulina (*Arthrospira platensis*) in their diet (5.0–10.0 g spirulina kg^{-1} diet), had higher red blood cell and white blood cell counts and nitro blue tetrazolium values (in macrophage respiratory burst assays) compared with control fish. The cumulative mortality was lower in fish fed with these probiotics after experimentally infecting them with *A. hydrophila* and *S. iniae* ($P < 0.05$).[144] In aquaculture, probiotics have been used to modulate the fish's immune response. *Bacillus subtilis* has been shown to improve innate immunity in Nile tilapia and decrease immunosuppression caused by stress associated with high stocking densities, with increased mean corpuscular haemoglobin and higher lysozyme and phagocytic activities of macrophages in treated fish.[145] Lactic acid bacteria and other probiotic bacteria can improve fish survival by modulating the host's immune functions. The probiotic *P. fluorescens* also reduced the level of mortality in Nile tilapia challenged with *A. hydrophila*.[146] When Aly et al.[147] used *B. pumilus* as a probiotic, which they fed to fish for 1 and 2 months, they saw a significant increase in total leucocyte counts, and lymphocyte and monocyte populations in the fish's blood.

Bacillus sp., *L. acidophilus* and *P. fluorescens* have been shown to improve the health status and disease resistance of Nile tilapia.[49,147,148] When these probiotics were fed to tilapia, the fish had a higher resistance to an infection by *S. agalactiae*.[149] As mentioned above, *P. fluorescens* also reduced mortality in Nile tilapia when challenged with *A. hydrophila*[49] and lower cumulative mortality was noted in tilapia fed with a combination of *B. subtilis*, *S. cerevisiae* and *A. oryzae* compared with untreated fish after infecting them with *A. hydrophila* or *S. iniae* ($P < 0.05$).[144] *Lactobacillus plantarum* subspecies *plantarum* JCM 1149 reduced a localized immune response in an ex vivo anterior sac from hybrid tilapia (*O. niloticus*♀ × *O. aureus*♂).[150] Previous research has shown that fish fed with probiotic gave the highest net return and the lowest total cost of production compared with the tilapia on a control diet.[151] Moreover, the use of probiotic in tilapia culture enhanced the immune and health status of the fish and improved their disease resistance.[147]

Prebiotics are non-digestible, complex carbohydrates such as inulin, fructo-oligosaccharides, short-chain fructo-oligosaccharides, mannan-oligosaccharides, galacto-oligosaccharides, xylooligo-saccharides, arabinoxylo-oligosaccharides, isomalto-oligosaccharides and GroBiotic.[152] They provide health benefits by stimulating the growth and activity of bacteria within the fish's gut. Prebiotics are metabolized by the gut bacteria and have the ability to stimulate the host's innate immune responses.[153] When combined with probiotics they are referred to as synbiotics, influencing the growth and activity of the probiotic. Cavalcante et al.[154] give an example of the use of probiotics, prebiotics and synbiotics in tilapia to improve protection against an infection by *A. hydrophila*.[154]

Immunostimulants are commonly used to increase the fish's resistance to disease at times of immunosuppression, with β-glucans being the most commonly used in aquaculture, especially β-glucan (β-1,3 and 1,6 glucans) derived from the cell wall of baker's yeast, *S. cerevisiae*.[155] This contains various immunostimulatory compounds that have the ability to enhance the immune responses of fish.[156] For example, mortality levels of Nile tilapia resulting from an infection by *A. hydrophila* were seen to decrease as the level of yeast increased in the fish's diet.[157] Moreover, supplementation of carotenoid product (Lycogen™) extracted from the photobacterium *Rhodobacter sphaeroides* WL-APD911 at 1.0% in feed can enhance tilapia (*O. mossambicus* × *O. niloticus*) growth performance through immune regulation.[158]

The application of probiotics and feed additives shows great promise in controlling disease in tilapia aquaculture, but extensive research is still required to optimize their use for this purpose. More information is needed to understand how these substances influence host/microbe interactions in vivo.

4.3 | Medicinal plants

To date, several medicinal plants have been used as feed additives using the whole plant, parts of the plant (leaf, root or seed) or extracted compounds and have been successfully used for the treatment of tilapia.[14] Medicinal plants are an important source of bioactive compounds and have been used as immunostimulants in traditional medicine for thousands of years.[159] These herbal medicines contain a wide range of phytoadditives, which are mainly alkaloids, terpenoids, lectins, polyphenolics, phenolics, quinones and polypeptides.[160] Medicinal plants are applied using the whole or parts of the plant, or as plant extracts, added to the water or the fish's diet. Plant extracts have been used as single compounds or in combination, or together with other bioactive compounds.[161] The use of herbs as natural feed additives has attracted great interest for tilapia aquaculture.[162,163] Many studies have shown that herbs, used as natural feed additives, have the potential to increase fish growth, immunity and health.[164,165] Although growth performance is an important parameter to be considered when assessing the potential of a feed additive for tilapia, improvement of the fish's immune system is also important to help prevent disease. Several studies have demonstrated that dietary herbal extract supplementation can improve disease resistance in

farmed tilapia. These include diets supplemented with elephant's foot (*Elephantopus scaber*),[166] cinnamon (*Cinnamomum zeylanicum*),[167] turmeric (*Curcuma longa*),[168] purslane (*Portulaca oleracea*)[169] and garlic (*Allium sativum*),[170] with treated fish displaying greater anti-bacterial activity against Gram-positive and Gram-negative bacteria.

It has also been shown that major bioactive compounds derived from these herbs can regulate the tilapia's immune system, particularly the proliferation of immune cells,[171,172] and have enhanced antioxidant properties.[173] Previous reports have shown that the mucosal immune response in tilapia can be triggered through the use of medicinal plants.[54,174] These studies indicate the potential of herbal medicine as an alternative approach for disease control by enhancing tilapia immunity, improving resistance to disease and helping to alleviate immunosuppression caused by husbandry practices. Therefore, the use of medicinal herbs as immunostimulants for tilapia might be a promising approach to reduce the risk of disease outbreaks and could reduce the use of chemicals during the course of production.

5 | VACCINATION

Vaccination is one of the most important tools for managing infectious diseases in fish culture,[175] because of the vaccine's ability to stimulate protective immunity and produce a memory response in vaccinated fish. The first commercial fish vaccine was licensed in 1976, and provides superior and long-lasting protection against bacterial infectious disease in salmonids.[176] Various bacterial vaccines are commercially available for tilapia. To date, only one commercially available streptococcosis vaccine (AQUAVAC® MSD Animal Health) is available, which has been widely applied in tilapia aquaculture.[177] Moreover, the emergence of viral pathogens, ISKNV and TiLV, have been spread across tilapia producing countries and it causes high mortalities leading to high economic losses. Regarding viral vaccines, only one ISKNV vaccine has been developed and commercialized (AQUAVAC® MSD Animal Health) so far. Besides ISKNV, recent studies have shown the effectiveness of vaccines against TiLV in tilapia.[94,178,179] Different types of vaccine are currently under development for TiLV, such as live-attenuated, inactivated, DNA and subunit vaccines.[94,178,179] Evidence suggests tilapia that have survived an infection by TiLV develop antibody responses and have protection against subsequent TiLV infections, thus indicating the potential of an effective vaccine to protect tilapia from TiLV disease.[180,181] A live-attenuated vaccine with a relative percentage survival of 55%–62% was the first vaccine to be developed. Subsequently, Mai et al.[94] developed both heat and formalin inactivated vaccines using whole virus, which gave a higher level of survival of between 81.3% and 86.3% compared with unvaccinated fish. After the first vaccination, the vaccinated tilapia produced 3.7 and 5.7 times more systemic and mucosal anti-TiLV IgM than the unvaccinated fish.[94] However, the anti-TiLV IgM levels dropped significantly after 3-weeks post-vaccination. Interestingly, fish receiving a booster vaccination elicited a strong antibody response 5.4 and 5.9 times higher than the unvaccinated fish.[94] In another study, using inactivated TiLV antigen combined with Montanide IMS 1312 VG adjuvant, injected tilapia had a survival rate of 86.7% against a TiLV challenge.[179] Moreover, a DNA vaccine and a subunit vaccine have also been developed to protect tilapia from TiLV. Both vaccines increase survival by up to 52.5% compared with unvaccinated fish. In contrast, when the DNA vaccine was used as the primary vaccine and the subunit protein as a booster vaccine, the survival rate increased to 72.5% in these fish.[178] Nevertheless, further research into the development of viral vaccines for tilapia is still urgently required to have an effective tool for managing viral diseases in tilapia aquaculture.

Recently, *S. agalactiae* is the main bacterial pathogen in cultured Nile tilapia, numerous vaccinations have been developed and can improve immune response against infection such as inactivated vaccines, subunit vaccines, DNA or live attenuated vaccines. The first vaccine against *S. agalactiae* was created in the 1930s.[182] With the development of the human *S. agalactiae* vaccine, studies and the use of *S. agalactiae* vaccine in tilapia have also advanced greatly in the past two decades. These vaccines are classified into replicative and non-replicative vaccines. Replicative antigen delivery systems used for the design of *S. agalactiae* vaccine for tilapia comprise live attenuated and DNA vaccines.[177] Pridgeno and Klesius[183] reported that an attenuated *S. agalactiae* vaccine, selected based on the bacterium's resistance to sparfloxacin, a fluoroquinolone antibiotic, provided 100% protection in both 3–5 g and 15–20 g tilapia administered via intraperitoneal (IP) injection. Another attenuated erythromycin-resistant *S. agalactiae* vaccine for tilapia presented 95%, 93.02% and 100% relative percentage survival (RPS) at 4, 8 and 16 weeks post-vaccination.[184] In 2019, Li et al.[185] produced a Δ2 mutant with a deleted D2 fragment that provided RPS values of 93.05% and 53.16% at 30 days post-IP injection and oral administration, respectively. DNA vaccines have been made using different surface proteins of *S. agalactiae* encoded in plasmid vectors. Surface immunogenic protein (SIP),[186] LPXT motif,[187] fibrinogen binding protein (FbsA),[188] alpha-enolase (ENO1), phosphoglycerate kinase (PGK),[189] ornithine carbamoyltransferase (OCT),[190] extracellular products 89 kDa protein (ECO89),[191] pyruvate kinase (PK)[190] and capsular polysaccharide protein E (CspE)[192] have been used as vaccine candidates for use in DNA vaccine production. Most of them showed higher protection than inactivated whole cell (IWC) vaccines.

No-replication antigen delivery systems include inactivated whole cell (IWC), subunit and extracellular protein (ECP) vaccines. The experimental data presented in the work by Avi et al.[193] showed that vaccines based on formalin-killed *S. agalactiae* strains are very effective at protecting tilapia against a lethal challenge with the bacterium. In order to enhance their immunogenicity, different adjuvants, including Freund's incomplete[194] and aluminium hydroxide gel[195] have been used in IWC-vaccine formulations for *S. agalactiae* in tilapia. He et al.[195] produced a subunit vaccine using the Sip protein that produced high protection in tilapia, while Yi et al.[188] showed high protection in tilapia using the subunit vaccines encoding the fibrinogen binding protein A (FbsA) and α-enolase antigens. An ECP vaccine was prepared by concentrating the cell-free fluid followed by 3% formalin inactivation. Research has shown that the RPS of ECP vaccines give a low level of protection against *S. agalactiae* challenge in tilapia; nevertheless, when the EPC vaccine was combined with the IWC-vaccine it

showed higher protection than either the IWC-vaccine or the ECP vaccine.[196] As such, the search for protective vaccines against *S. agalactiae* has significantly intensified alongside the rapid expansion of tilapia production in the last two decades.[177]

Factors that need to be considered during vaccine development for tilapia include the type of antigens and adjuvants used and the number of vaccinations that are required. Indeed, the route of vaccine delivery is important, as it can affect the wide-scale uptake of vaccine usage by the tilapia aquaculture industry. Immersion and oral vaccines are more practical for vaccinating large numbers of fish and are easier to give to small fish. Nonetheless, vaccines need to be used in combination with other farm management practices, including effective disease diagnosis and elimination of infected fish, as well as promoting fish health through alternative products and methods.

The time period from vaccination to potential exposure to the actual pathogen should be optimized in such a way that maximum protection coincides with the time of greatest risk of disease outbreak. Experimentally, formalin-killed cells of a single isolate of *S. agalactiae* have been reported to offer significant protection to 30 g tilapia, with a RPS of 80% in vaccinated fish at 30 days post-vaccination.[197] The polyvalent vaccine provided significant ($P < 0.001$) protection in both 3–5 g and 15–20 g Nile tilapia against challenges with 30 isolated of virulent *S. agalactiae*.[183] Overall, vaccination should be carried out some time before exposure to the actual pathogen, in order to give immunity sufficient time to develop. It is important to note that vaccination should not be carried out too early prior to the risk period, as the degree of immunity declines with time. Water temperature may be an important factor when deciding when to vaccinate, as well as the size of fish. These parameters are closely linked, and one cannot be settled without account being taken of the other.

6 | ECONOMIC CONSIDERATIONS

The decision of fish farmers to immunostimulate their tilapia is normally based on an economic consideration of benefits and costs. Of all the methods for enhancing fish immunity, the economy of vaccination is the most thoroughly studied. A model was developed and applied to calculate the point of break-even between costs of vaccination and losses due to disease.[198] It also considers labour costs for the vaccination procedure and losses resulting from side-effects. Due to vaccine licensing and other reasons, there has been no economic evaluation for *Streptococcus* vaccine for tilapia. Considering the economic benefits of the vibriosis vaccine used in Atlantic salmon, injection vaccine has been shown to be economically beneficial if the disease causes significant mortalities in non-vaccinated fish. An economic model was further developed by Thorarinsson and Powell and employed to evaluate the impact of disease risk, vaccine efficacy and market price of fish.[199] According to the results of the study, fish farmers should choose the vaccine giving the best level of protection, even if the price per dose is higher, due to the impact of vaccine efficacy on the economic outcome.

7 | CONCLUSION AND FUTURE DIRECTIONS

Recent epidemics due to emerging bacteria and viruses have caused mass mortalities in tilapia production, which have had serious implications for countries that rely on tilapia aquaculture for food security and socio-economic benefits. There are many strategies available to address the challenges brought about by risk of diseases affecting aquatic populations. These can range from effective biosecurity governance (at the farm, sectoral, industry and legislation/policy levels), good health management and responsible aquaculture practices (including responsible movement of live aquatic animals), and effective prevention technologies (e.g., use of clean seed through specific pathogen free stocks, vaccination) supported by sensitive and timely diagnostics, surveillance and emergency preparedness and contingency plans. The critical "control point thinking" and "risk mindset" can be applied throughout the value chain and combines good aquaculture and biosecurity practices to understand the risk, identify the hazards and manage the risk at each point of the chain.[200] Awareness and continuous capacity building are needed, especially targeting small-scale producers. A combination of good health, good nutrition and good genetics will create resilient hosts that can make farming of aquatic species for food and livelihood be sustainable.[201] Effective disease management is essential to control these disease outbreaks such as establishing a robust disease prevention program, including the use of probiotics and good management practice could have a beneficial impact on improving tilapia production.[202] Nevertheless, the decision to use products to enhance tilapia health will depend on multiple factors including farming practice, farmer perception and the overall cost–benefit of using such products. Importantly, creating a less stressful environment for fish can be achieved through effective husbandry management by maintaining high water quality, adequate nutrition and good biosecurity. Rapid response to epidemic events and the availability of rapid and accurate diagnostic methods are important to limit the damage caused by these diseases. Vaccination as a means of controlling infectious diseases is one of the most significant and successful health practices within the aquaculture industry, as seen in salmonids.

Strategies that enhance tilapia health are also beneficial for controlling disease. We have highlighted the current knowledge and strategies that are being used to improve tilapia health and disease resistance, such as the use of immunomodulators and herbs, and the supplementation of fish diets with probiotics and prebiotics. As well as enhancing the fish's immune response, these products help to promote a healthy balance of commensal bacteria within the gut microbiome and prevent an imbalance in the microbial taxa resulting from disturbance or perturbation of the microbiome due to environmental stressors or disease. We need a greater understanding of the role the gut microbiota has in key biological processes of the fish, such as its ability to modulate metabolic processes, protect the host from pathogen invasion through competitive colonization and the production of antimicrobial products, and in immune response development. Further development and implementation of effective vaccines is also needed

to reduce the negative impact of infectious diseases within tilapia farms.

New technologies to select strains of disease-resistant tilapia using marker-assisted selection (MAS) traits and next-generation sequencing will be used to increase the survival of fish during exposure to virulent pathogens.[203–206] Advances in sensor-based technology to monitor water quality, proper feed management and monitoring for the presence of pathogens in rearing environments will also increase the efficiency of tilapia health management and limit the spread of pathogens in the farms. Integration of these technologies together with the strategies summarized in this review will greatly improve tilapia health, survival of the fish, and a reduction in the use of antibiotics.

AUTHOR CONTRIBUTIONS

Win Surachetpong: Conceptualization; data curation; project administration; writing – original draft; writing – review and editing. **Bei Wang:** Conceptualization; writing – original draft; writing – review and editing. **Kim D. Thompson:** Conceptualization; data curation; writing – original draft; writing – review and editing. **Eakapol Wangkahart:** Writing – original draft; writing – review and editing. **Jidapa Yamkasem:** Data curation; writing – original draft. **Melba G. Bondad-Reantaso:** Conceptualization, Writing – original draft; writing – review and editing. **Puntanat Tattiyapong:** Data curation; writing – original draft. **Jichan Jian:** Writing – original draft; writing – review and editing.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Melba G. Bondad-Reantaso https://orcid.org/0000-0002-2380-3549

Win Surachetpong https://orcid.org/0000-0002-5707-3476

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REVIEW

REVIEWS IN Aquaculture

Improving tilapia biosecurity through a value chain approach

Brett MacKinnon[1] | Partho Pratim Debnath[2] | Melba G. Bondad-Reantaso[3] | Sophie Fridman[3] | Hao Bin[3] | Omid Nekouei[1]

[1]Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong SAR, China

[2]Center of Excellence in Fish Infectious Diseases Research Unit (CE FID), Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

[3]Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

Correspondence
Melba G. Bondad-Reantaso, Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, Rome, Italy.
Email: melba.reantaso@fao.org

Omid Nekouei, Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong SAR, China.
Email: omid.nekouei@cityu.edu.hk

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Abstract

Tilapia aquaculture is a major source of animal protein, with global production reaching over 6 million tonnes in 2020. The rapid growth of the tilapia sector has led to a number of emerging disease threats and subsequent production losses. Risk analysis can provide a targeted approach for improving biosecurity in the tilapia sector. The aim of this work was to describe the tilapia value chain and review the important infectious agents of tilapia that may affect the different points along the value chain; such points include input and service suppliers, producers (i.e., hatcheries, nurseries and grow-out farms), and processors, traders and marketers. We then describe how risk analysis can be used to identify critical controls points along the value chain and describe potential risk mitigation measures that may be implemented at those points. The control of diseases of tilapia requires a multi-faceted approach, with risk-based control measures chosen based on their feasibility, effectiveness and sustainability. The Progressive Management Pathway for Improving Aquaculture Biosecurity, as a risk-based, collaborative and progressive management approach combined with the systematic preventive principles of Hazard Analysis Critical Control Point, offers a strategic and practical way of improving biosecurity in the tilapia value chain.

KEYWORDS
aquaculture, HACCP, PMP/AB, risk assessment, tilapia, value chain

1 | INTRODUCTION

Tilapia is a very popular aquaculture commodity and is farmed in more than 120 countries and regions. It provides a good source of food and nutrition and positively contributes to livelihoods through domestic and export earnings. Global tilapia production is estimated to have reached about 6.8 million tonnes in 2020 (Figure 1). Of the overall total, tilapia capture fisheries produced about 0.7 million tonnes, accounting for only 10.8%, which has peaked and remained static since 2007. In the same period, tilapia aquaculture production has more than doubled to approximately 6.1 million tonnes in 2020, with an estimated value of USD 12.3 billion.[1]

In terms of global tilapia commodity trade (export, import, re-export), official Food and Agriculture Organization of the United Nations (FAO) data for 2020 reported a quantity of 1.1 million tonnes and a value of USD 3.2 billion.[2] The top 10 tilapia aquaculture producing countries in 2020 were China, Indonesia, Egypt, Brazil, Bangladesh, Viet Nam, the Philippines, Thailand, Colombia and Uganda, accounting for nearly 90% of the global aquaculture production. Only the top three producers reached around one million tonnes in 2020; 1.66 million tonnes for China, 1.23 million tonnes for Indonesia, 0.95 million tonnes for Egypt, respectively, totalling a share of around 63% of global tilapia aquaculture production.

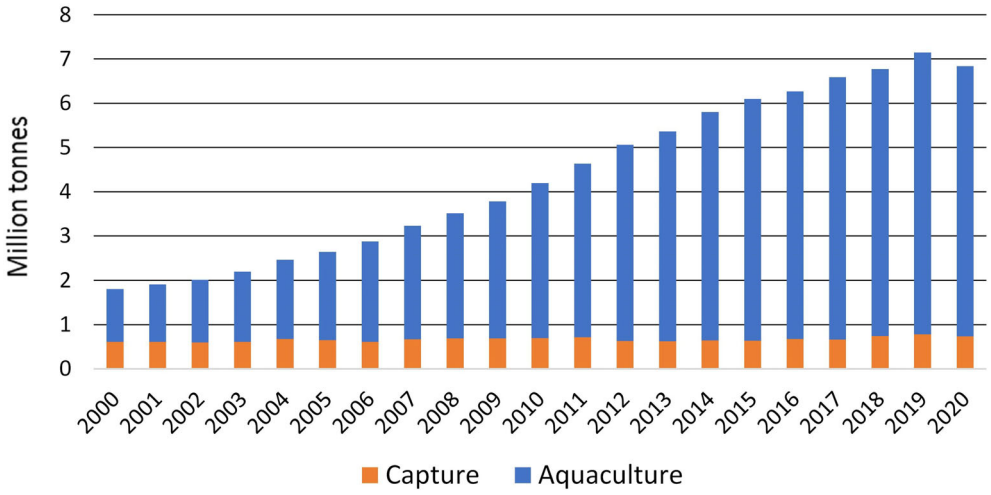

FIGURE 1 Global total tilapia capture and tilapia aquaculture production from 2000 to 2020. (source: www.fao.org/fishery/statistics/software/fishstatj/en)

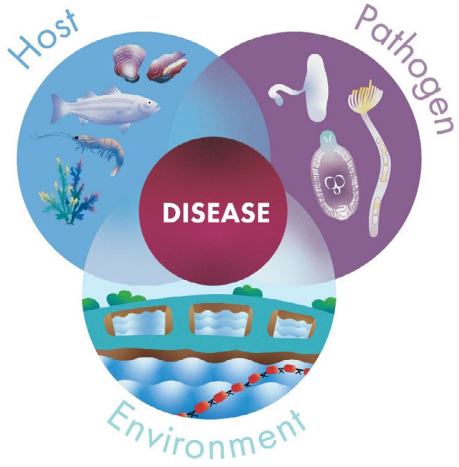

FIGURE 2 The Snieszko circle or epidemiological triad that shows the interplay between the pathogen, and susceptible host in a suitable environment that allows for transmission of the pathogen and development of disease in the population. (Figure credit: Paulo Padre)

In line with other food production systems, diseases in the aquatic sector represent a serious threat that must be addressed. Due to the intensification of the aquaculture industry, combined with the increase in the number and diversity of cultured species (more than 500 species of finfish, molluscs, crustaceans, amphibians and aquatic plants), the likelihood of the occurrence of new, emerging and re-emerging diseases has increased.[3–5] This is further exacerbated by the broad scope of culture environments, the production systems, types of management and scale of operations. Tilapia are tolerant to a wide range of environmental conditions; however, diseases, particularly those of bacterial and viral origin, remain one of the major limiting factors that hinder tilapia productivity, particularly when cultured in intensive farming systems.[3]

The emergence and spread of disease within an aquaculture system can be the result of a series of linked events reflecting the interaction between a number of biological factors, that is, host, infectious agent(s) and environment. The Snieszko circle,[6,7] also known as the epidemiological triad,[8,9] is commonly used to represent this paradigm. Representing the interplay between these variables, this triad highlights the interaction between pathogen and susceptible host in a suitable environment that allows for transmission of the pathogen and development of disease in the population (Figure 2). One approach to improving health management within the aquatic sector is to understand the sequence of events in a given production system, possible risks and pathways for pathogen transmission, and to identify interventions that may lead to improvements in the health status of farmed tilapia.[3]

An estimation of the risks to human health and safety as well as the identification and implementation of suitable mitigation measures can be carried out by risk analysis, with concurrent communication to stakeholders about the risks and measures employed.[10] Within an aquaculture context, these identified risk categories may include pathogen risks, food safety and public health risks, ecological (pests and invasive species) risks, genetic risks, environmental risks, economic risks and social risks.[11] In the rapidly expanding tilapia sector, risk analysis offers targeted improvements to biosecurity, a sound understanding of the tilapia value chain relevant to each region or country and knowledge of potential disease, all contributing to intervention and mitigation of disease risks.

The aim of this paper is to describe and review important infectious agents of tilapia that may affect the different points along the tilapia value chain. Also discussed is how risk analysis can be used to identify critical controls points within the value chain, and potential biosecurity measures that can be implemented at these points. Special attention is given to practical measures that can be implemented for smallholders in developing countries with limited capacity.

2 | UNDERSTANDING THE TILAPIA VALUE CHAIN

In general, a 'value chain' can be defined as a set of activities required to bring a commodity to market. To truly understand the tilapia value chain in a country, the stakeholders involved, and direction of activities, processes or movements of tilapia products should be well-understood.[12] The tilapia value chain can be mapped visually to describe the flow of commodities through points along the value chain, using a set of boxes and arrows, and includes both domestic and international trade activities (Figure 3). Information should be based on stakeholder consultations and published literature and reports to ensure the tilapia sector is accurately described. Wherever possible, this analysis should include socioeconomic information such as number of farms, commodity volumes and profits.

Through value chain mapping, we can describe the movements between points along the tilapia value chain, from input and service suppliers to tilapia producers, and from there onto wholesalers, processors, marketers, retailers and everything in between. Input and service suppliers provide producers with fish health services, transportation services, feed, pharmaceuticals, tilapia seed and broodstock. Tilapia producers have hatchery, nursery, or grow-out farms, with movement and trading often occurring between farms. Marketing and processing activities for live tilapia or their products may consist of markets, traders (i.e., import/export movements), dealers, wholesalers, processing plants, cold storage, or retail stores.

Relative to other actors in the value chain, smallholders or small-scale producers receive the fewest economic benefits, which may be due to lack of financial capital, infrastructure and organizational capacity.[13] From this perspective, they present the weakest link in the system and represent higher risk in the long value chain. In many developing countries that farm tilapia, the concept of value chain has not been fully applied and services such as certification, traceability, research on genetic improvements, enforcements of sanitary conditions and regulating best practice in aquaculture can be lacking.[14] Public institutions can strengthen and support the value chain; however, these can be limited due to a lack of policy, legal, or regulatory framework, or manpower and logistical difficulties.[15] Large-scale commercial businesses in the tilapia value chain generally have more limited risk exposure than small-scale companies in part due to significantly greater structural organization, either in the form of horizontally or vertically integrated operations, institutional support and economic benefits.[14] These advantages allow for large-scale producers, processors and retail markets to have a stronger ability to meet certification and quality assurance requirements related to fish health or food safety, and thus greater access to international markets.

3 | BIOSECURITY IN AQUACULTURE

Definition of biosecurity can vary. A broad definition of biosecurity was provided by FAO,[10] as a strategic and integrated approach that encompasses both policy and regulatory frameworks aimed at analysing and managing risks relevant to human, animal and plant life and health, including associated environmental risks. It covers food safety, zoonoses, introduction of animal and plant diseases and pests, introduction and release of living modified organisms (LMOs) and their products (e.g., GMOs), and the introduction of invasive alien species.

Biosecurity, in the context of the aquatic environment, is the sum total of a country's activities and measures taken to protect its natural aquatic resources, capture fisheries, aquaculture and biodiversity and the people who depend on them from the possible negative impacts resulting from the introduction and spread of serious transboundary aquatic animal diseases (TAADs). The concept of biosecurity as a collective term, referring to the application of appropriate measures, for example, proactive disease risk analysis, in order to reduce the probability of the spread of biological organisms or agents to an individual, population or ecosystem and mitigate the adverse effects was described by Subasinghe and Bondad-Reantaso.[16]

In aquaculture, the drivers of risk analysis include resource protection, food security, trade, consumer preference for high quality and safe products, production profitability as well as other investment and development objectives. Risk analysis is a core concept that can be applied to prevent and control the occurrence and spread of infectious diseases, and needs to be incorporated into governmental regulations as well as farm operational plans.[17] Effective governance at all levels (i.e., at both policy/legislation and farm levels) determines the sustainability of the aquaculture sector. Biosecurity is also a major player in the 'One Health' concept towards reducing antimicrobial resistance and zoonotic diseases from farmed aquatic organisms and their environment.

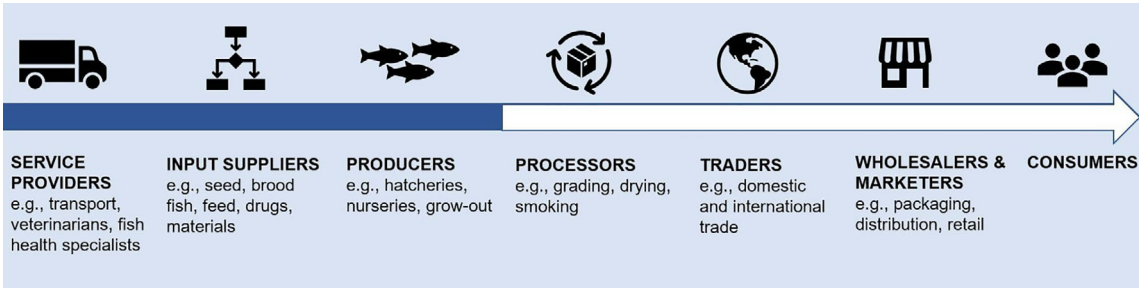

FIGURE 3 General representation of the tilapia value chain. (Figure credit: Brett MacKinnon)

The World Organisation for Animal Health (WOAH) (founded as OIE) defines biosecurity as a set of management and physical measures designed to reduce the risk of introduction, establishment and spread of animal diseases, infections or infestations to, from and within an animal population. The WOAH Aquatic Animal Health Code (Aquatic Code) establishes standards for the improvement of aquatic animal health worldwide,[18] and includes standards for the welfare of farmed fish and the use of antimicrobial agents in aquatic animals. It is used to develop measures for the early detection, internal reporting, notification, control or eradication of pathogenic agents in aquatic animals to prevent their spread via international trade. WOAH's Manual of Diagnostic Tests for Aquatic Animals[18] provides the rapidly evolving laboratory testing methods for the diagnosis of pathogenic agents that may adversely affect aquatic animals.

In the context of FAO's Progressive Management Pathway for Improving Aquaculture Biosecurity (PMP/AB), biosecurity refers to "the cost-effective management of risks posed by pathogenic agents to aquaculture through a strategic approach at enterprise, national and international levels with shared public-private responsibilities."[1] It follows the principles of being risk-based, progressive and collaborative.

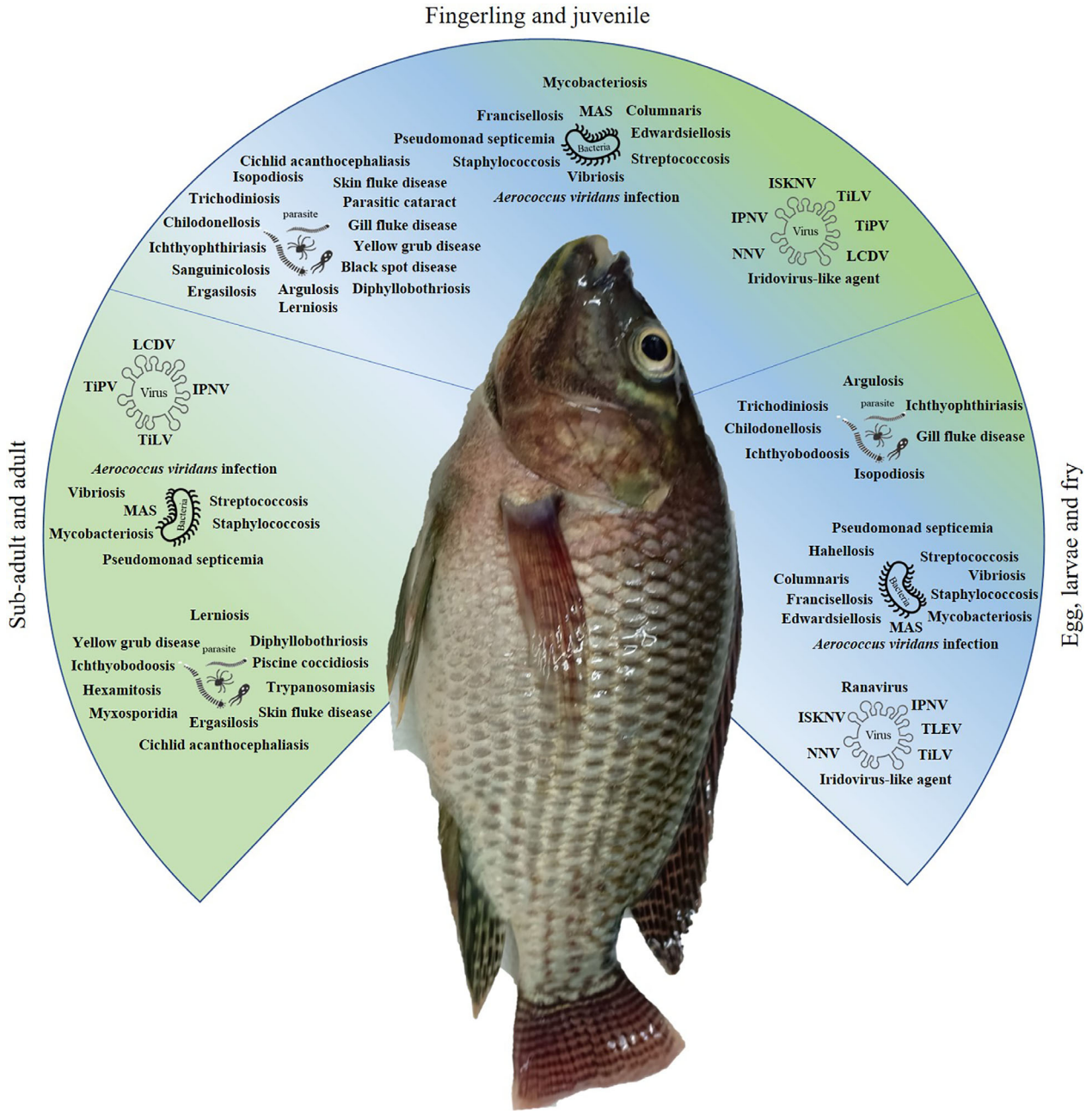

FIGURE 4 Diseases of tilapia reported to affect various stages of production. IPNV, Infectious pancreatic necrosis virus; ISKNV, Infectious skin and kidney necrosis; LCDV, Lymphocystis disease virus; TiPV, Tilapia parvovirus; TiLV, Tilapia lake virus; MAS, Motile *Aeromonas* septicemia; NNV, Nervous necrosis virus; TLEV, Tilapia larvae encephalitis virus. (Figure credit: Partho Dennath)

Implementation of the PMP/AB is expected to result in "sustainable reduction of burden of disease; improvement of health at farm and national levels; minimization of global spread of diseases; optimization of socioeconomic benefits from aquaculture; attraction of investment opportunities into aquaculture; and achievement of 'One Health' goals."

In this paper, biosecurity takes a broader dimension and combines a number of important principles and elements taken from the above definitions, whilst still taking the risk assessment approach and hazard analysis and critical control point (HACCP) approach in improving biosecurity in the tilapia aquaculture value chain.

4 | REVIEW OF INFECTIOUS AGENTS OF TILAPIA

Prior to implementing biosecurity measures on a tilapia farm or within the tilapia value chain, it is important to first identify and have a good understanding of all pathogens (i.e., viruses, bacteria, fungi, parasites) that could negatively impact the fish population. One or more stages of production may be affected depending on the infectious agent and aquaculture system (Figure 4). Ideally, serious diseases affecting the tilapia industry are reportable at the national level and have valid diagnostic tests for identification and merit the effort required to control their introduction and spread in the country, zone, or farm. These pathogens may already be present in the country, may be exotic or emerging, and may be listed under transnational reporting systems such as the World Animal Health Information System (WAHIS) of the WOAH (https://wahis.woah.org), the Animal Disease Notification System (ADNS) of the European Union (https://ec.europa.eu/food/animals/animaldiseases/not-system_en), the Emergency Prevention System for Animal Health (EMPRES-AH) of the FAO (http://www.fao.org/ag/againfo/programmes/en/empres/home.asp) and the Quarterly Aquatic Animal Disease (QAAD) Reports for Asia and the Pacific region administered by the Network of Aquaculture Centres in the Asia-Pacific (NACA).[19]

Below is a review of bacterial, parasitic, viral and fungal diseases that have been reported in cultured tilapia, identifying the potential risk factors involved, that is, the infectious agent, host or environmental characteristics specific to the geographic location or value chain.

4.1 | Parasitic diseases of tilapia

Wide ranges of parasite species have been reported to threaten tilapia production (Table 1). Several factors influence the occurrence and severity of parasitic infections, including the parasite load, culture system, and fish population density, species, sex, size and health status.[20] In both hatcheries and rearing facilities, protozoan parasites have been documented to cause severe mortality in wild and farmed tilapia.[20] Ectoparasitic infection by ciliated protozoans, such as *Trichodina* spp. and *Ichthyophthirius multifiliis*, has been extensively studied and characterized in tilapia.[21,22] In general, juvenile stages of tilapia are more vulnerable to protozoan infestations than adults.[20,23,24] In addition, higher temperatures have been reported to be a key risk factor for protozoan infestations on tilapia farms.[20,24–27]

Outbreaks with monogenean parasites may impact production on tilapia farms, especially in pond culture systems.[28] Monogenean infections are commonly associated with parasites belonging to the *Dactylogyridae* and *Gyrodactylidae* families, as well as the *Cichlidogyrus* genus.[24] Gyrodactylids, such as *Gyrodactylus cichlidarum*, are one of the most common monogenean species infecting young Nile tilapia (*Oreochromis niloticus*) and have the potential to cause substantial mortality in intensively farmed fish around the world.[29] Monogenetic trematodes are regarded as a bioindicator of the quality of culture conditions, since poor water quality, poor farm management, and toxic pollutants all contribute to parasitic abundance.[30] Digenetic trematodes are another major concern in tilapia farming, causing significant losses in fingerling and juvenile fish.[20] *Clinostomum* and *Euclinostomum*, for example, are two genera of digenetic trematodes reported to affect tilapia. There are no effective treatments for digenetic trematodes in tilapia, and infestations with these parasites are typically managed by removing snails and drying and liming ponds.[20]

Many species of nematodes, cestodes and acanthocephalans have been reported in both wild and cultivated tilapia, but little is known about their parasitic relevance.[31–35] There have been reports of tilapia diseases induced by these parasites, including diphyllobothriosis, heart worm disease and cichlid acanthocephaliasis.[26] The copepods *Ergasilus* spp., *Lernaea* spp., *Caligus* spp. and *Lamperoglena* spp., the branchiurans *Argulus* spp. and *Dolops* spp., and the isopod *Alitropus typus* are parasitic crustaceans that frequently infest wild and farmed tilapia.[36] Many of these parasites pose serious health risks to cultured tilapia, resulting in significant losses for producers.[36] Overstocking may lead to rapid transmission and replication of crustacean parasites, causing significant mortality in stocked fish, particularly in high water temperature, as their life cycles accelerate.[26] Overall, the life stage of the tilapia population is a critical indicator for managing infestations, with parasitic diseases primarily occurring in the larval, fingerling and juvenile stages. Apart from temperature fluctuations, other major risk factors for parasitic infections in tilapia include poor water quality management and inadequate biosecurity measures. It is important that these are addressed to control the parasitic load during early rearing stages.

4.2 | Viral diseases of tilapia

In recent years, there have been numerous reports of viral infections significantly impacting tilapia production. Nine viral diseases have been reported in tilapia, to date, including six DNA viruses and three RNA viruses (Table 2). Tilapia parvovirus (TiPV), reported on tilapia farms in China[37] and Thailand,[38] is the most recently discovered viral disease in tilapia. However, tilapia lake virus (TiLV) has had the most significant impact on the tilapia industry. TiLV was initially discovered in Israel[39] in 2014, and has now spread to 16 nations across four continents.[40] TiLV is a negative-sense single-stranded RNA virus that may infect tilapia at any life stage and cause massive mortalities as high as 90%.[39,41,42] Live fish translocation has been identified as a

TABLE 1 A description of diseases of tilapia caused by parasitic infections, including common species of tilapia affected, major clinical signs of infection and the life stages reported to be most impacted

Disease	Pathogen	Host species	Clinical signs	Life stages	Additional Information	References
Acanthocephalans						
Cichlid acanthocephaliasis	*Acanthosentis tilapiae*	*Oreochromis aureus* *O. niloticus* *Tilapia zillii*	Chronic heavy infestations; anorexia; mortality	Fingerling; juvenile; adult	Acanthocephalans serve as bioindicators of aquatic chemical pollution	24,147,148
Cestodes						
Diphyllobothriosis	*Diphyllobothrium dendriticum* *D. latum* *Eubothrium tragenna* *Polyonchobothrium* spp.	*O. niloticus*	Float on water surface; mortality	Fingerling; juvenile; adult	Cestodes are a public health concern via consumption of uncooked fish	24,26,33
Ciliates						
Chilodonellosis	*Chilodonella hexasticha*	*O. mossambicus* *O. niloticus*	Bluish-grey mucous on skin surface; gill damage; tissue hyperplasia	Larval; fry; fingerling; juvenile	Heavier infestations reported on tilapia during high temperatures	24,27,149
Ichthyophthiriasis	*Ichthyophthirius multifiliis*	*O. aureus* *O. mossambicus* *O. niloticus* *Sarotherodon galilaeus* *T. zillii*	White spots on skin, gills, fins and eyes; thick mucus on skin; erratic swimming and rubbing against hard surfaces; slow growth; high mortality	Larval stage more susceptible than fingerling/adult	Reproduce quickly at 20–25°C with high fish stocking density; heavier infestations on temperature-stressed fish; *I. multifilis* infection increased tilapia susceptibility to *Streptococcus iniae and Flavobacterium columnare* infection, with higher mortality	20,24,26,27,149–155
Trichodiniosis	*Paratrichodina africana* *Trichodina centrostrigata* *T. compacta* *T. heterodentata* *T. migala* *T. orthodens*	*O. aureus* *O. mossambicus* *O. mossambicus* × *O. niloticus* *O. niloticus* *S. galilaeus* *T. zillii*	Erratic swimming; rubbing against hard surfaces; anorexia, skin and gill damage; respiratory distress; mortality	Larval; fry; fingerling; juvenile	Hatchery and nursery phases may have heavy infestations and massive mortality rates; parasite load influenced by tilapia species, size, sex, and season; higher counts in the winter than summer; *S. iniae* vaccine efficacy reduction in fish infected with *T. heterodentata*, *G. cichlidarum*, and *I. multifilis*	20,24,26,34,149,156–164
Crustaceans						
Argulosis	*Argulus foliaceus* *A. indicus* *A. japonicus*	*O. niloticus* *O. mossambicus*	Erratic swimming; skin lesions; high mortality (heavy infestations)	Larvae, fry, fingerling and juvenile stages	Prevalent in stagnant water; can predispose to opportunistic bacterial or fungal infections	20,24,165–167

TABLE 1 (Continued)

Disease	Pathogen	Host species	Clinical signs	Life stages	Additional Information	References
Ergasilosis	*Ergasilus* spp.	*O. niloticus*	Excessive mucus on gills; high mortality (heavy infestations)	Fingerling; juvenile; adult	*Ergasilus* spp. are gill parasites; higher abundance in low salinity and high temperatures; heavy infestations on tilapia in high stocking densities may lead to high mortality rates; can predispose to opportunistic infections with *Branchiomyces* spp. and *Saprolegnia* spp.	24,149
Isopodiosis	*Alitropus typus* *Nerocila bivittata* *N. orbignyi* *Renocila thresherorum*	*O. niloticus* *T. zillii*	Bulged operculum and erosions; pale atrophied gills; excessive mucus secretions; haemorrhages, scale loss, and severe skin erosions and ulcers on the external body surface; pale liver	Larval; fry	Peak prevalence during summer and monsoons; prevalent in high salinity, high temperatures, and in waters with excessive food; can predispose to opportunistic bacterial infections	24,149,168
Lerniosis anchor worm infestation	*Lernaea arcuata* *L. cyprinacea* *L. hardingi* *L. lophiara* *L. oryzophila* *L. polymorpha* *L. tilapiae*	*O. niloticus* *S. galilaeus* *O. mossambicus* *T. heudeloti* *T. zillii*	Skin irritation; excessive mucus secretion; erratic swimming; scale detachment and skin lesions; growth reduction; high mortality	Fingerling; juvenile; adult	Only females are parasitic, while males are free-living stages	24,165,167,169–171
Digenetic trematodes						
Black spot disease	*Neascus metacercariae* *Posthodiplostomum cuticula*	*O. niloticus* *S. galilaeus*	Black spots on body surface of fish, especially on scales	Fingerling; juvenile	No specific medications have been proposed for controlling digenetic trematodes in tilapia	20,24,172
Parasitic cataract	*Diplostomum compactum* *D. spathicum* *D. tregenna*	*O. mossambicus* *O. niloticus* *O. urolepis* *S. galilaeus*	Increased ocular opacity (blindness); emaciation; anorexia; mortality	Fingerling and juvenile stages	Infection prevalence varies with host species and size, stocking density, and water quality; risk factors include early life stages, higher stocking densities, and high abundance of the snails	24,33,167,172–174
Yellow grub disease	*Clinostomum* spp.	*O. leucostictus* *O. niloticus* *S. galilaeus* *T. zillii*	Grubs (yellow or white) on the skin; excess mucus and inflammatory reaction on the sites of attachment; mortality	Fingerling; juvenile; adult	Snails and fish are intermediate hosts and birds are definitive hosts; problematic to tilapia culture and can cause high mortality rates among fingerling and juvenile fish	22,24,149,167,175

(Continues)

TABLE 1 (Continued)

Disease	Pathogen	Host species	Clinical signs	Life stages	Additional Information	References
Sanguinicolosis	*Sanguinicola* spp.	*O. niloticus*	Acute mortality from severe infections; anaemia and emaciation in chronic infections; slow growth; hypoxia; blood-tinged gill mucus; gill marbling	Fingerling; juvenile	Snail removal from ponds and bird nets to control infection	20,24
Flagellates						
Ichthyobodosis	*Ichthyobodo necator*	*O. mossambicus* *O. niloticus*	Flashing; rubbing against hard surfaces; lethargy; inactive on the bottom; anorexia; blue-grey thick mucus patches on skin	Larval; fry; adult	The most dangerous external protozoal infection in Nile tilapia hatcheries; significant threat to tilapia farming; survive at 2–30°C	24–26,157,176,177
Internal protozoans						
Hexamitosis	*Hexamita africans* *H. intestinalis*	*O. niloticus*	Severe anaemia; emaciation; degeneration of liver, kidney and spleen; bloody ascitic fluid	Adult	This disease affects the gut, gallbladder, and blood	24–26
Piscine coccidiosis	*Eimeria* spp.	Wild and farmed cichlids including *O. niloticus*	Emaciation, lethargy, and general poor health	Adult	Outbreaks observed mainly in spring and summer seasons	24,149,178
Monogeneans						
Skin fluke disease	*Gyrodactylus cichlidarum* *G. elegans* *G. malalai* *G. niloticus*	*Haplochromis* spp. *O. niloticus* *S. galilaeus* *T. zillii* *Tristramella* spp.	Irritated skin; slow moving; flashing; rubbing against hard surfaces	Fingerling; juvenile; adult	High-risk parasite on tilapia farms; heavier infestations in warmer water temperatures; poor water quality, poor farm management, and chemical pollution significantly contribute to the impact of infection; co-infection with *Aeromonas hydrophila* led to higher mortality in summer season	20,24,28,179–183
Gill fluke disease	*Cichlidogyrus dossoui* *C. halli* *C. sclerosus* *C. tilapiae* *Dactylogyrus vastator* *Scutogyrus longicornis*	*O. niloticus* *T. zillii*	Gill irritation; gasping at surface; accumulation at water inlet; rapid opercular movements	Larval; fry; fingerling; juvenile	Risk factors include poor sanitary conditions, water quality (higher temperatures, and low dissolved oxygen, contaminated sediments) and pond management (overstocking)	20,24,28,34,183,184
Sporozoans						
Myxozoans	*Myxobolus agolus* *M. beninensis* *M. branchiophilus* *M. dossoui*	Wild and farmed cichlids including *O. niloticus* *S. galilaeus*	Increased ocular opacity; head cysts; frontal skin ulcers; anorexia; stomach swelling; congestion, and thickening of intestines; mortality	Fry	This protozoan parasite has a two-host life cycle, involving fish and aquatic ring worms; high mortality rates observed during co-infection	20,24,150,157,172,185–193

TABLE 1 (Continued)

Disease	Pathogen	Host species	Clinical signs	Life stages	Additional Information	References
	M. exiguous *M. fomenai* *M. israelensis* *M. microcapsularis* *M. ovariae* *M. sarotherodoni* *M. tilapiae* *M. zillii* *Ortholinea africanus* *Triangula egyptica* *Zschokkella nilei*	*S. melanotheron* *T. zillii*			with *F. columnare* and *M. tilapiae*; higher impact on production in intensive earthen ponds; no known effective treatment for myxosporidian infections in tilapia	
Protozoan blood parasites						
Trypanosomiasis	*Trypanosoma choudhuryi* *T. mukasai* *T. tilapiae*	*O. exculenta* *O. lencosticta* *O. mossambicus* *O. niloticus* *O. variubilis*	Skin darkening; skin haemorrhage; multifocal aggregation of melanomacrophages in the liver, pancreas, spleen, and kidney	Early life stages	Higher prevalence in spring and summer seasons; main risk factor is increased stocking density; ectoparasitic invertebrates spread the disease between fish	20,24,149,194–196
Nematodes						
Heart worm disease	*Amplicaecum* spp. *Contacaecum* spp.	*O. leucostictus* *O. niloticus* *S. graham*	Pale skin; abdominal distention; nervous manifestations	Adult	Male fish have been reported to be more affected than females; highly prevalent when fish fed with natural zooplankton	24,31,197,198

TABLE 2 A description of viral diseases of tilapia, including common species of tilapia affected, major clinical signs of infection and the life stages reported to be most impacted

Disease/pathogen	Host species	Clinical signs	Life stages	Additional information	References
Bohle virus infection; family *Iridoviridae*, genus *Ranavirus*	*Oreochromis mossambicus*	Rapid swimming in a spiral; sinking to the bottom; gasping at the surface; dark coloration of skin; fin clamping; mortality within 24 h of behavioural signs	Larval; fry	No molecular characterization or PCR detection in tilapines has been published	199,200
Infectious pancreatic necrosis virus (IPNV); family *Birnaviridae*, genus *Aquabirnavirus*	*O. niloticus* *Tilapia mossambicus*	Irregular swimming; skin discoloration; gill pallor; swollen abdomen; white faecal casts; ascites	Not reported	No outbreaks have been reported in tilapia; IPNV has been reported to be pathogenic to tilapia, with experimental infection causing 25% mortality; subclinical infections of IPNV have been reported, with vertical transmission from carrier broodfish to fertilized eggs; the possibility of transboundary transmission was reported; the sharing of harvesting nets may be a possible source of transmission to tilapia	29,201–209
Infectious spleen and kidney necrosis virus (ISKNV) disease; family *Iridoviridae*, genus *Megalocytivirus*	*Apistogramma cacatuoides* *Astronotus ocellatus* *Etroplus maculatus* *Heros severus* *Laetacara curviceps* *Mikrogeophagus ramirez* *O. niloticus* O. niloticus × O. mossambicus *Pelvicachromis pulcher* *Pterophyllum scalare* *Tropheus duboisi*	Lethargy, gill pallor, anorexia, melanosis, mucus hypersecretion, haemorrhage, ascites, high mortality rate	Fry; juvenile	Iridoviruses have high pathogenicity, a wide host range and extensive geographic spread; early life stages of fish are more prone to ISKNV infection with mass mortalities ranging from 80–100%; in 2015, the first reported case of ISKNV occurred in Nile tilapia in Thailand, followed by the United States in 2016; ISKNV can be transmitted horizontally and vertically, with significant risk to commercial aquaculture; a number of studies have demonstrated the ability of ISKNV to cross species boundaries; strict adherence to broodstock management and biosecurity measures, as well as sensitive pathogen identification techniques, are recommended to limit the spread of ISKNV; emerging disease cases should be reported internationally to avoid further transboundary spread	46,199,210–235

TABLE 2 (Continued)

Disease/pathogen	Host species	Clinical signs	Life stages	Additional information	References
Iridovirus-like agent; family *Iridoviridae*, genus unknown	*O. niloticus*	Lethargy; slow swimming; exophthalmia; gill pallor; dark coloration; severe abdominal ascites; pale liver with petechial haemorrhagic	Fry; juveniles	Possible transboundary transmission of the virus was reported during fish importation into Canada from Florida	226
Lymphocystis disease virus (LCDV); family *Iridoviridae*, genus *Lymphocystivirus*	*Ciclilasoma synspilum* *Haplochromis* spp. *Tilapia amphimelas* *T. esculenta* *T. variabilis*	Hypertrophied lymphocystis on the tail of infected fish	Juvenile; adults	Not reported to be fatal to infected fishes; a molecular diagnostic test for LCDV in tilapia is yet to be reported	200,208,236,237
Tilapia lake virus (TiLV) disease; *Tilapia tilapinevirus*; family *Amnoonviridae*, genus *Tilapinevirus*	*Aulonocara* spp. *Oreochromis* spp. *O. mossambicus* *O. niloticus* *O. niloticus* × *O. aureus* O. niloticus × O. mossambicus *S. galilaeus*	Anorexia; lethargy; erratic swimming; haemorrhagic skin; skin lesions; scale protrusion; pop eye; enophthalmos; increased ocular opacity; swollen abdomen	Any life stage (earlier life stages more vulnerable)	TiLV was first reported in Israel in 2014 and since then has been reported by 16 countries from 4 continents; TiLV is the most commonly reported viral disease in tilapia; two distinct genotypes (Israeli and Thai strain) were identified after assessing the complete genome sequence; both vertical and horizontal transmission has been reported; subclinically infected tilapia can act as potential reservoirs; live fish translocation is a major risk of disease spread	39–43,47,48,131, 238–256
Tilapia larvae encephalitis virus (TLEV) disease; family *Herpesviridae*	*O. aureus* *O. niloticus* *O. niloticus* × *O. mossambicus* *S. galilaeus*	Whirling; dark pigmentation initially beginning on the fins and quickly extending over the body	Larval	TLEV infects larval stages during hatchery production, but other stages are reported as potential disease carriers; male and female fish have been identified as probable viral carriers with obvious vertical transmission; horizontal spread of the virus was detected in a cohabitation experiment with infected and non-infected tilapia; TLEV infection in tilapia has only been reported in Israel	46,208,257

(Continues)

TABLE 2 (Continued)

Disease/pathogen	Host species	Clinical signs	Life stages	Additional information	References
Tilapia parvovirus (TiPV); taxonomic classification is pending	*O. niloticus* *O. niloticus* × *O. mossambicus*	Abnormal swimming; body pallor; exophthalmia; ocular lesions; scale protrusion; skin haemorrhage; gill pallor; haemorrhage in the dorsal musculature; ascites, splenomegaly; intestinal enlargement with fluid accumulation	Juvenile; adult	First report of parvovirus causing diseases in teleosts; described as a new emerging virus and needs further investigation; outbreaks occur between 28–30°C; highly contagious and lethal to all sizes of cage-cultured adult tilapia; parvoviruses are particularly resistant to heat and desiccation, and their persistence in the environment allows for widespread transmission and infection	37,38,258–261
Nervous necrosis virus (NNV); family *Nodaviridae*, genus *Betanodavirus*	*O. niloticus*	Skin darkening; lethargy; anorexia; abnormal swimming behaviour; ocular opacity; corkscrew-like swimming pattern; mortality rate up to 100%	Larval; juvenile	Viral nervous necrosis (VNN) disease has been recorded in over 50 species of freshwater and marine fishes, including *O. niloticus*, across various geographical locations; Betanodaviruses isolated from tilapia in four different countries (France, Thailand, Indonesia, and Egypt) were closely linked to the red-spotted grouper nervous necrosis virus (RGNNV); mass mortalities from NNV infection have been reported in tilapia hatcheries; vertical or horizontal transmission; tilapia may be a potential carrier of VNN; poor water quality is a risk factor for VNN outbreaks	45,262–272

TABLE 3 A description of bacterial diseases of tilapia, including common species of tilapia affected, major clinical signs of infection, and the life stages reported to be most impacted

Disease	Pathogen	Host species	Clinical signs	Life stages	Additional information	References
Aerococcus viridans infection	*Aerococcus viridans*	*Oreochromis niloticus*	Exophthalmia; spiral swimming; congestion in the gills and abdomen; swollen gallbladder	Not reported	There has only been a single report of *A. viridans* infection in tilapia, which resulted in 30–40% mortality rate	273,274
Columnaris	*Flavobacterium columnare*	*Oreochromis* sp. *O. niloticus*	Discoloration of the skin; fin erosion; respiratory disorder; heavy mucus secretion; gill erosion; skin and muscles lesions, commonly extending over the back and sides of the fish like a 'saddle back'	Fry; fingerling	Any life stage of fish may become infected; however, outbreaks are most commonly detected in small fish; *Oreochromis* sp. fry reported to be highly susceptible to *F. columnare*; risk factors are related to stressful events, such as high stocking density, handling (particularly during the transfer of nursery fish to grow out), and poor water quality, which includes high or low water temperature, as well as high ammonia levels; *F. columnare* is resistant to sulfa drugs; Columnaris disease has been reported to negatively impact production on farms due to slower growth in infected fish	59,61,62,68,79,149,167,275–295
Edwardsiellosis	*Edwardsiella* spp. *Edwardsiella anguillarum* *E. ictalurid* *E. piscicida* *E. tarda*	*Oreochromis* sp. *O. niloticus*	Edema; abdominal distension; haemorrhage; exophthalmia; white dots in the spleen and head kidney	Larval; fingerling; juvenile	*E. tarda* is a zoonotic pathogen that infects a wide range of species; *E. ictaluri*, *E. piscicida*, and *E. anguillarum* are considered to be the most significant aquatic pathogens in aquaculture worldwide; multi-drug resistant strains have been reported; *E. ictaluri* is an emerging pathogen in Southeast Asian tilapia farming; due to the similarity in clinical signs to Francisellosis, Edwardsiellosis infections in tilapia may be under reported	60,63,64,296–306

(Continues)

TABLE 3 (Continued)

Disease	Pathogen	Host species	Clinical signs	Life stages	Additional information	References
Francisellosis	*Francisella orientalis*	*Oreochromis* sp. *O. niloticus* *O. niloticus* × *O. mossambicus*	Exophthalmia; anorexia; anaemia, pale gills with white nodules; erratic swimming; visceral white spots (liver, kidney); mortality	Fry; fingerling	Francisellosis is one of the most important emerging diseases threatening the aquaculture sector, owing to its rising prevalence, high infectivity rates, and wide variety of fish hosts; outbreaks have been reported since the early 1990s, with mass mortality up to 90%; horizontal and vertical transmission of the disease has been reported; disease incidence is higher in farmed tilapia when water temperature is <26°C; efforts are being made to produce vaccines for *F. columnare* protection in Nile tilapia, but none have been licensed for commercial use	65,66,74–79,83,85,307–314
Hahellosis	*Hahella chejuensis*	*Oreochromis sp.* *O. niloticus*	Red eggs	Eggs prior to hatching	Hahellosis is thought to be an emerging infectious disease in tilapia eggs; Mainly reported during periods of colder weather (< 24 °C); vertical transmission in tilapia has been hypothesized	80
Motile Aeromonas septicemia (MAS)	Gram-negative motile *Aeromonas spp.* *Aeromonas dhakensis* *A. hydrophila* *A. jandaei* *A. salmonicida* *A. schubertii* *A. sobria* *A. veronii*	*O. aureus* *O. mossambicus* *O. mossambicus* × *O. niloticus* *O. niloticus*	Dark colouration of skin; anorexia; ulcers or hyperaemia; fin rot; ocular opacity; exophthalmia	Any life stage	MAS has a multimillion-dollar impact on aquaculture across the world; *A. hydrophila* is a zoonotic pathogen that may rarely result in moderate to severe disease in humans; *A. hydrophila* has been reported to be multi-drug resistant; risk factors include an abrupt fluctuation in water temperature, aggressive handling, high levels of toxic ammonia, low levels of dissolved oxygen, and epidermal irritation caused by	35,67–73,167,315–330

TABLE 3 (Continued)

Disease	Pathogen	Host species	Clinical signs	Life stages	Additional information	References
					fungal or parasite infections; various vaccines have been produced against *A. hydrophila*, but a review study found that only intraperitoneal doses were effective in tilapia	
Mycobacteriosis	*Mycobacterium* spp. *Mycobacterium chelonae* *M. fortuitum* *M. marinum*	*O. mossambicus* *O. niloticus* *O. niloticus* × *O. mossambicus* × *O. aureus* *Sarotherodon andersonii* *Tilapia sparrmanii*	Skin lesions that progress to ulcers; foci on the fins or body; ulcers in the mouth; progressive fin necrosis; body deformity; exophthalmia	Not reported	Dietary or environmental pollution, stress associated with overstocking, poor nutrition, and poor water quality are all factors that may lead fish to mycobacterial infection; *Mycobacterium* spp. are zoonotic pathogens	24,81,82,84,167,331–342
Pseudomonas	*Pseudomonas aeruginosa* *P. anguilliseptica* *P. fluorescens* *P. mosselii* *P. putida*	*Oreochromis* sp. *O. aureus* *O. niloticus* *O. mossambicus* *S. niloticus*	Lethargy; erratic swimming; skin discoloration; scale loss; exophthalmia; lesions in the liver, spleen, kidney and gills; inflamed swim-bladder	Not reported	Pseudomonas infection is one of the most prevalent bacterial infections in fish; opportunistic infection in freshwater fish reared in an intensive environment; *Pseudomonas* spp. are extremely adaptable and can survive in a variety of environments; the highest mortality in infected tilapia was reported at temperatures 15–20°C	190,301,330,343–347
Staphylococcosis	*Staphylococcus* spp. *Staphylococcus aureus* *S. epidermidis* *S. galinarum* *S. xylosus*	*O. niloticus* *O. aureus*	White nodules and lesions in the spleen and anterior kidney	Not reported	Infected tilapias are more susceptible to *Streptococcus agalactiae* infection	348–355
Streptococcosis	*Streptococcus agalactiae* *S. iniae*	*Oreochromis* sp. *Oreochromis andersonii* *O. aureus* *O. macrochir* *O. niloticus* *O. niloticus* × *O. mossambicus* *O. tanganicae* *T. rendalli*	Anorexia; lethargy; erratic swimming; exophthalmia; distended abdomen; melanosis of the skin; haemorrhagic lesions on the body and fins; granulomas in the brain; discoloured liver; enlarged spleen; gill pallor	Any life stage	Streptococcus spp. comprise 90% of pathogens isolated from dead farmed tilapia each year; Streptococcosis may result in 30–90% mortality rate in affected tilapia farms; zoonotic disease in humans; opportunistic infection during stressful events such as poor water quality conditions and high stocking density;	49–58,132,356–387

(Continues)

TABLE 3 (Continued)

Disease	Pathogen	Host species	Clinical signs	Life stages	Additional information	References
Vibriosis	*Vibrio harveyi* *V. mimicus* *V. vulnificus*	*Oreochromis sp.* *O. aureus* *O. niloticus* *O. spilurus*	Lethargy; dark coloration; dermal necrosis; skin lesions; exophthalmia; scale loss; haemorrhage; increased mucus secretion	No specific report on pathogenesis at specific life stage	Transmission of *V. vulnificus* from eels to tilapia has been reported; *V. vulnificus* is a zoonotic pathogen	388–392

possible risk factor for the rapid spread of TiLV to several countries.[43] Recently, co-infections of TiPV and TiLV have been reported in tilapia farms in Thailand.[44]

In tilapia production, the disease risks associated with viruses vary greatly depending on the life stage of the fish. Several viral infections have been documented in tilapia fry, including betanodavirus and tilapia larvae encephalitis virus (TLEV), which often result in significant mortalities in hatcheries.[45,46] The majority of viruses affecting tilapia, namely TiLV, infectious spleen and kidney necrosis virus (ISKNV), TLEV, infectious pancreatic necrosis virus (IPNV) and nervous necrosis virus (NNV), may spread via vertical and horizontal transmission. TiLV, for example, was detected in subclinical broodstock sourced from different hatcheries and readily transmits between farms.[47,48] For the majority of viruses affecting tilapia (excluding TiLV), little information is known regarding their geographical distribution or impact on production. No treatment is available for these viral diseases; therefore, good biosecurity protocols should be followed during every stage of production.

4.3 | Bacterial diseases of tilapia

Tilapias are susceptible to a wide range of bacterial infections (Table 3). Among them, streptococcosis is one of the most common bacterial infections, which led to USD 40 million in economic losses in China in 2011.[49] *Streptococcus* spp. can transmit both vertically and horizontally[50,51] and outbreaks have been associated with high stocking densities and poor water quality, such as high temperatures, excessive ammonium and nitrite levels, and low dissolved oxygen levels.[50,52–55] The disease has been found in fish of any size or age.[50,55–57] *Streptococcus* spp. may also present a public health risk, which is a major concern in humans.[58]

Numerous other bacterial infections, such as columnaris, francisellosis and edwardsiellosis, have been reported as the most prevalent diseases in the tilapia industry, causing severe infection in fry and fingerling stages.[59–66] Columnaris infection, caused by *Flavobacterium columnare*, can significantly impact productivity on farms.[59] This disease produces necrotic lesions on the gills and surface of the skin, often affecting the back and sides of the fish with a 'saddle back' lesion. Outbreaks are often initiated after periods of environmental stress caused by poor water quality, excessive handling and high stocking density. Columnaris can affect tilapia at any stage of development; however, fry and fingerlings tend to be more severely impacted by this disease.[59,62]

Motile aeromonas septicemia (MAS) is another major threat to the tilapia industry.[67–70] Most commonly caused by *Aeromonas hydrophila*, this disease frequently results in high mortality rates and affects both juvenile and adult fish. Infected fish are often treated with antibiotics but there are many reports of antimicrobial resistant strains on farms around the world.[71] Several vaccines against MAS are available and range in their efficacy.[72] Multiple risk factors are well known that may lead to an increase in outbreaks on tilapia farms. These include management related factors known to stress tilapia—such as

aggressive handling, high stocking density and poor water quality. Abrupt fluctuations in water temperature or concurrent fungal or parasitic infections may also increase their susceptibility to developing MAS.[73]

Francisellosis, caused by *Francisella orientalis*, is a significant disease affecting the tilapia sector.[74] Disease outbreaks have been documented as early as the 1990s, and high prevalence of infection with this pathogen has continued to be reported on tilapia farms over the past decade.[75–77] Both horizontal and vertical transmission may occur, and the infection is typically chronic with high morbidity and mortality (<90%) on tilapia farms.[66,78] To date, no commercial vaccines against francisellosis are licensed for use in tilapia.[79]

The novel bacterial disease hahellosis, caused by *Hahella chejuensis*, reportedly affects eggs and leads to red egg syndrome prior to hatching.[80] Disease outbreaks with this marine pathogen are more likely to occur in colder seasons, with an increase in mortality rates when water temperatures drop below 24°C.[80]

In general, several stressors, such as fluctuating water temperature, pH and salinity, low levels of dissolve oxygen, increasing levels of ammonia, higher stocking density, improper fish handling and poor management may increase the risk of bacterial disease outbreaks in tilapia populations.[73,81–85] Antibiotics have historically been used to treat bacterial diseases in aquaculture, however, there is growing public concern regarding the use of antimicrobials for disease management due the introduction and spread of antimicrobial resistant genes and detection of drug residues.

4.4 | Fungal diseases of tilapia

Fungal infections are classified as opportunistic because they thrive in necrotic tissues linked with injuries, bacterial or parasitic infections, dead and rotting eggs, and suboptimal culture conditions.[86] A variety of fungal species have been identified from wild and farmed tilapia, with the following diseases being the most common and well-documented.[87,88] Saprolegniasis, caused by the water mould oomycete *Saprolegnia ubiquitous*,[89] appears as cottony white, grey, brown, red, or greenish masses on affected fish.[88] Haemorrhagic ulceration, erosion of the skin, fins, gills and muscles, systemic mycosis of the liver, spleen, eyes and kidney, and elevated mortality were reported as major clinical indications of saprolegniasis.[88] This pathogen causes notable infections, usually during larval growth in hatcheries,[90] resulting in fatalities and considerable economic losses, particularly in aquaculture facilities.[91–93] Low temperature (<15°C) was reported as the key influencing factor for the rapid spread of this pathogen.[94] Branchiomycosis (gill rot) is another fungal infection in tilapia that affects the gills and is caused by two oomycetes: *Branchiomyces sanguinis* and *B. demigrans*.[89] *Branchiomyces* spp. infection is sometimes referred to as 'bad-management disease,' since it thrives in low-quality water with large amounts of organic debris. Another fungal disease, Ichthyophoniasis, is one of the most economically and environmentally devastating diseases that affects farmed Nile tilapia.[95,96] Infection with *Ichthyophonus* spp. is more common throughout the winter.[96,97] Aspergillomycosis is a fungal infection in tilapia caused by *Aspergillus niger*.[24] The growth of this mould is caused by poor storage of fish feed.[20] There have been no reports describing the life stages of tilapia affected by ichthyophoniasis and aspergillomycosis.

5 | IMPLEMENTING BIOSECURITY MEASURES IN THE VALUE CHAIN

Any aquaculture biosecurity programme should include risk analysis as a fundamental component for disease control.[10] For the purposes of this paper, we focus on disease risk, in which 'risk' is represented by one or more infections/diseases. Risk analysis allows for a greater understanding of the tilapia sector in a particular country or region and can be used to identify the diseases that impact production and sustainability of fish farms. Using this systematic approach, risk-based biosecurity strategies can be developed to control the introduction and spread of infectious diseases within the tilapia value chain in an effective and sustainable manner for the industry.

The general framework for risk analysis typically consists of four major components: (i) hazard identification—the process of identifying hazards that could potentially produce consequences; (ii) risk assessment—the process of evaluating the likelihood that a potential hazard will be realized and estimating the biological, social and/or economic consequences of its realization; (iii) risk management—the seeking of means to reduce either the likelihood or the consequences upon occurrence; and (iv) risk communication—the process by which stakeholders are consulted, information and opinions are gathered, and risk analysis results and management measures are communicated. Risk analysis is a process that provides a flexible framework within which the risks of adverse consequences resulting from a course of action can be evaluated in a transparent, evidence-based manner. The risk analysis approach permits a defendable decision to be made on whether the risk posed by a particular action or 'hazard' is acceptable or not, and provides the means to evaluate possible ways to reduce the risk from an unacceptable level to one that is acceptable. Its structure and components will vary considerably depending on: (i) the sector (technical, social or financial), (ii) the user (government, company or individual), (iii) the scale (international, local or entity), or (iv) the purpose (to gain understanding of the processes that determine the risk or to form the basis for legal measures). It can be qualitative or quantitative.[17] As a decision-making tool, risk analysis can be used to identify and prioritize the points or indicators that may pose a risk for disease transmission along the tilapia value chain, offering flexibility in its use.

'Risk' is defined as an estimation of the likelihood (i.e., probability) of introduction and spread of a pathogen, and the consequences of this occurring.[98] Risk assessments are conducted as part of the overall risk analysis process to estimate the level of risk at each point along the value chain and typically follow the WOAH's methodology, consisting of an entry assessment and exposure assessment, followed by a consequence assessment.[18]

Specifically, entry and exposure assessments describe the risk pathways necessary for the entry of a pathogen into a particular environment and exposure of a susceptible population to the pathogen, followed by an assessment of the likelihood of the processes occurring.[99] These pathways should be targeted when implementing new biosecurity measures or enhancing existing measures. In developing countries with limited resources, risk pathways around points in the tilapia value chain with the highest levels of risk can be prioritized.

Risk pathways are stepwise in nature and assist in identifying all conditions required for a disease outbreak to occur at points in the value chain. It is important that all steps within the risk pathway are plausible and evidence-based, considering the epidemiology of pathogens and diseases of concern and specific processes and activities occurring within the value chain.[12] The networks and linkages in tilapia value chains provide opportunities for disease transmission within or between farms, and diseases are often spread by movements of infected fish or their products. Contaminated influent water or effluent water are other major routes of pathogen introduction to farms. Disease transmission can also occur through movements of contaminated input materials (e.g., feed, seed), vectors (e.g., people, birds, vermin), and equipment, vehicles and other fomites (i.e., non-living objects that can be contaminated with pathogenic agents) during transport. All potential transmission routes in which an infectious disease may enter a point along the value chain should therefore be considered.

For each step of the risk pathway, it is necessary to identify all known factors influencing the probability of that step occurring. Risk factors increase the likelihood of disease introduction and spread along the value chain (Table 4). These factors can be related to the infectious agent (e.g., virulence, pathogenicity, survival), host (e.g., immunity, species of tilapia), or environmental characteristics specific to the geographic location or value chain (e.g., management practices, seasonal changes, volume of commodity).[100] Based on this knowledge, the likelihood of each step occurring along the pathway is typically estimated qualitatively or semi-quantitatively.[12] It is especially important to engage with key stakeholders in the tilapia industry throughout the risk analysis process to increase compliance, and ensure results are realistic and biosecurity measures are feasible.

Critical control points are points in the risk pathways where efforts can be focused to minimize or completely eliminate the risks of disease introduction and spread. In other words, these points can be targeted for the implementation of risk management, a major component of the risk analysis process.[99] This is also in line with the HACCP system that has been adopted by the Codex Alimentarius Commission.[101] Particularly in developing countries with limited resources, it is important to select control measures that are practical, effective, economical and sustainable. Biosecurity measures should consider the likelihood estimates and risk factors identified at each step of the pathway. Multiple critical control points may be identified, but in many cases, they should be prioritized based on multi-stakeholder discussions.

In the sections below, we briefly discuss potential risk management measures that may be implemented at critical control points to mitigate the risk of disease introduction and spread along the tilapia value chain (i.e., input and service suppliers; producers at hatcheries, nurseries and grow-out farms; and processors, traders and marketers). It is important that biosecurity measures are first selected based on their effectiveness in preventing or minimizing the risks associated with known risk factors (Table 4), and then prioritized based on their practicality, cost-benefits and sustainability associated with implementation.

5.1 | Input and service suppliers

Input and service suppliers in the tilapia sector provide seed, brood fish, feed, drugs, equipment and materials, often serving multiple clients in a particular region. During these visits, their vehicles, farm equipment, boots or other outerwear may act as fomites and pose a risk for the introduction of infectious diseases onto farms. The risks increase if multiple sites are visited within a short period of time, particularly if hatcheries are attended following grow-out site visits. Disease management solutions that can be used in the tilapia sector include handwashing, footbaths and the cleaning and disinfection of protective clothing, vehicles, boats, nets and other equipment and materials between site visits.[18] Disinfectants should be safe for use around aquaculture systems and sufficient to kill pathogenic agents of concern on the farm. Commonly used disinfectants include iodophors (e.g., Wescodyne, Betadine) and Virkon Aquatic, which are effective against most aquatic bacterial and viral pathogens affecting tilapia.[102] Strict security should also be enforced at both in-land and cage culture systems to restrict the movement of visitors and ensure compliance with biosecurity protocols.[18]

A number of problems tend to arise when there is limited access to trained fish health service providers; major consequences include misdiagnosis and improper treatments on farms, which often leads to reduced production and treatment failure.[103] Implementation of fish health management and surveillance programmes are vital to support the early diagnosis of emerging diseases.[19] In countries or regions where fish health services are provided by non-aquatic veterinarians, one possible solution is to ensure fish health specialists are qualified through education (post-graduate qualification), fish health programmes recognized by the Competent Authority, a professional certification scheme that involves standardized training and assessments.[11] This would provide a mechanism for farmers to identify competent fish health professionals to attend their sites. A few of these certification schemes exist and are being explored by FAO as a means to build capacity in aquatic animal health in developing countries.[104] In countries with limited fish health diagnostic expertise, formal collaborations can be established with international accredited laboratories to provide diagnostic testing for diseases of national concern.

The introduction of fish seed or broodstock with an unknown health-status is another major pathway of disease introduction in the tilapia sector. To mitigate the risks, seed or broodstock should be sourced from specific pathogen free (SPF) stocks that are certified-free from pathogens of concern.[3] In many cases, local hatcheries supply tilapia seed over relatively short distances, which reduces the likelihood of transport-related stress and trauma that often predispose fish to opportunistic infections.[105] Transportation containers should be disinfected before use, and eggs should be disinfected prior to

TABLE 4 A generalized list of potential risk factors for disease introduction and spread at major points along the tilapia value chain

Point in value chain	Potential risk factors
Input and service suppliers	
Fish health services	• Inadequate fish health services (e.g., limited training, misdiagnosis, lack of surveillance) • Inadequate fish health diagnostic laboratory • Multiple farm visits within a short time period • Inadequate cleaning and disinfection of fomites (e.g., nets, boots, outerwear, boats, vehicles, equipment) between rearing units or site visits
Transportation services	• Multiple visits by different transportation services at farms • Inadequate cleaning and disinfection of fomites between visits (e.g., boots, boats, vehicles, nets) • Inadequate disinfection of effluent water • Inadequate disposal of mortalities or offal • Mixing of species • Mixing of life-stages • Lack of traceability of fish • Inadequate governmental biosecurity regulations or enforcement
Equipment and materials supplier	• Multiple visits by different suppliers at farms • Inadequate cleaning and disinfection of fomites between visits (e.g., boots, boats, vehicles) • Inadequate cleaning and disinfection of used equipment and materials
Feed and pharmaceutical suppliers	• Multiple visits by different suppliers at farms • Inadequate cleaning and disinfection of fomites between visits (e.g., boots, boats, vehicles) • Unreputable feed supplier • Using live feed • Using non-commercial feed • Unreputable pharmaceutical supplier • Using unlicensed, expired, or fraudulent drugs • Inadequate governmental biosecurity regulations or enforcement
Seed and broodstock suppliers	• Multiple visits by different suppliers at farms • Inadequate cleaning and disinfection of fomites between visits (e.g., boots, boats, vehicles, nets) • Inadequate disinfection of effluent water • Inadequate disposal of mortalities • Lack of health certification or traceability of seed or fish • Collection of seed from the wild • Inadequate disinfection of eggs • Inadequate governmental biosecurity regulations or enforcement
Producers	
Hatchery, nursery, or grow-out	• Inexperienced farmer • Lack of standard operating procedures or improper training of staff • Lack of security or visitor control to sites • Visiting multiple sites within a short time period • Visiting other sites prior to the hatchery or nursery within a short time period • Inadequate cleaning and disinfection of fomites (e.g., boots, outerwear, equipment, vehicles) between visits • Sharing equipment (e.g., nets) between sites • Inadequate cleaning and disinfection of tanks • Dirty or damaged nets • Untreated influent water or lack of a pathogen-free water source • Poor water quality • Inadequate cleaning and disinfection of ponds • Lack of fallowing of ponds, cages or net-pens • Multiple farms in a small geographic area • Seasonal fluctuations in weather • Shared water with infected wild fish populations • Accidental introductions of wild species • Introduction of fish of unknown health status • Mixing of stock from different sources • Mixing of stock from different age-classes • Mixing of species • Overstocking of fish • Excessive fish handling • Movement of fish between rearing units

(Continues)

TABLE 4 (Continued)

Point in value chain	Potential risk factors
	• Inadequate egg disinfection • Providing treatments without consultation of a fish health expert • Lack of vaccination • Use of contaminated or spoiled feed • Inadequate protection from predators • Inadequate disinfection effluent water • Inadequate disposal of mortalities or waste • Use of contaminated fertilizers in or around ponds • Presence of snails within sites • Inadequate governmental biosecurity regulations or enforcement
Processors, traders and marketers	
Processing plant	• Receiving fish from multiple sources • Lack of traceability of fish • Inadequate cleaning and disinfection of equipment • Inadequate disposal of fish or offal • Inadequate disinfection effluent water • Poor hygiene of staff • Improper storage of fish or their products • Inadequate protection from vermin and scavengers • Inadequate governmental biosecurity regulations or enforcement
Distributors, markets or retail	• Mixing of stock from different sources • Mixing of species • Lack of traceability of fish • Inadequate disposal of fish or offal • Inadequate disinfection effluent water • Poor hygiene of staff • Improper storage of fish or their products • Inadequate protection from vermin and scavengers • Inadequate governmental biosecurity regulations or enforcement

entry into hatcheries to minimize contamination with pathogenic agents.[18] At the country level, strict aquaculture biosecurity regulations should be in place regarding inspection and health certification of imported live fish or their products to ensure freedom from infectious pathogens.[106] These regulations also often include proper disinfection and disposal of effluent water, mortalities or waste related to transport from both locally or internationally sourced animals.

It is important that tilapia producers source their feed from reputable companies, as feed quality and nutrition are integral to fish health. Trash fish can carry numerous pathogenic agents and pose a risk for disease introduction, particularly in cage culture settings.[98] Commercial feeds that follow good manufacturing practices and HACCP guidelines undergo intensive processing that inactivates pathogenic agents, making these products a safe choice for farmed fish. However, feeds that are not stored in cool, dry and secure containers are at risk for contamination with pathogenic bacteria or fungi.[107] In the case of premixes and medicated feeds, proper storage is especially important to maintain the stability of active ingredients.

Antimicrobials and other drugs are commonly used in tilapia aquaculture production and should be used responsibly under the direction of an aquatic veterinarian or a fish health specialist.[108] Fish populations treated with expired or fraudulent antimicrobial products are at risk of treatment failure, which can result in sub-therapeutic levels of treatments and significant production losses. In some cases, the risks are exacerbated with the development of antimicrobial resistant genes.[109] It is therefore important to source medications and medicated feeds from reputable suppliers. However, as evident in recent studies in Asia, even commercially purchased antimicrobial products may be of poor quality with limited active ingredients, limiting their efficacy.[110,111] In the case of antibiotics, their efficacy can be confirmed through diagnostic testing that can be performed in most laboratories. Susceptibility testing via minimum inhibitory concentration (MIC) panels can be used to confirm the effectiveness of antibiotic products against targeted bacterial isolates; this test is a very cost-effective and practical method that can be used in laboratories with limited capacity. High-performance liquid chromatography (HPLC) can also be used to confirm the concentration of active pharmaceutical ingredients in antimicrobial products.[110] This test may be particularly useful in developing countries with limited regulations or enforcement for veterinary drugs. Many countries have strict regulations in place to ensure pharmaceutical products used in aquaculture are safe for use in food fish, and have strict withdrawal times and complete transparency regarding active ingredients.[112]

5.2 | Producers

5.2.1 | Hatcheries

Hatcheries are the first phase of the tilapia production cycle and should begin with healthy broodstock. If eggs or fry are produced on

site, broodstock are usually raised in ponds, hapas, or tanks where egg laying, spawning and fertilization occurs. At this stage, eggs may be collected and transferred to hatching units, or be left to hatch naturally alongside the brood fish. Fry are collected and transferred to tanks or hapas until they reach fingerling size. Infectious diseases are a major issue in hatcheries and can lead to massive mortality in larval populations.[113] For example, the majority of parasitic infections in tilapia occur during the early life stages of the fish; therefore, special attention should be paid to the prevention and treatment of parasites in hatcheries.

Disease risks cannot be mitigated by simply using SPF broodstock; however, stocking clean stocks is already a good starting point. Pathogens can enter a hatchery through a number of transmission routes, including risky practices associated with input and service suppliers, as described above. It is much easier to prevent disease outbreaks in a closed aquaculture system compared with pond or hapa-based culture—as discussed further in Section 5.2.2. Although not always practical, tank culture allows for easy observation of fish for early signs of disease and a high degree of control over water conditions. Regardless of the culture system, newly introduced brood fish or seed into the hatchery should first be quarantined and monitored for the presence of infectious diseases.

Although tilapia can be reared in a wide range of environmental conditions, poor water quality can lead to elevated mortality and reduced production on farms.[114] In tank culture systems, the use of contaminated influent water is a major risk, which can be mitigated with a water treatment system or by supplying tanks with pathogen-free ground water or de-chlorinated municipal water.[18] If lake or river water is used, screening and filtration should be in place to prevent the entry of wild fish, which can be carriers of infectious agents. These mitigating measures are not possible in ponds or hapas.

Subclinical infections are common in adult fish and remain undetected in a population without regular surveillance.[19] Furthermore, the immature immune system of tilapia larvae makes them particularly vulnerable to infections.[112] Biosecurity strategies should therefore be in place to minimize horizontal transmission from brood fish to these early stages within a hatchery.

In flow-through systems, water should first pass through the broodstock units and then the eggs, before supplying the early larval stages. Other ways to minimize horizontal transmission to tilapia seed include separation of life stages, designated equipment and staff for each rearing unit, proper disposal of waste and limited visitors to the hatchery.[18] Broodstock vaccination is another option that can transfer both specific and non-specific immunity to their offspring.[115–117] In a recent study, vaccination of tilapia brood fish against *Streptococcus agalactiae* provided enhanced protection in their seed.[118] Fry or juvenile tilapia can also be vaccinated before transfer to grow-out farms.

As with any aquaculture system, the goal is to reduce exposure to potential infectious agents through the removal of mortalities and regular cleaning and disinfection of nets, tanks, equipment and other materials. Tilapia producers should strive for early disease detection and treatment on farms by training all farm staff and working with an experienced fish health specialist. It is important that only healthy fingerlings are transferred to nurseries or grow-out sites to minimize the spread of disease within a farm.

5.2.2 | Nurseries and grow-out farms

Tilapia fingerlings are usually raised in nurseries for 2 to 3 months until they reach an advanced size.[119] Nurseries may consist of tanks, ponds, or hapas. Once fingerlings reach grow-out, pond culture is most common; however, cage, tank and raceway culture systems also exist. Any newly introduced fingerlings with an unknown health status should be contained in a separate quarantine unit to ensure freedom from infectious agents.

Water serves as the greatest risk for pathogen introduction in open aquaculture systems, particularly in cage culture. Disease risks can be minimized, but not completely prevented, by implementing biosecurity measures aimed to improve the immunity of the population, and reduce the level of exposure and pathogenicity of infectious agents. Several risk management measures may be used to reduce the potential pathogen load in nurseries or grow-out farms. Early treatments may reduce the severity of disease and limit transmission within a farm.[120] Therefore, farms should be monitored daily to allow for rapid diagnoses during outbreaks.

Proper sanitary conditions are very important, cost-effective and fairly easy to implement. In cage or hapa culture, regular removal of dead fish and cleaning of nets can improve water quality and reduce exposure to infectious agents in pens.[121] A fallowing period should also be implemented for several days between harvest and stocking.[122] In pond farming, ponds should be drained, cleaned, limed and dried before the next production cycle.[123] This will disinfect the pond and kill wild aquatic species that may serve as potential vectors for disease transmission to tilapia. For example, snails are the intermediate host of parasitic trematodes.[124]

Vaccination is widely promoted as a first line of defence against infectious diseases in tilapia production. Fingerlings should be vaccinated prior to grow-out. A number of vaccines have been developed to address major disease challenges in tilapia, such as streptococcosis, francisellosis, MAS and TiLV infection.[72,125,126] Autogenous vaccines can also be produced against various pathogens for individual farms.

Stressful environmental conditions weaken the fish immune system, increasing the occurrence of opportunistic infections.[127] Overstocking is a major stressor in tilapia culture and can lead to an increase in competition and aggression in the population. El Nouman et al.[128] reported that tilapia fingerlings reared in floating cages had the best performance at a medium density of 240 fish-m^3. Excessive handling, which can occur during transportation, vaccination or treatments, is also very stressful for fish.[129]

Fluctuations in weather can be problematic for open aquaculture systems. Rising water temperatures increase the risk of infectious disease outbreaks on tilapia farms.[121,130] For example, 'summer mortality syndrome' was reported on Egyptian tilapia farms in 2015 and potentially linked to TiLV.[131] For early detection of diseases, active

surveillance should be conducted regularly during seasons of elevated disease risk.[19] Storm events can stress fish, tear nets, or in severe cases, destroy farms entirely. Nets should be checked regularly and replaced, if necessary, to prevent the entry of wild fish, birds, or other predators, which may be vectors or carriers of disease.

5.3 | Processors, traders and marketers

Once live tilapia or their products reach the processing, trading or marketing points in the value chain, it is important to understand what typical managing practices are being followed, the quantity of commodity being moved and the transportation routes and final destination. These factors affect the risk of contamination occurring along this part of the value chain. To better estimate the level of risk, the effects of processing, storage, or transport on the pathogens of concern should be well-understood. Infectious agents may be reduced or inactivated during the handling, processing or storing (e.g., freezing) stages.[98]

In general, the tilapia industry is dominated by small-scale farms. The majority of tilapia production is marketed in rural areas and consumed locally, especially in developing countries. Local markets usually sell dried, smoked, live, freshly killed or chilled tilapia directly to consumers.[132] Food safety risks associated with tilapia products being sold in local market settings should also be considered. The surface of the fish may be contaminated with pathogens from the water, particularly in cases of sewage contamination, or through exposure during handling, processing, or transportation.[133] Strict hygiene measures should be taken to minimize the risks of foodborne illness, including handwashing, preventing cross-contamination from people/fomites and cooking raw fish products prior to consumption. Fresh tilapia products must be chilled immediately to prevent spoilage. Temperature control of fresh fish is dependent on the availability of ice or refrigeration in the region. Smoked fish may contain a high concentration of polycyclic aromatic hydrocarbons (PAHs), which may also present carcinogenic risks when consumed.[134]

In some cases, tilapia producers sell their fish directly to traders, who then sell the product to wholesalers or retailers. It is common, particularly in Southeast Asia, to see live tilapia in aquariums in supermarkets and restaurants. At these locations, the fish may be sourced from multiple producers within a local region, which represents a potential risk of disease transmission to farms via fomites or vectors, or even effluent water in the case of farms in very close proximity to markets.

In addition to the domestic markets, tilapia is highly traded on a global scale. Therefore, it is important that proper handling and processing is implemented to maintain high quality products that meet standards required by external trade partners.[132] Processed fish are in high demand in global markets, which includes, freshly frozen fillets, dried and salted fish, minced fish or fish balls. Large tilapia processors may sell their products to wholesalers or directly to retailers; or in some instances, these products are exported to international markets.

Governmental regulation and control of fish processing plants reduce the risk of microbial, chemical or physical hazards entering the tilapia value chain.[98] This includes the implementation of an operational HACCP plan and other food safety related risk management practices. Processing plants that are located near fish farms should ensure any effluent or waste is treated prior to disposal, especially if they are located near bodies of water. Movements through the processors to distributers should be traceable in order to identify the source of infection in the event of an outbreak.

6 | DISCUSSION AND PERSPECTIVES

Biosecurity risks should be a common responsibility among relevant authorities and stakeholders along the aquaculture value chain.[135] Indeed, capacity building in risk analysis and adaptive management at all levels—from farms to oversight bodies of the public and private sectors—is becoming essential in order to rapidly assess the threats and uncertainties from new species and innovations.

The diseases of concern to the tilapia sector should continuously be reviewed and updated based on changes in the national, regional and international situation. The prevalence and distribution of diseases affecting tilapia can be determined through national surveillance and monitoring programmes and unofficial data sources, such as peer-reviewed journals, news articles and public reports. It is fundamental to have a good understanding of the infectious agents affecting tilapia prior to implementing any mitigating measures on farms; this includes clinical signs, pathology, and epidemiology of diseases, as well as diagnostic testing and pathogen characteristics.

In general, the main purpose of aquaculture biosecurity is to protect a population of aquatic organisms against identified disease risks. Biosecurity measures can be applied at the national level or local farm level and includes mitigating the risk of disease introduction into a farm and preventing the spread between rearing units within a farm. The control of diseases in the tilapia sector requires a multi-faceted approach and may involve a combination of multiple biosecurity measures at numerous points along the value chain.[12]

In most cases of tilapia production, the complete elimination of disease risks along the value chain is not realistic. This is especially true in open aquaculture culture systems (i.e., ponds or net pens) that present many opportunities for disease transmission into a farm; and in certain cases, no control measures may be identified for a particular risk pathway. In these situations, a surveillance programme can be implemented as an early detection method for disease spread.[12,19] Contingency planning for diseases of high concern to the tilapia sector should also be a major part of any biosecurity programme, especially when appropriate control measures cannot be identified.[136] This is often the case when interactions between cultured and wild stocks cannot be prevented. A strong surveillance and contingency planning programme may promote a rapid disease response, which can reduce the impacts associated with outbreaks and lower the overall risk. It is also important to monitor for changes in value chains that could affect

disease risks. For example, risk factors may change depending on the season or if production systems develop and expand over time.

Smallholders often have unorganized or dispersed value chains stemming from multiple factors including informal trade and marketing practices, inadequate infrastructure and inaccessibility to required aquatic health services.[14] This poses an increased risk due to more opportunities for disease introduction and spread between points.[12] As small-scale operations expand, there will be increased incidence both of production-related diseases and the rapidly spreading emerging infectious diseases that will need to be managed at the farm level. The overarching aim should be firstly to increase resilience of small-scale producers to allow for a greater level of self-sufficiency in recognizing and mitigating risks within their sector. Small-scale producers can organize themselves into cooperatives and producer associations, which can then enable them to increase their representation and their interest as well as bargaining power, resulting in more active participation in the value chain. In addition, reducing the number of steps within value chains, if at all possible, will in turn reduce these risks.

Improving risk management along the production, marketing and governance chains may provide some solutions. Basic biosecurity such as good hygiene, sound sanitary practices and early detection of disease with appropriate responses should be a routine application at the farms. It is necessary that all levels of the sector, from small-scale farmers through to government policy makers, have a good understanding of the critical points in the system which need to be controlled, as well as the means to manage them. Addressing such issues in a systematic manner will reduce the impacts of diseases.

Access to reliable, effective and practical pond-side diagnostic tools and timely information are needed by fish farmers (especially smallholders) through revitalized extension and diagnostic services reaching often remotely located aquaculture facilities. Through the use of cluster management, the empowerment of individuals will develop leadership and foster ownership, creating economies of scale through collective action that can lead to improved governance and management of the sector, including biosecurity challenges.[137]

Developing countries with limited or no aquaculture biosecurity regulations should strive towards building capacity in their national legal framework related to aquatic animal health and aquaculture biosecurity. This includes regulations to safeguard the country against the introduction and spread of reportable diseases of tilapia through the importation and movement of live fish and their products. The often injudicious and inappropriate use of antimicrobial products to treat diseases in cultured tilapia has serious consequences, that include the introduction and spread of antimicrobial resistant genes and residues. Recognizing the risks associated with these imprudent practices and minimizing their usage is vital for promoting the sustainability of the tilapia industry. This requires the development of regulations and strict enforcement in regard to the labeling and marketing of veterinary drugs.

It is especially important that we ensure that the value chain for the tilapia sector can continue to operate, even with new biosecurity measures in place. Risk communication is a vital step of the risk analysis process and is a way for stakeholders to share information regarding identified risks and discuss major decisions prior to the implementation of new biosecurity policies. Public-private partnership (PPP), that is, active stakeholder engagement and collaboration, is strongly encouraged for this process and allows for transparent sharing of information among value chain actors. PPP should be formed early in the risk analysis process and serves the purpose of exchanging information to support the risk assessment and disseminating information during the risk management step prior to policy making.

Newly implemented control measures should be chosen based on their practicality, effectiveness and sustainability. It must be recognized that suitable control measures may vary depending on the tilapia species, culture conditions and environment, and may vary according to the country or development of the sector, so must be tailored accordingly. In developing countries with limited resources, it is essential to identify and prioritize the riskiest pathways for disease spread. It is therefore important to consult with key stakeholders in the tilapia industry prior to making decisions regarding risk management in order to improve compliance and build trust.

The PMP/AB, a pathway that builds on existing frameworks and is supported by appropriate tools (via the PMP/AB toolkit), offers a four-stage, step-wise risk management approach that introduces the building blocks for aquaculture biosecurity capacity that are relevant to national needs at every stage.[104] It typically includes the following elements: defining the risks, for example, pathogen, antimicrobial resistance (AMR), ecosystem risks; developing a long-term national aquaculture biosecurity strategic plan; implementing the plan; and monitoring and evaluating the plan. It can be tailored to meet either sector- or disease-specific risks and encourages PPP, thus promoting a greater recognition of the important role of biosecurity in aquaculture health management.[138]

Since the PMP/AB is risk-based, the concept of HACCP is very relevant. Indeed, the application of risk analysis to minimize disease risks within a value chain not a novel approach and has also been widely used in the terrestrial animal sector over the past decade. The Codex Alimentarius' HACCP system is the most recognized tool for developing food safety standards worldwide,[139] offering a logical framework and following a systematic approach, to identify, assess and control food safety related hazards throughout the food supply chain. Widely used in the food-processing sector, however, this approach still provides a good framework for the planning and implementation of biosecurity at the farm level. It is based on seven principles, (i) conduct hazard analysis to identify pathogens; (ii) determine critical points, that is, contamination pathways; (iii) establish critical limits, that is, water quality; (iv) establish monitoring system for critical points, for example, disinfection of intake water, movement of stock and disinfection of equipment and outerwear; (v) establish corrective actions when failures in biosecurity are detected; (vi) establish verification procedures to ensure the HACCP is working effectively; and (vii) maintain efficient record keeping appropriate to these principles and their application.[104]

The PMP/AB is based on similar frameworks that have been applied in the terrestrial animal production sector. In 2011, FAO developed detailed guidelines for applying the combination of value chain and risk analyses in a HACCP-like approach to control disease risks in livestock production[12]; this approach has since been promoted

by the European Commission for the Control of Foot-and-Mouth Disease (EuFMD) for the development of risk-based control strategies for foot-and-mouth disease and a number of transboundary animal diseases affecting livestock.[140] Several projects led by FAO have also used this approach to develop more effective risk mitigation measures[141]; for example, value chain mapping and risk assessment of cross-border chicken trade between Viet Nam and China was successful in developing stronger policies related to movement controls and trade mechanisms. More recently, a number of publications feature value chain analysis and risk assessment methodology to develop disease interventions in the pig[142–144] and poultry[145,146] production sectors.

7 | CONCLUSION

The prevalence of diseases affecting cultured tilapia is growing, as is the global distribution of emerging diseases causing concern to the industry. It is well recognized that their management and control requires a multi-faceted approach, with risk-based control measures chosen based on their feasibility, effectiveness and sustainability. The PMP/AB, as a risk-based, collaborative and progressive management approach combined with the systematic preventive approach of HAACP, offers a strategic and practical way of improving biosecurity in the tilapia value chain. Understanding and embracing the use of the risk analysis process to identify critical control points is encouraged, as it is not always possible to know and precisely predict every potential source of harm and its pathways. A flexible approach should be taken in order to implement potential risk mitigation processes.

In many developing countries that farm tilapia, small-scale producers may not have access to a functional value chain and public institutions should strive to address these risks. Small-scale producers can organize themselves into cooperatives and producer associations, which can then enable them to increase their representativeness and their interest as well as bargaining power, thus having more active participation in the value chain.

AUTHOR CONTRIBUTIONS

Brett MacKinnon: Conceptualization; data curation; formal analysis; investigation; methodology; validation; writing – original draft; writing – review and editing. **Partho Pratim Debnath:** Data curation; investigation; validation; visualization; writing – original draft. **Melba G. Bondad-Reantaso:** Conceptualization; formal analysis; funding acquisition; methodology; project administration; supervision; validation; visualization; writing – original draft; writing – review and editing. **Sophie Fridman:** Visualization; writing – original draft; writing – review and editing. **Hao Bin:** Data curation; validation; visualization; writing – original draft. **Omid Nekouei:** Resources; writing – review and editing.

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DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

ORCID

Brett MacKinnon https://orcid.org/0000-0001-7475-1542
Partho Pratim Debnath https://orcid.org/0000-0002-8107-7880
Melba G. Bondad-Reantaso https://orcid.org/0000-0002-2380-3549
Sophie Fridman https://orcid.org/0000-0002-0159-0474
Hao Bin https://orcid.org/0000-0002-5284-7946
Omid Nekouei https://orcid.org/0000-0002-2427-4159

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REVIEW

REVIEWS IN Aquaculture

A global review of problematic and pathogenic parasites of farmed tilapia

Andrew P. Shinn[1,2] | Annemarie Avenant-Oldewage[3] | Melba G. Bondad-Reantaso[4] | Armando J. Cruz-Laufer[5] | Adriana García-Vásquez[6] | Jesús S. Hernández-Orts[7] | Roman Kuchta[7] | Matt Longshaw[8] | Matthijs Metselaar[9] | Antoine Pariselle[10,11] | Gerardo Pérez-Ponce de León[12] | Pravata Kumar Pradhan[13] | Miguel Rubio-Godoy[6] | Neeraj Sood[13] | Maarten P. M. Vanhove[5] | Marty R. Deveney[14]

[1]INVE (Thailand), Nonthaburi, Thailand

[2]Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University, Townsville, Australia

[3]Department of Zoology, University of Johannesburg, Johannesburg, South Africa

[4]Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, Rome, Italy

[5]Hasselt University, Centre for Environmental Sciences, Research Group Zoology: Biodiversity and Toxicology, Diepenbeek, Belgium

[6]Instituto de Ecología A.C, Xalapa, Veracruz, Mexico

[7]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

[8]Calysta (UK) Ltd., The Wilton Centre, Wilton, Redcar, UK

[9]Aquatic Vets Ltd., Stirling, UK

[10]ISEM, CNRS, Université de Montpellier, IRD, Montpellier, France

[11]Laboratory "Biodiversity, Ecology and Genome", Faculty of Sciences, Mohammed V University in Rabat, Rabat, Morocco

[12]Escuela Nacional de Estudios Superiores Unidad Mérida, and Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

[13]ICAR-National Bureau of Fish Genetic Resources, Lucknow, Uttar Pradesh, India

[14]South Australian Research and Development Institute and Marine Innovation Southern Australia, SARDI Aquatic Sciences, West Beach, South Australia, Australia

Correspondence
Andrew P. Shinn, INVE (Thailand), 471 Bond Street, Bangpood, Pakkred, Nonthaburi 11120, Thailand.
Email: a.shinn@inveaquaculture.com

Marty R. Deveney, South Australian Research and Development Institute and Marine Innovation Southern Australia, SARDI Aquatic Sciences, West Beach, South Australia, Australia.
Email: Marty.Deveney@sa.gov.au

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Abstract

Over the past 80 years, tilapia have been translocated globally for aquaculture; active production is recorded in >124 countries. Of 7 million tonnes of tilapia produced in aquaculture, 79% is from 79 countries outside the natural range of tilapia. Capture fisheries account for a further 723,627 tonnes of tilapia, and >47% of this is landed from established invasive populations outside Africa. Tilapias host a rich fauna of parasites, many of which have been translocated with their hosts. This review summarises >2500 host–parasite records from 73+ countries and >820 recorded tilapia translocations (provided in the supplementary materials). This work focuses on the notable pathogens that threaten the health of cultured populations of tilapia, providing a description of their pathology and includes species that also have substantial impacts on wild tilapia

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populations, where relevant. For each major parasite taxonomic group, we highlight which parasites have been translocated or have been acquired from the new environments into which tilapia have been introduced, together with remarks on standard treatment approaches and research on them and their management and control. Regarding the theme 'Tilapia health: *quo vadis*?', Africa has enormous potential for aquaculture growth, but substantial knowledge gaps about tilapia parasites in many African states remain, which creates associated production and biosecurity risks. For each parasitic group, therefore, the risks of parasite translocation to new regions as tilapia aquaculture industries expand are highlighted.

KEYWORDS
aquaculture, global translocation, host–parasite record, pathogenicity, production

1 | INTRODUCTION

Cichlids belonging to the genera *Coptodon* Gervais, 1848 (31 species), *Oreochromis* Günther, 1889 (33 species) and *Sarotherodon* Rüppell, 1852 (13 species) are endemic to Africa and the Middle East, while those belonging to the genus *Tilapia* Smith, 1840 (four species) have distribution across southern parts of West Africa. Of these, 12 species and one hybrid of 'tilapia' are cultured intensively, namely *Coptodon rendalli* (Boulenger, 1897); *C. zillii* (Gervais, 1848); *Oreochromis andersonii* (Castelnau, 1861); *O. aureus* (Steindachner, 1864); *O. leucostictus* (Trewavas, 1933); *O. macrochir* (Boulenger, 1912); *O. mossambicus* (Peters, 1852); *O. niloticus* (Linnaeus, 1758); *O. aureus* × *O. niloticus* cross; *O. shiranus* Boulenger, 1897; *O. spilurus* (Günther, 1894); *Sarotherodon galilaeus* (Linnaeus, 1758); and *S. melanotheron* (Rüppell, 1852).[1]

The aquaculture production of tilapia approaches 7 million tonnes of which a staggering 4,866,563 tonnes (79.01%) is produced in 79 states outside their native range (Tables 1 and S1).[2] The collective production of tilapia of 6,192,963 tonnes valued at USD 12.342 billion, from 124 countries currently registering production, ranks first in all production categories above that of grass and silver carps, while Nile tilapia alone with a global production of 4,590,292 tonnes, ranks third. Production trends based on FAO,[2] and current to 2019, indicate that *O. niloticus* has the fastest industry growth, increasing at 4.11% year-on-year (2015–2019), when compared to the other top four fish species, that is, grass carp (*Ctenopharyngodon idella* [Valenciennes, 1844]; 2.61%), silver carp (*Hypophthalmichthys molitrix* [Valenciennes, 1844]; 1.09%), common carp (*Cyprinus carpio* Linnaeus, 1758; 2.78%) and bighead carp (*Hypophthalmichthys nobilis* [Richardson, 1845]; 1.26%). The average growth rate of all cultured tilapia across the same period is 3.73% year-on-year.

Tilapias are farmed in 79 territories outside their native range, mainly in China (1,641,662 tonnes), Indonesia (1,257,000 tonnes) and Bangladesh (350,258 tonnes), these producers accounting for 66.76% of all tilapia grown. Production of cultured tilapia surpassed the volumes landed from capture fisheries in 1993 and currently represents 89.54% of total tilapia production. Of the 723,627 tonnes derived from capture fisheries, 358,025 tonnes (47.71%) of the take is from 24 countries outside the native range of tilapia. Of these, Mexico (136,820 tonnes), Indonesia (68,650 tonnes) and Sri Lanka (51,810 tonnes) are the top three producers (Table S1).

The earliest recorded translocations of tilapia out of Africa were to South East Asia in the late 1930s with the purported unintentional introduction of *O. mossambicus* into the Serang River, Java in 1939,[3] and in the early 1940s with shipments of *O. mossambicus* to Hong Kong, Indonesia, Malaysia and Singapore, followed by consignments of *O. niloticus* to Argentina in 1940 and of *C. zillii* to Mexico and Antigua in 1943–1945 (Table S1). Tilapia host a rich fauna of metazoan parasites and eukaryotic microbial pathogens (protists), many of which have been translocated with the global movement of tilapia or have been acquired from resident fish and environments into which they have been introduced (Tables 1 and S1).

This review provides a list of recorded parasites (metazoans and protists) of tilapia (Tables S2 and S3) and focuses on the notable pathogens that threaten the health of cultured populations of tilapia. It provides comments on their pathology and effects on their hosts, including where relevant, references to the pathogens that also have substantial impacts on wild tilapia. For each major parasite taxonomic group, we provide comments on the translocation of parasites with fish and parasites from these new environments that parasitise tilapia, together with remarks on standard treatment approaches, where these exist, and research towards their management and control.

2 | PARASITIC INFECTIONS OF TILAPIA

The ensuing parasite sections follow the phylogenetic classification of eukaryotes proposed by Adl et al. and Burki et al.[4,5]

2.1 | Amoebozoa Lühe, 1913 (Amorphea: Amoebozoa)

2.1.1 | Taxonomic identity

Amoebozoa is a group of amoeboid protists often possessing blunt, fingerlike pseudopods and tubular cristae. At least seven genera of

TABLE 1 A summary of commercially important tilapia species in aquaculture and global capture fisheries

Species	Common name	Aquaculture tonnes (2019)	Producing countries (2019)	Number of countries					Capture fisheries tonnes in 2019 (from intro stocks)	No. of countries (2019)
				Native	Introduced	Intro. but not established	ID questionable	Misident.		
C. rendalli	Redbreast tilapia	2999	4	11	28	0	3	0	0	0
C. zillii	Redbelly tilapia	6	2	28	23	3	3	0	0	0
O. andersonii	Three spotted tilapia	4793	3	6	4	0	0	0	0	0
O. aureus	Blue tilapia	3100	4	10	35	4	0	0	1804 (1799)	2 (1)
O. aureus × *O. niloticus*	Blue-Nile tilapia, hybrid	4,10,553	2	0	2	1	0	0	0	0
O. leucostictus	Blue spotted tilapia	0	0	2	4	0	0	0	0	0
O. macrochir	Longfin tilapia	1800	1	5	18	5	2	0	0	0
O. mossambicus	Mozambique tilapia	74,435	10	7	93	8	2	1	21,450 (21,450)	2 (2)
O. niloticus	Nile tilapia	45,90,292	75	22	6	77	0	0	281,644 (73,337)	13 (7)
O. shiranus	Tilapia shiranus	4711	1	3	0	1	0	0	1082 (0)	1
O. spilurus	Sabaki tilapia	300	1	3	10	0	1	0	0	0
O. urolepis	Wami tilapia	0	0	1	2	0	0	0	0	0
Oreochromis spp.	Tilapias nei	10,99,860	51	39	55		0	0	451,159 (224,283)	23 (11)
S. galilaeus	Mango tilapia	19	2	26	3	1	1	0	255 (0)	1
S. melanotheron	Blackchin tilapia	95	1	15	5	2	0	0	2234 (0)	1
T. sparrmanii	Banded tilapia	0	0	11	2	0	0	0	0	0
Total		61,92,963							723,627 (320,869)	

Note: For each species, the total tonnage and the number of countries supplying returns to FAO in 2019 are provided; for capture fisheries, the tonnages resulting from native stocks are presented alongside those from stocks (in parentheses) that have been introduced and established in the wild. Figures are calculated from the FAO FishStatJ (2021) and Fishbase (Froese and Pauly, 2021) databases and the wider literature.

Abbreviations: BR, brackish water; C, *Coptodon*; FW, freshwater; nei, not included elsewhere; O, *Oreochromis*; S, *Sarotherodon*; T, *Tilapia*.

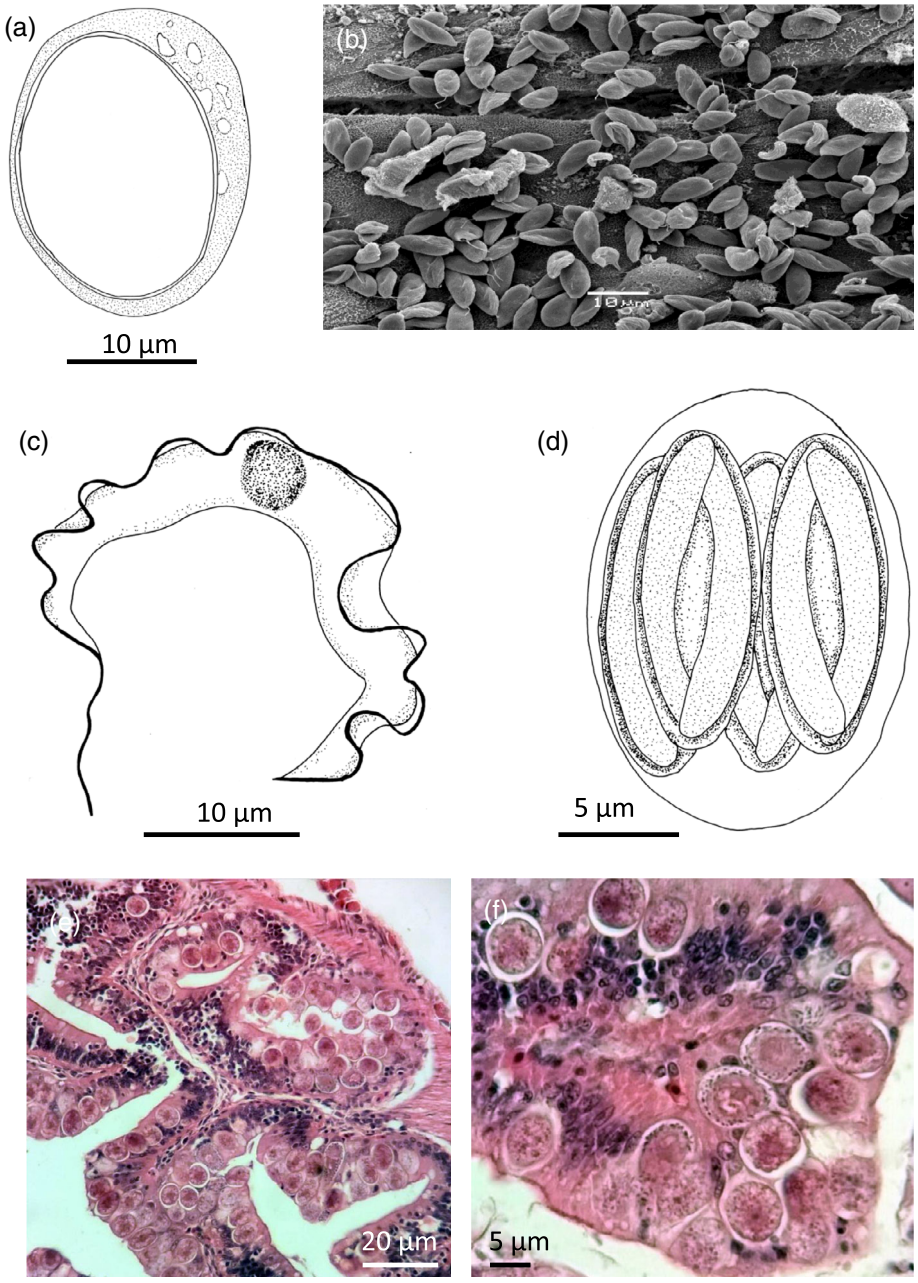

FIGURE 1 Protista. (a) Line drawing of *Dermocystidium aegyptiacus* reported from the intestines of *Oreochromis niloticus* cultured in Egypt. (b) Scanning electron microscope image of *Ichthyobodo necator* on epithelial surfaces. (c) Line drawing of *Trypanosoma mukasai* reported from the blood of a number of farmed and wild tilapia species. (d) Line drawing of *Goussia vanasi*. (e, f) Unidentified coccidian infection within H&E sections through the intestine of juvenile *O. niloticus* reared in lined tanks receiving water from a natural earthen reservoir in Brazil. Image (a) after El-Mansy (2008), image (c) after Baker (1960), image (d) after Molnár et al. (2004), images (e) and (f) courtesy of Leo Galli

free-living amoebae are reported in farmed tilapia, including *Rosculus* Hawes, 1963, *Mayorella* Schaeffer, 1926, *Platyamoeba* Page, 1969 and *Vermamoeba* Cavalier–Smith et Smirnov, 2011, from farmed *O. niloticus* in the Czech Republic,[6] *Acanthamoeba* Volkonsky, 1931, *Naegleria* Alexeieff, 1912 and *Vahlkampfia* Chatton et Lalung–Bonnaire, 1912 from farmed *O. aureus* and *O. niloticus* from the USA[7] and *Vernamoeba* from the intestines of farmed *O. niloticus* from the Philippines[8] and *O. niloticus* from Brazil.[9] Amoebae are single-celled organisms that can alter their overall shape, usually through the extension and contraction of pseudopodia. They are identified using a combination of morphology, transmission electron microscopy, histology of host tissues and culture methods.[6,7] Descriptions should also include molecular data following Milanez et al.[8] to confirm identity. There are no records of amoebae infecting wild tilapia, but this may reflect a lack of studies.

2.1.2 | Pathogenicity

Although Dyková et al.[6] noted granulomas in the pancreas of *O. niloticus* experimentally infected with *Vermamoeba* (syn. *Hartmannella*)

vermiformis (Page, 1967), no correlation was found between the presence of amoebae and lesions in farmed fish in the Czech Republic. A presumptive *Acanthamoeba* sp. was isolated from the intestine, gills and peritoneal fluid of a kill of invasive *O. aureus* in the USA[7] in which the intestinal mucosa, associated with the amoeba infection, was severely eroded but with limited inflammatory response.

2.1.3 | Global translocations

Infections reported in farmed tilapia appear to be of free-living amoebae normally found in the areas where tilapia were farmed. It is unlikely that they were translocated but it does not preclude the possibility that cryptic infections could be translocated to new areas with infected fish.

2.1.4 | Research

Reports of amoeba infections in tilapia are sporadic and due to either specific studies on amoeba or findings from mortality investigations. Screening fish for infections, confirming species identity, conducting host susceptibility trials and assessing pathogenicity in new hosts could clarify the role of amoebae in disease of farmed tilapia.

2.2 | Euglenozoa Cavalier–Smith 1981 (Excavata: Euglenozoa)

2.2.1 | Taxonomic identity

Euglenozoa are a group of flagellates, mostly with two flagella. Four genera of the group Kinetoplastea: *Cryptobia* Leidy, 1846, *Ichthyobodo* Pinto, 1928, *Trypanoplasma* Laveran et Mesnil, 1901 and *Trypanosoma* Gruby, 1843 and one genus in the class Euglenida: *Phacus* Dujardin, 1841, are reported in farmed and wild tilapia. Euglenozoa was reviewed by Kostygov et al.,[10] including data on phylogeny, life-cycles and identification methods. De Jesus et al.[11] provide further methods for the description of trypanosome infections of tilapia which include morphometric body measurements, DNA sequencing, blood smears and histology to localise and characterise infections. Kinetoplastids are characterised by one or more flagella arising from the body and a kinetoplast within the cytoplasm. A flagellated *Phacus* sp. from the rectum of *O. mossambicus* in India has green pigment in the cytoplasm.[12] *Cryptobia* spp. are recorded from farmed *O. niloticus* from the Philippines, Kenya and Indonesia and *O. niloticus* × *O. aureus* from Israel.[13–21] *Ichthyobodo necator* (Henneguy, 1883; syn. *Costia necatrix*) and *Ichthyobodo* sp. (Figure 1b) are found on a wide range of fish hosts, including farmed *O. niloticus* from Saudi Arabia,[22] Uganda,[17,19,23,24] Kenya,[17,19,20] Costa Rica[25] and Nigeria,[26] *O. niloticus* × *O. aureus* from Israel,[14] *Sarotherodon* sp. from Mexico[27] and *C. zillii* from Iraq.[28] Kinetoplastids are usually found on the gills and occasionally the skin and in the blood. A *Trypanoplasma* sp. is reported from *O. aureus* in Puerto Rico.[29] Three *Trypanosoma* species are recorded from *Oreochromis* spp. *T. mukasai* Hoare, 1932 (syn. *T. choudhuryi*; Figure 1c) occurs in farmed *O. mossambicus* in India[12,30] but has also been reported in a range of wild tilapia in Africa.[31–34] *Oreochromis niloticus* is also infected by *T. tilapiae*[35] and an undescribed *Trypanosoma* sp. in Brazil, Egypt and Sudan.[11,36,37] *Trypanosoma* sp. is also reported from wild tilapia including *Trypanosoma* sp. in *O. andersonii* from Botswana[38] and from Namibia,[39] in *C. rendalii*, *O. macrochir* and *T. sparrmanii* from Namibia,[39] *C. zillii* from Egypt,[40] *O. niloticus* from Kenya,[41] *T. cyanophilum* Mohammed, 1978 and *T. mansouri* Mohammed, 1978 in *C. zillii* from Egypt.[40]

2.2.2 | Pathogenicity

De Jesus et al.[11] noted mortalities in farmed *O. niloticus* infected with trypanosomes in Brazil. Infected fish darkened and had epidermal haemorrhages. Histologically, gills were oedematous with inflammatory infiltration and lamellar fusion while necrosis and infiltration were also noted in the liver, spleen and kidney.

2.2.3 | Global translocations

Ichthyobodo and *Cryptobia* spp. are widespread, but it is difficult to determine if these parasites have been translocated with tilapia or if their range is broad. *Trypanosoma* spp. typically have narrow host specificity and require a leech intermediate host for transmission. Given the relatively wide geographical range of some *Trypanosoma* spp. it is possible, however, that they have been translocated with their fish hosts.

2.2.4 | Research

The identifications of kinetoplastids in tilapia should be confirmed to determine the extent of translocations and the host specificity of those reported. Given the potential pathogenicity of the group, further studies should be directed towards development of suitable mitigation measures such as identifying effective treatments and life cycle intervention strategies.

2.3 | Metamonada Grassé, 1952 (Excavata: Metamonada)

2.3.1 | Taxonomic identity

Metamonads including diplomonads are flagellated protists with anaerobic metabolism. The diplomonads are flagellated protists normally composed of two symmetrical cells with two nuclei and four flagella and include recognised pathogens of fish. An unidentified species of *Spironucleus* Lavier, 1936 infecting farmed red tilapia (*O. mossambicus* × *O. aureus*) in Thailand was described by Supamattaya et al.[42] using a combination of light and electron microscopy. Another pathogenic *Spironucleus* sp. was reported by El-Khatib and El-Hady[43] in the intestine

of cultured *O. niloticus* from Egypt and was described using morphology and experimental trials, and cultured using Eagle's Minimum Essential Medium supplemented with 10% bovine serum (MEM 10% BS) culture media. An unidentified species of *Hexamita* Dujardin, 1838 was identified in *O. niloticus*, *O. niloticus* × *O. aureus* and *S. galilaeus* in Israel and Africa by light microscopy.[14,44] Use of transmission electron microscopy and molecular methods is likely to identify these parasites as *Spironucleus*.[45,46] A diplomonad of concern for human health, *Giardia intestinalis* Kulda et Nohýnková, 1995, is a zoonotic parasite found in a range of animals. Ghoneim et al.[47] identified the human strain of *G. intestinalis* in the faeces of farmed *O. niloticus* in Egypt using a strain-specific polymerase chain reaction (PCR) assay. The fish host was considered to contribute to contamination of water and may play a role in the epidemiology of giardiasis.

2.3.2 | Pathogenicity

No pathology was reported for the infections with *Hexamita* sp. and *G. intestinalis*. *Oreochromis mossambicus* × *O. aureus* infected with *Spironucleus* sp. were emaciated and presented with white nodules in the skin. Infected fish were attacked by healthy individuals in the same ponds and died from the resultant wounds.[42] *Spironucleus* sp. infections cause leukocyte infiltration, necrosis of infected tissues and muscle degeneration. *Oreochromis niloticus* infected with *Spironucleus* were dark, with excessive epithelial mucus production, had enteritis, skin lesions along the lateral lines and focal lesions on the surface of the liver.[43]

2.3.3 | Global translocations

Spironucleus spp. are rare in tilapia; it is unclear if these have not been observed due to a lack of appropriate sampling or if they are geographically restricted.

2.3.4 | Research

To understand disease risk, there is a need to confirm the identity of *Spironucleus* and *Hexamita* in tilapia and studies on the role of fish in the epidemiology of giardiasis are likely to inform human health risks.

2.4 | Apicomplexa Levine 1980 (SAR: Alveolata: Apicomplexa)

2.4.1 | Taxonomic identity

Apicomplexans are parasitic alveolates which mostly possess an apicoplast and an apical complex. They are transmitted directly or through an intermediate host and are found in a wide range of terrestrial and aquatic animal hosts, including farmed and wild tilapia. The typical morphology of a coccidian is shown in Figure 1d, which shows two sporozoites within each of the sporocysts which are contained within the oocysts; the number of sporozoites within each sporocyst and the number of sporocysts within each mature oocyst is used to determine the genus within the group. In addition to morphology, molecular methods are used extensively to confirm identity. Most infections in tilapia are of *Goussia cichlidarum* Landsberg et Paperna, 1985 in the swimbladders of *C. zillii* and *O. aureus* from Egypt[48] and Israel,[49] of *O. aureus* × *O. niloticus* and *S. galilaeus* from Israel[49] and of *O. niloticus* from Egypt[48] and Kenya.[17] *Goussia* (syn. *Eimeria*) *vanasi* (Landsberg et Paperna, 1987; Figure 1d) has been reported from the intestine of farmed *O. aureus* × *O. niloticus* and *S. galilaeus* from Israel,[50–53] and of *O. mossambicus* from South Africa,[50] and wild *T. sparrmanii* from South Africa.[50,54] Undescribed coccidian infections are reported in farmed *O. niloticus* from the Philippines,[55] Iraq[56] and Kenya.[19] A coccidian infection in the intestine of farmed *O. niloticus* reared in Brazil is shown in Figure 1e,f. *Cryptosporidium* spp. have been reported from the intestine and stomach of farmed *C. zillii* from Iraq,[56] of *O. niloticus* from Papua New Guinea[57] and Egypt,[58] and of *O. aureus* and *O. aureus* × *O. niloticus* from Israel.[59] Although Paperna and Vilenkin[60] proposed the name *Piscicryptosporidium* for species occurring in fish, this has not been widely accepted.[61,62] The intraerythrocytic *Babesiosoma* (syn. *Dactylosoma*) *mariae* (Hoare, 1930) occurs in numerous tilapias including *Oreochromis* spp. in Uganda,[31,63] Namibia[39] and Botswana.[64] It is not reported in farmed fish, but it may have been overlooked because of its cryptic habitat. The intraerythrocytic, haemogregarine in farmed *O. niloticus* reported by El-Asely et al.[65] may be conspecific with *B. mariae*.

2.4.2 | Pathogenicity

Goussia cichlidarum occurs in the swim-bladder of its hosts where it causes lesions in the thick tissue lining and hypertrophy of the cells surrounding the gas gland. Sloughing, necrosis and degeneration of the swimbladder were associated with developing stages of the parasite.[48] Intestinal infections with *G. vanasi* cause emaciation, growth retardation and occasionally mortality of juvenile *Oreochromis* spp.[50]

2.4.3 | Global translocations

Apicomplexan infections are restricted largely to the African subcontinent and there is limited evidence of translocation. It is unclear if the records of coccidians in the Philippines, Papua New Guinea and Vietnam represent translocations because the organisms associated with these records were not identified to species.[55,57,66,67]

2.4.4 | Research

Wild fish have been surveyed for apicomplexans,[64,68] but research on coccidians of farmed tilapia is limited. Determining the global distribution of these parasites and confirming the taxonomy of the group would inform better surveillance and understanding of their

pathogenesis. Understanding life-cycles and identifying methods of control would decrease farm losses and improve management efficiency.

2.5 | Dinoflagellata Bütschli, 1885 (SAR: Alveolata: Dinoflagellata)

2.5.1 | Taxonomic identity

Dinoflagellates are unicellular algae with two dissimilar flagella arising from the ventral side. Three dinoflagellate genera are reported in tilapia: *Amyloodinium* Brown et Hovasse, 1946, *Piscinoodinium* Lom, 1981 and *Pfiesteria* Steidinger et al., 1996. *Amyloodinium ocellatum* Brown et Hovasse, 1946 was noted on the gills, skin and fins of farmed and wild *O. aureus* and *O. mossambicus* in the USA.[69–72] *Amyloodinium ocellatum* is found globally in numerous hosts from saline environments.[73] *Piscinoodinium* sp. and *P. pillulare* (Schaperclaus, 1954) are reported from the skin, fins and gills of farmed *O. niloticus* from Brazil,[74–77] the Philippines[55] and Thailand[78] and *O. mossambicus* from India[79] and Puerto Rico.[29] *Pfiesteria shumwayae* Glasgow et Burkholder, 2001 is reported from *O. mossambicus* in the USA[80] and *P. piscicida* Steidinger et Burkholder, 1996 is reported from *O. aureus*, *O. mossambicus* and *O. niloticus* in laboratory aquaria in the USA.[81,82]

2.5.2 | Pathogenicity

Piscinoodinium spp. are pathogens of their fish hosts and are responsible for mortalities in *O. mossambicus* in India[79] and in *O. niloticus* in Brazil[74,76] and were associated with high mortality in young (<1-year-old and less than 13 cm in length) *O. mossambicus* in the hypersaline Salton Sea, California, USA.[71] Infected fish gasped for air at the water surface, leapt out of the water and lost their equilibrium before dying.

2.5.3 | Global translocations

The dinozoan infections reported in tilapia also occur in native species and it is therefore difficult to determine if tilapia are responsible for any translocations. Wilson et al.[83] considered, however, that *Piscinoodinium* sp. infections in invasive *O. mossambicus* in Australia were co-introduced with its host, and *Piscinoodinium* is also considered invasive in its new habitat.

2.5.4 | Research

Understanding the role of tilapia in the distribution of dinozoans globally would aid determining if they have caused or exacerbated infections in new areas and new hosts. Information on impacts on native hosts, including susceptibility are lacking and should be addressed. Development of improved control methods would improve farm productivity.

2.6 | Ciliophora Doflein, 1901 (SAR: Alveolata: Ciliophora)

2.6.1 | Taxonomic identity

Ciliates are protozoans characterised by small hair-like organelles (cilia). Ciliates from nine orders—Chlamydodontida Deroux, 1970, Endogenida Collin, 1912, Mobilida Kahl, 1933, Ophryoglenida Canella, 1964, Pleurostomatida Schewiakoff, 1896, Prorodontida Corliss, 1974, Sessilida Stein, 1933, Tetrahymenida Fauré-Fremiet in Corliss, 1956 and Vestibuliferida de Puytorac et al., 1974 are reported from farmed and native and invasive tilapia across their geographical range. Most records are from Mobilida and Sessilida, reflecting the pathogenic importance of these two orders. Identifications are based on morphology including the unifying presence of cilia, although molecular techniques allow the elucidation of cryptic species and confirm the identity of species. Although most identifications are correct, caution should be exercised in inferring translocations of ciliates with tilapia due to uncertainty over some of the identifications made in the literature.

Members of the genus *Chilodonella* Strand, 1926 (Chlamydodontida), including *C. hexasticha* (Kiernik, 1909), *C. piscicola* (Zacharias, 1894) (syn. *C. cyprini*) and *Chilodonella* sp. are recorded on the skin and gills of *C. rendalli* from South Africa[84,85] and Turkey,[86] *C. zillii* and *O. aureus* from Israel[85] and from Turkey,[86] *Oreochromis* sp. and *O. mossambicus* from Vietnam,[67] *O. mossambicus* from South Africa,[84] *O. niloticus* from Bangladesh,[87] Brazil,[88] Costa Rica,[25] Egypt,[89] Indonesia,[21] Kenya,[20] Mexico,[90] Saudi Arabia[91] and Turkey,[86] and *S. galilaeus* from Turkey.[86] These parasites have been identified using morphology rather than molecular methods, which are considered necessary for correct identification.[92,93]

Using histology, Afifi et al.[22] identified *Capriniana* (syn. *Trichophrya*) sp. (Endogenida) in *O. niloticus* reared in saline water in Saudi Arabia. This is the only record of this genus and order occurring in tilapia and because the identifications appear to be based on histology only, there is a need to confirm this identification. Similarly, the solitary reports of *Tetrahymena corlissi* Thompson, 1955 (Tetrahymenida) from the gills of *O. niloticus* in Indonesia[21] and of *Tetrahymena* sp. in *O. niloticus* from Nigeria requires confirmation because this ciliate is typically systemic, occurs rarely on the gills[94–96] and is probably a complex of cryptic species.[97] Experimental infections of *O. mossambicus* with *Cryptocaryon irritans* Brown, 1951 (Prorodontida) were used to demonstrate immunity in the host to the parasite.[98,99] Molecular methods were used to confirm the identity of the ciliate infection in *O. mossambicus* although histological methods were used to demonstrate the presence of *Cryptocaryon* sp. in farmed *O. niloticus* from Saudi Arabia.[22] The ubiquitous white spot parasite *Ichthyophthirius multifiliis* Fouquet, 1876 (Ophryoglenida) is recorded from the skin, fins and gills (Figure 2a) of

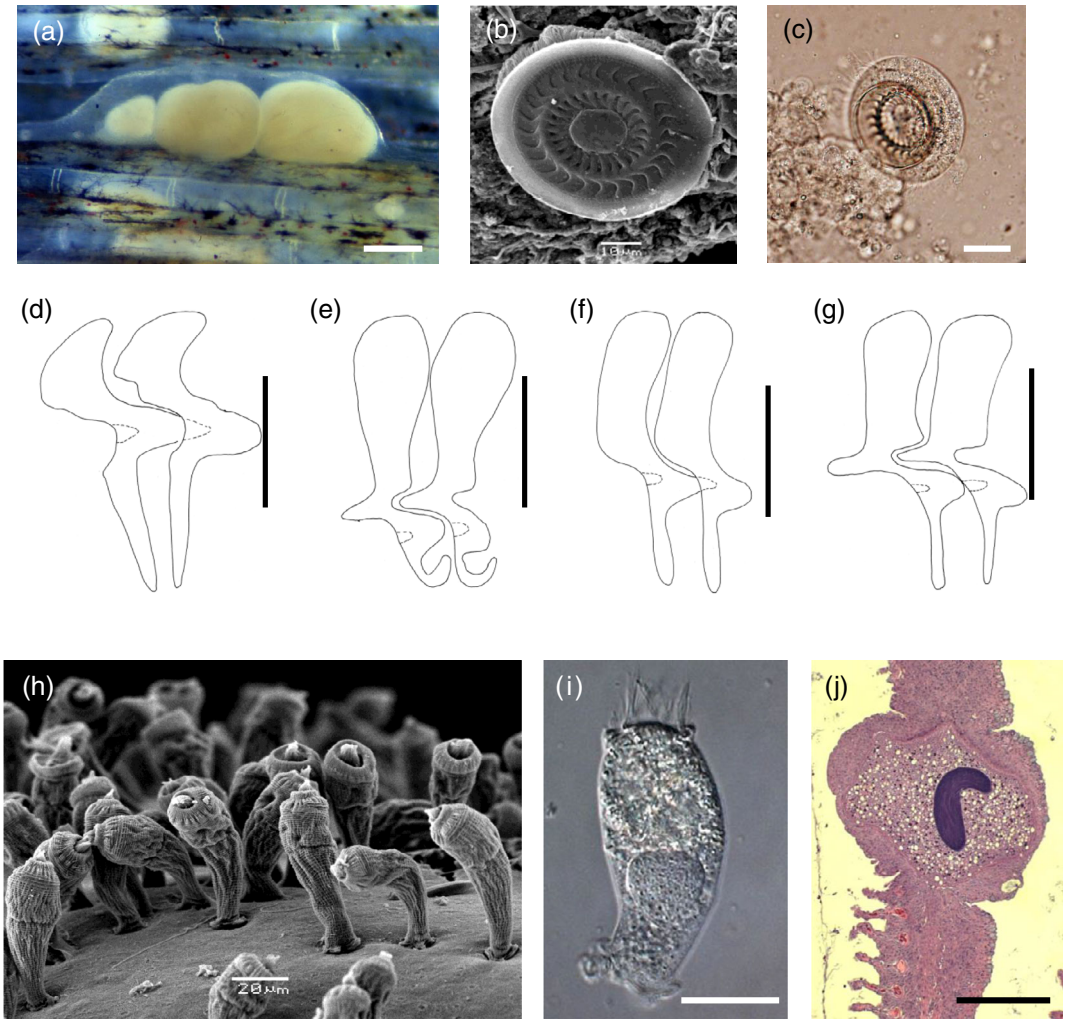

FIGURE 2 Ciliophora. (a) Photomicrograph of *Ichthyophthirius multifiliis* Fouquet, 1876 trophonts in the fin epithelium and (b) scanning electron microscopy image of the aboral surface of an unnamed *Trichodina* sp. collected from farmed *O. niloticus* from Veracruz, Mexico. Note the denticles in a radial pattern. (c) Photomicrograph of an unnamed *Trichodina* sp. from a *O. niloticus* fingerling and (d) line drawing of the denticles of a representative *Trichodina* sp. (e) Line drawing of the denticles of a representative *Trichodinella* sp. (f) Line drawing of the denticles of a representative *Paratrichodina* sp. (g) Line drawing of the denticles of a representative *Tripartiella* sp. (h) Scanning electron microscope image of a group of peritrichous ciliates on the epithelium of its host. (i) Photomicrograph of a solitary *Apiosoma* sp. (j) Histological section of a gill infected with an *Ichthyophthirius multifiliis* trophont. Images (a, i, j) Andrew Shinn, (b) courtesy of Greta Hanako Rosas Saito, (c) courtesy of Dong Ha Thanh, (d–g) after Basson and Van As (1989), (h) courtesy of Giuseppe Paladini. Scale bars: a, j = 300 μm; c, h, i = 20 μm; b, d–g = 10 μm

cultured *C. zillii* from the USA,[100] *Oreochromis* sp. from Vietnam,[67] *O. aureus* from the USA[100,101] and Mexico,[102,103] *O. mossambicus* from Puerto Rico,[29] from South Africa,[84] from the USA,[100] from Vietnam[67] and the Philippines,[16] *O. mossambicus* × *O. urolepis* from the USA,[100] *O. niloticus* from Brazil,[74,77,104–106] Egypt,[107–109] Greece,[110] Indonesia,[21] Nigeria,[26] the Philippines,[16,55] the USA[111] and Vietnam,[66,67] *O. niloticus* × *O. aureus* from Israel,[14] and *O. niloticus* × *O. mossambicus* from Thailand (Table S2).[112] *Ichthyophthirius multifiliis* is considered native in most freshwater systems worldwide and it is possible but unlikely that tilapias are responsible for translocating or exacerbating infections on wild, native fish.

Mobilida (Figure 2b,c) contains the genera *Trichodina* Ehrenberg, 1830 (Figure 2d), *Trichodinella* Srámek-Husek, 1953 (Figure 2e), *Paratrichodina* Lom, 1963 (Figure 2f) and *Tripartiella* (Lom, 1959) (Figure 2g), representatives of which are parasitic and recorded from farmed tilapia. The bulk of these infections occur on the skin, fins, or gills of their hosts. A checklist of trichodinids on tilapia species is provided by Islas-Ortega et al.[113] and Basson and Van As[114]; Van As and Basson[115] provided diagnostic keys to the genera of Mobilida. *Paratrichodina africana* Kazubski et El-Tantawy, 1986, simultaneously described from *O. niloticus* in Egypt and an unidentified tilapia in Africa has been translocated on *O. niloticus* and its hybrids to Brazil,[103,105,116–118] Mexico,[103] China,[119] Egypt[120] and Argentina.[113] It is possible that the record of *P. incissa* (Lom, 1959), described from European minnows from the skin of *O. niloticus* in Vietnam and included in the country summary[67] is a misidentification of *P. africana*. At least 20 *Trichodina* species have been described from tilapia, mostly from *O. niloticus*, with some from *O. mossambicus* (Table S2). *Trichodina* spp. are reported from most areas where tilapias are farmed and on native and invasive wild fish. The taxonomy of the genus is

relatively stable although some important species have been synonymised including *T. hypsilepis* (syn. *heterodentata*) Wellborn, 1967, and the record of *T. pediculus* Ehrenberg, 1831 recorded by Basson et al. (1983) was subsequently redescribed as *T. magna* Van As et Basson, 1989.[114,121] *Trichodinella epizootica* (Raabe, 1950) and an undescribed *Trichodinella* are recorded from the gills of farmed *O. niloticus* from Mexico,[113] Egypt,[120] Kenya,[17] Brazil[118] and Uganda,[17] and from *O. mossambicus* and *C. zillii* from the Philippines.[16] At least six species of *Tripartiella* are reported from *O. mossambicus* from Taiwan Province of China,[122] *O. niloticus* and hybrids from Vietnam,[67] the Philippines,[16,123] Brazil,[118,124] Mexico[103] and China[119] and *C. zillii* from the Philippines.[16,125]

At least six genera of Sessilida (Figure 2h) are recorded in tilapia, including *Ambiphrya* Raabe, 1952, *Apiosoma* Blanchard, 1885 (syn. *Scopulata* in part), *Epistylis* Ehrenberg, 1830, *Heteropolaria* Foissner et Schubert, 1977, *Riboscyphidia* Yankovskij, 1980 (syn. *Scyphidia*) and *Vorticella* (Li, 1767). *Ambiphyra ameiuri* Thompson, Kirkegarrd et Jahn, 1974 has been reported from the gills, skin and fins of *O. mossambicus* from Puerto Rico[29] and *O. niloticus* from Saudi Arabia[126]; unidentified *Ambiphrya* spp. have been noted in *O. niloticus* farmed in Indonesia,[21] Mexico,[90] Peru[127] and the Philippines.[55] At least seven *Apiosoma* spp. (Figure 2i) are described from tilapia, along with numerous records of unidentified species. *Scopulata* Viljoen et Van As, 1985 is considered a junior synonym of *Apiosoma*. *Apiosoma constricta* (Viljoen et Van As, 1985), *A. dermatum* (Viljoen et Van As, 1985) and *A. epibranchialis* (Viljoen et Van As, 1985) were described from the skin of farmed *O. mossambicus* and *C. rendalli* from South Africa,[128] *Apiosoma* sp. are reported from *O. mossambicus* from South Africa,[84] from *O. niloticus* from Costa Rica,[25] Indonesia,[21] the Philippines[13,15,16] and Israel,[14] *A. minutum* Chen, 1961 was reported from *O. niloticus* and *Oreochromis* sp. from Vietnam,[67] *A. phiala* Viljoen et Van As, 1985 was reported from *O. mossambicus* from South Africa,[128] *A. piscicola* (Blanchard, 1885) was reported from *O. aureus* and *O. mossambicus* from Puerto Rico,[29] and from *O. mossambicus* from South Africa[128] and Vietnam[67] and *A. viridis* Viljoen et Van As, 1985 was reported from *O. mossambicus* from South Africa.[128] *Epistylis colisarum* (Foissner et Schubert, 1977) was reported on the skin of *C. rendalii*, *O. aureus*, *O. mossambicus*, *O. mossambicus* × *O. urolepis* and *O. niloticus* farmed in Puerto Rico[29] and undescribed *Epistylis* spp. are recorded on *O. niloticus* from the Philippines,[16] Brazil,[74,75,77,88] Egypt[89] and Thailand[78] and on *O. mossambicus* from the Philippines[16] and South Africa.[84] The reports of *Heteropolaria* sp. from farmed *O. niloticus* from Costa Rica[25] and *Riboscyphidia* from *O. mossambicus* in South Africa[84] need confirmation due to their rarity and the potential confusion with other genera. An undescribed *Vorticella* sp. on *O. niloticus* have been recorded from Mexico,[13,129] the Philippines[15] and Saudi Arabia[126] and unidentified peritrichous ciliates have been noted on *O. niloticus* from Kenya[18] and Uganda.[17]

2.6.2 | Pathogenicity

Despite ciliates being known pathogens, there are few reports of mortality or pathology associated with these parasites on farmed tilapia. *Coptodon* spp. and *O. aureus* infected with *Chilodonella hexasticha* displayed emaciation, lethargy and some skin abrasions and the gills had extensive degeneration, necrosis and hyperplastic epithelia[85]; similar responses were noted in *O. niloticus* infected with *I. multifiliis* (Figure 2j).[109] Inflammatory responses, increased lymphocyte counts and reduced neutrophil counts were noted in *O. niloticus* infected with *Epistylis* sp.[130] Heavy infections with trichodinids may lead to lesions and sloughing and erosion of the epidermis.[127,131,132]

2.6.3 | Global translocations

Their direct life-cycles mean that ciliates are readily translocated with their hosts; discrepancies in parasite identifications can, however, complicate understanding translocations. *Ambiphrya* spp. and *Apiosoma* spp. of tilapia are recorded from several countries as noted above but, due to lack of specific identification, translocations cannot be confirmed. Evidence for the translocation of trichodinid infections is clearer. *Paratrichodina africana*, originally described from Israel and Africa, has been translocated to Bangladesh, Argentina, Brazil, Mexico and China.[105,113,116,118–120] *Trichodina acuta* Lom, 1961, *T. centrostrigeata* Basson, Van As et Paperna, 1983, *T. hypsilepis* Wellborn, 1967, *T. siluri* Lom, 1970 and *T. velasquezae* Bondad-Reantaso et Arthur, 1989 and *Tripartiella clavodonta* Basson et Van As, 1987 and *T. tilapiae* (Duncan, 1977) occur in several countries and are considered to have been introduced to the Philippines with fish from Thailand and Israel.[123] Trichodinid ciliates are likely to have been introduced broadly through fish translocations.

2.6.4 | Research

Species identities need to be confirmed using modern methods to understand the role that these hosts have had in translocating pathogens worldwide. Methods to treat infections and to render hosts safe for translocation need to be identified to minimise their impact and further spread.

2.7 | Myxozoa Grassé 1970 (Obazoa: Opisthokonta: Metazoa: Cnidaria: Myxozoa)

2.7.1 | Taxonomic identity

The myxozoans are obligately parasitic cnidarians comprising one or a few cells that have a spore comprising valve cells in the life-cycle. Myxozoans are found in marine and freshwater fish in almost all organs and show variable host and organ specificity. Life-cycles typically involve alternating vertebrate and invertebrate hosts. Often the invertebrate is an annelid or bryozoan but few life-cycles are documented. In rare cases, direct transmission is demonstrated. Myxozoans are multicellular, spore-forming obligate parasites possessing polar capsules containing extrudable polar filaments akin to cnidarian nematocysts. Identification is based on a combination of morphology (including number and arrangement of spore valves and polar

capsules), size, and use of molecular tools. Methods for identification include the use of light and electron microscopy, smears and tissue squashes as well as histology for understanding tissue tropism and pathogenicity. Seven genera of myxozoans are reported from farmed tilapia; *Enteromyxum* Palenzuela, Redondo et Alvarez-Pellitero, 2002 has been transmitted experimentally[133] and two genera (*Ortholinea* Shulman, 1962 and *Triangula* Chen et Hsieh, 1984) are reported in wild tilapia. Sporadic reports of myxozoans in farmed *O. niloticus* include an undescribed intestinal *Ceratomyxa* sp. from Indonesia,[134] *Sinuolinea niloticus* Rodrigues, Francisco, Biondi et Araújo Júnior, 2016 (Figure 3a) from Brazil,[135,136] *Sphaerospora melenensis* Fomena, Marques et Boiux, 1993 (Figure 3b) and *S. tilapiae* Fomena, Marques et Boiux, 1993 (Figure 3c) from Cameroon,[137,138] and an undescribed *Sphaerospora* sp. from Kenya and Uganda.[17,19] *Oreochromis mossambicus* from China have intestinal infections of *Thelohanellus talipiae* Chen et Ma, 1998 (Figure 3d) and *Zschokkella tilapiae* Chen et Hsieh, 1984 (Figure 3e).[139] *Zschokkella nilei* Abdel-Ghaffar, El-Tokhy, Al-Quraishy, Al-Rasheid, Abdel-Baki, Hegazy et Bashtar, 2008 (Figure 3f), *Ortholinea africanus* Abdel-Ghaffar, El-Tokhy, Al-Quraishy, Al-Rasheid, Abdel-Baki, Hegazy et Bashtar, 2008 (Figure 3g), *Thelohanellus valeti* Fomena et Bouix, 1987 and *Triangula egyptica* Abdel-Ghaffar, El-Tokhy, Al-Quraishy, Al-Rasheid, Abdel-Baki, Hegazy et Bashtar, 2008 (Figure 3h) are described from wild *O. niloticus* in Egypt.[44,140–142] Undescribed *Henneguya* spp. (Figure 3i) were noted in the gills of farmed *O. niloticus* from Brazil[104] and Saudi Arabia[22]; it is not clear if they are conspecific.

The most speciose myxozoan genus is *Myxobolus* Bütschli, 1882 with over 40 species reported or described from *Coptodon*, *Oreochromis* and *Sarotherodon* spp. Some reports are considered dubious and need re-evaluating including those reported in *O. niloticus*, such as *M. ellipsoides* Thelohan, 1892, which was originally reported from tench, *Tinca tinca* (Linnaeus, 1758), in Europe[143] but was also recorded from Egypt and Cameroon.[89,144,145] The record of *M. exiguus* Thelohan, 1895, which was originally reported from mugilids in Europe[146] but noted from Vietnam,[66,67] *M. dermatobius* Ishii, 1915, originally reported in eels from Japan but noted in Egypt,[147] *Myxobolus cyprini* Doflein, 1898 originally reported on European carp species but reported in *C. zillii* and *O. niloticus* from Nigeria in an undated report by Bello-Olusoji et al., and *M. pseudodispar* Gorbunova, 1936, originally reported in cyprinids in Europe[148] but noted in Cameroon[144,145,149] also require re-evaluation. *Myxobolus* spp. are reported from tilapia cultured in Cameroon,[137,138,150,151] Israel,[152,153] Nigeria,[154–156] Senegal,[138] Benin,[157,158] Egypt,[36,65,120,159–166] Vietnam,[66,67] Ghana,[167] Kenya,[17,18] Uganda[17,23,24] and Burkina Faso[168] (Figure 3j–o). *Myxobolus* spp. occur in a range of organs with some species showing organ specificity, with the bulk of these infections being noted in *O. niloticus*.

2.7.2 | Pathogenicity

Myxozoans are recognised pathogens of fish, and several species are responsible for mortalities in farmed and wild fish. Ovaries of tilapia infected with *M. dahomeyensis* (Siau, 1971) contain a suppurating thick liquid that replaced mature oocytes and infection was considered to sterilise the host.[156,157,169] *Oreochromis niloticus* with ocular infections of *M. sarigi* (Landsberg, 1985) showed exophthalmos.[156] The gills of *O. niloticus* infected with myxozoans typically display hyperplasia or hypertrophy.[65,107,153,161] In *O. niloticus*, inflammation, degeneration and necrosis of the kidney and the spleen have been noted in *Myxobolus* spp. infections,[156,159,160,162,164] in *Sphaerospora* sp. infections of *O. niloticus* from Kenya, Uganda and Ethiopia[17] and in *Sinuolinea niloticus* infections of *O. niloticus* from Brazil.[135]

2.7.3 | Global translocations

The obligate requirement for a specific alternate host limits the likelihood that myxozoans will establish in new geographical areas. *Myxobolus agolus* Landsberg, 1985, *M. brachysporus* (Baker, 1963), *M. camerounensis* Fomena, Marques et Boiux, 1993, *M. equatoralis* (Landsberg, 1985), *M. heterosporus* (Baker, 1963; Figure 3m), *M. homeosporus* (Baker, 1963; Figure 3o), *M. israelensis* Landsberg, 1985, *M. kainjiae* (Obiekezie et Okaeme, 1990), *M. sarigi* (Landsberg, 1985; Figure 3n), *M. tilapiae* Abolarin, 1974 and *M. zillii* Sakiti, Blanc, Marques, Boiux, 1991 are widespread across Africa and Israel, but have not been translocated, probably due to the absence of a suitable intermediate host in new localities. The reports of non-tilapia myxozoans such as *M. exiguus*, *M. pseudodispar*, *M. dermatobius* and *M. ellipsoides* likely represent misidentifications rather than evidence of parasite spillback or infections in other hosts.

2.7.4 | Research

Determining the distribution of myxozoans in tilapia across their range using a combination of molecular and morphological methods and including an assessment of pathogenicity would benefit aquaculture industries by informing responses to detection. Host specificity in the intermediate host has been little studied and would be key to estimating risk of establishment in new geographical areas. Although there are limited apparently pathogenic species in tilapia, efforts should be made to identify mitigation strategies to minimise impacts including development of pharmaceutical treatments, vaccines, environmental manipulation approaches and use of functional feeds.

2.8 | Oomycetes Winter, 1897 (Now Peronosporomycetes Dick, 2001) and Ascomycota Cavalier–Smith, 1998 (SAR: Stramenopiles: Peronosporomycetes and Obazoa: Opisthokonta: Nucletmycea: Ascomycota)

2.8.1 | Taxonomic identity

Oomycetes, commonly known as water moulds, are filamentous heterotrophic microorganisms that reproduce sexually and asexually. Oomycetes are more closely related to chromophyte algae (e.g. brown algae,

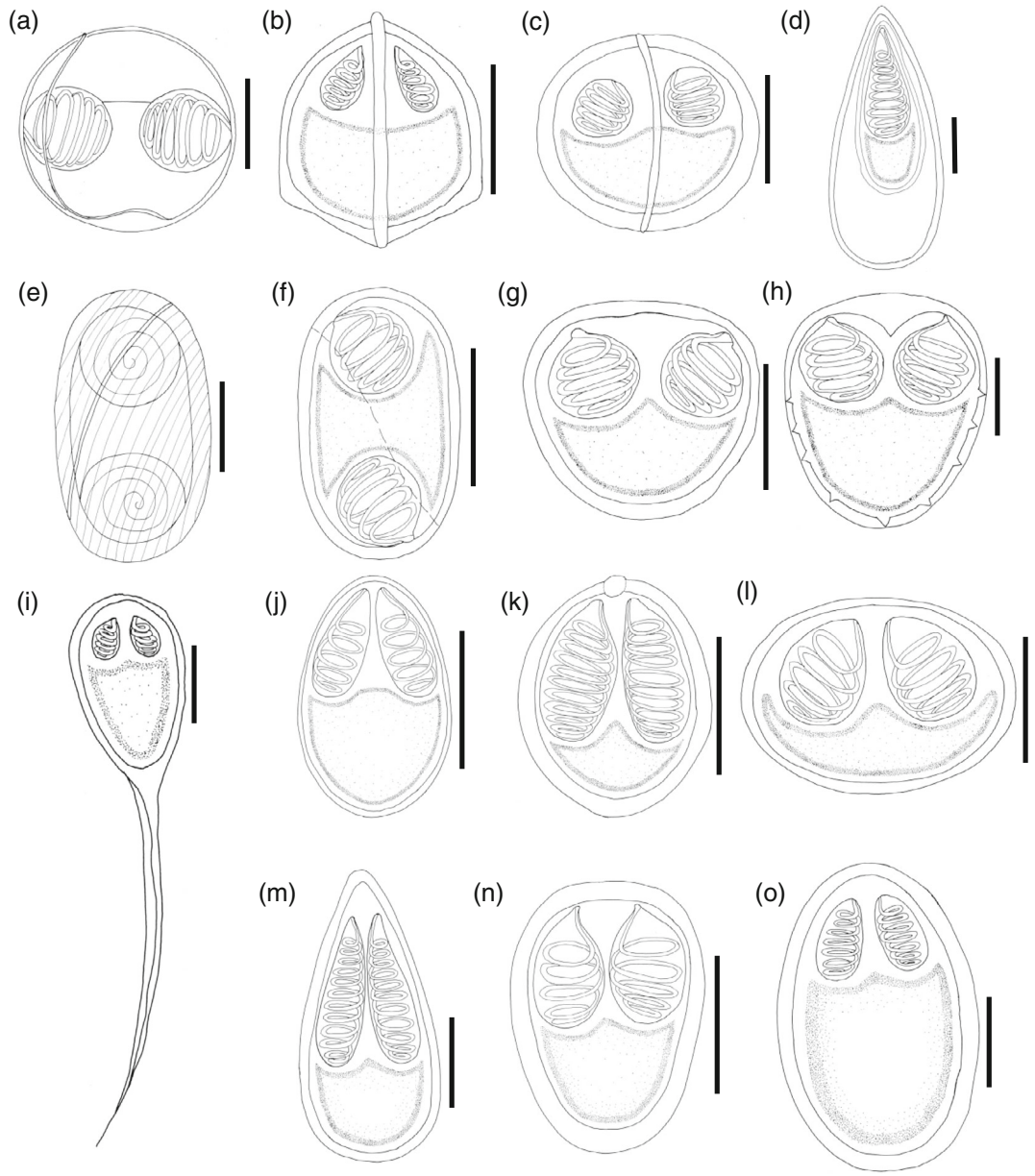

FIGURE 3 Myxozoa. Line drawings of various myxozoan spores reported in tilapia. (a) *Sinuolinea niloticus*, (b) *Sphaerospora melensis*, (c) *Sphaerospora tilapiae*, (d) *Thelohanellus talipiae*, (e) *Zschokkella tilapiae*, (f) *Z. nilei*, (g) *Ortholinea africanus*, (h) *Triangula egyptica*, (i) *Henneguya sarotherodoni*, (j) *Myxobolus bejeranoi*, (k) *M. agolus*, (l) *M. brachysporus*, (m) *M. heterosporus*, (n) *M. sarigi* and (o) *M. homeosporus*. Image (a) after Rodrigues et al. (2016), images (b, c, i, l, m, o) after Fall et al. (2000), images (k, n) after Landsberg (1985), image (d) after Chen and Ma (1998), image (e) after Matsche et al. (2020), images (f-h) after Abdel-Ghaffar et al. (2008), image (j) after Lövy et al. (2018). Scale bar: 5 μm

xanthophytes, diatoms, chrysophytes) than to the kingdom Fungi, as indicated by their heterokont ciliary pattern.[170] Most of the animal-pathogenic oomycetes belong to the subclass Saprolegniomycetidae, consisting of the orders Saprolegniales Fisch, 1892 and Leptomitales Kanouse, 1927.[171] In the Saprolegniales, species of *Saprolegnia* Nees, 1823, *Achlya* Nees von Esenbeck, 1823, *Aphanomyces* de Bary, 1860 and *Branchiomyces* Plehn, 1912 are known to infect finfish.[172–177] This group of pathogens has low host specificity and therefore, can infect a diverse range of fish.[178,179] The oomycetes are ubiquitously distributed, form motile zoospores, and their cell walls are composed of cellulose and glycans rather than chitin.[180,181] Oomycete infections in tilapia are recorded from *C. rendalli*, *C. zillii*, *O. andersonii*, *O. macrochir*, *O. mossambicus*, *O. niloticus*, *O. shiranus*, *Tilapia ruweti* (Poll et Thys van den Audenaerde, 1965) and *T. sparrmanii* (Table S2). Diseases caused by oomycetes and ascomycetes are considered second only to bacterial diseases in economic impacts on aquaculture.[182,183] Among these, diseases caused by oomycetes are more common,[184] although diseases caused by Mesomycetozoea (Ichthyosporea) and true fungi are also important.[185]

2.8.2 | Diseases caused by Oomycetes

Oomycete infections reported from tilapia include *Achlya americana* Humphrey, 1892 from *T. zillii* in Nigeria[186]; *A. bisexualis* Coker, 1927 from *O. niloticus* in Thailand[187,188] and from *O. mossambicus* in India[174]; *A. diffusa* Harvey, 1942 from *T. zillii* in Nigeria[186] and from *O. niloticus* in Thailand[188]; *A. dubia* Coker, 1923 from *O. niloticus* in Thailand[188] and *T. zillii* in Nigeria[186]; *A. hypogyna* Coker et Pemberton, 1908 from *T. zillii* in Nigeria[186]; *A. klebsiana* Pieters, 1915 from *T. zillii* in Nigeria,[186] and from *O. niloticus* in Egypt[173,189] and Thailand[188]; *A. megasperma* Humphrey, 1893 from *T. zillii* in Nigeria[186]; *A. prolifera* Nees von Esenbeck, 1823 from *T. zillii* in Nigeria[186] and *O. niloticus* in Thailand[188]; *A. proliferoides* Coker, 1923 from *O. niloticus* in Egypt[189] and *O. mossambicus* in India[174]; and *A. racemosa* Hildebrand, 1867 from *T. zillii* in Nigeria.[186] It is important to mention that most *Achlya* infections have been reported from the skin and only a few cases regarding infection of the fins. In addition to infection with *Achlya* sp., there are reports of infection with *Allomyces arbuscular* Butler, 1911 from *T. zillii* in Nigeria[186]; *Dictyuchus monosporus* Leitgeb, 1870 from *O. niloticus* in Egypt[189]; *D. sterile* Coker, 1923 from *T. zillii* in Nigeria[186] and *O. niloticus* in Egypt[189]; and a species of *Pythiopsis* de Bary, 1888 from *O. mossambicus* in India.[174] Infections with *Saprolegnia* sp. have been reported from tilapia and these include: *S. aenigmatica* Sandoval-Sierra et Diéguez-Uribeondo, 2015 from an undescribed species of tilapia in Brazil[190]; *S. diclina* Humphrey, 1892 from *O. mossambicus* in India,[174] from *O. niloticus* in Egypt[173,189] and *T. zillii* in Nigeria[186]; *S. ferax* Kützing, 1843 from *O. niloticus* in Egypt[189,191] and from *T. zillii* in Nigeria[186]; *S. litoralis* Coker, 1923 from *T. zillii* in Nigeria[186]; and *S. parasitica* Coker, 1923 from *O. mossambicus* in India,[174] from *O. niloticus* in Egypt,[175,176,191] and from *T. zillii* in Nigeria.[186] In addition to these, there are also reports of infection by an undetermined species of *Saprolegnia* Nees, 1823 from *O. niloticus* in Egypt[189,192] and of *Thraustotheca clavata* Humphrey, 1892 from *T. zillii* in Nigeria.[186]

Infection with *Aphanomyces laevis* de Bary, 1860 has been reported from *O. mossambicus* in India,[174] from *O. niloticus* in Egypt[173,189] and *T. zillii* in Nigeria[186] whereas there is a report of infection with *A. stellatus* de Bary, 1860 from *T. zillii* in Nigeria.[186] As with the *Achlya* species, *A. laevis* infections are reported from the skin. Additionally, *A. invadans* David et Kirk, 1997, the causative agent of epizootic ulcerative syndrome (EUS) has been reported from *C. rendalli* in Namibia, Zimbabwe and Botswana; *O. andersonii* from Namibia, Zambia and Zimbabwe; *O. macrochir* from Namibia; *O. mossambicus* from Zimbabwe; *O. shiranus* from Malawi; and *T. sparmanii* from Namibia and Botswana.[177,193] The *A. invadans* infections are generally observed in the skin and the underlying musculature of the infected fish. Importantly, *O. niloticus* is resistant to infection with *A. invadans*.[194] Other reports include those of *Branchiomyces demigrans* Wundsch, 1929 and *B. sanguinis* Plehn, 1912 from the gills of *O. niloticus* from Egypt,[195,196] whereas infection by an undetermined species of *Branchiomyces* Plehn 1912 has been reported in the gills of *O. niloticus*, *O. mossambicus* and *O. aureus* from Europe, Asia, the Middle East, Australia and North America, and also in *O. niloticus* × *O. mossambicus* hybrids and *O. niloticus* × *O. aureus* hybrids from Israel (Table S2).[172,197]

Oomycetes are transmitted by zoospores released from zoosporangia that develop from the hyphae in fish tissues at the body surface. A lack of nutrients and/or a sudden drop in temperature induces sporulation.[205] Zoospores can encyst on a host, forming primary cysts and subsequently releasing secondary zoospores,[206] which are more motile than primary zoospores and crucial for infection.[207] Zoospores exhibit positive chemotactic responses to amino acids in exudates and metabolites from tissues of susceptible hosts.[208,209] Subsequent contact of the zoospore with the host triggers encystment, which in turn initiates germination and results in infection. During infection, oomycetes secrete effector proteins that modulate its host's immune responses or inhibit the host's cell functions to the advantage of the pathogen.[210–215] Species of the genera *Saprolegnia* and *Achlya* infect the gills, skin, fins and eggs of fish (Figure 4a,c,d).[187,206,216,217] The infection progresses to the development of large wounds on the body surface leading to impaired osmoregulation and haemodilution; extensive lesions in the gills cause respiratory failure; both can lead to mortality. In susceptible fish, *A. invadans* hyphae invade the fish skin and skeletal muscles causing ulceration, often resulting in death.[218–220] In resistant fish, such as *O. niloticus*, *A. invadans* hyphae are unable to proliferate and lesions are restricted to the site of infection (Figure 4b). Conversely, infections with species of *Branchiomyces* which primarily affect the gills (Figure 4e), result in respiratory distress with associated high mortalities,[221] particularly when infections occur in waters exceeding 20°C.[172]

Ascomycetes fungi produce non-motile spores with a chitinous cell wall, which can survive in unfavourable conditions, and the resistance of the spores is an important adaptation strategy to infect susceptible hosts.[242] These spores play a crucial role in dispersal between hosts and dissemination within hosts.[185] Infection with species of *Fusarium* Link, 1809 causes skin ulcers or can become systemic causing kidney and brain necrosis.[180,243] In *O. niloticus*, infection with *F. oxysporum* (Schlecht. emend. Snyder et Hansen, 1940) has been reported to be associated with subcutaneous mycoses.[244] *Candida albicans* Berkhout, 1923 has been reported to colonise the epithelial surface of fish, expanding and invading tissues. During the invasion, morphogenesis of the pathogen from ovoid yeast to a filamentous hypha is important in causing tissue damage and mortality.[245] *Aspergillus* Micheli, 1729 infection in the gills causes damage to gill lamellae with subsequent respiratory distress,[246] but systemic infections from feed contaminated with *Aspergillus* sp. primarily present with high mortality.[247] *Paecilomyces* sp. infections commence with ingestion of the fungal spores in the water by the fish.[248] *Purpureocillium lilacinum* (Thom, 1910) infection has been associated with tilapia wasting disease in wild and farmed tilapia in Puerto Rico.

2.8.3 | Global translocations

Oomycetes and ascomycote fungi are emerging pathogens with increasing geographic distribution.[222,223] These pathogens have a broad host range including nonfish hosts, and this could be responsible for their wide dissemination.[185] A major contributor to the global

spread of oomycetes and ascomycotes is international trade in live aquatic animals.[224,225] *Aphanomyces invadans* can be transported and introduced along with resistant exotic hosts such as *O. niloticus*, introducing *A. invadans* to new ecosystems.[171] This pathway likely played a major role in the spread of *A. invadans* in Africa.[226,227] The transport water containing infective spores is further regarded as a pathway for dispersal of this pathogen.[226] Birds have been speculated to play a role in spread of *A. invadans* infection in South Africa.[228] The movement of infected fish and/or encysted oomycetes through interconnected water bodies has increased the geographic range of the pathogen,[226] and boats and contaminated fishing equipment have mechanically spread of the oomycete spores to unaffected regions.[227,228] Once introduced to a new ecosystem, the low host specificity of oomycetes and fungi increases the likelihood that disease outbreaks will occur in native species that have not been recorded as hosts for these pathogens.[185,229]

2.8.4 | Research

Oomycete and ascomycote diseases are difficult to control. The use of malachite green, considered the most effective treatment until the 1980s, was proscribed in most countries because of its carcinogenicity and persistent residues. Formalin immersion treatments are considered effective but may also be proscribed by regulatory processes.[185] There is therefore an urgent need to enhance our understanding of the basic biology of these pathogens to develop alternative methods to control these diseases. It is unclear if oomycetes can infect only wounded or immunocompromised animals or if they can cause infection in healthy fish.[209] In addition, the survival of oomycetes outside the host and during periods between outbreaks is poorly understood. It is also unknown if fish that recover can act as reservoirs of infection, if oomycetes can survive in sun-dried or smoked fish, or if the trade of these fish products can spread infections.[226,230] Genomic studies have mainly focussed on plant–pathogenic oomycetes, and little is understood about oomycete pathogens of aquatic animals. Genomic and proteomic studies of *S. parasitica* and *A. invadans* provide insights into molecular pathogenesis, particularly virulence factors and host gene expression.[212,213,231,232] The identification of complementary genes and proteins involved in the immune response of fish would provide an understanding of how to prevent oomycete diseases through pathogen-informed programmes that breed for resistance. Elucidating the role of virulence genes and identifying pathogen proteins that manipulate host immune systems would aid development of novel control strategies including vaccines.[209,233] It is important to mention that surveillance is key for early detection and disease control. Therefore, surveillance of oomycete and ascomycote pathogens should include natural habitats and reservoirs of infection. Since these diseases are associated with declines in wild fish populations, therefore, it is important to understand their ecological impacts for improving conservation strategies.[179,233] It is, furthermore, important to identify environmental drivers of fungal and oomycete diseases for better understanding of the ecological risks of disease emergence.[234]

2.9 | Mesomycetozoea Mendoza et al., 2002 (Now Ichthyosporea Cavalier–Smith, 1998) (Obazoa: Opisthokonta: Holozoa: Ichthyosporea)

2.9.1 | Taxonomic identity

The Mesomycetozoea (or Ichthyosporea) are an enigmatic group of parasitic organisms that are phylogenetically grouped with the fungi.[4,198] Mesomycetozoans have spherical spores and occur in a range of tissues. Methods for identification, along with a host–parasite list, are included in Rowley et al.[199] The group includes recognised animal pathogens including *Rhinosporidium seeberi* (Wernicke, 1903), species of *Ichthyophonus* Plehn et Mulsow, 1911, *Sphaerothecum destruens* Arkush, Mendoza, Adkison et Hedrick, 2003 and *Dermocystidium* Pérez, 1908. *Dermocystidium* spp. are pathogens of fish and are typically identified based on a combination of culture, tissue tropism, host identity, morphology and molecular techniques.[199] An undescribed *Dermocystidium* sp., which may represent more than one species, is reported from a range of organs in *O. niloticus* and *O. aureus* × *O. niloticus* cultured in Brazil, Egypt and Israel.[200–203] *Dermocystidium aegyptiacus* El-Mansy, 2008 was described from the intestines of *O. niloticus* farmed in Egypt (Figure 1a).[204]

2.9.2 | Diseases caused by Mesomycetozoea (Ichthyosporea)

Mesomycetozoeans are parasitic opisthokonts with large spherical or ovoid spores. Infection with *Ichthyophonus hoferi* Plehn et Mulsow, 1911 is principally transmitted by plasmodia which are formed by the fragmentation of multinucleated schizonts, the most common stage of *Ichthyophonus* in live fish.[235] The pathogen mainly affects internal organs, namely liver, kidneys, spleen and heart (Figure 4f).[236] Infection causes enlargement and the formation of raised nodules in these organs.[221,237] The resulting tissue damage can cause high mortality.[238,239] In case of infection with *Dermocystidium* sp., the zoospores encyst and enlarge to form spherical multinucleate cells with distinct wall inside the host,[198] leading either to gross cutaneous cysts[240,241] or chronic systemic lesions.[204]

2.9.3 | Pathogenicity

Dermocystidium sp. infections of the gills cause hyperplasia and fusion of the gill lamellae, although they do not appear to cause mortality.[202] Mortalities of *O. aureus* × *O. niloticus* cultured in Israel were associated with a *Dermocystidium* sp. infection in the liver which manifested as focal granulomas that occasionally contained a necrotic core.[200] Lesions

FIGURE 4 Oomycete and fungal diseases of tilapias. (a) *Oreochromis niloticus* showing cotton-wool like growths on the body surface following an experimental infection with *Saprolegnia parasitica* Coker, 1923 (image courtesy of Shimaa Ali, WorldFish, Egypt). (b) *Oreochromis niloticus* showing a superficial lesion following infection with *Aphanomyces invadans* (image courtesy of Supranee Chinabut, Thailand). (c) Gross appearance of *O. niloticus* infected with *Achlya* Nees von Esenbeck, 1823, showing ulcers and cotton-like growths on the body surface and caudal peduncle. (d) *Oreochromis niloticus* with prominent hyphal growth on the head, dorsal fin and caudal peduncle following experimental infection with *Achlya klebsiana* Pieters, 1915. Images (c) and (d) courtesy of Panchai, Nakhon Ratchasima Rajabhat University and Hanjavanit, Khon Kaen University, Thailand. (e) Fish gills displaying a marble appearance representing an advanced stage of *Branchiomyces* Plehn, 1912 infection. (f) *Oreochromis niloticus* with an enlargement of the liver with dark grey nodules infected with *Ichthyophonus* Plehn et Mulsow, 1911. Figures (e) and (f) provided courtesy of Heba H. Mahboub and Adel A. Shaheen, Zagazig University, Egypt)

were limited to the liver, unlike other *Dermocystidium* sp. infections. Systemic *Dermocystidium* sp. infection of *O. niloticus* was described by Mahboub and Shaheen,[203] including field sampling and experimental challenges. Infected animals were sluggish, darkened and exhibited scale loss and ulceration, as well as skin and fin damage. Multifocal cysts with minimal inflammatory response were observed in the liver, spleen, stomach and intestines. Ruptured cysts distributed spores into surrounding tissues, with concomitant infiltration of macrophages and lymphocytes.

2.9.4 | Global translocations

The lack of information on species identity makes it impossible to identify likely translocations of *Dermocystidium* spp. with tilapia. Given the variable tissue tropism and limited records, it is unlikely that *Dermocystidium* spp. have been translocated widely with tilapia. These parasites could be translocated to new localities, however, and, particularly for the pathogenic *Dermocystidium* sp. described by Mahboub and Shaheen,[203] there is disease risk associated with its translocation.

2.9.5 | Research

To mitigate risks and improve management, research should aim to improve control, confirm species identities, and assess if transboundary *Dermocystidium* spp. movements have occurred.

2.10 | Microsporidia Balbiani, 1882 (Obazoa: Opisthosporidia: Microsporidia)

2.10.1 | Taxonomic identity

Microsporidia are obligate spore-forming intracellular parasites whose spores contain an extrusion apparatus that has a coiled polar tube ending in an anchoring disc at the apical part of the spore. Molecular data identify microsporidians as basal fungi.[249,250] Their proliferation in cells, undergoing merogonous and sporogonous development leading to the production of thick-walled spores, results in an enlarged cell termed a xenoma. Generic and specific identification is based on

morphological characteristics of the coiled polar filament, the number of nuclei and spore dimensions[251,252] and molecular data of 16S rDNA sequences.[253,254] While biorefringent spores can be detected in haemotoxylin and eosin sections, quicker methods that negate the need for tissue embedding and sectioning, including the use of Giemsa or phloxin B can facilitate the rapid detection of spores in fresh material.[255,256] Spores range from 1 to 20 μm in length and their differentiation from cellular debris in some preparations is difficult. Calcofluor white specifically binds to chitin in the spore wall, which, with fluorescence microscopy, facilitates their identification in host tissues.[257] Spores can also be identified using immunohistochemistry[258] and/or in situ hybridization.[259–261]

Microsporidian infections from tilapia in aquaculture include *Loma camerounensis* Fomena, Coste et Bouix, 1992 from the intestinal tract of farmed *O. niloticus* in Cameroon[262]; from farmed *O. niloticus* in Kenya[20] and from the kidneys of *O. aureus* and hybrids in Israel[200]; systemic infection with *Neonosemoides* [syn. *Nosemoides*] *tilapiae* Faye, Toguebaye et Bouix, 1996 in wild *C. zillii* and *S. melanotheron* in Benin[263,264] and *Nucleospora braziliensis* Rodrigues, Francisco, David, da Silva et Araújo Júnior, 2017 infecting wild and farmed *O. niloticus* in São Paulo State, Brazil.[265] Microsporidian infections are recorded from other tilapia including a species of *Glugea* Thélohan, 1891 in invasive *O. niloticus* in Indonesia[266]; a species of *Pleistophora* Gurley, 1893 in the swimbladder of wild *Haplochromis angustifrons* Boulenger, 1914 and *Haplochromis elegans* Trewavas, 1933 from Uganda[19,201,267]; *N. tilapiae* from the stomach of wild *Coptodon guineensis* (Günther, 1862) cited as *T. guineensis* from Senegal,[264] as a systemic infection from the same host from Benin[263]; and in wild *Coptodon nyongana* (van den Audenaerde, 1971) cited as *T. nyongana* from Benin, Cameroon and Senegal.[262] Details of the host–parasites records of tilapia are summarised in Table S2. Taxonomic keys to Microsporidia are provided by Larsson,[268,269] Sprague et al.,[270] Canning et al.[271] and Cali et al.[272] These cases serve as a useful resource for facilitating identification and supporting the management of infections following the discovery of further microsporidian infections of cultured tilapia.

2.10.2 | Pathogenicity

Records of microsporidians in tilapia mostly note only their presence and seasonal prevalence in hosts, but Paperna[200] reported that *Pleistophora* sp. infections in *H. angustifrons* and *H. elegans* caused thickening of the swimbladder walls which contained abundant pansporoblasts. Rodrigues et al.[265] described *N. braziliensis* at 87%–100% prevalence in *O. niloticus* (av. 230–540 g) reared in reservoirs in Brazil. These infections caused xenomas in the gills with hyperplasia and telangiectasis and skin melanisation and inflammation, exophthalmos, stomach congestion with marked inflammatory responses associated with lesions, necrosis and liquefaction of infected muscle and kidney, hepatomegaly, splenomegaly and hepatic haemorrhages.[265] Sakiti and Bouix[263] observed *N. tilapiae* infections in *C. zillii*, *T. guineensis* and *S. melanotheron* and found xenomas in the gills and in the mesenteries, gut wall and liver, but without apparent clinical effect on the fish.

2.10.3 | Global translocations

There are insufficient data to understand transboundary movement of microsporidian infections with tilapia translocations. *Nucleospora braziliensis* is not reported in Africa, and a horizontal transfer from a resident fish in Brazil is the most likely source of infection, but Rodrigues et al.,[265] did not examine resident fish in the reservoirs.

2.10.4 | Research

Research on microsporidian infections of fish centres on the development of diagnostic methods for detection at low abundance that might be overlooked by histology, understanding routes of transmission and factors facilitating infection, development of *in vivo* challenge models to better understand host–parasite interactions, and the efficacy of management and control strategies.[273–275] There are few effective chemotherapeutic agents for treatment of microsporidians. A range of products have been assessed in fish including albendazole,[276] beta-glucans,[277] monensin[278] and quinine hydrochloride[279] against *L. salmonae*, and fumagillin and toltrazuril against *Glugea anomala* (Moniez, 1887).[280] While feed trials with monensin significantly reduced xenoma number, the effective dose of 1000 ppm for up to 3 weeks is above the *Oncorhynchus mykiss* (Walbaum, 1792) 96-h LC_{50} of 1.88 mg.[281] Trials with albendazole, beta-glucans and fumagillin also reduced the abundance of xenomas, while quinine hydrochloride delayed xenoma formation, and toltrazuril destroyed xenomas. Worldwide, there are, however, no veterinary medicines licenced for use against microsporidians in aquaculture. An experimental vaccine using freeze-killed spores of a low-virulence strain of *Loma salmonae* (Putz, Hoffman et Dunbar, 1965) given intraperitoneally, resulted in 85% fewer xenomas in the gills of *O. mykiss*.[282] The study indicates that non-treatment based approaches can be developed for control of microsporidian infections in fish.

2.11 | Monopisthocotylea Odhner, 1912 (Monogenea Carus, 1863) (Obazoa: Opisthokonta: Metazoa: Platyhelminthes)

Monogeneans are flatworms, primarily ectoparasitic on fish, characterised by possessing a haptor (opisthaptor), a specialised structure that uses hooks or clamps to maintain attachment of the parasite to the host.

2.11.1 | Taxonomic identity

Species of the genus *Gyrodactylus* von Nordmann, 1832 are small (100–200 μm long), transparent, ectoparasitic monogeneans that colonise the external surfaces and buccal-opercular cavities of their hosts (Figures 5–7a). The sclerites of the haptor include a pair of anchors (hamuli) linked by a thin dorsal bar which articulate over an

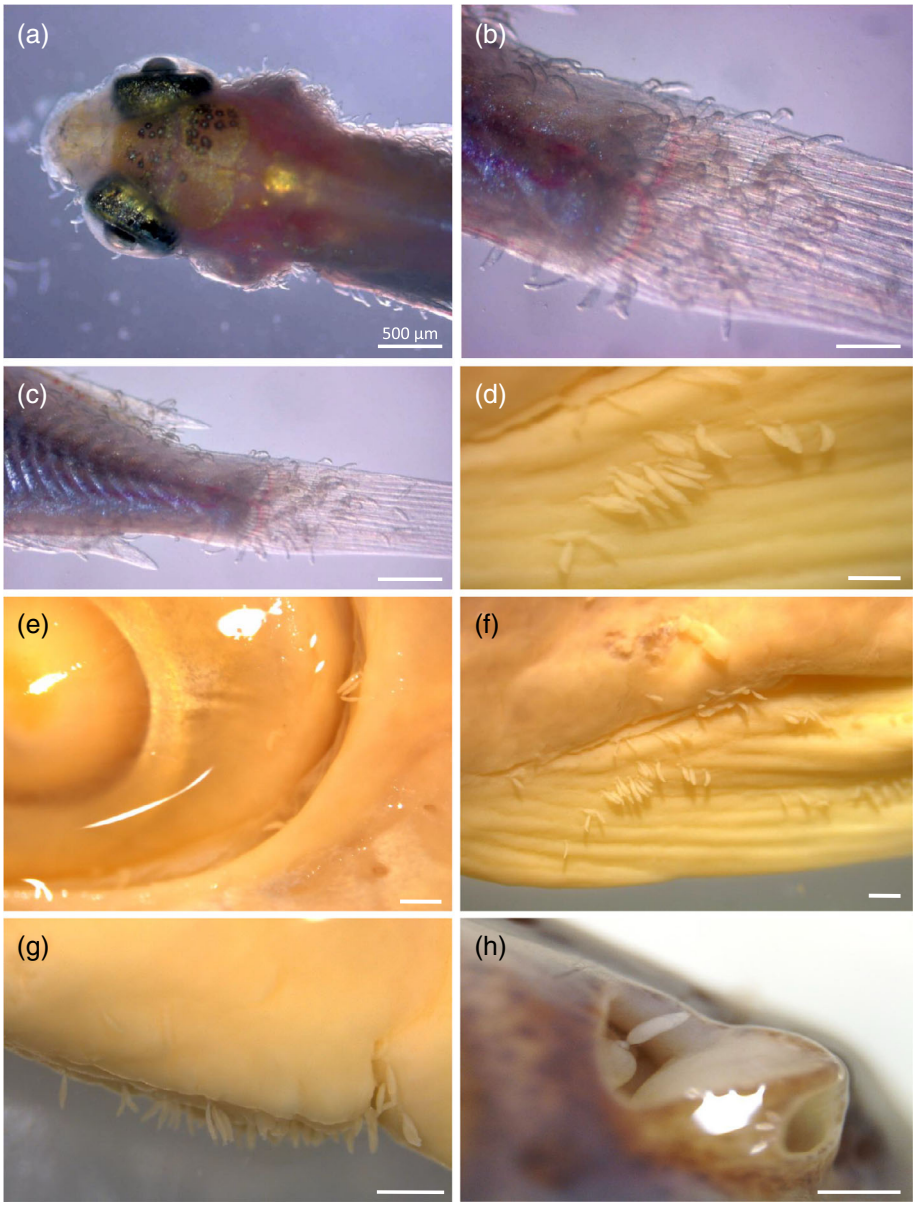

FIGURE 5 Light microphotographs of *Oreochromis niloticus* larvae (~2 cm) with a heavy infection of *Gyrodactylus cichlidarum* Paperna, 1968. (a) Head, (b) Caudal fin, (c) Caudal peduncle, (d, f), Eye, (e), Anal fin, (g) Ventrum and (h) Nares. All scale bars = 500 µm

approximately triangular-shaped ventral bar (Figure 7b–d). Eight pairs of marginals hooks (Figure 7e,f), typically of one morphological type and size, which are distributed around the periphery of the haptor function as the principal means of attachment. *Gyrodactylus* do not have eye spots, have a bi-lobed head with a pair of head organs that aid in anterior attachment to the host and are epidermal grazers. *Gyrodactylus* spp. notably are viviparous polyembryonous and progenetic; the large uterus contains an embryo at birth. Individuals develop a male copulatory organ (a muscular organ armed with small spines) after their first parturition. At least 15 species of *Gyrodactylus* infect tilapia (Table S2; Figure 8)—although this undoubtedly represents an underestimate given that seven new species have been described since 2000,[283–287] that cichlids and gyrodactylids are speciose, and that tilapia—*Gyrodactylus* host–parasite associations have not been extensively studied in Africa. Two *Gyrodactylus* spp. are widely distributed and associated with aquaculture mortalities: *Gyrodactylus cichlidarum* Paperna, 1968 described from *S. galilaeus* in Ghana, but now with a global distribution on numerous hosts,[287,288]; and *G. yacatli* García-Vásquez, Hansen, Christison, Bron et Shinn, 2011 described from *Oreochromis* spp. and *Vieja fenestrata* (Günther, 1860) in Mexico, but originating in Africa, and recorded from Kenya, and possibly Zimbabwe and China.[286,287]

Dactylogyrids possess a haptor with two pairs of anchors or hamuli, seven pairs of marginal hooks and four eyespots (Figure 9a); the configuration of the haptoral elements, the morphological shape of these and the reproductive organs (i.e., vagina and male apparatus) facilitate the identification of genera and species. They infect the gills and intestine of their hosts. There are 72 species of dactylogyrid

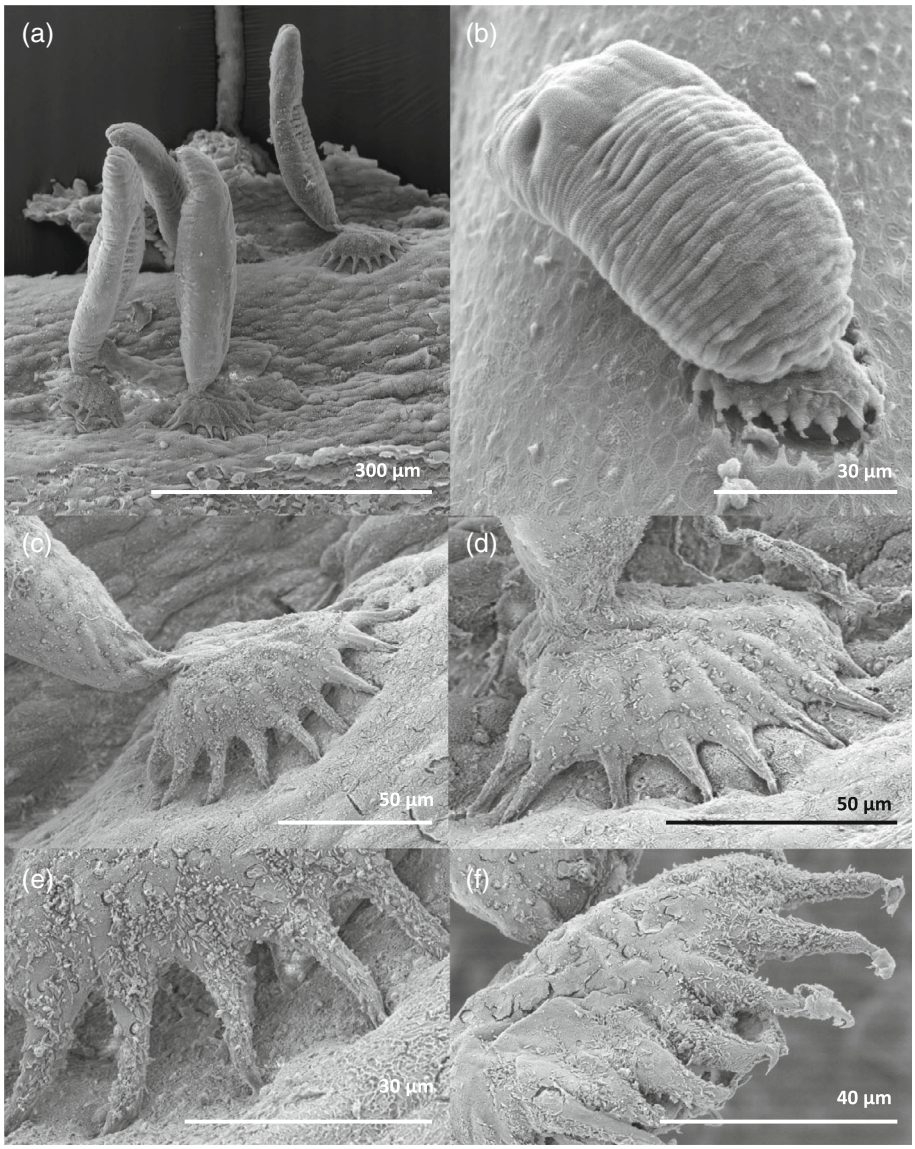

FIGURE 6 Scanning electron micrograph of *Gyrodactylus cichlidarum* Paperna, 1968. (a–e) Attachment on fish skin; (f) Haptoral structure of the worm after detachment from *Oreochromis niloticus*. Images a, c-f courtesy of Mrs Greta Hanako Rosas Saito, Instituto de Ecología A.C., Xalapa, Mexico. Image b courtesy of Giuseppe Paladini, Institute of Aquaculture, University of Stirling, Scotland, UK.

monogeneans described from tilapia (Figure 9a–i) in the genera *Cichlidogyrus* Paperna, 1960 (Figure 9a–d), *Enterogyrus* Paperna, 1963 (Figure 9f) and *Scutogyrus* Pariselle et Euzet, 1995 (Figure 9e). Morphological identification of dactylogyrids is typically based on the hard parts of the haptor (see below) and copulatory organs (Figure 9g–i).

The gill-infecting dactylogyrids of tilapia belong to *Cichlidogyrus* and *Scutogyrus*, and are characterised by a haptor bearing two pairs of anchors (whereas there is only one pair in gyrodactylids), a V-shaped ventral transversal bar (in contrast to the ventral bar of members of *Gyrodactylus*, which possesses a membrane), a dorsal transversal bar with two auricles (in contrast to the simple dorsal bar in *Gyrodactylus*) and seven pairs of marginal hooks (compared to 8 in *Gyrodactylus*; Figure 9c–e, g). Cruz-Laufer et al.[289] identified that numerous gill-infecting species have been co-introduced outside continental Africa and are reported in the peer-reviewed literature from Latin America, Asia, Australia or Madagascar. Five of these have been mentioned as co-introduced in at least 15 publications. Notable species that have been translocated include *C. halli* (Price et Kirk, 1967), *C. sclerosus* Paperna et Thurston, 1969, *C. thurstonae* Ergens, 1981, *C. tilapiae* Paperna, 1960 and *Scutogyrus longicornis* (Paperna et Thurston, 1969). The three species for which only one co-introduction is reported outside Africa have a limited natural distribution: *C. levequei* Pariselle et Euzet, 1996 on *Coptodon coffea* (Thys van den Audenaerde, 1970) in Guinea is reported from *O. niloticus* in China[290]; *C. quaestio* Douëllou, 1993 in Lake Kariba, Zimbabwe[291] and the Congo Basin[292] is reported from *O. niloticus* introduced into Mexico[129]; and *C. rognoni* Pariselle, Bilong Bilong et Euzet, 2003 from Senegal[293] and from cultured tilapia in Côte d'Ivoire[293] is reported from *O. niloticus* introduced into Brazil. Their limited distribution decreases their likelihood of translocation.[294]

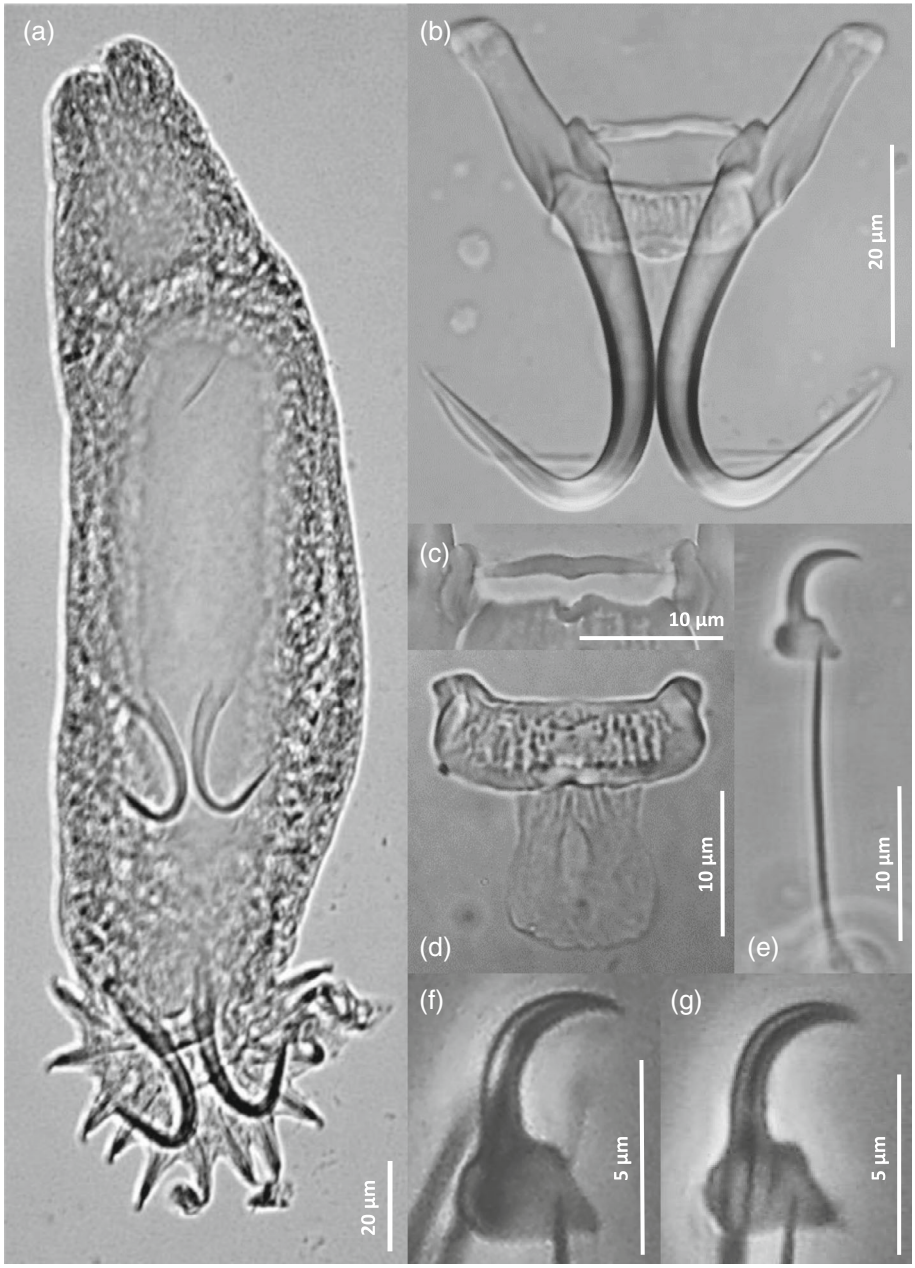

FIGURE 7 Light microphotographs of *Gyrodactylus cichlidarum* from *Oreochromis niloticus*. (a) Whole mount, (b) Hamuli, Ventral and dorsal bars, (c) Dorsal bar, (d) Ventral bar, (e) Marginal hook and (f, g) Marginal hook sickles

Species of *Enterogyrus* infect the stomach of their hosts and can be identified by the shape and configuration of their haptoral elements (Figure 9f).[295,296] They possess seven pairs of marginal hooks, a simple transverse bar, and two pairs of anchors of differing morphologies—the smaller-sized ventral anchors have prominent inner and outer roots, while the significantly larger dorsal anchors have a morphology closely resembling that of the marginal hooks. Of the 12 described species, nine infect tilapia. Some of these species have been co-introduced with tilapia outside Africa, such as *E. cichlidarum* Paperna, 1963,[297] *E. coronatus* Pariselle, Lambert et Euzet, 1991, *E. foratus* Pariselle, Lambert et Euzet, 1991 and *E. malmbergi* Bilong Bilong, 1988[298] in Brazil and Mexico[299]; and *E. coronatus* and *E. malmbergi* in China[300] and Cuba.[301]

Pariselle and Euzet[295] provided the most recent morphological identification key for dactylogyrid monogeneans including those parasitising tilapia, but numerous species have been described subsequently.[302] *Cichlidogyrus mbirizei* Muterezi Bukinga, Vanhove, Van Steenberge et Pariselle, 2012 is the only dactylogyrid described after Pariselle and Euzet[295] that commonly infects commercially important tilapia and has been translocated broadly (Table 1).[300,303–305] New host–parasite records continue to be made from wild populations of commercially important tilapia. *Cichlidogyrus papernastrema* Price, Peebles et Bamford, 1969 was recorded from native *C. rendalli* in the Upper Congo Basin and *C. berradae* Pariselle et Euzet, 2003, *C. cubitus* Dossou, 1982 and *C. flexicolpos* Pariselle et Euzet, 1995 were

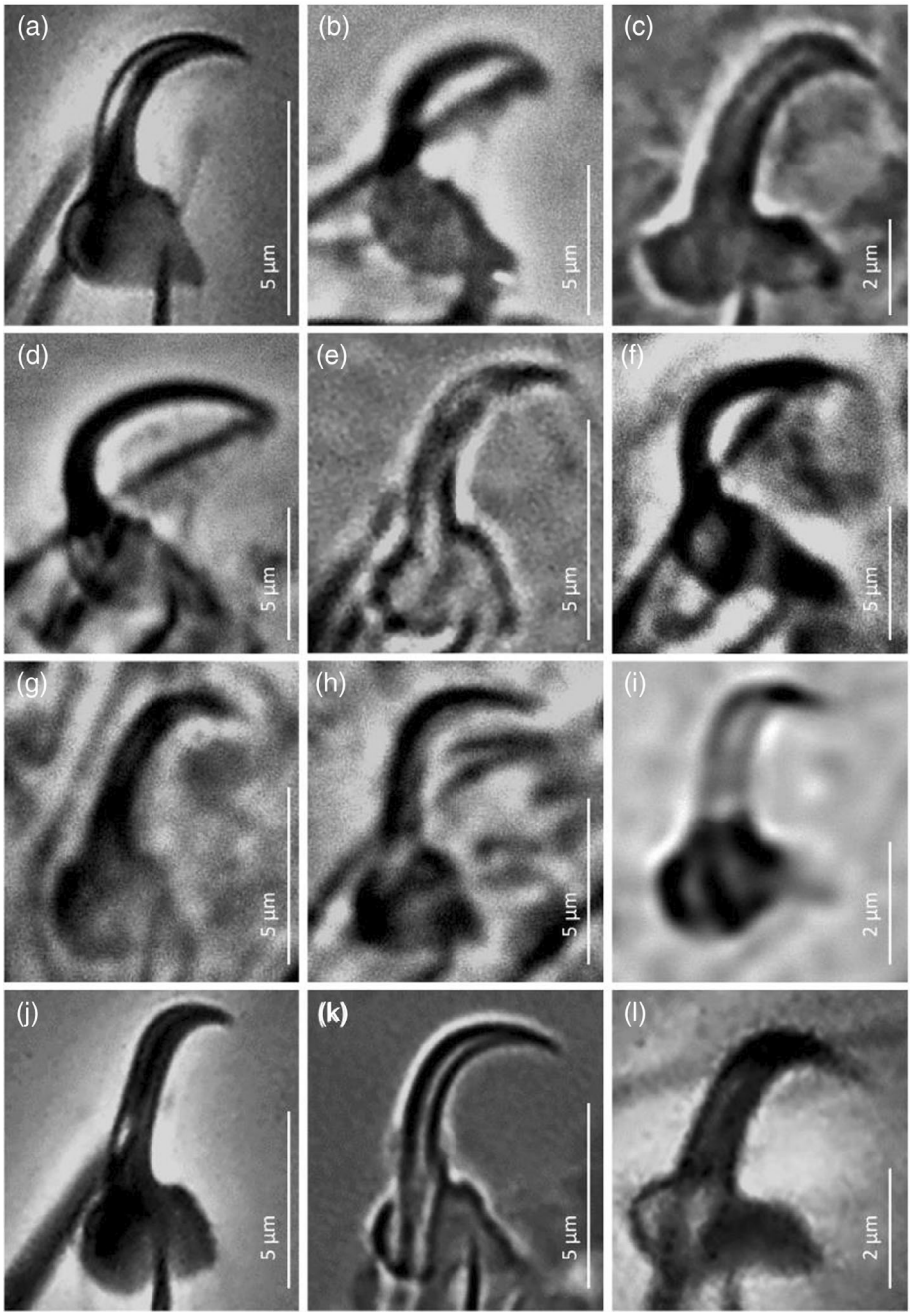

FIGURE 8 Light microphotographs under phase contrast of the marginal hook sickles of *Gyrodactylus* species infecting different species of tilapia. (a) *Gyrodactylus cichlidarum* Paperna, 1968. (b) *Gyrodactylus ergensi* Prikřylová, Matějusová, Musilová et Gelnar, 2009. (c) *Gyrodactylus hildae* García-Vásquez, Hansen, Christison, Bron et Shinn, 2011. (d) *Gyrodactylus malalai* Prikřylová, Blažek et Gelnar, 2012. (e) *Gyrodactylus niloticus* Cone, Arthur et Bondad-Reantaso, 1995 (syn. *G. cichlidarum*). (f) *Gyrodactylus nyanzae* Paperna, 1973. (g) *Gyrodactylus occupatus* Zahradníčková, Barson, Luus-Powell et Prikřylová, 2016. (h) *Gyrodactylus parisellei* Zahradníčková, Barson, Luus-Powell et Prikřylová, 2016. (i) *Gyrodactylus shariffi* Cone, Arthur et Bondad-Reantaso, 1995. (j) *Gyrodactylus shinni* García-Vásquez, Pinacho-Pinacho, Guzmán-Valdivieso, Calixto-Rojas et Rubio-Godoy, 2021. (k) *Gyrodactylus ulinganisus* García-Vásquez, Hansen, Christison, Bron et Shinn, 2011. (l) *Gyrodactylus yacatli* García-Vásquez, Hansen, Christison, Bron et Shinn, 2011

recorded from introduced *C. rendalli* in the Lower Congo Basin.[292] New species also continue to be described from wild populations of economically important tilapia, such as *Enterogyrus mashegoi* Luus-Powell, Madanire-Moyo, Matla et Přikrylová, 2020 and *E. multispiralis* Luus-Powell, Madanire-Moyo, Matla et Přikrylová, 2020 from the stomach of *O. mossambicus* in South Africa,[296] and *C. flagellum* Geraerts et Muterezi Bukinga, 2020, *C. lobus* Geraerts et Muterezi Bukinga, 2020 and *C. maeander* Geraerts et Muterezi Bukinga, 2020 from the gills of *T. sparrmanii* in the Democratic Republic of Congo.[306] Even for well-studied tilapia species, further dactylogyrid diversity is

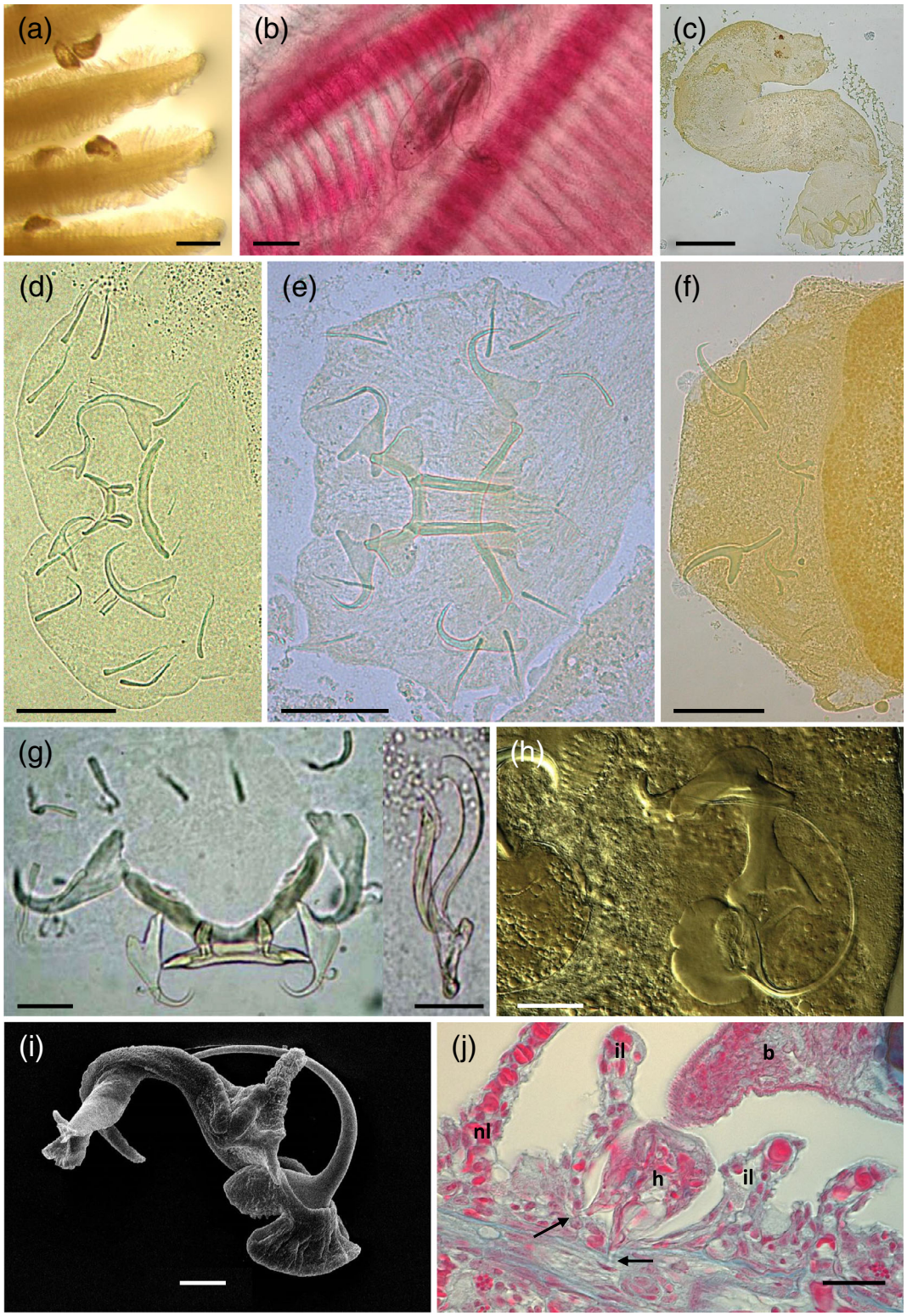

FIGURE 9 (a) *Cichlidogyrus* spp. on the gills of *Sarotherodon melanotheron* (photo A. Pariselle). (b) *Cichlidogyrus* Paperna, 1960 sp. on the gill of *Oreochromis niloticus* from Thailand (photo T. Limakom). (c) *Cichlidogyrus dossoui* Douellou, 1993 (in toto, glycerine ammonium picrate (GAP) medium) (photo A. Pariselle). (d) *Cichlidogyrus tiberianus* Paperna, 1960 (haptor, GAP medium) (photo A. Pariselle). (e) *Scutogyrus gravivaginus* (Paperna et Thurston, 1969) (haptor, GAP medium) (photo A. Pariselle). (f) *Enterogyrus malmbergi* Bilong Bilong, 1988 (haptor, GAP medium) (photo A. Pariselle). (g) *Cichlidogyrus halli* (Price et Kirk, 1967) (haptor, left side; male copulatory organ (MCO), right side, digested material; photo A. García-Vásquez). (h) *Cichlidogyrus agnesi* Pariselle et Euzet 1995 (MCO, phase contrast) (photo V. Sarabeev). (i) *Cichlidogyrus tiberianus* (MCO, scanning electron microscopy) (photo W. Fannes). (j) Histological section of *Cichlidogyrus philander* Douëllou, 1993 on the gills of *Pseudocrenilabrus philander* (Weber, 1897; photo P.C. Igeh/A. Avenant-Oldewage). b, parasite body; h, parasite haptor; il, impacted lamellae; nl, normal lamellae; black arrow: anchor deeply pushed in the gill lamellae. Scale bars: a = 250 μm; b, c = 100 μm; d–f, j = 50 μm; g = 25 μm; h = 20 μm; i = 5 μm

likely to be discovered. Some widespread tilapia monogeneans such as *C. halli* and *C. tilapiae* represent species complexes,[307] complicating identification. Morphological and molecular investigations of *C. halli* in the Upper Congo Basin indicate that introduced and native *O. niloticus*, and local other native tilapia harbour different species.[308,309]

Monogenean specimens are dissected with the hook-bearing haptor and the anterior genital organ-bearing parts used to facilitate morphological studies and vouchering of specimens, while the parts of the monogenean not bearing hard structures are used for molecular studies. Molecular identification of gyrodactylid and dactylogyrid monogeneans is largely based on nuclear ribosomal DNA (rDNA) markers.[310] Ek-Huchim et al.[311,312] designed primer combinations within the nuclear rDNA for non-invasive identification of monogeneans on tilapia.

2.11.2 | Pathogenicity

Attachment of gyrodactylids to fish involves the 16 marginal hooks simultaneously perforating the epithelium and causes damage to the epidermis.[313,314] The two large hamuli contribute to marginal hook attachment by lifting the centre of the haptor (see Figure 7 of the haptor of *G. cichlidarum*) but can also perforate the epithelium. These parasites also use their muscular pharynx to grab mucus and epidermal tissue which creates feeding wounds. *Gyrodactylus* spp. damage and erode the fins of infected fish leading to reduced swimming capacity and increased mortality.[315,316] High parasite burdens cause numerous superficial perforations that cause physiological and histological disturbances that can induce osmoregulatory failure.[317] *Gyrodactylus cichlidarum* is associated with mortality of farmed tilapia worldwide including Scotland,[286] Egypt,[318] Mexico[287] and various Latin American countries.[319] The combined physical damage from attachment and feeding constitutes an important breach to the primary, innate defensive barrier the skin provides, and renders hosts more susceptible to opportunistic pathogens. *Gyrodactylus cichlidarum* feeding and attachment activity damages the epidermis, increasing the susceptibility of fish to bacterial infection, including with *Streptococcus iniae* Pier, 1976[320] and *Aeromonas hydrophila* (Chester, 1901) with subsequent mortality.[318]

In *Cichlidogyrus* or *Scutogyrus*, the sclerites of the haptor penetrate the gill epithelium.[321,322] Attachment of *Cichlidogyrus philander* Douëllou, 1993 in a non-tilapia cichlid caused epithelial rupturing, disturbance and distortion of blood cells, blood cell puncture, distortion and sometimes penetration of the extracellular cartilaginous matrix in the gills, surface deformation of gill lamellae, erosion of epithelial cells, increased mucus production, neutrophilaemia, hyperplasia and fusion of gill lamellae (Figure 9j).[322,323] A humoral immune response[324] and changes in blood biochemistry[325] are observed in *O. niloticus* injected with extracts of *Cichlidogyrus* spp. The role of the marginal hooks of the haptor in attachment to the gills is, however, debated.[323,326,327] In *Enterogyrus*, attachment creates shallow epithelial perforation, damage and compression of the stomach epithelium, nuclear anomalies, metaplasia, hyperplasia, pleomorphism and vacuolation at the attachment site.[321] The apparently moderate pathology explains the lack of observed morbidity or mortality associated with these parasites. Noga and Flowers[328] observed a cultured population of *O. mossambicus* with specimens of *E. cichlidarum* attached to abnormal sites such as the gills, cranial bones, heart, blood vessels, liver, perirenal area, peritoneal cavity and liver with sign of systemic host immune response and severe morbidity and mortality.

Species of *Gyrodactylus* and *Cichlidogyrus* commonly co-occur in fish farms[129]; co-infection induces host immunosuppression and facilitates infection by both parasites.[329] Fish concurrently infected with species of *Gyrodactylus*, *Trichodina* and *I. multifiliis* do not develop immunity after vaccination for *S. iniae* and have higher mortality than uninfected fish.[330] Concurrent infection with *Gyrodactylus* sp. and *Cichlidogyrus* sp. has negative effects on hosts; high parasite burdens correlate with low host condition factor with an estimated 12%–15% decrease in profit margin.[331] Igeh and Avenant-Oldewage[322] outlined that natural infections of *Cichlidogyrus* are not very harmful. Sandoval-Gío et al.[324] noted little direct evidence for dramatic effects on cultured tilapia. Paperna[200] described no ill effects of tilapia dactylogyrids in Africa or Israel. Abundances of up to 800 *Cichlidogyrus* spp. on *C. guineensis* in Ébrié Lagoon (Côte d'Ivoire) had no apparent negative effect on the host (A. Pariselle, pers. obs.). *Cichlidogyrus* spp. are, however, potentially problematic in aquaculture.[332,333] Kabata[334] reported serious gill pathology in tilapia infected with *C. sclerosus* in the Philippines. Concurrent infections with species of *Cichlidogyrus* and *Scutogyrus* induce anaemia and decrease fish condition.[335] These impacts combined with their high prevalence and direct life-cycle caused Akoll et al.[23,24] to assess *Cichlidogyrus* spp. as high-risk parasites for aquaculture.

2.11.3 | Global translocations

Gyrodactylus cichlidarum, *Cichlidogyrus* spp., *Scutogyrus* spp. and other monogeneans have been translocated worldwide with tilapia for aquaculture (Tables S1 and S2)[287,288,300,319,336,337] and infect native fish, mainly cichlids, but also poeciliid fish in Mexico,[287,336,338] in areas where tilapia and their parasites are introduced. *Gyrodactylus cichlidarum* is the most common translocated gyrodactylid of tilapia and has been established in fish farms in Mexico for decades.[287,339] *Gyrodactylus yacatli* is recorded in Mexico and Kenya,[287] and probably in China[300] and Zimbabwe,[285] although more extensive sampling and accurate identification of specimens is needed. Translocation of tilapia parasites has also occurred in Africa, including *G. nyanzae* Paperna, 1973, which was transferred from introduced *O. niloticus* to *C. rendalli* in the Upper Congo Basin,[340] and *G. cichlidarum* and *G. malalai* Přikrylová, Blažek et Gelnar, 2012, which were introduced with *O. niloticus* to Lake Victoria, Kenya where they infect local native fish.[341]

There are many widely cointroduced dactylogyrid tilapia parasites. Of these, some have transferred to cichlid hosts in continental Africa (*C. sclerosus*, *C. tilapiae*),[340] to Malagasy cichlids (*C. halli*, *C. thurstonae*, *C. tilapiae*),[337] to American cichlids (*C. sclerosus*, *C. tilapiae*, *S. longicornis*, *E. malmbergi*),[336,342] and to members of the

cyprinodontiform families Aplocheilidae in Madagascar (*C. tilapiae*)[337] and Goodeidae in Mexico (*C. sclerosus*).[336] The transmission of monogenean parasites to new hosts is rarely reported, and reports mostly contain little evidence of translocation or transmission routes; information is too limited to assess frequency or probability of transfer for given monogeneans. It is clear, however, that the dactylogyrids that establish outside their native range can exploit a phylogenetically broad host range. Fannes et al.[343] described that *C. dossoui* Douëllou, 1993 and *C. tiberianus* Paperna, 1960, which normally infect coptodonine tilapia occur on other tilapia and cichlids in their introduced range. These changes in host range can occur wherever tilapias are translocated, because ecological opportunity and host phylogenetic history determine the host range.[289,344] Introduced populations can be free of gill monogeneans, such as *O. mossambicus* in New Caledonia, because of genetic bottlenecks, salinity changes, single introduction events or treatment of translocated stock.[345] Tilapia-infecting monogeneans may become the most widespread tropical freshwater fish parasites, given the ubiquity of tilapia and the prevalence of their monogeneans. Forty helminth species have been introduced to Mexico with introduced fish; 33 of these are monogeneans; of which 14 were introduced with tilapia.[336]

2.11.4 | Research

Most studies focus on epizootiology and ecology of the tilapia-gyrodactylid association. Aquaculture research focuses on the search for natural treatments and products to improve the ability of fish to respond to infections and the immune response elicited by infection. The identification of host immune genes that are activated by *G. cichlidarum* infection[346] and identification of major histocompatibility complex II α alleles associated with parasite resistance make genotype-assisted selection of resistant fish strains possible.[347] Bioinformatic analysis of monogenean parasite excretory/secretory proteins (secretomes) also provides a novel approach to identify potential drug targets.[348] The lack of reports of detrimental effects may be a consequence of a lack of study[349] and greater attention paid to pathogens of more immediate concern. More functional–biological research on monogenean life history and infection dynamics, physiology, including host detection and environmental tolerance, and pathogenicity such as attachment and histopathology, and facilitation of secondary infections would benefit aquaculture.[302] Monogenean phenotyping often focuses on the haptor. In cichlid-infecting dactylogyrids, rapid morphological adaptation of the haptor associated with host-switches is observed,[350] as is haptoral variation within monogenean species infecting populations of the same[351] and different[352] hosts. Morphological variation is observed in *G. cichlidarum* from different hosts and/or geographical regions, although this gyrodactylid displays limited molecular variation.[287] Accurately understanding monogenean translocations and host-switches requires population-level approaches of hosts and parasites. Understanding the influence of phenotypic diversity in the haptor and its role in pathogenicity and host-specificity could aid in predicting and understanding risk and impacts of lateral parasite transfer after tilapia translocations. Better understanding of why some tilapia monogeneans are more tolerant of translocation and more likely to establish could also aid in understanding translocation risks. How anthropogenic translocations alter the geographic and host range of tilapia parasites is a major question in cichlid parasitology, and improved baseline surveys and infection experiments are needed to address it.[353] Absence of parasites should also be more systematically published: reports are rare in the literature, and published accounts often do not explicitly state whether hosts were inspected for a parasite taxon that was not reported, probably because of publication bias against negative results.[302,345] Climate change may help sustain or expand invasive tilapia populations,[354] although its expected impact on directly transmitted aquatic parasites is unclear.[355] A better understanding of any aspect of the physiology and infection dynamics of tilapia monogeneans would aid understanding how global change will influence the poorly understood mechanisms behind monogenean pathology, distribution, host range and host-switching.

Chemotherapeutic agents are expensive, may leave residues in fish tissues and have negative environmental effects. Therefore, assays have been conducted to evaluate the effectivity of various plant extracts, essential oils and other natural compounds to control infections, for example, garlic extract, saponins and other products.[356] Leaf extracts of *Mitracarpus scaber* Zucc. (Rubiaceae), a plant commonly used in West African traditional medicine, improve growth, non-specific immunity and resistance of Nile tilapia to *G. malalai*.[357] *Leucaena leucocephala* (Lam.) (Fabaceae), a plant commonly used as an anthelminthic in cattle, has also shown promising results in controlling gyrodactylid infection of tilapia fingerlings.[358] Dotta et al.[359] found that combined dietary supplementation with bee propolis and *Aloe barbadensis* Miller (Asphodelaceae) extracts reduced abundance of dactylogyrid monogeneans infecting the gills of *O. niloticus*. De Oliveira Hashimoto et al.[360] found that essential oils of a hybrid mint *Mentha piperita* Linnaeus (Lamiaceae) were effective against these parasites.

Taxonomic identification of *Gyrodactylus* spp. and dactylogyrids is time consuming and requires detailed morphometric analysis of microscopic structures of the parasite attachment and/or copulatory organs and specialist knowledge: a practical alternative in aquatic veterinary medicine could be screening fish mucus using PCR to identify parasite molecular markers.[312] The relevance of this approach is limited to situations where precise taxonomic identification is required to meet quarantine regulations for export permits, or where parasite life-cycles are well documented and approaches to strategic control are established. Most reports of farmed tilapia mortality, furthermore, are associated with *G. cichlidarum*. Recent phylogeographic work indicates that genetic structure and host-specificity of *Cichlidogyrus* spp. differ between hosts.[309,352] Molecular markers will be crucial in disentangling the mechanisms that structure monogenean populations, because they are consequential for colonisation dynamics. The wild relatives of tilapia and their parasites are likely to be excellent disease models.[302,361] *Cichlidogyrus berminensis* Pariselle, Bitja Nyom et Bilong Bilong, 2013, for example, infects multiple *Coptodon* spp. in Lake

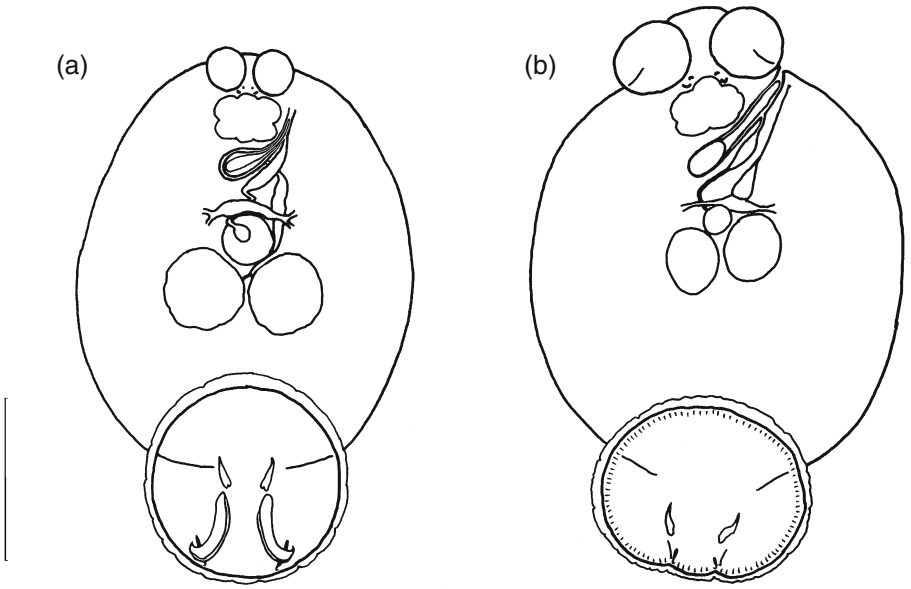

FIGURE 10 Capsalids from tilapia. (a) *Neobenedenia girellae* drawn from Queensland Museum specimen G218281 showing circular haptor, small anterior attachment organs and absence of a vagina. (b) *Benedenia monticellii* drawn from specimens on Hebrew University of Jerusalem slide HUJ-MONO1.0 showing laterally ovoid haptor with posterior notches, large anterior attachment organs and vagina with opening posterior to common genital pore. Scale bar = 750 μm

Bermin, Cameroon,[362] and closer scrutiny could increase our understanding of potential and achieved host-range. More variable markers than the currently widely used nuclear rDNA fragments are needed, for instance, the mitochondrial cytochrome *c* oxidase subunit 1 gene (COX1), which is highly variable in flatworms and therefore currently not widely applicable in monogeneans.[310] Mitogenomics of monogeneans infecting African cichlids[363–365] are likely to facilitate the application of mitochondrial markers to monogenean parasites of tilapia.

2.12 | Capsalidae Baird, 1853 (Obazoa: Opisthokonta: Metazoa: Platyhelminthes)

These monopisthocotylean monogeneans are reported from tilapia grown in brackish and marine systems.

2.12.1 | Taxonomic identity

Capsalids are monogeneans that primarily parasitise the external surfaces of fish; possession of accessory sclerites is a synapomorphy for the family.[366] From tilapia, *Benedenia monticellii* (Parona et Perugia, 1895) was recorded from *O. aureus* in Israel[367] and *Neobenedenia melleni* (MacCallum, 1927) was recorded from *O. aureus* in Cuba,[368] as *Benedenia* sp.,[369,370] *O. mossambicus* in Hawaii,[367,371] *O. niloticus* × *O. aureus* in Martinique,[372] *O. aureus* × *O. mossambicus* in Jamaica[333,373] and *O. aureus* × *O. mossambicus* in the Bahamas.[374–377] A *Neobenedenia* sp. was reported from *O. mossambicus* and *O. niloticus* hybrids in Mexico's Atlantic coast.[378] Invasive *O. mossambicus* and *Tilapia mariae* in brackish water in Australia are parasitised by *N. girellae* (Hargis, 1955; M. Deveney, unpublished data; Figure 10a).

Benedenia monticellii (Figure 10b) possesses a vagina and a transversely ovoid haptor with a muscular periphery and marked indentations in its posterior edge at the approximate positions of the posterior hamuli and large anterior attachment organs.[369] A key to *Benedenia* spp. was provided by Deveney and Whittington,[379] but species have been described subsequently and the key will misidentify some undescribed species. *Neobenedenia* spp. lack a vagina, have an almost circular haptor and small anterior attachment organs.[380] *Neobenedenia* Yamaguti, 1963 has a long and convoluted taxonomic history, but Brazenor et al.,[381] using molecular data, resolved distinct clades within morphologically similar *Neobenedenia* spp. and concluded that aquaculture infections were *N. girellae*. Molecular analyses are needed to identify *Neobenedenia* spp. and some specific tools have been developed for this purpose.[382]

While the life-cycles of benedeniine genera vary, complicating strategic control, all capsalids are susceptible to standard treatments such as freshwater (for marine farmed fish), oxidising agents including hydrogen peroxide, reducing agents such as formalin and anthelmintics including praziquantel, decreasing the importance of precisely identifying these parasites in aquaculture.

2.12.2 | Pathogenicity

Capsalids are important causes of disease in aquaculture: *Neobenedenia* spp. are regarded as notorious[383] and insidious[384] pathogens of cultured fish. Infections damage the epidermis[385] and eyes,[386]

decrease epidermal thickness,[387] facilitate secondary infections and can lead to fish death by compromising osmoregulation.[386]

2.12.3 | Global translocations

Capsalids are not recorded as translocated with their hosts; furthermore, capsalids infect tilapia only in brackish and marine systems. Stress associated with osmoregulation in seawater aquaculture systems increases the susceptibility of tilapia to capsalid infections.[367] These parasites are part of the fauna that infect tilapia from the environment when they are translocated, but it is noteworthy that *Neobenedenia* spp. are invasive and have been broadly translocated.[388]

2.12.4 | Research

There are substantial bodies of work on capsalid taxonomy,[381] biology and pathology.[386] Capsalid infections increase cost of production, decrease fish growth and cause mortality with substantial economic impacts on aquaculture.[389] Life-cycle parameters are used as a basis for temperature and salinity dependant strategic control[390,391] that aims to disrupt life-cycles.[386] Although freshwater is an effective, safe treatment,[367,392] substantial efforts have been made to optimise praziquantel[393] and hydrogen peroxide[394] treatments for capsalids and to identify effective natural products.[395] Parasite management is aided by shading[396] and increasing the depth at which fish are held,[397] which both decrease infection. Kishimori et al.[371] noted a specific antibody response to *Neobenedenia* in *O. mossambicus*, although Rubio-Godoy et al.[378] found that injecting purified worm extracts did not decrease *Neobenedenia* infection in tilapia. Ongoing research is likely to focus on management and decreasing the effects of infections on cultured fish and of control on the costs of production.[386]

2.13 | Digenea Carus, 1863 (Obazoa: Opisthokonta: Metazoa: Platyhelminthes)

2.13.1 | Taxonomic identity

Trematodes are parasitic flatworms with a ventral disc or a ventral and an oral sucker. Trematodes infecting 'tilapia' of the genera *Tilapia*, *Coptodon* and *Oreochromis* in their native range in Africa, as well as in at least 10 countries where they have been introduced, are represented by at least 45 taxa. Most of them are metacercariae (larvae) occurring in different tissues and organs of the fish, that is, skin, muscle, gills, operculum, liver, kidney, heart and mesentery. Only two adults were reported from Africa in one study [*Allocreadium ghanensis* Fischthal et Thomas, 1972 and *Alloglossidium corti* (Lamont, 1921) by Simon-Oke][398] and another three species were reported from Latin America [*Crassicutis cichlasomae* Manter, 1936 by Salgado-Maldonado (2006),[399] *Saccocoelioides sogandaresi* Lumsden, 1963 by Salgado-Maldonado et al.,[400] and *S. cichlidorum* (Aguirre-Macedo et Scholz, 2005) by Aguirre-Macedo and Scholz],[401] and the validity of these reports requires confirmation. All these metacercariae require fish to be consumed by a fish-eating bird or mammal including man to complete their life-cycle. Twenty-seven of the 42 metacercariae are identified up to species level. Tilapias are mainly parasitised by metacercariae of the orders Diplostomida (families Diplostomidae Poirier, 1886–13 spp., Clinostomidae Lühe, 1901–1908 spp.) and Plagiorchiida (Heterophyidae Leiper, 1909–15 spp.). The clinostomid *Euclinostomum heterostomum* (Rudolphi, 1809) is widely distributed in African 'tilapia' (Figure 11), although the diplostomids occurring on the skin (*Uvulifer* Yamaguti, 1934 and *Bolbophorus* Dubois, 1935) and in the brain and eyes (*Diplostomum* von Nordmann, 1832 and *Austrodiplostomum* Szidat et Nani, 1951) are of major concern for aquaculture. Among the members of the family Heterophyidae parasitising tilapia, at least six species are considered economically or medically important as fish-borne zoonotic trematodes (FZT). Among them, *Heterophyes heterophyes* (Siebold, 1853), *Haplorchis pumilio* (Looss, 1896) and *Centrocestus formosanus* Nishigori, 1924 are the most important.[403]

2.13.2 | Pathogenicity

Trematode metacercariae may be free in organs such as brain and eyes or encysted in different parts of the fish body. The condition caused by metacercariae of diplostomids in the eye of fish (eye humours, retina and lens) is known as diplostomiasis; fish develop impaired vision associated to clinical signs such as cataract formation, exophthalmia, lens dislocation and eventually blindness. Grobbelaar et al.[404] reported high prevalence of infection by free-moving metacercariae of diplostomids in the aqueous and vitreous humours of *T. sparrmanii* and *C. rendalli* in the Okavango River, Botswana (Figure 12). Histopathological analyses revealed the rupture of the inner eye lining. In another study, in individuals of *O. mossambicus* and *O. aureus* infected with the metacercariae of *Austrodiplostomum compactum* (Lutz, 1928) in Mexico, García-Márquez et al.[406] reported lesions as diffuse corneal edema, severe diffuse eosinophilic optic neuritis, eosinophilic iridiocyclitis, conjunctivitis and severe cortical cataracts. Furthermore, the metacercariae encysted on the skin, gill filaments and heart are also of major concern for fish health. For instance, *C. formosanus*, considered as a parasite originally from Asian cyprinids and co-introduced with their hosts across the globe, causes pathological alterations on the gills leading to respiratory distress and in severe infections causes mortality (Figure 11).[402] The heterophyid *H. pumilio* is of special interest because some studies of experimental infections of tilapia with cercariae of *H. pumilio* evidenced severe pathological effects as haemorrhages in skeletal muscles in heavily infected fish because the cercariae migrate through connective tissue and the final localisation of the metacercariae is in skeletal structures (Sommerville, 1982). Finally, the condition caused by metacercariae of *Uvulifer* spp. encysted on the skin of the fish causing an external melanised host inflammatory response is known as black spot disease; this

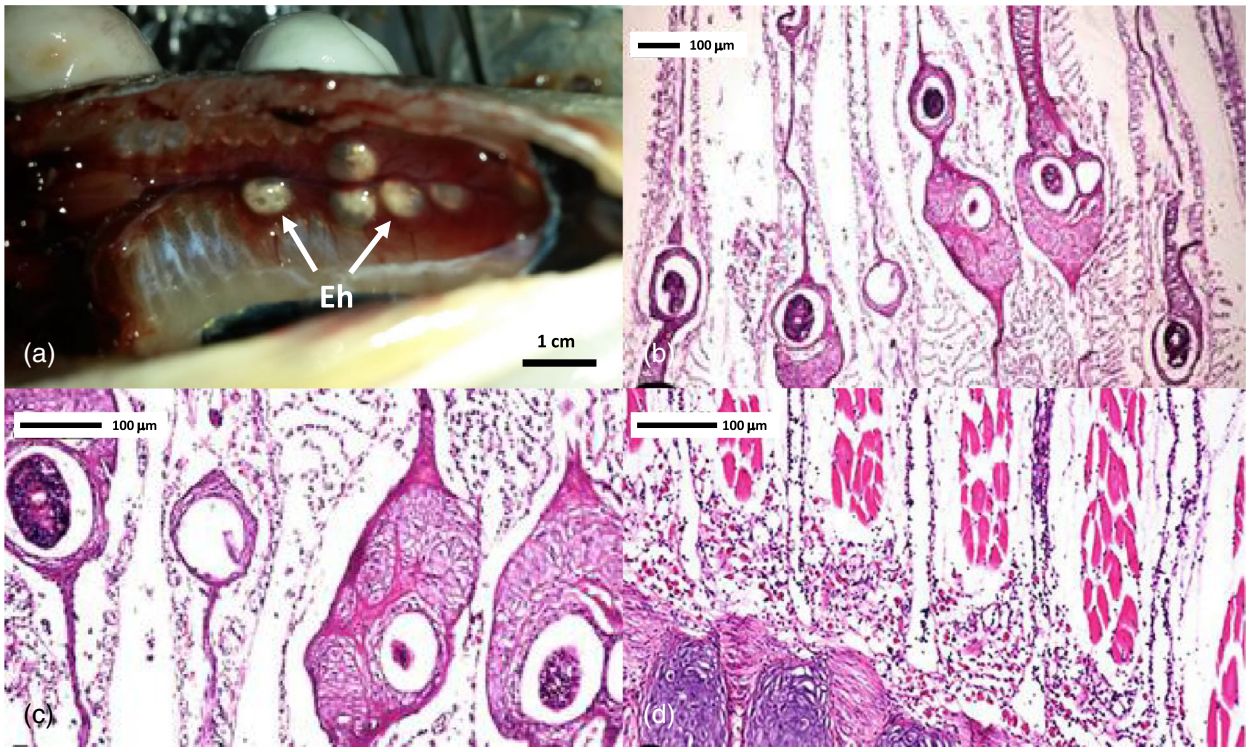

FIGURE 11 (a) Encysted metacercariae of *Euclinostomum heterostomum* (Rudolpi, 1809) (Eh) in the body cavity of *Oreochromis niloticus* (image courtesy of Liesl Van As and Andri Grobelaar from the Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, Republic of South Africa and the Editorial Office of African Zoology). (b) Photomicrograph of histological gill sections of *O. niloticus* infected with numerous metacercarial cysts of *Centrocestus formosanus* (Nishigori, 1924) within the gill filament. (c) Photomicrograph of gills showing expansive proliferation of cartilage of gill filament which surrounds the metacercariae of *C. formosanus*, with subsequent distortion of the normal gill architecture; (d) Photomicrograph of histological infected *O. niloticus* gill sections showing extensive edema associated with congestion of the blood vessels and intense inflammatory cell infiltration (images courtesy of Mahmoud Abou-Okada from the Faculty of Veterinary Medicine, Cairo University, Egypt reproduced from Abou-Okada et al.[402])

disease causes slow growth, deformities and increases the mortality rate of freshwater fish (Figure 12).[405] These authors analysed the effect of black spot disease in *O. niloticus* in Egypt, and even though they did not report large mortalities or morphological deformities of fish, apparently harvest weight of fish declined as severity of infection increased. They also observed that females were more susceptible to *Uvulifer* infections than males, although their loss of harvest weight on severe infection levels was greater than females.

2.13.3 | Global translocations

In sharp contrast with the pattern shown by monogeneans and the spillover across the globe along with the introduction of tilapia, trematodes associated with this group of cichlids have not been translocated; these parasites are less host-specific in the second intermediate host, but they possess complex life-cycles which involves three hosts and greater specificity may occur in the first and definitive hosts. Even though tilapia act as the second intermediate host harbouring the metacercarial stage, and fish-eating birds or mammals serve as their definitive hosts increasing the potential of dispersal, no species of trematode found thus far in their native range in Africa has been found in places where tilapia have been introduced. The lack of the same species of first intermediate host (a mollusc) in the areas where tilapias are introduced may preclude the completion of their life cycle. For instance, the metacercariae of *E. heterostomum* are widely distributed in Africa; yet, they have never been found in tilapia introduced in Asia or in the Americas. Even other clinostomids such as *Clinostomum phalacrocoracis* Dubois, 1930, *C. cutaneum* Paperna, 1964 and *C. tilapiae* Ukoli, 1966 are also found exclusively in Africa.[407] The fact that species of heterophyids such as *C. formosanus* (originally described from Taiwan Province of China) and *H. pumilio* (first described in Egypt) are found everywhere in the world is not related to the translocation of tilapia; most likely the widespread distribution of these species is due to the translocation of the first intermediate host, the gastropod, *Melanoides tuberculata* (Müller, 1774) or definitive hosts. In addition, the metacercariae of both species are considered as invasive alien species[408–410]; they display extremely wide host specificity and are also found in a wide variety of fish species across the globe. Conversely, tilapia introduced to the Americas show evidence of host-switching events of trematodes from native cichlids (and other freshwater fish) to farmed tilapia. For instance, the metacercariae of *A. compactum*, a diplostomid trematode parasite of cormorants and widely distributed across the Americas and the

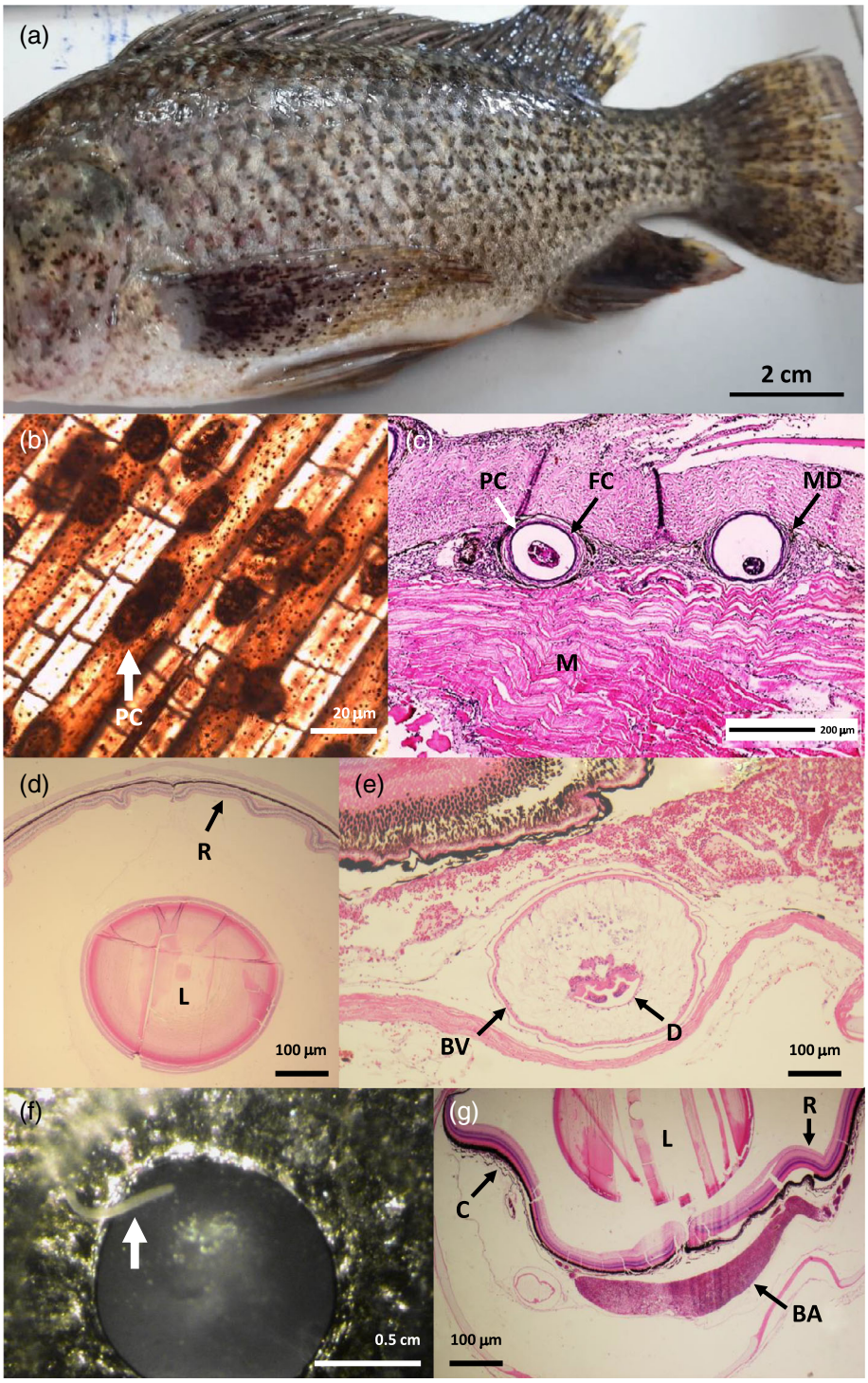

FIGURE 12 (a, b) Gross and microscopic examination of *Oreochromis niloticus* heavily infected with metacercariae of *Uvulifer* sp. (Trematoda: Diplostomidae) (black spot disease). (c) Photomicrograph of a histopathological section of *O. niloticus* skin and muscle infected with *Uvulifer* sp. showing the encysted metacercariae; FC, fibrous capsule; M, muscle; MD melanin deposits; PC, parasite cyst. Images courtesy of Harrison Charo-Karisa, Shimaa E. Ali and John A.H. Benzie from WorldFish, Abbassa, Egypt and Penang, Malaysia. Images reproduced from Charo-Karisa et al.[405] (d) Photomicrograph of a normal, non-infected fish eye with the retinal layers intact; (L) lense. (e) An encapsulated diplostomid (D) within a blood vessel (BV). (f) Gross examination of the eye of *Coptodon rendalli* showing a free-moving diplostomid metacercariae. (g) Photomicrograph of an infected eye of *C. rendalli* showing the accumulation of blood (BA), which has torn the retina (R) and choroid (C) from the sclera. Images courtesy of Liesl Van As and Andri Grobelaar from the Faculty of Natural and, University of the Free State, Bloemfontein, Republic of South Africa. Images reproduced from Grobelaar et al.[404]).

causative agent of diplostomiasis is commonly found in native species of cichlids[411]; however, it has been reported in wild and farmed Nile tilapia of Mexico and Brazil.[412,413]

2.13.4 | Research

Ongoing research on trematodes of tilapias includes the taxonomic report of their presence as a part of their parasite fauna in fish farms, or in aquatic environments where tilapia have been disseminated globally.[407,414,415] Investigations that can be applied practically in aquaculture are designed to assess the epidemiology of FZT using *O. niloticus* as a model because they are highly consumed in several countries. Some studies evaluate the risk of FZT because they are potentially transmissible to humans.[416] FZT are highly prevalent in countries where food traditions include eating raw or improperly cooked fish, such as Thailand, Cambodia, Laos, Vietnam or Korea.[403,417] *Oreochromis niloticus* and their parasites have, furthermore, been used for biomonitoring. Some studies have addressed the relationship between some parasitic infections, including those by trematode metacercariae, and the immunological health condition of *O. niloticus* through gene expression analysis and the assessment of the toxicity of some heavy metals.[166] The use of antiparasitic agents has been assessed to control *O. niloticus* infected with *C. formosanus*, which causes respiratory distress due to pathological alterations to the gills. Abou-Okada et al.[402] assessed the efficacy of acriflavine on *O. niloticus* infected with *C. formosanus* (and with *Trichodina centrostrigeata* Van As et Paperna, 1983) and found that application of 10 mg/L acriflavine for 7-days provided a 91% reduction in metacercariae colonising the gills. There are, however, regulatory limitations on use of acriflavine and it appears to have limited efficacy in the treatment of established infections.

2.14 | Cestoda Carus, 1863 (Obazoa: Opisthokonta: Metazoa: Platyhelminthes)

2.14.1 | Taxonomic identity

Cestodes are flatworms with no digestive system, many of which are elongated with multiplicated genital organs. Adult tapeworms are rare in tilapia except for the Asian fish tapeworm *Schyzocotyle acheilognathi* (Yamaguti, 1934), one of the most successful invasive freshwater fish parasites.[418] This invasive parasite is distributed across all continents except Antarctica, has been reported from >300 freshwater fish species and has been reported to cause mortality in naïve endemic hosts.[418] This parasite has an indirect life-cycle and uses copepods as its intermediate host and freshwater fish as its paratenic or definitive host.[418] In addition, there is another finding of another adult tapeworm, *Proteocephalus bivitellatus* Woodland, 1937 from *Tilapia* sp. (probably *C. zillii*) from Sierra Leone by Woodland.[419] This appears to be a valid species, but no other records of adult proteocephalids in tilapia have been reported to date.

Cestode larvae (metacestodes) are more commonly reported from tilapia, however, as their second intermediate or paratenic host. Most belong to the family Gryporhynchidae (Cyclophyllidea) that use fish-eating birds as their definitive host and copepods as their first intermediate host.[420] The larval stage of gryporhynchids, a merocercoid, is typically encysted or, rarely, free-moving, and is typically small in size at around 1–2 mm (with the exception of the non-encysted *Amirthalingamia macracantha* (Joyeux et Baer, 1935) which can grow up to 17 mm) and can be easily overlooked among internal organs including mesenteries, intestinal and stomach wall, liver, and/or gall-bladder.[421–424] Gryporhynchid merocercoids are easily recognised by the presence of a scolex armed with two rows of rostellar hooks and four suckers.[422,423] The identification of these merocercoids is based almost entirely on morphology and the number of rostellar hooks, but accurate identification depends on the proper flattened preparation of larvae for microscopic evaluation.[422,423]

There are several other cestode larvae (plerocercoids) that have rarely been detected in tilapia. These include the bothriocephalid plerocercoids of *Tetracampos ciliotheca* Wedl, 1861 (syn. *Polyonchobothrium ciliotheca*) or *P. polypteri* (Leydig, 1853) from *O. niloticus* (Figure 12),[425–427] but Eissa et al.[428,429] reported the presence of adult *T. ciliotheca* in 1%–6% of *O. niloticus* specimens and their hybrids from Egypt. This record is unusual and may be incorrect, because *T. ciliotheca* typically matures almost exclusively in catfish of the genus *Clarias* and those of *P. polypteri* occur almost exclusively in bichirs of the genus *Polypterus*.[430] Larvae (plerocercoids) of *Proteocephalus glanduligerus* (Janicki, 1928) (Onchoproteocephalidea) were found in the intestine of *O. mossambicus* from the Ndumo Game Reserve in South Africa (O. Kudlai; personal obs.). Tilapia most likely serve as accidental or paratenic hosts of this tapeworm that matures in clariid catfish.[431] Additional, clearly erroneous records include those of plerocercoids of the human broad tapeworm *Dibothriocephalus latus* (Linnaeus, 1758) (syn. *Diphyllobothrium latum*) from the intestine of *O. niloticus* reported from Côte d'Ivoire,[432] Kenya[433] and Nigeria[434] without supporting evidence. The broad fish tapeworm is not able to infect tilapia and moreover, does not occur in the tropics.[435] In addition, *Cyathocephalus* sp. (Spathebothriidea) has been reported from the internal organs and body cavity of *O. shiranus* in Malawi,[436] a cestode which has a natural distribution only throughout the Palearctic.[437] Likewise, the report of *Caryophyllaeus* sp. (Caryophyllidea) from tilapias in Nigeria (Ukpai 2001) which also is naturally distributed only in the Palearctic[438] or *Wenyonia* sp. (Caryophyllidea) from the intestines of *O. aureus* and other tilapia species in Nigeria[439] maturing exclusively in catfish in Africa[440] also represent clear misidentifications.

2.14.2 | Pathogenicity

The pathological effects *S. acheilognathi* exerts on cultured tilapia have not been documented, but it is likely that this cestode causes mechanical damage and inflammation of the intestinal mucosa, resulting in anorexia, weight loss, abdominal distension, anaemia and, behaviourally, a tendency to swim at the water surface.[319,441–443] This tapeworm is easily identified by its characteristic heart-shaped, unarmed

scolex bearing two deep, sucker-like bothria (Figure 13a).[418] *Schyzocotyle acheilognathi* is not a typical parasite of tilapia but may be infective to tilapia because of its low specificity, and the global, cosmopolitan distribution of both.[418]

While the risk posed by gryporhynchid merocercoids to the health of farmed tilapia may be minor, the tissue tropism of some species for the liver can have serious negative effects on host health, if present in sufficient numbers and particularly in juvenile fish.[424] Some unidentified merocercoids are reported from the intestinal wall of cultured and wild *O. niloticus* from Ethiopia, Kenya and Uganda, typically with low prevalence except in a wild population of *O. niloticus* in Kenya (14%).[17] These merocercoids from the intestinal wall of tilapia are represented by several species, including *Cyclustera magna* (Baer, 1959), *Parvitaenia macropeos* (Wedl, 1855) or *Neogryporhynchus lasiopeius* Baer et Bona, 1960 (Figure 13c).[423] Florio et al.[17] examined the histopathology of these larvae and showed encysted merocercoids surrounded by epithelioid cells, sometimes by fibroblasts and lymphocytes. The cyst showed serrated margins with cell detachments and the presence of red blood cells, possibly due to mechanical erosion caused by the larvae. The wall around merocercoids was hypertrophic and chronically inflamed. The cysts were frequently observed to protrude on the outer surface and/or into the lumen.[17]

2.14.3 | Global translocations

Schyzocotyle acheilognathi has been reported from both wild and cultured populations of *O. mossambicus* and *O. niloticus* from South Africa,[200] Cuba,[319] Mexico[399,441] and Nigeria[444] (Table S2).

The first record of a gryporhynchid from a tilapia was *A. macracantha* (Figure 13b) reported by Bray (1974) who isolated specimens from the liver of *O. niloticus* and the intestine of a reed cormorant, *Microcarbo africanus* (Gmelin, 1789), in Sudan. More recently, this species has also been detected in cultured *O. aureus* × *O. niloticus* hybrids in Israel.[424] To date, approximately 10 species have been recorded from domesticated and wild populations of tilapia, mainly from Africa, but also from Israel and Puerto Rico.[319,445]

2.14.4 | Research

There are no detailed studies on tapeworms in tilapia, because almost exclusively only gryporhynchid larvae are reported and tapeworms mostly are not important pathogens of tilapia.

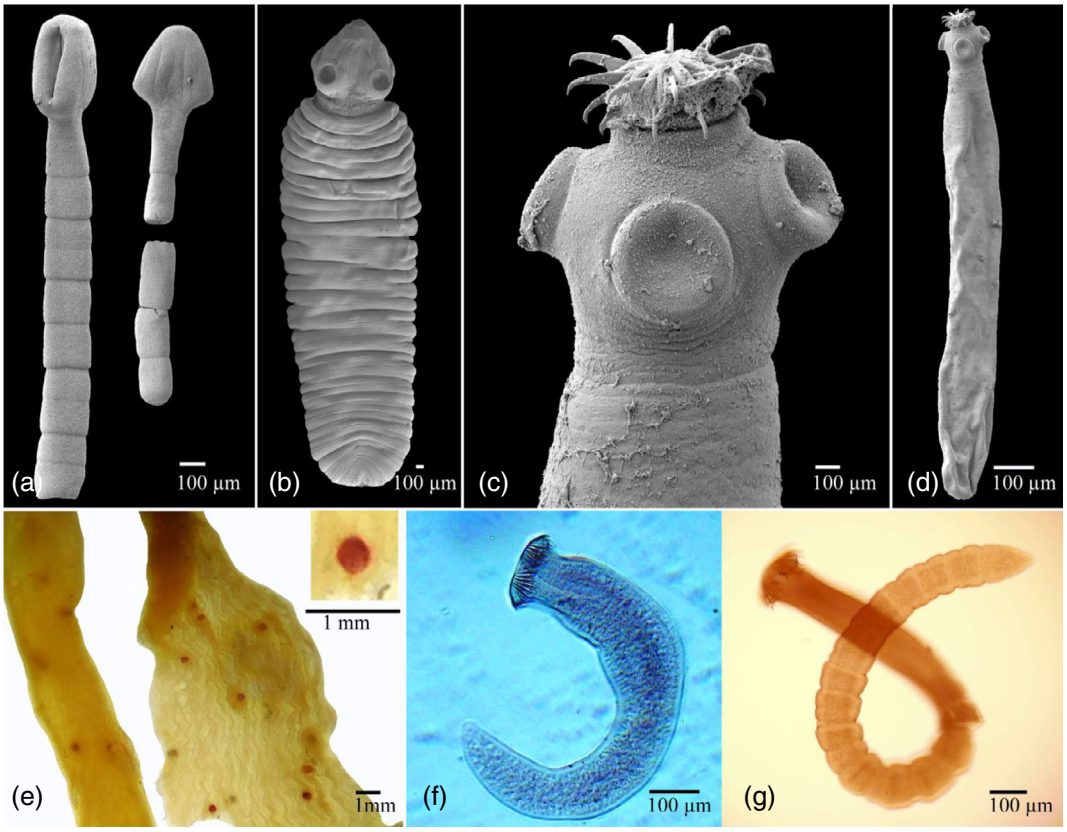

FIGURE 13 Cestodes of tilapias. (a) Immature *Schyzocotyle acheilognathi* (Yamaguti, 1934) from *Symphysodon aequifasciatus* from culture in the Czech Republic. (b) Larval *Amirthalingamia macracantha* (Joyeux et Baer, 1935) from tilapia hybrids in Israel. (c, d) Scolex and whole larva of *Neogryporhynchus lasiopeius* Baer et Bona, 1960. (e) Intestine of *Oreochromis mossambicus* from South Africa infected with gryporhynchid larvae (red). (f) Larva of *Tetracampos ciliotheca* Wedl, 1861 from the intestine of *Clarias gariepinus* from Malawi. (g) Larva of *Polyonchobothrium polypteri* (Leidig, 1853) Lühe, 1900 from the intestine of *Lates niloticus* from Kenya.

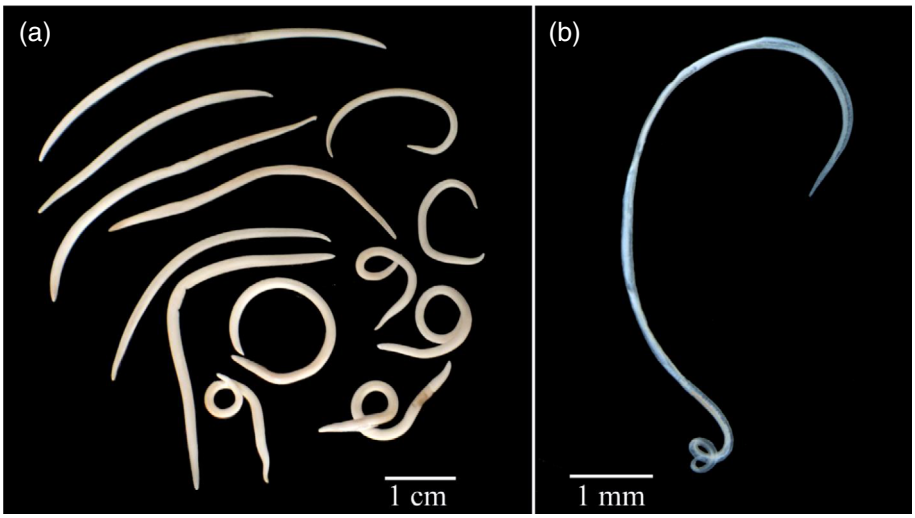

FIGURE 14 Nematodes of tilapias. (a) *Contracaecum* Type 2 larvae from *Oreochromis niloticus* from Egypt. (b) Stage L4 larva of *Rhabdochona* (*Globochona*) *paski* Baylis, 1928 from *O. niloticus* from Lake Victoria, Kenya

2.15 | Nematoda Diesing, 1861 (Obazoa: Opisthokonta: Metazoa: Ecdysozoa)

2.15.1 | Taxonomic identity

Nematodes are slender, cylindrical helminths characterised by a tubular digestive system and are covered by a cuticle. There are several records of nematodes in tilapia, but only a few of adult worms. Most records of adult nematodes are from their non-native range, such as *Goezia nonipapillata* Osorio-Sarabia, 1981 (Anisakidae) reported from *C. zillii*, *O. aureus* and *O. mossambicus* in Mexico (Michoacan),[446,447] *Rhabdochona kidderi texensis* Moravec et Huffman, 1988 (Rhabdochonidae) from *O. mossambicus* in the USA (Texas), and unusual reports of European *Schulmanela petruschewskii* (Shulman, 1948; Capillariidae) from cultured *O. aureus* in Cuba.[447,448] Only *Gendria tilapiae* Baylis, 1930 (Quimperiidae) was described from native *S. galilaeus* from Mali, but this species has not been reported from tilapias since Moravec.[449] There are records of camallanid nematodes such as *Paracamallanus cyathopharynx* (Baylis, 1923), *Paracamallanus laeviconchus* (Wedl, 1861) and *Procamallanus* (*Spirocamallanus*) *spiralis* Baylis, 1923 from *O. niloticus* or *O. mossambicus* in Egypt,[428] Nigeria,[450] the Republic of Benin[451] and South Africa,[452] *Procamallanus* (*S.*) *rebecae* (Andrade-Salas, Pineda-López et García-Magaña, 1994) from *O. aureus* in Mexico[447] and *Procamallanus* (*Spirocamallanus*) sp. from *O. niloticus* in Pakistan.[453] Identification of these species may be unclear because these nematodes are not specific parasites of tilapia.[449] Other reports of adult nematodes from tilapia represent misidentifications, such as *Hysterothylacium habena* (Linton, 1900) (Raphidascarididae) reported from *O. aureus* in freshwater in Mexico (Michoacan),[446,447] because it is a marine nematode whose distribution does not include Mexico.[454] *Aplectana chamaeleonis* (Baylis, 1929) (Cosmocercidae) was reported by Chen[455] from *O. niloticus* in Lake Langano, Ethiopia, but this species is a specific parasite of reptiles. Moravec[449] mentioned that this finding was probably a misidentification of a *Labeonema* sp. (Atractidae), but no species of this genus has been reported in tilapia and this finding may represent a post-cyclic infection in an atypical host.

Larval nematodes are frequently reported from various tissues of tilapia worldwide. Most of these reports refer to third-stage larvae (L3) of *Contracaecum* spp. (Anisakidae; Figure 14), which use fish-eating birds as their definitive hosts. These parasites are important because they have zoonotic potential. These larvae are mostly encapsulated in the internal organs and body cavity of tilapia with prevalence that can reach >50% and infection intensity that can reach up to 117 individuals per fish.[456] These L3 larvae have been frequently found in wild and farmed *O. niloticus* and *O. mossambicus* from Egypt,[457] Ethiopia,[456,458] Kenya,[459] South Africa,[452] Uganda[17] or Zimbabwe,[460] and also farms in Brazil, Mexico, El Salvador[17] and Peru.[461,462] Identification of *Contracaecum* spp. is complicated, but Moravec and Scholz[458] designated specimens from African tilapia as *Contracaecum* Type 2, although this may include several species.

There are reports of *Gnathostoma* spp. (Gnathostomatidae) from the musculature and internal organs of tilapia. Awosolu et al.[463] detected *G. spinigerum* Owen, 1836 in 17% of *O. niloticus* examined from Igbokoda River, Nigeria. Most reports are from tilapia in Mexico (Oaxaca, Puebla, Sinaloa and Veracruz) where three species, including *G. turgidum* Stossich, 1902, were reported from *O. aureus*, *O. mossambicus* and *O. niloticus*[464] and from *O. mossambicus* from Thailand.

Third-stage (L3) larvae of *Anguillicoloides crassus* (Kuwahara, Niimi et Itagaki, 1974) (Anguillicollidae), a typical eel pathogen, were found in the peritoneum and abdominal muscles of cultured *O. niloticus* in Belgium[465] and Egypt.[466] Tilapia can serve as a paratenic host for nematode larvae of this species. The fourth-stage (L4) larva of *Rhabdochona* (*Globochona*) *paski* Baylis, 1928 (Rhabdochonidae) was reported from *O. niloticus* from Lake Victoria, Kenya and DR Congo (Zaire) as *Rhabdochona congolensis* (Campana-Rouget, 1961) by Moravec[449] (Figure 14b). Species of *Amplicaecum* Baylis, 1920 (Ascarididae), *Camallanus* sp., *Capillaria* sp. (Capillariidae), *Cucullanus* sp. (Cucullanidae), *Eustrongylides* sp. (Dioctophymidae), *Procamallanus* sp., *Rhabdochona* sp., *Spiroxys* sp. (Gnathostomatidae) or even *Necator americanus* (Stiles, 1902)

(Ancylostomatidae) and *Porrocaecum* sp. (Toxocaridae) have been reported from *O. niloticus* and *O. mossambicus* in Africa (Table S2)[439,449,463,467] and the Neotropics,[447] but some of these findings could be misidentifications or accidental infections.

2.15.2 | Pathogenicity

Nematode larvae can invade any tissue of the host, including the pericardium, which can have negative effects on the health of the host. Tissue response to infection by *Contracaecum* spp. larvae includes the formation of epithelioids, the fibrous encapsulation of larvae, which can lead to mesenteric infections with extensive fibrosis and visceral adhesions in larger fish.[468,469]

Gnathostoma spp. use a wide range of vertebrates as paratenic hosts, including humans, where they can cause a serious disease, gnathostomiasis, while the adults parasitise in the stomach of mammals.[470] Humans become infected by eating raw or undercooked fish infected with L3 larvae. The disease is characterised by migratory inflammatory edema with larvae encapsulated in the stomach or ocular cavity. Most human cases (about 25,000 reported cases) are caused by *G. binucleatum* Almeyda-Artigas, 1991 in the Neotropical region and *G. spinigerum* Owen, 1836 with a cosmopolitan distribution, including Africa.[470]

2.15.3 | Global translocations

The nematodes of tilapia are not well studied or understood. Most records of adults are not from the native range of tilapia but from the introduced range,[447] except for a few camallanid species, but their identification should be verified.[449] Nematode larvae (mainly those of *Contracaecum* spp.) are also more frequently reported from the introduced range of the fish, but their identification requires the use of specific molecular markers which were not applied in the initial studies.

2.15.4 | Research

The most economically important nematodes in tilapia aquaculture are L3 of *Contracaecum* spp., which are widespread. Their control is difficult because they are associated with fish-eating birds. In addition, the larvae of the genus *Gnathostoma* have zoonotic potential and tilapia infected with them can also infect humans.

2.16 | Acanthocephala Koelreuter, 1771 (Obazoa: Opisthokonta: Metazoa: Gnathifera: Syndermata: Acanthocephala)

Acanthocephalans are a small group of endoparasites closely related to rotifers (Wey-Fabrizius et al. 2014).[471] They are characterised by a spiny eversible proboscis that anchors the parasite to the intestine of their definitive vertebrate hosts. Acanthocephalans have an indirect life-cycle with an intermediate invertebrate host.

2.16.1 | Taxonomic identity

Few species of acanthocephalans are reported from tilapia. These parasites can be identified based on morphological characteristics of the proboscis, the size, shape, number and distribution of proboscis hooks, the shape and dimensions of the trunk and the presence and distribution of trunk spines.[472] Most records of adult acanthocephalans in tilapia involve *Acanthogyrus* (*Acanthosentis*) *tilapiae* (Baylis, 1947; Quadrigyridae), a widely distributed intestinal parasite of >40 freshwater fish species in Africa.[473,474] This species has been reported from domesticated and wild populations of *C. rendalli*, *C. zillii*, *O. andersonii*, *O. aureus*, *O. leucostictus*, *O. macrochir*, *O. niloticus*, *O. spilurus*, *S. galilaeus* and *S. melanotheron* from Burkina Faso, Chad, Egypt, Ethiopia, Kenya, Madagascar, Nigeria, Congo, Senegal, Uganda and Zambia (Table S2).[473] Adult *Acanthogyrus* (*A.*) sp., most probably *A.* (*A.*) *tilapiae*, are reported from farmed and wild *C. zillii*, *O. macrochir*, *O. niloticus*, *O. mossambicus* and *S. galilaeus* from Egypt, Ethiopia, Kenya, Nigeria, Uganda and Zimbabwe (Table S2).[467,475–477] Tilapia become infected with *A.* (*A.*) *tilapiae* after consuming its unidentified invertebrate intermediate host, copepods of the genus *Cyclops* Müller, 1785.[478] *Acanthogyrus* (*A.*) *tilapiae* has a short cylindrical proboscis armed with 24 hooks arranged in three circles of eight hooks each and a trunk armed only anteriorly with circles of spines that are usually dorsally incomplete.[479] This acanthocephalan can occur at high prevalence in cultured (>65%)[480] and wild (>78%)[481] tilapia.

Other adult acanthocephalans are infrequently reported from cultured and wild tilapia in Africa, Asia and Oceania. An unidentified species of *Paragorgorhynchus* Golvan, 1957 (Rhadinorhynchidae) was reported in the intestine of wild *C. zillii* from Kenya[477] and a *Telosentis* sp. (Illiosentidae) was detected in wild populations of invasive *O. mossambicus* in Australia.[83] Adult *Neoechinorhynchus* (*Neoechinorhynchus*) *rutili* (Müller, 1780) (Neoechinorhynchidae) were recorded from wild *C. zillii*, *O. niloticus* and *S. galilaeus* from Nigeria,[482–484] and poorly described specimens ascribed to *N.* (*N.*) *quinghaiensis* Liu, Wang et Yang, 1981 were recorded from wild *O. niloticus* from the Philippines.[485] *Neoechinorhynchus* (*N.*) *quinghaiensis* was reported as *Neoechinorhynchus* sp. or *Acanthogyrus* sp. from cultured and wild *O. niloticus* in the Philippines.[486–489] Records of *N.* (*N.*) *rutili* and *N.* (*N.*) *quinghaiensis* in tilapia seem to be erroneous, because most species of *Neoechinorhynchus* are morphologically difficult to distinguish.[490] *Neoechinorhynchus* (*N.*) *rutili* mature in fresh- and brackish water fish from the northern Holarctic Region,[491] while *N.* (*N.*) *quinghaiensis* infects cyprinids from China,[492] placing doubt on these identifications.

Immature *Pallisentis* (*Pallisentis*) *nandai* Sarkar, 1953 (Quadrigyridae) were reported in the liver of farmed *O. niloticus* from Bangladesh.[493] In this unusual site of infection this acanthocephalan could not produce eggs.[494] *Pallisentis* (*P.*) *nandai* occurred at 23% prevalence in farmed *O. niloticus* from Bangladesh,[493] but this parasite appears unusual in tilapia.

The infective larval cystacanths of acanthocephalans are not typical parasites of farmed or wild tilapia. Infective stages of *Polyacanthorhynchus kenyensis* Schmidt et Canaris, 1967 (Polyacanthorhynchidae) use tilapia as paratenic hosts, but the identities of the intermediate and definitive hosts remain unknown.[495] Cystacanths of *P. kenyensis*

are reported encapsulated in the liver of 27% of wild *C. zillii* and 44% of *O. leucostictus* from Kenya.[496] Cystacanths of *Polymorphus spindlatus* Amin et Heckmann, 1991 (Polymorphidae) were observed free in the intestine of wild *O. niloticus* from Peru.[497] This species uses black-crowned night herons (*Nycticorax nycticorax* [Linnaeus, 1758]) as its definitive host,[498] and represents the only acanthocephalan species reported in tilapia from the Americas. Briones et al.[485] ascribed a single specimen of *Bolbosoma* sp. (Polymorphidae) from the mesenteries of *O. niloticus* from the Philippines, but it is obvious from their figure 3 that this worm is a species of *Southwellina* Witenberg, 1932, which mature in fish-eating birds.[499] Cystacanths of *Bolbosoma* spp. are, furthermore, recorded from marine fish and adults infect cetaceans.[500]

2.16.2 | Pathogenicity

The penetration of the proboscis of *A.* (*A.*) *tilapiae* into the intestine destroys the mucosal folds, causes lacerations of the intestinal villi from the proboscis hooks and provokes severe degeneration and necrosis of the mucosal epithelium.[327,481] Other changes observed in infected tilapia include desquamation of the mucosa, interstitial oedema and enteritis.[481,501,502] Aggregated infiltration of eosinophils, fibroblasts, lymphocytes and macrophages occurs at the site of attachment in response to chronic inflammation of the tissue.[481,502] Little is known about the pathological effects of adults of other acanthocephalan species in cultured tilapia, but it is likely that the submucosal lesions, loss of the mucosal layer, decreased haematocrit and red blood cell counts observed in *N.* (*N.*) *quinghaiensis* (syn. *Acanthogyrus* sp.) infections in *O. niloticus* in the Philippines[489] are typical.

The threat that immature *P.* (*P.*) *nandai* poses to the health of cultured tilapia is unknown, but these parasites probably cause mechanical damage, local necrosis, and hepatic inflammation.[503] Pathogenesis of *P. kenyensis* in tilapia is unknown, but its cystacanths may cause local necrosis and inflammation of the liver like that caused by cystacanths of other acanthocephalan species in other fish.[503]

2.16.3 | Global translocations

There is insufficient evidence to indicate that acanthocephalans have been translocated with tilapia, but Golvan[504] suggested that *A.* (*A.*) *tilapiae* was introduced to Madagascar with non-native cichlids from mainland Africa. Translocation of this parasite to native cichlids of Madagascar was not confirmed in the thorough survey there by Šimková et al.[337]

2.16.4 | Research

To better understand the diversity, distribution and life-cycles of acanthocephalans of tilapia, molecular and morphological approaches on larval and adult stages need to be integrated. Sequences of nuclear and mitochondrial genes are necessary to clarify the identity of acanthocephalans[505,506] because of interspecific homogeneity of morphological characters.[507] Future research includes histopathological investigation of alterations caused by larval cystacanths and adult acanthocephalans in tilapia to identify threats to cultured fish and better understand if treatment would be beneficial.

Metabarcoding using high-throughput sequencing technology has advanced our understanding of the endoparasite diversity of fish.[508] Using this technology, Elsaied et al.[509] detected a *Neoechinorhynchus*-like operational taxonomic unit (OTU) in the gut content of wild *O. niloticus* from Lake Nasser, Egypt. The taxonomic assignment of this OTU as *Neoechinorhynchus* by Elsaied et al.,[509] however, appears erroneous; *Acanthogyrus* (*A.*) *tilapiae* is the only acanthocephalan reported from tilapia in Lake Nasser.[502,510] Elsaied et al.[509] extracted DNA from eggs released by gravid females to the lumen of infected *O. niloticus*, and this approach has merit for non-destructive detection and identification of all endoparasites.

2.17 | Pancrustacea Zrzavý et Štys, 1997 (Crustacea) (Obazoa: Opisthokonta: Metazoa: Ecdysozoa: Pancrustacea)

Four groups of parasitic crustaceans (Copepoda, Branchiura, Pentastomida and Isopoda) can infect tilapias. Copepods have short cylindrical, segmented bodies. Branchiurans are obligate parasites with hooked maxillae or sucking discs. Pentastomids are elongate segmented crustaceans with five anterior protruberances; two pairs of hooks for attachment and the mouth. Isopods have rigid, segmented exoskeletons, two pairs of antennae, seven pairs of jointed limbs on the thorax and five pairs of branching appendages on the abdomen that are used in respiration. Crustaceans are mostly ectoparasites with direct life-cycles, and only pentastomids are endoparasites of internal organs with complex life cycles using some fish (including tilapias) as their intermediate host and tetrapods (e.g. crocodiles) as their definitive hosts. Some Copepoda Edwards, 1840 and all Branchiura Thorell, 1864 are ectoparasites of fish including tilapia and inhabit the gills, fins and skin.[511,512] The morphology of adult copepod parasites is adapted for attachment with appendages that are modified into hooks and suckers or cuticular outgrowths of the carapace. They are loosely host specific, most species infecting more than one host species.

The most important crustaceans for tilapias are copepods and branchiurans.

2.18 | Copepoda Edwards, 1840 (Obazoa: Opisthokonta: Metazoa: Ecdysozoa: Pancrustacea)

The parasitic copepods are a diverse group, and some are highly modified as adaptations to parasitism. Common features are a

complete or partial loss of segmentation, paired egg sacs that hang from the genital somite of the adult females for the duration of embryonation, some instars are lacking in the larval development and sexual dimorphism occurs (Figure 15b–d, k–m). Larval stages in some families have morphology like their free-living relatives and are free-living for much of their lifecycle. The simplest adaptations to parasitism are observed in Ergasilidae, where adult females have grasping appendages and retain segmentation of the thorax. In the highly modified Lernaeidae, however, in females the second antennae are extensively modified, segmentation is lost, the ovaries have attained enormous proportions and the thorax has consequently enlarged, elaborate attachment structures have developed, and the second maxillae are transformed into powerful attachment structures.

The adult females of *Ergasilus* von Nordmann, 1832, *Lernaea* Linnaeus, 1758, *Opistholernaea* Yin, 1960 and *Lamproglena* von Nordmann, 1832 attach permanently to a host after insemination. A single female produces up to 30 eggs at a time in egg sacs (Figure 15b–d, k–m). No intermediate hosts are required; a single egg-bearing female or two larvae introduced via transportation of infected fish or water can establish an infection.

Adult female *Lamproglena monodi* Capart, 1944 attaches with modified maxillulae on the gills of their hosts (Figure 15a–c). Capart[513] reported it from *Serranochromis thumbergi* (Castelnau, 1861) from Lake Mweru, and later also from *Haplochromis nubilus* (Boulenger, 1906) from the Molindi River, *Haplochromis macrops* (Boulenger, 1911) from the Rutshuru River, *Haplochromis eduardii* Regan, 1921 and *Haplochromis serridens* Regan, 1925 from Lake Edward, *Pseudocrenilabrus philander* (Weber, 1897) from the Kafubu River and *Hemichromis fasciatus* Peters, 1857 from the Legide River, the Congo. In Lake Victoria, Gobbin et al.[514] reported *L. monodi* from 14 sympatric Lake Victoria cichlids [*Mbipia lutea* (Seehausen et Bouton, 1998), *M. mbipi* Lippitsch et Bouton, 1998, *Neochromis gigas* (Seehausen et Lippitsch, 1998), *N. omnicaeruleus* (Seehausen et Bouton, 1998), *Neochromis* sp., *N. rufocaudalis* (Seehausen et Bouton, 1998), *Pundamilia pundamilia* Seehausen et Bouton, 1998, *P. nyererei* (Witte-Maas et Witte, 1985), *Pundamilia* sp., *Lithochromis* sp., *Haplochromis cyaneus* (Trewavas, 1935), *Parachromis chilotes* (Boulenger, 1911), *P. sauvagei* (Pfeffer, 1896) and *Parachromis* sp.].

In Egypt, Ibraheem and Izawa[515] reported this species from *O. niloticus*, *S. galilaeus* and *C. zilli*. In Brazil, Martins et al.[516] reported a *Lamproglena* sp. in the Guandu River, State of Rio de Janeiro and in the State of Santa Catarina. It was later identified and redescribed as (co)introduced *L. monodi* present on the indigenous *Astronotus ocellatus* (Agassiz, 1831) and *Cichla ocellaris* Bloch et Schneider, 1801, and Azevedo et al.[517] reported this species from introduced *O. niloticus* and *T. rendalli* (Boulenger, 1897). In the Philippines, Yambot and Lopez[518] reported *L. monodi* from cultured *O. niloticus*.

Ergasilus species infect tilapia in Africa.[511] They attach to their host's gills or skin with modified antennae and feed on tissue (Figure 15d–g). They are not strictly host specific.

The anchor worms, *Lernaea barnimiana* Hartmann, 1865, *L. hardingi* Fryer, 1956, *L. lophiara* Harding, 1950, *L. palati* Harding, 1950 and *L. tilapiae* Harding, 1950 have been reported on tilapia species in Africa[511] but these species have not been recorded outside Africa, except for a report of *L. lophiara* on a translocated population of *O. mossambicus* in Thailand.[334] Female *Lernaea* spp. can be observed macroscopically, and the head and anterior part of the thorax are embedded in the host muscle, under scales and on fins (Figure 15h). It attaches firmly with cuticular outgrowths forming anchors (Figure 15i), the minute head appendages scrape host tissue into the mouth, while the egg-string bearing thorax and the abdomen protrude from the host.[519]

Opistholernaea laterobranchialis (Fryer, 1959) is reported from *O. niloticus*, *O. andersoni* and *O. macrochir* from the Nile and Zambesi rivers.[520–522] The parasite head embeds in the palate of the fish and grows through the bony tissue to protrude through the eye socket, where it forms a capsule. The egg-bearing thorax and abdomen hangs from the roof of the buccal cavity of the host. Grobler[522] reported that the parasite may reach 18 mm in length and can be removed only after dissection of the bony tissue.

2.18.1 | Pathology

Ibraheen[523] described the pathological changes caused by *L. monodi* on the gill lamellae: attachment of the females is followed by proliferation of gill epithelium with fusion of adjacent filaments in heavy infections (Figure 15c). *Lamproglena monodi* feed on blood and the filament tip may become necrotic when blood supply is interrupted.[523]

Ergasilus spp. feed on gill tissue. Following attachment to the gills, the antennae may fuse in some instances.[524] Encirclement of a gill filament by the antennae causes compression of the gill tissue,[525] which in some instances constricts blood flow in that gill filament leading to its eventual atrophy.[519] Epithelial hyperplasia is seen in the region close to the point of parasite attachment; tissue changes at these points lead to the eventual loss of functionality with subsequent negative impacts on gas exchange (Figure 15d).[525]

Lernaea infections cause irritation that induces agitation in hosts that manifests as rubbing their bodies on objects in their environment. Adult females can be observed macroscopically and the area surrounding the attachment site usually displays an approximate 1 cm diameter field of haemorrhagic skin, impacting fish marketability negatively.[526] Individual *Lernaea* remain attached to the site which they colonise and feed using their appendages to scrape host tissue towards their buccal cavity. Lesions without parasites are commonly observed where parasites have been dislodged or have died; these sites remain inflamed until the wound has healed. Intense infections cause host fish to become sluggish and chronic infection results in the production of proliferative hyperplastic connective tissue that can encapsulate the parasite or may protrude from the skin surface of the host. Infected fish have reduced haematocrit and condition.[334,527] The epidermis surrounding the lesion is spongiotic with eosinophilic granular cells (EGCs) and lymphocytes, and infection sites often

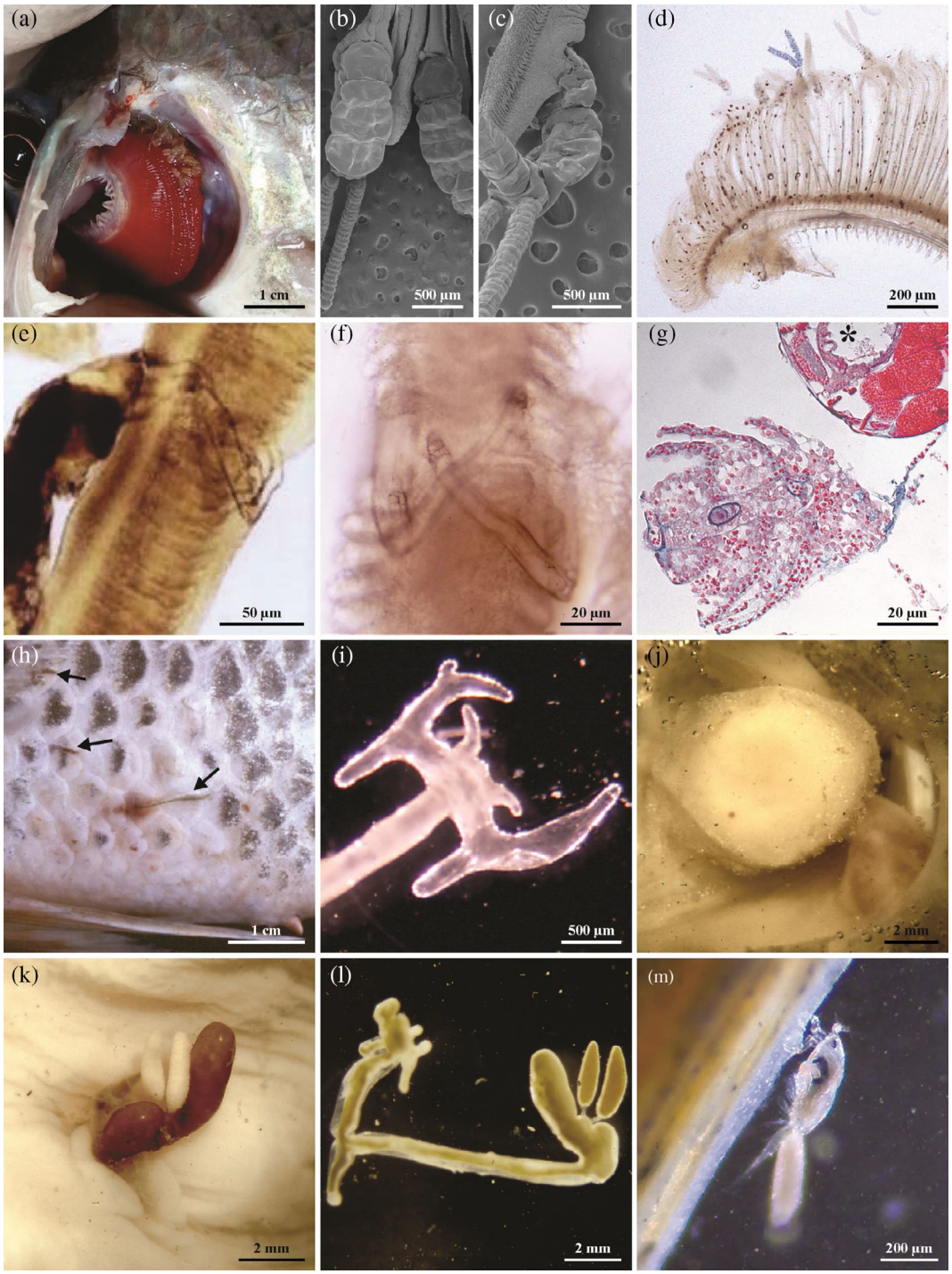

FIGURE 15 Crustacean copepod parasites of cultured tilapias. (a) Macrograph of *Lamproglena monodi* Capart, 1944 females on the gills of *Oreochromis niloticus*. (b) Scanning electron micrograph of *L. monodi* with paired egg sacs on the gills of *O. niloticus*. (c) Scanning electron micrograph of *L. monodi* feeding on the gills of *O. niloticus*. (d) Adult *Ergasilus sarsi* Capart, 1944 females attached to the gill filaments of its host. (e) *Ergasilus mirabilis* Oldewage et Van As, 1987 using their modified antennae in attachment to their host's gill filaments. (f) The antennae of *E. mirabilis* as seen from a different aspect. (g) Cross-section through a gill filament with *E. sarsi* with evident proliferation of the epithelia of the gill lamellae as well as the host tissue (*) in the parasite's intestinum. (h) Adult females of *Lernaea cyprinacea* L., 1758 (arrowed) on the skin of *Oreochromis mossambicus*. (i) Anterior of *L. cyprinacea* displaying its anchors. (j) Capsule housing the anterior of *Opistholernaea* Yin, 1960. (k) The posterior region of *Opistholernaea laterobranchialis* (Fryer, 1959) protruding into the buccal cavity. (l) *Opistholernaea laterobranchialis* released from the enclosing tissues of its host. (m) *Neoergasilus japonicus* (Harada, 1930) attached to the gills of *O. mossambicus* using their modified antennae. Images (a) and (b) are provided courtesy of Nehemiah Rindoria; images (i)–(k) are provided courtesy of Johan Theron and image (m) courtesy of Dr Quinton Dos Santos

become secondarily infected by bacteria and fungi. Blood may ooze into the water behind the parasite from the attachment lesion.[526,528] Larval infections can occur on the gills and cause respiratory distress, epithelial hyperplasia and telangiectasis.[334] Decreased haematocrit is caused by intense lymphocytopenia, neutrophilia and infiltration of immature leucocytes, haemorrhaging and haemodilution because of the ingress of water through the permanently open wound created by the parasite.[528] In small fish this parasite can penetrate the internal organs and cause mortality.[334] Fish that recover resist infection and if infected, the lesions are markedly smaller, probably due to an anamnestic immune response elicited by memory cells.[528]

The pathology associated with *Opistolernaea laterobranchialis* was described by Grobler.[522] The head and thorax, up to the level of the second pair of thoracopods, are surrounded by a large (0.7 mm diameter) bulbous granuloma consisting of three layers; areolar tissue, granular connective tissue and a multilayered epithelium. The remainder of the parasite thorax is covered by a simple, thin connective tissue sleeve containing melanocytes.[522]

2.18.2 | Treatment

Embedded *Lernaea* females are difficult to treat; eradication can require the use of products with strong negative environmental effects. Insecticides are effective but, in many countries, their use is not permitted due to the environmental effects of discharge; they are non-specific, kill non-target organisms and leave residues that can affect human health.[529] Infections can be managed by eradicating copepodite stages with organophosphate trichlorphon at 0.25 ppm with repeated treatments at the duration of the infective larval stages; trichlorphon kills copepodites but not nauplii.[334] Treatment with the carbamate 2-isopropoxyphenyl-N-methylcarbamate (Baygon™) elicited the emergence of resistance in four generations.[530] Sodium chloride eradicates all *Lernaea* at 20–40 mg/L at pH >6, is non-residual and is relatively environmentally benign; conditions for its use may be defined by local regulatory authorities including timing of treatments, volume and concentration used, discharge conditions, dilution and so forth. In recirculation systems, sodium chloride also kills the bacterial populations in biofilters, leading to nitrate build-up that needs to be managed while the bacterial population in the biofilter re-establishes.[531]

Woo and Shariff[532] reported that 50% of parasite eggs collected from fish that recovered from infection with *Lernaea* were viable, indicating a reduction in parasite viability when reinfection occurs. Fish recovering from infection recovered from subsequent infections faster, while parasites on fish that had recovered lost more egg sacs than *Lernaea* on first infection fish. If no naïve fish are introduced into closed aquaculture systems, infective larvae will decline with time and eventually the system should be safe for restocking. This indicates acquired immunity in the recovered fish. Shariff et al.[530] recommend that the parasite could be managed by removing all fish from a pond for 7–9 days because the absence of hosts would result in the loss of all larval stages of the parasite.

2.18.3 | Global translocations

Lernaea cyprinacea L., 1758 is one of the most invasive fish parasites and has spread to all continents,[533] and it is reported from *O. mossambicus* in South Africa[534] and *O. mossambicus*, *Oreochromis placidus* and *T. rendalli* in Zimbabwe.[535]

Neoergasilus japonicus (Harada, 1930) attaches predominantly to the base of the fins of their hosts but also on the operculum (Figure 15m). It was originally described from Asia,[536] but Hudson and Bowen[537] noted that it spread through aquaculture and the aquarium trade over 20 years. Its occurrence is recorded in Alabama, USA,[538] Cuba,[539] Mexico,[540] Peru[541] and South Africa.[542] It displays little host specificity and has been recorded from a wide variety of freshwater fish including cyprinids, percids, centrarchids, ictalurids and cichlids. In Japan *N. japonicus* is reported from redbelly tilapia (*C. zillii*), Mozambique tilapia (*O. mossambicus*) and Nile tilapia (*O. niloticus*).[543]

2.18.4 | Research

Parasitic copepod research focuses on parasite taxonomy, biodiversity and distribution. Research on *Lernaea* and other copepod parasites is complicated by their taxonomy being based on the limited morphological traits. In *Lernaea* the morphology of the anchor is used as a taxonomic character and many nominal species are probably synonyms; experimental infections show that anchor morphology and growth are affected by host anatomy.[544,545] Pallavi et al.[546] found that 18S and 28S sequences from four *Lernaea* specimens assigned to four different morphological species showed that all specimens were *L. cyprinacea*. Hua et al.[547] similarly concluded that *L. cyprinacea* and *L. cruciata* Lesueur, 1824 are conspecific based on their molecular data.

Copepod parasites are good bioindicators of metal and organic pollution.[548,549] Crustacean parasites can be collected from living hosts without harming them, providing further advantages over helminths and fish as indicators of pollution. It may also be possible to use data on the effect of adverse water quality to inform treatment of crustacean parasites provided safety margins are understood.

Development of treatment for crustacean parasites is focusing on natural compounds and application of nanocomposites. The *Lernaea* 1 h/LC_{50} for chitosan-silver was 5.495 ppm. When infected fish were exposed to the LC_{50} concentration for 24 h, it caused pathological changes to the *Lernaea* cuticle that dislodged all females and was followed by rapid healing of parasite-induced wounds.[526]

2.19 | Branchiura Thorell, 1864 (Obazoa: Opisthokonta: Metazoa: Ecdysozoa: Pancrustacea)

The Branchiura (fish lice) are covered by a dorsal carapace that is round to oval, with two carapace lobes and a bilobed abdomen. On average they are 3–7 mm in length, although gravid females may reach 10 mm. Females are larger than males and the sexes can be distinguished by the presence of two round spermathecae in the

abdomen of females, whereas males have one testis per abdomen lobe and peg and socket copulatory structures are present on the third and fourth thoracopods. The mouth is carried on a mouth tube, which extends ventrally, with the mandibles situated just inside the opening.

Males and females of *Argulus* Müller, 1785 and *Dolops* Audouin, 1837, as well as all life stages apart from the eggs, are parasitic on fish hosts. Branchiurans attach with maxillules that are modified to form suckers (*Argulus*; Figure 16a) or strong hooks (*Dolops*; Figure 16c) to the skin, in the buccal cavity or in the gill chamber (*Dolops*; Figure 16c). These parasites retain their ability to swim through life and can switch hosts; adults and larvae can survive without a host for up to 9 days.[550] Many species have been reported to have low host specificity.[512,551] *Argulus japonicus* Thiele, 1900, *A. foliaceus* (Linnaeus, 1758) and *A. coregoni* Thorell, 1866 are pathogenic and can reach high numbers in impoundments[552,553] or aquaculture.[551,554] Branchiurans deposit their eggs in rows on a substrate. A single female can deposit hundreds of eggs[555] which typically hatch within 21 days at 25°C, but the time to hatching is temperature dependent.[556]

Dolops spp. are recorded from South America, Africa, and Tasmania. *Dolops ranarum* (Figure 16c) occurs in Africa and infects *O. mossambicus* in the Zambezi River (Fryer, 1960), various rivers in the Limpopo River system in South Africa (Avenant and Van As, 1985) and the Okavango River, Botswana,[557] *Oreochromis variabilis* and *Oreochromis esculentus* (Graham, 1928) in Lake Victoria, Uganda[558] and *Serranochromis* sp. in the Kafue River, Zambia.[558] It was also recorded from *O. niloticus* in Lake Tana, Ethiopia (Fryer, 1965).

The *Argulus* species reported from tilapia are *Argulus africanus* Thiele, 1900, *A. cunningtoni* Fryer, 1965, *A. fryeri* Rushton-Mellor, 1994, *A. jollymani* Fryer, 1956, *A. kosus* Avenant-Oldewage, 1994, *A. monodi* Fryer, 1959, *A. rhipidiophorus* Monod, 1931, *A. striatus* Cunnington, 1913 and *A. tristramellae* Paperna, 1967 is recorded from *Tristramella* sp.[559] *Argulus japonicus* was introduced into Africa with cyprinids and is also reported from *O. mossambicus*.[552,560]

Argulus species transmit viruses, skrjabillanid and daniconematid nematodes.[519,561–563] In Mexico, Moravec et al.[447] reported *Argulus mexicanus* Pineda, Paramo et del Rio, 1995 collected from the cichlid *Mayaheros urophthalmus* (Günther, 1862; syn. *Cichlasoma urophthalmus*) as an intermediate host for daniconematid nematodes. The prevalence of infection was low at 1.29% with an intensity of 1–6 nematode larvae/*Argulus*. This highlights the role of argulids in the transmission of nematodes and the need for a detailed examination of *Argulus* specimens collected from the commercial species of tilapia.

2.19.1 | Pathology

Branchiuran parasites feed on the blood and tissue of their hosts.[512] Avenant-Oldewage[564] described the pathology caused by *Dolops ranarum* (Stuhlmann, 1891). *Dolops* spp. attach by inserting the hooks on the maxillules (Figure 16c), which causes local inflammation, disrupting osmotic control and providing a route of entry for secondary pathogens such as *Aeromonas* Stanier 1943 and opportunistic fungi. Avenant-Oldewage[564] showed that feeding by *D. ranarum* removes the epidermis of the host, leaving the dermis exposed (Figure 16d–f). Tavares-Dias et al.[565] reported that in *D. carvalhoi*, parasite intensity of 3–30 was not correlated to reduced haematocrit but was associated with increased thrombocyte and white blood cell counts and lower plasma glucose and serum electrolyte levels in infected fish.

In *Argulus*, the pre-oral and buccal spines are supplied by glands.[566] The parasites release digestive enzymes onto the host surface,[567] subsequently ingesting the predigested host tissue and blood. The process of feeding creates open wounds and although fish tolerate low and moderate levels of *Argulus* with few signs of disease, localised inflammation and damage at the affected site may lead to secondary infections. The parasite's high reproductive rate,[556] gravid females laying between 1 and 9 strings of eggs with 5–226 eggs per string, and the ability of eggs to overwinter two seasons[568] can quickly escalate an infection. Severe infections (i.e., hundreds of parasites per fish) cause extensive skin damage and inflammation which debilitates the host and reduces the ability of the host to osmoregulate.[569] Although the records of Kruger et al.[552] and Avenant-Oldewage[560] refer to the infection of wild tilapia in the Olifants River System, argulids are noted pathogens of fish held under culture conditions. *Argulus africanus* infection was common on *O. niloticus* in tanks at Kigera Dam, Lake Kainji, Nigeria (prevalence of 15%) and their presence resulted in disruption to the gill rakers.[156]

2.19.2 | Treatment

Fish remove and consume *Dolops* specimens from each other[519] and occasionally prey on free-swimming *Argulus* individuals.[570] Mechanical removal of parasites was suggested as a control method by Benz et al.[571] but is impractical for large-scale aquaculture. Hakalathi et al.[572] successfully reduced the number of parasites in ponds by deploying wooden egg laying plates in fishponds and removing them before the *Argulus* hatched, reducing the number of juvenile parasites in the ponds. Parvez et al.[573] painted chlorinated rubber onto the plates, which attracted more females to the plates, and increased removal of eggs and improved reduction in infection intensities.

The effect of pesticides on the environment prompted a focus on natural treatments. Sahoo et al.[574] analysed the full transcriptome of *Argulus siamensis*, which could direct development of plant-derived targeted treatments. The number of eggs per oviposition and their hatching success in *Argulus bengalensis* was decreased by exposure to 15 mg/L of an aqueous extract of neem, *Azadirachta indica* A. Juss (Meliaceae).[575] Development of *A. japonicus* eggs was disrupted after exposure to *Moringa oleifera* Lam. (Moringaceae) extract.[576]

Essential oil of lemon grass, *Cymbopogon citratus* (de Candolle) (Poaceae) against adult *Argulus* sp. and *Dolops discoidalis* was maximally effective at 140 μg/L. The LC50-24 h for *Argulus* sp. was 83.98 μg/L and the LC50-24 h for *D. discoidalis* was 82.48 μg/L,[577] suggesting that plant products have promise for management of these parasites. They, furthermore, reported that the eggs also lost their sticky cover after 30 days, dislodged from the substrate, sunk to the bottom and that altered anatomy of the ommatidia (eyes) occurred. These studies show promise for alternatives that consider the environmental impact.

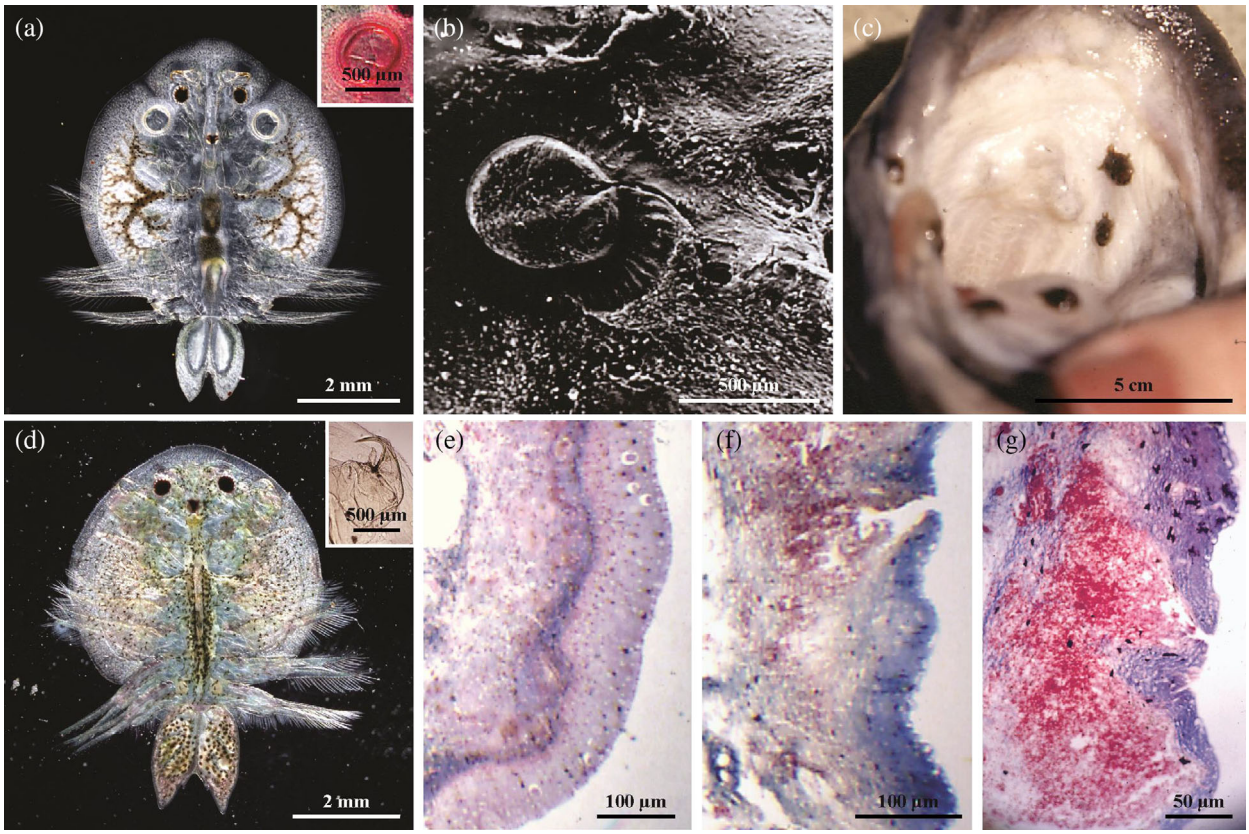

FIGURE 16 Crustacean branchiuran parasites of cultured tilapias. (a) Male *Argulus japonicus* Thiele, 1900. The inlay shows an enlarged image of an attachment sucker. (b) Scanning electron micrograph of the damage to host tissue inflicted by *A. japonicus*. The image shows an imprint of the suckers, destruction of the epithelium and open feeding wounds. (c) *Dolops ranarum* (Stuhlmann, 1891) in situ within the buccal cavity of *Oreochromis mossambicus*. (d) *Dolops ranarum*. The inlay shows the enlarged hook on the maxillulae. (e) Normal skin condition of *Clarias gariepinus* Burchell, 1822. (f) The skin of *C. gariepinus* showing denudement of the epithelium and inflammation as a consequence of *D. ranarum* attachment and activity. (g) The attachment of *D. ranarum* has resulted in extensive damage and haemorrhaging of the host's epithelium

2.19.3 | Global translocations

It is unclear if branchiurans have been translocated with tilapia, but several spillback infections have occurred from the environments to which tilapia are translocated. *Argulus japonicus* is a cosmopolitan species.[578] It infects *O. mossambicus* in South Africa,[552,560] while *A. coregoni* infects red *Oreochromis niloticus* × *Oreochromis mossambicus* in Malaysia[579] and *Argulus indicus* Weber, 1892, red *Oreochromis niloticus* × *Oreochromis mossambicus* in Thailand.[580] There is only one report of an introduction of *Dolops*, that is from Brazil to Japan and is that of *Dolops carvalhoi* Lemos de Castro, 1949 with gulper catfish, *Asterophysus batrachus* Kner, 1858.[581]

2.19.4 | Research

Research on Branchiura investigates biodiversity and new or improved treatment regimes. Morphological differences in descriptions of branchiurans are not conclusive and frequently poorly documented. There is, therefore, a drive to clarify the taxonomy[582] concurrent with the description of new species[583–585] and new hosts in South America.[586] If described species are sequenced, synonymies can be identified provided DNA sequences are included more frequently in descriptive studies. Saurubh et al.[587] reported that *Argulus* infection suppresses alpha-2 macroglobulin, serum complement activity response and ceruplasmin levels, indicative of stress. Ruane et al.[588] reported a humoral response to *Argulus foliaceus* antigens in trout and effective vaccines for *Argulus* are a focus for development.

2.20 | Pentastomida Diesing, 1836 (Obazoa: Opisthokonta: Metazoa: Ecdysozoa: Pancrustacea)

Pentastomids are dioecious flattened, segmented crustaceans ranging from 1 to 16 cm in length (males 1–2 cm; females 2–16 cm), and are covered in a chitinous cuticle, with five anterior appendages: a mouth and four hook-bearing appendages. The members of the order Cephalobaenida Heymons, 1935 have two pairs of appendages that lie behind one another while in Porocephalida Heymons, 1935 have hooked appendages aligned in a single row beside the mouth. Adult pentastomids are obligate parasites of the respiratory tract of vertebrates where they feed on blood or mucus and epithelial cells. Fish are common intermediate hosts that are infected by ingesting eggs or are actively infected by free-living larvae such as in *Subtriquetra subtriquetra* (Diesing, 1835) Sambon, 1922 which

attach to the skin, break through the epithelium, and ultimately encyst in the target organ. Larval development in *S. subtriquetra* includes seven moults over 70+ days. The final host is infected by ingesting the intermediate host, after which the pentastomid then crawls into the respiratory tract.

2.20.1 | Taxonomic identity

Four porocephalid pentastomid genera are recorded from tilapias.[589,590] *Alofia* Giglioli in Sambon, 1922, *Leiperia* Sambon, 1922, *Sebekia* Sambon, 1922 (Sebekidae Sambon, 1922), and *Subtriquetra* Sambon, 1922 (Subtriquetridae Fain, 1961), which use freshwater fish as their intermediate hosts and typically crocodilians as their final hosts. In South Africa, *Alofia* sp. Giglioli in Sambon, 1922 is recorded from the swimbladder of *O. mossambicus*,[590] *Leiperia cincinnalis* Sambon, 1922 from the mesentery of *C. rendalli* and *O. mossambicus*,[589] *Sebekia minor* (Wedl, 1861) (syn. *S. wedli*) from the swimbladder of *C. rendalli* and *O. mossambicus*,[589] and *Subtriquetra rileyi* Junker, Boomker et Booyse, 1998 from the swimbladder of *C. rendalli*[589] and *O. mossambicus*.[590] *Leiperia cincinnalis* is also recorded from *O. niloticus* from Africa (unspecified locality)[591] and from the Upper Nile.[592]

2.20.2 | Pathogenicity

Detailed descriptions of pathology in tilapias are lacking, notably of those associated with non-encysted infective *Sebekia* larvae within the swimbladder. Boyce et al.[593] observed that encapsulated nymphs in *Gambusia affinis* (Baird et Girard, 1853) tissues surrounding the gastrointestinal tract, liver, pancreas and mesentery caused a mild inflammatory response whereas nymph infections in *Xiphophorus helleri* Heckel, 1848, caused extensive traumatic damage, granulomatous inflammation with haemorrhage, myositis and myodegeneration. It should be noted that the observed pathologies in the latter resulted from encapsulated larvae and the trauma associated with migrating larvae. Infections are rarely reported from the swimbladder and mesentery of wild *O. niloticus*, *O. mossambicus* and *C. rendalli* from South Africa (Table S2).[590] This group has little impact on tilapias and appears to have no impact on farmed tilapias.

2.20.3 | Global translocation

There is no evidence that pentastomes have been translocated with tilapia.

2.21 | Isopoda Latreille, 1817 (Obazoa: Opisthokonta: Metazoa: Ecdysozoa: Pancrustacea)

2.21.1 | Taxonomic identity

Isopods are crustaceans that are dorsoventrally flattened with the body composed of a head, thorax and an abdomen. The head, containing paired eyes, antennae, antennules, mandible, maxillae and maxillipeds, is fused to the first thoracic somite. The thorax is comprised of six or seven somites, each possessing a pair of swimming legs while the abdomen is made up of five pleonites and a pleotelson that possesses a pair of uropods. All isopods reported from tilapia (Figure 17) are members of the superfamily Cymothoidea and include the families Aegidae, Corallanidae, Cymothoidae and Gnathiidae. Six species are reported from tilapia, including *Alitropus typus* H. Milne Edwards, 1840 (family Aegidae) from *O. niloticus* cultivated in the Philippines[16,594] India[595–598] and Thailand[599] and from *O. mossambicus* from India,[595,598] *Corallana nodosa* Schioedte et Meinert, 1879 (family Corallanidae) from *O. mossambicus* and *O. niloticus* hybrids cultivated in Malaysia,[600] *Braga* syn. *Philostomella cigarra* (Szidat et Schubart, 1960) experimentally transmitted to *O. niloticus* in Brazil,[601] *Nerocila bivittata* (Risso, 1816) and *Nerocila orbignyi* (Guérin-Méneville, 1832) on wild *C. zillii* in Egypt,[602–604] *Renocila thresherorum* Williams et Bunkley-Williams, 1980 (family Cymothoidae) on wild *C. zillii* from Egypt[604,605] and unidentified larval forms from the family Gnathiidae on *O. niloticus* from the Philippines.[13,16] Adult members of this group are typically identified using morphological methods.

2.21.2 | Pathogenicity

Although parasitic isopods can be pathogenic to their host, there are few examples of pathogenic isopods on tilapia. Typically, they are parasites of the surface and fins, but some species also invade the buccal and gill cavities, which can have negative effects on the host, including mortality and some species can attach to the tongue of the fish.[16] Mass mortality associated with *A. typus* on farmed Nile tilapia from the Philippines[594] and from Thailand[599] are reported; in Thailand, mortalities were estimated more than 50% of tilapia measuring 50 g each. Mortalities were noted in wild *C. zillii* from Egypt infected with *N. orbignyi* associated with erosion and haemorrhaging of the gills.[602]

2.21.3 | Global translocations

There is no evidence of translocation; *A. typus* has a wide distribution throughout Indo-China and the infection of tilapia appears to be opportunistic.

2.21.4 | Research

Given the relatively large size and ease of identification of parasitic isopods, it is assumed that the low number of records of isopods on tilapia reflect a genuine rarity of infections on these hosts. Caution should be exercised, however, because isopods can be transient on their host and caution should be exercised to minimise loss during examination of potential hosts. Targeted studies, designed to minimise

FIGURE 17 Infection of *Nerocila orbignyi* in the opercular cavity of *Oreochromis mossambicus* from Egypt. Image courtesy of Shimaa El Sayed Mohamed Ali and Mamdouh Yousif Abd Elaziz Elgendy from WorldFish, Egypt

parasite loss, may increase the number of records. Despite few reports of pathogenic species, studies to identify mechanisms to control infections should be considered.

2.22 | Hirudinea Lamarck, 1818 (Obazoa: Opisthokonta: Metazoa: Lophotrochozoa: Annelida: Clitellata: Hirudinea)

There are few reports of leech infections of tilapia (Table S2) and/or their treatment. This is probably driven by low prevalence and impacts.

Leeches are segmented, muscular, clitellum-bearing, hermaphroditic, parasitic hematophagous or predatory annelids possessing an anterior and posterior sucker. The possession of a proboscis, the number of eyes, gastric and intestinal caeca, testisacs, body annulation, patternation, presence of papillae and whether species produce cocoons or eggs that are brooded are features that are used to classify species.

2.22.1 | Taxonomic identity

Blood-feeding leeches belong to two orders, the Arhynchobdellida Blanchard, 1894 (proboscis-less leeches) and the order Rhynchobdellida Blanchard, 1894 (proboscis-bearing leeches). The Rhynchobdellida contains three families, the Ozobranchidae Pinto, 1921 (leeches of turtles), the Glossiphoniidae Vaillant, 1890 (leeches of freshwater fish) and the Piscicolidae Johnston, 1865 (leeches of freshwater and marine fish). Glossiphoniid and piscicolid leeches are vectors of several viral, bacterial and flagellated protistan pathogens of fish.[606]

Of the glossophoniid leeches infecting farmed tilapia, *Batracobdelloides tricarinata* (Blanchard, 1897) is recorded from *O. niloticus* in Egypt,[607] and a species of *Helobdella* Blanchard, 1896 is recorded from *O. niloticus* in Brazil (M. Metselaar pers. obs.; Figure 18). Two piscicolid leeches infect *O. niloticus*: a species of *Myzobdella* Leidy, 1851 in Malaysia[608] and *Piscicola geometra* (L., 1761) from Nigeria.[609] The ozobranchid, *Ozobranchus branchiatus* (Menzies, 1791) is reported from *O. aureus* in Puerto Rico from a public aquarium and probably infected the tilapia from a turtle that was also held in the system.[610] Arhynchobdellid leeches are recorded from farmed tilapia: *Hirudo michaelseni* Augener, 1936 and an unidentified species of *Hirudo* L., 1758, and a species of *Limnatis* Moquin-Tandon, 1827 was described from freshwater *O. niloticus* farms in south-eastern Côte d'Ivoire.[611] Unidentified leeches are reported to infect farmed *O. mossambicus* in Indonesia[334] and *O. niloticus* in Tanzania.[612] Given that leeches display low host specificity,[613,614] it is likely that species additional to those documented here and in Table S2 can also infect tilapia. *Zeylanicobdella arugamensis* de Silva, 1963 (Piscicolidae) is reported from invasive *O. mossambicus* in brackish water in Japan[615] and Sri Lanka,[616] a species of *Placobdella* Blanchard, 1893 (Piscicolidae) was recorded from invasive freshwater populations of *O. mossambicus* and *O. niloticus* in Thailand,[334] and *Piscicolaria reducta* Meyer, 1940 (Piscicolidae) is reported from invasive populations of *O. aureus* in freshwater in the USA.[617]

2.22.2 | Pathogenicity

The abundance of leech parasites is often inversely proportional to the size of the host.[607,609] Leeches are often considered to not be pathogenic, but infections can, however, cause mortality from physical trauma and blood loss, predisposing hosts to secondary infections, and transmitting pathogenic viruses, bacteria and flagellated haemoprotistans.[334] Leeches are more common in earth pond systems that more sophisticated aquaculture systems. Some leeches attach to their hosts temporarily and leave after taking a blood meal, while others attach for an extended period and take successive blood meals before detaching to lay their cocoons. Leeches that attach for extended periods can elicit a substantial host tissue response at the attachment site, and severe epidermal erosion may occur in heavy infections. Feeding by rhynchobdellid leeches can cause localised petechial haemorrhages and blood loss from damage to epithelia by the proboscis.

Williams et al.[610] described mortality of *O. mossambicus* in Puerto Rico infected with *Myzobdella lugubris* Leidy, 1851, but this was complicated by bacterial infections including *Vibrio vulnificus* (Reichelt et al., 1976). Pathology associated with *Myzobdella* infections was detailed by Volonterio et al.[618] who found that an infection (av. 12.5 leeches per fish) of *M. uruguayensis* (Mañé-Garzón et Montero, 1977) on the gills of *Rhamdia quelen* (Quoy et Gaimard, 1824; av. wt. 633 g) in Uruguay caused haemorrhages and formation of fibrin plaques at the sites of leech attachment. Gill infections were associated with oedema, hyperplasia and telangiectasis of nearby lamellae.

Glossiphoniid and piscicolid leeches are noted vectors of a range of fish pathogens. Feeding by *Piscicola geometra* can

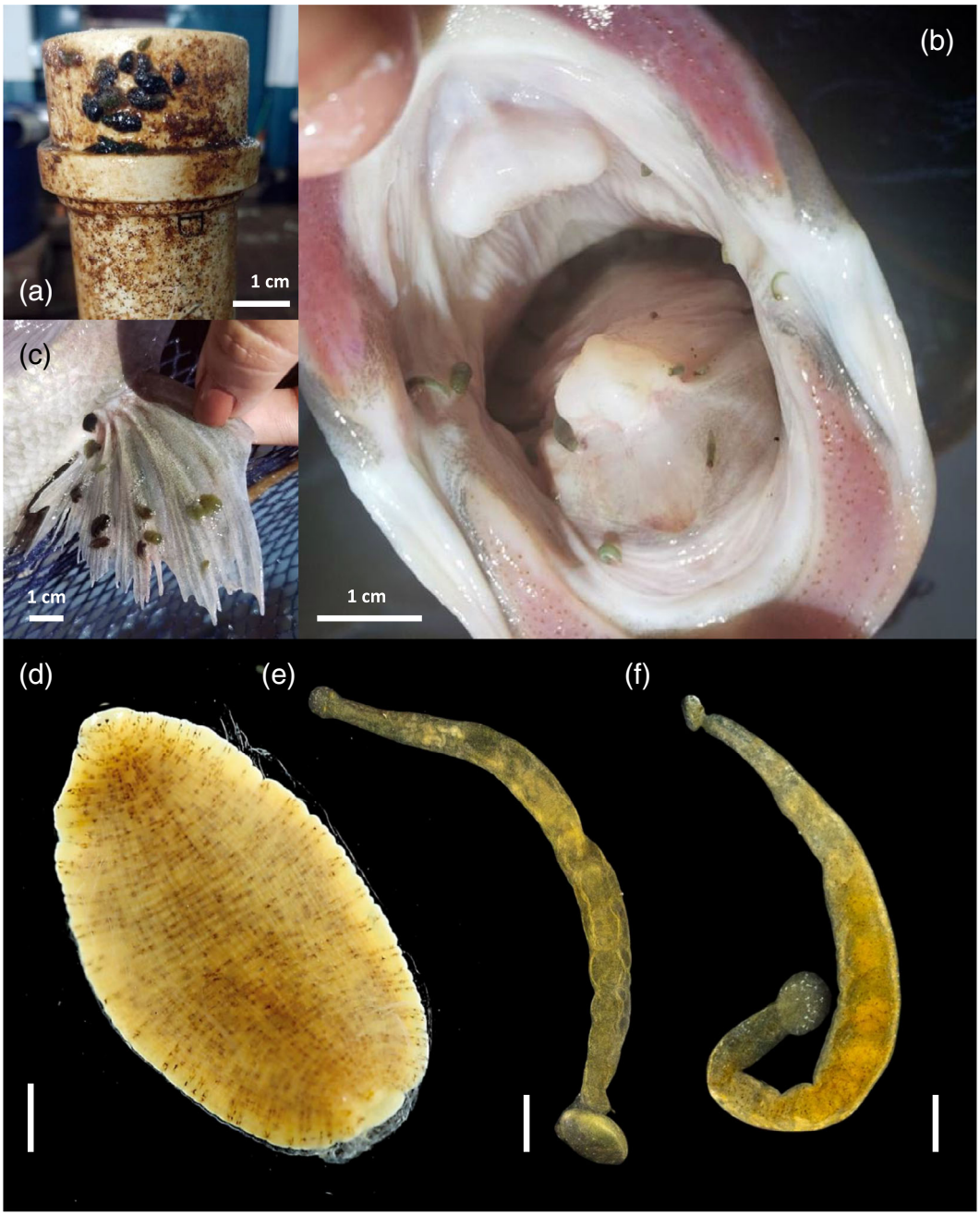

FIGURE 18 Leech infections of *Oreochromis niloticus*. (a–d) *Helobdella* sp. from stock cultured in Brazil showing leeches on (a) the system pipework, (b) attached to the inner lining of the oral cavity, (c) attached to the pectoral fin and (d) an ethanol fixed specimen of *Helobdella* sp. and (e,f) *Zeylanicobdella arugamensis* de Silva, 1963. Scale bar = 1 mm

mechanically transmit spring viraemia of carp virus (SVCV) which causes an acute, contagious haemorrhagic viraemia.[619] *Piscicola geometra* has a wide distribution throughout freshwaters across the Holarctic and Neotropic regions; Soliman et al.[620] reported isolating SVCV from *O. niloticus* from Egypt[621] but did not describe if leeches may have transmitted the virus. A range of bacterial pathogens are isolated from leeches: *Streptococcus* sp. was isolated from *B. tricarinata* in Natal, South Africa[613]; *Pseudomonas punctata* (Zimmermann, 1890) was isolated from *P. geometra*,[622] and Negele[623] reported *Aeromonas hydrophila* (Chester, 1901) from *P. geometra*. *Streptococcus agalactiae* Lehmann et Neumann, 1896 and *A. hydrophila* are significant pathogens of farmed tilapia with large economic impacts.[624] Negm-Eldin and Davies[32] demonstrated in an experiment that *B. tricarinata* could transmit the apicomplexans *Babesiosoma mariae* (Hoare, 1930) and *Cyrilia nili* Wenyon, 1909 from *O. niloticus* to *Clarias gariepinus* (Burchell, 1822).

Leeches are also common vectors of trypanosomes. Davies et al.[38] isolated *Trypanosoma mukasai* from *O. andersonii* from Botswana. Smit et al.[34] subsequently characterised trypanosomes

isolated from the blood of South African fish including *C. rendalli* and *O. mossambicus* and from *B. tricarinata*, and found that the trypanosomes resembled *T. mukasai*, suggesting a link between *T. mukasai*, *B. tricarinata* and tilapia.

Zeylanicobdella arugamensis is a problematic leech of cultured fish in South-East Asia[625,626] and is recorded from *O. mossambicus* reared in brackish water in Japan[615] and Sri Lanka.[616] It is a vector of *Haemogregarina curvata* Hayes, Smit, Seddon, Wertheim et Davis, 2006 and fish trypanosomes in South Africa.[627] This broad distribution and history of vectoring serious pathogens highlights the biosecurity risk this leech may have in brackish and marine water tilapia aquaculture.

2.22.3 | Global translocations

Leeches are unlikely to be translocated with fish, but most pre-export health inspections prescribe a sample size that is too low to detect typical prevalences of <2.5%.[607] While leeches are readily seen on the surface of fish, they frequently attach to the gills, buccal and opercular cavities and may be missed by visual inspection.

2.22.4 | Research

The treatment of leeches in aquaculture has largely been neglected. Much of this is due to the scale of earth culture systems, the large volumes of chemotherapeutant required, the environmental concerns regarding the use of certain products and the resistance of cocoons to treatment and need for repeat treatments.[628] For earth pond systems, leech infections were traditionally controlled using undesirable, regulated products such as metrifonate or trichlorfon or by drying and calcium oxide liming ponds to kill leeches and their cocoons.[334,606,629] Strategies for management and control of leech infections in aquaculture facilities have therefore focused on exploring alternative control strategies including the use of non-chemical traps to remove leeches and their cocoons (B.C. Kua, unpublished), in addition to implementing good basic biosecurity and sanitary practices.

2.23 | Mollusca Linnaeus, 1758 (glochidia) (Obazoa: Opisthokonta: Metazoa: Lophotrochozoa: Mollusca)

Glochidia are a microscopic larval stage of some freshwater mussels (Bivalvia) of the family Unionidae Fleming, 1828 and Margaritiferidae Haas, 1940. These parasitic larvae are armed with hooks that allow them to attach to fish (mainly the gills) for a period before detaching and falling to the substrate. *Cristaria plicata* Leach, 1815 (Unionidae) is listed from *O. niloticus* from the Philippines (Luzon) in the checklist of Arthur and Lumanlan-Mayo.[16] Few details regarding this infection of tilapia are available.

2.24 | Treaties, standards and guidelines in international trade of live aquatic organisms and their products

There are policies, legislation and guidelines, obligatory and voluntary, about health management and movement of live aquatic animals.[630–632] These controls are frequently revised and therefore change constantly. This is necessary to respond to rapid worldwide developments in aquaculture and culture-based fisheries, improved knowledge of diseases of aquatic animals and improved or new diagnostic tools and procedures. Trade patterns change to reflect the political, social, industrial and economic environments of countries and regions and contribute to the dynamics of risk and its sensitivity to assessment.[630]

The World Trade Organisation (WTO) Agreement on the Application of Sanitary and Phyto-Sanitary Measures (SPS)[633] is the main regulatory instrument governing health in relation to international trade. The three main international standard setting bodies are the Codex Alimentarius Commission of FAO/WHO for food safety; the World Organisation for Animal Health (formerly the Office International des Epizooties [OIE]) for animal (including aquatic animal) health; and the International Plant Protection Convention (IPPC) for plant health. Other relevant international agreements are the Convention on Biological Diversity[634] and the Convention on International Trade of Endangered Species (CITES). Voluntary agreements or guidelines include that of the International Convention for the Exploration of the Sea,[635] the European Inland Fisheries Advisory Commission[636] and FAO guidelines such as the Code of Conduct for Responsible Fisheries Technical Guidelines on Responsible Movement of Live Aquatic Animals[637] and regional guidelines.[638] Voluntary international guidelines are often incorporated into national legislation and can therefore become locally mandatory.[632]

Health certification is an element of national strategies for health management and aquaculture biosecurity.[637] The objective of certification is to facilitate trade of live aquatic animals while decreasing the risk of spreading infectious diseases to an acceptable level. It also protects captured fisheries, unexploited species and other natural and built assets managed by governments. Health certification is relevant to reportable or notifiable pathogen lists, risk assessment, diagnostics and surveillance of these strategies.[639]

Application of these instruments often does not capture the relevance of parasites whose inclusion may be warranted as pathogens of concern. National, regional and international lists of aquatic pathogens or diseases include few parasitic and fungal pathogens. This is because these eukaryotic pathogens do not fulfil the criteria for disease listing, despite their economic impacts. Redirecting efforts and studies towards understanding the disease burden, impacts and costs of management of these agents, the risks posed and development and application of better generic approaches to managing their translocation will increase attention to this important group and decrease ongoing costs of management. Implementation of basic biosecurity to farm management can aid in controlling numerous serious and production-affecting pathogens, and its uptake should be encouraged. Taxonomic studies are important, and their value will be more significant, if placed

in the context of disease control studies and biosecurity implementation.

3 | DISCUSSION

This review summarises the parasite fauna of tilapias from at least 73 countries and 3 major international lake systems—45 of which are in territories away from the native range of tilapias. These global movements of tilapia are associated with numerous transboundary introductions of parasites and spillback infections where local parasites have infected tilapia in their introduced environments (Table S1). More than 2500 host–parasite records are provided, raising awareness about the distribution of parasites and their capacity to spread with translocated fish. Table S2 presents information on 153 protists and 284 metazoan species summarised by country in Table S3. These distributional data highlight gaps in knowledge of the parasite fauna of tilapias in jurisdictions with large aquaculture industries, notably Cambodia, Guatemala, Lao PDR, Myanmar and Zanzibar (Tanzania), each with annual aquaculture production exceeding 10,000 tonnes in 2019 (Tables S1 and S3).[2] Table S3 further highlights additional countries and regions with limited information about the parasite fauna but with large tonnages (i.e. >10,000 in 2019) of tilapia being landed from aquaculture (e.g. Colombia, Costa Rica, Ecuador, Honduras, Taiwan Province of China and Tanzania) and capture fishery activities (e.g. Niger and Sri Lanka; Tables S1 and S3).[2] Gambia (1814 tonnes) and Togo (4507 tonnes) landed modest volumes from capture fishing activity in 2019, but no parasites are recorded from these countries.

Among the parasites, protists appear to be under-represented, with no reports from 31 of the 73 countries where parasites are documented from tilapia, suggesting that many have been overlooked or ignored. It is, however, appreciated that most diagnosed infections are treated to manage the infection and to prevent stock losses without identifying the species or reporting it scientifically. While we aimed to provide a comprehensive coverage of records, it should be noted that it includes some evident misidentifications[428,429,433,434,436,439,440]; where these were identified they are indicated. The identification of some species requires revisiting to confirm their translocation, mostly notably the records of 'introduced' coccidians and myxosporeans; in the absence of reference material, confirming identification of these must unfortunately await resampling.

There is no evidence of an introduced tilapia parasite having had a serious impact on indigenous fish fauna. Of translocated parasites, the most significant mortality event was caused by *A. ocellatum* from May 1997 to October 1998 in the hypersaline (46 psu) Salton Sea, California with massive mortality of young (1–13 cm TL) *O. mossambicus* in the shallows.[71] Assuming an average mortality of 20%–50% of the total 11 kg ha^{-1} biomass,[640] the value of the loss was estimated at US$ 6.77–16.93 M.[389] Other mortality events are reported but are in small populations of fish where losses due to a fungal infection of c. 200 variously sized juvenile *O. mossambicus* in India[174]; and c. 500 × 80 ± 10 g *O. niloticus* due to an oomycete infection in Egypt.[176]

There is also a paucity of information about the impact on introduced tilapia from endemic pathogens/infectious agents in receiving waters; such events have received less attention and are more likely to be regarded as caused by translocation stress, poor stock quality or mishandling.

Infections of *G. cichlidarum* on juvenile *O. niloticus* are common and associated with substantial losses of nursery and pond-reared tilapia in Egypt, Israel, Mexico, Scotland and Thailand.[286,339] Estimating parasite-associated losses in the early phases of production is complicated by the interplay of numerous environmental and management factors that are all difficult to assess. Shinn et al.,[641] however, estimated that the economic losses of juvenile tilapia attributable to parasites were USD 5.13–7.05 M at the swim-up stage, USD 5.84–8.02 M at the 21-day post-monosex stage, and US$ 4.84–6.66 M at the one-inch post-nursery stage in the 4.82 million metric tonnes per annum industry.

Records of host switching events such as *A. compactum* infecting tilapia from native Mexican cichlids and *A. crassus* infecting tilapia from eels in Belgium, although tilapia may be a paratenic host, highlight the vulnerability of translocated tilapia to infectious organisms in receiving waters. Translocation risks have focussed on obvious exotic pathogen introductions that infect indigenous hosts in environments conducive to establishment and spread. This review, however, has not detailed the 'spread' of specific parasites that have been introduced but instead collates infection records.

3.1 | Parasite species of global concern

A question that naturally emerges from this review, is 'which parasite species pose the greatest threat to the security of sustainable tilapia production?' While the mortality caused by *Amyloodinium ocellatum* in the Salton Sea represents the largest documented parasite-caused fish kill, and although other *A. ocellatum* infections are reported (Table S2),[69,70,72] only 17.40% (c. 1,076,612 tonnes) of tilapia in 2019 were cultured in brackish water and <0.002% (115.2 tonnes) in seawater. Of the parasites infecting tilapias grown in freshwater (c. 5,109,230 tonnes),[2] the monogenean genera *Cichlidogyrus*, *Gyrodactylus* and *Scutogyrus* have a wide geographic distribution, low host specificity and are pathogenic. Numerous species, furthermore, are found outside of their native range: *G. cichlidarum* (13); *C. tilapiae* (12); *C. sclerosus* (11); *C. thurstonae* (8); *S. longicornis* (8); *C. halli* (7); *C. longicornis* (3); *C. dossoui* (2); *C. haplochromii* (2); *C. mbirizei* (2); *C. tiberianus* (2); and *G. shinni* García-Vásquez, Pinacho-Pinacho, Guzmán-Valdivieso, Calixto-Rojas et Rubio-Godoy, 2021 (2); and *G. yacatli* (2). Within tilapia, the broad host specificity of the top six are: *G. cichlidarum* (6 hosts); *C. tilapiae* (9 hosts); *C. sclerosus* (9 hosts); *C. thurstonae* (6 hosts); *S. longicornis* (4 hosts); and *C. halli* (9 hosts) (Table S2). All have been recorded from hosts in *Coptodon*, *Oreochromis* and *Sarotherodon* species, except *S. longicornis* which is known from three *Oreochromis* spp. and one *Sarotherodon* species.

Although there are insufficient data to define the relative successes of each species, the non-obligate ciliated ectocommensals such as the trichodinids, may be among some of the most successful

colonisers. It is not a specific parasite that directly poses the greatest risk to tilapia aquaculture, but rather their role in facilitating the infection of pathogens of significance such as *S. iniae* and *A. hydrophila*,[318,320,642] their role in increasing stress and decreasing production efficiency and their effective transmission and increased pathogenicity in aquaculture. *Trichodina* spp. in pond systems serve as an appropriate example. In pond systems with high(er) stocking densities, low-to-zero flush or water exchange rates, or in low-tech input systems without additional aeration and waste management, or in systems where feeding regimes attempt to maximise growth, high organic loads, pronounced fluctuations in daily water chemistry and elevated stress combine to facilitate elevated parasite abundance and prevalence and increases the probability of disease and mortality.

3.2 | Parasites of tilapias: status quo

The parasite fauna of tilapias from Africa is unexplored in many regions and studies that have been made need increased visibility.[44] Much ground-level aquatic parasitology remains unknown. Our knowledge of coccidian, myxosporean and nematode infections of tilapias remains poor, the role of leech infections in parasite life cycles is implied but undefined, and these knowledge gaps remain as threats to production.

The global importance of tilapias (i.e. USD 2000 t^{-1} for Nile tilapia and USD 1721 t^{-1} for tilapias nei) in aquaculture while having a lower farm gate value than cyprinids (i.e. USD 2326 t^{-1} for bighead carp, USD 2050 t^{-1} common carp, USD 2291 t^{-1} grass carp, USD 2147 t^{-1} for silver carp), salmonids (i.e. USD 6524 t^{-1} for Atlantic salmon) and shrimp (i.e. USD 5911 t^{-1} for white leg shrimp)[2,643] creates a self-reinforcing problem in health management where low-profit margins from production reduce the likelihood of thorough investigations and the scope of treatments available to either non-chemical changes to farm practices or to regimes that can be afforded in low-income systems and compete with other needs in health and biosecurity. The investment in point-of-care diagnostics (e.g. microscopes, etc.) and capacity to recognise parasite infections may be lower and 'acceptable' levels of stock loss may be higher. Thus, the balance between the costs of health intervention versus profit gain on the number of fish surviving to harvest may be tipped in favour of taking fewer active steps to manage tilapia health. At the same time, there is also a need to develop and manage local, regional, and national fish health strategies to improve diagnostics and veterinary care to support producers.

3.3 | Parasites of tilapias: quo vadis?

Global tilapia production has been growing at 3.73% year-on-year (2015–2019) and applying a logarithmic trend to 2000–2019 production, it is expected to rise to c. 9.6 million tonnes by 2030. Africa has huge potential for aquaculture; tilapias are biologically suitable and socially acceptable and could help meet protein demand for growing populations. With increased African production, an increase in movement of tilapia including genetically improved strains is likely—increasing the risk of parasite translocations, disease events and, indeed, of negative impacts on native fauna and biodiversity in Africa. Over the coming decade, tilapia aquaculture will continue to face risks from known and emerging pathogens.

The discovery since 2000 of 25 new parasites from *O. niloticus* worldwide (*Cichlidogyrus mbirizei*; *C. rognoni*; *Dermocystidium aegyptiacus*; *Diplostomum tilapiae* Zhokhov, 2014; *Gyrodactylus ergensi* Přikrylová, Matejusová, Musilová et Gelnar, 2009; *G. hildae* García-Vásquez, Hansen, Christison, Bron et Shinn, 2011; *G. malalai*; *G. occupatus* Zahradníčková, Barson, Luus-Powell et Přikrylová, 2016; *G. parisellei* Zahradníčková, Barson, Luus-Powell et Přikrylová, 2016; *G. shinni*; *G. yacatli*; *Myxobolus bejeranoi* Lovy et al., 2018; *M. branchiophilus* Abdel-Ghaffar et al., 2008; *M. cichlidarum* Abakar-Ousman et al., 2006; *M. fomenai* Abdel-Ghaffar et al., 2008; *M. mapei* Fonkwa et al., 2017; *M. nounensis* Fomena et Bouix, 2000; *M. saintlouisiensis* Diamanka et al., 2007; *M. tchadanayei* Abakar et al., 2006; *Nucleospora braziliensis*; *Ortholinea africanus*; *Saccocoelioides cichlidorum* (Aguirre-Macedo et Scholz, 2005); *Sinuolinea niloticus*; *Triangula egyptica*; and, *Zschokkella nilei*), highlights that there is still much to discover. Translocations to new locations for aquaculture, without the appropriate biosecurity measures in place, reinforce that new host–parasite interactions will increase health threats to both the introduced tilapia and the native fish in receiving systems.[639]

There is also potential for the growth of Mozambique tilapia in coastal aquaculture. From 2015 to 2019, global production increased 26.5% year-on-year from 37,900 tonnes in 2015 to 74,400 in 2019.[2] Nile tilapia over the same period increased 3.2% year-on-year but the size of the industry was 4,590,300 tonnes in 2019.[2] Given global concerns regarding saltwater encroachment and competition for land and freshwater resources, the expansion of aquaculture of saline-tolerant tilapia may have production and environmental benefits. The potential threat from pathogenic marine species such as *Neobenedenia* spp. (Table S2) needs to be recognised and expanding industries will produce a concomitant need for investment in biosecurity and disease mitigation including selective breeding for parasite resistance, vaccine development and parasite management and control strategies.

The ongoing COVID-19 pandemic and disrupted global supply chains highlight the need for increased local and national food security. The pandemic is likely to drive increased consumption of domestically produced seafood and tilapia likely have a place in providing this, but increased production comes with substantial risks that should be mitigated to achieve the potential improvements in local food production and utilisation.

AUTHOR CONTRIBUTIONS

Andrew P. Shinn: Conceptualization; data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Annemarie Avenant-Oldewage:** Data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Melba G. Bondad-Reantaso:** Conceptualization; formal analysis; writing – original draft. **Armando J. Cruz-Laufer:** Data curation. **Adriana García-Vásquez:** Data curation; formal analysis; resources; writing – original

draft. **Jesús S Hernández-Orts:** Data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Roman Kuchta:** Data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Matt Longshaw:** Conceptualization; data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Matthijs Metselaar:** Data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Antoine Pariselle:** Data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Gerardo Pérez-Ponce de León:** Data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Pravata Kumar Pradhan:** Data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Miguel Rubio-Godoy:** Conceptualization; data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Neeraj Sood:** Data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Maarten P.M. Vanhove:** Data curation; formal analysis; resources; writing – original draft; writing – review and editing. **Marty R. Deveney:** Conceptualization; data curation; formal analysis; resources; writing – original draft; writing – review and editing.

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DATA AVAILABILITY STATEMENT

Data is openly available in a public repository that issues datasets with DOIs.

ORCID

Andrew P. Shinn https://orcid.org/0000-0002-5434-2685
Annemarie Avenant-Oldewage https://orcid.org/0000-0001-8820-7679
Melba G. Bondad-Reantaso https://orcid.org/0000-0002-2380-3549
Armando J. Cruz-Laufer https://orcid.org/0000-0003-1370-4739
Adriana García-Vásquez https://orcid.org/0000-0003-1076-2941
Jesús S. Hernández-Orts https://orcid.org/0000-0002-8177-7959
Roman Kuchta https://orcid.org/0000-0002-4219-6924
Matt Longshaw https://orcid.org/0000-0003-0099-284X

Matthijs Metselaar https://orcid.org/0000-0002-5000-9594
Antoine Pariselle https://orcid.org/0000-0002-1591-7423
Gerardo Pérez-Ponce de León https://orcid.org/0000-0001-6472-5113
Pravata Kumar Pradhan https://orcid.org/0000-0002-6793-1972
Miguel Rubio-Godoy https://orcid.org/0000-0002-9743-4416
Neeraj Sood https://orcid.org/0000-0002-2046-7957
Maarten P. M. Vanhove https://orcid.org/0000-0003-3100-7566
Marty R. Deveney https://orcid.org/0000-0001-6528-3601

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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REVIEW

REVIEWS IN Aquaculture

Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance

Olga L. M. Haenen[1] | Ha Thanh Dong[2] | Truong Dinh Hoai[3] | Margaret Crumlish[4] | Iddya Karunasagar[5] | Timothy Barkham[6] | Swaine L. Chen[7] | Ruth Zadoks[8] | Andreas Kiermeier[9] | Bing Wang[10] | Esther Garrido Gamarro[11] | Masami Takeuchi[12] | Mohammad Noor Amal Azmai[13] | Belén Fouz[14] | Rolando Pakingking Jr.[15] | Zeng Wei Wei[16] | Melba G. Bondad-Reantaso[11]

[1]National Reference Laboratory for Fish Diseases, Wageningen Bioveterinary Research, Lelystad, The Netherlands

[2]School of Environment, Resources and Development, Asian Institute of Technology, Pathum Thani, Thailand

[3]Faculty of Fisheries, Vietnam National University of Agriculture, Hanoi, Vietnam

[4]Institute of Aquaculture, University of Stirling, Stirling, UK

[5]Technology Enabling Center, Nitte University, Mangalore, India

[6]Department of Laboratory Medicine, Tan Tock Seng Hospital, Singapore, Singapore

[7]Genome Institute of Singapore, National University of Singapore, Singapore, Singapore

[8]Sydney School of Veterinary Science and Marie Bashir Institute, University of Sydney, Sydney, Australia

[9]Statistical Process Improvement Consulting and Training Pty Ltd, Adelaide, Australia

[10]College of Agricultural Sciences and Natural Resources, University of Nebraska-Lincoln, Lincoln, Nebraska, USA

[11]Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

[12]Food Systems and Food Safety Division, Food and Agriculture Organisation of the United Nations (FAO), Rome, Italy

[13]Department of Biology, Faculty of Science & Aquatic Animal Health and Therapeutics Laboratory, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

[14]Departamento de Microbiología y Ecología & Instituto en Biotecnologia i Biomedicina (BIOTECMED), Universitat de València, Burjassot, Valencia, Spain

[15]Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC AQD), Tigbauan, Iloilo, Philippines

[16]Guangdong Provincial Key Laboratory of Animal Molecular Design and Precise Breeding, School of Life Science and Engineering, Foshan University, Foshan, People's Republic of China

Correspondence
Olga L. M. Haenen, NRL for Fish Diseases, Wageningen Bioveterinary Research, P.O. Box 65, 8200AB Lelystad, The Netherlands.
Email: olga.haenen@wur.nl

Melba G. Bondad-Reantaso, Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, Rome 00154, Italy.
Email: melba.reantaso@fao.org

Abstract

Tilapia culture is an important source of income and nutrition to many rural families. Since 2000, the production of tilapia increased and reached domestic and global markets. Major farmed species is Nile tilapia (*Oreochromis niloticus*), in earthen ponds and cage cultures. Intensification contributed to global tilapia disease outbreaks, with bacterial infections causing mortalities and morbidities, threatening sustainable production. At tilapia farms, high nutrient concentrations, water temperature and fish

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densities enhance bacterial growth including virulent bacterial clones and potential zoonotic bacteria. Global warming favours this. This review respectively provides a comprehensive overview of the most common and emerging bacterial pathogens, diseases, clinical presentations and diagnostics of tilapia, including bacteria and diseases with zoonotic potential. First, common bacterial disease outbreaks, including streptococcosis, motile *Aeromonas* septicaemia, francisellosis, columnaris disease and vibriosis are described. Then, information on emerging bacterial infections of concern for tilapia, like edwardsiellosis through *Edwardsiella ictaluri* and *E. tarda*, as well as *Aeromonas schubertii* is provided. Reports of infectious bacterial tilapia disease outbreaks from other bacteria, including *Lactococcus garvieae*, *Aerococcus viridans*, *Pseudomonas* spp., *Mycobacterium marinum* and *Chlamydia* spp., and others are reviewed. Furthermore, bacteria with zoonotic potential, like *Streptococcus agalactiae* ST283, *S. iniae*, *Aeromonas* sp., *E. tarda*, *Vibrio vulnificus* pathovar (pv) *piscis* and *M. marinum* are included in the review, to provide the most current overview of the disease risks affecting production and post-harvest stages. Additionally, the status and risks of antimicrobial resistance in bacteria from tilapia and other cultured fish through imprudent use of antibiotics, and its future at a global level are provided.

KEYWORDS
AMR, bacterial disease, diagnosis, tilapia, zoonosis

1 | INTRODUCTION

Diseases of aquatic organisms seriously constrain the expansion and development of sustainable aquaculture. Globally, in aquaculture, the trend is that a previously unreported pathogen that causes a new and unknown disease will emerge, spread rapidly, including across national borders, and cause major production losses approximately every 3–5 years.[1]

The capability to manage health of aquatic organisms has significantly increased during the last three decades. However, such capacity did not match the rapid growth of the aquaculture sector.[2] Many of the most serious infectious disease agents affecting cultured species in aquaculture are bacteria. Because they rarely act as primary pathogens and they occur most commonly as opportunistic pathogens in already damaged or severely immunocompromised hosts, there is low attention given to this pathogen group. In fact, in the OIE (now known as WOAH) list of notifiable aquatic animal diseases, there are very few bacterial pathogens.[3]

However, bacteria may cause severe losses in tilapia farming. Bondad-Reantaso et al.[4] compiled a list of bacterial species or species groups affecting cultured finfish, crustaceans and molluscs. Their importance is growing, thus the need to pay more attention is there, not only in the context of its impact on production, but also of its zoonotic potential and contribution to development of antimicrobial resistance (AMR) through misuse of antibiotic treatments.

Farming of tilapia is primarily done in Asia; additional production comes from Africa and the Americas. The most predominant species is Nile tilapia (*Oreochromis niloticus*) with a 2019 production of 4.6 million tonnes.[1] From subsistence farming, tilapias are now commercially produced and tilapia products are traded globally. At a global level, the top three producers in 2019 are (i) China (1.6 million tonnes), (ii) Indonesia (1.3 million tonnes) and (iii) Egypt (1.1 million tonnes).

This article is part of a compendium of papers of a Special Issue in *Reviews in Aquaculture* which resulted from a virtual webinar event: 'Tilapia health: *quo vadis*', organized by the Food and Agriculture Organization of the United Nations (FAO), held from 1–3 December 2021. The objective is to review the most important bacterial pathogens and bacterial diseases affecting tilapia, including those that have zoonotic potential and understand ways to reduce bacterial disease risk for both fish and humans, with general recommendations of therapeutic and prevention strategies against the related pathogens, and pointing to the risk of development of antimicrobial resistance through imprudent use of antibiotics.

For this literature review, the authors used a systematic approach to the review, which included the use of relevant keywords (e.g. streptococcosis and tilapia) in the following databases of literature: *Web of Science*, *Scopus*, *PubMed*. The scientific literature included peer review journals, book chapters, health organism's reports, and so forth, with an initial search covering the last 10 years. Where little data was available, the temporal search was expanded as appropriate. An inclusive approach was adopted, where each of the authors took responsibility for a section and worked with those that had most expertise/experience in each of the sections or bacterial species. This was then shared with the authors and cross-revised accordingly. Preference was given to literature that included tilapia, and other fish species were included where data in tilapia was more limited.

The review work was divided among the authors, per expertise. Each expert read their database-acquired collection of papers and made

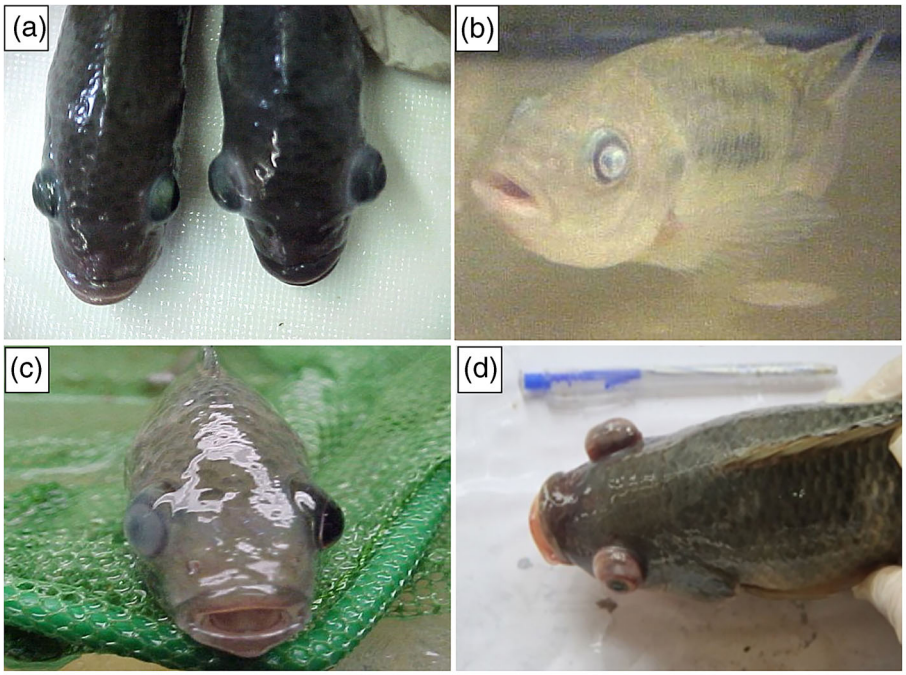

FIGURE 1 (a–c) Streptococcosis by *S. iniae*/*S. agalactiae* in diseased tilapia in the USA. The tilapia shows exophthalmos and cataract. They may have a C-shaped body, which causes them to swim spirally (b). Pictures (a–c): Courtesy Dr Joyce Evans, USDA-ARS, Aquatic Animal Health Research Unit, Auburn, Alabama, USA. Picture (d) Diseased tilapia from an outbreak of *S. agalactiae* in tilapia in Vietnam. The fish shows exophthalmos and a congested belly from full sepsis. Courtesy Dr Truong Dinh Hoai (co-author)

a draft text, which was altogether reviewed by the co-authors. Although most of the information was found on bacterial diseases of other fish than tilapia, we focused as much as possible on tilapia bacterial diseases.

2 | REVIEW

In total, 370 references have been cited for this review paper, from the years 1970–2022.

2.1 | Current bacterial diseases of significant importance

Tilapia may be infected with various bacteria, including species of the genera *Vibrio*, *Aeromonas*, *Pseudomonas* and *Streptococcus*,[5] whereas some genera may be present also on healthy fish, like species of the genera *Pseudomonas Aeromonas*, and *Plesiomonas*.[6] In general, most fish diseases are induced by a stress factor, like a suboptimal environment, for instance, bad water quality, and this allows opportunistic bacteria including *Aeromonas hydrophila* to infect tilapia and cause disease.[7–9] Moreover, many bacterial diseases are multifactorial.[10] We should keep this in mind, when trying to understand the cause of and finding a way to cure a bacterial fish disease.

The current bacterial tilapia diseases of significance (related to fish-welfare, economy and society) are streptococcosis, aeromonasis, francisellosis, columnaris disease and vibriosis.

To compare economic losses in USD due to bacterial disease in tilapia farming with those in other fish culture species is difficult, as costs are dependent on the value of the fish species, the production system, the country, the currency and so forth. A comparison in terms of % of fish production lost might be meaningful but there is not sufficient data that is collected in a consistent manner to allow for such comparisons across studies, countries, fish species and production systems.

2.1.1 | Streptococcosis

Outbreaks of streptococcosis have been widely reported in farmed tilapia species globally,[11–13] described as a septicaemic infection due to the bacterial species of *S. iniae* or *S. agalactiae*.[14] These facultatively anaerobic, Gram-positive bacteria are described as non-motile and non-spore forming, presenting with varied degrees of haemolysis dictated by species and strain variation.[15] In cultured tilapia, high prevalence of *S. iniae* and *S. agalactiae* infection was usually observed during hot and dry seasons when the water temperature is ≥27°C.[16,17]

Streptococcus agalactiae may cause acute[15] or chronic disease[18] in tilapia. Clinical presentations of the acute form include, but are not restricted to erratic swimming, c-shaped body of the fish, uni- or bilateral exophthalmia (with or without corneal opacity), distended abdomen and haemorrhages[15] (Figure 1). Meningoencephalitis has been reported in infected tilapia, as the bacteria cross the blood brain barrier[19,20] and similar clinical signs of disease were reported[21] in

tilapia both naturally and experimentally infected with either *S. iniae* or *S. agalactiae*. In the chronic form, yellow or dark red nodules were seen in the musculature near the vertebra of Nile tilapia.[18] An outbreak or cumulative mortality during chronic, persistent streptococcosis in tilapia can reach 80%,[22] while monthly prevalence of isolation ranged from 0% to 32% throughout the year.[23] Since *S. agalactiae* and *S. iniae* may be zoonotic,[24,25] in case of a chronic infection, the fish farmers may have a longer exposure to the bacterium, without very clear clinical signs.[18] This imposes a risk for the fish farmers, the fish processors, and the consumers.

Concurrent *Streptococcus* infection with other bacteria and tilapia lake virus (TiLV) has been reported in cultured tilapia.[26,27] The estimated economic impact of *S. iniae* and *S. agalactiae* infections in tilapia was around USD 150 million annually in 2000 and further increased to USD 250 million annually in 2008, representing approximately 5.7% and 6.7% of the total global value of tilapia, respectively.[14] However, no updated value on the economic impacts of streptococcosis in cultured tilapia is available.

Streptococcus isolated from fish are identified using a combination of phenotype (biochemical tests), serotype (agglutination test) and genotype (PCR, multi-locus sequence typing and whole genome sequencing). Barnes et al.[28] serologically and morphologically typed *S. iniae* isolates from tilapia (*Oreochromis* sp.) and hybrid striped bass (*Morone saxitilis* × *M. chrysops*) from the USA. Serologically distinct isolates of *S. iniae* identified as serotype I (ADH + ve) and II (ADH-ve) were isolated from natural disease infections in Thai tilapia farms.[29] Imperi et al.[30] reported 10 serotypes of *S. agalactiae* based on the composition of the capsular polysaccharide, where serotypes Ia, Ib and III are the most commonly reported strains in global tilapia outbreaks.[31,32] Genotyping studies using multi-locus sequence typing and whole genome sequencing have improved the understanding of pathogenesis of both *S. iniae* and *S. agalactiae*.[33]

In piscine streptococcosis, three major factors influence the pathogenesis; the virulence of the agent, the environmental stressors and the susceptibility of the host. Genetic virulence associated with genes that encode several protein molecules have been identified.[34] Buchanan et al.[35] identified the enzyme phosphor-glucomutase as the virulence factor for *S. iniae*. This enzyme inter-converts glucose-6-phosphate and glucose-1-phosphate, which play important roles in the production of polysaccharide capsule of *S. iniae* that enhances the bacterial virulence. In *S. agalactiae*, virulence gene profiles revealed that *S. agalactiae* serotype Ia ST7 lacked *lmb*, *scpB*, *pavA*, *fbsB*, *cyl*, *bca*, *cspA* and *bac* genes, which were present in serotype III ST283.[36] Varied routes of transmission have been reported in tilapia infections including cohabitation of infected and non-infected fish.[16] Transmission of *S. agalactiae* from a hatchery to a grow-out farm also has been documented.[23] Pradeep et al.[37] reported the first evidence demonstrating parents-to-offspring, vertical transmission of streptococcosis in tilapia.

Regarding vaccination, Shelby et al.[38] tested passive immunization of tilapia (*O. niloticus*) with intraperitoneal (i.p.) injection of anti-*S. iniae* whole sera, and this proved to be highly effective. Evans et al.[39] produced a *S. agalactiae* (Group B) vaccine for tilapia, which worked best after i.p. injection. Vaccination through i.p. injection with a re-attenuated strain of *S. agalactiae* (TFJ-ery), from the natural low-virulence *S. agalactiae* strain TFJ0901 as basis, gave almost 100% protection of tilapia.[40]

Regarding genetic resistance it is difficult to disentangle the role of tilapia species or strain, environmental conditions, pathogen prevalence and fish husbandry in susceptibility to different pathogens because most descriptions of disease are observational and not based on systematic comparison under controlled condition. Hence, any apparent association with species or breed may be due to underlying, uncontrolled, risk factors. There is, however, opportunity to breed for resistance to certain pathogens, as demonstrated recently for *S. agalactiae*, where a reduction in mortality of >50% could be achieved.[41,42] The impact of breeding for disease resistance on other desirable traits, for example, growth rate or flesh quality, is yet to be assessed.

2.1.2 | Aeromoniasis

Aeromonas spp. are ubiquitously found in freshwater environments and are described as infectious and opportunistic organisms, which may cause fish disease when stress factors are present in a diverse range of aquatic farming systems.[8] It has been shown, that *A. hydrophila* is one of the main pathogenic bacteria in tilapia culture, which not only causes high mortality and disease to cultured fish, but also causes similar problems to wild fish, resulting in huge economic losses, to both tilapia and wild fish.[43–45] It has been reported that aquatic animals infected with *Aeromonas* may suffer acute and chronic diseases, including haemorrhagic septicaemia, skin ulcers, and enteritis, with an average mortality rate of 30%.[46,47]

The taxonomy of the genus *Aeromonas* is subject to constant change, currently comprising 36 recognized species. The aeromonad fish pathogens are all motile with the exception of *A. salmonicida* subspp.[44] Generally, they are all described as Gram-negative, oxidase positive, facultative anaerobes.[48–50] They are non-spore forming, rod-shaped bacteria of approximately 1–3 μm[51,52] in length, capable of fermenting glucose and characterized by tolerating increasing concentrations of NaCl varying from 0.3% to 5%.[51]

A diverse range of motile aeromonads are reported as opportunistically pathogenic, especially under stressful environmental circumstances, resulting in clinical disease outbreaks leading to high levels of morbidity and mortality in a wide range of tilapia farming systems.[43,45] The most common species associated with natural disease outbreaks in farmed tilapia include *A. hydrophila*,[27,53–55] *A. sobria*,[56] *A. dhakensis*,[57–59] *A. veronii*[26,60,61] and *A. jandaei*.[60] The *A. hydrophila* and *A. veronii* had the highest prevalence of bacteria isolated from the liver, spleen, and other organs of infected tilapia.[60,62,63] Tilapia infected by these two species of bacteria showed lethargy, and apathy, ulcerations, pale spots, and haemorrhages along their body.[43,45,60,63] In addition, co-infections of *Aeromonas* with other bacteria is one of the important reasons for mass mortalities of tilapia, such as co-infection with *A jandaei* and *A. veronii*[60] (Figure 2),

FIGURE 2 Nile tilapia (*Oreochromis niloticus*), co-infected with *Aeromonas veronii* and *A. jandaei*. Courtesy Dr H. T. Dong (co-author)

Aeromonas sp. and *Streptococcus* sp.,[64,65] and of *A. veronii* and *F. columnare*.[26] Furthermore, co-infections with TiLV,[66] and with *S. agalactiae* and TiLV[27] (Figure 3) have been described.

The non-motile *A. salmonicida salm.* may cause furunculosis in salmonids and the atypical *A. salmonicida* is known to cause ulcer disease or erythrodermatitis in cyprinids[67] and in marine flatfish.[44] Experimentally induced infection of tilapia of 40 g through i.m. and i.p. injection of tilapia with atypical *A. salmonicida* at 28°C caused darkening, ulcers on the dorsal musculature and trunk region, gill congestion, exophthalmus and haemorrhages in the eyes, and reached 100% mortality at an i.m. dose of $\sim 1 \times 10^8$ CFU/fish. Internally, a congested liver and kidney were recorded.[68] Atypical *Aeromonas salmonicida* has been isolated from tilapia in Oman, but experimentally induced infection by intraperitoneal (i.p.) and intramuscular (i.m.) injection of 0.1×10^8 colony forming units (cfu) per 30 g tilapia at 26°C did not cause any disease or mortality.[69] In Bangladesh, a study was done on the presence of typical *A. salmonicida* in swamp water where tilapia is cultured, and its pathogenicity to tilapia of 10g after i.p. injection.[70] Results indicated, that the swamp water contained on average 3.3×10^6 CFU/ml. The injected tilapia showed 20% mortality at an i.p. dose of 3.3×10^6 CFU/g, and up to 80% mortality at an i.p. dose of 3.3×10^8 CFU/g at 20–25°C. They concluded, that natural average bacterial load of 3.3×10^6 CFU/ml or below in tilapia culture water did not produce significant mortality in *Oreochromis mossambicus*.[70] Overall, *A. salmonicida* may be harmful, but, like with motile aeromonads especially to injured tilapia under stressful conditions.

Identification of *Aeromonas* strains to species level is still a challenge because of the genetic heterogeneity of this genus.[71] Phenotypic identification of *Aeromonas* strains is done by physiological, morphological and biochemical characteristics.[48,72,73] Classic phenotypic characteristics that identify the genus *Aeromonas* are Gram-negative staining, the presence of cytochrome oxidase, and growth in nutritive broth at 0% NaCl in the presence of the vibriostatic factor O/129.[48,73] Commercial, fast identification systems, such as API 20E, Vitek, BBL Crystal, MicroScan W/A and others, have commonly been used to identify *Aeromonas* spp.[74] However, conventional methods based on the phenotypic properties and automated systems are of limited utility in identifying some *Aeromonas* spp.,[73] and their accuracy is affected by constant reclassification among components of this genus.[75]

Molecular biological techniques are the best option for the precise identification and taxonomic classification of the genus *Aeromonas*, through amplifying constitutive housekeeping genes (*gyrB* and *rpoD*) genes through polymerase chain reaction and sequencing the amplified products.[75] The 16S rRNA typing method, generally used in bacteriology[76,77] is also accurate for identification of *Aeromonas* spp.[78–80] Dong et al.[60] identified *A. jandaei* and *A. veronii* based on phenotypic features and homology of 16S rRNA. However, for certain species of *Aeromonas*, 16S rRNA alone will not adequately distinguish them, as additional sequencing of housekeeping genes such as *gyrB* is needed.[81]

Nile tilapia juveniles, after being exposed to transport-induced stress, appeared to have 19 responsible isolates of *A. hydrophila* in their body, as identified by 16S rRNA testing.[9] The *A. dhakensis* was firstly identified by phenotypic and 16S rRNA sequencing from diseased Nile tilapia.[57] Additionally, other molecular methods, such as the enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR), and the amplified fragment length polymorphism (AFLP) are also used for identification and genotyping of *Aeromonas*.[82–86] The ERIC-PCR is one of the most popular methods for genotyping *Aeromonas* because it is easy to carry out, does not require any expensive equipment, and is highly reproducible.[87]

Aeromonas virulence is complex since several factors contribute significantly to the development of the infection process.[88,89] These virulence factors such as structural components, extracellular products, secretion systems and proteins associated with metals acting jointly or individually enable the microorganisms to adhere to and invade host cells, evade host immune defences and compete for nutrients, resulting in an infection that generates the disease.[46,48,71,90–94] Four secretory systems have been reported in the genus *Aeromonas*, being types II, III, IV and VI. They are responsible for releasing virulence factors produced by bacteria into the extracellular environment or even directly into the host cell, which is extremely relevant to the host cell damage and infection processes.[46,50]

At present, there are no specific data on the transmission mechanism of *Aeromonas* in fish, but there are data on its transmission in humans. Holmberg et al.[95] studied the clinical and epidemiological characteristics of human enteritis caused by *Aeromonas* and believed that drinking untreated water was the most likely mode of infection for patients, supported by Moyer[96] in a study of *Aeromonas* isolated from diarrhoea patients. Ghenghesh et al.[97] proposed water and food transmission in their research on *Aeromonas* infections in humans in developing countries, which has certain limitations, compared to in fish. However, overall, it is recognized, that the transmission routes of *Aeromonas* are horizontal, via water, food and faeces.

Certain *Aeromonas* strains are serious pathogens of tilapia, devastating this industry worldwide. Therefore, proper preventive and control measures are necessary. Generally, antibiotics are the most effective and often the main option for tilapia farmers. An example of antibiotic susceptibility was published for tilapia in Ethiopia.[5] However, antibiotic therapy should always be based on an antibiogram, to be sure, the therapy is effective. Moreover, frequent use of antibiotics results in development of antibiotic resistant strains, bio-accumulation, changes in the physiochemical properties of water and imbalance of bacterial microbiota in the fish bodies or the habitat.[63,98,99]

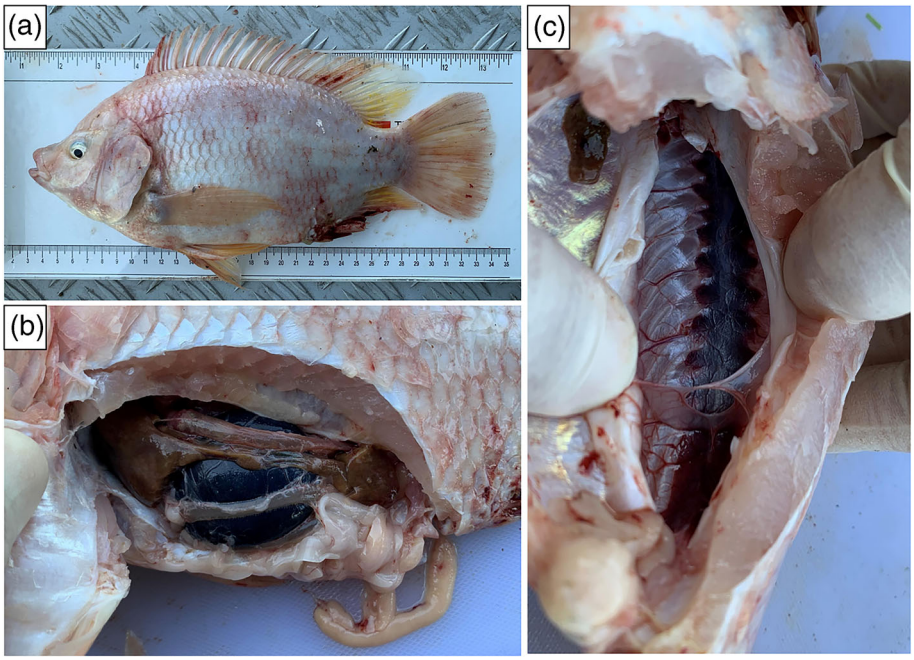

FIGURE 3 Clinical signs and gross lesions of red hybrid tilapia naturally co-infected with *Aeromonas hydrophila*, *Streptococcus agalactiae* and tilapia lake virus (TiLV). (a) Red skin with haemorrhages in the operculum, body and base of anal fin. (b) Enlarged gall bladder and brownish liver. (c) Haemorrhages of kidney. Photos: Courtesy: Mohammad Noor Amal Azmai (co-author)

Good Aquaculture Practice for tilapia,[100] and more specifically, vaccination may be the choice for prevention and treatment of *Aeromonas* infections. Formalin whole cell inactivated live vaccine was successfully used for the first time in tilapia in 1986, and the relative protection level of the vaccine was 100%, within 2 weeks after inoculation.[101] Since then, many researchers have been engaged in the research of fish vaccine against *Aeromonas* and obtained many achievements. Pridgeon and Klesius[102] prepared live vaccines against different virulent strains of *A. hydrophila*, with 100% protection at 14, 28 and 56 days post-vaccination (dpv). Pridgeon et al.[103] attempted live vaccines against *A. hydrophila*, *E. tarda*, *S. iniae* and *S. agalactiae* in tilapia and catfish. After bacterial challenge, the relative percentage of survival (RPS) of tilapia inoculated at 14 and 28 dpv were 100% and 92%, respectively. Aly et al.[104] developed an inactivated *A. hydrophila* vaccine for tilapia. An effective bivalent inactivated vaccine for tilapia brood stock against *S. agalactiae* and *A. hydrophila* resulted in 73.81% RPS after challenge by *A. hydrophila*.[105] Monir et al.[106] proposed an alternative method to reduce the main infectious diseases of tilapia, namely feed-based vaccination, and conducted experiments with four different forms and control groups of bivalent inactivated vaccines against *S. iniae* and *A. hydrophila* of hybrid red tilapia. The results showed that bivalent vaccines caused significant non-specific and specific immune responses to hybrid red tilapia, and had a high protective effect. This newly developed feed-based bivalent vaccine is an effective and large-scale fish immunization technique in aquaculture.[106] Some researchers developed recombinant fish vaccines to solve the serotype specificity issue.[107] The surface proteins Omp38 and OmpF of *A. hydrophila* were presented as vaccine candidates against *A. hydrophila*.[108] An S-layer protein-based vaccine for tilapia demonstrated a high protection against *A. hydrophila*.[109] Although some recombinant vaccines have been developed, these vaccines induce lower protection than whole-cell killed vaccines under the same conditions.[107]

Therefore, further works on recombinant vaccines should focus not only on optimizing and improving the protective efficacies, but on cost-effectiveness for commercial-scale to enable it as a viable solution to motile aeromonad septicaemia. At present, some studies have found that adding specific plant extracts to feed can prevent and treat some bacterial diseases in fish. Hardi[110] found that when combined extracts of *Boesenbergia pandurata* (BP), *Solanum ferox* (SF) and *Zingiber zerumbet* (ZZ) were added to fish diets, in particular, SF50/ZZ50 (50 mg SF extract/kg feed with 50 mg ZZ/kg feed) had positive effects on the immune system of tilapia in the treatment and prevention of bacterial infection. Adding ZLP (*Ziziphus mauritiana* leaf powder) into the tilapia diet enhanced the immune and antioxidant capacity to effectively control *A. hydrophila* infection of Nile tilapia.[111] Plant extracts carvacrol and cymene at concentrations of 100 or 200 ppm were used as effective oral treatment of experimentally infected *Oreochromis niloticus* with atypical *A. salmonicida*.[68] Kuebutornye used *Bacillus* isolated from tilapia, and Phumkhachorn used bacteriophages to control *A. hydrophila* infections in tilapia (*O. niloticus*).[54,112]

2.1.3 | Francisellosis

Francisella orientalis, formerly known as *F. noatunensis* subsp. *orientalis*,[113,114] has been recognized as one of the most serious pathogens of tilapia (*Oreochromis* spp.) and other fish species such as three-line grunt (*Parapristipoma trilineatum*) and hybrid striped bass

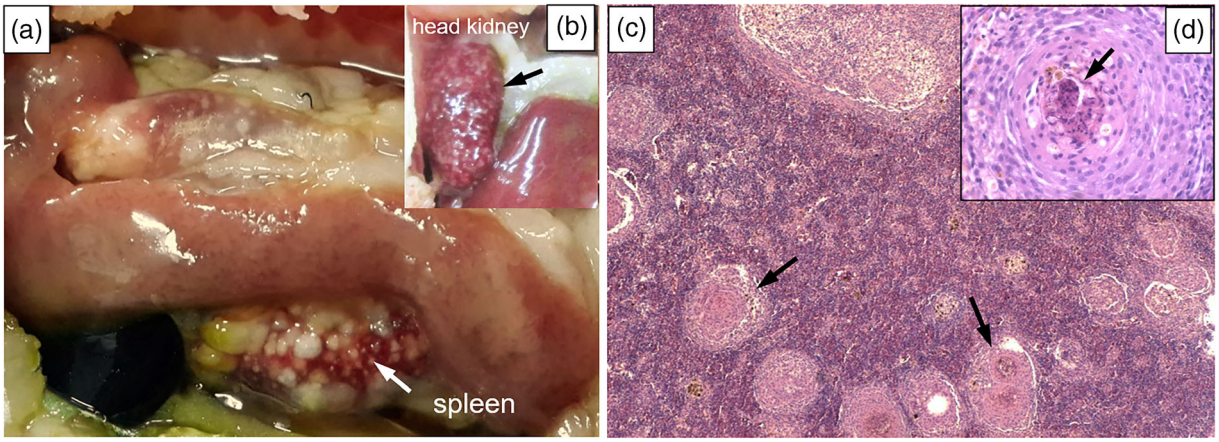

FIGURE 4 Francisellosis in tilapia. (a) Granuloma in head kidney of *F. orientalis* infected tilapia. (b) Same fish: Granuloma in spleen. (c, d) Haematoxylin–Eosin stained histological sections of the spleen of a tilapia from indoor recirculation aquaculture in the Netherlands showing granuloma from a systemic and chronic *Francisella*-infection. (c): 40× magnification. (d): 400× magnification. Pictures (a, b): courtesy Dr H. T. Dong (co-author); (c, d): courtesy Dr O. Haenen (leading author)

(*Morone chrysops* × *M. saxatilis*), both farmed and wild, from various geographical regions worldwide.[115–120] Occurrence of francisellosis in farmed tilapia has been documented in Brazil,[121] China,[122] Costa Rica,[123,124] Indonesia,[125] Taiwan Province of China,[126] Thailand,[127] United States[128] and United Kingdom.[129] Initially considered a *Rickettsia*-like[117,126,130] or *Piscirickettsia*-like organism,[130] the pathogen was later confirmed as a ɣ-Proteobacteria in the family *Francisellaceae*, order *Thiotrichales*.[131]

The typical gross pathological signs of francisellosis in tilapia and other species such as three-lined grunt and hybrid striped bass have been commonly manifested as granulomatosis (Figure 4) causing renomegaly and splenomegaly typically ascribed to multiple whitish nodules with comparable lesions in the gills, muscle or liver.[126,127] Furthermore, pale body coloration, the presence of numerous white granulomas on gills and internal organs including the spleen, liver, kidney and intestine have been noted in tilapia infected with *F. orientalis*.[127] Francisellosis could induce 50%–60% mortality in cultured tilapia which usually occurs in cool season, that is, when water temperature ranges from 23°C to 26°C.[127] Notably, coinfection of *F. orientalis* and the ciliate parasite *Ichthyophthirius multifilis* could lead to more severe mortality compared to the single infection with either *F. orientalis* or *I. multifilis*.[132]

Francisella spp. are strictly aerobic, facultatively intracellular, nonmotile, Gram-negative coccobacilli to pleomorphic spherical measuring 0.1–1.5 μm in size.[131] Members of the genus *Francisella* are fastidious in their requirements for growth on laboratory media and require specific media for in vitro culture. Isolation of *F. orientalis* from the blood, spleen, kidney or granulomatous lesions of infected fish has been successfully attained using enriched blood agar plates supplemented with 0.1% cysteine and 1% glucose, cysteine heart agar with 5% sheep blood (CHAB) or cysteine heart agar with 1% haemoglobin (CHAH) or Thayer–Martin Media,[118,124,131] with optimal growth of *F. orientalis* on these enriched blood agar plates observed at 28–30°C.[124] The addition of polymyxin B (100 μg/mL) with or without ampicillin (50 μg/ml) to selective agars was successfully used for the isolation of *F. orientalis*.[124] Additionally, nucleic acid-based detection methods including conventional polymerase chain reaction (PCR),[113,115,124,127,133] quantitative real-time PCR (qPCR),[133–137] duplex PCR, *in situ* hybridisation,[138] recombinase polymerase amplification (RPA),[139] and loop-mediated isothermal amplification (LAMP)[37] have been applied for the detection of *F. orientalis* in tilapia.

Nile tilapia experimentally infected with *F. orientalis* via immersion challenge exhibited the highest number of bacteria, that is, quantified as *F. orientalis* genome equivalents by qPCR, in their surface mucus at 3 h post-infection. Moreover, at 96 h post-infection, septic fish had marked increases of *F. orientalis* genome equivalents in their gills, anterior and posterior kidney, spleen, liver, heart, gastrointestinal tract and gonads which corresponded with the appearance, size and number of granulomas typical of francisellosis.[140] Homologues of virulence genes associated with the serious, zoonotic pathogen *F. tularensis*, detected in various cold and warm-blooded animals and humans,[141] have also been identified in *F. orientalis* including the intracellular growth locus (IGL; *iglA*, *iglB*, *iglC* and *iglD*) genes associated with the type 6 secretion system present on the *F. tularensis* pathogenicity island.[142] Soto et al.[142] reported that a functional *iglC* gene of *Fno* was crucial for intramacrophage survival, although *iglC* gene played no role in protection from serum killing. The *iglC* gene is by far one of the most extensively studied genes within the *Francisella* pathogenicity island owing to its marked expression during intracellular growth, demonstrating its significance for pathogenicity and virulence.[143] Also, serum complement and host cell mannose receptors have been recognized as vital for internalization of *F. orientalis* in macrophage.[130] Horizontal transmission of *F. orientalis* via the waterborne route has been demonstrated by Soto et al.[140] in Nile tilapia fingerlings under experimental condition.

Additionally, Pradeep et al.[37] documented that apparently healthy red tilapia (*Oreochromis* spp.) broodstock who were asymptomatic

carriers of *F. orientalis* could vertically transmit the pathogen to the fertilized eggs. Evidence of vertical transmission was subsequently confirmed in a controlled laboratory challenge.[144] Therefore, utilization of *F. orientalis* negative tilapia broodstock is an important strategy to prevent vertical transmission of *F. orientalis* to their offspring.

Although commercial vaccines are currently unavailable, there are promising results from research. In 2019, developed *F. noatunensis* subsp. *orientalis (Fno)* whole-cell vaccines were developed for tilapia.[145,146] A whole-cell formalin-inactivated autogenous vaccine was developed using the highly virulent isolate STIR-GUS-F2f7 and the oil-based adjuvant Montanide™ ISA 763A VG showing 100% RPS (relative percentage of survival) rates in red tilapia after i.p. injection with 4.0×10^3 CFU/fish.[145] Shahin et al.[146] compared a 100% RPS giving *Fno* vaccine with inactivated whole-cell injection vaccines of *Fno*, using bacterial strains from various geographical regions in heterologous and homologous infection trials by i.p. injecting nile tilapia. They found RPS values of 65.9%–82.3%, with the highest in homologous trials.[146]

Pulpipat et al.[147] demonstrated recently the efficacy of a formalin-killed *F. orientalis* vaccine in cultured tilapia via intraperitoneal injection. Vaccinated fish experimentally challenged with *F. orientalis* via intraperitoneal injection and immersion at 6 weeks post-vaccination led to production of potent antibodies and relative percent survival (RPS) of 71% and 76%, respectively. Transcripts of proinflammatory cytokines and immune-related genes, including interleukin-1β (IL-1β), tumour necrosis factor-alpha (TNFα), C-X-C motif chemokine ligand 8 (CXCL8) and interleukin-17C (IL-17C), were significantly upregulated after vaccination. Additionally, vaccinated fish had lower bacterial loads in the blood and lower granuloma intensities in the kidney, spleen, liver and gill compared with the unvaccinated fish. Antibiotic administration of in-feed oxytetracycline and florfenicol to naturally and experimentally infected tilapia resulted in lower mortalities[148] suggesting efficacious antibiotic treatment. Furthermore, antibiotic treatment was particularly noted to be effective during the acute stage of infection.[148] Accordingly, in the event of an outbreak, it is prudent to depopulate fish and disinfect the facility with disinfectants that are effective against planktonic and biofilm forms of *F. orientalis*.[149]

2.1.4 | Flavobacteriosis

Flavobacteriosis, and in this case, columnaris disease caused by *F. columnare* (also known as myxobacterial disease, peduncle disease, saddleback, fin rot, cotton wool disease or black patch necrosis) is one of the oldest known diseases of freshwater fish species worldwide.[150,151] The *F. columnare* associated with (or isolated from) tilapia was recently renamed to *F. oreochromis*.[152]

The disease affects various fish species culturing in both cold and warm water, including tilapia (*Oreochromis* spp.).[153–155] The earliest report of columnaris disease in farmed Nile tilapia was documented in Egypt[156] but remained relatively unrecognized until recent reports in Brazil[154] and Thailand.[155,157] The disease affects fish in both hatcheries and grow-out systems, and resulted in 10%–70% cumulative mortality in natural outbreaks.[155] Experimental challenge resulted in variable levels of mortality ranging from 0% to 100% in hybrid red tilapia (*Oreochromis* sp.) fry and juveniles.[157,158] Major gross signs of disease fish were discoloration, fin and skin erosion and gill necrosis[155–157] (Figure 5).

Flavobacterium columnare is a Gram-negative, slender filamentous bacterium. This bacterium produces flexirubin pigment and forms yellow rhizoid colonies on culture media due to the characteristic of gliding motility on solid surface.[150,153] Dong et al.[155] reported that the isolates from tilapia exhibited homologous phenotypic characteristics, but high genetic diversity. Based on the restriction fragment length polymorphism of the 16S rRNA gene (16S-RFLP), a scheme for genetic typing *F. columnare*,[159] the isolates from tilapia were classified into three genomovars (I, II and I/II) with predominance of genomovar II.[155,160] Phylogenetic analysis based on the 16S rRNA suggested that majority of tilapia isolates belong to a unique genetic group.[155,161] Comprehensive genomic comparison of *F. columnare* isolates derived from different host species revealed extensive sequence diversity where the unique strains from tilapia were thought to represent the forthcoming novel taxa or subtaxa in the genus *Flavobacterium*.[162] In 2022, this was confirmed, as many *F. columnare* strains were genetically reclassified by phylogenetic analyses of 16S rRNA and *gyrB* genes, and this resulted in four genetic groups, with proposed names of 4 species: Genogroup 1 = *F. columnare*, Genogroup 2 = *F. covae* sp. nov. (AL-02-36$^{\text{Type strain}}$), Genogroup 3 = *F. davisii* sp. nov. (90-106^{T}), and genogroup 4 = *F. oreochromis* sp. nov (Costa Rica 04-02-TN^{T}), with at least the last species being a tilapia pathogen.[152]

Apart from gross pathological signs, examination of long rod-shaped filamentous bacteria through wet-mount and/or rapid Gram-staining for smeared lesions are useful for presumptive diagnosis of columnaris disease in tilapia. Bacterial isolation was successful using selected media such as Anacker and Ordal's agar (AOA), modified Shield agar (MSA) or tryptone yeast extract salts (TYES) agar supplement with antibiotics either tobramycin or neomycin and polymyxin B.[150,155] Specific PCR,[163,164] LAMP,[165] and *F. columnare*-monoclonal antibodies[166] have been used for rapid diagnosis of *F. columnare* from clinical samples and bacterial culture. Sequencing of 16S rRNA and/or whole genome represents common approach for identification and characterization of this bacteria.[155,161,162]

The tilapia isolates form two different colony morphotypes (rhizoid *vs.* non-rhizoid). The rhizoid morphotype is highly pathogenic while the non-rhizoid morphotype has non- or low pathogenicity.[157,158] Comparative studies of *F. columnare* revealed that the adhesion ability to the gill surface, biofilm formation and the production of capsular polysaccharide are significantly associated with the highly pathogenic strain of *F. columnare*.[157] Like other *F. columnare* infections, the disease in tilapia affects the skin, gills and muscle and is rarely found in the internal organs.[26,155,167] Coinfections of *F. columnare* and other pathogens have been recorded which may contribute to increasing disease severity.[26,158,168,169] Horizontal transmission through waterborne routes have been demonstrated by experimental immersion studies for both Nile tilapia and hybrid red tilapia.[138,156,157] It is unclear whether *F. columnare* transmits vertically. However,

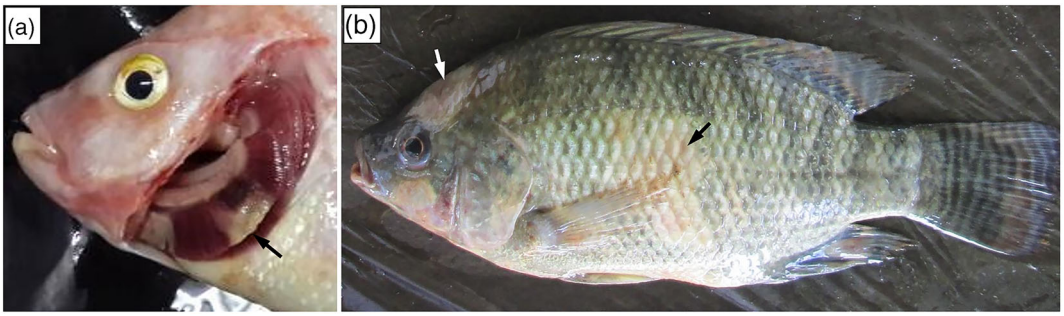

FIGURE 5 Tilapia (*Oreochromis* sp.) infected with *Flavobacterium columnare* (new, proposed name *F. oreochromis*[152]), showing (a) Gill necrosis (arrow), and (b) Superficial skin necrotic lesions all over the body, with deslimed areas (arrows). Pictures courtesy Dr H. T. Dong (co-author)

detection of *F. columnare* in reproductive organs of apparently healthy tilapia broodstock, fertilized eggs and newly hatched fry suggested possible maternal transmission.[165]

Effective antibiotic therapy against flavobacteriosis in tilapia is difficult, as mostly other factors, like stress play a role in the disease. Moreover, findings on antibiotic susceptibility differ. Various fish strains of the salmonid pathogen *F. psychrophilum* were found susceptible to ampicillin, erythromycin, streptomycin, tetracycline, trimethoprim-sulphate, with resistance against neomycin and polymyxin.[170] The oxytetracycline-treated group showed significant reduction in these lesions and the treated fish appeared normal. Use of a probiotic, *Bacillus subtilis* was tested in water and in fish feed as prophylaxis and was effective in amelioration of lesions caused by *F. columnare* in Egyptian freshwater fish.[171] They also stated that oxytetracycline was effective to treat columnaris disease.[171] In an Egyptian Master thesis[172] strains of *F. columnare* were found susceptible to tetracycline, nalidixic acid, trimethoprim, erythromycin, streptomycin and doxycycline with high resistance to neomycin. Studied 20 strains of *F. columnare* of Nile tilapia were tested for *in vitro* susceptibility to amoxicillin, amoxicillin, clavulanic acid, amikacin, cefixime, ciprofloxacin, novobiocin, neomycin, norfloxacin, nitrofurantion, poly mixin B and tetracycline: They found multi-resistance in >18/20 strains.[173] A paper on the development of genetic-resistant strains of Nile tilapia against *F. columnare* presented promising results as a longer-term alternative to antibiotic treatment.[174]

2.1.5 | Vibriosis

Fish vibriosis is referred to as a systemic infection caused by a number of *Vibrio* spp., including *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum* and *V. vulnificus*.[175,176] The genus includes Gram-negative, oxidase-positive rod-form bacteria with polar flagella, ubiquitous in marine and estuarine ecosystems. Although vibriosis has multiple clinical manifestations, depending on the host and bacterial species, in all cases the acute form is a septicaemia that can lead to death, mainly in immunocompromised hosts.[177–179]

Vibriosis is commonly associated with brackish and marine aquaculture, and therefore, tilapia cultured in these environments are

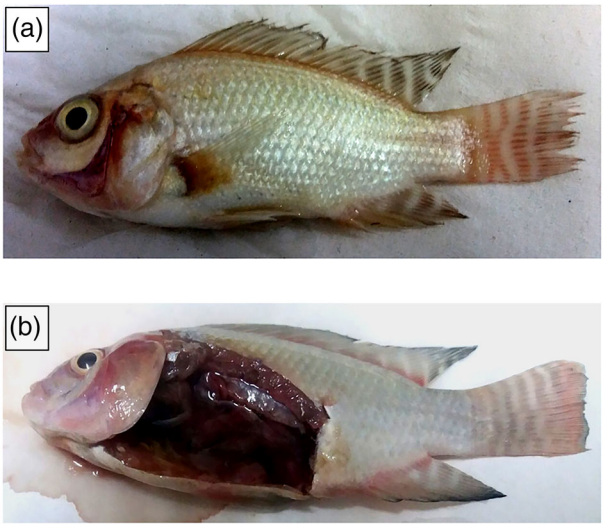

FIGURE 6 Vibriosis caused by *Vibrio vulnificus* pathovar *piscis* in Nile tilapia *(Oreochromis niloticus)*. Images correspond to moribund tilapia after being challenged by immersion. Clinical signs mirror those of the natural disease, a septicaemia characterized by haemorrhages in (a) the mouth, head and fins and in (b) the intestine, abdominal cavity and muscle. Pictures courtesy of Dr B. Fouz (co-author)

susceptible. Although sporadic cases of some and related *Vibrio* spp. have been isolated from diseased tilapia (*V. parahaemolyticus* or *Photobacterium damselae* subsp. *damselae*, *Phdd* [formerly *V. damsela*]),[180,181] *V. vulnificus* is the major pathogenic *Vibrio* spp.[175,179] It is important to highlight that, within this species, only pathovar *piscis* (pv. *piscis*; formerly Biotype 2) is considered as fish pathogenic,[182] and the disease is known as warm-water vibriosis (WWV).[179,183]

Pv. *piscis* strains possess a conjugative fish virulence plasmid (pFv) absent in other strains of the species, and group in several clades/serovars, Ser E and the recently described Ser T proving zoonotic potential.[184,185] Different authors have reported *V. vulnificus* as the causative agent of infectious episodes/outbreaks in Japan,[186] Taiwan Province of China,[187] Bangladesh,[188] India,[189] or eastern Mediterranean farms.[185,190] In all cases, the bacterium was mostly isolated from the diseased fish blood, kidney, liver, spleen, and brain. Diseased fish showed dark coloration, external haemorrhagic areas, exophthalmia and skin ulcers. Internally, a pale liver

with haemorrhagic lesions, oedematous brain or splenomegaly were observed. Moreover, some authors have experimentally induced infections and disease in Nile tilapia after challenges with pv. *piscis* strains (specifically serovars/clades E, A and the new one described T)[185,191–193] (Figure 6).

Simple and rapid methods to identify *Vibrio* spp. causing disease in cultured fish are essential in order to take fast preventive and curative decisions. Individuals with clinical signs of septicaemia compatible with vibriosis should be analysed microbiologically by bacterial isolation, using a general medium such as TSA-1 (1% NaCl concentration), together with thiosulfate-citrate-bile salts-sucrose (TCBS) and/or *V. vulnificus* medium (VVM) agar.[179] However, since *V. vulnificus* is recovered as a pure culture from diseased tilapia, also media, like sheep blood agar plates may be used to isolate *V. vulnificus*. Pure cultures could be tentatively identified to species level using the commercial phenotypic API 20E system (bioMérieux). Afterwards, PCR- or protein-based (like MALDI-TOF) methods should be used to confirm species[194] or subspecies identification.[195]

In the case of *V. vulnificus*, PCR targeting *vvhA*, *fpcrp* and *seq61* genes allows to identify strains to species, pv. *piscis*, and zoonotic Ser E, respectively.[196] *V. vulnificus* strains could be subtyped for public health hazard by a PCR that amplifies a variable region located within the gene *pilF*.[197] Although *V. vulnificus* is generally sensitive to most antimicrobials permitted on fish farms in the EU and the USA, an antibiogram must be performed to select the most effective antibiotic to start the treatment as soon as possible.

Fish pathogenic *Vibrio* spp. exhibit different virulence factors such as capsular polysaccharides, adhesive factors, cytotoxins, lipopolysaccharides and flagella.[198] In bacterial pathogenesis, the adherence to the host surface is considered a critical step and can be favoured by flagella, capsules or loose slime. Resistance to phagocytosis and complement-mediated killing together with efficient iron acquisition systems allow bacteria to colonize the host and multiply. Moreover, toxins and exoenzymes are responsible for host lesions. *V. vulnificus* pv. *piscis* initially colonizes the gill/skin mucus, being protease VvpE and the capsule involved in this process and invasion is favoured by local damage and destruction of phagocytes by excreted toxins (mainly toxin RtxA1).[179] When bacteria enter the bloodstream of the fish, they are able to survive, proliferate, and therefore, induce the fatal septicaemia. Under iron restriction, the bacterium over-expresses the haemolysin VvhA and RtxA1 toxins as well as the outer membrane proteins Fpcrp (fish phagocytosis and complement resistance protein) and Ftbp (fish transferrin-binding protein), which constitute a 'survival in fish blood kit',[199] encoded by plasmidic genes. pFv and closely related plasmids have probably been acquired in fish farms by different clones which have been amplified after successive outbreaks.[182,185]

Vibriosis is a water-borne infection, meaning that the etiological agent uses the water column as its natural transmission medium. In fact, experiments with eels and tilapia artificially infected with pv. *piscis* by different routes revealed that immersion in water followed by ingestion is the primary route for the transmission of WWV.[183,185,191–193] The virulence of the strain is strongly dependent on the water salinity (maximum at 0.5%–1.5%, depending on the serovar) and temperature (maximum at 28°C).[183,193] Similar observations were reported in transmission of vibriosis caused *by Phdd*,[200] another potential pathogen for Nile tilapia. Therefore, since *Vibrio* spp. can be transmitted horizontally, either from open lesions or as secretion in the faeces of infected fish and carriers, pathogenic strains can be easily transferred among fish in the nearby area using water as transport medium.

Finally, efficient preventive measures in tilapia farms against *V. vulnificus* pv. *piscis* are considered necessary, including both manipulation of physicochemical parameters (use of freshwater and temperature below 26°C) and specific vaccination. In fact, a patented vaccine called *Vulnivaccine* has proven to be highly effective against WWV at eel farms.[179]

2.2 | Emerging bacterial diseases of concern

2.2.1 | Edwardsiellosis

Edwardsiella is well known as a genus hosting severe pathogenic bacteria affecting global aquaculture with various fish species, including tilapia.[201–203] The genus comprises Gram-negative, rod-shaped bacteria belonging to the family Enterobacteriaceae and the order Enterobacteriales.[204] The bacterium is a facultative intracellular pathogen that can survive inside fish phagocytes such as macrophages and neutrophils.[205,206] Since recently, the genus comprises five species, and three of them have been reported to infect and cause mortality in Tilapia including *E. ictaluri*, *E. tarda* and *E. anguillarum*.[201,202,203,207,208]

2.2.2 | *Edwardsiella ictaluri*

Edwardsiella ictaluri, is the causative pathogen of enteric septicemia in channel catfish[209] and freshwater catfish species *Pangasianodon hypophthalmus*.[210] It now has a less restricted host range causing disease in various catfish species[167,211–215] and non-catfish species such as zebrafish, and wild ayu in Japan.[216–218] Natural disease outbreaks reported in several fish species showed that this pathogen produced 40%–90% mortality,[207,219] while experimental infection resulted in up to 100% mortality,[207,216,220,221] indicating that *E. ictaluri* is a pathogenic bacteria of multiple freshwater fish species. The first detection of *E. ictaluri* in tilapia was in the Western hemisphere.[203] Natural disease cases of *E. ictaluri* in red tilapia raised in open floating cages were first detected in Southeast Asia in 2016,[207] and truly have become an emerging disease, widespread to a large region in Vietnam, with high risk of further national and international spread.[208] *E. ictaluri*-affected tilapia did not exhibit recognizable external signs, causing misleading presumptive disease diagnostics and untimely treatment efforts under active surveillance. Early diagnostic screening and biosecurity measures are highly recommended to prevent for transboundary spread and negative impact of this pathogen.

Gross signs with white spots appearing on the spleen and head kidney are critical features for the first detection (Figure 7). In

FIGURE 7 Diseased tilapia by *Edwardsiella ictaluri* from an experimentally induced infection. Courtesy Dr Truong Dinh Hoai (co-author)

addition, pale gills due to anaemia and the liver due to the reduced fat reserve in the liver are also helpful for screening affected fish.[203,207,208] Wet-mount with gram staining with the presence of Gram-negative, rod shape, the intracellular bacterium could be the first step to confirm the presence of *E. ictaluri* from fresh fish tissue such as kidney, spleen. A distinguishing test should be performed between francisellosis through *F. noatunensis* and *F. orientalis*, and edwardsiellosis through *E. ictaluri* because the clinical signs of visceral white spots had always been linked to these diseases. PCRs should be developed, but currently, the wet-mount technique could help to distinguish them, since *F. noatunensis* and *F. orientalis* have a different shape as coccobacillus bacteria.[133] *E. ictaluri* grows as typical whitish pinpoint colonies on culture media. Biochemical characteristics of *E. ictaluri* from tilapia were identical to a strain isolated from catfish, except for the Voges–Proskauer test which was variable among isolates.[207,208,210] Thus, the combination of sequencing of 16S-rRNA, house-keeping genes such as *gyr*B for phylogenetic analysis[222,223] and specific PCR-based assay[224] were accurate for identifying *E. ictaluri*. To discriminate *E. ictaluri* from tilapia from other different hosts and geographic origins, parallel and combined techniques such as rep-PCR, 16S, *gyr*B and sequencing plasmid or whole-genome has been recommended.[225,226]

Regarding pathogenesis, varieties of virulence factors for *E. ictaluri* have been identified, such as extracellular capsular polysaccharide, fimbriae-like structures, chondroitinase, lipopolysaccharides O side chain and outer membrane protein. Other known pathogenicity islands such as the type III secretion system (T3SS) gene *esrC*, the putative T3SS effector *eseI* and its chaperone *escD*, the type IV secretion system (T4SS) gene *virD4*, the type VI secretion system (T6SS) gene *evpC* and *ureA-C* of the urease operon have been determined also as the virulence factors of this pathogen. However, the distribution of virulent factors varied between species.[214] The screening of six virulence genes from *E. ictaluri* isolated from tilapia outbreaks revealed that the presence of *esrC*, *evpC* and *ureA-C* genes were in all strains, but they did not have *virD*, *eseI* and *escD* genes which were present in strains of channel catfish.[208,225] The completed pathogenicity test conducted by the latest study from outbreaks in southeast Asia showed that the lethal dose LD50 of the Asian strain is very low, $<10^2$ CFU/fish, to kill 50% of the tilapia population. The results suggested that new, hyper virulent *E. ictaluri* strains are circulating and spreading in this region.[208] Thus, the mechanism and virulent gen distribution of *E. ictaluri* strains infecting tilapia need to be clarified in further studies.

The pathogen could be transferred horizontally between fish and spreads by the water flow. The disease outbreak has existed in both freshwater ponds[203,208] and in floating cage farms on the rivers and reservoirs.[207,208] However, the data from the survey showed that the open tilapia culture system has a higher risk for the disease than culture ponds do.[208] Disease outbreaks have occurred from fingerling fish to marketable size,[203,207] but fish less than 350 g were more sensitive to this pathogen. The mortality rate from the outbreaks ranged from 30% to 65%.[207,208] *E. ictaluri* can attach and penetrate host mucosal membranes rapidly and establish a systemic infection. It is also a facultative intracellular pathogen, which may survive inside phagocytic cells, which could be a mechanism of dissemination. This characteristic plays a vital role in the rapid spread of the disease. The disease appeared in the temperature range of 23–29°C. The detection of *E. ictaluri* associated with disease outbreaks from two different continents (America and Asia) highlights the risk of transboundary spread and potential impact on the tilapia industry.

Although the serious fish disease caused by *E. ictaluri* was first detected in farmed tilapia in Asia only 5 years ago, the isolated *E. ictaluri* show already high levels of antibiotic resistance.[208] Nevertheless, alternatives to antibiotics should be further explored to tackle this emerging, highly pathogenic bacterium. Current studies investigate the presence of homologous strains from outbreaks. Thus, an autogenous vaccine might be the best option to combat this emerging disease in the present time before a better vaccine candidate for a wider region is discovered.[207,208]

2.2.3 | *Edwardsiella tarda*

Edwardsiella tarda is a Gram-negative, motile, short, rod-shaped bacterium (1 μm × 2–3 μm) of the family Enterobacteriaceae. It is a severe pathogen for a variety of fish.[168,227] Principally, *E. tarda* have been isolated from different aquatic water environments and affected fish are common intestinal carriers of this pathogen, thereby resulting in possible contamination of fish carcasses during fish processing. They have been found in the intestines of infected humans, after consumption of contaminated fish. This pathogen is often responsible for septicaemic fish disease, causing mass mortalities (up to 70%) and high economic losses in fish farms of freshwater and marine fish in many countries.[228,229] Tilapia is one of the susceptible fish to *E. tarda* and disease cases have been reported in several countries[201,230] in Nile tilapia (*O. niloticus)* and red tilapia.[231,232]

The clinical, gross and microscopic changes caused by *E. tarda* have been relatively well characterized for a range of different fish species, especially catfish. For tilapia, gross disease signs include corneal opacity and loss of the eyes, reddening of the anal papilla and marked pallor of the gills (Figure 8). Internally, the kidney and liver may be pale and seeded with white nodules. The swim bladder and kidney existed of flocculent material, with congestion and

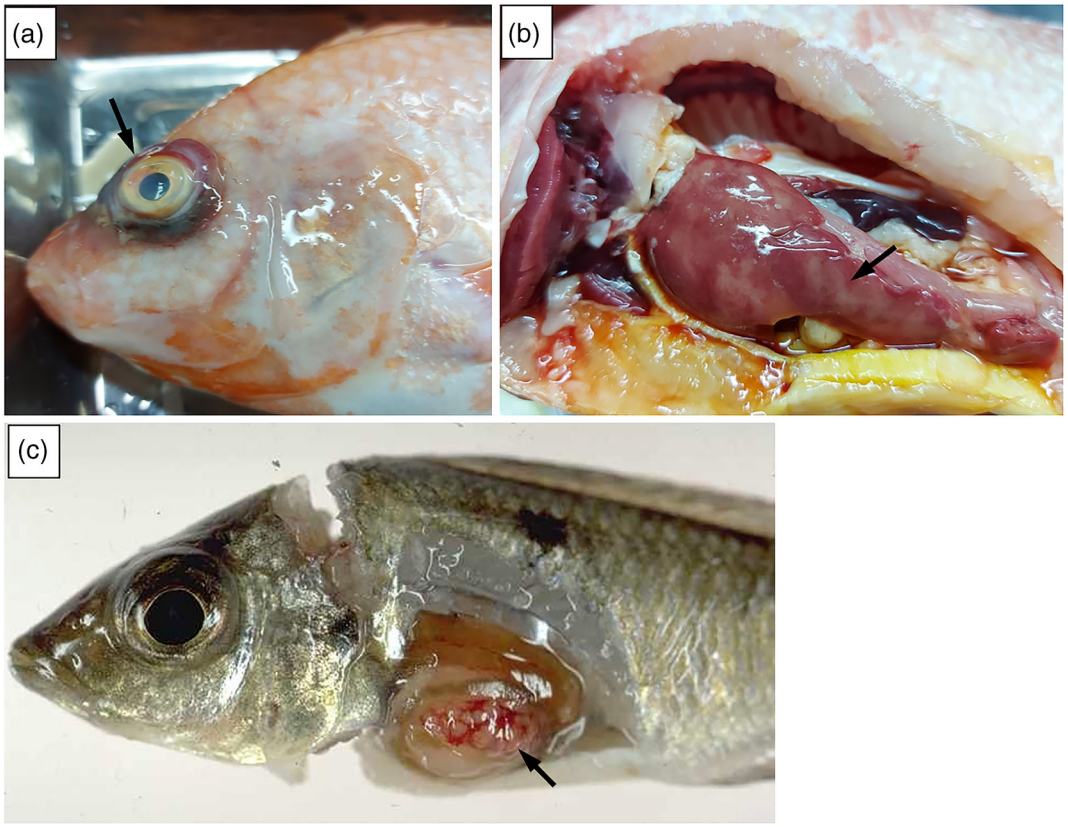

FIGURE 8 Edwardsiellosis by *Edwardsiella tarda* in tilapia or cichlids. (a) Corneal opacity, inflammation and loss of the eyes. (b) Pale organs with white nodules. (c) Cichlid from a zoo with a systemic *E. tarda* infection: anorexia and bacterial nodules (arrow) can be seen. Pictures courtesy (a, b): Dr H. T. Dong (co-author); (c) Dr O. Haenen (leading author)

haemorrhage on the intestine.[233] Microscopic lesions in the brain and lymphoid organs of tilapia were also demonstrated.[233,234]

Edwardsiella tarda is usually identified based on its unique biochemical characteristics after isolation on brain-heart infusion agar or tryptone soya agar from infected fish. PCR-based detection with *gyrB* gene was developed for *E. tarda* from fish species and successfully modified to nested PCR and applied to detect affected tilapia using tissues samples.[233,235] Since 2013, *E. tarda* has been subdivided into three genetically distinct species regarding infecting fish, *E. tarda*, *E. piscicida* from various fish,[236] and *E. anguillarum* (from eel),[237] based on several identification techniques including sequencing analysis of *gyrB* and *sodB* genes, nested PCR, rep-PCR and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF), proven effective for *E. tarda* identification.[238,239] However, the above techniques have almost not yet been used for tilapia isolates of *E. tarda*, and further assessment needs to be done. Also, we should realize, that published casus with identifications of *E. tarda* from tilapia from before 2013 may have represented causes of *E. anguillarum*, or perhaps of *E. piscicida*.

Virulent factors of *E. tarda* were well characterized in fish species including type III secretion systems (TTSS apparatus protein *Esa*B-V, TTSS chaperone protein *Ecs*A-C, TTSS effector protein *Ese*B-G and TTSS regulator protein *Esr*A-C), type IV secretion systems (*Evp*A-P) and other proteins including autotransporter protein (AidA), α-hemolysin-modulator like protein (HhaEt), hemolysin A, B (EthA, EthB), DNA-binding transcriptional regulator and sensor protein QseC (QseB, QseC), component regulator and sensor proteins (PhoP and PhoQ).[229] In tilapia, the role of regulator FucP regulation of the T3SS in *E. tarda* has been demonstrated to contribute to pathogenesis.[240] *E. tarda* isolated from diseased Southern flounder (*Paralichthys lethostigma*) has been demonstrated to be virulent to Nile tilapia.[241]

Edwardsiella tarda could be transferred horizontally between fish via the faecal–oral route. The wide range of hosts such as invertebrates, amphibians, reptiles, birds, a variety of fish, mammals and humans indicated that it has a wide geographical distribution and is an important pathogen in terms of public health as an epizootic and zoonotic bacterium. In aquaculture, this pathogen commonly exists in the environment, pond water and sediment. High temperature, poor water quality and high organic load increase the risks of infection.[242] In addition, cross-contamination may occur during manipulation of fish skin, handling and preparing fish seed, or in integrated farming where tilapia are reared in conjunction with other animals, or the cross-infection between other fish species and tilapia in the polyculture system.[242,243]

A variety of chemicals have been tested and demonstrated to be effective disinfectants against this pathogen, including ethyl alcohol (30%, 50% or 70%), benzyl-4-chlorophenol/phenylphenol (1%), sodium hypochlorite (50, 100, 200 or 50,000 mg/L), n-alkyl dimethyl benzyl

ammonium chloride (1:256), povidone–iodine (50 or 100 mg/L), glutaraldehyde (2%) and potassium peroxy–monosulphate/sodium chloride (1%). However, using chemicals may raise concerns about toxicity to the environment, costs and human health risks, and is impractical in a large volume of water or cage culture in rivers or lakes.[242]

Antibiotics have been used popularly for the treatment of the disease. However, overuse of antibiotics has accounted for a major antibiotic resistance of *E. tarda* in tilapia.[232,244,245] Alternatives to chemical and antibiotic use have been investigated against *E. tarda* in tilapia, including use of natural compounds (carvacrol and cymene),[246] glucose, polysaccharides, yeast oligosaccharide,[247–249] essential oils,[250] ascorbic acid, α-tocopheryl acetate and selenium,[251] kugija *Lycium chinense*[252] and probiotics.[253–255] Another affordable alternative to antibiotics is the use of vaccines. Several developed vaccine candidates were investigated, including the vaccines *E. tarda* ghost,[256] live cells of *E. tarda*[257] and a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) vaccine from *E. ictaluri* against *E. tarda*.[258]

2.2.4 | *Edwardsiella anguillarum*

Edwardsiella anguillarum shares similar characteristics to other *Edwardsiella* isolates, such as the growth capability under anaerobic conditions; however, its non-motile nature differentiated it from other groups.[259] *E. anguillarum* was the last group to be distinguished from the *E. tarda* group and demonstrated virulence to a variety of fish species, including tilapia in Costa Rica and Korea.[202,260]

2.2.5 | *Aeromonas schubertii*

Aeromonas schubertii is a Gram-negative, short rod-shaped bacterium with a single polar flagellum required for its motility.[261] *A. schubertii* infection-causing multi-organs necrosis is considered an emerging tilapia disease.[261,262] Diseased fish usually showed haemorrhages in the caudal, pectoral and dorsal fins. Internally, affected fish exhibited visceral white spots in internal organs (i.e. liver, kidney and kidney),[261] similar to clinical signs caused by *F. orientalis* or *E. ictaluri* infection.

Natural disease outbreaks in both farmed and wild Nile tilapia were reported in China,[261,262] after its emergence in snakehead fish in 2012.[263–265] Although there is no evidence of disease outbreak in tilapia in other countries, active transferring live tilapia for aquaculture highlights a potential risk of its transboundary spread and broader distribution. Increased awareness and active surveillance are required to gain a better understanding of disease prevalence and impact on tilapia farming countries that have relied on imported tilapia stocks.

Presumptive diagnosis is based on observing visceral white necrotic foci and the presence of short rod-shaped bacteria in smeared tissue stained with Diff-Quick.[261] Previous studies employed trypticase soy agar supplemented with 5% sheep blood[261] or Luria–Bertani (LB) agar[262] for bacterial isolation. An approached combination of phenotypic tests, sequencing of 16S rRNA and several housekeeping genes (e.g. *gyrB*, *rpoB*, *ela* and *dnaJ*) has been used for bacterial identification.[261,262] Recently, Liu et al.[40] reported a highly sensitive TaqMan MGB probe fluorescence real-time quantitative PCR for detecting and quantifying *A. schubertii* from snakehead fish. This method might be helpful for early screening of an infection in tilapia.

Experimental infection revealed that *A. schubertii* was capable to induce disease and acute fish mortalities by both intraperitoneal and intramuscular injection. In contrast, immersion and oral challenges have resulted in no or low mortalities.[262] Zebrafish is a susceptible model fish to study the disease pathogenesis of this bacterium.[261] Histopathological changes described in diseased fish include vacuolization in the liver, haemorrhage in the spleen, and swelling capillaries in the brain. Necrotic lesions filled with a large number of short rod-shaped bacteria were also found in the liver, spleen and kidney.[261,262]

Little is known about the transmission of *A. schubertii* in tilapia. Ren et al.[262] suggested that the damages on the body surface and/or digestive tract might be natural routes of *A. schubertii* infection.

2.3 | Other bacterial diseases

2.3.1 | Lactococcosis (*Lactococcus garvieae*)

Lactococcus garvieae is a facultatively anaerobic, non-motile, non-spore-forming, Gram-positive, ovoid cocci bacteria belonging to the family Streptococcaceae. *L. garvieae* is a significant pathogen of both freshwater and marine aquaculture species, such as rainbow trout (*Oncorhynchus mykiss*), yellowtail (*Seriola quinqueradiata*)[266–268] and tilapia (*Oreochromis* spp.).[269]

In tilapia, *L. garvieae* infections were reported as an emerging disease during the last decade in several countries such as Egypt, Zambia, Brazil, and Singapore.[269–272] The experimental challenge of tilapia showed that the infected fish exhibited ocular opacity, exophthalmia, haemorrhages and cataract, skin erosion and scale detachment.[270,271,273] Lamellar congestion with necrosis of respiratory epithelium of primary and secondary gill lamellae, mild fatty degeneration of hepatocytes with multiple cell necrosis, sinusoidal congestion and necrosis in the spleen has been reported.[270]

To date, the studies on *L. garvieae* infection in tilapia focused on isolation, identification and confirmation of suspicion of the disease.[269,271,273] Further studies should investigate the prevalence of this pathogen in tilapia, the risk factors and geographical distribution of this pathogen, as well as its pathogenesis. On the other hand, comparative analysis of *L. garvieae* strains from different fish hosts may shed light on the evolution of this bacterium in tilapia.

2.3.2 | Aerococcosis (*Aerococcus viridans*)

Aerococcus viridans is a Gram-positive coccoid, order Lactobacillales, phylum Firmicutes. It is facultatively anaerobic and forms tetrads and pairs. The bacterium does not grow well on agar. *A. viridans* causes greening (alpha haemolysis) on rabbit or horse blood agar. The Gram-positive tetrads (four bacteria together) are visible by microscopy.

Also, the co-agglutination technique of Saxegaard and Håstein[274] or the API-Zym may be used for diagnosis. For better understanding of this disease, further investigation on its prevalence and disease pathogenesis in tilapia are recommended.

In aquaculture, *A. viridans* var. *homari* is known to cause gaffkemia in farmed European lobster (*Homarus gammarus*) and American lobster (*H. americanus*).[275,276] Ke et al.[277] described for the first time a tilapia disease outbreak in 2010 caused by *A. viridans* in China, with a loss of 30%–40%. The diseased fish showed congested gills and abdomen, a swollen gall bladder and a severe diffusion in the liver. *A. viridans* infections have been subsequently reported in Indonesian,[278] and in Egyptian tilapia farms,[279,280] always in combination with other bacteria. In Indonesia, the bacterium was isolated in a screening of water from a tilapia pond and in faeces of tilapia, and was identified by biochemistry.[278] In Egypt, the bacterium was isolated as one of 17 in a multibacterial infection of wild caught tilapia from the Nile River,[279] and it was isolated from diseased tilapia from two tilapia farms, in combination with *Enterococcus faecalis*,[280] and the *A. viridans* were identified by molecular methods, like 16SrRNA typing.

2.3.3 | Pseudomonasis

Pseudomonas spp. are aerobic motile Gram-negative rods and are representatives of the order Pseudomonadales.[281] Most *Pseudomonas* spp. are non-pathogenic, but some cause diseases in fish. *Ps. anguilliseptica* is the most pathogenic species, especially to Japanese and European eel, in which it may cause red spot disease or 'Sekiten byo'.[282–284] It has also been isolated from diseased tilapia with *Ps. fluorescens*,[7] and together with *Ps. fluorescens*, *Ps. putida* and *Ps. aeruginosa*.[285] The diseased tilapia showed clinical signs of pseudomonas septicaemia, including reddening of the whole body, abdominal swelling, cloudiness of eyes, loosening scales and congested gills.[285] In another study, *Ps. anguilliseptica* caused disease in Nile tilapia, showing anorexia, darkening, petechial haemorrhage on the body and at the base of fins, loose scales, eroded and erected fins, with some fish showing slight abdominal distension, exophthalmia and pale gills. At post-mortem enlarged kidneys and spleen were seen.[286]

Pseudomonas fluorescens is more often described as an opportunistic pathogen of tilapia (*Oreochromis* spp.) especially under stressful environmental circumstances.[7,230,287–290] Miyazaki et al.[291] described an outbreak of *Ps. fluorescens* in Nile tilapia in Japan. The systemically infected fish showed exophthalmia, darkening, spotty or nodular lesions in the liver, spleen, kidney and gills, and an inflamed swim-bladder. By histopathology, abscess formation in eyes, spleen and swim-bladder and focal necrosis in the liver, gills and kidney were seen in some of the diseased fish. Some other fish showed granuloma formation in all infected lesions.

Several disease cases in cultured tilapia (*O. niloticus)* associated with other *Pseudomonas* spp. were also reported, including on *Ps. aeruginosa*. The tilapia showed darkening of the body, loss of scales, tail rot and congestion of all internal organs.[292] *Pseudomonas aeruginosa* is however not considered to be a primary pathogen for tilapia.

Pseudomonas spp. are found in the aquatic and terrestrial environment at a global level. Although *Pseudomonas* infections occur globally, the *Ps. fluorescens* cases were described in Japan,[230] Philippines,[287] Kingdom of Saudi Arabia,[288] Egypt[289] and Guatemala.[290] *Pseudomonas mosselii* was described as a fish pathogen of Mozambique tilapia (*O. mossambicus*) in Mexico.[57] The disease is transmitted horizontally, via water, gear and by direct fish-to-fish contact.

Pseudomonas fluorescens produces fluorescein. After inoculation of blood or TSA agar, or *Pseudomonas* F agar at 22–28°C, the cream/white fluorescent colonies will appear. Apart from biochemical identification, API 20E or API 20NE may be used,[281] or molecular- or protein-based diagnostic methods. More research is needed, like screenings and artificially induced infections studies, to estimate the real impact of *Pseudomonas* infections in tilapia culture.

Regarding therapy of pseudomonasis, in general, an antibiogram is best to test the susceptibility of the isolate. *Ps. anguilliseptica* from Nile tilapia was found susceptible to ciprofloxacin, erythromycin, gentamycin, oxytetracycline, streptomycin and trimethoprim and sulphamethoxazole.[286] Additionally, they found the bacterium sensitive to methanolic extracts of *Anabaena wisconsinense* and *Oscillatoria curviceps* (blue-green algae or cyanobacteria), and ciprofloxacin and a methanolic extract of *Anabaena wisconsinense* were highly effective in the experimental treatment of pseudomonas septicemia at a dose of 10 mg per kg body weight, after i.p. injection.[286] In another study, lime oil nano-emulsion was tested *in vitro* and *in vivo* against *Ps. aeruginosa* infection in *O. mossambicus*, with good results.[293]

2.3.4 | Mycobacteriosis (*Mycobacterium marinum*)

Mycobacterium marinum is one of the fish mycobacteria, Gram-positive, acid–alcohol-fast, non-motile, non-spore forming rods which may cause stress-induced chronic and lethal 'fish tuberculosis' in warmwater fish, including in tilapia all over the world, in warmwater fish from freshwater, brackish and marine waters.[294,295] Sonda-Santos & Lara-Flores[296] and Lara-Flores et al.[297] reported disease and significant mortality of tilapia (*O. niloticus*) in Mexico through *M. marinum*. Skin discoloration, non-appetite, lethargy, abnormal swimming, cutaneous ulcerations or erosions, ascites, reduced growth, exophthalmia, grey or white nodules (granuloma) in internal organs, and hypertrophy of spleen, kidney and liver are signs of the disease by *M. marinum* in warmwater fish.[295] Also in indoor warm recirculation systems of fish culture *M. marinum* may occur, and clinical signs may only be noted after weeks, whereas internal disease already caused granuloma in organs.[298] As a consequence, fish may show mortality, morbidity and, also in case of subclinical infection, decreased feed uptake and growth rates, and is subsequently less marketable.[299] Granulomatous melano-macrophage centres have been described in Nile tilapia in its spleen.[300]

Diagnosis of mycobacteriosis starts with making a fresh smear of the inside of fish organs like liver, preferably taken at the site of nodules or granuloma, fixing the smear 3× through a flame, and staining the smears Ziehl–Neelsen, after which the smear is read by light

microscopy with a 100× (oil immersion) objective lens for presence of pink, rod-form bacteria, a sign of the acid-fast mycobacteria.

Identification of mycobacteria in fish was traditionally done based on time-consuming isolation (weeks, to max 2 months of incubation to declare a mycobacterial isolation negative) and on biochemical methods. Dong[301] however isolated the *M. marinum* within days from betta fish, *Betta splendens*. Currently, fast and accurate molecular methods are used for identification of the disease and phylogenetic studies.[297,302] Therapy of infected fish requires months of costly antibiotic treatments, and therefore this is not applied for edible fish, also, because high concentrations of residues of antibiotic will accumulate in the fish, which is then not marketable for consumption.[295] There is no vaccine available for *M. marinum*. The transmission of *M. marinum* from fish to fish is not yet clear, and is at least horizontal, via oral uptake of infected dead fish, contact with infected fish skin or through gills.[303]

Mycobacterium marinum is known as a potential contact-zoonotic bacterium, causing 'swimming pool granuloma', 'fish tank granuloma', 'fish handlers/fanciers disease' or 'fish TB' after entry in the skin of humans through injuries for instance.[304] It is not a food zoonosis, as the bacterium often does not grow at 37°C or above, although there are exceptions.[305] As hospitals incubate at 37°C or above, the diagnosis may be missed.[298]

2.3.5 | Epitheliocystis (*Chlamydia* spp.)

Epitheliocystis is a fish disease caused by obligate intracellular bacteria (most of them *Chlamydia*).[306] The disease is characterized by enlarged infected epithelial cells of mostly the gills and skin, which can be seen as tiny white cysts in the gill or skin epithelium. The disease has been reported in over 90 fish species, freshwater, marine and in cold to tropical areas. Characteristic is the presence of a basophilic inclusion in the cytoplasm of an enlarged cell. Severe infection of the gills results in inflammation and respiratory distress.

Although the disease epitheliocystis is widespread, the causative agents in most species of fish so far found are unique, and therefore isolates appear to be very host species specific. *Chlamydia*-like organisms (CLOs) have been the main agents related to this disease.[306] Epitheliocystis has been diagnosed in most regions worldwide in saltwater and freshwater fish. The specific agents causing epitheliocystis, however, appear more regionally restricted.[307] In Brazil, histologically epitheliocystis was found in rare cases in cultured Nile tilapia.[308,309]

Individual cysts from skin and gills up to 400 μm can be seen in wet mounts of gill clippings. Histologically cysts are seen as basophilic inclusions in infected epithelial cells, with a thickened membrane. Sometimes a host response is seen, as a cell proliferation, which even worsens the respiratory inefficiency of the affected gills. The pleomorphic development cycle of epitheliocystis in organisms obtained from *Tilapia mossambica* and *T. aurea* × *T. nilotica*, and the connection between epitheliocystis organisms and known chlamydial organisms of (in)vertebrates are discussed.[310] Epitheliocystis may be confirmed by molecular methods, like amplification of the 16S rRNA gene and sequencing.[311]

More research is needed, like screenings, to judge the real impact of *Chlamydia* infections in tilapia culture. Because there is no established way to culture *Chlamydia* in most fish disease labs, there are hardly data on host range or ways of transmission. At least there is horizontal transmission, from fish to fish, or via water, fish gear and so forth.[312] For this pathogen however also vertical transmission via eggs may be the case, since genomic presence of the pathogen in pre-hatched eggs, and in subsequent generations of barramundi suggested this.[313] Treatment of epitheliocystis with antibiotics is not possible, since it is caused by an intercellular bacterium. Prevention is through good farming management, at least by keeping the environmental factors optimal.[100]

2.3.6 | Nocardiosis (*Nocardia* spp.)

Nocardia is a genus of Gram-positive rod-shaped bacteria of the Order Mycobacteriales, Family *Nocardiaceae*, which show a weak Gram-staining, and are catalase-positive.

Labrie et al.[314] described cases of nocardiosis in freshwater tilapia (*Oreochromis* spp.). In general, fish with nocardiosis may show lethargy, multiple skin ulcers, and red spots. Brownish or haemorrhagic gills, abscess inside the operculum, a greyish or haemorrhagic liver with white nodules, fibromatosis in the abdominal cavity, spleen necrosis associated with the presence of macroscopic white nodules, ascites, haemorrhagic brain and swollen kidney often associated with the presence of white nodules may be seen. On-farm mortality is mostly chronic and may in cases reach 30%.[314]

Nocardiosis in fish is caused by *N. asteroides* and *N. seriolae*, and results in septicaemia in many marine species with serious mortality in some.[315] *Nocardia* in tilapia has been reported in large (>100–600 g) freshwater tilapia in Indonesia,[314] were it could be isolated from the skin and gills, brain, spleen and liver.

Isolation of the pathogen can be accomplished by taking samples from fresh lesions and culture them on nutrient-rich media, like Eugon agar, for *N. seriolae*. Colonies may appear matt to velvety and dry, with a granular surface, irregularly shaped edges, and are light brown. Impression prints represent a fast and reliable method to demonstrate the presence of *Nocardia* sp.[314] Histopathology may also be used, showing typical granuloma.[314] PCR can be used to confirm the identity up to species,[314,316,317] while LAMP (loop-mediated isothermal amplification) can be used as well for detection of *N. seriolae*.[318]

Nocardia asteroides can be found in soil, but can also be found in lake and marine sediments, like scum-activated sludge.[319] It can be transmitted via fresh fish feeds to a fish population, and has a horizontal transmission.

As nocardiosis is a chronic disease, which is often discovered in a late stage only, months of antibiotic treatment would be needed. This is costly and non-effective, and implies risk of AMR-development. Therefore, prevention through good husbandry and good management practices is the best approach for nocardial infections.[100,320] One of the aspects is to avoid the use of uncooked fish feeds (live, raw or frozen) when rearing fish, as these may transmit the pathogen.

Diagnosis of nocardiosis is not easy, as special media are necessary, and more research should focus on artificially induced infections, to estimate the real impact of *Nocardia* on tilapia culture. Thereby, the possibility, that nocardiosis may be zoonotic should be considered, and therefore prevented for, through good hygiene.

2.4 | Zoonotic potential of tilapia bacterial pathogens

Tilapia is cultured in relatively warm water.[100] Some of the pathogenic bacteria of tilapia grow well at these temperatures of 20–30°C, and may be contact-zoonotic, that is, also harmful to humans, after direct skin contact with the infected fish or fish-water, especially when humans have an injured skin, and are immunocompromised.[298] Although this risk is present in open tilapia (pond) culture, in infected warm water recirculation aquaculture systems, including aquaponics systems this may be even a bigger risk, as infected water is recirculated and bacteria may accumulate, being a risk to the fish culture professionals.

Some of the tilapia pathogenic bacteria described in paragraphs above may cause bacterial contact–zoonotic infections in humans, as a few of these bacteria have been isolated from wounds, superficial soft tissue, or even from invasive systemic infections in humans. Often those diseases were connected to a spine, puncture or exposure event, or after humans ingested the bacterium, the latter being a food zoonosis. A choice of potential contact- or food-zoonotic bacteria are *S. agalactiae* ST283, *S. dysgalactiae* subsp. *equisimilis*, *S. iniae*, *A. hydrophila*, *E. tarda*, *M. marinum* and *V. vulnificus*.[298,304,321–323]

2.4.1 | *Streptococcus agalactiae* ST283

Early evidence for association between fish consumption and *S. agalactiae* colonisation came from a prospective longitudinal cohort study among college students living in a dormitory in United States.[25] This study showed that fish consumption increased the risk of *S. agalactiae* colonisation with capsular types 1a and 1b combined 7.3-fold.[25] Group B *Streptococcus* (GBS) has been associated with superficial and invasive infections in immunocompromised non-pregnant adults, and is the main cause of neonatal sepsis. Invasive infections in non-pregnant adults without comorbidities came to light after the 2015 fish-associated outbreak in Singapore involving at least 146 people manifesting as bacteraemia, septic arthritis and meningitis. Through various researches and official investigations, it was revealed that this 2015 GBS foodborne outbreak in Singapore was caused by Sequence Type 283 (ST283) belonging to serogroup III-4, as explained below, and case-control studies found the outbreak to be associated with the consumption of raw freshwater fish.[324,325]

While there are different methods that classify GBS types in different ways, Capsular typing (serotyping) and multi-locus sequence typing (MLST) are the major typing systems.[22] Serotyping, which is based on the capsular type of the organism and can be conducted using antibodies or primers targeting the capsular operon, recognizes 10 types (Ia, Ib, II–IX). In fish, three major serotypes of *S. agalactiae* are recognized, that is, type Ia, Ib and III.[32] MLST, which is a standardized method based on the DNA sequence of seven conserved housekeeping genes,[326] recognizes some 2000 Sequence Types (STs) and hence provides more discriminatory identification of *S. agalactiae* strains across host species and countries. The major serotypes of *S. agalactiae* found in fish largely correspond with three STs: isolates of serotype Ia belong to ST7 or closely related ST, isolates of serotype Ib belong to ST260 or closely related ST, and isolates of serotype III belong to ST283 or closely related ST.[32] The fish-specific serotype Ib/ST260 clade has never been detected in humans, whereas the serotype Ia/ST7 clade has been detected in fish, dolphins and humans.[32,327] There is no evidence, however, of direct fish-to-human transmission. Such evidence only exists or serotype III (subtype 4)/ST283: Molecular epidemiological studies revealed that GBS ST283 isolated from freshwater fish (food) samples and infected patients were identical, supporting the hypothesis of foodborne transmission of GBS ST283.[328–330]

Barkham et al.[331] showed that GBS ST283 had been present in human blood cultures in Singapore since 1998. Data and collections of GBS associated with invasive infections were retrieved from other South-East Asian countries. Taken together, 29% of human GBS from Hong Kong, Thailand, Lao PDR, Vietnam and Singapore turned out to be ST283: the earliest known isolate was from Hong Kong in 1995. 97% of patients with ST283 were adults and 36%–80% did not have comorbidities. The prevalence of ST283 in invasive GBS infections varied from 11% in Hong Kong to 73% in Thailand and 76% in Lao PDR.[329] However, none of 18 isolates from Malaysia and only 5/4198 (0.1%) of GBS isolates from mainland China, Africa, Europe, North and South America belonged to ST283.[22] FAO convened an expert group which found insufficient data for a full risk analysis, but published a risk profile detailing gaps in knowledge that would benefit from more research.[22]

Identification of GBS ST283 in freshwater fish has been reported from a number of species such as grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*H. nobilis*), Nile tilapia (*Oreochromis niloticus*), red and black tilapia (*Oreochromis* sp.), Mekong giant catfish (*Pangasianodon gigas*), freshwater frogs (*Hoplobatrachus rugulosus* and *H. chinensis*) and marine species, Asian seabass (*Lates calcarifer*).[37,332–334] The outbreak in Singapore was controlled after advising the public against consumption of raw freshwater fish. It is well known that consumption of raw fish is associated with risk of infection with bacterial, viral and parasitic infections. Data indicates that *S. agalactiae* can be inactivated by pasteurization and therefore adequately cooked tilapia and other fish would be safe for consumption.[22]

Remarkable to add, in exceptional cases, fish may get infected from humans as well, so, in an anthroponosis: Experimental induced infection of Nile tilapia *(O. niloticus)* with a human isolate of GBS (serotype Ia, ST7) was able to cause disease and mortality in the tilapia.[335]

2.4.2 | *Streptococcus dysgalactiae*

Only incidental reports have been published on seafood as source of *S. dysgalactiae* subspp. zoonosis in humans, especially percutaneous

injuries, like upper limb cellulitis in humans after skin spine or puncture while cleaning seafood.[336,337] Based on genomic sequencing, *S. dysgalactiae*, subsp. *dysgalactiae* (SDSD) is associated with ruminants, whereas *S. dysgalactiae* subsp. *equisimilis* has been found in humans, companion animals (e.g. dogs and horses) and fish. Subspecies identification based on data from individual genes may not be accurate, resulting in some inaccurate reporting of species identity.[323]

Streptococcus dysgalactiae has been isolated from diseased farmed Nile tilapia in Brazil showing septicaemia and subcutaneous abscesses in the caudal peduncle region[338,339] and from tilapia in Egypt.[279] In Brazil, induced infection experiments with the isolated strain of *S. dysgalactiae* were performed, causing reproduction of disease in adult Nile tilapia, showing anorexia, lethargy, tachypnoea and darkened skin, rapidly leading to mortality rates up to 100% and 83% after intramuscular and intraperitoneal injection, respectively, with re-isolation of bacteria from diseased tilapia.[338]

2.4.3 | *Streptococcus iniae*

Streptococcus iniae has not been assigned to any Lancefield group, but 16S rRNA sequencing indicates that these are closely related to GBS. Human infections have been reported in elderly people and individuals with underlying conditions like diabetes mellitus, rheumatic heart disease or cirrhosis handling fresh fish. Infections following fish consumption have not been reported so far. The disease may manifest as cellulitis following soft tissue injuries while handling fresh tilapia (*Sarotherodon galilaeus*), also known as St. Peter's fish or Hawaiian sunfish.[24] But complications such as arthritis, meningitis, endocarditis and osteomyelitis may also develop.[340] Most infections have been associated with people of Asian origin, possibly due to the habit of handling whole tilapia. Studies in Canada using pulse field gel electrophoresis (PFGE) showed that strains causing fish infections and human infections belong to same clone.[341] *S. iniae* infections in humans may be under-reported since identification of this pathogen in clinical laboratories is hampered by the limitations of the commercial identification systems.[342]

2.4.4 | *Aeromonas* spp.

Aeromonas spp. are Gram-negative rods. The motile *Aeromonas* spp., like *A. hydrophila* and *A. sobria*, are opportunistic bacteria and can be found everywhere, in- and outdoor, in soil and in fresh to brackish water, as aquatic commensals and secondary pathogens.[343,344] In humans, *Aeromonas* spp. originating from various fish species may cause acute haemorrhagic diarrhoea. It may also cause invasive skin and soft tissue infections, after aquatic injuries through spines, punctures and bites of animals. Within 24 h after infection, infected wounds may show erythema, oedema and purulent discharge, which may develop into fever in untreated or improperly treated cases, which may progress into invasive infections, especially in the immunocompromised patients, with necrotizing fasciitis, necrotizing myositis and osteomyelitis.[304,322,343]

Aeromonas isolates isolated from human infections were found to be susceptible to various antibiotics, of which sulphonamids were less effective.[48] In serious cases, besides wound drainage and debridement, *Aeromonas* wound infections should be treated initially with either a fluoroquinolone or a third-generation cephalosporin, possibly plus an aminoglycoside until culture and antibiotic sensitivity results are known, and rule out *Pseudomonas* coinfections.[48,345]

2.4.5 | *Edwardsiella tarda*

Edwardsiella tarda is a Gram-negative rod of the family Enterobacteriaceae. It is known as pathogen of various fish, like eel, tilapia and it causes emphysematous putrefactive disease of catfish.[322,346] It may cause 'fish gangrene', 'emphysematous putrefactive disease of catfish' or 'red disease of eels', referred to as *Edwardsiella* septicaemia (ES), a systemic disease of fish.[298]

Edwardsiella tarda from cold-blooded animals like marine, brackish and freshwater fish, reptiles and amphibians may also cause disease in humans.[347] Slaven et al.[348] described various zoonosis cases in the 1990s in humans in Louisiana by *E. tarda*: 11 extraintestinal infections, with five wound infections (three with exposures to marine fish or fish bones), five abscesses requiring surgical drainage and one case of bacteraemia. In severe and scarce cases, extensive myonecrosis and fatal septic shock in immunocompromised patients, especially in patients with chronic liver disease was seen. Therapy recommended consisted of antibiotics, like ampicillin, cepahalosporins, such as cefazolin and ceftazidime, aminoglycosides and fluoroquinolones.[348]

2.4.6 | *Vibrio vulnificus*

Vibrio vulnificus is a multi-host fish pathogen that inhabits coastal ecosystems in temperate, subtropical and tropical areas (>18°C) and likes low to moderate salinities.[179,349] It is a zoonotic agent as vibriosis can be transmitted directly from diseased fish to humans by contact.[184,322,350] In humans, *V. vulnificus* may cause a range of diseases with variable clinical manifestations, like acute gastroenteritis from eating undercooked shellfish, progressing into acute sepsis, or, in rare cases, primary sepsis and severe wound infections from marine injuries and water exposures, which may develop into life-threatening necrotizing fasciitis.[179,184,298,322,350–353] Historically, the species was divided into three biotypes (Bt), all of which contained human pathogenic strains. Pathovar *piscis* (pv. *piscis*; formerly Bt 2), is considered as primary fish pathogen and is subdivided into several clades/serovars, from which Ser E and Ser T have proven zoonotic potential and thus represent a risk to also aquaculture professionals.[184,185]

Regarding zoonosis through *V. vulnificus* infected tilapia, several clinical cases have been reported. Chan et al.[354] described a case of a septicaemia that progressed into necrotizing fasciitis after the patient experienced a puncture by the dorsal fin of an infected tilapia. Nudelman et al.[355] and Bisharat et al.[356] described wound infections after

injuries in extremities by the sharp spines of infected tilapia in Israel. Vinh et al.[357] also reported a fatal case of *V. vulnificus* sepsis developed in a patient with chronic hepatitis B and chronic renal failure after handling and ingesting tilapia.

Other authors experimentally challenged Nile tilapia with the zoonotic pv. *piscis* Ser E and fish developed a haemorrhagic septicaemia similar to eel vibriosis, warning that this bacterium could constitute a serious health hazard for tilapia and, indirectly for humans.[191,193] Interestingly, there have been reports of the isolation of *V. vulnificus* from diseased tilapia cultured in Indian and eastern Mediterranean farms, all of them potentially dangerous for humans.[185,189,190] Moreover, it has been demonstrated that human clinical isolates which had not been linked to fish vibriosis or to zoonosis cases, also belong to pv. *piscis*, demonstrating their zoonotic nature.[185] Thus, apart from the risk for tilapia, these facts might also imply a risk to humans and, thus, the species should be higher estimated as a zoonotic pathogen.

Therefore, tilapia farm environments, with high nutrient concentrations and host densities, may clearly contribute to an increase in *V. vulnificus* populations and provide advantageous conditions for the emergence of genetically more diverse and more virulent strains and/or the expansion of particular lineages/clades, including the zoonotic ones.[185,188] Moreover, under the climate change scenario, the increased water temperatures may favour these events.[179,188,358]

Regarding therapy of diseased humans, prompt intervention with antibiotics should be performed, as sepsis and fasciitis necroticans may be fatal within 48–72 h. The U.S. Centers for Disease Control and Prevention[359] recommended a third-generation cephalosporin, especially ceftazidime, plus doxycycline, as initial empiric antibiotic combinations for suspected *V. vulnificus* infections; see their website. Other cephalosporins can be used as well, as well as fluoroquinolones like ciprofloxacin, see CDC.[359] The treatment may include early surgery for wound debridement and monitoring for compartment syndromes, as these increase the survival rate when a systemic human infection is the case.

Development of effective control and preventive measures in fish farms against *V. vulnificus*, the most infectious of all zoonotic *Vibrio* spp., is considered highly necessary, including development of effective vaccines.

2.4.7 | *Mycobacterium marinum*

Mycobacterium marinum is one of the fish mycobacteria, Gram-positive, acid–alcohol-fast, non-motile, non-spore forming rods that may cause chronic and lethal fish tuberculosis in warmwater fish, including tilapia.[294,303]

In humans, *M. marinum* may cause 'swimmer granuloma', 'fish tank granuloma' or 'fish handler's disease',[294,298,303,304,360–362] which may be chronic infections of hands and feet, but not easily lethal (Figure 9). *M. marinum* has an optimum temperature of 30°C (Haenen, own findings), and is inhibited at 37°C. This means, in humans, almost exclusively, skin infections occur in extremities, which are cooler. The incubation time for mycobacteriosis in the skin is 7–21 days after skin injury.[322]

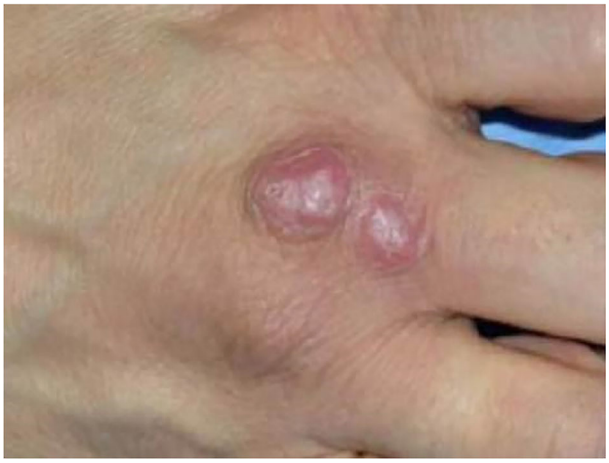

FIGURE 9 Swimmer granuloma on the right hand, after infection by *Mycobacterium marinum* through skin contact with warmwater fish and fish water. Picture courtesy Dr Cassetty and Dr Sanchez, 2004; details in Dermatology Online Journal[361]

In a later phase, granulomatous nodules will develop on the skin, which may become secondary infected. Also deeper, invasive infections may develop, like septic arthritis, bursitis, tenosynovitis and osteoarthritis.[363] Yacisin et al.[364] monitored *M. marinum* skin or soft tissue infections cases at Chinese markets in New York City, and concluded, the highest risk of acquiring the zoonosis was through skin injury of the finger or hand during fish handling.

Fast preliminary diagnostics is done by acid-fast staining smears of nodules and lesions, and through culture from nodules. PCR identification *M. marinum* is confusing[322] and requires more than one PCR. Only chronic treatments are considered effective.[365] According to Aubry et al.[366] clarithromycin, cyclines and rifampin were the most commonly prescribed antibiotics, with an effective cure of 87% of the 63 patients. *M. marinum* is susceptible to macrolides like clarithromycin, sulfonamides/trimethoprim-sulfamethoxazole, ethambutol and rifampin/rifabutin.[367] A typical treatment consists of a combination of two of these drugs (i.e. clarithromycin plus ethambutol, or clarithromycin plus rifampin) for approximately 3–4 months, to be ended only 4–8 weeks after symptoms have vanished.

2.5 | Status of antimicrobial resistance in fish culture through imprudent antibiotic use, and its future

In semi-intensive and intensive aquaculture, access to safe and effective veterinary medicines or drugs is essential to a successful operation. However, if used imprudently, antibiotics used to treat bacterial diseases may be ineffective and may lead to unacceptable residue levels in aquaculture products that can result in bans on importation, import rejections and detentions.[2] Misuse of veterinary medicines may lead to the development of antibacterial-resistant genes in bacteria, and this may therefore cause antimicrobial resistance (AMR). This

consequence happens across all food-producing sectors, including aquaculture. There are many examples, like a joint 97% antibiotic resistance to ampicillin, erythromycin, and oxytetracycline in 173 bacterial isolates from apparently healthy tilapia in Trinidad.[6] Therefore, if antibiotics are to be used, the choice of antibiotic must always be based on the results of an antibiogram, to be sure, the therapy is effective.

There is increased global attention through various assemblies, meetings and conferences where AMR has been specifically mentioned as a vital and growing problem. The Global Action Plan (GAP) on AMR (with contributions from FAO and OIE) was adopted during the 68th World Health Assembly in 2015.[368] In the same year, the World Assembly of the OIE delegates adopted the strategy, and the 39th FAO Conference adopted Resolution 4/2015. A political declaration was made during a high-level meeting on AMR at the 71st United Nations General Assembly (UNGA, September 2016). The UNGA called upon the Tripartite (i.e. FAO as global leader for food and agriculture, the OIE as global leader for animal health and welfare and the World Health Organization [WHO] as global leader for human health) and other intergovernmental organizations to support the development and implementation of National Action Plans (NAPs) and AMR activities at the national, regional and global levels under the One Health platform. The FAO, OIE and WHO agreed to step up a joint action to combat health threats associated with interactions between humans, animals and the environment.

A memorandum of understanding was signed in May 2018 to strengthen their long-standing partnership, with a strong focus on tackling AMR. In addition, the United Nations Secretary-General convened the Interagency Coordination Group (IACG) on AMR in May 2017 in consultation with Tripartite members to provide guidance on approaches for ensuring sustained global action on AMR, and reported back to the Secretary-General during the 73rd General Assembly in 2019. This mandate included making recommendations on enhancing coordinated action across sectors and countries, building political momentum, future governance and mobilizing stakeholders.[2]

Countries are now encouraged to develop National Action Plans (NAP) on AMR. In the development of the aquaculture component of a country's NAP on AMR, understanding and increasing knowledge of bacterial diseases affecting the sector, how they are being managed, complexities associated with AMR in the aquatic environment and how to achieve One Health goals are essential.[369]

These developments should now serve as a signal of the urgent need for aquaculture countries, especially those with substantial aquaculture production and food security objectives through aquaculture, to pay high attention to the emergence of antimicrobial-resistant organisms that can result from antimicrobial (specifically antibiotics) imprudent and irresponsible use in the aquaculture sector.

Hanson[370] provided practical management measures to minimize AMR from bacterial diseases of finfish by reducing the use of antibiotics and ensuring its prudent use when it is needed. Good husbandry (good seed, adequate nutrition, good water quality and environment, minimizing stress, etc.) and biosecurity practices (e.g. health monitoring, rapid action on first signs of abnormal observations or clinical signs of disease, vaccination, breaking disease transmission pathways) through all phases of production should be part of normal practice. Disease prevention can be achieved by managing the environment and host, by pathogen avoidance and by having a biosecurity plan, as parts of Good Aquaculture Practice.[2,100]

FAO[2] listed several biosecurity measures that may reduce or eliminate AMR. These include avoidance, using clean facilities, use of immunostimulants to enhance innate immunity, inclusion of probiotics in feeds, vaccination, phage therapy via feeds and the use of plant extracts. Of these, vaccines have been widely used against fish infections. Avoidance of AMR can also be achieved by farming high-value SPF (Specific Pathogen Free) fish species in a controlled way.

3 | CONCLUSIONS

There are many microbial agents in the aquatic environment, some of which are potential pathogens to tilapia, depending on a variety of factors specific to the host, pathogen and environment.

Since decades, some bacterial species, belonging to at least four genera, are considered important pathogens for tilapia: *S. agalactiae*, *S. dysgalactiae* and *S. iniae*, motile *Aeromonas* species, *F. orientalis*, *F. columnare* (new name: *F. oreochromis*) and *V. vulnificus* pv. *piscis* and some other *Vibrio* species. Additionally, at least two bacterial tilapia diseases are emerging, edwardsiellosis through *E. ictaluri* and *E. tarda* as well as disease by *A. schubertii*. Furthermore, bacteria with zoonotic potential, like *S. agalactiae* ST283, *S. dysgalactiae* subsp. *equisimilis*, *S. iniae*, *Aeromonas* sp., *E. tarda*, *V. vulnificus* pv. *piscis* and *M. marinum* are included in the review, to provide altogether the current overview of the disease risks affecting production and post-harvest stages.

Various other bacteria may be opportunistic and pathogenic to tilapia as well, especially under favourable conditions of the environment (water at a high temperature, with high loads of organic material, low oxygen and other stress factors), and vulnerable fish (low in immune status, in too high stocking densities, too variable in size, etc.), like *L. garvieae*, *A. viridans*, *Pseudomonas* spp. and *Chlamydia* spp.

The important role played by aquaculture in providing high-quality nutrition, improving livelihoods, stimulating and creating decent work and economic growth and alleviating poverty, particularly in low-income food-deficit countries will be only possible, if disease challenges (including bacterial diseases) affecting production can be addressed in a decent way. It is of utmost importance to train the tilapia farmers in good aquaculture practices (GAP), including hygiene at the fish farm, to avoid spread of fish bacterial disease and fish mortality. For this, it is very important to educate fish health professionals for field work, to be able to control bacterial diseases in tilapia farming and avoid spread.

Regarding bacterial zoonosis, cases from tilapia culture are mostly not recorded on a global scale. For sure they occur, from mild (mycobacteriosis, swimmer granuloma, i.e. chronic skin infections by *M. marinum*) to serious (necrotic fasciitis through systemic infection by *V. vulnificus*), depending on the patient's immune status, and they can be

prevented for through good hygiene. Awareness of *One Health* and Good Hygiene Practice should be in place in aquaculture, including in the whole tilapia production chain up to the consumer. This means avoiding direct contact of potential zoonotic pathogens with the human skin, and avoid inhalation and ingestion of those pathogens. At tilapia farms, slaughter facilities and packing sites this means special clothing, wearing gloves and face masks and regularly wash hands and skin with soap after any contact with fish and fish water. It also means, that when professionals would develop signs of a contact or food safety zoonosis they should mention to the medics, that they work with warmwater fish, and may have acquired a zoonotic infection from the fish.

Regarding antimicrobial resistance (AMR), responsible use of antimicrobial agents is an important part of farm biosecurity to ensure that pathogen challenges are minimized, that the natural defence mechanisms of the cultured stocks are maximized, and that disease and mortality, including costs of containing, treating and/or eradicating diseases, are reduced.[2] Therefore, the use of antimicrobial agents should be minimized, and be consistent with established principles of prudent use, to safeguard public and animal health.[2] Furthermore, apart from Good Aquaculture Practice (GAP), development and use of effective and economically favourable vaccines is recommended.

AUTHOR CONTRIBUTIONS

Olga Haenen: Conceptualization; data curation; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing – original draft; writing – review and editing. **Ha Thanh Dong:** Conceptualization; data curation; investigation; resources; writing – original draft; writing – review and editing. **Truong Dinh Hoai:** Resources; writing – original draft; writing – review and editing. **Margaret Crumlish:** Conceptualization; data curation; investigation; methodology; writing – original draft; writing – review and editing. **Iddya Karunasagar:** Conceptualization; data curation; investigation; methodology; writing – original draft; writing – review and editing. **Timothy Barkham:** Writing – original draft; writing – review and editing. **Swaine L. Chen:** Conceptualization; investigation; writing – original draft; writing – review and editing. **Ruth Zadoks:** Conceptualization; investigation; writing – original draft; writing – review and editing. **Andreas Kiermeier:** Conceptualization; investigation; methodology; writing – original draft. **Bing Wang:** Writing – original draft; writing – review and editing. **Esther Garrido Gamarro:** Conceptualization; funding acquisition; investigation; project administration; resources; writing – original draft; writing – review and editing. **Masami Takeuchi:** Conceptualization; funding acquisition; investigation; project administration; resources; writing – original draft; writing – review and editing. **Mohammad Noor Amal Azmai:** Data curation; formal analysis; investigation; methodology; supervision; validation; writing – original draft; writing – review and editing. **Belén Fouz:** Conceptualization; data curation; investigation; resources; validation; visualization; writing – original draft; writing – review and editing. **Rolando Pakingking Jr.:** Conceptualization; data curation; investigation; writing – original draft; writing – review and editing. **Zeng Wei Wei:** Data curation; investigation; writing – original draft; writing – review and editing. **Melba G. Bondad-Reantaso:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST

The views expressed in this publication are those of the author(s) and do not necessarily reflect the views or policies of the Food and Agriculture Organization of the United Nations.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study. Existing literature was used, Open and Restricted Access papers, peer review journals, book chapters, health organisms reports and so forth.

ORCID

Olga L. M. Haenen https://orcid.org/0000-0002-5569-9803
Ha Thanh Dong https://orcid.org/0000-0002-7190-757X
Truong Dinh Hoai https://orcid.org/0000-0002-2271-849X
Margaret Crumlish https://orcid.org/0000-0002-7810-8172
Iddya Karunasagar https://orcid.org/0000-0001-8783-1269
Timothy Barkham https://orcid.org/0000-0003-0975-2244
Swaine L. Chen https://orcid.org/0000-0002-0107-2861
Ruth Zadoks https://orcid.org/0000-0002-1164-8000
Andreas Kiermeier https://orcid.org/0000-0001-6240-2919
Bing Wang https://orcid.org/0000-0003-0174-2252
Esther Garrido Gamarro https://orcid.org/0000-0002-1000-5265
Masami Takeuchi https://orcid.org/0000-0002-7594-5303
Mohammad Noor Amal Azmai https://orcid.org/0000-0002-7664-9821
Belén Fouz https://orcid.org/0000-0001-9751-0143
Rolando Pakingking Jr. https://orcid.org/0000-0002-0914-4874
Zeng Wei Wei https://orcid.org/0000-0003-1770-957X
Melba G. Bondad-Reantaso https://orcid.org/0000-0002-2380-3549

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REVIEW

REVIEWS IN Aquaculture

From the basics to emerging diagnostic technologies: What is on the horizon for tilapia disease diagnostics?

Ha Thanh Dong[1] | Thawatchai Chaijarasphong[2,3] | Andrew C. Barnes[4] | Jérôme Delamare-Debouteville[5] | Peiyu Alison Lee[6] | Saengchan Senapin[2,7] | Chadag Vishnumurthy Mohan[5] | Kathy F. J. Tang[8] | Sharon E. McGladdery[9] | Melba G. Bondad-Reantaso[10]

[1]School of Environment, Resources and Development, Asian Institute of Technology, Pathum Thani, Thailand

[2]Center of Excellence for Shrimp Molecular Biology and Biotechnology (Centex Shrimp), Faculty of Science, Mahidol University, Bangkok, Thailand

[3]Department of Biotechnology, Faculty of Science, Mahidol University, Bangkok, Thailand

[4]The University of Queensland, School of Biological Sciences and Centre for Marine Science, Brisbane, Queensland, Australia

[5]WorldFish, Penang, Malaysia

[6]GeneReach USA Inc., Lexington, MA, United States of America

[7]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand

[8]Yellow Sea Fisheries Research Institute, Qingdao, China

[9]118 Parr Street, St. Andrews, New Brunswick, Canada

[10]Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

Correspondence
Ha Thanh Dong, School of Environment, Resources and Development, Asian Institute of Technology, Pathum Thani, Thailand.
Email: htdong@ait.ac.th

Melba G. Bondad-Reantaso, Fisheries and Aquaculture Division, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
Email: melba.reantaso@fao.org

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Abstract

Tilapia is an affordable protein source farmed in over 140 countries with the majority of production in low- and middle-income countries. Intensification of tilapia farming has exacerbated losses caused by emerging and re-emerging infectious diseases. Disease diagnostics play a crucial role in biosecurity and health management to mitigate disease loss and improve animal welfare in aquaculture. Three continuous levels of diagnostics (I, II and III) for aquatic species have been proposed by Food and Agriculture Organization of the United Nations (FAO) and the Network of Aquaculture Centers in Asia and the Pacific (NACA) to promote the integration of basic and advanced methods to achieve accurate and meaningful interpretation of diagnostic results. However, the recent proliferation of cutting-edge molecular methods applied

Abbreviations: AI, artificial intelligence; ARG, antimicrobial resistant gene; AST, antimicrobial susceptibility test; AuNP, gold nanoparticles; Cas, CRISPR-associated protein; CPA, cross-priming amplification; CRISPR, clustered regularly interspaced short palindromic repeats; Ct, cycle threshold; dPCR, digital polymerase chain reaction; eDNA, environmental deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; eRNA, environmental ribonucleic acid; HAD, helicase-dependent amplification; IAM, isothermal amplification method; iiPCR, insulated isothermal PCR; IR, infra-red; ISH, in situ hybridization; LAMP, loop-mediated isothermal amplification; LFIA, lateral flow immunoassay; LoD, limit detection; MLST, multilocus sequence typing; mNGS, metagenomic next generation sequencing; NASBA, nucleic acid sequence-based amplification; NGS, next generation sequencing; ONT, Oxford Nanopore Technologies; PCR, polymerase chain reaction; POCT, point-of-care testing; qPCR, quantitative real-time polymerase chain reaction; RCA, rolling circle amplification; RPA, recombinase polymerase amplification; RT-LAMP, reverse transcriptase loop-mediated isothermal amplification; RT-PCR, reverse transcriptase-polymerase chain reaction; RT-qPCR, reverse transcription quantitative real-time polymerase chain reaction; RT-RPA, reverse transcriptase recombinase polymerase amplification; SMRT, single molecule real-time; TEM, transmission electron microscopy; TGS, third generation sequencing; WGS, whole genome sequencing; WOAH, the World Organisation for Animal Health.

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in the diagnosis of diseases of aquacultured animals has shifted the focus of researchers and users away from basic approaches and toward molecular diagnostics, despite the fact that many diseases can be rapidly diagnosed using inexpensive, simple microscopic examination and that most emerging diseases in aquaculture were discovered by histopathology. This review, therefore, revisits and highlights the importance of the three levels of diagnostics for diseases of tilapia, particularly the frequently overlooked basic procedures (e.g., case history records, gross pathology, presumptive diagnostic methods and histopathology). The review also covers current and emerging molecular diagnostic technologies for tilapia pathogens including polymerase chain reaction methods (conventional, quantitative, digital), isothermal amplification methods Loop-mediated Isothermal Amplification (LAMP), recombinase polymerase amplification (RPA), clustered regularly interspaced short palindromic repeats (CRISPR)-based detection, lateral flow immunoassays, as well as discussing what is on the horizon for tilapia disease diagnostics (next generation sequencing, artificial intelligence, environmental Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA) and point-of-care testing) providing a future vision for transferring these technologies to farmers and stakeholders for a sustainable aquatic food system transformation.

KEYWORDS
disease, basic diagnostics, emerging technologies, tilapia

1 | OVERVIEW OF TILAPIA AQUACULTURE, DISEASES AND IMPORTANCE OF DIAGNOSTICS

Aquatic foods, both farmed and caught, distributed through various supply chains, have significantly contributed to the improvement and diversification of diets, as well as the promotion of nutritional well-being for many people.[1] Recognition of the critical role of aquatic foods in nourishing nations and transforming food systems is increasing with the recent tabling of a discussion paper by the United Nations (UN) on the role of aquatic foods for nutrition gaining global attention.[2] In addition to underpinning local nutritional needs and livelihoods for tens of millions of people, aquatic commodities are some of the most traded food products in the world. The value of global fish exports increased from USD 7.8 billion in 1976 to USD 164 billion in 2018.[3] Producing aquatic foods that are safe, healthy, accessible and affordable is the need of the hour to meet the nutritional needs of millions of people. This is where the farming of carps, tilapias and catfish assumes significance and presently supply 35.84% of world aquaculture production with a value of 83 billion dollars.[3,4]

Tilapia, by virtue of their overall resilience, have been species of choice for farming in a diverse range of farming systems, from simple backyard/homestead ponds to highly intensive raceways. Today, tilapia is the second most commercially important finfish group after carps, farmed in over 140 countries.[3,5–7] In 2018, global tilapia production by volume was estimated at 6.5 million metric tonnes (MMT) with the top four producers being China (1.78 MMT), Indonesia (1.11 MMT), Egypt (0.88 MMT) and Bangladesh (0.32 MMT).[3] The global tilapia industry and its associated value chains are currently estimated to be worth about US$ 7.9 billion.[8,9] Access to genetically improved elite strains of Nile tilapia (*Oreochromis niloticus*) is further fuelling the growth of the tilapia industry across the globe. Members of the genus *Oreochromis* are important not only for providing food and employment for local people, including women and youth, but also for earnings from domestic market and international export.[10–12] Today, Nile tilapia has become the third-most produced fish of all finfish species, representing a major source of affordable protein nutrients for multitude of consumers in many low- and middle-income countries (LMICs) across Asia, Africa, America and the Pacific.

Infectious diseases remain a serious bottleneck for aquaculture development, particularly in Asia where over 89% of the global production takes place.[3] Globally, disease-related losses in the aquaculture sector were estimated to exceed USD 6 billion in 2017.[13,14] Finfish aquaculture alone suffered annual losses ranging from USD 1.05 to USD 9.58 billion per year.[15,16] For many years, tilapia was perceived as hardy and disease resistant but this has changed in the face of intensification, climate change and global trade of live aquatic species, where global tilapia farming is now affected by serious disease problems caused by parasites (e.g., protozoan, monogenean), bacteria (e.g., *Streptococcus* spp., *Aeromonas* spp., *Edwardsiella* spp., *Flavobacterium columnare*, *Francisella orientalis*) and viruses (e.g., tilapia lake virus [TiLV], infectious spleen and kidney necrosis virus [ISKNV], tilapia parvovirus [TiPV] and nervous necrosis virus [NNV]) that are impacting the performance of the industry globally. The true economic cost of diseases in the tilapia industry is hard to estimate, but based on selected case studies[15,17–19] disease-related losses could run up to several billion dollars annually. For example, the value of 300,000 tonnes of tilapia lost due to disease caused by

Streptococcus spp. infections was estimated at USD 500 million.[20] Disease is also seen as a primary driver for increased use (misuse) of antimicrobials contributing to antimicrobial resistance (AMR) problems in aquatic food systems.[21–25]

Global outbreaks of Streptococcosis and recent outbreaks caused by TiLV and ISKNV in farmed and wild tilapia have drawn the attention of aquatic health specialists and policy makers worldwide to call for more research and better understanding of diseases and their management in tilapia aquaculture. Adoption of disease management practices such as routine diagnostics and biosecurity measures with other disease prevention approaches are going to be central to ensure sustainability of tilapia farming. Compared with high value salmonids and shrimp, the global research and development investment toward disease diagnostics and health management in low value but affordable species like tilapia, carps and catfish is less. As a result, adoption of effective health management and biosecurity practices relatively weak in LMIC undertaking farming of low value species.

Diagnostics may be defined as the determination of the cause or nature of a disease through the examination of signs, symptoms and diagnostic tests.[26] Diagnostic tests include both straightforward, pond-side methods and more advanced laboratory-based techniques requiring a high level of expertise and infrastructure. Disease diagnostics play three essential roles in aquaculture health management and disease control.[27,28] Firstly, diagnostics for screening healthy animals to ensure that they are not inapparent carriers of pathogens is aimed at disease prevention and is typically used to identify populations that have tested negative for specific pathogens as required for domestic or international translocation. This helps to limit the risk of disease transmission from farm to farm at national and international levels. Diagnostics play a crucial role in avoiding the transboundary transmission of a significant number of pathogens between countries and continents. Secondly, diagnostics have been used for routine health monitoring of farmed animals in order to detect infection/illness at an early stage. This facilitates timely intervention on the host–pathogen–environment complex to avoid a scenario of disease outbreak and substantial economic losses. Thirdly, diagnostics are used to diagnose diseases in animals that have clinical signs of illness. In this scenario, determining the cause(s) quickly and accurately is crucial for implementing appropriate management actions (e.g., treatment decisions, emergency harvest, etc.) to limit the negative impact on aquaculture farms in the short- and long-term. Diagnostics is particularly important in national disease monitoring programs, which provide the scientific foundation for development of national policies, emergency responses, risk management and biosecurity measures.[28–30] Such policies protects the sector from disease risks underpin international trade agreements in biological commodities.

The Snieszko circle,[26,31] also known as the epidemiological triad,[32,33] shows the relationship between the host, the pathogen and the environment in disease development (Figure 1a). However, in the triad, anthropogenic factors are incorporated into the environment circle of the Venn diagram which underplays their importance in the onset and outcomes of infectious disease, particularly in modern aquaculture. In 2013, Shields updated the triad to an epidemiological tetrad to reflect the significant anthropogenic drivers behind outbreaks of lobster diseases in Long Island Sound, The United States.[34] These included extensive eutrophication leading to hypoxia, exposure to metals and pesticides and various fisheries induced stressors.[34] Here we adapt the tetrad to reflect farmed rather than wild animal disease investigation, although there is substantial overlap (Figure 1b). There are many human impacts on farm animal health. These include actions of the farmer such as water management, animal handling, stocking practice, feed storage and feeding regimes.[35,36] There are directly connected actors such as feed companies, where diet provided to farms may not be optimally

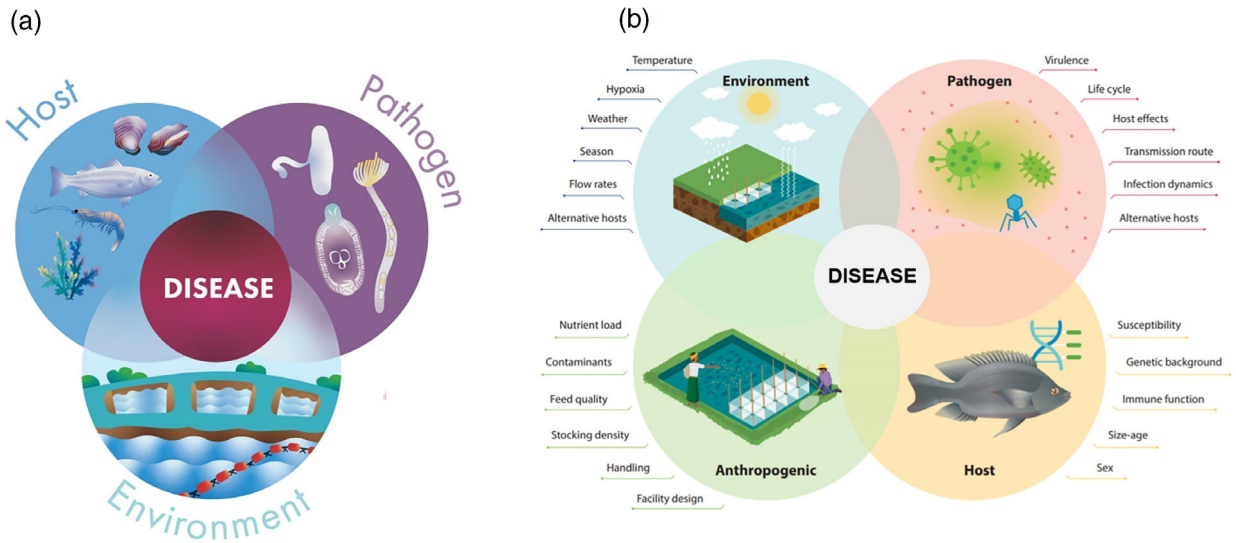

FIGURE 1 (a) The epidemiological triad[31] and (b) the epidemiological tetrad modified from Shields (2013).[34] The tetrad is based upon the original triad of Sniezsko,[31] but is modified to separate anthropogenic drivers of disease outbreaks from those that are purely environmental. This is an important consideration for disease investigation in fish farms where multiple stakeholders may have direct and indirect influence on farming conditions and consequently animal health. It highlights the importance of a broad based framework for diagnostic investigation and subsequent mitigation of disease. (Image A by M.G. Bondad-Reantaso and Paulo Padre, image B by A. C. Barnes and J. Delamare-Deboutteville.)

formulated leading to immune compromise.[36] Finally, agriculture and urban water that are indirectly connected through shared water resource that may adversely impact the water available to the farm in terms of quantity (leading to inadequate water exchange and resulting hypoxia) and quality. Indeed, many pesticides and other pollutants are known to suppress the immune systems of aquatic organisms leading to disease.[34,37] The importance of the tetrad to disease diagnosis lies in the emphasis of a broad based investigation to establish cause and effect. The outcome of diagnosis, ultimately, is establishment of cause for effective treatment and prevention.

Diagnostics is an important element of a national strategy on aquatic animal health[38,39] (now called national aquatic organism health strategy) (Figure 2) and supports the other elements such as for example, policy, legislation and enforcement, risk analysis, pathogen list, border inspection, health certification, quarantine, farm-level biosecurity and health management, use of veterinary drugs, disease surveillance, emergency preparedness and contingency planning and others.

Availability of accurate diagnostic tools is an important criterion for listing of diseases in the OIE (currently known as the World Organisation for Animal Health, WOAH) Aquatic Animal Health Code.[40] Article 1.2.1 of the WOAH Aquatic Animal Health Code lists four criteria for listing an aquatic animal disease. These are: (i) significant production losses, negative affect on wild populations, zoonotic; (ii) infectious aetiology proven, strong association; (iii) capacity for international spread and (iv) diagnostic methods exist.

Diagnostic testing is an essential part (checklist no. 6) of a 12-point surveillance checklist for surveillance of diseases of aquatic organisms.[28] The choice of diagnostic technique needs to account for the following:

- analytical sensitivity which refers to the limit of detection for a disease agent
- analytical specificity which refers to the ability to distinguish the targeted disease agent from another
- diagnostic sensitivity which refers to the probability of test to correctly identify diseased individuals
- diagnostic specificity which refers to the probability of test to correctly identify non-diseased individuals

Quality assurance of a diagnostic system is also an essential part (checklist no. 11) of the surveillance checklist. Diagnostic laboratories that support surveillance could be any accredited laboratory recognized by the competent authority as having the appropriate technical competence in disease diagnostic work. Thus, proficiency ring tests, accreditation and analytical methods are all essential components of an overall quality assurance system.[28] ISO 17025 is the accepted international standard by which laboratories are accredited as being technically competent for specific diagnostic analyses.

Due to their significant benefits in terms of short turnaround time, high specificity and sensitivity, molecular diagnostic methods (e.g., polymerase chain reaction [PCR], quantitative real-time PCR [qPCR], digital droplet PCR [dPCR], loop-mediated isothermal amplification [LAMP], recombinase polymerase amplification [RPA] and others) have emerged as important technologies for improving disease diagnosis in aquaculture. Disease diagnosis in aquaculture was mainly reliant on clinical observation, rapid microscopic inspection by wet-mount and/or quick staining of smears or imprinted tissue, histopathology and culturing of infectious agents prior to the expansion and adoption of molecular

FIGURE 2 Important elements or components of a national aquatic organism health strategy where each element is not a stand-alone component but rather supports each other. (Image by M.G. Bondad-Reantaso and Paulo Padre)

methods in the early 2000s.[41,42] Importantly, many infectious diseases in fish and shrimp are initially discovered and diagnosed based on Level I (see Section 2, below) gross clinical observations and traditional histopathology (Level II). For example, disease caused by TiLV was detected and defined for the first time as syncytial hepatitis of tilapia (SHT) based on a pathognomonic lesion identified in the liver of sick tilapia using classical histology.[43] Similarly, an unknown viral disease, scale drop syndrome, in Asian sea bass was discovered based on gross pathology and histopathological findings of viral inclusion.[44] The shrimp microsporidian *Enterocytozoon hepatopenaei* (EHP) was discovered as a novel microsporidian based on histopathological observation of cytoplasmic spores and multinucleated plasmodia in the shrimp hepatopancreas.[45] In these cases, the disease was identified histopathologically before its causative agent became known to science and before any molecular diagnostic procedures were available for the causative agents. Recent widespread use of molecular diagnostics (Level III) in aquaculture has shifted the focus of diagnostic application away from observational approaches (Level I and II). However, there has been some evidence indicating that molecular diagnostic methods, including those from published papers, The WOAH recommended protocols and commercial kits, sometimes give false-positive results (see Refs. [46–48]). Clinical observations and microscopic examination, on the other hand, are useful for presumptive diagnostics, which guides the choice of the appropriate level II diagnostic test(s) and serves as a clinical judgment in diagnostic error(s). Thus, disease diagnostics should involve a combination of fundamental and sophisticated procedures, including macroscopic, microscopic and molecular investigation, to achieve accurate and meaningful results. In this review, we therefore revisit and emphasize the necessity for fundamental diagnostic procedures for tilapia diseases. Furthermore, current and emerging molecular diagnostic methods are discussed, and their future prospects are critically addressed.

2 | BACK TO BASICS: THREE LEVELS OF DIAGNOSTICS FOR INFECTIOUS DISEASES IN AQUACULTURE

Disease diagnostics is the procedure by which the causative agent of an infectious disease is identified. The Food and Agriculture Organization of the United Nations (FAO) and the Network of Aquaculture Centers in Asia and the Pacific (NACA) have long promoted the use of levels I, II and III for disease diagnosis[39,49] (Figure 3). The principle being that none of the three diagnostic levels function in isolation. They form a continuum of observations (Figure 3) with strong linkages needed for accurate and rapid diagnosis (e.g., for general health surveillance, health certification of import stock and to reduce the risk of disease introduction into disease-free areas) so that appropriate and effective management measures can be rapidly applied.

Level I provides the foundation and is the basis for accurate interpretation of results obtained from Levels II and III laboratory findings. It also sets the foundation for 'presumptive' and 'confirmatory' diagnostic test reporting. Presumptive tests establish if a sample is not infected by a pathogen, or that it is likely infected by a pathogen. In the latter case, it may remain presumptive where the test cannot distinguish pathogenicity (just presence/absence) or the exact identity of the pathogen (e.g., endemic from exotic strain/species). Confirmatory tests are then required to confirm (or refute) the presumptive analysis. Level I may be sufficient for recurrent, pathognomonic

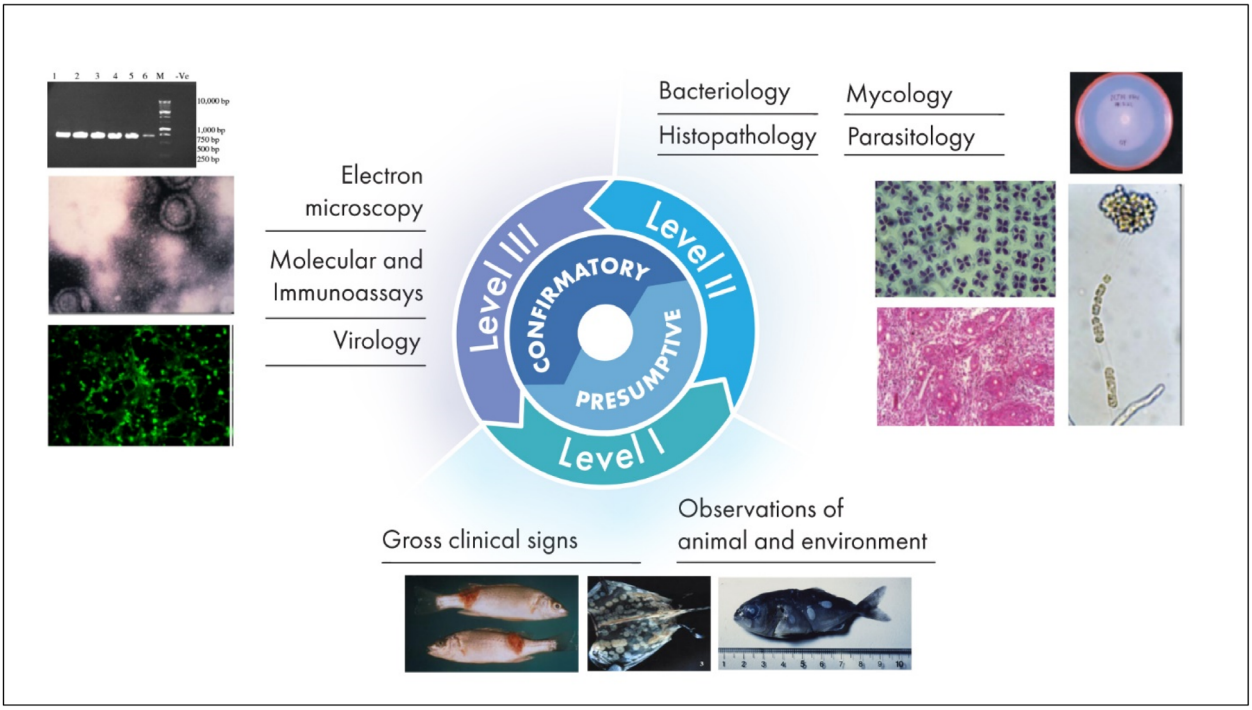

FIGURE 3 The three diagnostic levels (I, II and III) are a continuum of observations; each level builds on the other and contributes valuable data and information to build a diagnostic case for optimum diagnostic accuracy. (Image by M.G. Bondad-Reantaso.)

(i.e., clinical signs are specific to a particular pathogen or environmental stressor) infection. However, confirmatory diagnoses, most commonly, require Level II or III equipment and expertise to distinguish significant pathogens from more benign, infectious species or strains.

Level II laboratories include the equipment and experienced personnel to undertake analyses that can detect and/or identify a range of pathogens. Level II laboratory personnel can perform parasitology, histopathology, bacteriology and mycology examinations. Level II, particularly histopathology, remains the gold standard, especially for unknown and emerging diseases.

Level III diagnostics encompass techniques that target a specialized pathogen or group of pathogens or require highly specialized equipment. Level III laboratories are highly specialized and many such laboratories are accredited nationally or by the WOAH as 'Reference Laboratories'.[50] These laboratories can also be used to confirm disease-freedom to reinforce national health certification for import-export purposes. Use of Level III techniques support Koch's postulate to prove that a particular organism causes a particular infectious disease is important, especially for first time diagnosis of an unknown disease in a country.

Level III diagnostics rarely consider interactions at the host–pathogen interface (pathogenicity) as it relies on detection of molecular signals of these interactions and does not take environmental parameters into account. Thus, any correlation falls on linkage with Level I or II diagnostic observations. The increasing availability of field rapid test kits has been a major advantage for field extension officers, aquatic animal health specialists and farm veterinarians, but brings into play the risk of false/negative results without adequate user training and interpretation. Thus, the importance of conclusive diagnoses being based on more than a single test cannot be under-estimated, and is now clearly outlined by the WOAH[50] for their listed diseases. Accuracy of results is significantly augmented by two or more consistent results, especially of new or previously unknown disease outbreaks.

Three levels of diagnostics can be flexibly applied for infectious diseases of tilapia including bacterial, viral, parasitic and fungal diseases (Figures 4 and 5). At level I, presumptive diagnostics comprises observation of abnormal behaviours, clinical presentation, historical record, environmental parameters and preservation of samples for subsequent analyses in levels II and III. Fish from an affected pond/cage usually exhibit abnormal swimming behaviour, such as failure to school, with separation of sick individuals in the corner or bottom of the pond or cage. Diseased fish may show pale colouration, dark colouration, reddish gill opercula, skin haemorrhage and scale protrusion. Internally, clinically sick fish can exhibit a pale, watery and necrotic liver, accumulation of yellow ascetic liquid in the peritoneal cavity and gas in the intestine.[11,43,51] At level II, presence of syncytial hepatitis is considered pathognomonic for TiLV infection, while intracytoplasmic inclusion bodies may also, occasionally, be observed.[43] More recently, liver tissue smears stained with Haematoxylin and Eosin (H and E) has been found to be a simple and effective approach for rapid screening of syncytial hepatitis (or giant cells) (experience from HT Dong, see Figure 4). Several molecular techniques including reverse transcription PCR (RT-PCR), nested or semi-nested RT-PCR, RT-qPCR, RT-LAMP and in situ hybridization (ISH)[11,47,52–57] culturing of virus using cell line,[11,56,58] TEM[11,43,59] and enzyme-linked immunosorbent assay (ELISA)[60] have been applied for diagnostics of TiLV at level III.

Similarly, bacterial diseases (e.g., Streptococcosis, Columnaris, Edwardsiellosis, Francisellosis and Aeromonasis) in tilapia can be

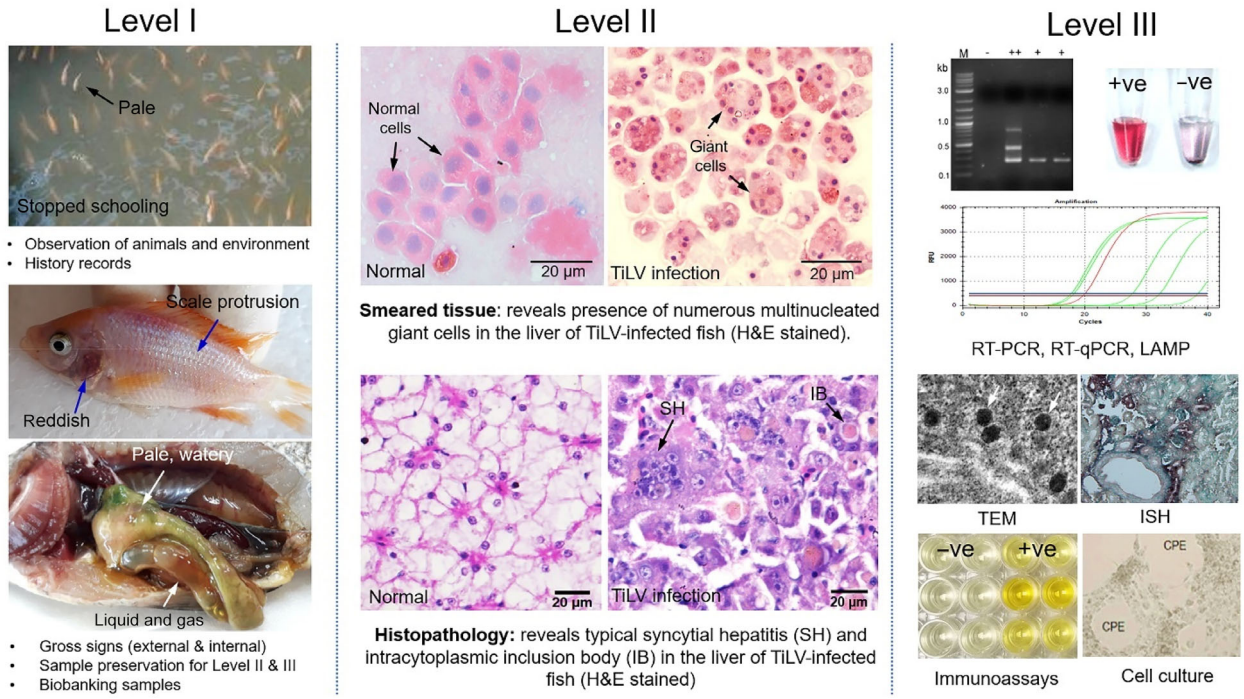

FIGURE 4 Illustration of three levels of disease diagnostics for tilapia lake virus disease in tilapia. (Image by H.T. Dong.)

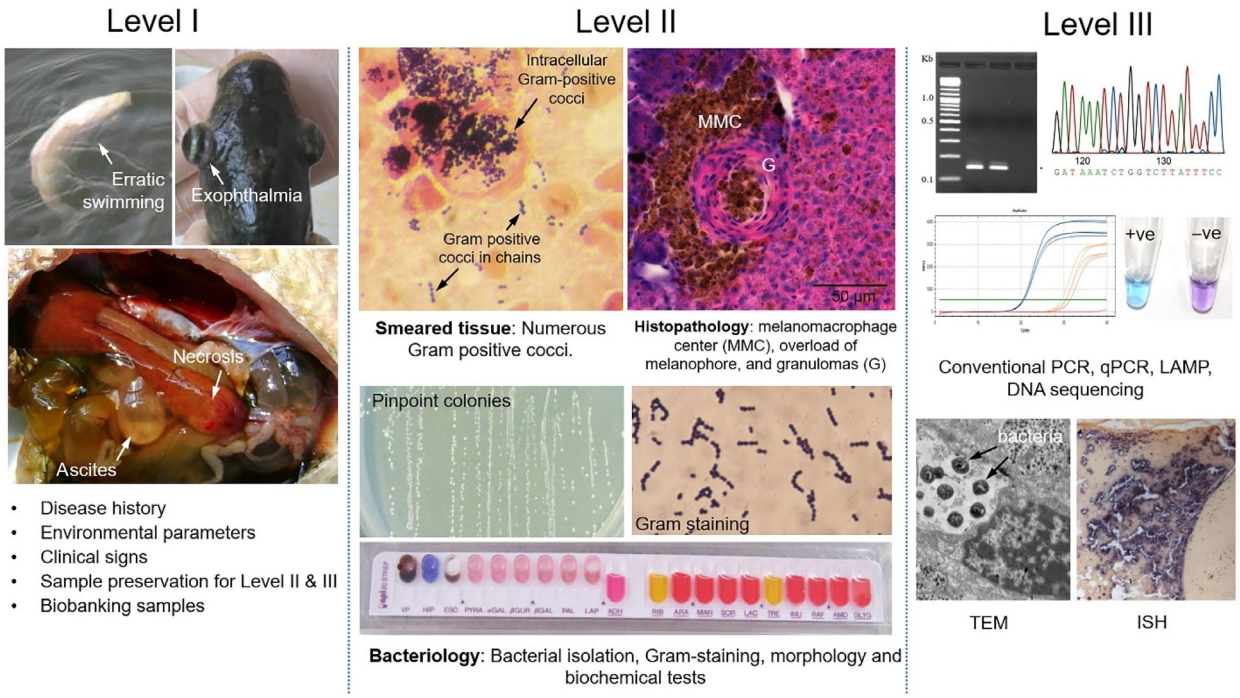

FIGURE 5 Example of three levels of disease diagnostics for Streptococcosis in tilapia. (Image by H.T. Dong.)

presumptively diagnosed using levels I and II, including clinical signs, wet-mount and smeared tissues stained with Giemsa or Gram stain. Further analysis using level II (histopathology, bacterial culture, biochemical screens) and level III (molecular methods) approaches is usually employed for confirmatory diagnosis of suspected bacterial diseases.[61,62] An example of three diagnostic levels in the context of Streptococcosis is shown in Figure 5. At level I, erratic swimming and exophthalmia were considered important clinical signs for presumptive diagnostic of *Streptococcus* sp. infection. Internally, diseased fish presented with ascites, accumulation of liquid in the intestine and dark brown and necrotic liver. At Level II, Gram or Giemsa-stained tissue smears from head kidney was useful for visualization of extra- and intracellular Gram-positive cocci. Histopathologically, diseased fish exhibited increasing melanomacrophage centres and granulomatous inflammation with overload of melanophores in the liver. *Streptococcus* sp. could be isolated from diseased fish using general culture medium such as blood agar (BA), nutrient agar (NA), tryptic soy agar (TSA) or brain heart infusion agar (BHIA), morphologically identified by microscopy of Gram stained samples and biochemically characterized by commercial processes such as API 20 Strep or Vitek. Several methods can be employed at level III for confirmatory diagnostic of Streptococcosis including conventional specific PCR,[63] qPCR,[64] LAMP,[65–67] sequencing of 16S rRNA,[68] ISH and TEM.

In reality, it is unlikely that disease outbreaks in tilapia farms in LMIC are currently diagnosed in a timely manner by rigorous diagnostic tests. Therefore, level I diagnostics should be considered through observation of clinical signs,[69] case history records, outbreak description as part of the syndromic surveillance to support early presumptive diagnosis and also to make informed evidence-based decisions on appropriate further sampling and diagnostic approaches, as well as immediate management actions. Preservation of biological samples (biobanking) might be useful for retrospective diagnostics as well as epidemiology and evolution of infectious agents.[70–72] In the context of tilapia disease diagnosis, the term 'biobanking' refers to the systematic preservation of biological materials in a suitable manner for later examination using advanced diagnostic methods. Fixed tissues or blood (e.g., in ethanol 95% or RNA later for molecular testing) and nonfixed frozen tissues or serum (e.g., storing at minus 80°C or liquid nitrogen for later recovery of infectious agents) are examples of these samples. The biological samples also include pathogens (isolates/strains) recovered from diseased animals, extracted genetic materials (DNA or RNA) and paraffin-embedded samples. Appropriate biobanked samples provide the necessary materials for interconnecting three diagnostic levels (I, II and III) which are required to progress from presumptive to conclusive diagnoses.

3 | CURRENT AND EMERGING MOLECULAR DIAGNOSTIC TECHNOLOGIES

The field of molecular diagnostics has, in recent years, developed rapidly and contributed substantially to our ability to detect and identify microbial pathogens of aquatic organisms, most importantly the detection of sub-clinical carriers. Various nucleic acid-based amplification techniques are commonly used in detecting aquatic pathogens, including conventional PCR, qPCR, dPCR, LAMP and CRISPR. The strengths and limits of each technology, and their current and potential application for disease diagnosis in tilapia aquaculture is discussed below.

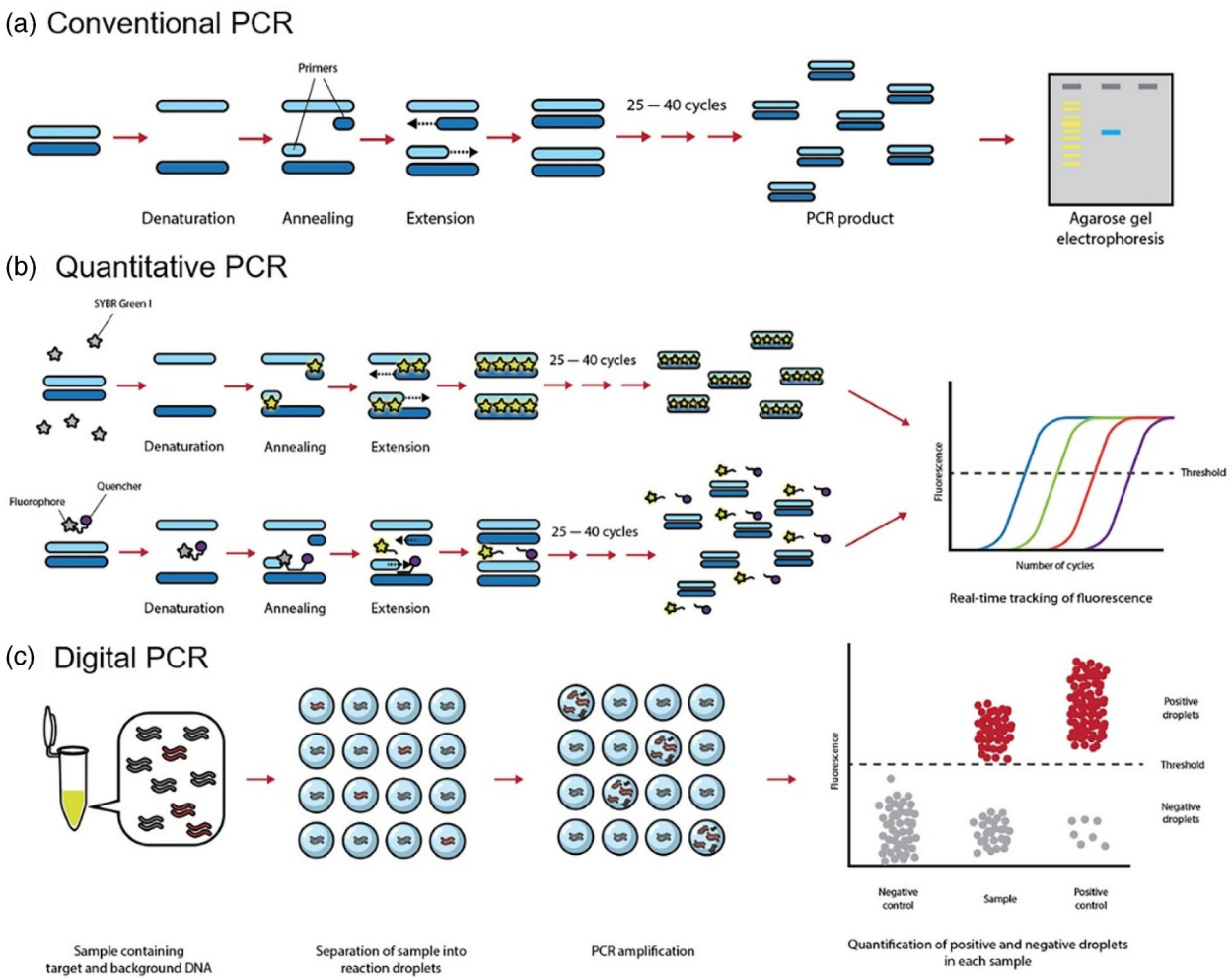

FIGURE 6 Illustrations depicting the backbones of conventional PCR (a), quantitative PCR (b) and digital PCR (c). (Images by T. Chaijarasphong and H.T. Dong.)

3.1 | Polymerase chain reaction

3.1.1 | Conventional polymerase chain reaction

Polymerase chain reaction (PCR) is a method that employs a thermostable polymerase to amplify a specific region of DNA defined by a pair of primers. PCR relies on thermal cycling; the DNA templates are exposed to repeated cycles of heating and cooling to permit DNA melting, annealing of the primers and DNA synthesis by the polymerase. This reaction generates large numbers of DNA synthetic copies from a small amount of DNA template (Figure 6a). When the reaction is 100% efficient, approximately 10^9 copies of DNA target can be produced per template after 30 cycles. As primer and deoxynucleotide triphosphate (dNTP) are consumed during the reaction, single-step PCR has limited sensitivity. Nested PCR, two successive PCR reactions, using second round primers specific to the first-round amplicon, provides increased sensitivity and specificity, and has been developed for detecting pathogens in sub-clinically infected animals. The PCR procedure involves extraction of DNA (or RNA) from host tissue samples, followed by amplification of target DNA. A Taq polymerase, a major component of PCR, will not work on an RNA template, so PCR cannot be used to directly amplify an RNA molecule. For detecting RNA viruses, extracted RNA must be first transcribed into its complementary DNA (cDNA) by the enzyme reverse transcriptase (RT). This method of RNA amplification is called reverse transcriptase-polymerase chain reaction (RT-PCR).

Advantages of PCR-based diagnosis include their high sensitivity and specificity, rapid turnaround, elimination of the need for prior isolation or culturing of microorganisms and relatively low cost. The method is especially useful for detecting pathogens in inapparent infected individuals and in identifying pathogens that are unculturable, such as shrimp or molluscan viruses, or difficult to culture, such as intracellular bacteria. However, PCR requires trained technicians for optimization and reliable diagnostic results, along with well-equipped facilities with strict protocols for nucleic acid extraction and processing. PCR is susceptible to contamination and requires strict consistency of procedures for high throughput automation.

Conventional end-point PCR/RT-PCR (including single, semi-nested, nested PCR, duplex and multiplex PCR) has been commonly used for the detection of infectious pathogens in tilapia such as TiLV,[11,47,57,73] TiPV,[74] NNV,[75] *S. agalactiae*,[76,77] *S. iniae*.[78,79] In tilapia, *S. agalactiae* and *S. iniae* are the two most frequently detected

bacteria that cause streptococcosis. Both cause similar clinical signs, thus a duplex PCR using two primer pairs and a differential PCR using a single primer pair were developed for detecting and differentiating these two bacteria.[63,80] Multiplex PCR was also developed for serotyping of *S. agalactiae*.[81]

PCR methods have also been developed for detecting *F. orientalis* and *F. columnare*,[82,83] which are fastidious bacteria that require time-consuming, complex culture media and biochemical assays for non-molecular infection diagnosis in tilapia.

Edwardsiellosis and motile *Aeromonas* septicemia (MAS) are among the most prevalent bacteria detected following mortality in freshwater fish, including tilapia. PCR-based methods were developed for detecting of *E. ictaluri* and *E. tarda*[84] and applied for tilapia.[85] For *Aeromonas* bacteria, PCRs targeting the virulence-associated genes, hemolysin and aerolysin, were developed to identify *A. hydrophila* isolated from tilapia with MAS.[86]

3.1.2 | Quantitative real-time PCR

Quantitative real-time PCR (qPCR) is a well-established method for diagnosis of aquatic animal diseases. Amplification of target nucleic acids can be detected in real-time during PCR through the use of either sequence-specific fluorescent-labelled oligonucleotide probes (e.g., TaqMan), or sequence-independent fluorescent dyes (e.g., SYBR Green I).[87] The presence and quantity of a target DNA can be determined by its cycle threshold (Ct) value, which corresponds to the cycle number when the fluorescence level is significantly above a pre-defined, experimentally determined threshold (Figure 6b). This method eliminates post-PCR gel electrophoresis and thus reduces the risk of cross-contamination between samples during loading of the gel. Usually, a cut-off Ct value is determined based on a limit of detection established experimentally. This helps to eliminate false positives based on non-specific amplifications, and the test is interpreted as positive if the Ct value is less than the cut-off Ct. If the Ct value is greater than the cut-off value (i.e., below the limit of detection), the test may be interpreted as negative.

Quantitative real-time PCR measures fluorescence intensity and can be used to quantify the number of copies of target nucleic acids present in a tissue sample to determine the viral (or other microorganism) loads. Quantification of a specific virus in tissues of infected animals is one of the most important means of monitoring the progression of a disease. Cell culture-based methods of quantifying pathogens are time-consuming and not applicable to some aquatic organisms, such as shrimp, for which cell culture systems have not been developed. qPCR has the advantages of rapid, high-throughput and a wide dynamic range (7–8 $\log_{10}$) for quantification; it can be multiplexed to detect several targets in a single reaction.

RT-qPCR or qPCR procedures have been developed and optimized for the detection and quantification of viral or bacterial loads in infected tilapia. Target pathogens include TiLV,[55,56,88] ISKNV,[89] TiPV,[74] *S. agalactiae*[64,90] and *F. orientalis*.[91–93] Several multiplex TaqMan qPCR assays have also been developed to detect and quantify three to four pathogen species in a single PCR test, such as *F. orientalis*, *S. iniae* and *S. agalactiae*[94]; *A. hydrophila*, *A. veronii* and *A. schubertii*[95]; and *E. ictaluri*, *E. tarda*, *E. anguillarum* and *E. piscicida*.[96]

3.1.3 | Digital PCR

Digital PCR (dPCR) uses the same analytical process as qPCR, but is used to quantify the absolute number of target DNA molecules.[97] In dPCR, the DNA template and reagents (identical to the qPCR reaction mixture, including pathogen-specific primers and probe) are mixed and then partitioned, either in emulsion droplets or in wells, on a nanofluidics chip. dPCR amplification is then performed on each of the partitions. At the end of the dPCR, each partition is read, and the absolute quantification of DNA template is calculated with Poisson statistical analysis. The process is 'digital' in that each partition is scored as either 1 (positive) or 0 (negative) (Figure 6c). It is important that the DNA template be adequately diluted, as most partitions contain one or no target DNA molecules.[98,99]

Digital PCR has advantages over qPCR in that dPCR does not require a standard curve for quantifying the DNA template and provides more accurate quantitative results, because the presence of PCR inhibitors has little effect. There are two disadvantages to dPCR: (1) it is laborious and has a lower throughput, and (2) it has a smaller dynamic range than qPCR, so samples need to be diluted within a specific range to generate accurate results.

Digital PCR is relatively new to aquaculture so has only been applied to few fish pathogens. dPCR methods are available for ISKNV[100] and *S. agalactiae*.[101] The detection limit of ISKNV dPCR was determined to be 1.5 copies/μl, which is substantially lower than the 34 copies/μl of a TaqMan qPCR. This assay was used to detect ISKNV in mandarin fish (*Siniperca chuatsi*) and shown to have a higher positive rate (65%) than that of qPCR (30%).[100] Similarly, the latter method was developed for absolute enumeration of *S. agalactiae* in tilapia tissue which is more sensitive than conventional plate count method and qPCR.[101] These results suggest that dPCR presents a promising diagnostic platform for other tilapia pathogens.

3.2 | Isothermal amplification

Isothermal amplification methods (IAM) present a powerful class of nucleic acid detection analytics that provide streamlined workflows and rapid turnaround times, while preserving the diagnostic merits of conventional PCR. By using polymerases capable of replicating nucleic acids at a constant temperature, IAM avoid the thermal cycling associated with PCR, making them ideal for on-site diagnosis in areas lacking scientific resources and manpower. To date, a plethora of IAM have been developed and implemented with varying degrees of success, including loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), nucleic acid sequence-based amplification (NASBA), helicase-dependent amplification (HDA), rolling circle amplification (RCA) and cross-priming amplification (CPA). This section of the review will focus on two IAM that show potential for rapid detection of tilapia diseases: (1) LAMP, by far the most frequently used

IAM, and (2) RPA, which has grown in use over the last decade as a result of its improvements over LAMP's shortcomings. Additionally, we will discuss the combination of isothermal amplification and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) diagnostic analyses, which open up new potential applications currently not feasible with IAM alone.

3.2.1 | Loop-mediated isothermal amplification

A typical loop-mediated isothermal amplification (LAMP) reaction consists of a DNA template, four to six primers targeting six to eight distinct regions along the target DNA, and the large fragment of *Bacillus stearothermophilus* (*Bst*) strand-displacing DNA polymerase.[102,103] The reaction is typically completed within an hour at a temperature of 60°C–65°C and progresses exponentially through characteristic, dumbbell-shaped DNA intermediates, eventually generating concatemers of various sizes harbouring the target sequence (Figure 7a). This size heterogeneity of LAMP products manifests as ladder-like bands when analysed by agarose gel electrophoresis. To detect RNA viruses such as TiLV, reverse transcription step by reverse transcriptase (RT) must be incorporated into the procedure to produce cDNA from the RNA target prior to LAMP. Alternatively, a newer generation of *Bst* polymerase, *Bst* 3.0, that shows high reverse transcriptase activity, could be utilized for single-enzyme RT-LAMP protocols.[104] LAMP platforms have been described for the detection of tilapia pathogens, including *Streptococcus agalactiae*,[65–67] *Flavobacterium columnare*,[105] *Shewanella putrifaciens*,[106] ISKNV[107] and TiLV.[54,108,109] These applications exhibit high sensitivity and specificity for their respective targets, with the lowest limit of detection reported at 1 viral copy per reaction, approximately 100 times lower than that typically obtained using conventional PCR.[54]

While different LAMP assays use similar nucleic acid amplification procedures, they vary in their visualization methods, which range from being laboratory oriented to field compatible. Agarose gel electrophoresis, for example, is commonly employed in laboratory settings, but it is time-consuming and requires extensive reagent preparation and handling. While it can be used to validate amplicon size in PCR, this advantage is lost in LAMP due to the products' ladder-like appearance precluding direct size comparison. To further streamline detection, colorimetric dyes such as SYBR Green,[109] calcein[105,106,108] and hydroxynapthol blue[107] can be incorporated to LAMP reactions. The use of these reporters enhances field deployability of the assay, but they are sequence-independent and

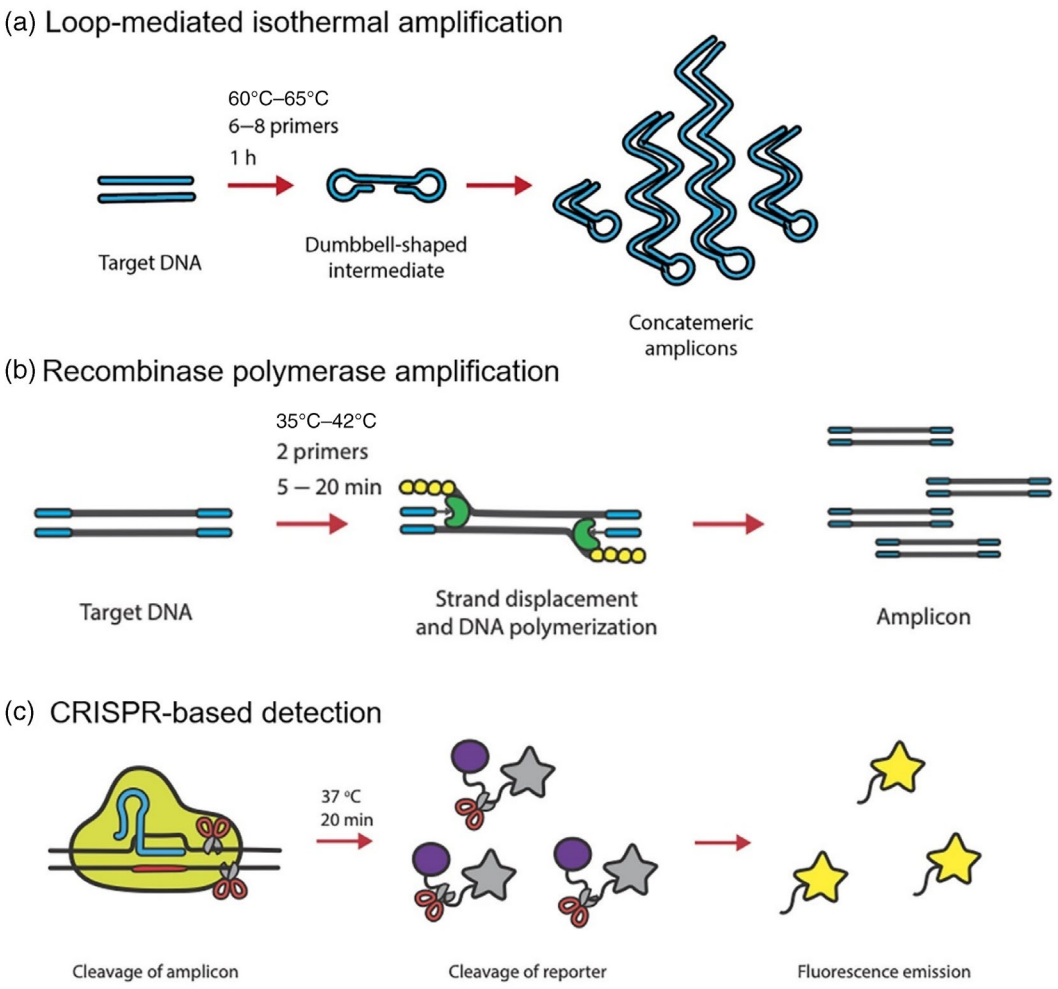

FIGURE 7 Illustrations depicting the backbones of RPA (a), LAMP (b) and CRISPR-based detection (c). (Images by T. Chaijarasphong.)

thus incapable of discriminating between specific and spurious amplification products. To exclude non-specific amplicons, sequence-specific hybridization probes, such as gold nanoparticles (AuNP) coated with single-stranded DNA (ssDNA), can be used.[54] Upon denaturing and reannealing of LAMP products, the gold-conjugated ssDNA hybridizes to its complementary region in the valid amplicon, preventing AuNP from aggregating in the presence of high salts. As a result, a positive reaction retains the pink colour of dispersed AuNP, while a negative sample precipitates AuNP and loses its solution colour.

3.2.2 | Recombinase polymerase amplification

While LAMP has substantially improved the convenience of tilapia disease detection, the technique still faces a number of limitations including the large number of primers required, which increases the likelihood of primer–dimer formation, and the reaction temperature that, while constant, is still sufficiently high to require a heating device.[110] In comparison, a relatively recent IAM called recombinase polymerase amplification (RPA) requires a relatively low temperature between 35° and 42°C that can be supplied instrument-free, has a short reaction time of 5–20 min, and requires only two primers, similar to PCR.[111] This assay relies on a bacterial recombinase protein to partially unwind the target DNA duplex and enable primer annealing to the complementary regions.[112] The reaction also contains single-stranded DNA-binding proteins that sequester the displaced DNA strand and prevent it from reannealing (Figure 7b). With primers in place, DNA polymerase initiates exponential DNA amplification and generates a large amount of daughter DNA that can be visualized by agarose gel electrophoresis, fluorescence or lateral flow detection.[113,114] To detect RNA, a preincubation step with reverse transcriptase at 42°C can be directly incorporated into an RPA reaction, yielding an RT-RPA workflow. Thus far, RPA methods have been used to detect a number of pathogens affecting tilapia, such as *Aeromonas hydrophila*, *Flavobacterium columnare* and *Francisella noatunensis* subsp. *orientalis*, with an analytical sensitivity of up to 15 DNA copies per reaction.[115–118]

3.2.3 | CRISPR detection

Due to their low reaction temperatures, IAM like LAMP and RPA are intrinsically susceptible to primer dimer formation and non-specific amplification. Additionally, the sensitivity of the assays is highly target-dependent, with challenging targets needing extensive, iterative optimization to enhance sensitivity. Integrated with CRISPR detection, specificity and sensitivity of IAM can be raised in a plug-and-play manner.[119,120] The CRISPR detection method begins with an RNA-guided CRISPR-associated protein (Cas) endonuclease, such as Cas12a or Cas13a, recognizing and cleaving the target nucleic acid (e.g., IAM amplicon). This on-target cleavage induces a conformational change in the Cas protein, causing it to indiscriminately digest the ssDNA (in case of Cas12a) or ssRNA (in case of Cas13a) that connects a fluorophore and its quencher in the synthetic reporter, resulting in unquenching and consequent fluorescence emission[121,122] (Figure 7c). Thus, the presence of the positive amplicon is converted into a fluorescent signal observable by the naked eye, or, with some modification, a colorimetric signal on a lateral flow dipstick.[123,124] It should be noted that Cas13a, which exclusively targets ssRNA, requires the addition of RNA polymerase and nucleoside triphosphates (NTP) as well as the presence of a promoter sequence in one of the IAM primers to allow transcription. This CRISPR detection step may be preceded with practically any IAM, although RPA is most commonly chosen due to its optimal temperature being close to that of Cas proteins (37°C).[125] On the other hand, the choice of Cas proteins is restricted to a small number of Cas homologues capable of carrying out reporter cleavage in the manner described above.[119,120,126] Indeed, Cas9, the most widely used homologue for genome editing, lacks nonspecific secondary cleavage activity so cannot readily be repurposed for diagnostic applications.[119,127,128]

Along with providing several modes of simple visual detection, integration with CRISPR may improve the sensitivity and specificity of IAM. The diagnostic Cas endonucleases are capable of increasing sensitivity owing to their multiple-turnover kinetics, whereby the cleavage of a single target DNA/RNA molecule activates Cas protein for digestion of several reporter molecules, resulting in signal amplification.[119,123] Nonetheless, this sensitivity enhancement effect is not always observed and is more frequently found with Cas13a than Cas12a, presumably due to the superior reporter cleavage kinetics of the former.[124,129–131] In terms of increasing specificity, by tailoring the CRISPR assay to target an area within the correct amplicon, it is possible to filter out nonspecific amplification products from IAM.[129] Moreover, Cas endonucleases are exceptionally stringent in their target recognition—a 2-bp mismatch between guide RNA and target nucleic acid has been shown to drastically reduce the cleavage activity.[120,132] This low mismatch tolerance can be used to genotype closely related pathogen strains whose sequences may be too similar for traditional PCR or IAM alone to differentiate. CRISPR detection, therefore, may allow for easy identification of geographical isolates or genotypes of RNA viruses such as TiLV, which may grow more diverse in sequence and virulence in the future due to their fast mutation rates. While CRISPR detection has been extensively applied to high-impact pathogens such as SARS-CoV-2, it has not yet been harnessed for disease detection in tilapia, highlighting an untapped opportunity for improving the efficacy and utility of the present diagnostic toolbox.[133,134]

3.3 | Lateral flow immunoassays

Although nucleic acid detection approaches are highly sensitive and specific, they are limited by long processing time, extensive liquid handling and the requirement for scientific instruments. While IAM have simplified overall procedures, some liquid handling and wait time remain necessary. In comparison, lateral flow immunoassays (LFIA) allow the user to simply apply the analyte to a ready-to-use strip and

wait for 5–10 min before reading the result, which is colorimetric and interpretable by eye. The analytic materials also have long shelf-life and can be stored at room temperature. Therefore, despite their generally lower sensitivity and specificity than comparable nucleic acid detection technologies,[135–138] the convenience of LFIA greatly aid screening of diseases and presumptive disease diagnosis in tilapia, as well as adoption by stakeholders who may be hesitant to use more laborious, time-consuming, diagnostic platforms.

To perform an LFIA, the sample must first be isolated from the source specimen. The extraction protocol varies depending on the target organ, but generally involves briefly homogenizing the tissue in a lysis buffer and collecting the supernatant.[139,140] The supernatant is then applied to a sample pad on a membrane-bound strip, before it is immersed in a running buffer. Alternatively, some LFIA kits use the lysis buffer for strip development, obviating the need for a dedicated running buffer and reducing liquid handling steps. Through capillary action, the analyte is drawn up the strip and comes into contact with different antibodies along the way. In the 'sandwich' assay format—the most used type—the analyte interacts with the first monoclonal antibody at the conjugate pad. This antibody binds to an antigenic site on the analyte with high affinity, and is labelled with a reporter, commonly gold nanoparticles (AuNP). The antigen-antibody complex and unbound labelled antibody travel to the first detection line (test line) where another monoclonal antibody is embedded. This antibody targets a different epitope on the analyte, causing the latter to become sandwiched between two antibodies and yielding an intense purple band (colour of nanogold) at the test line. Excess AuNP-tagged antibody, on other hand, continues migrating to the second detection line (control line) and gets captured by the embedded antibody specific for the labelled antibody (Figure 8a). Thus, a positive sample generates two coloured bands on the strip, whereas a negative sample produces only one band at the control line. If the control band is not visible, the result is deemed invalid.

If two monoclonal antibodies to the analyte are not available, or if the analyte is too small to be bound by two antibodies simultaneously, the 'competitive' assay format can be employed. In this format, the test line is coated with the target analyte instead of an antibody. If the analyte is present in the sample, it sequesters the labelled antibody and prevents it from interacting with the embedded analyte at the test line. In contrast, when the target analyte is absent, the labelled antibody is free to bind to the embedded analyte. Consequently, in this format, a positive result is represented by

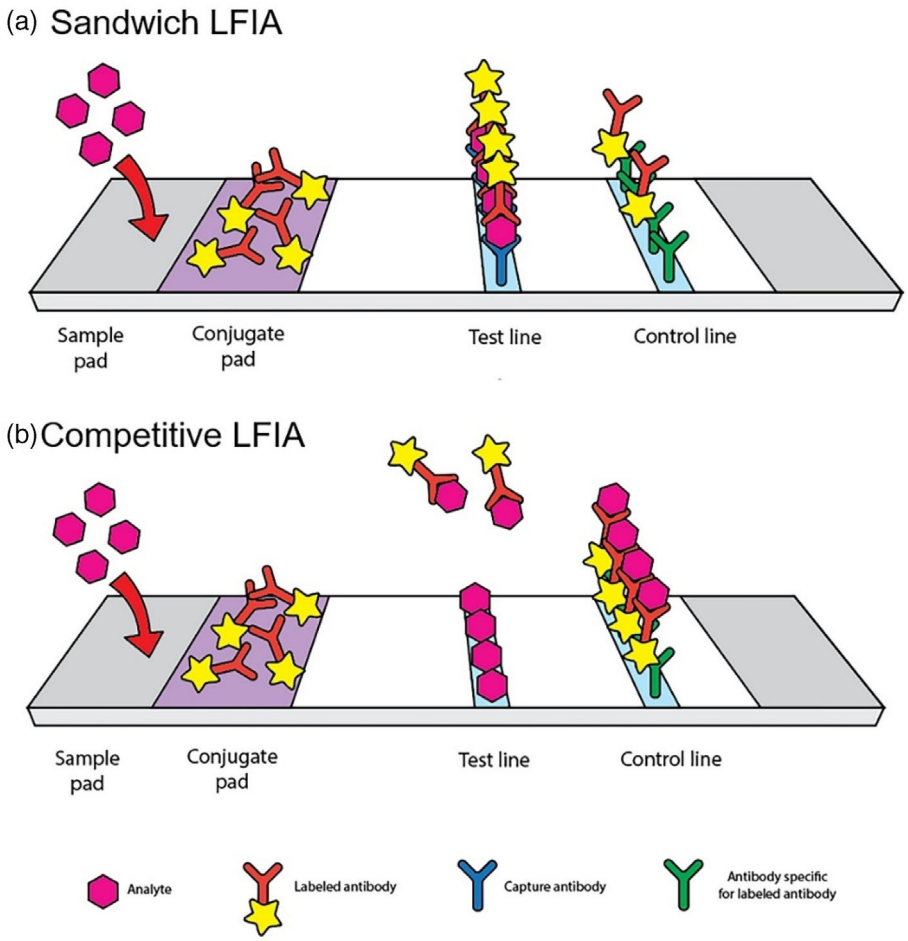

FIGURE 8 Schematic showing the composition and mechanism of sandwich (a) and competitive (b) LFIA. (Images by T. Chaijarasphong.)

TABLE 1 Pros and cons of available sequencing technologies

Technology	Read length	Total data	Pros	Cons
Illumina iSeq, MiniSeq, MiSeq and NextSeq, Novaseq*	2 × 150 bp 2 × 250 bp*	1.2–6000 Gbp	Becoming 'standard' for short reads. Accurate data, random error can be polished out. Established and well-validated, open source/community data analysis tools	Short reads High capital cost Requirement for laboratory infrastructure even for 'benchtop' units
Ion Torrent Personal Genome Machine (Thermo Fisher)	200 or 400 bp	30 Mbp to 2 Gbp	Fast output (2.3–7.3 h) Moderately priced	Short reads Limited community analysis tools Requirement for lab infrastructure
Pacbio Sequel II, Sequel IIe	30–40 kbp	160 Gbp per SMRT cell	Long reads, low systematic error rate (~0.1% for HiFi reads)	High capital cost Large footprint Requirement for lab infrastructure High run cost
Oxford Nanopore Technologies MinION	Up to 2.3 Mbp[164]	~30 Gbp per MinION flow cell (~10 Gbp per cell per day)	Long reads Low cost Pocket sized instrument No requirement for lab infrastructure or mains power Consensus error rate <0.005% (R10.4 flow cell) Open source/community data analysis tools	Systematic error rate ~5% for raw reads

Note: Data from manufacturers' websites, October 2021.

a single band at the control line while a negative result yields two bands on the strip (Figure 8b).

To date, LFIA tests have been developed for detection of diseases in a variety of fish species,[140–142] but so far only two are for tilapia pathogens, *Streptococcus agalactiae* and *Edwardsiella tarda*.[140,143] Although some pathogens, such as TiLV and *Flavobacterium columnare*, lack dedicated LFIA, effective antibodies against them have been identified and utilized to develop other immunoassays such as immunohistochemistry, enzyme-linked immunosorbent assay (ELISA) and fluorescence microscopy.[144,145] In addition, antibodies capable of recognizing host antibody directed against a specific pathogen have been identified, which may be useful for interrogating the present and past infection statuses of a fish population.[60,146] While these antibodies may serve as a good starting point for future development of LFIA, further optimization may be required, as an antibody that performs well in one type of assay may not perform well in another, due to differences in antibody affinity and concentration, chemical modification and microenvironment.

Although LFIA tests show great promise for routine disease diagnosis in the tilapia farming industry, there are still some issues that require attention. Currently available for only two tilapia pathogens, the cost of lateral flow strips constitutes a large fraction of the LFIA price per assay. Multiplex LFIA, capable of testing several pathogens at once, will significantly reduce cost. With greater utility and economic viability of the technology, LFIA should become more accessible and of greater use to tilapia farmers for disease diagnosis, improving protection from delayed detection or misdiagnosis of disease outbreaks.

3.4 | Next generation sequencing for fish disease diagnosis and epidemiology

Next generation sequencing (NGS) targeting molecular information from infectious organisms for diagnostic purposes has a long history, with the majority of standard methods for determination of infection status in humans, animals and plants now dependent on thoroughly validated PCR tests. These methods target highly specific loci of differentiation within the target pathogen, but provide little information beyond a well-defined case-positive or -negative within specified detection limits. Whole genome sequencing (WGS), on the other hand, provides the total information encoded in the genome of the pathogen, which contains a wealth of clinically relevant data; from antimicrobial susceptibility[147] to high resolution strain identity, that is valuable for epidemiology assessment and related disease control.[148–151] The value of such epidemiological detail has been highlighted through the global severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, where genomic information, provided in near real-time, was employed to identify case origins, and define quarantine controls which, in some cases, prevented further spread.[149,152,153] Indeed, epidemiological use of genomic data has attained global awareness as a result of daily updates from public health authorities.

In addition to targeted genome sequencing, NGS technology also lends itself to non-targeted or metagenomic NGS (mNGS), where total nucleic acid from a sample is sequenced directly, or generic regions such as 16S ribosomal RNA (16S rRNA) are amplified and then sequenced.[154] The resulting pool of sequence data can be de-noised,

assembled and analysed for presence and prevalence of possible pathogens. The non-targeted nature of mNGS makes it particularly useful for pathogen discovery. Indeed, the complete genome sequence of TiLV was first identified from metagenomic data derived from Illumina sequencing,[52] while a novel tilapia parvovirus HMU-HKU-1 was also discovered by Illumina sequencing from metagenomic libraries enriched for viral nucleic acids.[155]

While the advantages of genomic and metagenomic information to disease diagnostics and epidemiology are evident, they are relatively recent additions to the clinician's toolkit, largely due to the cost and time required to generate the information. Sequencing costs have fallen dramatically in the last two decades, greatly out-pacing Moore's law.[156] For example, it is estimated that the first human genome cost in excess of $100 million US. In contrast, the sequencing cost for the whole human exome (about 6 giga base pairs [Gbp] of data) is now around $1500 US. Sequencing cost is somewhat proportional to the amount of data required. Bacterial genomes are 1000 times smaller than a human exome at 2–6 megabase pairs (Mbp), and viral genomes even smaller, just 2–3 kilobase pairs (kbp) for nodaviruses, and 9–10 kb for TiLV.[157] Consequently, with adequate multiplexing, it is possible to generate sequence data for a bacterial genome for substantially less than $100 US. However, sequencing is only one part of the cost, so there is a strong tendency to underestimate the true cost of generating useful clinical genomic information, including sample preparation and downstream bioinformatics analysis.[158]

Current NGS technologies can be separated into two paradigms: (1) short-read and (2) long read sequencing.[159] There are pros and cons to each of the technologies and instruments currently in general use (Table 1). Short read sequencing is now dominated by the Illumina platform with very well-established laboratory preparation protocols and a wide range of well tested, open-source, data analysis tools and complete pipelines for mapping and assembly.[160] Moreover, there are excellent open-source tools for variant calling and clinically relevant typing, much of which can be performed directly from Illumina read data without need for time consuming assembly.[161,162] Short reads become problematic when structural elements need to be correctly resolved.[163] These might include critical plasmids, transposons or structures of long variant regions such as lipopolysaccharide (LPS) O-antigen and capsular polysaccharide (CPS) where rearrangement can lead to clinically relevant serotype switching. Pacbio Single Molecule Real-Time (SMRT) and Oxford Nanopore Technologies (ONT) nanopore sequencing are the major 'third generation sequencing' (TGS) platforms for generation of long read data that can fully resolve genomes to chromosome level. Because SMRT is polymerase-based, read-length is constrained by the enzyme chemistry and currently generates up to 30 kbp reads. SMRT provides high consensus accuracy due to effectively re-sequencing the same circular DNA constructs by the immobilised polymerase within the SMRT cell waveguide enabling highly accurate chromosome-level closure of genomes.[163] Nanopore directly sequences DNA molecules by actively drawing them through a biological pore in a solid state membrane while measuring the charge across the pore. The length of read generated is therefore only limited by the integrity of the DNA loaded, with the longest read recorded to date being 2,272,580 base pairs (bp).[164] The compromise with nanopore sequencing is relatively high systematic sequencing error (~5%) in raw reads, as the electrical resistance across the pore is influenced by several bases in the pore and their methylation state.[165] Nevertheless, the latest version of the nanopore MinION flow cells chemistry (R10.4), coupled with continuously improving base-calling algorithms, can provide a consensus accuracy of 99.995% from nanopore sequencing runs. The major advantage of the nanopore platform is the very low Minion instrument cost ($1000 US), and capability to operate the instrument under field conditions to generate clinical data in real time.[166]

3.5 | Application of WGS to fish disease diagnosis and epidemiology

In infectious disease investigation, genomic data is most useful for the high resolution it can deliver for epidemiology. The origins of disease introduction and most likely routes of transmission have been well-illustrated by WGS for some fish pathogens. For example, the trans-Atlantic dissemination of *Renibacterium salmoninarum* was postulated by genomic investigation,[167] while presence of serotype O2 *Yersinia ruckeri* in Tasmania and likely transmission of serotype O1b with salmonid eggs from Tasmania to Chile was also identified using NGS.[168] Introduction of piscine *Streptococcus agalactiae* serotype Ib into Australia, probably with imported tilapia in the 1970s and 1980s, and subsequent dissemination and evolution in wild marine fish populations was determined using Illumina short read sequencing.[169] However, NGS platforms have utility beyond WGS. Often, useful epidemiological and clinical information can be derived by sequencing amplicons generated by diagnostic PCR methods. For example, nanopore-based sequencing was recently employed to sequence diagnostic PCR amplicons for rapid genotyping of TiLV isolated from disease outbreaks in farmed tilapia.[170] The ability to conduct the sequencing locally and in near real time may be particularly advantageous in evidence-based outbreak control. Thus, a simple workflow for field application of nanopore sequencing in aquaculture may become a useful tool in the near future (Figure 9). In addition to simple field sample collection and processing protocols, utility of the technology will depend upon user-friendly interfaces that can interpret and correct read-data in real-time direct from the instrument and provide clinically relevant information back to the user, for example via a smartphone.

4 | WHAT IS ON THE HORIZON FOR EMERGING TECHNOLOGIES AND TILAPIA DISEASE DIAGNOSTICS?

4.1 | Artificial intelligent and machine learning

The rapid evolution of sequencing capabilities and costs, coupled to simplified analytical workflows, makes them accessible to fish disease

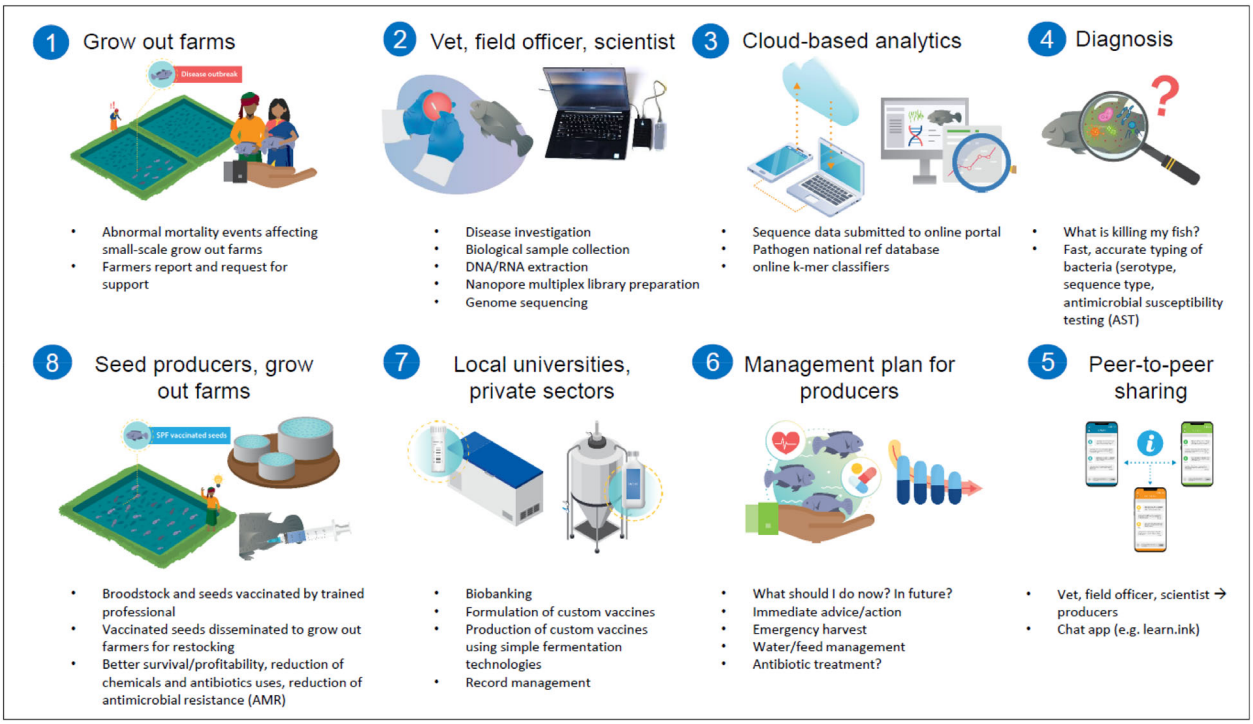

FIGURE 9 A hypothetical workflow for real-time field diagnostics using Oxford Nanopore sequencing. (Images by A. C. Barnes and J. Delamare-Deboutteville.)

diagnostics with capacity to generate a mass of genomic data. Translating such data into clinical decisions or, at least, to information that is useful to clinical decision making by personnel on the ground, however, remains challenging. In the human genome context, the '$1000 genome and the $100,000 analysis' has been discussed.[171] Solutions, or partial solutions to this problem may lie in increasing use of artificial intelligence (AI). AI can be divided into expert systems and machine learning. Expert systems are devised around pre-defined sets of rules derived from clinical or veterinary experts to create a knowledge base that is mined by the expert system to provide computer-aided decision support.[172] However, as scenarios become more complex, as indeed they are in the diagnosis of infectious diseases in aquaculture environments, expert systems are clearly limited by the information in the knowledge-base. Machine learning overcomes this constraint by employing algorithms that devise and refine their own sets of rules from data, allowing them to learn as more data become available. Ensuring the quality of the training data then becomes the major limitation.[172] Machine learning is already integrated into the ONT' base calling algorithms for interpretation of the current signal into bases, with several available nanopore community and open source bioinformatics post-processing applications, all based on artificial neural networks.[165] To get from sequence to clinically relevant actionable information is more challenging. For example, predicting antimicrobial susceptibility to enable rapid evidence-based therapeutic intervention is feasible from whole genome or metagenomic data using neural networks.[173] Predicting antibiotic susceptibility direct from raw nanopore sequencing reads was an early application of the technology.[174] To provide comprehensive clinical and epidemiological information on infectious agent, serotype, sequence type and antimicrobial susceptibility, direct from sequence reads is feasible by taking a k-mer approach.[175] Although there is a high computational overhead to k-mer based analysis as datasets become large, by using an application-specific database (e.g., fish pathogens) and binning k-mers into differentially descriptive subsets,[175] a classifier based on this approach is highly feasible for fish infectious disease diagnostics. Indeed, there is an open access development release of a k-mer classifier and associated database for pathogens of aquatic organisms including tilapia available from WorldFish.[176] This is a field that is moving very rapidly and the choice of online tools that are easy and free to use is growing. The Danish Technical University provides a suite of online tools and databases through their Centre for Genomic Epidemiology portal including, for example, pathogen identity, antimicrobial resistant genes (ARG) prediction and multilocus sequence typing (MLST) direct from raw sequence data.[177,178]

Artificial Intelligence may also become applicable to Level I and Level II diagnostics through interpretation of real-time environmental and behavioural cues (level I) to alert to potential problems, although perhaps not to the level of specific disease diagnostics. Sensor arrays for water and environmental monitoring, measuring and controlling feed intake and in-tank/cage camera systems for morphometric analysis are already widely deployed throughout salmonid aquaculture for automation. Coupling to AI is therefore highly plausible to provide computer-assisted level I diagnostic alerts. For tilapia aquaculture, the costs of sensor infrastructure will need to fall substantially to enable adoption in the most important producing nations. Level II diagnostics

are already assisted by AI in human clinical medicine, particularly cancer diagnostics, where screening of histopathological samples may be aided by deep neural network-based machine learning algorithms.[179] It is also possible to combine AI with other more rapid Level II laboratory methods such as infra-red (IR) spectroscopy (30 min)[180] or flow cytometry (3h)[181] to provide same-day antimicrobial susceptibility test (AST) results and predict bacterial abundance. Indeed biomarkers from blood measured by IR-spectroscopy coupled to artificial neural networks can provide rapid non-invasive diagnosis of *Helicobacter pylori* infection in children.[182]

4.2 | High throughput diagnostic systems

For local diagnostic testing of fish farm disease outbreaks, high throughput of sample numbers is not a major factor, and high capacity instrumentation can be expensive to operate when not used at capacity. However, there is a use-case for high throughput diagnostics in pathogen surveillance for biosecurity. For example, the screening of broodstock and seedstock to certify specific pathogen free (SPF) status, or for the screening of live or uncooked seafood prior to international shipping for compliance with trade legislation to limit transboundary spread of endemic diseases. For advances in high sample number throughput for pathogen detection, we return to the SARS-CoV-2 pandemic. Here, rapid testing of hundreds of thousands of samples per day by health authorities and the private sector has informed lockdowns and tracked dissemination of new virus variants.[149,183] For aquaculture biosecurity, the need is somewhat different, in that testing fewer samples for a cohort of pathogens of concern is more important than testing high sample numbers for a single pathogen. But there are important advances made during the pandemic that can be applied equally well to fish disease diagnostics. For example, one of the major constraints (and costs) of diagnostics is in sample preparation with many recommended molecular assays stipulating particular extraction kits.[184] Recent findings indicate that for qPCR-detection of SAR-CoV-2 from clinical samples, the extraction process can be substituted for a short high temperature treatment without adversely impacting sensitivity.[184] Molecular assays lend themselves very well to high throughput as the small reaction volumes that are required facilitate use of microwell plates (e.g., 384 wells) and array type technologies. qPCR methods are standardised for many pathogens of fish and are readily multiplexed by using different fluorochromes in probe-based qPCR such as TaqMan. For tilapia, multiplexed qPCR detection of common bacterial pathogen, *Francisella* spp., *Edwardsiella* spp. and *Streptococcus* spp. was effectively used for disease surveillance on hatcheries in Costa Rica.[94] The extent to which assays can be multiplexed in this way is quite severely limited by the range of fluorochromes and the number of channels on the instrument that can detect the differing wavelength emissions. Once internal controls are accounted for, four to five pathogens per sample is the limit to which the assay can be multiplexed. This problem can be reduced by coupling the qPCR to electrospray mass spectrometry, in which the qPCR amplicons from the multiplexed primer reaction are fed to a mass spectrometer which then identifies which amplicons are present in each sample by mass, eliminating the need for fluorochrome probes.[185] This method may enable quantitative detection of 13 or 14 different pathogens per sample in a single reaction and is limited by the biochemistry of the qPCR reaction with higher numbers of multiplexed primer sets. For increased pathogen multiplexing, microarray-based chips may include thousands of genetic loci with potential to identify tens to hundreds of pathogens to variant level.[186] Such arrays are quite costly but have been used in human medicine for screening blood samples,[187] and DNA microarray genus-species 16S rRNA analysis for multiplexed detection of key pathogenic bacteria have been explored in aquaculture.[188] High throughput microarray methods for tilapia disease diagnosis are limited but may offer future perspectives to cover all key pathogens of tilapia including bacteria, viruses and parasites.

4.3 | Environmental DNA and RNA for early detection of pathogens from water

Environmental DNA and RNA (eDNA and eRNA) refer to genetic materials found in environments such as water, soil, sediment, snow or even the air. eDNA/eRNA include those within or shed and excreted from any living or dead organisms, from viruses to unicellular and multicellular organisms.[189] Sample collection for eDNA/eRNA investigation can be done once, or on a regular basis at a certain timeframe and location for continuous monitoring. Following that, the samples are treated to appropriate concentration processes (commonly filtration, centrifugation or coagulation) before DNA, RNA or total nucleic acid are extracted[190,191] (Figure 10). The obtained eDNA/eRNA is then subjected to either a metagenomic NGS (or metabarcoding) approach, in which the contribution of organism taxa can be identified simultaneously, primarily at the genus level, or a target-specific conventional or quantitative PCR for detection of species of interest[189,192,193] (Figure 10). Application of eDNA/eRNA has played an increasingly important role in both common and unusual circumstances in aquatic ecosystems and aquaculture. Monitoring eDNA, for example, can be used to look at organism diversity in the context of natural conservation or to assess the biological impact of climate change, changes in environmental parameters and anthropogenic activities (e.g., oil spill, drilling and mining).[194,195] eDNA/eRNA can be applied for disease screening to ensure free status of any pathogens of concern particularly for biosecurity in the fish/shrimp trade.[196] eDNA monitoring can help identify invasive species and assess endangered species in aquatic habitats.[197–199] Furthermore, eDNA/eRNA has been used to assess the distribution and abundances of waterborne pathogens, as well as the presence of pathogenic agents in the environment.[191,200]

The application of eDNA/eRNA for tilapia disease diagnosis is still limited, however, a straightforward approach for TiLV detection and quantification from water that employed a simple iron flocculation method for viral concentration coupled with a probe-based RT-qPCR has been described.[55] TiLV nucleic acid was detected and quantified in water collected from affected ponds/cages as well as sewage, and a reservoir. This approach might be effective for noninvasive monitoring of TiLV in aquaculture environments, and allow suitable biosecurity

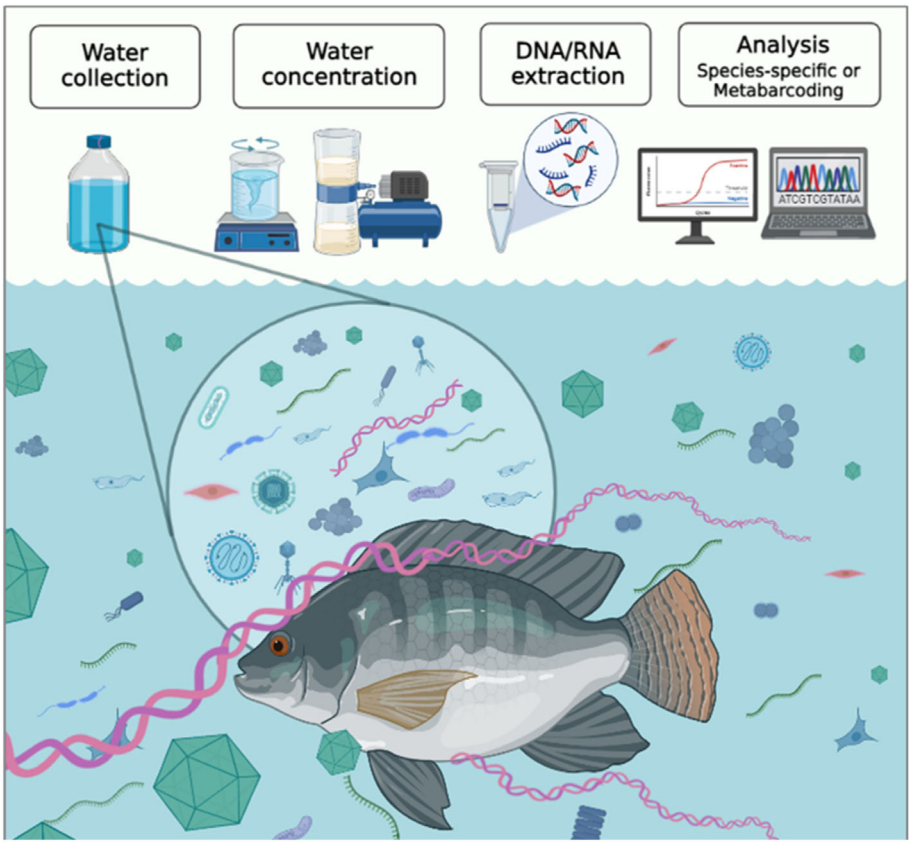

FIGURE 10 eDNA/eRNA application in tilapia disease diagnosis. Pathogen(s) collected with water samples from fish culture systems are usually concentrated prior to nucleic acid extraction. Pathogen(s) of concern can be detected by species-specific or metabarcoding approaches. (Images by S. Senapin and S. Taengphu created in BioRender.com.)

interventions.[201] Potential applications of eDNA/eRNA in disease diagnosis have also been described in other fish species and their pathogens, including the use of pathogen-specific detection approaches, metabarcoding strategies and a combination of both. For instance, the detection and quantification of red sea bream iridovirus (RSIV) in a challenge model with Japanese amberjack (*Seriola quinqueradiata*) and farmed red sea bream (*Pagrus major*) revealed high viral loads at least 5 days before fish mortality, suggesting potential application of eDNA assay for early forecast of disease.[55,202,203] Multiple target pathogens were detected using eDNA samples collected from Atlantic salmon (*Salmo salar*) farm sites to assess the potential of pathogen transmission from domesticated to wild fish populations sharing the same habitat.[204] The use of universal metabarcoding markers (e.g., mitochondrial genes, internal transcribed spacer (ITS) sequences and small-subunit ribosomal RNA gene) as potential monitoring tools for harmful parasites and microalgae in cultured fish have been described.[205] A synergistic association of bacterial microbiome and abundance of the parasitic ciliate *Chilodonella hexasticha* with mortality in barramundi (*Lates calcarifer*) has been demonstrated using a combination of metabarcoding- and targeting-based approaches.[206] eDNA assays, on the other hand, have indicated an antagonistic effect between bacterial loads and viral pathogens.[207,208] As aquaculture is an interactive complex system, environmental parameters together with host and pathogen factors should be taken into account for eDNA/eRNA data analysis and interpretation. The advancement of technology in the eDNA/eRNA methods described in other fish species can easily be used for tilapia health monitoring and disease diagnosis. Availability of curated genomic sequence databases of tilapia pathogens and other aquatic organisms characterized from healthy and diseased tilapia culturing environments will support accurate eDNA/eRNA species-level identification and interpretation of complex microbial assemblages. In the near future, more accessible and inexpensive NGS and qPCR/dPCR facilities and services will promote a rise in the use of eDNA/eRNA for early diagnoses and disease forecasting in tilapia farming systems.

4.4 | Point-of-care or pond-side testing

The term 'point-of-care testing' (POCT) describes diagnostic tests, or any other tests, that are not confined to a laboratory setting and, thus, can be conducted close to/in the direct proximity of the testing subjects, typically by people without professional training. Different circumstances may require different POCT solutions involving different testing devices or regimes. For fish farmers, POCT allows

anyone to easily and quickly perform accurate testing close to or at the pond side. POCT may also be undertaken in many locations such as fish processing plants, wet-markets or by customs biosecurity officers for monitoring and screening purposes. In summary, its relevance in aquaculture may include 'pond-side testing, 'point-of-need testing', 'remote rapid testing' or 'decentralized testing'.[209,210]

Accurate diagnostics for effective treatments are not available for many infectious diseases in tilapia, making good farm practices and prevention the best strategies for achieving optimum performance results. A rapid, accurate and reliable diagnosis allows the farmers to make immediate and informed decisions and take appropriate actions in the fastest manner possible to better manage and control diseases, especially at early stages when clinical signs may not be easily identified by the farmers.[211] However, most tilapia farms exist in relatively remote locations with limited accessibility to laboratory testing facilities. Sending clinical samples to specialized laboratories has the drawback that it usually takes a long time (days to weeks) to obtain test results. For diseases that quickly lead to high morbidity and/or mortality, having results one or two weeks after sample submission is not optimal. Therefore, POCT tools that provide quick and reliable testing results at the tilapia farm level are much needed to shorten the test turn-around time for timely decision-making.[14]

An ideal POCT should meet the 'ASSURED' guidelines (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Delivered)[212] put forward by the WHO.[213] POCT should provide test sensitivity and specificity comparable to those of laboratory testing in a short time under a wide range of conditions. The equipment, when needed, should be compact, work with a simple operating protocol, with battery and built-in calibration and provide means for data management (such as test results, date, time, sample, operator, location, quality control and device info).[214] Ideally, the reagent should be provided in single-dose and ready-to-use format and require no cold chain for shipping and storage. Concerns about POCT focus mainly on risk of poor test performance due to oversights, such as potential user errors, insufficient quality control and inadequate storage of reagents and maintenance of devices (if any). Therefore, clear instruction guides, user training, user-friendly design and usability validation are some measures known to ensure correct use of POCT.

Lateral flow immunoassay (LFIA) is the most widely commercially available POCT platform. Working with little or no supporting infrastructure, LFIA has advantages of being simple, rapid and cost-effective. These features are very useful, especially in settings with low resources, to improve decision making and turn-around time. LFIA can be used to screen for infection and antibiotic resistant markers to facilitate responsible and prudent use of antimicrobial agents.[215–217] However, to the best of our knowledge, no LFIA tests for infectious diseases of tilapia are commercially available at time of print.

Current LFIA tests are, in general, not consistently sensitive and specific enough to meet the needs of early disease detection, especially sub-clinical infections.[218] Therefore, tests based on molecular technologies are considered more reliable with greater sensitivity for this purpose. Laboratory molecular technologies mentioned in the previous sections, such as PCR, LAMP, RPA, CRISPR and the Nanopore MinION sequencing platform, have all been automated into single use commercial POCT assays prepacked with required reagents for diagnosis of COVID-19 infection.[219] The automated steps include sample preparation, nucleic acid extraction, amplification, signal detection, recording and processing and result interpretation and presentation. Besides being able to improve test specificity with its ability for strain identification with single-nucleotide specificity through CRISPR base-pair matching, CRISPR-based diagnostics for pathogen detection also hold great promise in facilitating equipment-free diagnostics to allow POCT to be easily accessible to more users.[220,221] However, the majority of available CRISPR-based platforms require an amplification step to enhance sensitivity, significantly lengthening test turn-around time.[222] Although a number of PCR, LAMP and RPA assays have been reported for detection of TiLV, ISKNV, *S. agalactiae*, *S. iniae*, *L. garvieae* and *F. columnare*,[67,105,107,223] to the best of our knowledge, only one POCT RT-PCR test that works on the compact POCKIT platform (GeneReach, USA) is commercially available for TiLV detection in tilapia. Designed to work with the fluorescence-based insulated isothermal PCR (iiPCR) technology,[224,225] this compact platform provides fast binary (positive/negative) results. Based on this platform, semi-automated (POCKIT Combo) and fully automated (POCKIT Central) systems are available for pond-side PCR testing at different settings. The semi-automated POCT system generates results within two hours with a protocol requiring minimal manual steps; one nucleic acid extract can be used flexibly for simultaneous PCR testing of different pathogens. The sample-in-answer-out POCT system, on the other hand, fully automates the nucleic acid extraction and iiPCR steps and works with preloaded single-use cartridges to provide results within 90 min, meeting particularly the needs of settings with limited human resources.

The TiLV POCT RT-iiPCR assay is available in a lyophilized format for easy shipping and storage. On the fully automated POCKIT Central system, LoD 95% (limit of detection) of the POCT assay was determined to be 12 genome equivalents. The POCT assay was comparable to a reference semi-nested RT-PCR assay[47] in analytical and clinical performance. The two RT-PCR assays have similar analytical sensitivity as their detection end points were within one log in a test using a serial dilution of a TiLV-positive sample. A study testing 92 tilapia liver, brain, gill, muscle or mixed samples showed that diagnostic performance of the two assays was also comparable. Positive percentage agreement and negative percentage agreement were 94.44% (95% CI, 78.72%–100%) and 95.95% (95% CI, 90.4%–100%), respectively (GeneReach Biotechnology Corporation data).

Development of commercial LFIA tests for the tilapia industry may consider incorporating dedicated LFI readers and alternative detection methods (fluorescence, chemiluminescence, electrochemical signals, surface-enhanced Raman spectroscopy).[216,226] These technologies have potential to improve test sensitivity and enable

quantitative testing. The use of readers also makes data digitalization, tracking, storage and transmission possible.

The functions of POCT are being improved to enhance its usability for different applications at various point-of-care settings. First, the ability of multiplexing is favourable to improve testing efficiency of POCT.[227,228] Second, integration of easy, or no, sample preparation enhances user-friendliness of POCT.[229] Thirdly, miniaturized integrated devices are being developed to enhance test portability and user-friendliness.[230] In the last decade, huge progress has been made in microfluidics and microfabrication technologies that enable automated pipetting, mixing, separation and amplification in a single miniaturized device, with significant reduction in sample and reagent volume, test turn-around time, energy consumption and waste production.[230] Fourthly, improving connectivity of POCT to allow integration of accurate outbreak reporting systems via a mobile app or computer connections, can help with timely and accurate reporting of outbreaks to competent authorities.[214] Moreover, cloud-based reporting and artificial intelligence (AI) have potential to further bridge what scientists and aquatic health professionals can offer to meet the needs of tilapia farmers at remote locations.[231] The momentum accumulated in the last decade in amplification, multiplexing, microfluidics and data connectivity technologies, could be integrated realistically and in different ways to build cost-effective POCT for the tilapia aquaculture industry in the near future.

Continuous research and enhancement of POCT with the goal of providing end-users with better and simpler access to biodetection techniques will assist farmers in disease management and control enhancing future tilapia productivity. Currently available techniques are not widely used in aquaculture settings, owing mostly to their relatively expensive prices, thus, efforts are also required to reduce the costs of POCT.

5 | CONCLUSION

In aquaculture, diagnostic techniques are constantly evolving and becoming more complex. The level I-III approach established over 20 years ago highlights the importance of the diagnostic continuum as a quality control mechanism, especially for exotic or previously unreported mortality events. They remain meaningful in light of diagnostic technology advances and increasing recognition of the role of the aquatic environment on both host physiology and pathogen virulence. Accurate diagnosis of a disease can rarely be achieved by a single test. A presumptive diagnosis, indicating a strong likelihood of disease identification, is usually made with multiple tests to be considered for confirmatory diagnosis (100% certainty of identification of the causative pathogen). In order to reduce the risk of misdiagnosis, inclusion of three levels of diagnostic observations and use of a matrix of results gives the most solid foundation possible for accurate diagnosis. This is essential for effective risk assessments at the farm, regional, national and international levels of aquaculture production, as well as for effective disease response and control. Accurate diagnosis forms the basis for determining what the disease condition is, the severity and cause(s) of the condition. Inaccurate diagnoses can lead to ineffective or inappropriate control measures, delay treatment and may cause severe economic loss. The choice of diagnostic technique should follow the principles of being 'fit-for-use, fit-for-purpose' with defined sensitivity and specificity and cost-effectiveness within the pathogen–host–aquatic environmental interaction framework. Diagnostic challenges to detect 'unknowns' and 'emerging diseases' will persist, however, our increasing molecular databases and analytical tools should enhance our capability to detect and identify these new pathogenic agents more rapidly and accurately in the future compared with the present.

The intrinsic qualities of tilapia, as well as its biology, farming needs and nutritional values, give it the inherent potential to become one of the world's most important future food fish groups. The inter-relationship of human, animal and environmental health enshrined in the One Health philosophy, that is beginning to underpin global health policy, means that the future of tilapia aquaculture must centre on sustainable health management and biosecurity. There has been a rapid proliferation in the development of novel diagnostic methods, with many technical challenges having been overcome. The major hurdle that faces the adoption of such powerful aids to diagnosis is likely to be the rigorous validation required for them to be accepted for transboundary animal movement and product entry into supply chains. We recognize the potential for misapplication of new technologies in aquaculture disease diagnostics, including tilapia, in the absence of other diagnostic information and we emphasize the importance of three continuous levels of disease diagnostics that incorporate fundamental (Level I and II) and advanced (Level III) approaches to optimize the diagnostic data value. It is likely that LAMP, and NGS methods for tilapia pathogens will be validated and join WOAH standard diagnostic tests, such as qPCR, in the near future. We also expect to see incorporation of artificial intelligence, machine learning, high throughput diagnostic systems and POCT into diagnostic workflows in the relatively near future. Non-invasive sampling using eDNA, in conjunction with highly sensitive diagnostic technologies such as qPCR and dPCR for early pathogen detection and disease forecast, should also be incorporated in the coming years. Regulatory and socio-economic hurdles aside, the technology for fast, easy, accurate and farmer-accessible diagnostic tools for future sustainable aquatic food is already here.

AUTHOR CONTRIBUTIONS

Ha Thanh Dong: conceptualization; investigation; methodology; writing – original draft; writing – review and editing. **Thawatchai Charijarasphong:** Formal analysis; investigation; methodology; visualization; writing – original draft. **Andrew Barnes:** Investigation; methodology; visualization; writing – original draft; writing – review and editing. **Jerome Delamare-Deboutteville:** Investigation; methodology; visualization; writing – original draft; writing – review and editing. **Peiyu Alison Lee:** Investigation; methodology; writing – original draft. **Saengchan Senapin:** Investigation; methodology; visualization; writing – original draft. **Kathy F. J. Tang:** Conceptualization;

investigation; methodology; writing – original draft; writing – review and editing. **Sharon E. McGladdery:** Investigation; methodology; writing – original draft; writing – review and editing. **C.V. Mohan:** Investigation; methodology; writing – original draft. **Melba G. Bondad Reantaso:** Conceptualization; investigation; methodology; writing – original draft; writing – review and editing, resources.

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CONFLICT OF INTEREST

The views expressed in this publication are those of the author(s) and do not necessarily reflect the views or policies of the Food and Agriculture Organization of the United Nations.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created in this review.

ORCID

Ha Thanh Dong https://orcid.org/0000-0002-7190-757X
Thawatchai Chaijarasphong https://orcid.org/0000-0003-1302-0863
Andrew C. Barnes https://orcid.org/0000-0002-3990-8070
Jérôme Delamare-Debouteville https://orcid.org/0000-0003-4169-2456
Peiyu Alison Lee https://orcid.org/0000-0002-2551-6615
Saengchan Senapin https://orcid.org/0000-0002-5599-4343
Chadag Vishnumurthy Mohan https://orcid.org/0000-0002-2574-284X
Kathy F. J. Tang https://orcid.org/0000-0003-1513-3239
Sharon E. McGladdery https://orcid.org/0000-0003-2846-4141
Melba G. Bondad-Reantaso https://orcid.org/0000-0002-2380-3549

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