ARTICLES FOR FACULTY MEMBERS

ADVANCED TECHNOLOGIES FOR SUSTAINABLE AQUACULTURE

Title/Author	**Advances in nanotechnology for sustainable aquaculture and fisheries / Shah, B.R. and Mraz, J.**
Source	***Reviews in Aquaculture*** **Volume 12 Issue 2 (May 2020), Pages 925-942** **https://doi.org/10.1111/raq.12356** **(Database: Wiley Online Library)**

Title/Author	**Applications of genotyping by sequencing in aquaculture breeding and genetics / Robledo, D., Palaiokostas, C., Bargelloni, L., Martínez, P. and Houston, R.**
Source	***Reviews in Aquaculture*** **Volume 10 Issue 3 (Aug 2018) Pages 670-682** **https://doi.org/10.1111/raq.12193** **(Database: Wiley Online Library)**

Title/Author	**Aquaculture industry prospective from gut microbiome of fish and shellfish: An overview / Diwan, A. D., Harke, S. N., Gopalkrishna, & Panche, A. N.**
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ADVANCED TECHNOLOGIES FOR SUSTAINABLE AQUACULTURE

Title/Author	**Boosting Immune Function and Disease Bio-Control Through Environment-Friendly and Sustainable Approaches in Finfish Aquaculture: Herbal Therapy Scenarios / Seyed Hossein Hoseinifar, Yun-Zhang Sun, Zhigzhang Zhou, Hien Van Doan, Simon J. Davies & R. Harikrishnan**
Source	***Reviews in Fisheries Science & Aquaculture*** **Volume 28 No. 3 (March 2020) Pages 303-321** **https://doi.org/10.1080/23308249.2020.1731420** **(Database: Taylor & Francis Online)**

Title/Author	**Environmental impacts and imperative technologies towards sustainable treatment of aquaculture wastewater: A review / Abdul Latif Ahmad, Jing Yi Chin, Mohd Hazarel Zairy Mohd Harun, Siew Chun Low**
Source	***Journal of Water Process Engineering*** **Volume 46, April 2022, 102553,** **https://doi.org/10.1016/j.jwpe.2021.102553** **(Database: ScienceDirect)**

Title/Author	**Harnessing genomics to fast-track genetic improvement in aquaculture / Houston, R.D., Bean, T.P., Macqueen, D.J. et al.**
Source	***Nature Reviews Genetics*** **Volume 21 (July 2020) Pages 389–409** **https://doi.org/10.1038/s41576-020-0227-y** **(Database: Nature Reviews Genetics)**

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Title/Author	**Progress in valorisation of agriculture, aquaculture and shellfish biomass into biochemicals and biomaterials towards sustainable bioeconomy / Wan Mahari, W. A., Waiho, K., Fazhan, H., Necibi, M. C., Hafsa, J., Mrid, R. ben, Fal, S., el Arroussi, H., Peng, W., Tabatabaei, M., Aghbashlo, M., Almomani, F., Lam, S. S., & Sillanpää, M.**
Source	***Chemosphere*** **Volume 291 Part 2 (March 2022), 133036** **https://doi.org/10.1016/J.CHEMOSPHERE.2021.133036** **(Database: ScienceDirect)**

Title/Author	**Subtopic: Advances in water and wastewater treatment harvesting of Chlorella sp. microalgae using Aspergillus niger as bio-flocculant for aquaculture wastewater treatment / Mohd Nasir, N., Mohd Yunos, F. H., Wan Jusoh, H. H., Mohammad, A., Lam, S. S., & Jusoh, A.**
Source	***Journal of Environmental Management*** **Volume 249 (Nov 2019) 109373** **https://doi.org/10.1016/J.JENVMAN.2019.109373** **(Database: ScienceDirect)**

Title/Author	**The role of the gut microbiome in sustainable teleost aquaculture / Perry, W. B., Lindsay, E., Payne, C. J., Brodie, C., & Kazlauskaite, R.**
Source	***Proceedings of the Royal Society B: Biological Sciences*** **Volume 287 Issue 1926 (May 2020)** **https://doi.org/10.1098/RSPB.2020.0184** **(Database: Royal Society Publishing)**

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Advances in nanotechnology for sustainable aquaculture and fisheries

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Abstract

In recent years, aquaculture is considered a fastest-blooming global food industry, playing a crucial role in fulfilling the increased demand for animal protein requirements. However, disease prevalence, chemical contamination, environmental degradation and ineffective feed utilization are the factors that drastically hinder the outcome of this sector in aiding to achieve global food security. In this regard, new avenues have been paved in science and technology to cope with these challenges in aquaculture. Among these, nanotechnology has emerged a tremendous potential to improve aquaculture with novel nanotools. This review critically analyses the advances in the application of nanoparticles and emulsion-based systems to fish disease prevention, water purification and delivery of nutrients. On the other hand, as the use of antibiotics and other chemical antimicrobial agents, synthetic compounds as growth enhancers not only leads to aquaculture pollution but also consumer's reluctance. Therefore, the importance of ecofriendly, non-toxic natural strategies to promote sustainable aquaculture has also been highlighted.

Key words: nanotechnology, environment, toxicity, aquaculture, bioactive compounds, sustainability.

Introduction

With increase in world population and rapid economic growth, the demands for protein are on rise. Due to positive health effects and important food features of composition, aquatic protein resources are highly appreciated and therefore, global aquaculture has grown at impressive rate recently. Currently, aquaculture accounts for 50 percent of the world's fish that is used for food. Global fish production peaked at about 171 million tons in 2016, with the total first sale value of fisheries and aquaculture production estimated at US$ 362 billion. In per capita terms, food fish consumption grew from 9.0 kg in 1961 to 20.2 kg in 2015, at an average rate of about 1.5 percent annually and was estimated for further growth of about 20.3 and 20.5 kg, for 2016 and 2017 respectively (FAO, 2018). Nevertheless, along with development, the industry is still under uncertainty in terms of putting a question mark on its sustainability where the effect of ever-increasing aquaculture waste has bad impact both on productivity inside aquaculture systems and on the ambient aquatic ecosystem. There is a huge gap to be filled in technical innovation for the drug use, disease treatment, water quality management, production of tailored fish for suiting better health, productivity drive by epigenetic and nutrigenomic interaction, better breeding success by efficient delivery of maturation and spawning inducing agent, nutraceutical delivery for rapid growth promotion and culture time reduction, successful use of auto-transgenics and effective vaccine in this area. (Aklakur *et al.*, 2016). To overcome these challenges, a combined approach of understanding, integrating and deploying new strategies in science and technology in maintaining a desirable aquaculture is indispensable. At this point, the aquaculture sector undergoes new scientific and technological innovations to produce more qualified end products. Among the recent advancements in science, nanotechnology is fast emerging as the new science and technology platform for the next generation of development and transformation of agri-food systems (Kumari *et al.*, 2014; Rodrigues *et al.*, 2017).

Although the U.S. National Nanotechnology Initiative (NNI), defines nanotechnology as; 'understanding and

control of matter at dimensions of roughly 1–100 nm where unique phenomena enable novel applications'. More elaborately it may be defined as 'the study, design, creation, synthesis, manipulation and application of functional material devices and systems through control of matter at the nanometre scale (1-100 nanometres, one nanometre being equal to 1×10^{-9} of a meter) that is at the atomic and molecular levels, and the exploitation of novel phenomena and properties of matter at that scale'. Nevertheless, herein, it should be noted that there is no specific and comprehensive definition of nanomaterials and so far a number of definitions have been proposed by government, industry and standards organizations (Nature Nanotechnology Release, 2019). The definition of 'nano' which is based on size is still under debate. Because the aforementioned definition of nanomaterials may lead to misinterpretation as in particulate form, these particles may be present either as single particles or as agglomerates or aggregates having external dimensions well beyond 100 nm and may not be considered as nanomaterials. But these agglomerates and/or aggregates yet retain specific physicochemical properties of the nanomaterials. And hence in addition to size other elements, for example agglomerates and aggregates, distributional thresholds, novel properties, solubility and so on are also worth consideration (Boverhof *et al.,* 2015).

The so-called nanoparticles (NPs) are being used in different forms and shapes such as nanospheres (Donbrow, 1991), nanocapsule (Torchilin, 2006), carbon nanotubes (Reilly, 2007), dendrimers (Aulenta *et al.,* 2003; Gillies & Frechet 2005; Wu *et al.,* 2015) and so on and have been reported with many advantages, for example dose reduction, tissue-specific targeting, reduction in the toxic or secondary adverse effects, increase bioavailability and efficacy of the drug. (Toyokawa *et al.,* 2008; Xu *et al.,* 2018a).

So far, a variety of materials have been successfully used for the fabrication of these NPs; however, polymeric NPs have been extensively investigated as drug delivery carriers because of their multiple advantages, such as the ability of protecting of drug from degradation, improving the efficiency of drug utilization and controlling the drug release rate (Fan *et al.,* 2012). For example, chitosan (CS), a natural biodegradable, biocompatible and non-toxic biopolymer extracted from the shells of crustaceans has been extensively examined for medical and pharmaceutical applications especially in artificial organs, targeted drug delivery, drug transport, protein delivery gene transfer and so on (Sakai *et al.,* 2002; Lavertu *et al.,* 2006; Wei *et al.,* 2007).

In aquaculture, nanotechnology has a broad spectrum of applications from the sterilization of ponds, water treatment, detection and control of aquatic diseases, efficient delivery of nutrients and drugs (including hormones and vaccines) to the enhancement of fish potential in absorbing these substances (Bhattacharyya *et al.,* 2015; Huang *et al.,* 2015). To date, an ample evidence of published literature is available presenting a comprehensive overview of the applications of nanotechnology in aquaculture (Fig. 1) (Ji *et al.,* 2015; Bina *et al.,* 2016; Luis *et al.,* 2017; Masoomi Dezfooli *et al.,* 2018). However, contrary to the usefulness of this technology, there is likelihood that it itself may contribute to the pollution of aquaculture that is mostly either unknown or ignored so far (Mehboob *et al.*, 2014; Huang *et al.,* 2015). Furthermore, the use of excessive antibiotics for treating various diseases and other synthetic compounds as growth promoters exert adverse effects on the aquatic ecosystem (Awad & Awaad 2017; Baldissera *et al.,* 2018). These practices collectively give rise to alarming situations including growth and reproduction impairment, mortality and biochemical changes in both adult fish and embryos which can ultimately lead to huge economical losses in fishery (Khan *et al.,* 2015). On the other hand, most importantly, they raise potential concerns about safety to human health as well as the environment (Seaton *et al.,* 2009; Purohit *et al.,* 2017). Hence, this discussion implies that there are still gaps in terms of new methods of encapsulation in nanotechnology, safer and ecofriendly especially for lipophilic bioactive compounds which can be used as natural remedies instead of artificial. And therefore, the current article describes the advances in nanotechnology in general and these novel prospective avenues which are proposed to have a great influence on aquaculture and fisheries in particular (Table 1).

Current nanotechnology in aquaculture

Delivery of vaccines

The use of vaccines has been crucial in aquaculture as a defence mechanism against pathogens to protect the host from the infections by these pathogens. The most reliable and effective ways of vaccination in fisheries is either oral administration or by injection. The latter, a traditional adjuvant practice, requires vaccines to be prepared with oil/water formulations that results in many adverse effects. Generally, this kind of formulations along with the administration procedures may occasionally lead to the mortality of the fish (Ji *et al.,* 2015). To overcome these problems, the scientific community in recent years proposed nano-delivery system as an alternative strategy for vaccines delivery in fish that is regarded not only safer but also to enhance the efficacy. In this context, to date different encapsulation techniques have been developed and tried. Among these, alginate particles were regarded as the preliminary candidates for oral delivery of vaccines to aquatic animals (Joosten *et al.,* 1997). Alginate is a copolymer of β-D-mannuronic acid (M) and α-L-guluronic acid (G) that is found in different species of brown algae or as polysaccharide in some bacteria. It has been known for its mechanical

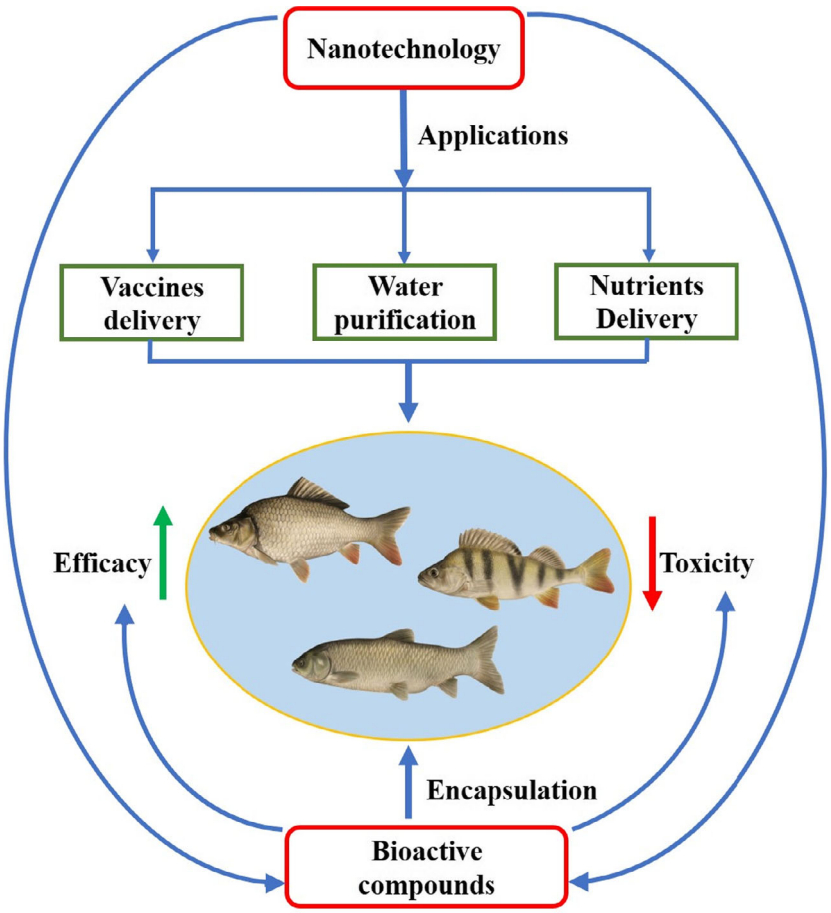

Figure 1 Schematic representation of nanotechnology applications in fishery.

and physical stability as well as mucoadhesive properties allowing its contact with the walls of epithelial cells, thus making it very attractive for oral administration (Gombotz *et al.*, 1998; Sosnik, 2014). For application in fish, alginate particles are generally produced by emulsification (Leal *et al.*, 2010; Ana *et al.*, 2010) that is one of the fastest methods for NP preparation and is readily scalable (Reis *et al.*, 2017), and to a lesser extent by other methodologies such as the orifice-ionic gelation and the spray method (BI, 2010). Reports from different researchers presented alginate as an antigen adjuvant (Tafaghodi *et al.*, 2007; Borges *et al.*, 2008), survival and weight promoter of fish (Fujiki *et al.*, 1994; Chiu *et al.*, 2008). Furthermore, alginate administration has also shown enhanced immune-stimulant response of carp (*Cyprinus carpio* L.) and the brown-marbled grouper (*Epinephelus fuscoguttatus*, (Cheng *et al.*, 2008; Huttenhuis *et al.*, 2006; Yeh *et al.*, 2008), as well as enhanced defence of the turbot (*Scophthalmus maximus* L.) against *V. anguillarum* (Skjermo & Bergh 2004), and the orange-spotted grouper (*Epinephelus coioides*) and brown-marbled grouper against iridovirus and *Streptococcus* sp. (Cheng *et al.*, 2008; Yeh *et al.*, 2008). Chitosan (CS) generally found in the exoskeleton of crustaceans and insects is considered as naturally occurring second most abundant biopolymer. Due to its inimitable biological nature, that is bioadhesive, biodegradable, biocompatible and non-toxic, CS-based formulations are predominantly used as drug carrier vehicles, bio-nanosensor, edible coatings as well as in different medical disciplines (dentistry, surgical procedures etc.) (Dutta *et al.*, 2004; Sogias *et al.*, 2008). To date, a line of literature is available highlighting the beneficial effects of CS in fisheries. In this context, Meshkini and colleagues described that CS-supplemented diet (0.25 CS kg^{-1}) boosted up the resistance of rainbow trout against environmental stress and immunological parameters thereby resulting in increased count of lymphocytes, and decreased counts of neutrophils and eosinophils (Meshkini *et al.*, 2012). On the other hand, keeping in view these characteristics, CS has been used as a carrier for different kinds of DNA and vaccines in fish through different routes of administration (orally or injection). For example, *Vibrio anguillarum* in Asian sea bass (*Lates calcarifer*) (Kumar

Table 1 Nanoparticle-based systems for removal of contaminants from water

System	Target contaminants	References
AgNP-Coated Polyurethane Foam	*E. coli*	Jain and Pradeep (2005)
$AgCoFe_2O_4$–GO nanocomposite	Pb(II), *E. coli*, *S. aureus*	Ma *et al.* (2015)
AgNPs impregnated Ceramic water filters	*C. parvum*	Abebe *et al.* (2015)
CSNPs, AgNPs and ZnNPs	*E. faecalis*	Motshekga *et al.* (2015)
Fe_2O_3 NPs impregnated ultrafiltration mixed matrix membrane	*E. coli*	Mukherjee and De (2015)
Ag/rGO hydrogel	*E. coli*	Zeng *et al.* (2015)
Polyelectrolytes/AgNPs self-assembled thin films	*E. coli*	Zarpelon *et al.* (2016)
AgNPs or CuNPs	Total coliforms and *E. coli*	Dankovich *et al.* (2016)
IAO/GO	F^{-1}	Liu *et al.* (2016)
3-D RGO hydrogel	Hg and F^{-1}	Wu *et al.* (2016)
GO-based magnetic nano-sorbent	Pb (II)	Ravishankar *et al.* (2016)
NCC	NO^{-3}	Azadbakht *et al.* (2016)
FeOOH-GO nanocomposites	F^{-1}	Kuang *et al.* (2017)
CMGO/nHA	AM	Kuang *et al.* (2017)
TiO_2 and TiO_2–SiO_2	F^{-1}	Zeng *et al.* (2017)

Ag, Silver; rGO, Reduced Graphene Oxide; NPs, Nanoparticles; *E. Coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *E. faecalis*, *Enterococcus faecalis*; Fe_2O_3, iron oxide; CS, Chitosan; Zn, Zinc; Cu, Copper; *C. parvum*, *Cryptosporidium parvum*; Pb, Lead; Co, Cobalt; F^{-1}, Fluoride; CM, Chemically modified; nHA, nanohydroxy apatite; AM, Aureomycine hydrochloride; IAO, iron–aluminium oxide; TiO_2, Titanium dioxide; SiO_2, Silicon dioxide; 3D, Three-dimensional; NCC, Nanocrystaline cellulose.

et al., 2008), major capsid protein (MCP) gene of *Lymphocystis Disease Virus (LCDV)* in Japanese flounder *(Paralichythys olivaceus)* (Tian *et al.*, 2008), *Philasterides dicentrarchi* in *Scophthalmus maximus* (León-Rodríguez *et al.*, 2013), *Vibrio parahaemolyticus* in *Acanthopagrus schlegelii* (Li *et al.*, 2013), dietary RNA in *Labeo rohita* (Ferosekhan *et al.*, 2014) have been successfully encapsulated and delivered in CS-based systems. Similarly, PLGA, Poly (D,L-lactic-co-glycolic acid), another biodegradable polymer that has been extensively used for encapsulation and delivery of different compounds in fish. As the name indicates, this polymer is produced from two monomers, that is lactic and glycolic acid in different shape and sizes. Behra et al., used PLGA for the delivery of *Aeromonas hydrophila* in rohu, and found significant immune-stimulatory and antibody response in these fishes compared to the control group (Behera *et al.*, 2010). Similar results were obtained by another research group in Japanese flounder, where DNA vaccine encapsulated in PLGA showed enhanced inducing effects on immunological parameters against lymphocystis (Tian & Yu 2011). Another formulation 'liposomes' which are composed of phospholipids have been extensively used in various research disciplines focusing on fish farming. In carp (*Cyprinus carpio*), liposomes-encapsulated *Aeromonas salmonicida* antigen showed improved survival rate (83%) and skin ulcers compared to the control group (Irie *et al.*, 2005). Furthermore, liposomes-loaded *A. hydrophila* antigens significantly elevated antibody counts in serum thereby boosted up the immunity of common carp (*C. carpio)* as demonstrated by improved protection against live *A. hydrophila* (Yasumoto *et al.*, 2006).

Water purification

Water treatment is one of the most important pillars required for sustainable aquaculture. In recent years, water contamination is regarded as the foremost health hazard globally which is continuously growing due to disposal of wastes materials from cities, industries, agriculture as well as abuse of antibiotics and other synthetic compounds in fisheries. The deterioration of waters in this way, not only affect human health directly by diminishing the resources of clean groundwater but also indirectly by affecting the aquatic animals–the consumption of which can lead to different kinds of food-borne illnesses. Apart from this, the fishery industry faces a huge economical loss caused by microorganisms and heavy metals in these waters leading to growth retardation and death of the fish. In aquaculture, nanotechnology has core applications for water treatment to provide favourable and safe habitat for fish breeding. In this perspective, scientific community endorses adsorption and photocatalysis as the most efficient and affordable approaches to purify water (Table 2). Also, Figure 2 illustrates the proposed mechanism how different NP-based photocatalytic adsorbents and hydrogel biofilms work practically in water purification herein with example of fluoride (F^-), nitrate (NO_3^-) and coliforms (*E. Coli*) removal from contaminated water.

Our previous research group developed magnetic konjac glucomannan (KGM) aerogels to decontaminate water from arsenite. The designed system was found to have pH-dependent capacity with green step characteristics (Ye *et al.*, 2016). However, in last few years, graphene oxide

Table 2 Potential application of curcumin in fish health

Experimental fish	Fish weight (g)	Course of treatment	Curcumin (Dose and Route)	Effect of Curcumin on physical and health status compared to control	References
Rohu, *Labeo Rohita*	10 ± 2	60 days	0.1, 0.5, 1.0 and 5.0 g kg^{-1} - orally supplemented feed	Significantly improved lysozyme activity, superoxide anion production and serum bactericidal activity.Enhanced protection against *Aeromonas hydrophila*	Sahu *et al.* (2008)
Anabas testudineus (Bloch)	40 ± 5	60 Days	0.5% - orally supplemented feed	A significant protective effect on fish lipid peroxidation.Improve in disease resistance, growth and survival rates in A. testudineus (Bloch)	Manju *et al.* (2008)
Carp, *Cirrhinus mrigala*	45 ± 5	30 days	100, 200, 300, 400, 500, 600 and 700 ppm (mg L^{-1}) -intramuscularly using 1 mL tuberculin syringe with a 24-G needle	Significantly enhanced the serum lysozyme activity (Ly), production of reactive oxygen species and reactive nitrogen species (RNS or NO) by peripheral blood leucocytes	Harikrishnan *et al.* (2009)
Labeo Rohita	30-40	42 days	1.5 mg, 150, 15 & 1.5 μg - intraperitoneal injection	Significantly increased some non-specific immune parameters such as respiratory burst, myeloperoxidase, haemagglutination, haemolytic and bacterial agglutination without any side effects at low doses.	Behera *et al.* (2011)
Carp (*C. carpio*)	54.39 ± 3.11	4 days	50 mg kg^{-1}- single intraperitoneal injection	Enhanced activity against the oxidative effects of Cadmium (Cd)A significant role in lowering the tissue contents of Cd	Sevgiler *et al.* (2011)
Anabas testudineus (Bloch)	40 ± 5	14 & 56 days	0.5 & 1% - orally supplemented feed	Improved antioxidant status and protein content facilitation growth of the fish.Decreased lipid peroxidation product.Significant liver proactive effects.	Manju *et al.* (2012)
Anabas testudineus (Bloch)	40 ± 5	180 days	0.5 & 1% - orally supplemented feed	Increased haemoglobin content, RBC count and haematocrit in the fish.Improved over all health status of the fish	Manju *et al.* (2013)
Common carp (*Cyprinus carpio carpio* L.)	54.39 ± 6 3.11	4 days	50 mg kg^{-1}- single intraperitoneal injection	Significantly lowered liver thiobarbituric acid reactive substances - (TBARS) levels.Lowered Cd concentration in the muscle of the Carp	Karaytug *et al.* (2014)
Jian carp	30 ± 1.0	60 days	0.1%, 0.5%, or 1.0% - orally supplemented feed	Significantly reduced CCl4-induced liver damage in Jian carp by upregulating hepatocyte antioxidative capacity and inhibiting NF-*k*B, IL-1β, TNF-α, and IL-12 expression.	Cao *et al.* (2015)
Nile tilapia	45 ± 5	30 days	2% - supplemented diet	Enhanced non-specific immune defence mechanisms of Nile tilapia against *Vibrio alginolyticus*	Elgendy *et al.* (2016)
Oreochromis mossambicus (Mozambique tilapia)	9.45 ± 0.63	35 days	0.5 & 1% - As food additive	Improved the activities of digestive enzymes.Modulates the expression of GH in brain and growth factorssuch as IGF-1 and IGF-2 in muscle of O. mossambicus	Midhun *et al.* (2016)
Crucian carp	76.3 ± 0.10	105 days	1 & 5 g kg^{-1} - orally supplemented diet	Significantly improved body weight (FW), percent weight gain (PWG), and feed efficiency (FE)Increased intestinal antioxidant capacity, digestive and absorptive ability, and promoted fish growth.	Jiang *et al.* (2016)
Oreochromis niloticus	12.91–12.94	56 days	2, 4 & 8 g kg^{-1} - orally supplemented diet	Exerted immunomodulatory effect through manipulation of lymphocyte count, IL- 2, IL-4 and antibacterial enzymatic activity (NO and lysozyme).Improved weight gain.	Abdelrazek *et al.* (2017)

Table 2 (continued)

Experimental fish	Fish weight (g)	Course of treatment	Curcumin (Dose and Route)	Effect of Curcumin on physical and health status compared to control	References
Tambaqui	2.06 ± 0.18	4 days	200, 300 or 500 μL L^{-1} - in solution in oil form diluted in ethanol	Essential oils of curcumin longa (EOCL) are recommended for anaesthesia and sedation of fish because in spite of inducing anaerobic metabolism, these EOs did not alter most biochemical parameters, reduced the lipid peroxidation LPO and increased the antioxidant capacity in vital tissues.	Saccol *et al.* (2017)
Channa punctatus	40-50	4 days	1, 2 and 3 mg L^{-1}- along with Chromium (Cr) in solution	Increased frequency of micronuclei induction in peripheral erythrocytes.Exerted significant antigenotoxic effect against chromium in time and dose dependent manner.	Prasad *et al.* (2017)
Tilapia	2.55 ± 0.003	84 days	50, 100, 150 or 200 mg kg^{-1} - orally supplemented diet	Improved growth performance, feed utilization, oxidative status, immune responses, and disease resistance in Tilapia.	Mahmoud *et al.* (2017)
Cirrhinus mrigala	10.5 ± 1.4	45 days	0.25, 0.5, 1, 1.5 and 2% - orally supplemented diet	Improved growth performance and increased disease resistance against *Edwardsiella tarda* infection in C. mrigala.	Leya *et al.* (2017)
Catfish	≈ 40	60 days	0.5 & 1% - orally supplemented diet	Enhanced performance of catfish and increase their disease resistance in reducing use of antimicrobials in fish farmin	Hafiz *et al.* (2017)
Silver catfish	205.55 ± 18.93	14 days	150 g kg^{-1} – orally supplemented diet	Exerted potent bactericidal action against *Streptococcus agalactiae*, presenting 100% of therapeutic efficacy.The occurrence of clinical signs of disease, as erratic swimming, corneal opacity, skin lesions in the fin and tail, and loss of appetite were prevented	Baldissera *et al.* (2018)
Oreochromis niloticus (Nile tilapia)	40 ± 0.2	14 days	10 & 20 g kg^{-1} - orally supplemented diet	Improved hepatic lesions in aflatoxin B infected fish.Significantly improved hepatosomatic index (HIS) values	Manal (2018)
Common cap fingerlings	4.82 ± 0.41	60 days	0.75 & 1.5 g kg^{-1} - orally supplemented diet	Significantly mitigated the toxicity of silver nanoparticles AgNPs.Showed enhanced protection of intestinal microflora against feed-born nanosilver particles.Showed high sensitivity to mesophilic and lactic acid bacteria.	Khorshidi *et al.* (2018)

(GO) and graphene nanosheets (GNs) attracted tremendous attention globally for its significant role in removing various kinds of contaminants from water (Motamedi *et al.*, 2014; Liu *et al.*,2016; Kuang *et al.*, 2017). The fabrication of hybrid GO-TiO_2 for various environmental and energy application such as adsorption, evacuating heavy metal ions and organic dyes from waste water has been particularly focused (Hu *et al.*, 2013; Atchudan *et al.*, 2017). Being non-toxic, chemically and biologically stable, low cost and efficient photocatalyst make TiO_2 as a potential candidate for wastewater treatment. Many studies related to the photocatalytic activities of TiO_2 for the inactivation of pathogenic organisms such as bacteria, viruses and algae have been documented (Hu *et al.*, 2006; Park *et al.*, 2013; Ouyang *et al.*, 2016). Thanks to scientific community for being concerned with this issue of water treatment and removal of these contaminants from water. Because if left untreated, their higher concentrations can exert adverse even life-threatening effects on human health by accumulating in the tissues of aquatic animals, particularly in fish which are at the end of aquatic food chain and the consumption of which is highly recommended to cope with cardiovascular diseases (CVDs) and cancer (Sioen *et al.*, 2007). Owing to feeding and residing in aquatic environment, fish are principally the most susceptible and most exposed aquatic animals that have no escape from the detrimental influences of these pollutants (Mahboob *et al.*, 2014; Saleh *et al.*, 2014). For example, there are some reports stating higher degrees of heavy metal (Hg, Cd and Pb) accumulation in the tissues of marine aquatic animals

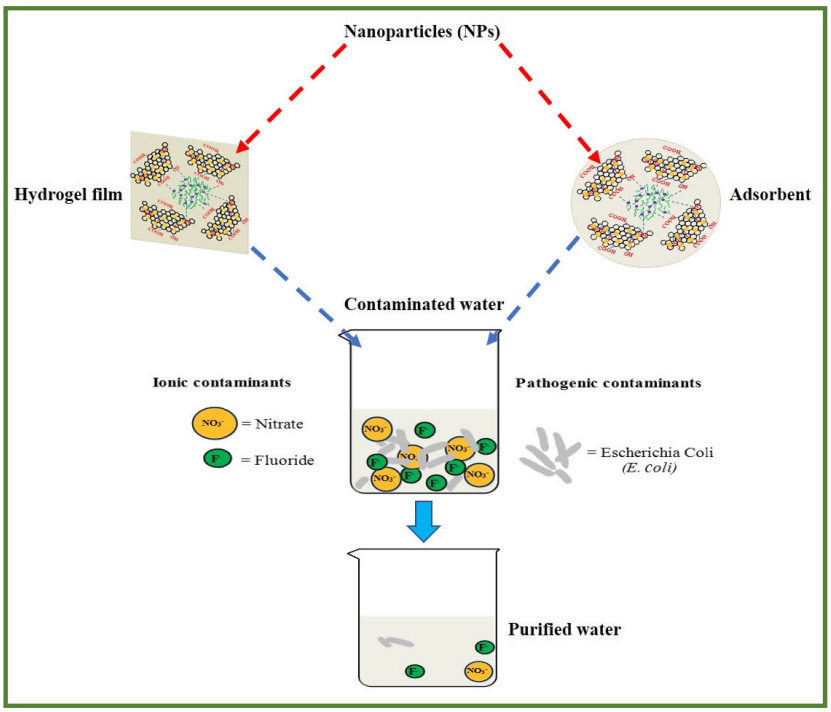

Figure 2 Mechanism of nanoparticle-based adsorbents and hydrogel films for removal of F^-, NO_3^- and coliforms *(E.coli)* from contaminated water.

due to natural processes (e.g. volcanic activity) or anthropogenic actions (Dugo *et al.*, 2006). Similarly, F^{-1} toxicity was found to be responsible for malfunctioning of enzyme actions, gastric function and immune system of the experimental fish (Manna *et al.*, 2007) and habitat degradation and destruction of freshwater snail *Physella acuta* (Camargo *et al.*, 2017). In this perspective, interestingly, Wu et al., used 3D RGO (three-dimensional reduced graphene oxide) hydrogel prepared by hydrothermal method for Hg and F^- removal from aqueous solution. Their results indicated significant potential of the aerogel for adsorption of $Hg^{+2}$and F^{-1} that reached to 185 and 31.3 mg g^{-1} respectively. They proposed the system as favourable one for environmental pollution management (Wu *et al.*, 2016). Azadbakht and colleagues synthesized nanocrystalline cellulose (NCC) for removal of NO_3^- from aqueous solution. Obtaining peak level removal of nitrate as 25% at pH 6, they concluded that bagasse-based NNC could be a useful approach for removal of nitrate from both water and wastewater reservoirs (Azadbakht *et al.*, 2016). Liu et al., prepared magnetic iron–aluminium oxide/graphene oxide (IAO/GO) NP-based selective adsorbent for water purification from F^{-1}. The absorbent was characterized to have enhanced selective adsorption capability for F^{-1}, stability in acid-base environment and super para-magnetism features. Therefore, they suggested that IAO/GO basedadsorbents could have promising applications for F^{-1} in natural water resources (Liu *et al.*, 2016). Another research group used TiO_2 and TiO_2–SiO_2 nanocomposite for the removal of F^{-1} from aqueous solution. The adsorbents so fabricated showed significantly high levels of F^{-1} adsorption reaching up to 94.3 mg g^{-1} by TiO_2 (Zeng *et al.*, 2017). After testing several nanosystems, Liu et al., showed nano net as one of the best that ensured 100% improvement in fish survival rate. Furthermore, they also found a significant decrease in water nitrite and nitrate levels in addition to improved pH and water quality (Liu *et al.*, 2008).

Delivery of nutrients

Undoubtedly nutraceuticals are known to play a significant role in scaling up growth and immunological parameters in fish. However, instead of minimal requirements, their incorporation requires higher costs. Therefore, intense care should be taken in their usage to avoid wastage and maximize their utilization (FOE, (Friends of the Earth) 2008). A huge body of literature is available supporting the role of nanotechnology in effective delivery of dietary supplements and nutraceuticals in fisheries. These systems are basically aimed to enhance the bioavailability, bio accessibility and hence efficacy of the nutrients by improving their solubility and protection from harsh environment of the gut. In this

perspective, it was found that adding 1 mg of nano-Selenium (Se) per kg of diet showed significant improvement in common carp *(Cyprinus carpio)* growth and antioxidant defence system as compared to the control ones (Ashouri *et al.,* 2015). Also, (Se), zinc (Zn) and manganese (Mn) NP supplementation in early weaning diets improved stress resistance and bone mineralization of gilthead seabream (*Sparus aurata*) (Izquierdo *et al.,* 2017). In comparison to a competitor 6-coumarin loaded pectin microparticles (MPs), a formulation of solid lipid (SL) NP-encapsulated 6-COUM showed enhanced uptake of the compound by two gilthead seabream (*Sparus aurata* L.) cell types, that is an established cell line (SAF-1 cells) and the primary cultures of head-kidney (HK). Thereby making SLNPs as suitable nanocarriers for the delivery of biologically active substances in fish (Trapani *et al.,* 2015). Diet supplemented with iron NPs and *Lactobacillus casei* as a probiotic significantly improved growth parameters in rainbow trout (Mohammadi *et al.,* 2015), whereas diet added with 16 mg kg^{-1} of MnO NPs significantly promoted growth and antioxidant defence system of freshwater prawn *(Macrobrachium rosenbergii)* (Asaikkutti *et al.,* 2016). Similarly, copper (Cu) NP supplementation at 20 mg kg^{-1} significantly elevated the growth, biochemical constituents, digestive enzyme activities, antioxidant, metabolic enzyme levels and non-specific immune response of the freshwater prawn, (*Macrobrachium rosenbergii)* post larvae (Muralisankar *et al.,* 2016) and red sea bream, (*Pagrus major*) (El Basuini *et al.,* 2017). Kunjiappan *et al.,* evaluated hepatoprotective and antioxidant effects of *Azolla microphylla*-based gold NPs (GNPs) against acetaminophen (APAP)-induced toxicity in a fresh water common carp fish (*Cyprinus carpio* L.). GNPs significantly ameliorated the levels of metabolic enzymes, hepatotoxic markers, oxidative stress markers, altered tissue enzymes, reduced hepatic ions, abnormal liver histology etc. Therefore, they recommended *Azolla microphylla* phytochemically synthesized GNaP as an effective protector against acetaminophen-induced hepatic damage in fresh water common carp fish (Kunjiappan *et al.,* 2015). Sharif Rohani and colleagues studied the effects of three different levels (0.5, 1 and 1.5% of the diet) of *Aloe vera* NPs on growth performance, survival rate and body composition of Siberian sturgeon (*Acipenser baerii*). Their results showed that diet supplemented with 1% *Aloe vera* NPs significantly promoted the growth indices of Siberian sturgeon as compared to control ones (Sharif Rohani *et al.,* 2017). In the same year, another research group evaluated the efficacy of ginger (GN) and GNNPs on performance, cognition capability, immunity and prevention of motile *Aeromonas* septicaemia (MAS) in *Cyprinus carpio* fingerlings. Fish fed with 1 and 0.5 g GNNPs per kg feed showed 100% relative percentage survival (RPS) whereas fish fed with 0.5 g GN per kg feed showed 20% mortality rate and 71% RPS. These results confirmed GNNPs as a successful formulation in the prevention of MAS more than GN (Korni & Khalil 2017). *Azadirachta indica* (neem) constructed AgNPs were synthesized to see their potential immunomodulatory activity in *Cirrhinus mrigala* fingerlings challenged with *Aeromonas hydrophila.* After observing significantly elevated functional activity of immunological parameters in fish treated with these NPs, it was concluded that they have a potential immunomodulatory and antibacterial activity (Rather *et al.,* 2017). Most recently Erdem and research group synthesized AgNPs from *Aeromonas sobria* to evaluate their antibacterial efficacy against different fish pathogens (*H. alvei, P. rettgeri, M. morganii subsp. Sibonii, C. braakii, E. hermannii, A. hydrophila, E. cloacae and E. coli).* Demonstrating highest efficacy against *A. hydrophila*, these NPs were believed to be a hope for possible application as a disinfectant or antimicrobial agent for better fish health management (Erdem *et al.,* 2018).

Toxicity

Besides their core application in the development and sustainability of aquaculture, nanotechnology-based materials and products are also known to exert adverse effects on environment and human health. Particularly, aquatic organisms due to their higher vulnerability are at an increased risk of exposure to the potential toxicity of these materials. Therefore, it is of prime importance to take into consideration the adverse and toxic effects of nanomaterials to the aquatic organisms (Wang *et al.,* 2008). Li et al., identified the toxicities of Nano-Se and selenite in selenium-sufficient Medaka fish. Approximately sixfolds higher liver accumulation and fivefolds stronger toxicity (in terms of LC50) of nano-Se was observed as compared to selenite. This hyper-accumulation of nano-Se was supposed to be responsible for increased oxidative stress responses in these fish (Li *et al.,* 2008). In 2012, Lee and colleagues investigated acute toxicity and oxidative stress of citrate capped AgNPs in common carp *(Cyprinus carpio).* After exposer to 200 μg L^{-1} of AgNPs, enzymatic activities in the brain of these fish were found to be significantly reduced. Also, their recovery rate was much slower than those treated with lower doses (Lee *et al.,* 2012). In the following year, same results were presented by Johari et al., who determined the acute toxicity of colloidal AgNPs during different life phases of rainbow trout. As compared to control, the treated-juvenile group showed reduced blood plasma chloride and potassium, and elevated cortisol and cholinesterase levels, thereby confirming AgNPs as toxic and very toxic candidates (Johari *et al.,* 2013). Similarly, iron oxide NPs (≥10 mg L^{-1}) were shown to develop toxicity in the embryos of zebrafish

(*Danio rerio*), causing mortality, hatching delay and malformation (Zhu *et al.,* 2012). Pathological findings were stated in the gill, gut, liver, kidney, brain and muscle of juvenile rainbow trout *(Oncorhynchus mykiss)* exposed to 20 or 100 μg L^{-1} of either CuNPs or $CuSO_4$ (Cu sulphate). As compared to the control, fish in the treatment group experienced organ injuries with either CuNPs or $CuSO_4$. Having said that, CuNPs were found to cause severe injuries in the intestine, liver and brain than the equivalent concentration of $CuSO_4$ (Al-Bairuty *et al.,* 2013). Connolly and coworkers determined tissue distribution and oxidative stress responses of dietary supplemented Zn in the form of ZnO NPs in rainbow trout *(Oncorhynchus mykiss)*. Administration of ZnO NPs at a dose of 1 g kg^{-1} feed for 10 days resulted in Zn distribution to the liver of fish. By experiencing oxidative stress-related biochemical disturbances in the liver and ethoxy-resorufin-*O*-deethylase (EROD) activity of these fish, they postulated that ZnO NPs or its ions may hinder cytochrome P450 metabolic processes (Connolly *et al.,* 2016). In order to study their organ pathologies (the kidney, liver, gill, and intestine), osmo-regulatory responses and immunological parameters, Tilapia *(Oreochromis niloticus)* were exposed to 10–30 nm ZnO NPs at two doses of 1 and 10 mg L^{-1}. The NPs at all concentration were found to ameliorate different pathological conditions in the selected organs (Kaya *et al.,* 2016). Afifi et al., assessed acute and sub-acute toxicity of AgNPs (4 mg L^{-1} and 2 mg L^{-1}) on brain tissues of *Oreochromis niloticus and Tilapia zillii.* Results from biochemical and molecular analysis conducted on tissue homogenates revealed that exposure to AgNPs at a dose 4 mg L^{-1} has lethal effects on brain antioxidant system of *O. niloticus and T. zillii* (Afifi *et al.,* 2016). Another study evaluated acute toxicity of AgNPs (0, 0.2, 1, 2, 6, 10 and 15 ml L^{-1}) in Roach *(Rutilus rutilus)* and Goldfish *(Carassius auratus)*. By calculating the mortality of the treated fish at different time intervals (24, 48, 72, 96 h), it was concluded that AgNPs have significant deleterious effects for fish species (Yalsuyi *et al.,* 2017). Juvenile *Piaractus mesopotamicus* ('pacú') exposed to different concentration of AgNPs (2.5, 10, and 25 μg L^{-1}) showed enhanced Ag accumulation in the brain than in the liver and gills at all concentrations. This led to the increased lipid peroxidation as well as DNA damage, evidencing detrimental effects of AgNPs in these fish (Bacchetta *et al.,* 2017). Most recently, Chupani and colleagues aimed to investigate chronic toxicity of dietary ZnO NPs in juvenile common carp *(Cyprinus carpio)*. Fish in the treatment group were fed with diet containing ZnO NPs at doses of 50 and 500 mg kg^{-1} of feed for 6 weeks. After analysing haematological, biochemical, histological parameters, and accumulation of Zn in tissues, it was concluded that ZnO NPs might hinder kidney and liver function in fish (Chupani *et al.,* 2018).

Natural bioactive compounds

The preceding discussion implies that it is inevitable to find out safe, ecofriendly and cost-effective compounds to be used in fishery as growth promoters, stress resistance boosters and immuno-stimulator. In this regard, phytochemicals which have been known for centuries as promising remedies for human therapies will be candidates of choice. Tannins, alkaloids and flavonoids, the secondary metabolite components of phytochemicals have a broad spectrum of shielding effects against different diseases (Pandey *et al.,* 2010). A profound insight of literature is available proving the potential role of these phytochemicals in fish which have been researched time to time in different species. For example, aqueous extract from the leaf of *Eclipta alba (E. alba)* (Bhangra) (oral administration as feed supplement) boosted up immune-stimulatory responses and disease resistance of tilapia (*Oreochromis mossambicus)* against *A. hydrophila* infection (Christybapita *et al.,* 2007). Similarly, oral administration of aqueous extracts from ginger (*Z. officinale*) and four Chinese herbs that is *Rheum officinale, Andrographis paniculata, Isatis indigotica* and *Lonicera japonica* were found to enhance the phagocytic activities of white blood cells (WBCs) in rainbow trout and carp respectively (Yin *et al.,* 2009). Ahilan et al., stated that herbal additives from *Phyllanthus niruri* and *Aloe vera* (Aloe) significantly promoted the growth performance and resistance against *A. hydrophila* infections in goldfish (*Carassius auratus)* (Ahilan *et al.,* 2010). *Azadirachta indica* (neem) leaves contain active compounds nimbin, azadirachtin and meliantroil and are known to have insecticidal and antiviral characteristics. In common carp *(Cyprinus carpio),* aqueous extract from the leaf of neem showed significant control against *A. hydrophila* infection. Furthermore, two bacterial strands (*Enterobacter* sp. and *E. coli* bacteria) isolated from marine fish (*Amphiprion sebae*) exhibited 15 mm zone of inhibition against neem extract (Abdul Kader Mydeen *et al.,* 2011). Besides, due to their lower toxicity, Indian almond (*Terminalia catappa*) and garlic (*Allium sativum*) were described to be effective substitutes for chemicals in treating fish ectoparasites, *Trichodina* sp. infections in tilapia (*O. niloticus*) fingerlings (Pandey *et al.,* 2012). However, in the past decades particular attention has been given to 'Curcumin' a naturally occurring polyphenolic yellow-pigmented compound derived from the rhizomes of turmeric (*Curcuma longa* L.). Curcumin has been remained an important and widely used compound in traditional Indian and Chinese medicines for many centuries. This efficacy of curcumin was further dug out by scientists who signified it for its vast range of pharmacological applications including anti-arthritic (Deodhar *et al.,* 1980), thrombosuppresive (Srivastava *et al.,* 1985), anti-human immuno-deficiency virus (Jordan *et al.,* 1996), myocardial infarction protective

(Nirmala & Puvanakrishnan 1996), hepato- and nephroprotective (Venkatesan *et al.,* 2000), hypoglycaemic (Arun *et al.,* 2002), anti-microbial (De *et al.,* 2009; Wang *et al.,* 2009a), anti-inflammatory (Aggarwal & Harikumar 2009), anti-tumour (Lee *et al.,* 2009), antioxidant (Pizzo *et al.,* 2010) and anti-parasitic (Yallapu *et al.,* 2010). And based on these information, most recently researchers around the globe took interest to evaluate these promising effects of curcumin in fish that gave tremendous results as summarized in Table 2. Unfortunately, previous literature shows that the potential role of curcumin is hampered by its hydrophobic nature and short biological half-life resulting in low bioavailability in plasma as well as tissues. In this perspective, nanotechnology has been employed in an attempt to elevate its retention span and enhance its bioavailability (Cui *et al.,* 2009). Therefore, to accomplish the task, curcumin was encapsulated in cyclodextrine (Baglole *et al.,* 2005), hydrogel (Shah *et al.,* 2008), liposomes (Letchford *et al.,* 2008), polymeric micelles (Takahashi *et al.,* 2009), surfactant micelles (Wang *et al.,* 2009b), NPs (Das *et al.,* 2010), phospholipids (Semalty *et al.,* 2010) etc. In our previous study, we successfully encapsulated curcumin in CS-TPP NPs stabilized Pickering emulsion that maintained good and long-term stability of the encapsulated curcumin (Shah *et al.,* 2016). So far Pickering emulsion is considered as the safest and long-term stable encapsulation approach for hydrophobic bioactive compounds (Matos *et al.,* 2016; Xu *et al.,* 2018b) and has been discussed in detail in the following section.

Pickering emulsion

Pickering emulsions are emulsions which are stabilized by solid particles instead of surfactants and were named after S.U. Pickering, who described the phenomenon in 1907, although the effect was first recognized by Walter Ramsden in 1903 (Ramsden *et al.,* 1904; Pickering, 1907). Pickering emulsions are much more advantageous over conventional surfactant stabilized emulsions in terms of having no or low toxicity (Dickinson, 2010), enhance resistance to coalescence (high stability) (Binks, 2007), good reproducibility, facile and scalable productivity and improved biocompatibility (Wu & Ma 2016). Furthermore, the design and formulation of these emulsions ensures sustained and controlled release of the encapsulated-bioactive compounds throughout the course of gut (Shani-Levi *et al.,* 2013). A simple preparatory mechanism of the emulsion system as a carrier has been schematically drawn in Figure 3.

To date numerous colloidal particles have been reported to be employed as stabilizing agents in the fabrication of Pickering emulsions. Marku and colleagues prepared starch-based Pickering emulsions as topical drug delivery vehicles with good stability during storage for 8 weeks and against mild centrifugation (Marku *et al.,* 2012). Similar system composed of starch-based Pickering emulsion was used by Cossu et al., for the encapsulation and delivery of antifungal natural phenolic compound thymol and amphotericin B. to evaluate their antifungal efficacy against *C. albicans.* Abstracting their results, it was concluded that starch-based emulsion could be a potential approach for the delivery of hydrophobic antifungal compounds in treating oral candidiasis (Cossu *et al.,* 2015). In the following year, cyclodextrins-based Pickering emulsions were synthesized for topical delivery of antifungal azole derivatives. Long-term stability and rheological behaviour showing its compatible nature for topical applications made these emulsions new and active formulations for antifungal econazole derivatives delivery (Leclercq & Nardello-Rataj 2016). Heat-treated soy glycinin stabilized gel-like Pickering emulsion was prepared for the delivery of β-carotene. In in vitro studies simulating intestinal digestion conditions, it was found that all the tested emulsions well protected the encapsulated β-carotene against degradation over the whole digestion process and ensured its sustained release (Liu & Tang 2016). Another similar study for intestine-targeted sustained released delivery of β-carotene through pea protein isolate stabilized Pickering emulsion was conducted by Shao and coworkers. The in vitro-simulated digestion findings of this study indicated that the release and stability of the β-carotene against degradation during digestion process were significantly modulated by its encapsulation in these emulsions at oil fraction ($\Phi = 0.6$) than at ($\Phi = 0.3$) (Shao *et al.,* 2016). In the same year, we also synthesized chitosan tripolyphosphate (CS-TPP) NPs stabilized Pickering emulsion for the delivery of curcumin. The system showed long-term storage stability, high stability against pH and salts (NaCl and $CaCl_2$) and ensured sustained release of curcumin over extended period of time (Shah *et al.,* 2016). In an attempt to enhance the oral bioavailability of silybin, its nanocrystal self-stabilized Pickering emulsion was synthesized using a high-pressure homogenization technique. The stability, in vitro release and in vivo bioavailability results revealed that Pickering emulsion of silybin could be stabilized by nanocrystals of silybin itself that was found to significantly enhance oral bioavailability of silybin. Therefore, the system based on drug nanocrystalline self-stabilized Pickering emulsion could be a promising oral drug delivery system for poorly soluble drugs (Yi *et al.,* 2017). Last year Matos et al., aimed to prepare and compare quinoa starch stabilized Pickering emulsion with non-ionic surfactant (Tween 20) stabilized emulsions for the encapsulation and delivery of a natural phenol 'resveratrol'. Interestingly their findings described Pickering emulsions, an appropriate resveratrol delivery system, more efficient than surfactant-stabilized emulsions,

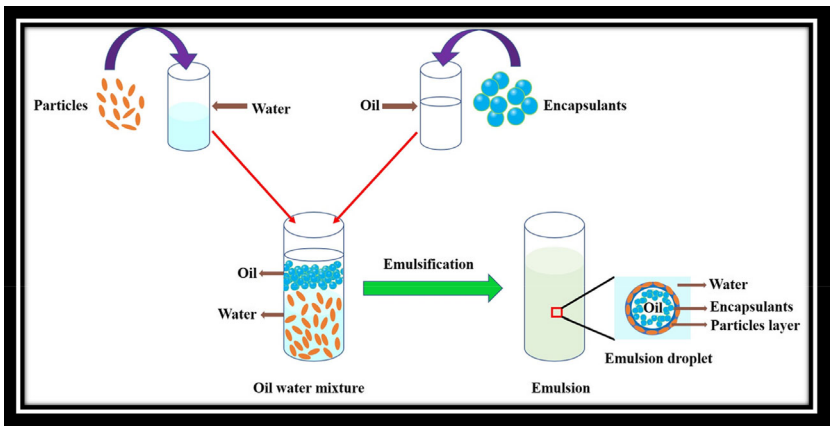

Figure 3 Facile representation of emulsion preparation as delivery system.

leading to a higher encapsulation efficiency (EE) of up to 98%, being more than twice that of the surfactant stabilized systems (Matos *et al.*, 2018). Similarly, another research group synthesized nanofibrillated mangosteen cellulose (NFMC) stabilized Pickering emulsion for the encapsulation and delivery of vitamin D_3. The in vitro digestion experiments were conducted by simulating gastrointestinal tract (GIT) model with mouth, gastric and intestinal phases that followed a decrease trend in rate, extent of lipid digestion and vitamin D_3 bioaccessibility with increase in NFMC concentration (Winuprasith *et al.*, 2018). Shao et al., used taro starch stabilized Pickering emulsion as a carrier for tea polyphenols. The system was found to have high stability and significant ability for encapsulating tea polyphenols with a retention rate of up to 67% (Shao *et al.*, 2018). Anticancer and antimicrobial efficacy of coumarin and curcumin was scaled up by their encapsulation in nanocellulosic-based NPs stabilized Pickering emulsion (Ngwabebhoh *et al.*, 2018). Furthermore, oregano essential oil (OEO) Pickering emulsion stabilized by cellulose nanocrystals was used to enhance the antimicrobial activity of OEO against four food-related microorganisms, that is *S. aureus*, *B. subtilis*, *S. cerevisiae and E. coli* (Zhou *et al.*, 2018). Also, Zein/gum Arabic nanoparticle-stabilized Pickering emulsion was fabricated with thymol as an antibacterial delivery system against *E. coli* (Li *et al.*, 2018).

Conclusion and future perspective

Nanotechnology for sure contributes a significant role in the development and sustainability of aquaculture. So far, different kinds of nanotechnology-based systems have been employed to strengthen the important pillars of aquaculture and fishery. However, there is a growing concern about the toxicity of NPs, excessive antibiotics and other synthetic compounds usage in this discipline. Therefore, applications of safe and ecofriendly approaches are inevitable. In this regard, recently natural bioactive compounds have attracted much attention, particularly curcumin which has shown a potent role in fishery. For example, as shown in Table 2, a lot of work has been done on curcumin recently in fishery but in its free-form rather than encapsulated form. And unfortunately, these kinds of hydrophobic bioactive compounds are known to have low bioavailability and retention time hampering their efficacy. Thus, to synthesize safe and efficient vehicles for their encapsulation and delivery will be of the greatest value. Pickering emulsion which is stabilized by solid particles (nutrients) instead of surfactants is being used as a carrier for numerous compounds due to its non-toxic and long-term stable characteristics. But to date scarce evidence is available on utilization of Pickering emulsion in fishery thereby leaving a huge gap in fishery in terms of using this approach. Based on these information, we strongly recommend that future work is needed in aquaculture and fishery to:

- Synthesize novel nanoparticle-based adsorbents and films for water purification from heavy metals and coliforms.
- Avoid excessive usage of antibiotics and nanoparticles with known toxicities.
- Identify new natural bioactive compounds that could be used against different diseases and as growth promotors
- Incorporate nutrient-stabilized Pickering emulsions as delivery systems for the encapsulation and delivery of these compounds to enhance their efficacy and ensure their targeted and sustained release.

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Conflict of interest

The authors declare no conflict of interest in this manuscript.

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Applications of genotyping by sequencing in aquaculture breeding and genetics

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Abstract

Selective breeding is increasingly recognized as a key component of sustainable production of aquaculture species. The uptake of genomic technology in aquaculture breeding has traditionally lagged behind terrestrial farmed animals. However, the rapid development and application of sequencing technologies has allowed aquaculture to narrow the gap, leading to substantial genomic resources for all major aquaculture species. While high-density single-nucleotide polymorphism (SNP) arrays for some species have been developed recently, direct genotyping by sequencing (GBS) techniques have underpinned many of the advances in aquaculture genetics and breeding to date. In particular, restriction-site associated DNA sequencing (RAD-Seq) and subsequent variations have been extensively applied to generate population-level SNP genotype data. These GBS techniques are not dependent on prior genomic information such as a reference genome assembly for the species of interest. As such, they have been widely utilized by researchers and companies focussing on nonmodel aquaculture species with relatively small research communities. Applications of RAD-Seq techniques have included generation of genetic linkage maps, performing genome-wide association studies, improvements of reference genome assemblies and, more recently, genomic selection for traits of interest to aquaculture like growth, sex determination or disease resistance. In this review, we briefly discuss the history of GBS, the nuances of the various GBS techniques, bioinformatics approaches and application of these techniques to various aquaculture species.

Key words: aquaculture, genotyping, next-generation sequencing, restriction-site associated DNA, selective breeding, single nucleotide polymorphism.

Background

Despite the critical role for aquaculture in global food security, the vast majority of world fish and shellfish production is based on stocks without advanced selective breeding programmes (Gjedrem *et al.* 2012; Janssen *et al.* 2016). Aquaculture breeding schemes tend to lag behind their terrestrial livestock counterparts in terms of the uptake of genomic technologies, and for many aquaculture species, molecular genetic tools are only applied for pedigree reconstruction (Chavanne *et al.* 2016). In comparison, most modern breeding programmes in livestock are now underpinned by genomic selection (GS, Meuwissen *et al.* 2001), the benefits of which are well-illustrated in dairy cattle (Hayes *et al.* 2009). GS typically requires genome-wide genetic marker data for a large number of individual animals. Up until a few years ago, obtaining genetic markers was costly and laborious; hence, large numbers of markers were only available for a handful of well-studied species. However, the recent advances in next-generation sequencing (NGS) have greatly reduced the cost of nucleic acid sequencing, and therefore also genetic marker discovery. This has opened the door for rapid generation of genome-wide genetic marker datasets, either via generation and application of SNP arrays, or directly via genotyping by sequencing (GBS) techniques (Davey *et al.* 2011). GBS techniques have revolutionized the field of evolutionary genomics (reviewed in Andrews *et al.* 2016) and have also led to several advances in genetics and breeding of aquaculture species, the subject of this review.

Due to the high fecundity of aquaculture species, the majority of breeding programmes are based on collection of trait data on close relatives (e.g. full siblings) of the selection candidates, particularly where the trait of interest cannot be measured on the candidates themselves (e.g. fillet quality, disease resistance). Without genetic markers, this set-up enables family selection, whereby family-level estimated breeding values (EBVs) for selection candidates are calculated using the data collected on the relatives. However, to utilize the within-family genetic variation in these traits, genetic markers are necessary to distinguish between selection candidates. Implementation of markers in breeding can broadly be split into two categories; marker-assisted selection (MAS) and GS. MAS is based on the use of targeted markers linked to major quantitative traits loci (QTL) affecting the trait, and one of the first examples in aquaculture was host resistance to infectious pancreatic necrosis virus (IPNV) in Atlantic salmon (*Salmo salar*, Houston *et al.* 2008; Moen *et al.* 2009). For traits with a polygenic architecture, GS is a more appropriate approach, whereby the relatives of the selection candidates become the 'training' population with genotypes and phenotypes, and those data are used to calculate genomic breeding values (GEBVs) for selection candidates with genotype data only. This application of genomic selection in aquaculture breeding is at a formative stage, and most examples to date have focussed on improved breeding for resistance to infectious diseases (e.g. Ødegård *et al.* 2014; Tsai *et al.* 2015, 2016b; Vallejo *et al.* 2016; Dou *et al.* 2016; Palaiokostas *et al.* 2016). The majority of high-resolution genetic studies in aquaculture species, and applications of genomic selection, have been underpinned by GBS techniques, either by directly providing genotype data or by discovering markers for the design of SNP arrays, which are currently only available for a handful of aquaculture species (e.g. Atlantic salmon, Houston *et al.* 2014; Yáñez *et al.* 2016; Pacific oyster, *Crassostrea gigas*, and European flat oyster, *Ostrea edulis*, Lapègue *et al.* 2014; channel catfish, *Ictalurus punctatus*, Liu *et al.* 2014; common carp, *Cyprinus carpio*, Xu *et al.* 2014a; rainbow trout, *Oncorhynchus mykiss*, Palti *et al.* 2015a).

The most common GBS techniques involve library preparation steps that result in deep sequence data at a repeatable subset of sites dispersed throughout the genome, typically using one or two restriction enzymes (RE), although also new GBS techniques based on targeted sequencing have been recently developed (i.e. GT-Seq, discussed below). The reason behind this genome complexity reduction is that high-coverage sequencing of a typical aquaculture species' genome with enough depth to confidently call genotypes is still prohibitively expensive for the number of animals required for high-resolution genetic studies and breeding programme applications. Genome complexity reduction via RE is fast and inexpensive. Indeed, RE-based techniques have been commonplace in genotyping for many years, with RFLP and AFLP being widely applied to generate genotyping assays for limited numbers of genetic markers. The marriage of these ideas with NGS has enabled a major breakthrough for genetic studies of complex traits in nonmodel organisms, and their application to improve aquaculture production.

RAD sequencing

Restriction-site associated DNA sequencing (RAD sequencing or RAD-Seq) covers a range of GBS techniques which combine the use of genome complexity reduction with REs and the high sequencing output of NGS technologies. RAD-Seq was first described by Baird *et al.* (2008), following on from a similar idea based on microarrays (Miller *et al.* 2007). Some of the main reasons for its instant success are that RAD-Seq does not require any prior genomic knowledge, it allows generation of population-specific genotype data (i.e. no ascertainment bias) and it offers flexibility in terms of desired marker density across the genome. The use of different REs or innovative modifications to the base technique allows a high level of control over the number of markers obtained for a specific study. RAD-Seq and similar techniques are also amenable tools for aquaculture breeding, where genetic markers have typically been used in family assignment and pedigree reconstruction (Vandeputte & Haffray 2014). Mass spawning species are common in aquaculture, where mixed rearing and unknown parental contribution necessitate the use of genotyping for family-based breeding. RAD-Seq potentially facilitates a single experiment whereby pedigrees are reconstructed, genetic diversity is quantified, QTL can be mapped and genomic breeding values calculated (Palaiokostas *et al.* 2016). Since the original RAD-Seq paper by Baird *et al.* (2008), several variants of this methodology have been described. Three of them have been extensively used in aquaculture genetics research: the original RAD-Seq (Baird *et al.* 2008), 2b-RAD (Wang *et al.* 2012) and ddRAD (Peterson *et al.* 2012). Other RAD-based techniques like ezRAD (Toonen *et al.* 2013) or SLAF-seq (Sun *et al.* 2013) introduced minor modifications, which do not confer a major advantage for aquaculture applications. All available RAD-based techniques have been recently reviewed in depth elsewhere (Andrews *et al.* 2016); therefore, here we have focused on those most relevant in aquaculture breeding. The main features of original RAD-Seq, 2b-RAD and ddRAD are shown in Table 1, and they are briefly described below.

Original RAD-Seq

In original RAD-Seq (Baird *et al.* 2008), genomic DNA samples from several animals are individually digested with

Table 1 Summary of the different genotyping by sequencing (GBS) techniques

Technique	Key features	Advantages	Disadvantages
RAD-Seq	Digestion with one RE	• Paired-end contigs • PCR duplicate removal	• Complex library preparation
2bRAD	Digestion with type IIB REs	• No size-selection step • High reproducibility • Easy library preparation • Strand bias detection	• Short fragments • Removal of PCR duplicates not possible
ddRAD	Digestion with two different REs	• Can multiplex many samples • Easy library preparation • Flexibility over SNP density	• Repeatability dependent on size-selection step

a RE of choice. The digested DNA is then randomly sheared and pooled after ligation of adaptors with nucleotide barcodes for unique identification of each sample. The resulting restriction fragments are selected for suitable size range (i.e. for Illumina sequencing, typically 300–600 bp), and after a subsequent polymerase chain reaction (PCR) step, the fragments are sequenced. The result is high-coverage sequence data for flanking regions of the RE cut sites, which are typically dispersed quite evenly throughout the genome. As such, a genome-wide genetic marker dataset can be produced across a population of individuals at a fraction of the cost of whole genome resequencing. Illumina sequencing of short fragments either involves sequencing one (one read, single end) or both (two reads, paired end) ends of each fragment and currently gives reads of up to 300 bp in length. Each flanking sequence of the RE cut site is referred to as a RAD locus (or RAD-tag), and the high coverage of RAD tags facilitates simultaneous SNP detection and genotyping. The number of RAD tags, and therefore SNPs, generated in the experiment is tuneable via the choice of rarer or more frequent cutting RE. The most commonly used enzyme to date is SbfI which has an eight base recognition site and therefore cuts relatively infrequently throughout the genome. Online tools are available to guide the choice of the most appropriate RE according to the requirements and budget of the study (Lepais & Weir 2014). In addition to sequencing and genotyping individuals, the approach is also amenable to genotyping pooled populations for bulk-segregant analysis (Baird *et al.* 2008; Hohenlohe *et al.* 2010). One of the main drawbacks of the original technique is that shearing by sonication is random and variable, potentially hindering the efficiency and the reproducibility of RAD-Seq (Davey *et al.* 2013). However, this random shearing step can also be a benefit, as the variable size of the genomic fragments anchored at the RE cut site facilitates the assembly of a contig based on the paired-end reads. This augments annotation of the RAD loci when there is no reference genome available, and also the design of specific primers for re-genotyping of targeted SNPs. In addition, the paired-end data from RAD-Seq allow identification and removal of putative PCR duplicates (reads originated from the same original DNA fragment, therefore presenting identical sequences), which can hinder analysis and interpretation of Illumina sequencing data (Schweyen *et al.* 2014). While there are several sources of potential bias and error in RAD-Seq techniques (see review by Andrews *et al.* 2016), several theoretical and empirical studies have demonstrated that RAD-Seq does render reproducible genotyping data across different laboratories, populations and even species (e.g. DaCosta & Sorenson 2014; Gonen *et al.* 2015).

2b-RAD

The first major modification of the original RAD technique was termed 2b-RAD (Wang *et al.* 2012). The main innovation in 2b-RAD is the use of type IIB REs, which share the feature of cutting the genomic DNA at both sides of the recognition site at a fixed distance, resulting in protruding noncohesive ends. The result is short genomic DNA fragments of identical size at each IIB RE site in the genome. Library construction in the 2b-RAD protocol is simple. Following DNA digestion, adaptors are ligated to the fragments, and specific barcodes are added to each sample through PCR amplification using degenerated linkers. Samples are then pooled and sequenced typically using Illumina technology, but allowing for runs of shorter read length due to the smaller size of the fragments in comparison to original RAD (2b-RAD fragments are 33–36 bp). The use of type IIB REs theoretically facilitates the sampling and sequencing of identical sites across individuals, circumventing the potential bias of RAD-Seq caused by the random shearing step. It also avoids the time-consuming and potentially error-prone size-selection step, which characterizes the majority of other RAD methods. Additionally, 2b-RAD is currently the only member of the RAD family that allows removal of loci exhibiting strand bias (Puritz *et al.* 2014a). The possibility to produce individually barcoded libraries allows targeted adjustment before pooling to obtain more equal representation of individual samples. The main caveat of this method is that it produces short sequencing reads (33–36 bp), which are less amenable for alignment to reference genome assemblies, and hinders follow-up applications such as the design of individual SNP assays (due to lack of SNP flanking sequence). However, this is not an issue if a draft genome sequence is available for the species, as is becoming the case in many aquaculture fish species.

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ddRAD

Peterson *et al.* (2012) developed a new RAD-Seq platform using a double digestion of genomic DNA with two REs (ddRAD), thus eliminating the shearing step of original RAD. The ddRAD protocol is more flexible than RAD-Seq or 2b-RAD in terms of targeted marker density; the number of fragments and SNPs can be readily tailored by combining different RE pairs. Due to the typical use of a rare and a common cutting enzyme, ddRAD results in fewer sequenced sites than RAD-Seq, facilitating higher sequence coverage and/or more individuals multiplexed within a single sequencing lane. Higher multiplexing is possible due to combinational multiplex indexing, whereby a first barcode is introduced in the ligation step and a second during the PCR. Therefore, a larger number of samples can potentially be sequenced in a single lane than with the other RAD techniques. Compared to the RAD-Seq protocol, the workflow of preparation of ddRAD libraries is simpler, quicker and also substantially cheaper. However, the workflow is still more complex than the 2b-RAD protocol and requires a size-selection step. To ensure repeatability of sampled ddRAD loci across samples and libraries, consistency of size selection is paramount (Andrews *et al.* 2016). A simplified variation of the initial ddRAD protocol, where both P1 and P2 adaptors with individual barcodes are ligated prior to size selection (Palaiokostas *et al.* 2015a), further reduces hands-on time for library preparation.

RAD bioinformatic analyses

The advent of NGS posed important challenges in terms of data storage, transfer and analysis, which necessitated the development of specialized hardware and software. Consequently, the improvement of NGS-based sequencing platforms occurred in tandem with continuous development and improvement of suitable bioinformatics tools to analyse the large datasets. A wealth of software is available for analysing data originating from the RAD family of techniques. In the current review, a general framework for data analysis will be described, rather than attempting to provide a comprehensive overview of all available tools. Accordingly, the most popular, straightforward to use and regularly updated of the available tools are highlighted in terms of a suggested order of usage that might form a complete RAD analysis pipeline.

Experimental design and simulation

Sequencing and library construction typically account for the bulk of the cost of any experiment utilizing NGS. This leads to a balancing exercise, whereby researchers strive to include as many samples as possible per sequencing lane (multiplexing), without compromising the read coverage required for accurate SNP genotype calling. Therefore, two key variables for a RAD experiment are the choice of the RE (affecting how many sites are sequenced), and the desired read coverage per locus. *In silico* simulation is a valuable tool for any well-designed RAD experiment. The R-based package SimRAD (Lepais & Weir 2014) can be utilized for simulation-based prediction of the expected number of loci for each RE (or their combination) and the genome of study. Although simulation estimates are likely to differ from the empirical data, valuable information can be gained to optimize experimental design before committing to the high cost associated with library construction and sequencing.

Demultiplexing libraries

The files that are generated by the sequencer (typically FastQ files) require demultiplexing into individual samples based on nucleotide barcodes. The most popular packages for this task include *Stacks* (Catchen *et al.* 2011) and *pyRAD* (Eaton 2014). Standard quality control procedure is to discard sequence reads below user-defined acceptable quality scores, erroneous barcodes and reads missing the characteristic sequence pattern obtained from the RE. Following demultiplexing, sequence files corresponding to each individual are generated for downstream analyses, including SNP calling and genotyping.

SNP identification

One of the key advantages of RAD-Seq approaches for non-model organisms (including many aquaculture species) is the ability to identify and genotype SNPs without requiring a reference genome for the organism under study. This approach, commonly defined in the literature as *de novo* assembly, can be performed using either *Stacks* (Catchen *et al.* 2011), *pyRAD* (Eaton 2014) or *dDocent* (Puritz *et al.* 2014b); however, the latter is limited to ddRAD or ezRAD data. The *de novo* approach involves identification and assembling of RAD loci in each individual, based on user-defined parameters related to read coverage required per locus, and sequence divergence between loci (Catchen *et al.* 2011). Identification of SNPs and inference of alleles within RAD loci is performed using a maximum-likelihood-based algorithm (Hohenlohe *et al.* 2010), which undertakes statistical tests at each nucleotide position to assess the likelihood of a particular diploid genotype. In doing so, the model implicitly estimates and accounts for sequencing error rate (Catchen *et al.* 2011). The *Stacks* software does not currently support SNP identification and genotyping in the paired-end (P2) read, unless anchored to a second RE (e.g. in ddRAD). Therefore, in original RAD experiments

using Stacks, the P2 read is typically used for quality control (e.g. removal of PCR duplicates), and for constructing paired-end 'mini-contigs' which facilitate BLAST alignment and genotyping assay design (Etter *et al.* 2011). The simultaneous use of P1 and P2 reads in the case of *dDocent*, and the application of an alignment-clustering algorithm in the case of *pyRAD*, allow the identification of insertion/deletion polymorphisms (indels) and identification of SNPs in the P2 reads.

Due to the decreasing cost of NGS, reference genome sequences are becoming available for many important aquaculture species. The number of species with reference genome assemblies is rapidly increasing (Atlantic cod, *Gadus morhua*, Star *et al.* 2011; Pacific oyster, Zhang *et al.* 2012; European sea bass, *Dicentrarchus labrax*, Tine *et al.* 2014; rainbow trout, Berthelot *et al.* 2014; Japanese eel, *Anguilla japonica*, Kai *et al.* 2014; half-smooth tongue sole, *Cynoglossus semilaevis*, Chen *et al.* 2014; common carp, Xu *et al.* 2014b; Northern pike, *Esox lucius*, Rondeau *et al.* 2014; Nile tilapia, *Oreochromis niloticus*, Brawand *et al.* 2015; Asian sea bass, *Lates calcarifer*, Vij *et al.* 2016; Mediterranean mussel, *Mytilus galloprovincialis*, Murgarella *et al.* 2016; turbot, *Scophthalmus maximus*, Figueras *et al.* 2016; Atlantic salmon, Lien *et al.* 2016; channel catfish, Chen *et al.* 2016), and new sequencing data will improve genome quality and annotation. Therefore, reference-guided RAD-Seq approaches are likely to be increasingly utilized. Both *Stacks* and *dDocent* can utilize reference genome information, using standard alignment tools followed by similar SNP calling algorithms to the *de novo* approach described above.

Potential bias and sources of error

While the bioinformatic pipelines for the RAD-like approaches are becoming increasingly standardized, there remains potential intrinsic barriers that must be overcome to ensure the generation of accurate and repeatable SNP datasets. One example that is particularly relevant to the aquaculture research community is distinguishing between genuine allelic SNPs and paralogous variants resulting from ancestral whole genome duplication. This is particularly a challenge for salmonid species, and strategies to account for this include (i) assessing read coverage for patterns suggestive of paralogous variation, (ii) checking for excessive heterozygosity at loci and (iii) sequencing (double) haploid individuals as the basis for filtering out paralogous sequence variants (e.g. Everett & Seeb 2014; Houston *et al.* 2014; Palti *et al.* 2015a,b). Another potential source of error for all RAD-Seq studies is the problem of RAD allele dropout (Gautier *et al.* 2013), where mutations within the recognition sequence for the RE segregating in the population are a common source of null alleles. The extent of the issue is related to the length of the RE recognition sequence, and it is therefore potentially more of a problem for ddRAD (which requires two REs) versus other methods (Gonen *et al.* 2015; Andrews *et al.* 2016). Both read coverage levels and assessment of segregation distortion in pedigreed crosses can assist in identifying and removing, or accounting for, these null alleles. Finally, the concept of PCR duplicates is raised above, and this is due to preferential amplification of certain clonal DNA fragments derived from the original genomic DNA fragments. PCR duplicates can give rise to the situation where one allele is overrepresented in the resulting sequence data and causes problems with differentiating homozygous and heterozygous individuals at that locus (Schweyen *et al.* 2014).

Applications of RAD sequencing in aquaculture

Since its first description by Baird *et al.* (2008), RAD-Seq has quickly spread through different fields of genetic research, and it has been used in different aquaculture species to construct genetic maps (e.g. Recknagel *et al.* 2013; Gonen *et al.*2014), for comparative genomics (e.g. Kakioka *et al.* 2013; Manousaki *et al.* 2015), for mapping genes associated with production traits (e.g. Houston *et al.* 2012; Shao *et al.* 2015; Fu *et al.* 2016), mapping sex determining loci (e.g. Palaiokostas *et al.* 2013a,b), studying population dynamics (e.g. Bradic *et al.* 2013), for fisheries management (e.g. Ogden *et al.* 2013), assembling reference genomes (e.g. Tine *et al.* 2014) or generating SNP resources for future SNP array development (e.g. Houston *et al.* 2014; Palti *et al.* 2014). A summary of the studies performed directly relevant for aquaculture is detailed below and in Table 2.

Genetic marker discovery for SNP array development

Early studies using RAD-Seq typically focussed on simply generating a genetic marker resource for nonmodel organisms. When the genome size of the target species is large, then whole genome (re)sequencing is arguably not cost-effective for SNP discovery across many individuals, and genome complexity reduction is advantageous. As such, RAD-Seq and similar techniques enabled a step change in the number of genetic markers (SNPs) available for several species (e.g. sturgeon, *Acipenser* genus, Ogden *et al.* 2013; or rainbow trout, Palti *et al.* 2014), and these have subsequently been used for several high-resolution genetic studies. SNPs generated by RAD techniques have also been applied to produce SNP arrays for several aquaculture species, including Atlantic salmon (Houston *et al.* 2014), rainbow trout

Table 2 Summary of aquaculture-oriented studies using restriction-site associated DNA sequencing (RAD-Seq)

Study	Species	Aim	Technique	Samples	SNPs	Families
Salmonids						
Houston *et al.* (2012)	*Salmo salar*	Disease resistance QTL (IPNV)	RAD	32	6712	Two families
Gonen *et al.* (2014)	*Salmo salar*	Linkage map	RAD	96	8257	Two families
Campbell *et al.* (2014)	*Oncorhynchus mykiss*	Disease resistance QTL (BCWD and IHNV)	RAD	456	4661	40 families
Palti *et al.* (2014)	*Oncorhynchus mykiss*	SNP resource	RAD (×2)	19	145 168	19 genetic lines
Palti *et al.* (2015b)	*Oncorhynchus mykiss*	Disease resistance QTL (BCWD)	RAD	252	5612/4946	Two families
Liu *et al.* (2015b)	*Oncorhynchus mykiss*	Cortisol response to crowding QTL	RAD	234	4874	One family
Liu *et al.* (2015b)	*Oncorhynchus mykiss*	Disease resistance QTL (BCWD) and spleen size QTL	RAD	301	7849	Two half-sib families
Vallejo *et al.* (2016)	*Oncorhynchus mykiss*	Genomic selection (BCWD)	RAD	711	24 465	81 families
Everett and Seeb (2014)	*Oncorhynchus tshawytscha*	Thermotolerance and growth QTL	RAD	422	3534	Six families
Larson *et al.* (2016)	*Oncorhynchus nerka*	Thermotolerance and growth QTL	RAD	491	11 457	Five families
Nonsalmonid fish						
Palaiokostas *et al.* (2013b)	*Oreochromis niloticus*	Sex determination QTL	RAD	88	3904/4477	Two families
Palaiokostas *et al.* (2015a)	*Oreochromis niloticus*	Sex determination QTL	ddRAD	372	1279	Five families
Palaiokostas *et al.* (2013a)	*Hippoglossus hippoglossus*	Sex determination QTL	RAD	93	7572/5954	2 half-sib families
Palaiokostas *et al.* (2015b)	*Dicentrarchus labrax*	Sex determination QTL	RAD	187	6706	4 + 4 half-sib families
Wang *et al.* (2015a,b)	*Scophthalmus maximus*	Sex determination and growth QTL	RAD	151	6647	One family
Brown *et al.* (2016)	*Polyprion oxygeneios*	Sex determination and growth QTL	ddRAD	59	1609	One family
Manousaki *et al.* (2015)	*Pagellus erythrinus*	Linkage map	ddRAD	99	920	One family
Shao *et al.* (2015)	*Paralichthys olivaceus*	Disease resistance QTL (*Vibrio anguillarum*)	RAD	218	13 362	One family
Palaiokostas *et al.* (2016)	*Sparus aurata*	Disease resistance genomic selection	2b-RAD	777	12 085	75 families
Wang *et al.* (2015a,b)	*Lates calcarifer*	Growth QTL	ddRAD	144	3349	One family
Fu *et al.* (2016)	*Hypophthalmichthys nobilis*	Growth QTL	2b-RAD	119	3323	One family
Invertebrates						
Jiao *et al.* (2014)	*Chlamys farreri*	Sex determination and growth QTL	2b-RAD	98	7458	One family
Li and He (2014)	*Pinctada fucata*	Growth QTL	RAD	100	1381	One family
Shi *et al.* (2014)	*Pinctada fucata*	Growth QTL	2b-RAD	98	10 577	One family
Tian *et al.* (2015)	*Apostichopus japonicas*	Growth QTL	2b-RAD	102	11 306	One family
Lu *et al.* (2016)	*Marsupenaeus japonicus*	Thermotolerance and growth QTL	RAD	152	9829	One family
Dou *et al.* (2016)	*Patinopecten yessoensis*	Genomic selection (growth)	2b-RAD	349	2364	Five families
Ren *et al.* (2016)	*Haliotis diversicolor*	Growth QTL	RAD	142	3317	One family

(Palti *et al.* 2015a) and Pacific oyster (Lapègue *et al.* 2014). With the reduction in sequencing costs over recent years, whole genome (re)sequencing (i.e. pool-sequencing, Schlötterer *et al.* 2014) has become increasingly viable. However, RAD-like techniques still hold a significant advantage for SNP discovery when (i) there is no reference genome available, and (ii) only a medium density SNP resource is required.

Linkage maps and reference genome assembly

Restriction-site associated DNA sequencing techniques have been widely used in aquaculture species for constructing genetic maps based on recombination events in defined crosses. Such medium density SNP linkage maps are useful tools for downstream applications such as QTL mapping, comparative genomic and gene mining, or population

genomic studies. For example, RAD-based linkage maps have been created for Atlantic salmon (Gonen *et al.* 2014), channel catfish (Li *et al.* 2014), Japanese flounder (Shao *et al.* 2015), turbot (Wang *et al.* 2015b) and Asian seabass (Wang *et al.* 2015a). Genetic maps based on RAD-Seq have also contributed to mapping and orientation of scaffolds for reference genome assemblies for key aquaculture species such as European sea bass (Tine *et al.* 2014), rainbow trout (Berthelot *et al.* 2014), Japanese eel (Kai *et al.* 2014), half-smooth tongue sole (Chen *et al.* 2014) and turbot (Figueras *et al.* 2016). While NGS technology has enabled rapid and cheap reference genome assemblies, they are typically fragmented and incomplete. Further, assembly errors are quite common, and linkage maps can also assist with resolving mis-assemblies (Fierst 2015; Tsai *et al.* 2016a). Aquaculture species typically have an amenable family structure for high-resolution linkage maps, due to the high fecundity resulting in large full and half sibling families. Linkage maps can also be used in conjunction with physical reference genome sequences to detect variation in recombination rates across the genome, with implications for downstream applications (e.g. LD between markers and QTL in association mapping studies).

Mapping QTL associated with traits of economic importance

The rate of application of genomic technology to aquaculture species tends to reflect the degree of scientific and commercial interest of those species. This is typically motivated by the interest of understanding the genetic basis of economically-important production traits, for example growth, disease resistance or sex determination. Researchers working in the high-value salmonid species were amongst the first to exploit RAD-Seq techniques, evaluating resistance to different pathogens causing high economic losses, including infectious pancreatic necrosis in Atlantic salmon (Houston *et al.* 2012), and infectious hematopoietic necrosis (Campbell *et al.* 2014) and bacterial cold water disease (Campbell *et al.* 2014; Liu *et al.* 2015a; Palti *et al.* 2015b) in rainbow trout. Based on early successes, and given the importance of disease resistance to modern aquaculture breeding programmes (Yáñez *et al.* 2014), large-scale projects have been established to apply RAD-like techniques to detect markers, and eventually the genes and causal mutations involved, for improving resistance. For example, the European Union funded FISHBOOST project (www.fishboost.eu) is using RAD sequencing techniques to genotype several thousand animals from large-scale disease challenge experiments in rainbow trout, common carp, European sea bass, gilthead sea bream (*Sparus aurata*) and turbot. These genotype and phenotype data will be used to estimate genetic parameters, map disease resistance QTL and evaluate genomic prediction approaches for disease resistance breeding.

In addition to disease resistance, RAD-Seq association studies have been widely applied for mapping QTL affecting a range of other production-relevant traits, particularly in salmonid species. These include spleen size (Liu *et al.* 2015a) and cortisol response (Liu *et al.* 2015b) in rainbow trout, and thermal tolerance and growth in *Oncorhynchus nerka*, the sockeye salmon (Larson *et al.* 2016). Out with the salmonid genera, RAD-Seq has been performed to map loci affecting disease resistance in olive flounder (*Paralychthys olivaceous*, Shao *et al.* 2015), and growth in bighead carp (*Hypophthalmichthys nobilis*, Fu *et al.* 2016) and turbot (Wang *et al.* 2015b). In addition, RAD-like techniques have been very popular for marker discovery and QTL mapping in bivalve shellfish including Chinese scallop (*Argopecten irradians*; Jiao *et al.* 2014), Akoya pearl oyster (*Pinctata fucata*; Li & He 2014; Shi *et al.* 2014), variously coloured abalone (*Haliotis diversicolor*; Ren *et al.* 2016; Yesso scallop (*Patinopecten yessoensis*; Dou *et al.* 2016) and have also been applied in the shrimp kuruma prawn (*Marsupenaeus japonicas*; Lu *et al.* 2016) and one echinoderm, the sea cucumber (*Apostichopus japonicus*; Tian *et al.* 2015). Interestingly, 2b-RAD has been the most common technique in bivalves, while in finfish, traditional RAD has been more widely utilized.

Using RAD to study sex determination

Sex determination (SD) is one of the most critical traits for many aquaculture species, as phenotypic sex is often not evident in juveniles and sexual dimorphism in growth rate is commonly observed. SD is complex in many fish species, often with polygenic control and an environmental component (reviewed in Martínez *et al.* 2014), and the application of large genotyping projects has been strongly recommended to screen for SD loci in fish (e.g. Pan *et al.* 2016). RAD-like techniques have clearly boosted our knowledge of SD in aquaculture, with studies in Nile tilapia (Palaiokostas *et al.* 2013a, 2015a), Atlantic halibut (*Hippoglossus hippoglossus*, Palaiokostas *et al.* 2013b), European sea bass (Palaiokostas *et al.* 2015b) and turbot (Wang *et al.* 2015b) finding putative sex determining loci. Controlling sex ratio is not only interesting to obtain higher growth rates, but also to avoid size dispersion or to delay sexual maturity. Further, there are some clear examples, like the sturgeon, where the commercial advantage of rearing fish of one sex over the other is obvious.

Genomic selection approaches

While QTL mapping and MAS approaches can be successful when the genetic architecture of a trait suggests a gene of major effect (e.g. IPNV resistance, Houston *et al.* 2008;

Moen *et al.* 2009), improvement of polygenic traits using genomic data is more effectively achieved using genomic prediction of breeding values (Meuwissen *et al.* 2001). Studies of genomic selection in aquaculture were first carried out in salmonid fish, with simulated (Sonesson & Meuwissen 2009; Lillehammer *et al.* 2013) and empirical (Ødegård *et al.* 2014; Tsai *et al.* 2015, 2016b; Vallejo *et al.* 2016) data, demonstrating the clear advantages over pedigree-based methods. Studies using varying marker densities for prediction in salmonids have highlighted that as few as a thousand SNPs may be adequate for achieving the gain in selection accuracy versus pedigree approaches (Ødegård *et al.* 2014; Tsai *et al.* 2015, 2016b). Therefore, it is reasonable to assume that RAD-like techniques may be useful for genomic selection in aquaculture breeding, as typical RAD SNP datasets comprise a few thousand SNPs. Indeed, the potential of this approach has already been highlighted for resistance to bacterial cold water disease in rainbow trout (Vallejo *et al.* 2016), for growth in Yesso scallop (Dou *et al.* 2016), and for resistance to pasteurellosis in gilthead sea bream (Palaiokostas *et al.* 2016).

Genetic traceability and aquaculture sustainability

One of the main concerns for aquaculture producers and consumers is to minimize the environmental impact of fish farming. In this sense, traceability tools are essential to assess the impact of aquaculture escapees in natural populations or distinguish between farmed and wild specimens. RAD-Seq has been utilized to obtain SNPs for sturgeon traceability and conservation (Ogden *et al.* 2013), which will contribute to enforce current legislation on aquaculture and fishing practices but also aid on the handling of wild stocks, critical for sustainable aquaculture. RAD-Seq is also the main tool of the European project AquaTrace (aquatrace.eu), the results of which have been recently presented in the European Aquaculture Society meeting in Edinburgh (Aquaculture Europe 2016). One of the AquaTrace objectives was to assess the impact of escapees on natural populations of European sea bass, gilthead sea bream and turbot, while also developing forensically validated tools for traceability purposes. The results highlighted the utility of RAD-Seq approaches to capture population or family specific variation making it a suitable tool for genetic traceability and conservation of natural populations. This is of the outmost importance for sustainable aquaculture growth, leading to lasting economic benefits, food safety and social acceptance.

RAD-Seq and SNP arrays, towards a peaceful co-existence

The development of NGS has greatly increased the amount of genomic resources available in the most important aquaculture species, including genome assemblies for many of them. Alongside RNA-Seq and whole genome sequencing, RAD-Seq has contributed significantly to the availability of abundant genetic markers compared to a few years ago. While RAD-Seq and similar techniques are likely to remain the genotyping method of choice for species with few genomic resources, several medium and high-density SNP arrays are already available for aquaculture species (Atlantic salmon, Houston *et al.* 2014; Yáñez *et al.* 2016; channel catfish, Liu *et al.* 2014; common carp, Xu *et al.* 2014a; rainbow trout, Palti *et al.* 2015a; Pacific oyster and European flat oyster, Lapègue *et al.* 2014), and many more are unpublished or currently being produced and validated.

Single nucleotide polymorphism arrays are a type of DNA microarray, where hybridization of allele-specific probes results in a fluorescent signal which can be measured to call a genotype in a given loci. They have both advantages and disadvantages over RAD-Seq approaches (Table 3). For instance, the experimental procedures and bioinformatic analyses are much simpler for the user of SNP arrays, requiring less technical knowledge and usually resulting in a faster turnaround. The genotype scoring method is more robust and amenable to automation, and therefore less prone to errors (Hong *et al.* 2012; Wall *et al.* 2014). The repeatability and reproducibility are higher for SNP arrays than RAD-Seq, and genotyped loci are known in advance. However, having a fixed set of loci on the chip is also a disadvantage, especially in species with strong population structure, because of ascertainment bias whereby the SNP set is biased to polymorphic markers in the discovery population(s). This presents a major issue where aquaculture strains for a specific species are highly variable, and the utility of a SNP array will vary hugely depending on the relationship to the discovery population. RAD-like approaches overcome this issue and also offer much greater flexibility to the researcher in terms of the targeted number of loci. Further, RAD-Seq captures variation that is specific to populations, families and individuals that is likely to be missed from SNP array, which are typically biased towards common variants. Another putative advantage of RAD-like

Table 3 General comparison of restriction-site associated DNA sequencing (RAD-Seq) and single nucleotide polymorphism (SNP) chips

	RAD-Seq	SNP arrays
Sample processing	Laborious	Straightforward
Bioinformatic analysis	Complex	Negligible
Turnaround time	Long	Medium
Accuracy	Medium-high	High
Repeatability	Medium	High
Design	Adjustable	Fixed
Cost	Low	Medium

techniques is that the direct cost of the experiment is cheaper, although the additional time required for library preparation and bioinformatics analyses should be considered into any comparison.

In the near future, genomic selection (GS) is likely to be a key technique for breeding programmes of many aquaculture species, due to the demonstrable increase in selection accuracy versus current pedigree-based methods. SNP arrays are now routinely used in livestock breeding programmes for GS and are increasingly utilized in technologically advanced aquaculture breeding. Several studies have shown that only moderate SNP marker density is required for effective GS in salmon (Ødegård *et al.* 2014; Tsai *et al.* 2015, 2016b). Vallejo *et al.* (2016) compared both RAD-Seq and SNP arrays for GS to BCWD resistance in rainbow trout, finding similar selection accuracies for both techniques despite higher marker density from the SNP chip (~40k SNP array versus ~10k RAD-Seq). This may reflect high levels of linkage disequilibrium in typical aquaculture family selection programmes, whereby trait recording is often performed on close relatives of the selection candidates. Therefore, the higher marker density associated with SNP chips may be advantageous when predicting breeding values in animals more distantly related to the training population (Tsai *et al.* 2016b), or in species with greater effective population sizes and/or lower levels of linkage disequilibrium. However, given the relatively short genomes of many nonsalmonid aquaculture species (i.e. European sea bass – ~763 Mb, or turbot – ~658 Mb; Atlantic salmon – ~2970 Mb), the typical marker density generated by RAD-like techniques may be perfectly adequate for effective GS. However, this needs to be tested, as the recombination frequency and patterns of linkage disequilibrium across the genome are pertinent to the question of adequate marker density. Further reductions in marker density requirements are likely to be observed when genotype imputation approaches are used, for example genotyping parents at high density, and offspring for a small subset of the markers. As already mentioned, RAD methods allow for substantial flexibility in terms of number of genotyped markers. In addition, lowering average sequence coverage in the offspring with parents sequenced at high coverage could be used to generate genotype data at a much lower cost.

Targeted GBS techniques

Both RAD-Seq and SNP arrays will also have to compete with recently developed genotyping methods based on targeted genotyping by sequencing. For example, genotyping-in-thousands by sequencing (GT-Seq, Campbell *et al.* 2015) is a method of targeted sequencing which follows a multiplex PCR approach, where hundreds to thousands of loci (amplicons) are selected for genotyping. In this method, a multiplex PCR using loci-specific primers that also contain Illumina sequencing primers is used to amplify the targeted regions. Unique barcodes for each sample are added with a second PCR reaction, followed by pooling and sequencing of samples. Unlike RAD techniques, this method requires previous knowledge to design the assays, and the number of SNPs genotyped in a single run is limited to a few thousand. Similar technologies are now provided by major genotyping technology providers, and it appears likely to become one of the most cost-effective systems of genotyping targeted SNPs. Other GBS targeted-sequencing techniques have also been recently developed, for example RAD capture (Rapture), where preselected RAD tags are isolated using capture probes and then sequenced (Ali *et al.* 2016). These targeted GBS techniques have the potential to become major players in aquaculture breeding and genetics due to their simplicity and flexibility. However, in part, they suffer from the same limitation as SNP arrays that they require prior knowledge and selection of the SNPs that are useful in the population of interest.

Future outlook

Restriction-site associated DNA sequencing techniques have driven a major increase in the application of genomics to aquaculture species. While the catalogue of SNP arrays for aquaculture species will increase in the coming years, it is likely that RAD techniques will continue to be widely applied. We anticipate that both techniques will co-exist for several years, and the choice of RAD-Seq or SNP chip will depend on the species and project-specific factors. For example, it may be that high-value aquaculture species with larger genomes (e.g. salmonids) are more suitable for SNP arrays, while lower-value species with smaller genomes (and/or higher levels of LD) are more suitable for RAD techniques, although it will also depend on the resources available for each particular project. Targeted GBS techniques like GT-Seq are likely to find a niche in genotyping hundreds to several thousands of previously identified SNPs across many samples. Further, RAD techniques are likely to remain the gold standard for new aquaculture species and/or those produced on a smaller scale, where SNP arrays are not available, and genomic resources are scarce. Eventually the cost of generating and analysing sequence data may drop to a level where genome complexity reduction is no longer required, but it seems unlikely in the short term. Therefore, RAD sequencing will continue to flourish in aquaculture research in the following years and is likely to be routinely applied to deliver the benefits of genomic

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selection to selective breeding of many different aquaculture species.

Concluding remarks

The appearance of genotyping by sequencing technologies has provided the aquaculture research community with a hugely valuable method for identifying and concurrently genotyping large numbers of genetic markers in species with limited genomic resources. Further, these techniques have become multi-purpose tools for addressing several topics of research and commercial interest like genetic diversity, population and family structure, association analyses with traits of economic interest, and genomic selection. Despite the increasing availability of genomic resources and the increasing number of SNP arrays, RAD techniques will continue being important for aquaculture research and application to selective breeding in the next few years. RAD sequencing and other genotyping by sequencing currently offer unequalled versatility and cost-effectiveness for meeting the needs of many diverse research projects.

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REVIEW

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Aquaculture industry prospective from gut microbiome of fish and shellfish: An overview

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Abstract

The microbiome actually deals with micro-organisms that are associated with indigenous body parts and the entire gut system in all animals, including human beings. These microbes are linked with roles involving hereditary traits, defence against diseases and strengthening overall immunity, which determines the health status of an organism. Considerable efforts have been made to find out the microbiome diversity and their taxonomic identification in finfish and shellfish and its importance has been correlated with various physiological functions and activities. In recent past due to the availability of advanced molecular tools, some efforts have also been made on DNA sequencing of these microbes to understand the environmental impact and other stress factors on their genomic structural profile. There are reports on the use of next-generation sequencing (NGS) technology, including amplicon and shot-gun approaches, and associated bioinformatics tools to count and classify commensal microbiome at the species level. The microbiome present in the whole body, particularly in the gut systems of finfish and shellfish, not only contributes to digestion but also has an impact on nutrition, growth, reproduction, immune system and vulnerability of the host fish to diseases. Therefore, the study of such microbial communities is highly relevant for the development of new and innovative bio-products which will be a vital source to build bio and pharmaceutical industries, including aquaculture. In recent years, attempts have been made to discover the chemical ingredients present in these microbes in the form of biomolecules/bioactive compounds with their functions and usefulness for various health benefits, particularly for the treatment of different types of disorders in animals. Therefore, it has been speculated that microbiomes hold great promise not only as a cure for ailments but also as a preventive measure for the number of infectious diseases. This kind of exploration of new breeds of microbes with their miraculous ingredients will definitely help to accelerate the development of the drugs, pharmaceutical and other biological related industries. Probiotic research and bioinformatics skills will further escalate these opportunities in the sector. In the present review, efforts have been made to collect comprehensive information on the finfish and shellfish microbiome, their diversity and functional properties, relationship with diseases, health status, data on species-specific metagenomics, probiotic research and bioinformatics skills. Further, emphasis has also been made to carry out microbiome research on priority basis not only to keep healthy environment of the

fish farming sector but also for the sustainable growth of biological related industries, including aquaculture.

KEYWORDS
aquaculture, bioindustry, finfish, microbiome, shellfish

1 | INTRODUCTION

The current knowledge of microbiome composition in fish and shellfish is actually derived from the various works that have been carried by different workers and also from numerous scientific publications. From these reports, it appears that microbiome focus is on farmed finfish and shellfish and among fish; salmonids have been paid much attention. Attempts have been made to investigate the microbiome associated with finfish and shellfish by the number of workers and the focus of microbial studies in earlier years was mainly on the different parts of the body like skin, gills and gastrointestinal tract (Harrison, 1929). This is because these body parts are the major routes for the entry of microbes into fish and shellfish (Bøgwald & Dalmo, 2014; Chen et al., 2017; Granados et al., 2017; Ringø et al., 2007, 2008; Seetharam et al., 2015). Li, Ringø, et al. (2018) reported that microbial communities that are associated with different parts of the body including intestinal tract affect health of the fish. It is emphasized that mucosal adhesion particularly in the intestinal tract is very critical for bacterial infection during early phases of development of fish. Nayak (2010), while carrying out studies on the role of gastrointestinal microbiota in fish, summarized the most commonly reported bacterial organisms in salmon fish. However, current trends of the research are concentrated on micro-organisms living inside the digestive tract and these are linked with roles involving different physiological functions like defence against diseases, modulating immune response, affecting nutrient absorption, regulating metabolic processes and synthesizing vitamins (Granados et al., 2017; Gómez & Balcázar, 2008; Romero et al., 2014; Sullam et al.,2012). There are several other reasons why microbiome is important in maintaining optimal health and well-being of an organism. Gut microbes play a significant role in the absorption of essential nutrients and produce the energy they need throughout the day and without gut microbiome, digesting and breaking down compound molecules from food cannot be accomplished. The metabolic activity of gut microbiome also influences diet requirement, food cravings and sensation of a full appetite and even determines the nutritional pattern. For identification of microbiome species, the conventional methods used earlier were phenotypic and biochemical characteristics and a variety of culture media were also used to enumerate bacterial levels. In recent years due to availability of advanced genetic engineering tools like next-generation sequencing (NGS) technologies, researchers have been able to analyse community of the microbiome at greater depth with cost effectiveness (Tarnecki et al., 2017). This has resulted in undertaking many investigations in genome structural composition of microbial species in finfish and shellfish. This kind of genetic information of the microbes will facilitate to do any type of microbial manipulation to enhance their required functional properties like feed efficiency and disease resistance. Unfolding of the knowledge with regard to host–microbe relationships and identification of microbial community functions is very much essential for building a healthy microbiome environment in cultivable organisms and also in aquaculture farming systems (Tarnecki et al., 2017). Talwar et al. (2018) while reviewing fish gut microbiome carried out in-depth analysis on the diversity and functional aspects of the different microbes. Further, they emphasized that by studying functional aspects of microbiome, there is a possibility of enhancing aquaculture production on sustainable basis. Considerable studies covering these aspects have also been carried recently by Legrand et al. (2019), Perry et al. (2020) and Villamil et al. (2020). Legrand et al. (2019) while doing in-depth analysis of relationship involved between host and microbial system in fish, reported functional involvement of microbiota with host and their interplay mechanisms. Numerous drivers of microbiome diversity have been mentioned for fish including environmental factors, dietary and genetic factors too. They emphasized that in order to understand functional interactions and association between bacteria and other important microbes with host, more innovative procedures like metagenomics, metatranscriptomics, meta-proteomics or metabolomics are very much essential. Such techniques may provide vital information regarding functional interplay between micro-organisms and its host.

In the case of shellfish, particularly in shrimps, microbiome studies have been carried out earlier by conventional methods. In most of the studies, the presence of microbiome diversity observed was culture-based methods adopted in the shrimp farming system. Using these traditional methods, a number of attempts have been made in the past to investigate microbiome composition among penaeid shrimp species viz., *Penaeus monodon* (Rungrassamee et al., 2014), *Fenneropenaeus chinensis* (Liu, Wang, et al., 2011), *Penaeus penicillatus* (Wang et al., 2014) *Penaeus merguiensis* (Oxley et al., 2002) and *Litopenaeus vannamei* (Huang et al., 2010, 2014, 2019). Hepatopancreatic tissue and the digestive tract were the main target organs for microbiota analysis, and a comparison was made between the shrimp collected from the wild and those cultured in grow-out ponds (Xiong et al., 2016). There is a paucity of information on the microbiota in shrimp organs other than the intestine because the digestive tract is continuous one and well connected with hepatopancreatic tissues (Cheung et al., 2015). At present, there is only one report on the microbiome analysis of hepatopancreatic tissue in the shrimp *Neocaridina denticulate* (Cheung et al., 2015). Due to the outbreak of diseases caused by acute hepatopancreatic necrosis in

the shrimp, globally shrimp aquaculture has suffered heavy losses (Joshi et al., 2014). In order to minimize these losses, efforts are being undertaken to understand molecular basis of the disease and pathogens involved (Gomez et al., 2014).

Granados et al. (2017) carried out studies on microbiome composition of shrimp collected from different ecological niches, viz. wild, aquacultured and those that are exposed to acute hepatopancreatic necrosis disease (AHPND)/early mortality syndrome (EMS) outbreak conditions. In this study, comparison of differential bacterial community composition was made and focus was on the analysis of microbiota of hepatopancreatic tissue and intestine of shrimp from these three environmental situations and as well as from pond sediments of the hatcheries. Early studies utilized a variety of culture media to enumerate bacterial levels and used phenotypic and biochemical characteristics for identification of isolates but now due to the limitations of such approaches, the method adopted is sequencing of 16S rRNA as the gold standard for isolate identification and characterization of the composition of total bacterial communities. In this particular study, microbial analysis was carried out by using sequencing of seven hyper variable regions of the 16S rRNA gene. From analysis of the sequence data pooled from all the samples, it has been reported that the microbiota RNA gene and their functional metagenomics were completely different in the shrimp collected from different environmental niches. Similarly, the microbiota of hepatopancreatic tissues and intestines showed significant differences in their genome. However, mention has been made that there has been a lot of similarity of microbial diversity between sediment and intestine of the cultured shrimps. Cardona et al. (2016), while working on the relationship between bacterial community of seawater and intestine of shrimp *L. stylirostris* in a bio-flock system noticed that the similarity was not that significant. Similar observations were also made earlier by Johnson et al. (2008), while working on the microbial community of seawater, foregut and hindgut during different phases of growth of the shrimp *L. vannamei* in closed system aquaculture. Further, it is also reported that changes in the microbiota were observed in the shrimp of early developmental stages affected by AHPND/EMS disease. Healthy cultured shrimp were found to be associated with the dominance of bacterial species like *Faecalibacterium prausnitzii* and *Pantoea agglomerans*, whereas the diseased shrimp were found to be associated with bacterial communities enriched with *Aeromonas taiwanensis*, *Simiduia agarivorans* and *Photobacterium angustum* (Granados et al., 2017). Granados et al. (2017) further mentioned that when the shrimps are affected by disease, they lose the ability to select bacterial residents in stomach and in that process; the environmental factors become more important in shaping stomach microbiota. These findings are in agreement with similar observations made by Xiong et al. (2017) while working on gut microbiota of diseased shrimp.

Similar attempts were made by Chen et al. (2017) while working on microbiome dynamics of shrimp (*L. vannamei*) infected with acute hepatopancreatic necrosis disease (AHPND). This syndrome is generally known for high mortality when an outbreak of disease occurs in 'grow-out' ponds. It has been reported that some 62 samples of post-larvae of shrimp were collected from grow-out ponds with this infectious bacteria (AHPND) in pond water. The microbiota collected from shrimp's stomach and pond water were analysed for sequencing of 16S rRNA gene with Illumina sequencing technology. The results indicated that microbiota collected from stomach and pond water underwent varied dynamic modifications. There was a significant change in the bacterial diversity in stomach of shrimp infected with AHPND. The microbiome composition showed the presence of *Vibrio* and *Candidatus Bacilloplasma* as predominant in numbers, and further, it is mentioned that there was a change in species-to-species connectivity and complexity of the interaction network. From these observations, it was concluded that the AHPND disease outbreak in shrimp ponds can be minimized/controlled by studying microbiome dynamics (Chen et al., 2017). In this review, we have made efforts to present comprehensive information on various aspects of fish and shellfish microbiomes and how knowledge of microbiomes influences the development of the bio/aquaculture industry. Emphasis has been given to the gut microbiome diversity, functional aspects of different microbial species, microbiome relationship with disease control and health status of the animals. Other aspects covered in this review are the microbiome and its importance for the growth of sustainable aquaculture, prospects of microbiome-dependent aquaculture industry, microbial species as a source of manufacturing of innovative products and use of bioinformatics skills in microbiome research to accelerate the growth of the bio and aquaculture industry.

2 | MICROBIOME DIVERSITY AND METAGENOMICS

2.1 | Fish microbiome

The number of workers has classified microbial data in the gut system of animals depending upon duration of stay of the bacteria present either temporarily or on a permanent basis (Bhatt et al., 2018; Shade & Handelsman, 2012). The temporary microbiota generally comes through feed and does not last for a long time, whereas the resident or permanent microbiota lives in the host intestinal membrane, playing a role in symbiotic relationships (Zhang et al., 2016). For the identification of microbial species and enumerate them properly, a variety of culture media and biochemical characteristics were used in earlier studies. But now with the advent of the availability of modern genetic engineering tools like NGS technology, identification of bacterial isolates has become easy, precise and cost-effective (Wetterstrand, 2016). Several researchers have adopted the use of 16S rRNA sequencing as the gold standard for isolate identification and variability in the 16S rRNA to characterize the composition of total bacterial communities via culture-independent means (Clements et al., 2007; Gao et al., 2010; Kim et al., 2007; Llewellyn et al., 2014). In earlier years, many studies have been carried out on microflora present in different parts of the body of fish, including the digestive tract. Body parts like skin, gills and gastrointestinal (GI) tract are recognized as

major routes for entry of microbial communities in fish (Bøgwald & Dalmo, 2014; Ringø et al., 2007), and therefore, the majority of the research works targeted microbial communities are associated with these portions of the body. However, microbiome research has grown significantly in recent years due to expansion and continuous growth of the aquaculture industry and more focus is being now emphasized on exploration and survey of microbes in the digestive gut of several finfishes and shellfishes (Bentzon-Tilla et al., 2016; Bruijn et al., 2018; Leung & Bates, 2013; Murray & Peeler, 2005; Perry et al., 2020; Ringø et al., 2015; Romero et al., 2014; Tran et al., 2017; Villamil et al., 2020; Walker & Winton, 2010). While discussing teleost fish gut microbiome in the context of sustainable aquaculture, Perry et al. (2020) emphasized four important aspects viz, diet, immunity, artificial selection and closed loop system. The niche areas identified are use of insect-based feed, vaccination, understanding the mechanism involved in use of prebiotics and probiotics, artificial selection on the hologenome, role of bacteriophages in circulating aquaculture system and as a biomarker. In order to resolve the issues of comparability, reproducibility and meta-analysis of fish gut microbiome, it is suggested to use one marker and one sequence platform with many metabarcoding microbiome studies (Perry et al., 2020). It is now well-established fact that the health status of animals depends on the presence of a healthy microbiome environment in digestive tract of cultivable organisms (Sullam et al., 2015; Xiong et al., 2016; Zhu et al., 2016). Despite this, no adequate attention has been paid to gut microbiome of invertebrates, particularly with crustaceans' animals of aquaculture importance. It is important that the knowledge of host species-specific gut microbiome and actual gut microbiota present coupled with health status of aquatic animals will reveal the role of microbiomes in host health and the underlying ecological mechanisms (Perry et al., 2020; Sullam et al., 2012; Xiong et al., 2016).

Ringø et al. (1995) while working on microbial population in digestive tract of fish reported the presence of very dense population of microbes in entire gut system than the surrounding water. This indicates that digestive tract provides a very favourable ecological niche for such micro-organisms. Furthermore, they estimated the aerobic and anaerobic bacterial counts in intestinal tract of fish and the number estimated were 10^8 aerobic bacteria g^{-1} and approximately 10^5 anaerobic bacteria g^{-1}. Navarrete et al. (2009) while doing molecular analysis of microbes present in the digestive tract of the Atlantic salmon; *Salmo salar* found that 60% of the microbiota belonged to *Pseudomonas* bacteria. Roeselers et al. (2011) studied microbiome communities in digestive tract of zebra fish using NGS technology and reported that there was a significant difference in the diversity of microbiome of fish collected from wild and domesticated ones. While carrying out studies on faecal microbiome of invasive carp species in relation to different environmental locations, Eichmiller et al. (2016) mentioned that the primary composition of gut microbiome always depends on the type of environmental niche where fish inhabits. In support of this observation, Sullam et al. (2012) while investigating the composition of microbiome communities in digestive tract of freshwater fishes in relation to different ecological niches also reported similar findings and further mentioned that host trophic level, habitat and perhaps host phylogeny are also determinant factors for shaping the core gut microbiome diversity. Furthermore, it has also been reported that the composition of microbiome community in both freshwater fish and those inhabiting the marine environment is almost similar regardless of their phylogeny (Sullam et al., 2012). Tarnecki et al. (2017) while studying fish gut microbiome observed that *Aeromonas* species of bacteria were dominant in digestive tract of freshwater fish, whereas in marine fish, *Vibrio* species of bacteria were predominant. Many researchers have reported the presence of other groups of bacteria, viz, *proteobacteria*, *fusobacteria*, *firmicutes*, *actinobacteria* and *verrucomicrobia* in digestive tract of both freshwater fish and marine fish (Eichmiller et al., 2016; Larsen et al., 2014; Liu et al., 2016). The presence of one of the most important bacterial species viz. *Cetobacterium somerae* has been reported in gut of freshwater fish, which is known to produce large quantities of vitamin B12 and can prevent the growth of harmful pathogens (Etyemez & Balcazar, 2015; Gaulke et al., 2016; Larsen et al., 2014; Lyons et al., 2015). Further, it is mentioned that this particular microbiome species are difficult to culture under laboratory conditions and warrants future research. Several studies have pointed out that the presence of a common core microbiome in the gut of different fish species across environment and there are no relationships with the host genetics or phylogeny (Hennersdorf et al., 2016; Roeselers et al., 2011; Wilson et al., 2008). However, in order to build up this kind of hypothesis, more research is required on fish gut microbiome so that one will be able to manipulate genetic structure in bacterial communities to promote health and well-being in fishes. Number of workers has attempted to investigate gut microbiome of carps, because this fish group is dominating on priority of the aquaculture industry all over the world (FAO, 2018). Bacterial species like *Proteobacteria*, *Firmicutes* and *Fusobacteria* are found in abundance in gut system of carps, but density of population always differs according to environmental conditions (Eichmiller et al., 2016; Liu et al., 2016; Ni et al., 2014; Wu et al., 2013; Yan et al., 2016). Generally, in Asian carps, bacterial species belonging to the phylum Bacteroidetes and Cyanobacteria have been reported to be in abundance (Kashinskaya et al., 2015; Li et al., 2015; Ye et al., 2013) and are considered as core gut microbiome. Eichmiller et al. (2016) and Yan et al. (2016), while studying microbiome in gut system of grass carp reported that the genus *Cetobacterium* was found to be abundant in density. Similar observations have been made earlier in common carp and bighead carp by Kessel et al. (2011) and Li et al. (2015). Li et al. (2015) observed the dominance of *Aeromonas* species of bacteria in digestive gut of grass carp, whereas the dominance of other bacterial species, viz., *Veillonella*, *Rothia* and *Methylocystaceae* has been noted by several other workers (Wu et al., 2012, 2013). In Atlantic salmon, which is one of the major species of aquaculture in United States, number of workers has noted the dominance of *Proteobacteria* and *Firmicutes* in digestive gut system irrespective of whether it is cultured in freshwater or saltwater (Dehler et al., 2017; Gajardo et al., 2016; Schmidt et al., 2016; Zarkasi et al., 2016). Dehler et al. (2017) mentioned the dominance of bacterial species

like *Ruminococcaceae*, *Mycoplasmataceae* and *Pseudomonas* in gut microbiome of juvenile salmon in freshwater environments. In marine adult salmon during cold temperatures, Gajardo et al. (2016) found *Leuconostoc* and *Weissella* as dominant members of the bacteria in gut microbiome community, whereas in warmer waters, *Vibrio*, *Aliivibrio* and *Photobacterium* bacterial species were found to be dominant (Zarkasi et al., 2014). Desai et al. (2012) from their studies also observed a more varied microbiome community composition in gut of Atlantic salmon. From these results, it can be said that salmon fish have got the capacity to change their microbiome community according to changes in the environment. In Nile tilapia fish (*Oreochromis niloticus*) *Proteobacteria* and *Fusobacteria* have been reported as dominant phyla in the gut microbiome environment (Giatsis et al., 2015; Ran et al., 2016; Zhang et al., 2016), whereas Zhang et al. (2016) also observed the dominance of other bacterial groups such as *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* in the same fish reared in saline water. However, Standen et al. (2015) observed the presence of allochthonous microbiome of Nile tilapia and identified Firmicutes as the primary phylum. Yan et al. (2016) mentioned that the differences observed in microbiome community also depend on feeding habits of fish like herbivore or omnivore nature of the species.

Merrifield and Rodiles (2015), while working on the fish microbiome and their interaction with mucosal membrane, mentioned that there exists a large diversity in the microbiome community in fish gut and the microbes include protoctista, fungi, yeasts, viruses and members of the bacteria and archaea. Recent literature findings indicate that fish gut microbiome is almost similar to mammals (Ktari et al., 2012), and *Proteobacteria* are found to be the dominant groups among fish gut microbiome diversity (Rombout et al., 2011). Clements et al. (2014) described that the density, composition and function of the microbiota change in different parts of fish gut system. Das et al. (2014) while working on 'enzyme producing' bacteria in different parts of the digestive tract of four brackish water fish species (*Scatophagus argus*, *Terapon jarbua*, *Mystus gulio and Etroplus suratensis*) reported that density of these bacteria increased in gut from the proximal to distal portion. Several workers also found similar trends while working on the microbiota of different fish (Fidopiastis et al., 2006; Hovda et al., 2007; de Paula Silva et al., 2011; Ringø et al., 2006; Zhou et al., 2007). In contrast, while working on yellow grouper, *Mycteroperca venenosa*, Zhou, Liu, et al. (2009) reported that there was no such trend in the microbiome density in different parts of digestive tract. Even in Atlantic salmon, similar trend has been observed by Navarrete et al. (2009). Some researchers have mentioned that numerous factors are responsible for variability in fish gut microbiome and some have said that variations in the physiological environment between microbiota and digestive tract can also be possible causes (Clements et al., 2014). To assess the microbiome community, fish culture-based techniques have been used in the past, but in recent times, culture-independent techniques are being used to compare the microbiome community in different gut portions of fish, including stomach (Ringø, 1993; Ringø et al., 1998; Zhou, Shi, et al., 2009; Zhou et al., 2008). Paula Silva et al. (2011) and Estruch et al. (2015) reported the dominance of *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* in stomach of gilthead sea bream, *Sparus aurata*. They also reported the dominance of the presence of bacterial group belonging to the genera *Photobacterium* and *Corynebacterium*. In another study that included analysis of the adherent stomach microbiota, found greater diversity of bacteria in stomach of yellow grouper compared to other sections of the gut (Zhou, Liu, et al., 2009). Zhou, Shi, et al. (2009) while studying the microbiome diversity in the fish red snapper, *Lutjanus sebae*, mentioned that there were no significant differences in the composition of microbiome community in the stomach and other parts of digestive gut. However, Ringø et al. (2006) observed that dietary intervention changed the composition of microbiome community in Atlantic cod, *Gadus morhua*. In the fish fed with fishmeal diet, they observed that bacteria like *Psychrobacter* and *Brochothrix* were found to be dominant in foregut and mid-gut, while a bacterium belonging to the family Carnobacteriaceae was dominant in the hindgut microbiota. Interestingly, fish fed with soybean meal and bioprocessed soybean meal diets had *Psychrobacter* dominating throughout the gut. Hovda et al. (2007) studied the microbiota diversity in the different portions of the gut system of the fish Atlantic salmon and found that *Proteobacteria*, *Pseudomonas*, *Acinetobacter* and *Vibrio* were predominant in foregut, in mid-gut *Proteobacteria* species like, *Photobacterium phosphoreum* and *Pseudomonas* were in large numbers, while in the hindgut, it was *Vibrio* and *P. phosphoreum* that were present in higher numbers. Because of the different methods adopted for the investigation of microbiome diversity in fish, it becomes difficult to bring out a real true picture of the diversity analysis and even for comparison of the results obtained by different workers becomes difficult. Egerton et al. (2018), in their review of gut microbiome of marine fish, mentioned that microbiome genera analysed from thirty fishes indicated that *Vibrio*, *Photobacterium* and *Clostridium* are the most frequently reported and dominant genera. Sullam et al. (2012) also mentioned that *Vibrio* and *Photobacterium* accounted for 70% of the microbiota in the marine fish they studied. Xiong et al. (2019) while discussing the role of gut microbiota in controlling fish diseases and improving the immunity mentioned that whenever there is a dysbiosis of the gut microbiota due to any factor, these animals get infectious diseases. Further, it is also suggested that identification of fish diseases can be possible by developing biomarkers from gut microbes, which requires further investigation.

Holben et al. (2002) found that Atlantic salmon collected from two different locations showed the dominance of some other bacterial population in the digestive tract. For example, in fish collected from Scottish hatchery, *Mycoplasma* was dominant (81%), whereas in a Norwegian hatchery, *Acinetobacter* accounted for 55%. Similarly, Pond et al. (2006) and Michl et al. (2017) reported that the intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*) showed the dominance of *Clostridium* and *Aeromonas* bacterial populations. Similarly, Kim et al. (2007) also reported the dominance of *Clostridium* in gut microbiota of rainbow trout. Ley et al. (2008) mentioned that bacterial diversity is generally influenced by dietary habits of fish. Their observations were based on the studies on microbiome analysis of

digestive tract of salmon fed with carnivore, herbivore and omnivore diet. Ward et al. (2009) made similar observations while studying the microbiome of Antarctic fish. In contrast, Smriga et al. (2010) mentioned that the microbiota in the three fishes collected from the coral reef inhabiting different diet trophic levels showed a predominance presence of *Proteobacteria* in gut microbiome, followed by *Gammaproteobacteria*, *Fusobacteria Planctomycetes* and *Firmicutes*. The authors suggested that the observed differences among fishes may reflect gut microbiota and/or bacterial assemblages associated with different ingested prey. Some studies have reported the presence of yeast and protozoa as part of the microbiota in gut system of the fish (Gatesoupe, 2007; Grim et al., 2002; Li et al., 2009; Merrifield et al., 2011). However, further investigation is needed to find out the role of yeast and protozoa in maintaining fish health and nutrition. Clements et al. (2009), while working on microbiota of the herbivorous fish, reported that fish use the microbiome components to convert carbohydrates into short-chain fatty acids through fermentation and these fatty acids will be further absorbed by fish gut epithelial cells. Fidopiastis et al. (2006) while assessing the microbiota in the digestive tract, particularly the hindgut of zebra perch, *Hermosilla azurea*, using the technique of 16S rDNA and which have been fed with macroalgae diet reported that the bacterial count and the concentration of short-chain fatty acids were significantly on higher side. Furthermore, it is mentioned that the microbiota composition was dominated by *Proteobacteria*, *Enterovibrio* and *Desulfovibrio*. In contrast, Moran et al. (2005) reported that in another herbivorous fish, *Kyphosus sydneyanus*, the microbiota composition showed the dominance of the bacterial genus *Clostridium*. In still another study, microbiome composition of three herbivorous fishes using the technique of next-generation sequencing technology showed that the bacterial group belonging to *Clostridia* was dominant in the digestive tract (Mountfort et al., 2002). Variations and differences in the results of microbiome composition obtained by different workers may be again due to differences in the methodology adopted for identification of the microbes. Several studies have recorded the presence of lactic acid bacteria in the digestive gut system of various fishes (Gatesoupe, 2007; Lauzon & Ringø, 2012; Navarrete et al., 2010, 2012; Vazquez et al., 2005). Merrifield et al. (2010) reported that there is a large potential for the development of probiotics from such number of endogenous lactic acid bacterial strains. Gatesoupe (2007) reported the presence of yeasts in the digestive gut of several fishes from both freshwater and marine waters. However, there is no much information available on the role of yeast in fish health and nutrition. Kutty and Philip (2008) and Song et al. (2010) mentioned that marine yeasts participate in several ecological processes, particularly in the decomposition of aquatic plant nutrient recycling, biodegradation of oil and recalcitrant compounds. There are also reports that yeast produces number of vitamins, pigments, cell proteins, immunostimulants and enzymes (Chi et al., 2009). Future research is required to find out the exact role of yeast in fish microbiome.

Some of the researchers have reported that the microbiome organisms found in the host fish are not always found to be pathogenic, but they have different vital functions benefiting the organisms. For example, Samad et al. (2014) and Chen et al. (2015) found that bacterial species like *Vibrio alginolyticus can* work as probiotics in fish Atlantic salmon, protecting against pathogenic bacterial species viz. *Aeromonas salmonicida*, *Vibrio anguillarum and Vibrio ordalii* (Austin et al., 1995). It has been reported that the number of *Vibrio* species produces hydrolytic enzymes and helps in breaking down the dietary components in the digestive gut system. There are several reports mentioning about described bacterial strains producing enzymes like amylase, lipase, cellulose and chitinase (Gatesoupe et al., 1997; Itoi et al., 2006; Sugita & Ito, 2006). The presence of numerous strains of luminous *Photobacterium* in the digestive tract of the number fishes has been reported by Ward et al. (2009) and Smriga et al. (2010). There are also reports on the presence of non-luminous *Photobacterium* in gut system of cold water fishes (Onarheim et al., 1994; Urakawa et al., 1999). It has been indicated that, many *Photobacterium* species helps in chitin digestion in the host fishes (Itoi et al., 2006). Clements et al. (2007), Clements et al. (2009), while working on the microbiome of marine fishes, found that the bacterium species viz. *Clostridium* is predominantly present in digestive tract, particularly in hindgut of many herbivorous fishes and performs the function of host's nutrients supplying fatty acids and vitamins. In southern flounder, *Paralichthys lethostigma*, Ramirez and Dixon (2003) mentioned that *Clostridium* along with other Gram-negative bacteria displayed enzyme activities of acid and alkaline phosphatases. It has also been reported that some species of *Clostridium*, viz., *C. butyricum*, have been used successfully as probiotics in aquaculture, enhancing the resistance of rainbow trout to vibriosis and stimulating the immune response and improving survival in Japanese flounder (Taoka et al., 2006). The gut microbiome composition of different fish species is given in Table 1.

2.2 | Shellfish microbiome

The development of the commercial culture of shellfish, particularly shrimp, has been accompanied by the occurrence of infectious and non-infectious diseases. Many of the important microbial diseases are caused by organisms that are part of the normal microflora and fauna. These organisms are opportunistic pathogens that cause disease only under conditions that favour them over the host. Many organisms in this category are ubiquitous, and most have been reported from major shrimp culture areas around the world. Among this category of microbiome pathogens, filamentous bacteria, the peritrich protozoans, the invasive bacteria and the fungi are very common. Among the most important disease-causing microbiomes are viruses and these viruses may once have been limited in their geographic distribution in wild stocks, but they have become widespread in shrimp culture facilities. With the advent of the establishment of several commercial shrimp hatcheries, the shipment of brood stock and post-larvae from these culture facilities to other farms in different geographic regions has often resulted in the spread of these pathogenic microbiomes outside their normal range in wild populations. With respect to disease agents, the Global Aquaculture Alliance

TABLE 1 Gut microbiome composition of different fish species

Fish species	Gut microbiome composition profile (core groups)	Reference
Gadus morhua	*Vibrio/Aeromonas*, *Pseudomonas*, *Cytophaga/Flexibacter*, *Lactobacillus*	Strøm and Olafsen (1990), Strøm and Ringø (1993)
Hippoglossus hippoglossus	*Cytophaga/Flexibacter/Flavobacterium*, *Vibrio/Aeromonas Vibrio/ Aeromonas*	Bergh et al. (1994), Bergh (1995)
Solea solea	*Pseudomonas/Alcaligenes*, *Vibrio/ anaerogenic Aeromonas*, *Moraxella*, *Enterobacteriaceae*, *Flavobacterium/Cytophaga*, *Moraxella*, *coryneforms*	Campbell and Buswell (1983)
Scophthalmus maximus	*Vibrionaceae*, *Aeromonas*, *Acinetobacter* *Pseudomonas/Alcaligenes*, *Flavobacterium/Cytophaga*, *Enterobacteriaceae*, *Acinetobacter*, *Photobacterium*, *Moraxella Aeromonas*, *Vibrio*, *Enterobacteriaceae*, *Cytophaga*, *Micrococcus*, *Staphylococcus*, *coryneforms Oxidative Gram-negative rods*	Nicolas et al. (1989) Gatesoupe (1991) Blanch et al. (1997) Munro et al. (1993), Munro et al. (1994) Ringo et al. (1996) Blanch et al. (1997)
Clupea harengus	*Pseudomonas/Alteromonas*, *Flavobacterium*	Gatesoupe et al. (1997)
Sebastes schlegeli	*Vibrio*, *Pseudomonas*, *Acinetobacter*, *Flavobacterium/Cytophaga*	Hansen et al. (1992)
Pagrus major	*Aeromonas*, *Vibrio*, *Pseudomonas*, *Enterobacteriaceae*, *Cytophaga*	Tanasomwang and Muroga (1989)
Acanthopagrus schlegeli	*Aeromonas*, *Vibrio*, *Pseudomonas*, *Enterobacteriaceae*, *Cytophaga* *Pseudomonas*, *Vibrio*, *Enterobacteriaceae*	Muroga et al. (1987)
Chanos chanos	*Pseudomonas*, *Vibrio*, *Enterobacteriaceae*	Fernandez et al. (1996)
Dicentrarchus labrax	*Vibrio*, *Acinetobacter*, *Moraxella*, *Enterobacteriaceae*	Gatesoupe et al. (1997)
Silurus meridionalis	*Tenericutes*, *Fusobacteria*, *Proteobacteria*, *Bacteroidetes* *Firmicutes*, *Proteobacteria*,	Zhang et al. (2018)
Danio rerio	*Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes* *Planctomyctes*, *Fusobacteria*, *Verrucomicrobia*	Zheng et al. (2017)
Siganus fuscescens	*Proteobacteria*, *Cyanobacteria*, *Firmicutes*	Koo et al. (2017)
Gambusia affinis	*Proteobacteria*, *Planctomycetaceae*; *Flavobacterium*	Nielsen et al. (2017)
Oncorhynchus mykiss	*Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Fusobacteria* *Actinobacteria*	Carlson et al. (2017)
Carassius auratus	*Fusobacteria*, *Proteobacteria Bacteroidetes*	Michl et al. (2017)
Ctenopharyngodon idellus *Megalobrama amblycephala*, *Hypophthalmichthys molitrix* *H. nobilis*,	*Firmicutes*, *Proteobacteria*, *Tenericutes*	Li, Zhou, et al. (2017)
Salmo salar	*Firmicutes*, *Proteobacteria*, *Fusobacteria*, *Bacteroidetes*, *Actinobacteria*	Li, Zhou, et al. (2017)
Oreochromis niloticus	*Fusobacteria*, *Proteobacteria*, *Bacteroidetes*, *Firmicutes*	Dehler et al. (2017)
Dicentrarchus labrax	*Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Firmicutes*	Gajardo et al. (2017)
Siniperca chuatsi, *Silurus meridionalis*, *Carnis megalobramae*, *Cyprinus carpio*, *Canna micropeltes*	*Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia* *Cyanobacteria*	Zhai et al. (2017)
Ictalurus punctatus	*Bacteroidetes*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*	Gatesoupe et al. (2016)
Trematomus bernacchii, *Chionodraco hamatus*, *Gymnodraco acuticeps* *Pagothenia borchgrevinki*	*Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Thermi*, *Bacteroidetes* *Tenericutes*	Yan et al. (2016)
Pimephales promelas	*Proteobacteria*, *Bacteroidetes*, *Fusobacteria*	Narrowe et al. (2015)

(GAA) survey revealed that 60% of losses occurred due to viruses and about 20% due to bacteria. It has been reported that the first serious outbreak of microbial disease in the shrimp occurred during the 1980s in Taiwan and the epidemic was *Monodon baculovirus* (MBV) (Flegel et al., 2008). This was followed by infectious hypodermal and hematopoietic necrosis virus (IHHNV) in the United States (Lightner, 1996), yellow head virus (YHV) in Thailand (Flegel, 1997) and Taura syndrome virus (TSV) again in the United States (Brock et al., 1997).

During the period 1993–2003, when the shrimp industry was still struggling with MBV, IHHNV, YHV and TSV outbreaks, the industry faced another disaster with the arrival of white spot syndrome virus (WSSV). After its first appearance in China in 1992, it spread rapidly around Asia, creating devastating losses at many places in the world (Flegel et al., 2008). Gunalan et al. (2014) while doing the studies on disease occurrence in *L. vannamei* of pond culture system of different regions of India reported the occurrence of six microbial diseases in the shrimp including black gill disease, TSV, IHHNV, WMD (white muscle disease), white gut disease and muscle cramp disease. Bacterial diseases of shrimp have been observed for many years and the number of researchers has noticed that bacterial infection usually occurs when shrimps are weak or culture practices are unhygienic. Even sometimes, normal shrimps are also infected if conditions favour the presence and abundance of a particularly harmful bacterium. Shrimp body fluids are most often infected by bacterial groups named *Vibrio*. Bacteria also invade the digestive tract. When infestation is heavy, filamentous bacteria may also be present in large quantities in gill filaments (Johnson, 1995). Several workers have reviewed the bacterial diseases in shrimp and the range of problems associated with it, leading to mass mortalities. Over 20 species of bacteria associated with shrimp have been recognized, some of which are human pathogens (*Vibrio cholera*, *V. parahaemolyticus* and *V. vulnificus*), while some species are pathogens of aquatic animals (*V. harveyi*, *V. spendidus*, *V. penaecida*, *V. anguillarium*, *V. parahaemolyticus*, *V. vulnificus*) (Otta et al., 2001). Considerable work has been carried out on luminous bacteria like *V. harveyi*, mostly found in coastal and marine waters, in association with the surface and gut of marine and estuarine organisms as well as in shrimp pond water and sediment (Otta et al., 2001). There are reports of causing mass mortalities in shrimp due to the presence of *V. harveyi* (Karunasagar et al., 1994). It has been reported that certain strains of *V. harveyi* had high LD_{50} for *P. monodon* larvae (Karunasagar et al., 1994). Others have reported that strains of this bacterial species virulent to *P. monodon* formed a separate cluster in the protein profile, but no virulence factors have been established in this species (Harris & Owens, 1999; Liu et al., 1996). Brown gill syndrome in *P. monodon* has been reported due to the presence of *V. harveyi* (Pasharawipas et al., 1998), and further, it has been reported that the presence of these bacteria is not that critical for shrimp pathogenicity. Filamentous bacteria such as *Leucothrix mucor*, *Thiothrix* sp., *Flexibacter* sp., *lavobacterium* and *Cytophaga* sp. have been reported in shrimp-causing infections, particularly at the larval stages. Discoloration of gills, low growth and feeding and enhanced mortality are common signs of the disease. A high degree of infection may lead to necrosis in the gill tissue (Karunasagar et al., 2005). More than 20 viruses have been recognized among farmed shrimps as causative agents of various diseases in penaeid shrimps. These viruses can be classified as members of parvoviruses, baculovirus, picornaviruses, toga-like viruses and some of the newly identified virus families. Of these, seven viral pathogens have been listed by the Office International des Epizootics (OIE), considering the extent of damage caused by these viral pathogens (OIE, 2004). Ceccaldi (1997), while carrying out studies on the microbial composition of the hindgut in crustaceans mentioned that the digestive tracts, particularly the hindguts, are the most favourable environments for the presence of large numbers of micro-organisms. The importance of microbial flora in the digestive process varies considerably and bacteria in digestive tract can be a source of food, vitamins or perhaps produce digestive enzymes (Ceccaldi, 1997). The abundance of hindgut microflora was related to neither taxon of the host nor its habitat, but seems to be affected by feeding habits of the animals. Leaño et al. (1998), while working on the penaeid shrimp gut microbiome, reported that 90% of the gut microflora is composed of *Vibrio*. Otta et al. (1999) also observed that *Vibrio* is the most common flora in the natural environment, especially shrimp ponds. Yasuda and Kitao (1980) noticed that when the shrimps are in the zoea stage, they found the presence of high density of bacteria in the gut system. This density of the microbiota decreases in the advanced stages of development. From the mysis stage onwards, the larvae start to feed on larger organisms. This is indeed a pointer to the fact that probiotic organisms can be introduced into the larval stages of shrimp with beneficial effects.

Oetama et al. (2016) investigated the microbiome composition and detection of pathogenic bacteria as in case of the shrimp *P. Monodon*. In this study, they examined the faecal microbiota of the shrimp collected from highly polluted water and compared the same with less polluted waters. The microbiome composition of this kind of study indicated that *Proteobacteria* (96%) was found to be most predominant group, followed by *Bacteroidetes* (2.3%), *Fusobacteria* (0.96%) and *Firmicutes* (0.53%). In addition, they also noticed the presence of other pathogenic bacteria like *Vibrionales* and *Pseudoaltermonadales*. From these studies, it was observed that free-living shrimps from polluted and less polluted waters contain different microbial communities. Li, Chang, et al. (2018) carried out the work on composition of gut microbiome and its modulation for improving the performance and production of the shrimp *L. vannamei* in aquaculture farming. It has been reported that 111 bacterial strains have been isolated from the gut of this shrimp and these bacteria are categorized into 13 taxonomic groups. The dominant groups that represent are from the genera, *Photobacterium*, *Vibrio*, *Aeromonas*, *Xanthomonas*, *Agrobacterium* and *Bacillus* and the family Enterobacteriaceae (Wan et al., 2006a). It was observed that in gut microbiome composition, *Proteobacteria* was found to be the dominant core bacterial group, which is beneficial for promoting shrimp health and growth. Other than *Proteobacteria*, there are several other opportunistic pathogenic bacteria in gut system which changes their functional role with different developmental stages of shrimp so also with diet composition and environmental factors. Various opportunistic pathogenic bacteria have been reported in the intestine of *L. vannamei*, including those belonging to the genera *Pseudomonas*, *Flavobacterium*, *Escherichia*, *Aeromonas*, *Vibrio*, *Rickettsia*, *Shewanella* and *Desulfovibrio* and of the family Enterobacteriaceae. In the intestine of healthy *L.vannamei*, the abundance of these bacteria has been found to be either low or at a certain level that maintains a balance with the number of other microbes in shrimp (Cardona et al., 2016; Qiao et al., 2017; Sun et al., 2016; Zhang et al., 2016).

The role of various prebiotics and probiotics as diet supplements and their evaluation on the regulation of health and immune system and also in shaping the microbial community profile of gut of the shrimp has been assessed by a number of researchers (Li, Ringø, et al., 2018; Yukgehnaish et al., 2020). Several studies have indicated the changes in gut microbiome composition in relation to the incidences of diseases in the shrimp. In *L. vannamei*, the incidence of acute hepatopancreatic necrosis disease (AHPND) has been correlated with significant reduction in the bacterial diversity in hepatopancreatic tissues when compared to healthy individuals (Liu, et al., 2018; Restrepo et al., 2018; Liu et al., 2018, 2019). The gut microbiota of *L. vannamei* when infected with white spot syndrome virus (WSSV) showed significantly altered microbiome diversity when compared to normal and healthy individuals. The shrimps infected with WSSV showed an increase in *Proteobacteria* and *Fusobacteria*, including the pathogenic bacteria belonging to the *Arcobacter* genus. However, there was reduction in *Bacteroidetes* and *Tenericutes* counts in the infected shrimps (Wang et al., 2019). Huang et al. (2018), Huang, Guo, et al. (2020) reported the gut microbial diversity did not change much in *L. vannamei*, which has been infected with WSSV and cultured in a biofloc system. From these studies, it was concluded that the culturing such infected shrimps in biofloc systems will not lead to the development of healthy varieties resistant to WSSV infection. However, a recent literature review finds that biofloc technology can improve the health of shrimp microbiomes. It suggests that, if biofloc technology is widely adopted, the sector could reduce its environmental footprint, while improving the overall health of farmed shrimp. The researchers focused on microbial dynamics biofloc systems, identifying the heterogeneous bacteria and proteins in the bioflocs that consume shrimp waste and act as feed. They found that in well-managed biofloc systems, shrimp are surrounded by a healthy variety of microbes that suppress the growth of pathogens. The flocks also break down ammonia and nitrates in the water, creating a healthy culture environment. This allows the shrimp to acquire a resilient microbiome—making them healthier and leading to a productive farm cycle (Rajeev et al., 2021).

Holt et al. (2020) described about the occurrence of white faeces syndrome (WFS), which is characterized by white golden gut contents and white faecal strings and further mentioned that the reasons for this kind of syndrome are not known so far. Hou et al. (2018a, 2018b) reported that the gut microbiome composition in the WFS-infected shrimps totally differs from that of normal and healthy shrimp. It was noticed that in the infected shrimp, there was an increase in bacterial population like *Tenericutes and Firmicutes* and a decrease in *Proteobacteria* counts. Huang, Zeng, et al. (2020) later confirmed the above observations, while carrying out similar types of studies in the shrimps and mentioned that when intestinal microbiota transplant of the infected shrimps were done in healthy shrimps, the healthy ones also eventually became infected with the disease. Zhou et al. (2019) worked on cotton shrimp-like disease (CSL) which is associated with reduced growth, atrophy of hepatopancreatic tissues, empty digestive tract and softshell with white opaque muscle. While carrying out work on gut microbiome composition in relation to CSL infection, they found that there was an increase in the population of bacteria like *Tenacibaculum*. However, they also observed that the gut microbial composition of the infected CSL shrimps was almost similar to normal and healthy shrimps. Liang et al. (2020) reported blue body syndrome/disease (BBS) among the shrimp, which is characterized by blue colouration of the body associated with slow growth, reduced feed intake and lean body. Studies carried out on the gut microbiome composition of the BBS-affected shrimps and normal healthy ones indicated that there was lot of dissimilarity in the bacterial community of these two groups. However, same authors also observed that there was no significant difference in the gut microbiota composition of BBS affected and normal shrimp. This shows that more research studies are required in this field of microbiome.

Considerable efforts have been made to explore the microbiome community found in the Pacific oysters *Crassostrea virginica* (King et al., 2012; La Valley et al., 2009; Mayasich & Smucker, 1987) and *Crassostrea gigas* (Faury et al., 2004; Hernandez & Olmos-Soto, 2006). Oysters are well known for their commercial value and many attempts have been made to study their biology and ecology, including interactions with bacteria and other microbes in the environment. Much of the available literature has emphasized diseases (Wetz et al., 2002) and the presence of human pathogens, especially *Vibrio parahemolyticus* and *V. vulnificus* (Johnson et al., 2010; Sobrinho et al., 2010) in these animals. King et al. (2012) carried out studies on the microbiome community present in gut and stomach content of the oyster *C. virginica* and for characterization of the microbes they used high-throughput pyro-sequencing method. It has been reported that the stomach microbiome composition of oysters are dominated by bacteria like *Mollicutes* and *Planctomyctes*, whereas gut microbiome showed domination of bacterial assemblage like phylotypes. These results collectively revealed the presence of novel microbial communities within the oyster digestive system, where functions of oyster microbiomes are largely unknown. Mayasich and Smucker (1987), while working on oyster bacterial interaction identified a symbiotic bacteria viz. *Cristispira* closely associated with crystalline style of the digestive gut system. Number of workers has identified other bacteria like *Vibrio* and *Stappia* from the cultured oysters, *C. virginica* and *C. gigas* (Boettcher et al., 2005; Kueh & Chan, 1985). Romero et al. (2002) reported that there is a great diversity exists in the microbiome community of the oysters collected from the wild and those collected from the hatchery raised culture systems and found that *Proteobacterium Arcobacter*, as a major contributor to the microbial community of the Chilean oyster, *Tiostrea chiliensis*. From the literature, it appears that more studies are required on microbiome surveys of cultured aquatic animals, molecular identification at the species level, their relationship with number of biotic and abiotic factors and host microbial interaction should be the need and priority for future research areas. The gut microbiome composition of different shellfish is illustrated in Table 2.

TABLE 2 Gut microbiome composition of different shellfish species

Shellfish species	Gut microbiome composition profile (core groups)	Reference
Penaeus japonicus, adult	*Vibrio, Moraxella, Flavobacterium*	Sakata and Taruno (1987)
Penaeus japonicus, larvae	*Vibrio, Aeromonas, Pseudomonas, Staphylococcus, Flavobacterium*	Yasuda and Kitao (1980)
Penaeus aztecus, larvae	*Vibrio, Alcaligenes, Aeromonas, Chromobacterium, Pseudomonas, Xanthomonas, Alteromonas*	Dempsey et al. (1989)
Penaeus setiferus, Adults	*Vibrio, Alcaligenes, Aeromonas, Chromobacterium, Pseudomonas, Xanthomonas, Alteromonas*	Dempsey et al. (1989)
Penaeus indicus, Eggs, larvae	*Vibrio, Alteromonas, Alcaligenes, Aeromonas, Flavobacterium, Pseudomonas, Bacillus, Chromobacterium*	Hameed (1993)
Penaeus indicus, Juveniles	*Vibrio, Pseudomonas, Moraxella, Micrococcus, Bacillus, Acinetobacter, Staphylococcus, Enterobacteriaceae*	Singh et al. (1998)
Penaeus monodon	*Proteobacteria, Photobacterium, Vibrio, Aeromonas, Xanthomonas, Agrobacterium, Bacillus, Firmicutes, Bacteroidetes, Actinobacteria*	Rungrassamee et al. (2014)
Litopenaeus vannamei	*Photobacterium, Vibrio, Aeromonas, Xanthomonas, Agrobacterium, Bacillus, Firmicutes, Bacteroidetes, Actinobacteria*	Wan et al. (2006), Qiao et al. (2017)
Litopenaeus vannamei	*Pseudomonas, Flavobacterium, Escherichia, Aeromonas, Vibrio, Rickettsia, Shewanella, and Desulfovibrio, Acetobacter, Bacillus, Bacteroides, Bdellovibrio, Lactococcus, Rhodopseudomonas, and Streptococcus Firmicutes, Bacteroidetes, and Actinobacteria*	Li, Chang, et al. (2018)
Crassostrea virginica (Pacific oyster)	*Mollicutes, Planctomyctes, phylotypes, Cristispira, Vibrio, Stappia,*	King et al. (2012), Mayasich and Smucker (1987),
Tiostrea chiliensis Chilean oyster)	*Proteobacteria*	Romero et al. (2002)

2.3 | Metagenomics

Number of investigators has highlighted the importance of metagenomics studies because; the in-depth knowledge of metagenomics will provide better understanding of microbial diversity in aquaculture facilities. Metagenomics help to evaluate the bacterial communities in two ways, that is functional genomics and sequencing based metagenomics. Both these aspects are important in the sense that sequencing based metagenomics help to understand the microbiome diversity in gut microbiome environment in a better way and functional metagenomics throws light on various functional aspects of the microbial species. Since its inception, metagenomics has been widely implemented across an array of different environments and hosts (Gill et al., 2006). Quince et al. (2017) also mentioned that metagenomics is a powerful tool which provides suitable knowledge of gene function from microbial community and also helps in identifying genetic and physiological traits of the micro-organisms. From such studies, it has been suggested that metagenomics libraries can be constructed for future use in genomic manipulations of the bacterial species, whereas from functional metagenomics, new molecules can be discovered in the form of enzymes and other bioactive compounds which can be used for therapeutic purpose and other required applications in the fish farming system (Yukgehnaish et al., 2020). Another reason to study the metagenomics of gut microbiome is to develop appropriate probiotics, which is important to fish from nutritional point of view. By altering the microbial composition in the gut system, one can improve the metabolic and immunological performance in fish. However, more research studies are needed on metagenomics and factors influencing the composition of the gut microbiome and also the physiological effects of this on the host fish (Yukgehnaish et al., 2020). As far as fish and shellfish are concerned, there is a paucity of information on metagenomics.

Several research investigations have been carried out on microbiome diversity and composition in relation to both biotic and abiotic factors, but as far as functional aspects of microbiome are concerned, not much attention has been paid. In recent years, researchers are making attempts to understand functions of microbial species at genomic levels so that by altering gene structure, desired properties can be developed in microbes. Such studies are very much essential and quite useful for creating innovative products for the benefit of growth of various sectors including pharmaceutical/bioindustry. There are several reports also mentioning that by developing healthy gut microbiome in fish and shellfish, there is an improvement not only immune system but overall health and quality of farm produce (Gómez & Balcázar, 2008; Hanning & Diaz-Sanchez, 2015; Tarnecki et al., 2017). Tarnecki et al. (2017), while working on metagenomics of fish intestinal microbiome, described microbial structure of several commercial aquaculture species with the goal of manipulating microbes to increase feed efficiency and decrease disease susceptibility. Udayangani et al. (2017) also reported the data on metagenomics analysis in zebra fish, *Danio rerio*, and studied the effects of chitosan silver nanocomposites supplemented diet on different properties of the gut microbial communities particularly on goblet cell density, gut morphometry and mRNA expression

of immune-related and mucin-encoding genes. It is observed that when chitosan silver nanocomposites supplemented diet was given for 30 days, the gut microbiota composition showed reduction in the counts of *Proteobacteria*, while there was significant increase in the counts of *Fusobacteria* and *Bacteroidetes*. The fish gut also showed enhanced goblet cell density and also an increase in villi height in mid-gut portion, which probably indicates better improvement in the immune system when compared to control fish. Furthermore, it is also reported that there was significant up-regulation of mRNA expression of immune-related genes and mucin-encoding genes. These results suggest that chitosan silver nanocomposite-supplemented diet can be used as immunostimulants for the management of disease control in the aquaculture farming by developing healthy gut microbiome. Tyagi et al. (2019) investigated the gut microbiota composition of freshwater carp (*Labeo rohita*) using shotgun metagenomics method to understand taxonomic composition and functional capabilities of the microbial species. Using the taxonomic composition, it has been reported that, the gut microbiota of carp contains more than three-fourths of *Proteobacteria* and very low prevalence of commonly used probiotic bacteria (*Bacillus*, *Lactobacillus*, *Streptococcus* and *Lactococcus*). Considering the herbivorous habit of fish, it has been reported that gut microbiome had pathways that degrade cellulose, hemicellulose, chitin, pectin, starch and other complex carbohydrates. High prevalence of *Actinobacteria* and antibiotic biosynthesis pathways have also been noticed in gut microbiome which indicates that these microbes can produce antibiotics and this has been confirmed by identifying 51 different types of antibiotic resistance genes in the gut microbiota (Tyagi et al., 2019). Merrifield and Rodiles (2015), while working on fish microbiome and its interactions with mucosal tissue reported that, though fish contains complex communities of microbiome, which are usually influenced by the range of seasonal factors, however, in gut system which always contains core component of microbiome generally does not get affected even if fishes are reared in different environmental conditions. Further, it is also mentioned that microbiome can act in creating barrier function in the sense that, in the absence of certain microbiome species, fish larvae fail to develop properly. While studying microbiome composition in the skin mucosal surfaces of the eel fish, *Anguilla anguilla*, Carda-Diéguez et al. (2017) reported that this microbial composition is quite similar to the composition and functions of microbial species present in mammalians. To test this similarity, they compared the databases of metagenomics and also the genomic evidences of microbiome present in the skin mucosal surfaces of fish collected from different ecosystems and also the microbiome present in water surrounding the host. Furthermore, it has been reported that the microbial species present in the skin mucosal surfaces may have specific genes for specific functions like forming biofilms for attachment, genes specific for bacterial competence and communications and resistance to mucosal innate immunity. Yukgehnaish et al. (2020) while studying metagenomics of fish gut microbiota reported that the microbiome composition in the gut system has very important role in controlling metabolism, feeding behaviour and immune response. Development of probiotics in aquaculture industry for the management of diseases is probably the outcome of the knowledge of metagenomics of microbes that prevent infectious diseases. It has been also emphasized that the proper usage of prebiotics in aquaculture farming helps in developing healthy microbes in the fish gut system. Further, in their review paper, they have also mentioned that the new methods of genome sequencing technology and development of bioinformatics tools have supported proper analysis of metagenomics data of gut microbiota and their relevant functions (Yukgehnaish et al., 2020). In recent past, a number of workers have attempted to carry put research studies on metagenomics of microbial species in the fish gut system. New molecular tools such as 16s rRNA taxonomical markers and PCR techniques have greatly contributed in developing new information in metagenomics (Garza & Dutilh, 2015). From the literature analysis, it appears that the metagenomics research studies on microbiome of the fish gut system carried out so far, emphasized two main aspects, that is the factors that affect microbiota composition and how the fish reacts to this changed microbiome environment. There are several other studies also on the microbiome composition and its relationship with factors like nutritional status, water pollutants, reef settlement and trophic levels (Baldo et al., 2015; Brown-Peterson et al., 2015; Eichmiller et al., 2016; Estruch et al., 2015; Liu et al., 2016; Miyake et al., 2015). Furthermore, investigations have also been made to find out the physiological changes to gut microbiota and gene-related factors of the host fish on the impact of target fish gut microbiota and vice versa (Li et al., 2013; Smith et al., 2015).

3 | MICROBIOME AND HEALTH STATUS OF AQUACULTURE SPECIES

The relationship between the host's microbiome and health status of the organisms is now well established, and in recent years, much research is being carried out to manipulate the microbiome community to enhance immunity and health of the host. Considering substantial growth of the aquaculture industry all over the world, priority is now being given to manipulate microbiota in digestive tract of both finfish and shellfish to improve health and quality status of cultivable organisms. For manipulation of gut microbial community, the method adopted is changes in the feed formulations by adding more quality proteins and lipids in the diet as well as addition of probiotics and prebiotics (Desai et al., 2012; Geurden et al., 2014; Kotzamanis et al., 2007). Several researchers have reported how the microbiota changes with manipulation of the diet. Similarly, some have mentioned different type of microbiome in farmed fish and shellfish compared to their counterparts collected from the wild (Gilmour et al., 1976) and how these farmed species succumbed to infection due to poor quality of microbiome in their gut system (Olivier et al., 1981). Zarkasi et al. (2016) while working on Atlantic salmon found that by enhancing quantity of protein in the diet, alters the microbiome community in digestive gut tract, and further, it has been also mentioned that reduced protein levels in the diet shows more divergent microbial community in gut. Delcroix et al. (2015)

while studying the effects of dietary marine protein hydrolysates on the development of sea bass larvae *Dicentrarchus labrax* and associated microbiota found that microbiome composition proliferates in the digestive tract as growth of the larvae advances. Similar observations have also been reported by Ha et al. (2019), while working with South American catfish, *Rhamdia quelen*. Delcroix et al. (2015) also mentioned that fish feed containing protein hydrolysates and short-chain peptides can influence the gut microbiome composition in cultivable finfishes. It has been reported that the presence of peptides in the diet enhances antimicrobial activity and protect animals from pathogenic bacteria (Sila et al., 2014). Several workers have reported that the presence of amino acids and protein hydrolysates in fish feed diet can improve immunity in fish and reduce the intensity of the pathogenic bacteria in gut system (Bui et al., 2014; Egerton et al., 2018; Khosravi et al., 2015; Kiron, 2012). The role of lipid as macronutrients in the diet of fish and its relationship with microbial community in gut system has been studied by number of workers. Ringø et al. (2002) and Montero et al. (2010) reported that, when fish oils are replaced with plant oils in fish diet, the quality of microbial community gets affected and when plant oils are replaced with fish oils, there was significant improvement in the quality of gut microbiome and also resistance to pathogenic bacteria. Further, it is mentioned that, this happens because, the fish oils contain high amount marine polyunsaturated fatty acids, whereas in plant oils, the same is lacking (Merrifield et al., 2011). The relation between lipids in fish diet and how it influences gut microbiota is a matter of further investigation. Yoshida et al. (2016), while working on fish microbiota mentioned about the presence of bacteria not only in gut system which can synthesize polyunsaturated fatty acids, but such bacteria can be isolated from the seawater and sediments also. It is very important to investigate the use of such bacteria for the preparation of probiotics and their use for fish health and nutrition.

Several researchers have reported that the presence of beneficial microbiome in host organisms do not cause any diseases or disorders in the body system, but when there is disturbance in the balance of microbial community, the harmful pathogens will become more prevalent and can cause infections and diseases (Montalban-Argues et al., 2015; Moya & Ferrer, 2016; Romero et al., 2014; Turner et al., 2013). Li et al. (2016), while working on microbiome of fish found that in diseased affected fish samples, the population density of *Aeromonas* bacteria was higher in abundance compared to healthy individuals which indicates that in healthy fish the pathogenic expression of the *Aeromonas* was totally prevented due to the presence of healthy microbiome. It was also observed that imbalance in microbial community can be induced by several factors like changes in the environmental conditions and use of number of antibiotic compounds. Li et al. (2017) while working on carps of healthy and diseased condition also noticed that in healthy fish *Cetobacterium*, *Cyanobacteria* and *Clostridiaceae* were more abundant, whereas *Aeromonas*, *Vibrio* and *Shewanella* were more abundant in diseased individuals. Similar observations were also made by Llewellyn et al. (2016, 2017), while working on Atlantic salmon. Therefore, it is confirmed that the presence of commensal microbiomes in the gut system of fish and shellfish helps in maintaining the health and protection against harmful pathogens (Merrifield & Rodiles, 2015; Salinas, 2015). However, further investigation is necessary to establish this kind of concept.

For establishing the relationship between microbiota and disease, Seetharam et al. (2015) investigated the microbiome composition in the shrimp *L. vannamei* collected from different geographical locations. In their study, they used tail muscle of the shrimp as a sample tissue for microbiome analysis. The shrimps were collected from different locations of Central and South America and also from South-East Asia. For microbiome analysis, genomic DNA was actually sequenced from the tail muscle tissues of the shrimp and entire data of the DNA sequences of all shrimp collected from different locations was assembled and classified based on their similarity to the sequences, which are already available in the public domain. After analysing the data, it was reported that there is a high degree of correlation among the microbiota of the shrimp collected from disparate locations. Further, it is also mentioned that the presence of some DNA from the bacterial composition known to cause food poisoning in humans. In another study, Xiong et al. (2017) carried out investigation in the shrimp (*L. vannamei*) gut microbiota during entire culture operations starting from healthy phase of shrimp particularly in early stages of growth and later in diseased phases as the shrimp reaches adult size. In their study, they used null model and phylogenetic based taxon distance analysis. From the results obtained, it was concluded that the bacterial community in healthy shrimp was well organized and as the shrimp suffered from the severity of disease, the bacterial community was disrupted. Similar observations on the dysbiosis in gut microbial composition and its relationship with host diseases have been made by Berry et al. (2012) and Xiong et al. (2015). Currently, the relationship between the gut microbiota and health of the host fish has become an important topic for research investigation (Berry et al., 2012; Clemente et al., 2012; Subramanian et al., 2014). Few studies have indicated and quantified how the microbiota is affected by the emergence of disease under different aquaculture conditions (Xiong et al., 2017). Pathogens can interact with the host microbiota depending upon dominance of resources and release antimicrobial compounds or vice versa. Hence, dominance of pathogens can create microbiota dysbiosis. Due to the constant interaction between shrimp digestive system and its surrounding environment, the microbiota of the intestine and hepatopancreas is supposed to play important role in disease resistance mechanisms. However, there are no much research studies, describing changes in the microbiota in shrimp during an AHPND outbreak in closed system (Granados et al., 2017). Xiong et al. (2016) while working on shrimp disease control in pond water system mentioned that outbreak of diseases occur due to complex interaction between the hosts, surrounding quality of aquatic environment and corresponding microbiome community available at that time. It has been also reported that, due to eutrophication of pond water, the bacteria like *vibrio* along with other pathogenic bacteria generally invade the digestive system of many aquatic species creating gastrointestinal diseases (Johnson, 2013). In view of such findings, several

investigators have raised the issue of healthy and diseased stomach microbiome in penaeid shrimp. In controlled aquaculture farming system, it is important that, while cultivating the aquatic species the water quality of the ponds has to be maintained to build healthy microbiome in the gastrointestine of the animals (Lai et al., 2015; Xiong et al., 2014). While assessing the health of the shrimp during aquaculture farming, it has been reported that the composition of the gut microbiome has got very close relationship with health status. Li and Ding (2006), while working of microbial community of the shrimp *L. vannamei* in relation to infected and non-infected shrimp with white spot syndrome, observed that the total bacterial count was higher in infected shrimp compared to the same in uninfected individuals. Whereas the percentage of *Lactobacillus* in uninfected individuals was found to be higher than that in infected ones, but the percentage of *Aeromonas*, a common pathogenic bacterium in aquaculture was lower. Yang et al. (2016) noticed that in healthy *L. vannamei*, *Alphaproteobacteria* and *Firmicutes* were present in abundance in the intestinal tract, whereas in infected shrimps *Betaproteobacteria*, *Bacteroidetes* and *Actinobacteria* were found to be in lower abundance. From these results, it was concluded that sick shrimp develops imbalance between beneficial and pathogenic bacteria, and the higher abundance of pathogenic bacteria could be partly responsible for the occurrence of the disease. Ninawe et al. (2020) suggested the use of bacteriophage technology for controlling various bacterial and viral diseases for the cultivable aquaculture species. Though, the phage therapy has been advocated by several researchers because of its effectiveness in mitigating the infectious diseases; however, the technology needs further investigation prior to its commercial applications. Earlier also several workers have attempted the use of phage therapy to cure bacterial and viral infections in fish and shellfish, and it was mentioned that the phage therapy can replace the use of antibiotics and other antibacterial chemicals (Oliveira et al., 2012; Subharthi, 2015). Confirmatory investigations are still needed in such niche areas to advocate use of bacteriophage technology to mitigate infectious diseases in aquatic organisms.

In aquaculture farming, the microbiome that is available naturally has a vital role in maintaining the healthy aquatic environment but sometimes beneficial microbiomes are added artificially also to fulfil different functional roles. Therefore, monitoring and manipulations of microbial communities in the aquaculture system hold a great potential not only in the maintenance of soil and water quality but also controlling the infectious microbial pathogens in aquatic species. For this, it is very necessary to have in-depth knowledge about the microbiome community of healthy and diseased aquaculture environment, so that these microbes can be manipulated and engineered according to the need. This will help in reducing the use of chemicals and antibiotics in the aquaculture system. In recent studies, it has been demonstrated that the probiotics can control fish pathogenic bacteria present in the live fish feed and reduce the mortality of fish larvae significantly. However, successful management of the aquaculture through microbiome manipulation is currently hampered due to lack of knowledge of relevant microbial interactions and the overall ecology of these systems.

4 | MICROBIOME AND ITS ROLE IN CONTROLLING OUTBREAK OF DISEASE IN AQUACULTURE INDUSTRY

The increasing importance of the fish and shellfish microbiome in the biotechnology industry has become a source of investor excitement in recent times. The microbiomes or the genes of a community of microbial cells, which body possess, have unexpected benefits and implications of overall health, from regulating the immune system to enhancing growth and reproducing ability of an organism. The microbiomes found in the body system have the capacity to produce number of compounds through their genes and these compounds offer several benefits. It has been already reported that the microbiota found in the gut system produces vitamins and helps in digestion of food, storage of nutrients and promotion of a healthy metabolism. The microbiomes also help to maintain the integrity of cell connections in intestinal lining, entry of preventing harmful bacteria and toxins from leaking through intestinal wall. The gut microbiome is also closely linked to the immune system and the regulation of inflammation. As the microbiome genes produce several vital biochemical compounds, numbers of biotech and pharmaco-companies are taking lot of interest in manufacturing these products for launching into the market. All over the world, there are several companies like Enterome Bioscience, Second Genome, Rebiotix, Seres Therapeutics, Vedanta Biosciences and Microbiotica who have started manufacturing drugs from microbiomes, which can be used for the treatment of various disorders not only in humans but also in other organisms. Even some of the companies have started microbiome sequencing services in the last few years due to the growing support for research into genomics. This has benefited from the growing investment being driven into the life sciences sector and particularly genetic and genomic research. Aquaculture has long been recognized as an important activity in South East Asia and, therefore, continues to be the dominant region in the world in production terms. As per the estimates of South-East Asia State Fisheries and Aquaculture (SEASOFIA, 2017), the world's human population has increased from 6.1 billion in 2000 to 7.3 billion in 2014 and has become 7.7 billion in 2018 which has led to the world's increasing demand for fish to sustain food security requirements. From 2000 to 2018, the aquaculture production at global level has continued to grow at an average rate of more than 7% annually. But according to FAO (2018), the annual growth rate of aquaculture has been reported to the tune of 5.8 per cent during the period 2001–2016; however, aquaculture continues to grow faster than other major food production sectors. Also, the disparity in the level of sectoral development and uneven production distribution remain great among the countries within the regions and across the world. Today, the world aquaculture production has reached to the tune of 80.0 million tons. Asia is the biggest aquaculture producer with production that accounted for more than 90% of the world's total aquaculture production, out of which aquaculture production from the Southeast Asian countries accounted for around 22%. It has been reported that all over the world fish production from

capture fisheries is continuously declining, and in 2018, according to SEASOFIA's report, the production from capture fisheries has been shown to the tune of 79.30 million tons. In the Southeast Asian region also, capture fisheries continued to show declining trend, particularly for marine capture fisheries, whereas, aquaculture has shown steady increase in the production. Besides aquaculture's contribution to food security, it also plays very important role in terms of enhancing people's livelihood and generating income. Furthermore, it also helps in reforming the practice of using low-value fish as feed to produce higher value aquaculture products, thus enhancing the economic growth of all developing nations.

In aquaculture industry, infectious diseases caused by bacteria, viruses and parasites are primary concern in augmenting growth of the industry. Effective control of such diseases is one of the most critical issues in the management of successful aquaculture. When the aquaculture farming is being carried in captive condition, occurrences of finfish and shellfish diseases are more common and pose a major problem, particularly in tropical countries. From disease-induced mortalities in commercial aquaculture which have been reported earlier, it is concluded that outbreaks of diseases are more deadly in tropics than in temperate regions. Once the disease spreads, it can wipe out entire fish stocks in a relatively short time, with devastating consequences affecting the economy and food security for any nation. Overstocking, overfeeding and excessive use of antibiotics in aquaculture farming are some of the other important factors which influences the outbreak of diseases. As aquaculture continues to expand as a means of food production, achieving food security and bolstering the domestic economy, it becomes even more crucial to ensure that such ventures are protected from the impact of diseases (FAO, 2018). Bruijn et al. (2018) while discussing the use of microbial communities for the management of diseases in the aquaculture system reported that, for controlling various types of diseases and for protection of fish and shellfish from emerging harmful pathogens, only promising approach is introduction of beneficial microbes in the aquaculture farming. Further, they have also mentioned that the understanding of functional aspects of each individual microbe is very important for the future growth of the aquaculture industry. Holt et al. (2020) while discussing the role of gut microbiome for disease control and health management of the commercially important shrimp reported that there is a dominance of *Proteobacteria* in the gut microbiota of the many of the shrimps and these bacteria play a very important role in maintaining the health. These microbes present in the gut interact with their host and contribute to number of vital process including digestion and immunity. These authors have further advocated that, instead of using synthetic antibiotics for disease control in the shrimp, building healthy microbiome in gut system of these animals can be an alternate method not only for disease control but also for improving growth rate and survival of the shrimp. Earlier also several workers have investigated the gut microbiome composition of the shrimps like *P. monodon* and *L. vannamei* and found that *Proteobacteria* group was the dominant and this group comprises of *Vibrio* and *Photobacterium* spp (Rungrassamee et al., 2013, 2014; 2016; Zheng et al., 2017). In *P. monodon*, the gut microbiome composition also showed the presence of *Firmicutes*, *Bacteroidetes*, *Fusobacteria* and *Actinobacteria* (Rungrassamee et al., 2014). It has been mentioned that many *Vibrio* spp produce chitinolytic enzymes which will have decalcifying effects on the carapace of the shrimp resulting in several health problems (Jayasree et al., 2006). Huge losses have been reported by the shrimp industries due to *Vibrio* spp; however, despite this, many workers have described the presence of *Vibrio* spp as a dominant group within the shrimp gut microbiota (Manilal et al., 2010). While working on microbiome diversity and dysbiosis in aquaculture system, Villamil et al. (2020) also mentioned that there is always a close relationship between aquaculture productivity and microbial composition and suggested a gnotobiotic model. According to this model, changes in microbial composition of the digestive tract generally reflect on animal's health and performance (Duan et al., 2020; Fan et al., 2019; Villamil et al., 2019). However, there is no much information to confirm the concept of such model in aquaculture system (Zhang et al., 2020). Further, it is reported that dysbiosis whether in the form of loss of beneficial bacteria or the spread of harmful micro-organisms can be used as an indicator tool for productivity determination.

Shrimp farming has become one of the most important food production industries of the world and one of the most lucrative and widely traded aquaculture products, generating huge amount of revenue and foreign exchange, employing millions of people (FAO, 2015). The shrimp farming is highly beneficial to local communities as well as national economies of developing countries. Though the growth of this industry and its importance in the coastal economy is impressive, the aquaculture of shrimp has suffered huge losses due to outbreak of infectious diseases. Catastrophic infectious diseases hit shrimp farming since mid-1990s causing devastating losses in shrimp production all over the world. The enormous concentration of animals and their coprophagous behaviour imposed by intensive culture techniques have triggered the development of disease outbreaks, which are often explosive and sometimes leads to the loss of the complete stock. The development of the commercial culture of shrimp has been accompanied by the occurrence of diseases of infectious and non-infectious aetiologies. Many of the important diseases are caused by organisms that are part of the normal microflora and fauna. These organisms are opportunistic pathogens that cause disease only under conditions that favour them over the host. Many organisms in this category are ubiquitous, and most have been recognized and/or reported from each of the major penaeid culture areas of the world. Included among this category of pathogens are the filamentous bacteria, the peritrich protozoans, the invasive bacteria and the fungi. Among the most important disease-causing agents are the penaeid viruses. With the advent of commercial penaeid hatcheries, the shipment of brood stock and post-larvae from these culture facilities to others in different geographic regions has often resulted in the spread of these agents outside their normal range in wild populations. Other important diseases of cultured shrimps are related to the nutritional, physical and toxic disease syndromes. It has been reported that the first serious outbreak of disease in the shrimps occurred during mid-1980 in Taiwan and the epidemic

was *Monodon baculovirus* (MBV) (Flegel et al., 2008). This was followed by infectious hypodermal and hematopoietic necrosis virus (IHHNV) in United States (Lightner, 1996), yellow head virus (YHV) in Thailand (Flegel, 1997) and Taura syndrome virus (TSV) again in the United States (Brock et al., 1997). During the period 1993–2003, when shrimp industry was still struggling with MBV, IHHNV, YHV and TSV outbreaks, the industry faced with another disaster with the arrival of white spot syndrome virus (WSSV). After its first appearance in China in 1992, it spread rapidly around Asia creating devastating losses at many places in the world (Flegel et al., 2008). Gunalan et al. (2014) while doing the studies on disease occurrence in *L. vannamei* of pond culture system of different regions of India reported the occurrence of six diseases in the shrimp including Black gill disease, TSV, IHHNV, WMD, white gut disease and muscle cramp disease. The symptoms of each disease and their possible cure were also described in their report in details. Therefore, disease control and health management are of major importance in aquaculture and for growth of the shrimp industry.

5 | MICROBIOME AND DEVELOPMENT OF PROBIOTICS RESEARCH

In recent years, spectacular growth of the aquaculture industry using modern techniques and culture operations has resulted in inviting number of problems relating to proliferation of diseases and water quality issues. In order to control the spread of diseases, there has been use of enormous medicines and antibiotics to ensure the health of cultivable organisms as well as productivity of the farms. Research carried out so far has indicated that 76% of antibiotics used in aquaculture are for humans. Their indiscriminate use in the last two decades has created catastrophic effects on the aquaculture farming system. Among these, the prevalence of antibiotic-resistant microorganisms and imbalance in the gut microbiota, which affects fish health and residual deposition in the fish muscle, becomes a potential health risk to consumers. Several workers have suggested the use of probiotics as an alternative approach to prevent diseases in the cultivable organisms in the aquaculture industry and some others have suggested its use not only for disease control but also for promoting growth and reproduction (Dawood & Koshio, 2016; Fyzul et al., 2014; Hai, 2015). Seyed Hossein et al. (2015) also emphasized that administration of dietary prebiotics (mannaoligosaccharides, fructo-oligosaccharides, galacto-oligosaccharides and yeast by-products) in aquaculture system is very much effective for improving fish immune response. Probiotics can be also used as nutrient sources, providing enzymes for better digestion, modulating the immune system and increasing the immune response against pathogenic bacteria (Adel, El-Sayed, et al., 2017, Adel, Yeganeh, et al., 2017). The most common probiotics used in aquaculture, include lactic acid bacteria such as *Lactobacillus-sp*., *Bacillus-sp*., *Enterococcus-sp*. and yeast, *Saccharomyces cerevisiae* (Adel, El-Sayed, et al., 2017, Adel, Yeganeh, et al., 2017). The concept behind the use of probiotics is to feed adequate amount of healthy microbes, which replace the harmful microbes in the gut flora of fish and shellfish with result that these exogenous bacteria compete with pathogens, preventing their adhesion to the intestinal wall and secreting antibacterial substances. The proliferation of healthy microbes also helps in better digestion and absorption of food in the gut resulting in increased growth performance (Ringo et al., 2010). The success of probiotic supplementation in aquaculture appears to be dependent on fish species and size, as well as the feeding management adopted. This is evident from a series of studies on the use of probiotics alone or in combination with prebiotics (Ringo et al., 2010). It has been reported that there was an improvement in diet digestibility when *Pangasianodon hypopthalmus* juveniles were fed with the diets containing 45% soybean protein. In the case of *Channa striata*, it has been reported that the beneficial effects of probiotic containing *Lactobacillus acidophilus* were significant even after the withdrawal of supplementary food like yeast and other prebiotics. In *in-vivo* challenge studies using *Aeromonas hydrophila*, both fish species responded best in terms of resistance to infection and 100% survived, when *Lactobacillus acidophilus* was the candidate probiotic compared to live yeast *S. cerevisiae*. It is evident that, the benefits accorded by probiotics make it a viable alternative to the use of antibiotics and chemicals. However, adopting probiotics in large-scale aquaculture poses logistical problems. Their effectiveness is dependent on factors such as the proper management of strains, necessary provision of dosage and ensuring bacterial stability during production and storage, which potentially reduces the number of viable organisms. Furthermore, concerns about the safety of strains and the transmission of antibiotic resistance and virulent plasmids are issues that cannot be ignored (Williams, 2017). Abelli et al. (2009) pointed out the importance of the use of probiotics as an alternative to antibiotics in aquaculture farming system, which also restricts wide spread of chemicals in the aquatic environment. Akhter et al. (2015) while working on probiotics mentioned about the use of several microbiome species like Gram-positive and Gram-negative bacteria, bacteriophages, microalgae and yeasts for the preparation of probiotics, which can be used for fish culture. Some of the most frequently used probiotics include LAB Bacillus, Lactococcus, Shewanella and *Aeromonas* genera (Burr et al., 2005; Hagi et al., 2004; Merrifield & Carnevali, 2014). Some 61 published studies that investigated the administration of probiotics in teleost fishes have been reported by Carnevali et al. (2017). There are several studies, which indicated enhanced growth rate and improvement in the immune system in fish after manipulation of gut microbial composition using probiotics (Balcazar et al., 2006; Cordero et al., 2015; Huang et al., 2014; Lobo et al., 2014; Perry et al., 2020; Tahere et al., 2008). It has been also reported that, in aquaculture system successful application of probiotics can be difficult because of low viability of bacteria during processing and storage and loss from leaching in the water during feeding fish in culture operations. However, despite this, it has been observed that when probiotics are used, it will reduce the cost of fish farming through improvement in the fish growth and yield in minimal culture period (Merrifield et al., 2010). In recent past, several workers have addressed different aspects of probiotics and its impact on the growth of the aquaculture,

particularly in the management of infectious diseases, as growth promoters and immunostimulants and also as nutritional supplements (Ayisi et al., 2017; Hoseinifar et al., 2018, 2019; Ninawe & Selvin, 2009; Shefat, 2018; Soltani et al., 2019). Ringø (2020) has recently reviewed the use of probiotics, particularly for the growth of shellfish aquaculture industry. In his review he reported, the use of several probiotic species like *Lactobacillus*, *Enterococcus*, *Bacillus*, *Aeromonas*, *Alteromonas*, *Arthrobacter*, *Bifidobacterium*, *Clostridium*, *Microbacterium*, *Paenibacillus*, *Phaeobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodosporidium*, *Roseobacter*, *Streptomyces* and *Vibrio* for sustainable aquaculture development. Restrepo et al. (2021) while working on microbial community of the shrimp *P. vannamei* developed a probiotic using the ILI strain of *Vibrio diabolicus* bacterium which prevents the infection in the shrimp caused by AHPND virus. In this study, it was shown that gastrointestinal microbiome composition was totally different in healthy shrimp compared to the infected ones with AHPND. Upon detail analysis, it was observed that *Proteobacteria*, *Firmicutes* and *Tenericutes* were the most abundant in healthy shrimp, whereas in infected shrimp these groups of bacteria were reduced in numbers. These findings suggest that ILI strain of *V. diabolicus* can be used as probiotic to control the population of pathogens for AHPND in the shrimp. It is further mentioned that the aquaculture production of shellfish suffered many a times throughout the world due to the spread of infectious bacteria and viruses and the use of probiotics is good alternative method rather than the use of chemotherapies or antibiotics.

Some researchers reported that, instead of use of probiotics, prebiotics applications also help in improving the health benefits in the fish farming. Though, the prebiotics are difficult to digest but these products have been shown to enhance immune responses, improve nutrient uptake and increase growth and feed conversion ratios (Gibson et al., 2017; Milad et al., 2016). There are also some difficulties in successfully administering these supplements. Some of the prebiotics used in the fish farming are fructose-oligosaccharides, short-chain fructose-oligosaccharides, oligofructose, mannaoligosaccharides, trans-galacto-oligosaccharides, inulin, galacto-oligosaccharides, xylo-oligosaccharides, arabinoxylo-oligosaccharides and isomalto-oligosaccharides (Ringø et al., 2016). Rebaca et al. (2011) reported that the prebiotics are sometimes used in combination with probiotics to create better results. Seyed Hossein et al. (2020) studied the effect of microbial feed additives including prebiotic, probiotic and synbiotic on antioxidant enzymes in fish and found that by adopting different dietary approaches and microbial feed additives improve the level of antioxidant enzymes and defence system in fish. It is hypothesized that the probiotics are active in small intestine and prebiotics influence the microbiota of large intestine. However, the use of probiotics and prebiotics in aquaculture is a fast-growing area and further research is required to investigate how these components work together in the gut system to build up adequate microbiota. Daniels and Hoseinifar (2014) while reviewing prebiotic applications in shellfish mentioned that prebiotics in the form of different diets (mannaoligosaccharides fructo-oligosaccharides, isomalto-oligosaccharides, xylo-oligosaccharides, inulin) when given at different stages of the growth of the shrimp, prawns, crayfish and lobsters can not only improve the microbiome composition of the digestive tract but also influences the immune system. The prebiotics also help to modulate better digestion of food materials and improves food conversion ratio resulting in faster growth performance of the host species. It is also reported that the prebiotic efficiency is always dependent on a number of factors specific to the culture conditions and host species which requires future research investigations.

6 | BIOINFORMATICS SKILLS IN THE MICROBIOME RESEARCH FOR THE DEVELOPMENT OF THE AQUACULTURE SECTOR AND BIOINDUSTRY

The microbiome research has gained lot of importance through number of investigations on human and livestock health, where studies of this nature have been ongoing for many years, whereas microbiome research in aquaculture and bioindustry is in its relative infancy. In this context, so far no much attention has been paid in utilizing the knowledge of bioinformatics skills for the development of aquaculture farming and the bioindustry. When aquaculture industry started suffering losses due to outbreak of bacterial and viral diseases, to mitigate these challenges several measures have been taken and implemented. In recent years, in order to understand disease dynamics and its interaction with cultivable organisms, so also interaction of pathogens that are present in aquatic bodies and their relationship with aquatic animals, the knowledge of bioinformatics was brought in to address some of the disease management issues in aquaculture industry. For example, white spot syndrome virus (WSSV) is one of the most catastrophic pathogens that are destructive to shrimp aquaculture activity in commercial shrimp farms (Liu et al., 2009). In order to control this virus, more and more studies have been carried out in shrimp in last decade. Lan et al. (2006) reported the discovery of transcriptional profile of WSSV genes in shrimp with DNA microarray technique. Numbers of workers have identified many host genes and proteins responding to WSSV infection through large-scale approaches (Li et al., 2013; Liu et al., 2011; Wang et al., 2006; Zhao et al., 2007). From these studies, it is observed that lot of host genes and proteins are found up-regulated or down-regulated after WSSV infection. However, at present the information lacking on the direct interaction between WSSV and the host proteins for proper understanding of the pathogenesis of WSSV in shrimp. Under such situation, bioinformatics analysis will provide a highly effective approach for identifying genes and proteins involved in WSSV/shrimp interaction based on the public protein-protein interaction (PPI) databases (Zheng et al., 2014). Zheng et al. (2014) while working on bioinformatics prediction of WSSV-host protein–protein interaction in shrimp *F. chinensis* mentioned that it becomes possible to identify WSSV/shrimp interacting proteins with bioinformatics techniques by using the genome data of WSSV (Hulten et al., 2001; Yang et al., 2001) and the transcriptome data

from shrimp. Exploitation of the information with regards to protein–protein interaction with bioinformatics approaches provides an effective way to analyse high-throughput experimental data in typical organisms (Pattin & Moore, 2009; Remmerie et al., 2011). The predicted interaction between shrimp protein and WSSV protein which might be either independent interaction or synergistic interaction provides information on possible invasion approaches during WSSV infection to host cells. Moreover, these interactions could also lead to intracellular signalling pathways initiated by the host proteins. Number of workers has carried out the studies on host genes and proteins interaction to WSSV infection in different crustaceans animals through large-scale approaches (Li et al., 2013; Liu, Wang, et al., 2011; Wang et al., 2006, 2007; Zhao et al., 2007) and it has been reported that, after WSSV infection the host genes and proteins are found to be down-regulated, but further it is mentioned that there is no evidence on direct interaction between WSSV pathogen and the host proteins to reveal the mechanism of WSSV infection in the shrimp. There are several earlier researchers who have reported the involvement of host genes and proteins in WSSV infection in shrimp. Liu et al. (2009), while dealing with antiviral immunity in crustaceans mentioned that there is a cellular receptor called β-integrin which is involved in WSSV infection by interacting with WSSV envelope protein VP187. Although this particular data provide us some useful information about WSSV infection mechanism, it is still not very clear about the molecular mechanism of WSSV infection. From available literature, it is observed that there exits considerable information on the whole-genome sequences of different WSSV viruses, so also on transcriptome of some of the shrimp (Li et al., 2013; Hulten et al., 2001; Yang et al., 2001). Under availability of such information, bioinformatics analysis will provide a highly effective approach for identifying genes and proteins involved in WSSV/ shrimp interaction. Szklarczyk et al. (2011) in their paper while discussing the bioinformatics methods mentioned about the uses of different search tools for collecting PPI data bases. The most widely used PPI databases mainly include the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), the Database of Interacting Proteins (DIP) and Reactome. STRING is a database of known and predicted protein interactions based on the sources derived from the genomic context, high-throughput experiments, co-expression and previous knowledge (Szklarczyk et al., 2011). Xenarios et al. (2002) reported the use of DIP databases of interacting proteins as a search tool of bioinformatics for studying cellular networks of protein interaction. There is another database tool, mentioned by Joshi-Tope et al. (2005) called 'Reactome', to find out the biological pathways related to PPI information.

6.1 | Microbiome and bio/pharma industry

There is no much development on the use of gut microbiome particularly from fish and shellfish which has directly contributed for the benefit of bio/pharma industry. Lack of knowledge pertaining to metagenomics or the functional aspects of microbial species may be one of the main reasons attributed in this particular field. Therefore, in recent years, much emphasis is being laid down to investigate functional aspects of microbial species using molecular tools. The efforts made so far on the metagenomics of the microbial species of the fish gut system has been described elsewhere in this paper in metagenomics section. While discussing on fish microbiome, Sanchez et al. (2012) mentioned that the bacterial communities available in the fish gut particularly in the intestinal portion, acts as a new source of microbial and biosynthetic diversity for natural products discovery. It is further reported that there are several marine invertebrates and associated microbial communities which are the source of unique natural products with diverse array of biological activities. To understand these Sanchez et al. (2012) examined the intestinal microbiome community of the six varieties of fish and found that the bacterial strain, *Actinobacteria* which is predominant in gut systems of these fishes has been identified to contain a novel bioactive lipid component as sebastenoic acid. This particular lipid component has been found to possess antibacterial property against certain bacterial strains like *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecium* and *Vibrio mimicus*. Recent studies of invertebrate-associated microbial communities are revealing micro-organisms as the true producers of many of these compounds. More than 1000 new compounds have been reported from the marine environment, particularly from microbial species which are associated with marine invertebrates. Microbial species belonging to an order Actinomycetales have been reported to produce number of antimicrobial agents and perhaps the antibiotic drug discovery has been originated from these microbiomes (Trindade et al., 2015). Lot of work has been therefore carried out on the genome sequencing of these bacterial communities. For example, the genome sequence for *Streptomyces avermitilis* reveals gene clusters that code for the production of 37 secondary metabolites, yet only 13 natural products have been reported from this organism (Trindade et al., 2015). The extensive study of different natural products derived from fish microbial species has resulted in the isolation of several *Actinomycetales* bacterial species from fish intestines, as well as unique strains of *Firmicutes* and *Proteobacteria*. It has been reported that bacterial species show a wide range of biological activities against both Gram-positive and Gram-negative pathogenic bacteria and are able to inhibit the growth of a number of commercially important fish pathogens. In recent years, few biopharma companies have taken initiative in manufacturing of innovative products from different species of microbiome found in fish and shellfish under different commercial names for their use in the aquaculture industry. The products may be in the form of either probiotics or in the form of feed. These products have been proved to be quite useful in maintaining the quality of water and soil as well as health of the cultivable organisms in farming system. In order to promote the future prospects of the bioindustry, there is an urgent need of intensive research to be undertaken on fish and shellfish microbiomes, particularly in the areas of genomics and metagenomics. For the development of any commercial product from the bacterial species and its applications in the fish farming system, the industry has to face several challenges. Despite this, the aquaculture

TABLE 3 Fish and shellfish microbiome as a source of developing products for the growth of bio/pharma and aquaculture industry

Microbiome species of fish and shellfish	Possible product preparations	Reference
Lactobacillus, Enterococcus, Bacillus, Aeromonas, Alteromonas, Arthrobacter, Bifidobacterium, Clostridium, Microbacterium, Paenibacillus, Phaeobacter, Pseudoalteromonas, Pseudomonas, Rhodosporidium, Roseobacter, Streptomyces and vibrio	Probiotics	Ringø (2020)
Saccharomyces cerevisiae sp	Probiotics	Adel, El-Sayed, et al. (2017), Adel et al. (2017)
Cetobacterium somerae	Vitamin B12	Larsen et al. (2014); Etyemez and Balcazar (2015); Lyons et al. (2015); Gaulke et al. (2016)
Vibrio species	Hydrolytic enzymes Amylase, Lipase, Cellulose, and Chitinase	Gatesoupe et al. (1997, 1998), Itoi et al. (2006); Sugita and Ito (2006)
Vibrio alginolyticus	Probiotics	Samad et al. (2014) and Chen et al. (2015)
Photobacterium	Chitinase enzyme	Itoi et al. (2006)
Clostridium species	Fatty acids, Vitamins Enzymes-Acid and Alkaline phosphatases Probiotics	Ramirez and Dixon (2003)
Actinobacteria	Antibiotics, Sebastenoic acid	Tyagi et al. (2019), Sanchez et al. (2012)
Actinomycetales	Antibacterial agents	Trindade et al. (2015)
Streptomyces avermitilis	Secondary metabolites	Trindade et al. (2015)
Bacillus species	Aqua Pro F	Karen et al. (2017)
Bacillus amyloliquefaciens	Ecobiol	Karen et al. (2017)
Pediococcus acidilactici	Bactocell	Karen et al. (2017)
Bacillus ceres var. toyoi	TOYOCERIN	Karen et al. (2017)

industry has already taken up the commercial application of number of microbiome-based products in recent past and this has created a vast range of new enterprises especially in South East Asia, Central and South America and also in Europe. All over the world, efforts are being made to increase the aquaculture production at exponential rate and to achieve this, the aquaculture and bio and Pharma industries have to play a vital role in bringing novel products from the microbiome species. These novel products will address the issues of not only the health and quality of the fish produce on sustainable basis but also enhances the yield of the farming sector. However, the microbiome technology requires a high-level of expertise and has been widely regarded to be the modern core of the bioindustry. Exploring the research and development programmes on fish and shellfish microbiome has enormous potential as the basis for new science in academia and new business opportunities. The discovery of new compounds with new applications will be the starting point for further new enterprises. Table 3 illustrates fish and shellfish microbiome species as a source of developing innovative products for future growth of bio/pharma and aquaculture industry.

7 | CONCLUSIONS

Several attempts have been made to investigate microbiome composition of the gut system of the many commercially important fish and shellfish. It is now well speculated that the microbiome diversity can be correlated with the number of health issues of the animals like intensity of digestion of food in the digestive tract, energy generation and metabolism, issues related to infectious diseases and immune system, and also its impact on nutrition, growth and reproduction. It has also been suggested that the gut microbiome can be used as a biomarker of fish health and in that case, future research is required to understand the relationship between fish disease and specific taxa of microbial species, which may enable us to optimize gut microbiota composition to mitigate fish disease. However, more confirmatory research studies are required in such niche areas of science. There is also need of more research on microbiome composition, its diversity in relation to various factors and identification of these microbes up to the species level in several fish and shellfish. Due to the availability of gene-based molecular tools like DNA sequencing and

NGS technology including amplicon and shot-gun approaches, and associated bioinformatics skills, has now been possible to count and classify commensal microbiome at the species level. Development of these molecular techniques, encouraged many workers to undertake the studies on various functional aspects of microbes and in recent years the field of microbiome metagenomics has gained lot of importance. Efforts are being made to understand different functions of the microbial species at the genomic levels so that by altering the gene structure desired properties can be developed in microbes. Bio-industries including aquaculture firms are looking for such innovative research to develop new products for controlling outbreak of diseases in fish and shellfish and build a healthy and sustainable aqua-farming systems. In order to maintain health status of the aquaculture species, probiotics are now being practised instead of using medicines and antibiotics. It has also been suggested that use of probiotics not only controls the spread of diseases but promotes the growth and reproduction in cultivable organisms. The probiotics are manufactured from beneficial microbial species and proliferation of healthy microbes in the gut system generally keeps the animals healthy. Now in shrimp farming system, the probiotics are used very often for disease management but their usage for controlling bioremediation and bio-control strategies requires further studies. Lot more emphasis has to be given to explore marine beneficial microbes instead of using terrestrial strains as a potential source for manufacturing probiotics. In aqua-farming, disease management is one of the greatest hurdles and in order to understand the precise mechanism of infectious pathogens causing damage to the host, use of bioinformatics knowledge has been suggested by number of researchers. However, a lot of research is needed in this particular area to mitigate not only disease management but also other physiological disturbances in cultivable organisms. The bio-pharma and aquaculture industry are looking for such innovative research not only for their prospects but for extending support for the development of healthy and sustainable aqua-farming.

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CONFLICT OF INTEREST

None declared.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon request.

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REVIEW

Boosting Immune Function and Disease Bio-Control Through Environment-Friendly and Sustainable Approaches in Finfish Aquaculture: Herbal Therapy Scenarios

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ABSTRACT

Aquaculture offers a promising source of economic and healthy protein for human consumption and improved wellbeing. This has led to the development of the aquaculture industry, led through advanced production technologies and culture systems in many parts of the world. The intensification of fish production systems by farmers to meet consumer's needs, as well as to generate increased profits, creates a source of stress and the added occurrence of disease and subsequent loss. The negative environmental effects of chemical use have caused legislation to imposed regulations and restrictions to decrease their therapeutic or prophylactic use in aquaculture. As a result, dietary approaches have been suggested as an alternative. Medicinal herbs have been investigated for use in finfish diets to improve immune response and disease resistance. This review paper discusses the suggested modes of action of the effects of medicinal herbs on fish physiology and the immune systems. In addition, a comprehensive literature review on the effects on bacterial, viral, and parasitic diseases is also presented. An added objective was to address the gap between existing knowledge and future perspectives for the improvement of fish health and disease resistance through the use of natural products.

KEYWORDS

Herbs; immunostimulants; innate immunity; diseases resistance; herbal therapy

1. Introduction

The global population is set to increase to nearly 10 billion by 2050, with increasing pressures on resources, such as energy and food. However, food sources and proteins and food are not unlimited, as hunger remains a crisis that continues to propagate due to potential competition, conflict, and climate change. Aquaculture is a promising source of quality and healthy proteins for humankind (FAO, 2016), in which strategies to encourage the development of sustainable fish farming industries have been advocated (Carbone and Faggio 2016). Modern aquaculture addresses two primary concerns: the reduction of water used for culture; and increasing production output per unit. To meet market demands, farmers may increase stock densities, which can pose stress to fish and shellfish cultures in intensive operations (Van Doan et al. 2017). The stressful conditions that result in the weakening of the immune system of farmed aquatic species have been well-documented (Vatsos et al. 2010; Roosta and Hoseinifar 2016). The increasing risk of antimicrobial resistance in animal production has warranted more interest in organic farming of fish and shrimp, in which the use of antibiotics and chemotherapeutic agents to deal with established or emerging pathogens are avoided. Given the difficulties of disease treatment in so-called "green aquaculture" (antibiotic-free), strengthening the innate immune system is of great importance (Ringø et al. 2016). Among the available approaches, the administration of feed additives has been suggested as an environmentally friendly means of

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immunomodulation (Pohlenz & Gatlin III, 2014; Dawood and Koshio 2016). During the past few decades, increasing attention has developed in the direction of medicinal herbs in aquaculture (Wang et al. 2017; Wang et al. 2017). These natural phytobiotic agents have been used as preventative means of disease control. Several researchers evaluated the potential of herbs to control bacterial, viral, or parasitic diseases, with reported increases in protections in most of the studies (Harikrishnan et al. 2011; Jeney et al. 2015; Van Hai 2015). In the current review paper, a comprehensive literature review on existing knowledge regarding the application of medicinal herbs to increase resistance against various types of diseases was performed. Also, the mode of actions of the herbs intended to increase protection against infectious diseases was clarified in cases that were determined in the context of their applications and health management of various fish species. The gaps between existing knowledge and future perspectives are highlighted.

2. An overview of fish immunity and possible boosting using feed-additive

The immune response is defined as a homeostatic process or a sequence of dexterously balanced complex, multicellular, metabolic, or physiological processes that allow an individual to distinguish foreign material from "self" and deactivate and/or eliminate them (Magnadóttir 2006). The immune functions and their efficiency are affected by several exogenous and endogenous factors, which lead to either immunosuppression or immunostimulation processes in the organism (Trichet 2010; Pohlenz & Gatlin III, 2014; Caipang and Lazado 2015; Nawaz et al. 2018). The use of herbal immunostimulants is one of the most promising alternative eco-friendly means to achieve chemotherapy and vaccination as it facilitates the phagocytic cellular function and increases the bactericidal and fungicidal activities (Newaj-Fyzul and Austin 2015; Van Hai 2015). There are different types of leucocytes produced by hematopoietic stem cells in the bone marrow, which are commonly termed innate immune cells that provided a primary signal or first line of Defense which activates the innate immune system of fish when administered with herbal products (Manjrekar et al. 2000; Christybapita et al. 2007; Haniffa and Mydeen 2011) .They may also act as an interdependent mechanism of innate resistance and/or an adaptive immunity (Bhattacharyya et al. 2009). Neutrophils and monocytes circulate in the bloodstream and migrate into tissues that participate in the nonspecific immunity and kill pathogens, such as bacteria, through phagocytosis. Furthermore, nonspecific immune cells have an important function in the inflammatory response (i.e., through releasing cytokines). Additionally, the recognition of pathogens or epitopes is achieved by different lymphocytes (T cells and B cells) (Abbas et al. 2010). There are also other types of immune cells, such as monocytes, macrophages, dendritic cells, and neutrophils, which actively participate in removing pathogens through phagocytosis. They have actively recruited cells on infection sites via inflammatory responses. The ingestion of microbes into vesicles develops into phagosomes, which fuze with lysosomes that contain various proteases that kill microbes through proteolytic processes (Abbas et al. 2010). The activation of macrophages takes place when immune cells bind with the microbe. The receptors responsible for microbe recognition are TLRs, G protein-coupled receptors, antibody Fc, and complement C3 receptors, as well as receptors for cytokines, mainly IFN-γ. These receptors are capable of destroying the ingested microbes via receptor-mediated signal transduction (Palti 2011). There is a link between nonspecific immunity and acquired immunity, which is mediated by different types of cytokines; IL-1, IL-12, and TNF, which cause inflammation, secreted by activated macrophages (Zou and Secombes 2016). Respiratory burst (RB) or oxidative burst (OB) is the rapid release of important bactericidal reactive oxygen species (ROS) from the activated macrophages and neutrophils (Uribe et al. 2011). These activated cells produce phagocyte oxidase enzymes that form ROS form molecular oxygen. Inflammatory mediators are also capable of producing such enzymes (Abbas et al. 2010), such as lysosomal hydrolases, an acid phosphatase that can remove phosphate groups from other molecules. These molecules exist in phagolysosomes of macrophages (lysosomal acid phosphatases); and, when activated, result in an acidic environment that kills (Møller et al. 2006; Abbas et al. 2010).

The complement system (CS) is an important means of Defense in humoral and adaptive immunity, which involves clearing pathogens from the host (Uribe et al. 2011) through either classical or alternative pathways. CS activation involves the successive proteolysis of proteins and generates novel enzyme complexes that eventually cause lysis of the microbe by facilitation of opsonization (Yano 1995; Abbas et al. 2010). B lymphocytes contain membrane-bound antibody molecules which are capable of antigen

detection. Generally termed antigen receptors, they become engaged by other signals, thereby triggering multiplication and increased lymphocyte numbers. Their differentiation to mast cells allows them to secrete antibodies with distinct functions within the immune response (Wang et al. 2016). These antibodies bind to the microbes, commonly called antibody-dependent cellular cytotoxicity (ADCC), to prevent, kill, or neutralize microbes or infect cells by promoting opsonization and phagocytosis (Magnadóttir, 2010). Lysozyme is an essential enzyme in the immune function, which can lyse the cell wall (glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine) of pathogenic bacteria (Alexander and Ingram 1992). Macrophages are the main producers of lysozyme, and microbial lipopolysaccharide has been known to stimulate lysozyme production (Goethe and Phi-van 1998).

Several natural or synthetic immunostimulant agents, frequently described as immunostimulants, are capable of normalizing or modulating the pathophysiological processes (Puri et al. 1994). They can also modulate, suppress and/or stimulate any components of innate (nonspecific) or adaptive (specific) immunities, known as immunomodulators, immune restoratives, immunoaugmentors, or biological response modifiers. In general, immunostimulants stimulate phagocytosis and bacterial killing action through macrophages, complement pathways, lymphocytes, and innate cytotoxic cells, which results in enhanced disease resistance and protection to numerous pathogens. Immunostimulants, such as $\beta-1,3$ glucan positively affected cellular immune responses, such as the number of phagocytic cells or macrophages bactericidal activity in several fish species; like trout, Atlantic salmon, catfish, and carp (Rao et al. 2006); and through the production of superoxide anions via the macrophages (Jørgensen et al. 1993). Yeast-derived glucan has also been reported to increase phagocytosis or the production of ROS by phagocytes in Indian major carps (Karunasagar and Karunasagar 1999). Components of innate immunity in immunomodulation consist of an array of cells, including natural killer (NK T-cells, macrophages, neutrophils, eosinophils, and basophils, and dendritic cells); whereas, B-cells (naïve $CD4^+$ T-cells) differ from $CD4^+$ T-cells, and include helper T-cells (TH1, TH2, and TH17 cells), induced regulatory T-cells, and natural regulatory T-cells (Kaiko et al. 2008). Herbal immunostimulants, as an aquafeed supplement, also possess the same biological activity as above and are viewed as modern and promising substitutes for chemicals and vaccines (Van Hai 2015). The plant-derived natural products, which are generating a great deal of attention, are potentially safer immunomodulator sources, capable of providing a more secure alternative strategy to replace the use of antibiotics in aquaculture. Herbal medicines with multiple-component agents are expected to stimulate or suppress the components of both innate and adaptive immune systems in such a way to prevent the infection, rather than act as a treatment and cure of the disease (Hoseinifar et al. 2018). While limited information exists about the inherent attributes of immunostimulants on immune cells and immunity under *in vitro* condition, several *in vivo* studies have revealed the improvement of immune responses following the application of immunostimulants either, either via injection or feeding (Reverter et al. 2014; Caipang and Lazado 2015; Wang et al. 2017). Dietary additives as a potential for immunostimulants may enhance the innate defense mechanisms, thereby increasing resistance or protection from specific pathogenic challenges through the activation of leukocytes (Lundén and Bylund 2000). The increasing defense status of the organism can be due to the elevation of phagocytosis and secretion of cytokines from macrophages. Many herbal products function via a notable modulatory effect on a large number of multiple cytokines, which produce soluble extracellular proteins or glycoproteins in the form of interleukins (ILs), interferons, and chemokines. They act as critical components in both innate and acquired types of immunity through intermolecular "cross-talks", which maintain physiological stability (Mogensen 2009). A few studies have demonstrated the capabilities of herbal substances or bioactive single compounds to influence immunosuppression and/or immunostimulation activities in fish, as described in Table 1.

3. The mode of action of medicinal herbs and effects on fish physiology and immune system

Herbal substances present more biological activity than that of single bioactive compounds, due to their high selectivity and potency, and low toxicity for targeted molecule, cells, and diseases. Plants sources have a rich content of secondary metabolites (SM), like volatile oils, saponins, phenolic compounds, tannins, alkaloids, polypeptides, and polysaccharides, which initiate immunomodulatory activities; like anti-stress, appetite stimulation, antimicrobial function, and disease resistance. They can act positively in stress

Table 1. Immunostimulation of herbal substances or bioactive single compound enriched diet in fish.

Herbs	Parts	Solvent	Dose	Fish	Pathogen	Increase/Enhance/Modulation	Referemces
Jerusalem artichoke	Leaf	NA	20 g kg^{-1}	*Lates calcarifer*	*Aeromonas hydrophila*	Leucocytes, phagocytic activity, respiratory burst activity, lysozyme activity	Ali et al. (2017)
Rauvolfia tetraphylla	Leaf	Ethanol	5 g kg^{-1}	*Labeo rohita*	*Aphanomyces invadans*	Leucocytes, respiratory burst activity, complement activity, myeloperoxidase activity	Yogeshwari et al. (2015)
Siegesbeckia glabrescens	Leaf	Ethanol	10 & 20 g kg^{-1}	*Epinephelus bruneus*	*Vibrio parahaemolyticus*	ROS, reactive nitrogen intermediate (RNI), myeloperoxidase (MPO) production	Harikrishnan et al. (2012a)
Acanthopannax korearum, Glycyrrhiza vralensis, Panax ginseng	Stems	NA	5 g kg^{-1}	*Paralichthys olivaceus, Oplegnathus fasciatus*	-	ROS, lysozyme activity	Kim et al., (2012)
Liriope platyphylla	Leaf	Methanol, Ethanol	1.0 or 2.0 mg kg^{-1}	*P. olivaceus*	*Flexibacter maritimus*	Leucocytes, total protein and globulin, phagocytic activity, complement activity	Harikrishnan et al. (2012b)
Punica granatum	Fruit	Ethanol	10 g kg^{-1}	*P. olivaceus*	*Philasterides dicentrarchi*	Leucocytes, scuticocidal activity, respiratory burst, lysozyme activity	Harikrishnan et al. (2012c)
Suaeda maritima	Leaf	Ethanol	10 g kg^{-1}	*P. olivaceus*	*Miamiensis avidus*	Leucocytes, lysozyme activity, scuticocidal Activity, respiratory burst activity	Harikrishnan et al. (2012d)
Pueraria thunbergiana	Leaf	Ethanol	20 g kg^{-1}	*E. bruneus*	*Vibrio harveyi*	Leucocytes, total protein content and albumin, phagocytic activity, lysozyme activity, superoxide anion production, bactericidal activity, anti-protease activity	Harikrishnan et al. (2012e)
Alnus firma	Leaf	Ethanol	10 g kg^{-1}	*P. olivaceus*	*Tenacibaculum maritimum*	Leucocytes, respiratory burst activity, lysozyme activity	Harikrishnan et al. (2011)
Kalopanax pictus	Leaf	Ethanol	20 g kg^{-1}	*E. bruneus*	*Vibrio alginolyticus, Philasterides dicentrarchi*	Leucocytes, respiratory activity, lysozyme activity, bactericidal activity, total protein level, myeloperoxidase, α2-macroglobulin	Harikrishnan et al. (2011b)
Prunella vulgaris	Flower	Ethanol	10 g kg^{-1}	*P. olivaceus*	*Uronema marinum*	Respiratory burst activities, phagocytic activities, lysozyme activity	Harikrishnan et al. (2011c)
Eriobotrya japonica	Fruit	Ethanol	10 & 20 g kg^{-1}	*E. bruneus*	*Vibrio carchariae*	Leucocytes, total protein, albumin, and globulin, superoxide anion, lymphokines production, phogocytic activity, lysozyme activity, bactericidal activity, complement activity, agglutinating antibody titer	Kim et al. (2011)
Lactuca indica	Flower	Ethanol	10 & 20 g kg^{-1}	*E. bruneus*	*Streptococcus iniae*	Respiratory burst, phagocytic activity, total immunoglobulin, lysozyme activity	Harikrishnan et al (2011d) .
Styrax japonica	Flower	Ethanol	10 & 20 g kg^{-1}	*E. bruneus*	*Vibrio Harveyi, Uronema marinum*	Phagocytic activity, respiratory activities, complement activity, lysozyme activity, serum bactericidal activity, total protein level, antiprotease activity,	Harikrishnan et al. (2011e)

						myeloperoxidase, α2-macroglobulin	
Scutellaria baicalensis	Leaf	Water	10 g kg^{-1}	*Oplegnathus fasciatus*	*Edwardsiella tarda*	Leucocytes, lysozyme activity, complement, antiprotease activities, ROS, RNS production	Harikrishnan et al. (2011f)
Azadirachtin indica	Leaf	Ethanol	2 g kg^{-1}	*Cirrhina mrigala*	*Aphanomyces invadans*	Leucocytes	Harikrishnan et al. (2010a)
Punica granatum	Leaf	Ethanol, methanol	50 or 100 mg kg^{-1}	*P. olivaceus*	*lymphocystis disease virus*	Phagocytosis activity, respiratory burst activity, alternative complement activity, lysozyme activity	Harikrishnan et al. (2010b)
Avicennia marina	Leaf	Ethanol	20 & 40 g kg^{-1}	*Amphiprion sebae*	*Vibrio alginolyticus*	Leucocytes, serum lysozyme activity, respiratory burst assay, complement activity, phagocytic activity, disease resistance, gut bacteria	Dhayanithi et al. (2015a)
Cucurbita mixta	Fruit	Ethanol	4 & 6 g kg^{-1}	*Oreochromis mossambicus*	*Aeromonas hydrophila*	Complement activity, phagocytic activity, respiratory burst activity, lysosome activity	Musthafa et al. (2017)
Rhizophora apiculata	Leaf	Ethanol	50 or 100 g kg^{-1}	*Amphiprion sebae*	*Vibrio alginolyticus*	Lysozyme, respiratory burst, complement activity, phagocytic activity, superoxide anion production, anti-protease activity	Dhayanithi et al. (2015b)

mediation, due to handing, transport, grading, and poor water quality (Sakai 1999; Magnadóttir, 2010; Ringø et al. 2012). A plants SM skeleton contains several polar and non-polar functionally reactive groups; including aldehyde and SH-groups, epoxides, double bonds with non-configuration, and triple bonds; which can form through covalent bonds with proteins, peptides, and sometimes DNA (Wink 2005, 2008, 2012).

Secondary metabolites (SM) containing the aldehyde group are capable of establishing a Schiff base with amino/imino groups of proteins and amino acid residues. The epoxides are capable of binding with free amino groups, DNA bases, and SH-groups of proteins. The exocyclic methylene groups of terpenes, phenylpropanoids, allicin, or sesquiterpene lactones bind with SH-groups of proteins and glutathione. Proteins that generally act as receptors, enzymes, ion channels, transcription factors, or cytoskeletal proteins are the most affected molecules. The alteration of the biding site or catalytic site of receptors and enzymes prevent binding with the ligand or substrate to initiate a molecular response. The three-dimensional configurations of proteins or peptides can be affected by alkylation that influences protein-protein recognition, as well as binding or catalytic activity or turnover. Secondary metabolites can affect a large number of proteins (non-selectively), and can, therefore, be used as "multi-target drugs" for the treatment of diseases. It should be noted that alkylation of DNA bases by aldehydes, epoxides, and pyrrolizidine alkaloids can cause specific mutations, which might even lead to cancer (Wink 2004; Wink and Van Wyk 2008).

Van Wyk and Wink (2004) and Wink (2012) further reported that interaction of secondary metabolites with reactive functional groups or phenolic or polyphenolic components present in medicinal herbs that contain one or more hydroxyl (OH-) groups are capable of modulating proteins. These negatively charged groups can bind with amino acid residues (their positively charged amino groups) and, if occurring within catalytic sites, can impair their functions. Furthermore, the glycolisation of phenolic compounds with sugar molecules and subsequent interaction with proteins can indirectly affect gene expression (Pakalapati et al. 2009; Holtrup et al. 2011; El-Readi et al. 2013). The semipermeable bio-membrane of organisms principally acts as a penetration barrier, functioning as ion channels, receptors, and transporters; that permit the regulation of influx of external substances. The bio-membrane can be easily lysed, resulting in necrotic cell death (Van Wyk and Wink

Table 2. The SMs from different medicinal herbs and observed effects on disease resistance in various fish species.

Medicinal herb	Type of secondary metabolites (SM)	Immune parameters	Aquatic species	Pathogen challenge	Reference
Rheum officinale	Anthraquinon	Resp, Lys, Alp Hemolymph Pr	*M. rosenbergii*	*Aphanomyces invadans*	Liu (2010)
Azadirachta indica + *Ocimum sanctum* + *Curcuma longa*	Azadirachtin + camphor + curcumin	Resp, Lys, WBC	*C. mrigala*	*A. hydrophila*	Harikrishnan (2009)
Zataria multiflora	Essential Oil (Thymol)	Lys, WBC, RBC,	*Cyprinus carpio*	*Vibrio harveyi*	Soltani (2010)
Sargassum fusiforme	polysaccharide	SOD, Phen, RBC	*Fenneropenaeus chinensis*	*Vibrio alginolyticus*	Huang (2006)
Allium sativum	Allicin	Antibody, Lys, Phagocytosis	*O. mykiss*	*A. hydrophila*	
Rosmarinus officinalis	Cineol	Lys, Phagocytosis	*Oreochromis* sp.	Streptococcus iniae	Abutbul et al. (2004)
Cinnamomum zeylanicum	Cinnamaldehyde	Resp, Lys, Phagocytosis	*O. niloticus*	*Vibrio anguillarum*	Rattanachaikunsopon (2010b)
Quillaja saponaria	Saponin	RBC, Resp, Phen	*Litopenaeus Vannamei*	Streptococcus agalactiae	Su (2008)
Eichhornia crassipes	*Phenolic compound*	Lys, Alp, Ig, Protease SOD, CAT, Gpx	*O. mykiss*	Streptococcus iniae	Rufchaie (2020)

Resp: Respiratory burst activity; Lys: Lysozyme; Alp: Alkaline phosphatase; Pr: Protein; WBC: White blood cells count; RBC: Red blood cells count; Phen: Phenol oxidase activity; Ig: Immunoglobulin; SOD: Superoxide dismutase; CAT: Catalase; Gpx: Glutathione peroxidase.

2004; Van Wyk and Wink 2015). Table 2 presents the SM of different medicinal herbs, as well as their effects on disease resistance in various fish species.

Van Wyk and Wink (2004) and Van Hai (2015) reported that numerous mono- and sesquiterpenes could accumulate in membranes at high levels, which alters the fluidity of the permeability membrane; and, subsequently, the antimicrobial and cytotoxic activities. The production of ROS may lead to several prolonged health complaints, like diabetes, metabolic syndrome, and cardiovascular disease (Van Wyk and Wink 2015). Several phenolics or terpenoids capable of conjugating with double bonds inhibit ROS and other oxygen radical generation in cells and tissues (Van Wyk and Wink 2004; Van Wyk and Wink 2015). Interestingly, previous studies revealed the alteration of the membrane's permeability by terpenes (Wink 2007). This promotes the cytotoxic membrane's activities against a broad spectrum of bacteria and fungi (Tyler 1994; Wichtl 2004; Wagner et al. 2009; Van Wyk and Wink 2015). The monoterpenes contain phenolic hydroxyl groups; such as thymol and carvacrol, or terminal methylene groups in the camphene, pinocarvone or linalool; which can bind to SH groups of proteins, resulting in the disturbance of ROS production in cells; thus inhibiting the growth of bacteria and fungi (Teuscher and Lindequist 1994; Van Wyk and Wink 2004; Van Wyk and Wink 2015).

Phenylpropanoids are another class of plant compounds that bear several phenolic hydroxyl abilities, possessing anti-inflammatory and antiviral activity (Teuscher et al. 2004; Teuscher and Lindequist 2010), which prevents cell division and immune-modulation (Kuljanabhagavad and Wink 2009; Wink and Schimmer 2010; Su et al. 2015a, 2015b). Gallotannins are also the plant compounds that contain several phenolic hydroxyl groups which are capable of interaction with a broad spectrum of proteins in microbes and animals (Van Wyk and Wink 2004; Wink and Van Wyk 2008; Van Wyk and Wink 2015). Other components in medicinal herbs which are capable of triggering antimicrobial and immunostimulant activities include polyacetylenes or polyenes, such as in falcarinol (Marriott et al. 1999; Van Der Meijden 2001); which bind to SH groups bound in membrane proteins, receptors, and ion channels (Van Wyk and Wink 2004; Wink and Van Wyk 2008; Van Wyk and Wink 2015). Alkaloids and amines found in several plants also comprise one or several nitrogen atoms that bind to bacteria, fungi and, viruses (Ablise et al. 2004; Okwu 2004; Van Wyk and Wink 2004; Lee et al. 2008; Wink and Van Wyk 2008; Teuscher and Lindequist 2010; Van Wyk and Wink 2015). Another class of a compound is the lectins (highly specific sugar-binding proteins or glycoprotein substances) present in herbals that contain a crucial immune function that recognizes pathogens through the binding of carbohydrates exclusively located on pathogens (Dam and Brewer 2010). The mannose-binding lectin identifies the complex carbohydrate patterns which exist on the surface of various pathogenic microorganisms (Boshra et al. 2006; Van Kooyk and Rabinovich 2008).

Active components of herbs are believed to improve nutrient digestibility, absorption, assimilation capacity, and digestive enzyme secretion, as well as maintain healthy intestinal microflora in fish (Van Kooyk and Rabinovich 2008). Fish are frequently challenged by oxidative stress, caused by numerous

environmental stressors (abiotic and biotic), generally under rearing conditions. Therefore, to overcome these deleterious changes in the metabolic state, these stressors may be mitigated by applying various medicinal herbs in the feed containing rich sources of antioxidants, such as polyphenols and flavonoids that act synergistically, offering enhanced protection (Alok et al. 2014). Based on the above research, it can be inferred that herbal immunostimulants, in either crude or purified bioactive compounds, can significantly affect immune responses and subsequent disease protection, through the mechanisms or modes of action of herbal immunostimulants in fish have not yet been determined. The high antioxidant effects of their bioactive components can improve cell protection, such as immune cells (like leukocytes), and thereby benefit the fish immune system in general.

4. Medicinal herbs and treatment of bacterial diseases in finfish aquaculture

This section highlights several examples of medicinal herbs that have been evaluated in association with various fish bacterial diseases.

Indian ginseng (*Achyranthes aspera*): Rohu, *Labeo rohita*, fingerlings fed with a 0.2% Indian ginseng (*Achyranthes aspera*) enriched diet showed a 41% decrease in mortality after experimental exposure to the pathogenic *A. hydrophila*. Evaluation of the immune parameters was further supported by the stimulation of immune responses of fish fed *A. aspera* (Rao et al. 2006). Subsequently, Chakrabarti and Srivastava (2012) reported the dietary administration of 1, 2.5, and 5 g kg^{-1} of *A. aspera* to rohu (*L. rohita*) larvae, in which 5 g kg^{-1} of *A. aspera* prevented tissue and organ damage, provided protection against oxidative stress, and enhanced disease resistance when fish were injected intraperitoneally with live *Aeromonas hydrophila*.

Indian bael (*Aegle marmelos*): Pratheepa et al. (2010) reported that freshwater fish *Cyprinus carpio*, consuming a diet supplemented with *Aegle marmelos* leaf extract for 50 days resulted in significantly enhanced immune functions. After experimentally infected with *A. hydrophila,* a significant increase of resistance and survival was noticed in the treated fish, with the highest survivability in the 5 g kg^{-1} treatment.

Garlic (*Allium sativum*): In a 60-days feeding trial, Sahu et al. (2007) evaluated the anticipated disease protection properties of different levels (1, 5, and 10 g kg^{-1}) of dietary garlic (*Allium sativum*) in rohu fingerlings (10 ± 2 g). At the end of the feeding trial, fishs were exposed to the challenge experiment with *A. hydrophila* (Sahu et al. 2007). The results indicated that the *A. sativum* stimulates the immune system of the rohu, resulting in a higher resistance to the pathogen. Interestingly, the survival rate was 57%, 85%, and 71% of fish survived in 1, 5, and 10 g kg^{-1} treatments, respectively. Similarly, the dietary administration of mashed garlic (5 and 10 g kg^{-1}) in rainbow trout (*Oncorhynchus mykiss*) resulted in improved survival rates (96% in the treatment compared to 12% in control) when challenged with *A. hydrophila* (Nya and Austin 2009). The fish presented superior growth performance and demonstrated elevated specific immune parameters, such as white blood cells, lysozyme activity, and bactericidal activity. Talpur and Ikhwanuddin (2012) investigated the effects of different levels of garlic supplement (5, 10, and 20 g kg^{-1}) on the immune parameters, as well as resistance against *Vibrio harveyi* in Asian sea bass (*Lates calcarifer*) fingerlings. Data revealed that the levels of immune parameters, as well as the survival rate post *V. harveyi* challenge, were noticeably increased in garlic fed Asian sea bass.

Female ginseng *(Angelica sinensis):* Wang et al. (2011) studied the supplemental *Epinephelus malabaricus* diets (0.5 and 3 g kg^{-1}) of polysaccharide derived from *Angelica sinensis*. At the end of the feeding trial, both immune parameters and disease resistance were evaluated, which revealed the stimulation of cellular immunity and a higher protection against *Edwardsiella tarda*. Notably, the popularity and low price of ginseng in Asia make it an effective means of disease prevention in Asian aquaculture. The functionality of this herb should be tested on other important cultured species. Despite the published results, more experiments are needed to elucidate the exact mode of action of this medicinal herb on immunity.

Astragalus membranaceus: *A. membranaceus* is a common and widely employed herb in Chinese traditional medicine used as an enhancer of immune responses in both humans and animals. The polysaccharide is the main constituent of *Astragalus* root, acting as a biological function. Yin et al. (2009) administered *Astragalus* extract at a dose of 5 g kg^{-1} in common carp diet. They observed a significant elevation of fish resistance to *A. hydrophila* in comparison with that of the control group. Similarly, dietary administration of *A. membranaceus* extract significantly increased both the immune parameters (phagocytic and respiratory burst activities), as well as the survival rate after *A. hydrophila* challenge (Ardó et al. 2008). In a study with red drum (*Sciaenops ocellatus*),

Pan et al. (2013) investigated the effects of 20 g kg^{-1} of the medicinal herb *A. membranaceus*, and observed that improved stimulation of the immune parameters, as well as resistance against *V. splendidus*.

Neem (*Azadirachta indica*): Dip treatment of common carp with neem (*Azadirachta indica*) leaf extract notably elevated immune parameters in serum, which protected fish against pathogenic *A. hydrophila* (Harikrishnan et al. 2003). Talpur and Ikhwanuddin (2013) later reported that Asian seabass fingerlings fed neem leaf-supplemented diets (1, 2, 3, 4, and 5 g kg^{-1}) had significantly a higher immunity and resistance properties against *V. harveyi*. Similarly, Verma et al. (2013a) reported that supplementation of common carp fingerlings with neem leaf powder diets decreased the mortality rate (35%) following the experimental infection with *A. hydrophila*. Interestingly, the mortality rate of fish fed the basal diet was significantly higher (85%) than that of treated fish. Accordingly, given the positive effects of *A. indica* extract (150 mg L^{-1}) for the protection of *O. mossambicus* against *Citrobacter freundii*, Thanigaivel et al. (2015) suggested neem as an efficient alternative to antibiotics for mitigating infection.

Cinnamon (*Cinnamomum zeylanicum*): Dietary administration of cinnamon (*Cinnamomum zeylanicum*) at a dose of 10 g kg^{-1} in Nile tilapia diet demonstrated the antibacterial activity's protection against *A. hydrophila* infection (Ahmad et al. 2011).

Polypore mushroom (*Coriolus versicolor*): Wu et al. (2013a) added the extracted polysaccharides from polypore mushroom (*Coriolus versicolor*) to crucian carp (*Carassius auratus gibelio*) diets to investigate the effects on both the physiological parameters disease resistance. The results revealed that the inclusion of 1 g kg^{-1} CVP protected against *A. hydrophila* infection.

Pink mempat (*Cratoxylum formosum*): In a 30-days feeding trial, Rattanachaikunsopon and Phumkhachorn (2010a) supplemented Nile tilapia diets with pink mempat extract (1-15 g kg^{-1}), challenged with *S. agalactiae*. The results confirmed that motilities decreased in the treated fish. The survival rate in the control group (15%) rose to 88 and 90% in Nile tilapia fed 10 and 15 g kg^{-1} pink mempat extract, respectively. Concomitantly, the pink mempat extract was found to enhance several immune parameters, such as lysozyme, phagocytic, and respiratory burst activity.

Turmeric (*Curcuma longa*): In a 60-day feeding trial, rohu fingerlings were fed a series of diets containing varying levels of turmeric (*Curcuma longa*). After this, the fish was challenged with *A. hydrophila* (Sahu et al. 2008). The results showed that *C. longa* stimulated immune function and improved disease resistance of rohu. The best results were obtained when rohu fingerling treated with 1.0 g kg^{-1} *C. longa* (Sahu et al. 2008).

Loquat (*Eriobotrya japonica*): *E. japonica* is a medicinal plant native to China, and belongs to rosaceae family (Hoseinifar et al. 2018). Both the *in vitro* and *in vivo* studies revealed promising immunostimulatory and antioxidant effects of this herb (Alshaker et al. 2011). These beneficial effects were attributed to the presence of bioactive compounds; like roseoside, procyanidin B-2, triterpene acids, chlorogenic acid, and flavonoids (Zar et al. 2014). To the best of our knowledge, only two available studies exist regarding the administration of *E. japonica* in aquaculture. Kim et al. (2011) evaluated the disease protecting effects of *E. japonica* in kelp grouper (*Epinephelus bruneus*), revealing that fish treatments of 10 and 20 g kg^{-1} *E. japonica* extract increased immune responses as well as higher survival rate post-challenge. Likewise, supplementation of common carp diet with loquat leaf extract significantly increased serum immune parameters as well as immune-related gene expression (Hoseinifar et al. 2018). However, in that study, no challenge test was undertaken to determine whether this medicinal herb could protect fish against diseases.

Cats hair (*Euphorbia hirta*): Cat hair, or asthma weed, is a medicinal plant belonging to the family of Euphorbiaceae with a long history of administration for the treatment of diseases; such as asthma, diarrhea, and dysentery (Ogbulie et al. 2007). In the study with common carp, the dietary administration of *E. hirta* leave extract (50 g kg^{-1}) significantly elevated phagocytosis and resistance against *Pseudomonas fluorescens* (Pratheepa and Sukumaran 2011).

Lian Qiao *(Fructus forsythia):* This medicinal plant ranks among several famous herbs in traditional Chinese medicine. Recent studies demonstrated its anti-inflammatory, antioxidant, and antiviral activities (Ogbulie et al. 2007). However, in aquaculture, studies have been limited. A single dose (20 g kg^{-1}) of this herb was fed to red drum (*Sciaenops ocellatus*) (Pan et al. 2013), in which significant resistance against pathogenic *V. splendidus* was observed. Evaluation of immune parameters, such as lysozyme activity and phagocytic index, also revealed notable increases in the treated groups, demonstrating the potential of this herb as an aquacultural immunostimulant.

Jungle geranium (*Ixora coccinea*): In 30 days feeding trial, (Anusha et al. 2014) investigated goldfish diets supplemented with a single dose (400 mg kg^{-1}) of *I. coccinea* extract (either crude or purified), and

evaluated disease resistance and immune parameters. In the results, both crude and purified extracts improved bactericidal and phagocytic activities and decreased the mortality from 100% in the control group to 40% and 20% in the treated group.

Indian Lettuce (*Lactuca indica*): This herb is belonging to Asteraceae family, and has been used in the treatment of various diseases due to its anti-inflammatory, antibacterial, and anti-diabetic properties (Yeasmin et al. 2015). Harikrishnan et al. (2011) studied the immunostimulatory and disease protecting effects of this herb extract in kelp grouper throughout a four-week feeding trial. The results revealed that fish treated with either 10 or 20 g kg^{-1} *L. indica* produced significantly higher immune parameters as well as resistance against *Streptococcus iniae*.

Japanese honeysuckle (*Lonicera japonica*): It has been reported that the extract of the flower of this herb has several bioactive components that serve as a beneficial medicinal herb (Schlotzhauer et al. 1996). In aquaculture, there has been just one study on this herb, on Nile tilapia, conducted by Ardó et al. (2008). The results confirmed the immunomodulatory effects of this herb through the observed increase in immune parameters (e.g., phagocytic and respiratory bursts activities). Also, treated fish had significantly higher survival rates after challenge with *A. hydrophila*.

Lupin (*Lupinus perennis*): Lupin is a flowering plant belonging to the legume family. Awad and Austin (2010) administered this medicinal herb at a level of 10 g kg^{-1} level in rainbow trout diet, and then subjected them to the experimental challenge with *A. hydrophila*. Interestingly, while 32% mortality was recorded in the control group, no mortality occurred in the treated group. These results define the potential of this medicinal herb to prevent disease outbreak in aquaculture and to encourage further research involving the administration of this medicinal herb, such as dose, administration, and duration.

Mango (*Mangifera indica*): Mango is a flowering plant belonging to Anacardiaceae family, native to India. Numerous cultivars of this herb can be found in tropical areas (Pino et al. 2005). It delivers beneficial effects, due to the presence of bioactive components. In 14 days feeding trial, Awad and Austin (2010) studied the potential disease protecting effects of mango (10 g kg^{-1}) on rainbow trout. They reported a 28% lower mortality post *A. hydrophila* challenge. Additionally, the serum immune parameters, such as bactericidal, respiratory burst, complement, and lysozyme activities), were significantly higher in the mango treated trout.

Peppermint (*Mentha piperita*): This herb is a hybrid of watermint and spearmint, and native to Europe and the Middle East. Nonetheless, it can be found in many areas of the world. Talpur (2014) supplemented the diets of Asian sea bass (*Lutes calarifer*) with peppermint at incremental levels between 1–5 g kg^{-1}. After four weeks of feeding, improved immune function and increased resistance against pathogenic *V. harveyi* were obtained.

Night jasmine (*Nyctanthes arbortristis*): Night jasmine is a flowering plant belonging to Oleaceae family, and is known as a medicinal herb in India (Rani et al. 2012). To the best of our knowledge, it has been evaluated just in one study in aquaculture. Kirubakaran et al. (2010) supplemented tilapia diet with varying levels (1-10 g kg^{-1}) of *N. arbortristis* seeds chloroform extract. At the end of the trial, immune responses were assessed, and fish were subjected to experimental challenge with *A. hydrophila*. The results revealed a significant increase in immune parameters and disease resistance in treated fish, and dietary administration of 1 g kg^{-1} proved to be the optimum inclusion level of night jasmine seed extract for tilapia.

Holy basil (*Ocimum sanctum*): Holy basil or tulasi is an aromatic herb belonging to Lamiaceae family. This herb grows naturally in India and the tropical region of Southeast Asia. The essential oils and natural compounds of this herb have been found to contain beneficial properties, which made it a promising medicinal herb (Makri and Kintzios 2008). In aquaculture, the administration of holy basil *(Ocimum sanctum)* leaf extract, simultaneously with or after vaccination, positively affected the immune parameters, such as antibody production in *O. mossambicus*. Treated fish also demonstrated remarkably higher survival rates post-challenge with pathogenic *A. hydrophila* (Logambal et al. 2000). Similarly, methanolic extracts of *O. sanctum* notably enhanced serum immune parameters and resistance against *Vibrio harveyi* in juvenile greasy groupers (*E. tauvina*) (Sivaram et al. 2004).

American ginseng (*Panax quinquefolium*): A medicinal herb belonging to the ivy family and native to North America. Due to the presence of several bioactive components, the roots and leaves of this herb have long been used in traditional medicine (Kitts et al. 2000). Abdel-Tawwab (2012) tested the possible effects of different levels (0.50, 1.0, 2.0, and 5.0 g kg^{-1}) of American ginseng on growth performance and disease resistance in Nile tilapia. After 8 weeks of feeding, the *P. quinquefolium* supplemented diet

improved immune competence as well as protecting tilapia against *A. hydrophila* infection.

Selfheal (*Prunella vulgaris*): Selfheal, or heal-all, is a medicinal herb belonging to Lamiaceae family and is found in most of the world's temperate regions. An assessment of the application of Selfheal extracts supplementation (0.1, 1, and 10 g kg^{-1}) was conducted in olive flounder (*P. olivaceus*) for four weeks. At the end of the trial, serum immune parameters and resistance against experimental challenge with *Uronema marinum* was tested. The results revealed that administration of selfheal extract at either 1 or 10 g kg^{-1} improved 0.1% and 1.0% doses of olive flounder immune parameters as well as providing protecting against *U. marinum* (Harikrishnan, Kim, Balasundaram, and Heo 2011).

Common guava (*Psidium guajava*): Guava, also referred to as the guava tree, belongs to the myrtle family (Myrtaceae); and is widely found in subtropical regions. It has been considered as a medicinal herb due to the presence of bioactive components with antimicrobial, immunostimulatory, and anti-oxidant effects (Hoseinifar et al. 2019). A study of the ethanoic extract of fourteen medicinal herbs against *A. hydrophila* infection in *Oreochromis niloticus* was conducted, in which the highest protection was noticed in the case of common guava supplemented diets (Pachanawan et al. 2008). Similarly, guava leaves extract (GLE) protected rohu against *A. hydrophila* infection, as well as presenting an up-regulated cytokines gene expression in a later study by Giri et al. (2015). In a more recent study, Hoseinifar et al. (2019) supplemented common carp diets with varying levels of GLE, and reported notable increases of immune-related genes expression, as well as heightened mucosal immune parameters.

Kudzu vine (*Pueraria thunbergiana*): Kudzu vine is a medicinal herb belonging to the Fabaceae family. In aquaculture, a single study conducted by Harikrishnan et al. (2012), demonstrated the dietary administration of Kudzu vine (*Pueraria thunbergiana*) at 10–20 g kg^{-1} fed to kelp grouper (*Epinephelus bruneus*) over four weeks. Improved protection against challenge *V. harveyi* was observed, in which the evaluation of serum immune parameters revealed notable increases in the treated groups.

Rhubarb (*Rheum officinale*): Belonging to the Polygonaceae, Rheum is native to Europe, as well as several temperate regions throughout Asia, and has long been administrated for medicinal purposes (Xiao et al. 1984). Liu et al. (2012) studied the possible effects of this herb (anthraquinone extract) as a potential means of disease protection in Wuchang bream (*Megalobrama amblycephala*). They supplemented diet with a single dose (g kg^{-1}) for 10 weeks and then subjected the fish to *A. hydrophila* infection. The findings showed that mortalities decreased from 100% in control to 86.67% in the experimental group. Furthermore, rheum extract also stimulated several immune-related indices, as well as heat shock protein 70 (HSP70) expressions.

Rosemary (Rosmarinus officinalis): *Rosmarinus officinalis* is a woody herb belonging to the Lamiaceae, and is native to the Mediterranean region. Rosemary and its oil derivates have a long history in traditional medicine; however, limited information is available regarding applications in aquaculture. The effects of dietary administration for disease resistance in both dried leaves and leaf extracts were tested in tilapia. The results demonstrated a significant decrease in the mortality in fish challenged with *Streptococcus iniae* (Abutbul et al. 2004). This pathogen is of paramount importance as a causative agent of a widespread infection, mortality, and losses to the Asian tilapia industry. Therefore, this herb can be considered as biological and environment-friendly means of controlling this pathogen in tilapia aquaculture.

Baikal skullcap (*Scutellaria baicalensis*): Baikal skullcap or Chinese skullcap is a flowering plant belonging to Lamiaceae family. The root of this herb contains several bioactive compounds (baicalein, baicalin, wogonin, and norwogonin) with pharmacological implications, and is considered to be one of the fifty fundamental herbs used in traditional Chinese medicine (Zhang et al. 2011). In aquaculture, Pan et al. (2013) reported that the dietary administration of 20 g kg^{-1} Baikal skullcap in red drum (*Sciaenops ocellatus*) diets stimulated immune function and improved resistance against *V. splendidus* infection.

Solanum (*Solanum trilobatum*): Solanum is a medicinal herb belonging to the Solanaceae family, which can be found in various tropical counties. Divyagnaneswari et al. (2007) investigated the possible immunomodulatory and disease protecting effects of *S. trilobatum* (either water- or hexane extracts) in *O. mossambicus*. Administration through intraperitoneal injection was conducted at a rate of 4, 40, and 400 mg kg^{-1} of body weight. Regardless of the dose and extraction method, notable immunostimulation was noticed. Also, the survival rates post *A. hydrophila* challenge were significantly higher than of the control group (Divyagnaneswari et al. 2007).

Ku Shen (*Sophora flavescens*): This herb belongs to the Fabaceae family, and inhabits Asia, Oceania, and the Pacific Islands. Several species of the *Sophra* sp. have been used in traditional Chinese medicines (Zhang et al. 2011). Wu et al. (2013b) evaluated the effects of Ku Shen on the innate immune parameters, and disease resistance of tilapia fed experimental diets containing 0.25, 0.5, 0.1, 0.2, and 0.4 g kg^{-1} of *S. flavescens*. Upon the conclusion of the feeding trial, the treated groups, regardless of the inclusion level, presented higher innate immune parameters, which caused improved survival rate post *S. agalactiae* challenge (Wu et al. 2013b).

Heart-leaved Moonseed (*Tinospora cordifolia*): Also known as guduchi or giloy, this herb belongs to the Menispermaceae family, and is native to tropical regions of India, Myanmar, and Sri lanka. In aquaculture, the ethanol and petroleum ether extracts (0.8, 8.0, and 80 mg kg^{-1} body weight) of *T. cordifolia* were administrated in *O. mossambicus* diets as an immunostimulant and disease protector (Sudhakaran et al. 2006). Their data illustrated conclusively that ethanol and petroleum ether extract at 8 mg kg^{-1} stimulated the immune responses and afforded higher degrees of protection to fish against *A. hydrophila*.

Stinging nettle (*Urtica dioica*): The stinging nettle is a flowering plant belonging to the Urticaceae family. Awad and Austin (2010) supplemented rainbow trout diets with 10 g kg^{-1} of stinging nettle (*Urtica dioica*). Fourteen days later, the fish was subjected to an experimental challenge with *A. hydrophila*. Mortalities decreased from 32% in control to 4% in the supplemented group, and immune functions improved as well (Awad and Austin 2010). The results confirmed the potential of this medicinal herb for disease protection in rainbow trout.

Indian ginseng (*Withania somnifera*): The herb is also known as ashwagandha or winter cherry. Indian ginseng belongs to the Solanaceae family, which is highly cultivated in India. Sivaram et al. (2004) demonstrated that supplementation within diets of juvenile greasy grouper improved immune responses, as well as resistance against *Vibrio harveyi* infection. In accordance, Indian major carp (*Labeo rohita*) fed 2 g kg^{-1} *W. somnifera* root powder for a period of 42 days showed a significantly higher disease resistance against *A. hydrophila* infection (Sharma et al. 2010).

Ginger (*Zingiber offinale*): A flowering plant of the Zingiberaceae family, ginger has been shown to have beneficial effects as a medicinal herb (Stoilova et al. 2007). Interestingly, studies on fish have revealed its promising effects for disease protection. For instance, rainbow trout fed ginger (5-10 g kg^{-1}) developed a 64% survival rate, post-experimental challenge with *A. hydrophila* (Nya and Austin 2009). Similar results were reported by Talpur and Ikhwanuddin (2013) on Asian sea bass in which ginger strengthened nonspecific immunity and improved disease resistance against *V. harveyi*.

Herbal mixture: In addition to the administration of medicinal herbs mentioned above, several studies have examined the use of herbal mixtures for disease treatment (Reverter et al. 2014). Jian and Wu (2003) studies the possible immunomodulatory and disease protecting attributes in traditional Chinese medicine (TCM) of Astragalus Root (*Radix astragalin seu Hedysari*) and Chinese angelica root (*Radix angelicae sinensis*) in the large yellow croaker (*Pseudosciaena crocea*). The results revealed that the herbal mixtures improved nonspecific immunity and disease resistance to *Vibrio alginolyticus*. In a further study by Kumari et al. (2007); rohu fingerlings, fed a single dose of a herbal mixture (1.0 g kg^{-1}) containing holy basil, Indian ginseng (*Withania somnifera),* guduchi *(Tinospora cordifolia*), and Indian gooseberry (*Emblica officinalis*) revealed improved functions and greater protection against *A. hydrophila*.

Ardó et al. (2008) evaluated the effects of singular or combined administration of two Chinese medicinal herbs (0.1%); huángqí (*Astragalus membranaceus)* and Japanese honeysuckle (*Lonicera japonica)*; fed separately and/or in combination with and without 0.05% boron, in Nile tilapia. The results demonstrated that both singular and combined administration resulted in a significant increase in disease resistance against *A. hydrophila* and stimulation of immune responses (Ardó et al. 2008). Similarly, dietary administration of ethanol solvent extracts of three herbal leaves (*Azadirachta indica*, *Ocimum sanctum,* and *Curcuma longa*) improved immune responses and protected goldfish (*Carassius auratus*) against *A. hydrophila* infection. In an investigation of Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) on carp immunity, Yin et al. (2009) investigated the effects of fish fed dietary supplements of these combined herbs (5 g kg^{-1}). Interestingly, combined administration resulted in the highest protection (60% of survival rate compared with 10% survival in the control group).

In a study with sea bream (*Pagrus major*) larvae, Takaoka et al. (2011) tested the possible effects of herbal mixtures for disease protection. To this aim, fish were fed rotifer enriched with a herbal mixture of *Massa medicata, Crataegi fructus, Artemisia capillaries,* and *Cnidium officinale*. The results indicated improved growth performance, as well as protection

of the larvae against *V. anguillarum*. Verma et al. (2013) combined Indian banyan (*Ficus benghalensis*) and white lead tree (*Leucaena leucocephala*) for a test involving disease resistance in juvenile African Sharktooth catfish (*Clarias gariepinus*). The results showed that the fish treated with herbs had significantly higher serum immune parameters (including serum antibody titer, lysozyme activity, and phagocytic index); as well as higher survival rates post-challenge against *A. hydrophila* (Verma et al. 2013). Likewise, a herbal combination of *R. scutellaria*, *R. coptidis*, *Herba andrographis* (Ha), and *Radix sophorae flavescentis* (Rsf)) showed promising results in the protection of grass carp (*Ctenopharyngodon idellus*) against *A. hydrophila* infection (Choi et al. 2014). Reverter et al. (2014) reported the synergistic effect of mixed herbal extracts (Reverter et al. 2014). Nonetheless, it is not clear if the observed effects of mixed herbal extracts, however, it is not clear if the observed effects were due to isolated molecules, or rather a consequence of the synergistic between several molecules contained in the extracts (Harikrishnan et al. 2011); thus, creating an obvious area for future researches.

5. Medicinal herbs and viral diseases in finfish aquaculture

Viral diseases are the reason for mass mortality and other health-related issues in aquaculture. Unlike bacterial diseases, they are not easily treated (Dadar et al. 2017). The dietary administration of medicinal herbs has been suggested as a means of controlling or treating viral diseases, though in a limited number of studies, and very few on the study of finfish. In an *in vitro* study, the viral hemorrhagic septicemia virus (VHSV), a salmonid rhabdovirus, was inhibited by plant extract derived from olive tree leaf (*Olea europaea*) and the major compound oleuropein (Micol et al. 2005). Dietary administration of *Punica granatum* leaf extract at doses of 50 and 100 mg kg^{-1} enhanced innate immune responses and reduced the mortality of olive flounder (*Paralichthys olivaceus*) infected with the lymphocystis disease virus (LDV) (Harikrishnan et al. 2010a).

6. Medicinal herbs and parasitic diseases in finfish aquaculture

Parasitic diseases commonly occur in aquaculture; and, like viral diseases, are not easily treatable. In recent years, several researchers tested the administration of medicinal herbs as possible remedies or controlled parasitic diseases. In this section, we provide an overview of available literature in this field. To the best of our knowledge, the first study was performed by Ekanem et al. (2004), which tested the potential control of methanolic extract of *Mucuna pruriens* leaves and petroleum ether extract of *Carica papaya* seeds on parasitic *Ichthyophthirius multifiliis* infections in goldfish (*Carassius auratus auratus*). A 90% reduction in numbers of *I. multifiliis* in fish after bath treatments of plant extracts (200 mg l^{-1}). Similarly, methanolic extracts of *Piper guineense* seeds were effective in the treatment of monogenean diseases, as well as a higher efficacy of the effects of these extracts to goldfish parasites under *in vitro* conditions than under *in vivo* (Ekanem et al. 2004). Ji et al. (2012) investigated the treatment of fish with bupleurum root (*Radix bupleuri chinensis*), cinnamon (*Cinnamomum cassia*), Chinese spice bush (*Lindera agreggata*), and golden larch (*Pseudolarix kaempferi*) extracts; which demonstrated promising results in the protection of goldfish against monogenean *Dactylogyrus intermedius*. Similarly, Wu et al. (2011) reported that methanol, chloroform, and ethyl acetate extracts from *Radix Bupleuri chinensis* exhibit potential as preferred natural antiparasitics for the control of the *D. intermedius* in goldfish (*C. auratus*). Accordingly, Tu et al. (2013) conducted treatments with Indian sandalwood (*Santalum album*) for the protection of fish against *D. intermedius* and *Gyrodactylus elegans*. These authors observed that bath treatments with long exposures and multiple administrations were more effective and provided a higher protection against monogenean infections.

Chitmanat et al. (2005) evaluated the possibility of protecting tilapia against ectoparasites, *Trichodina* sp. via the treatment of garlic (*Allium sativum*) and Indian almond (*Terminalia catappa*). The results showed that crude extracts of both garlic or Indian almond at 800 mg/l significantly reduced *Trichodina* sp. infections in tilapia. Green tea extract can be used as an effective alternative to chemotherapeutic treatment in the control of *Trichodina* sp. infestations of *O. niloticus* fry in hatchery under natural conditions (El-Deen 2010). Harikrishnan et al. (2010a) reported that the treatment of olive flounder with *P. vulgaris* extract (either via oral or intraperitoneal administration) and traditional Korean medicinal (TKM) as triherbal extracts improved resistance against the parasite *Uronema marinum*. Harikrishnan et al. (2010b) determined that the enrichment of olive flounder diets with a herbal mixture containing *C. cinerariafolium*, *P. granatum,* and *Z. schinifolium*

extracts protected against *Philasterides dicentrarchi*. In two subsequent studies Harikrishnan et al. (2011c) and Harikrishnan et al. (2012b) revealed that dietary *K. pictus* extract afforded protection from a mixed infection by *V. alginolyticus* and *P. dicentrarchi* in olive flounder; and that the mortality rates of olive flounder infected with ciliate *Miamiensis avidus* decreased from 80% (the control diet) to as low 40% in fish fed diets enriched with *Suaeda maritima* extract at doses of 0.1% and 1.0%, respectively.

Militz et al. (2014) researched the effect of garlic extract (*Allium sativum*) immersion, with the active component allicin, on *Neobenedenia* sp. egg development, hatching success, oncomiracidia (larvae) longevity, infection success, and juvenile *Neobenedenia* survival; compared with both freshwater and formalin immersions. Garlic extract administered as a therapeutic bath was shown to deliver antiparasitic properties toward *Neobenedenia* sp. (Platyhelminthes: Monogenea) infecting farmed barramundi, *Lates calcarifer*. Militz et al. (2013) also fed farmed barramundi (*L. calcarifer*) two enriched garlic diets, and challenged them with *Neobenedenia* sp. After long-term supplementation (30 days), a 70% reduction was observed. These results proved far superior to those fed the control diet and those fed the short-term supplementation (10 days) and the control group.

7. Concluding remarks and further perspectives

This review of currently available scientific literature has revealed the promising role of medicinal herbs on disease resistance and improved health and welfare of farm-reared fish and shellfish. Therefore, it can be speculated that these environmentally friendly dietary supplements receive increasing interest as alternatives for antibiotic use in the aquaculture industry. However, it must be kept in mind that the aforementioned research presented the effects of herbal feed supplements are species-specific, and must be considered cautiously. Therefore, the optimum feed supplementation, administration dose, and duration must be determined individually for each cultured species, and each phase of production from fry to fingerling and grow-out stages. Furthermore, despite the promising effects obtained through the use of medicinal herbs on the systemic immune parameters, there is a paucity of available information on the interaction between medicinal herbs and mucosal immune responses. As a result, further research is required in the determination of the immunomodulatory capacity and benefits of medicinal herbs on mucosal surfaces, such as the gut, gills, and integument of fish and shellfish under various conditions. We, therefore, conclude that our present understanding of the exact modes of action of medicinal herbs on systemic and mucosal immune parameters merits further scientific study.

Lastly, it should be noted that natural therapeutic feed additives and supplements with medicinal claims are subject to stringent legislation in many areas of the world, especially in the UK and European Union (EU). Legislation may not permit their use in animals and fish under such terms and must adhere to strict definitions compliant with government and veterinary agencies.

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Environmental impacts and imperative technologies towards sustainable treatment of aquaculture wastewater: A review

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ABSTRACT

Aquaculture activities surge tremendously worldwide and intensively shifted the landscape of food consuming across the globe. As the fish catch production from natural environment has reached its limit, the public has begun to rely on farmed aquatic products for continuity of protein sources. The aquaculture industry is currently dominated by Asia and has evolved into multiple configurations to increase fish production. Nonetheless, the constituent in aquaculture wastewater (mainly from fish feed) and other pharmaceutical substances have raised public concern as those constituents are potent to jeopardize surrounding environment when released into the ecosystem. In order to minimize the impact of aquaculture wastewater, rigorous researches have been proposed and conducted to effectively treat aquaculture effluent. A thorough treatment mechanisms have been covered, of which the recirculation aquaculture system (RAS) is the most extensively implemented, other technologies introduced include biological, physical and chemical treatments. This review covers an in-depth analysis of these technologies, including their pros and cons, treatment efficacies and process intensifications. Bioreactor, bio-floc, wetlands and phytoremediation are among the biological treatment methods revealed in the discussion, while the physiochemical section encompassed an overview of adsorption, advanced oxidation processes (AOPs) and membranes technologies. Future recommendations are proposed in terms of aquaculture regulations to ensure sustainable aquaculture development.

1. Introduction

The necessity of aquatic farming has risen as a consequence of an increasing world population, because the fish catch production has reached its limits. In 2017, aquaculture accounted for 67.7% of the total fisheries production, reaching a staggering 53 million tons [1]. Compared to 2000, production of aquaculture has increased significantly. There was merely 20.8 million tons in 2000, accounting for only 26.3% of the total aquatic production [1]. To cope with the increasing demand for fish and other aquatic products, aquaculture activities have been intensified, which has led to a more intense competition for the fundamental needs of aquaculture development such as land, water and natural resources [2]. Aquaculture activities are usually carried out in a controlled environments, not limited to fish farming, but also includes mollusks, crustaceans, shrimps and many other aquatic species [3].

In addition to meeting global food demand, the aquaculture industry also brings a positive impact to the economy by providing more employment opportunities. According to Nasr-Allah et al. [4] and Ottinger et al. [5], the aquaculture industry directly or indirectly creates approximately 23 million full-time jobs, especially in developing countries. The most cultivated aquatic species is freshwater fish, which account for 56% of the total aquaculture production, followed by mollusks and crustaceans at 23% and 10% respectively [5]. From the analysis of the geographical distribution of the production of the top 10 aquaculture species, it can be seen that Asian countries, particularly China, dominates the production, which account for around 53% of the total global output [5]. However, the rapid development of aquaculture has led to land subsidence and mangrove destruction. The most critical issue is that it seriously pollutes the surrounding water bodies and has a negative impact on the environment [5]. Wastewater containing large amount of nutrients, suspended solids [6], chemicals and pharmaceutical products [7] are often discharged from the aquatic species farms.

In view of the escalating urge to mitigate the adverse effects of aquaculture on the environment, various treatment technologies have emerged. Treatment technologies introduced involved either biological or physicochemical treatment mechanisms. Biological methods use

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microorganisms to break down pollutants. Physical treatment usually involves separating contaminants from the aquaculture wastewater in their original form. Adsorption [8], membranes [9] and coagulation [10] are examples of the physical separation applications. As for chemical treatment process, undesired substances are degraded into by-products to minimize the harmful effects on the environment when the wastewater is discharged. The degradation of constituents often achieved through advanced oxidation process, which can transform parent compounds into miscellaneous by-products through a series of chemical reactions. Rather than standalone biological or physicochemical treatments, the wastewater purification operation performances can also be enhanced through integration of different modes of treatment mechanisms. Through process integration, the degradation and separation of the harmful substances are performed simultaneously to achieve better treatment efficiency.

With the information gathered, it is certain that the further growth of the aquaculture industry is inevitable, hence in-depth understandings and researches of aquaculture wastewater treatment technologies are crucial. Nonetheless, a thorough review of aquaculture wastewater treatment technology is still scarce. Therefore, this review serves the purpose to close the review gaps in assembling available treatment technologies for aquaculture effluent. The current aquaculture landscape and the impact of aquaculture on the ecosystem will be outlined to emphasize the importance of wastewater treatment, followed by the reviews of treatment technologies. The treatment mechanisms, advantages, disadvantages and efficacies of the treatment methods will be disclosed. Lastly, recommendations for sustainable aquaculture development were summarized to ensure that the ecosystem is protected despite having to cope with surging growth of the fish farming activities.

2. Current aquaculture landscape

Aquaculture is becoming more prevalent globally due to the increasing demand for food to address the nutritional imbalance of the population [11]. Several countries have started to improve their food security by establishing different development stages of aquaculture production. Fig. 1 Values of g depicts the global aquaculture production, both marine and inland, in 2019, summarized from the report: Fishery and Aquaculture Statistics: Global aquaculture production 1950–2019 [12]. The report shows that Asia yields about 110 million ton of aquaculture products annually, valued at approximately US$232 million. In the past 20 years, aquaculture production in other regions including Africa, Americas, Europe and Oceania has also seen rapid growth [12].

The global distribution of the aquaculture sector is influenced by many factors, including geography, market demand, infrastructure, human resources, technical capability and institutional system [13]. According to Bostock et al. [14], the main factor that promotes the development of aquaculture industry is market competition. Market competition has compelled culturists to seek out any alternative to meet

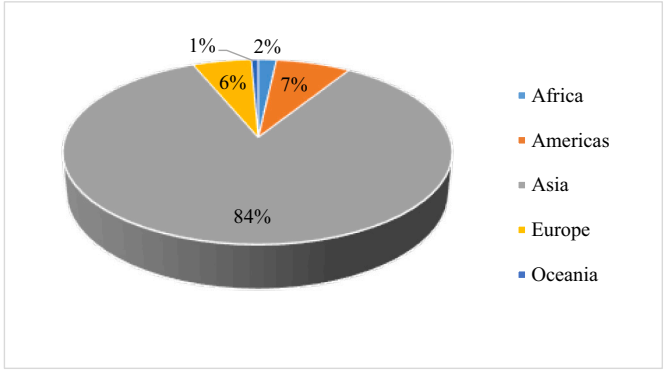

Fig. 1. Values of global aquaculture production in 2019 [12].

demand. Several strategies have been implemented to improve fish production through proper practices, technological development and regulations. Nowadays, most farmers have shifted to closed system technologies, which have been found to be more environmental friendly than open waters [15]. Common types of traditional closed aquaculture systems developed by farmers include pond, cages, raceways and recirculating aquaculture system (RAS). Each system has its own advantages and drawbacks, depending on the species and location of the culture. In addition, according to Anh et al. [16], in order to increase annual production, most of the culturists have widely adopted modern intensive, semi-intensive and improved intensive systems. The goal of these cutting-edge techniques is to boost fish production by concentrating additional nutrients and additives in the aquaculture environment [17]. The proliferation of pharmaceutical products as highly prescribed compound over-the-counter in the aquaculture industry can effectively prevent many diseases among farmed species. However, improper production practices, such as excessive use of antibiotics, are classified as illegal, unreported, and unregulated (IUU) fishing, which can lead to unsustainable fisheries [18]. Furthermore, hormones used to deliberately feminize fish may have a negative impact on the surrounding ecosystem. For instance, 17β-estradiol introduced in the aquaculture feed had improved the female eels species [19]. However, the residual of the 17β-estradiol in the water matrices even at low concentration of 1 ng/L become harmful towards environmental and health problems since it had proven to have a linked towards immune and reproductive system [20]. Fig. 2 shows the conceptual path of aquaculture wastewater to the surrounding environment. It shows that nutrient and pharmaceutical products are the two major components added to fish ponds, which will cause problems for aquaculture wastewater. Based on Fig. 2, various treatment technologies are potentially sustainable solutions for treating aquaculture wastewater and improving nutrient recovery, water recovery, sludge recycling and contaminants of emerging concern (CEC) removal [21]. This paper will review each stage involved in the aquaculture wastewater pathways, with particular emphasis on treatment technologies.

3. Impacts of aquaculture towards the ecosystem

Though aquaculture alleviates food supply issue and boosts the economy, the impacts of aquaculture on the ecosystem cannot be overlooked. This is because culture of aquatic species involves the addition of foreign constituents to the cultured water, and also exploitation of lands for fish farming. The artificially added constituents served different functions, with the main purpose to keep the fish healthy. However, it is usually not possible to be completely absorbed, leaving the residue in the water as contaminant. Besides, the metabolic excretion of fish will also lead to waste generation in aquaculture water [2].

3.1. Constituent of aquaculture wastewater

There are multiple waste sources in aquaculture, namely the feed, chemicals and pathogens. Feed is the main source of waste in aquaculture systems, and its impact on waste depends on several factors, such as the quantity of feed per unit time, composition of nutrient, whether to feed in pellet form, extruded form etc. On the other hand, chemicals are added to treat and control fish diseases. Some chemicals including antibiotics, vaccines, salts and lime are used to prevent microbial infections, adjust pH of aquaculture pond and relieve fish stress [2]. These sources subsequently lead to two types of waste, which are the solid wastes and the dissolved wastes.

Solid waste is mainly produced by feed, in the form of uneaten food or components that have not been digested and excreted as feces. Solid waste can be further categorized into suspended solids and settled solids [3]. While the settled solids will sink in short period of time and can be easily removed from the aquaculture water, the suspended solids are the

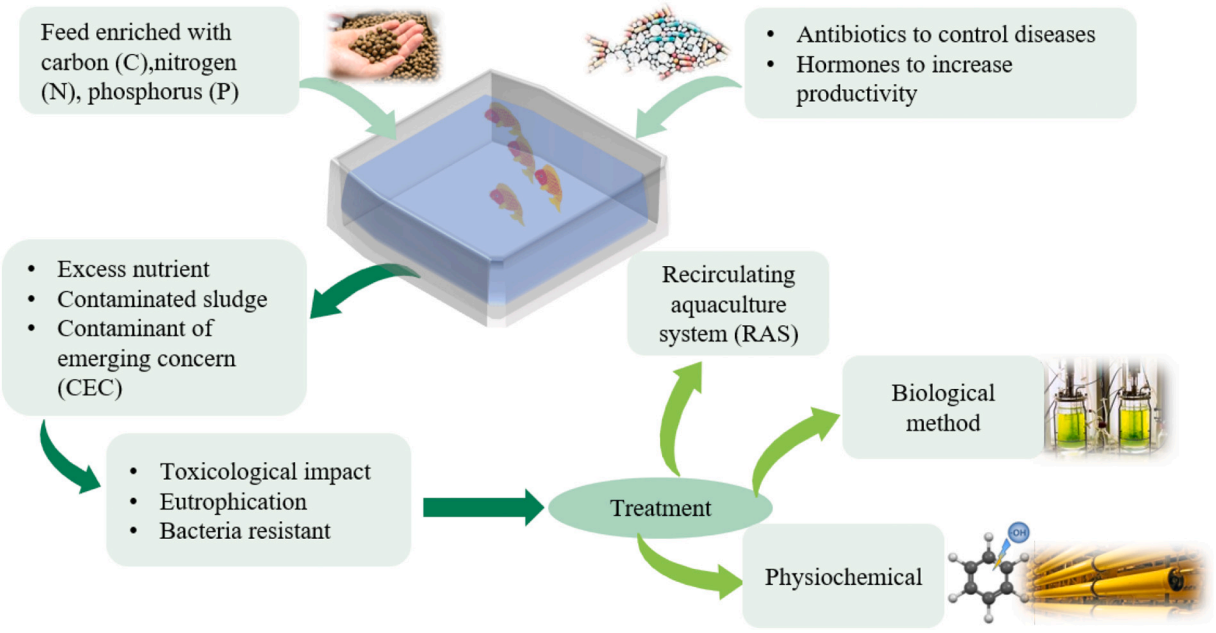

Fig. 2. Pathway of aquaculture wastewater towards surrounding environment and treatment technologies.

fine particles that are difficult to remove, causing pressing issues in all kinds of aquaculture [22]. Dissolved wastes, on the other hand, comprises of nutrients and pharmaceutical drugs. Nitrogenous product in the form of ammonia and phosphorus are the main dissolved contaminants in aquaculture water. N and P commonly originate from unconsumed feed and excreta from farmed fish. Table 1 shows the components found in aquaculture wastewater from various culture species. According to Crab et al. [23], 1 kg of fish biomass requires approximately 1 to 3 kg of dry feed, and 36% of the consumed feed will be excreted from the fish as organic waste due to indigestibility. A research by Ackefors and Enell [24] reported that assuming that fishmeal contains 7.2% N and 0.9% P with a conversion coefficient of 1.5, producing 1 ton of fish will release 75 kg N and 9.5 kg P.

CEC constitute another part of dissolved waste. In aquaculture, CEC in the form of antibiotics [25], disinfectants [3], vaccines [26], steroids [27] are usually added to control diseases, promote aquatic growth and productivity. The concentration of CEC in aquaculture is very low, ranging from 0.01 to 4000 ng/g of farmed fish. However, trace amount of CEC has raised public attention because they tend to accumulate in water bodies and aquatic products, thereby causing harm to consumers and the environment.

3.2. Impact of aquaculture wastewater towards environment

As discussed in Section 3.1, the wastewater discharged from the aquaculture sector contains excessive nutrients and CEC. These contaminants have a negative impact on the surrounding environment, including aquatic ecosystems, plants, and to a lesser extent, humans.

3.2.1. Toxicological impact

The excess nutrients and CEC residues in aquaculture wastewater have a negative impact on non-aquaculture ecosystems. According to Camargo et al. [15], nitrate is a toxic compound that will adversely affect the surrounding aquatic species. Nitrate reacts with hemoglobin to produce methemoglobin, which can hinder the breathing ability of aquatic organisms due to hypoxia [36]. Done et al. [37] reported that antibiotics such as oxytetracycline (OTC), 4-epioxytetracycline, sulfadimethoxine, ormetoprim and virginiamycin have exceeded the limits of the Food and Drug Administration (FDA). As a result, human consumption of these contaminated aquatic organisms can cause problems such as allergies and toxicity, which are difficult to diagnose due to the lack of information on antibiotic consumption [38]. Not to mention that the contaminated water in the surrounding environment also harms the aquatic plants due to the presence of a low concentrations of the pharmaceutical compounds [39]. According to Vilvert et al. [40], OTC has a concentration range of 0.5 μg/L to 25 μg/L, which can disrupt the physiological functions of aquatic plants after prolonged exposure. Therefore, proper treatment is needed to remove CEC in aquaculture wastewater to reduce the impact over time.

3.2.2. Eutrophication

Eutrophication occurs when the surrounding water environment is rich in phytoplankton due to increased nutrient availability. The accumulation of fish excreta, excessive nitrogen, phosphorus and ammonia

Table 1
Component of aquaculture wastewater at different culture environments.

Culture environment	Wastewater component				References
	NH_4^+-nitrogen (ppm)	NO_2^--nitrogen (ppm)	NO_3^-nitrogen (ppm)	Total phosphorus (ppm)	
Silver Sea Bass	5.59	0.125	12.22	6.75	[28]
Yellow catfish	2.35	0.13	0.51	0.23	[29]
Mixed culture fish	9.45	25	30.17	32.5	[30]
Mixed culture fish	0.63	0.17	0.38	0.07	[31]
Shrimp	289.1	66	101.3	–	[32]
Shrimp	1.25	–	–	4.50	[33]
Crabs	1.88		4.5	0.131	[34]
Mixed culture fish	8.21	3.27	2.96	0.93	[35]

from aquaculture discharges contains the nutrient source required for the rapid cyanobacterial activity [41]. According to Garmichael et al., cyanobacteria, also known as blue-green algae, is photosynthetic bacteria that can produce secondary metabolites that are toxic to organisms, such as cyanobacteria toxins [42]. Several studies reported that microcystins (MCs) produced by cyanobacteria have contaminated a variety of aquatic organisms into the polluted eutrophic waters [43]. In addition to excess nutrients in the surrounding water, climate may also cause algae to rapidly multiply in surface waters [44]. For instance, algal blooms have become more common in tropical climates, leading to increased MC level in water bodies [45]. In addition, because algae multiply in surface water, the aquatic organisms and plants in the water receive less light and to some extent depletion of the living resources. According to Cai et al. [46], the decomposition of dead algae and plants will increase the acidity of the surrounding water due to the presence of CO_2. Therefore, excessive nutrient levels in aquaculture wastewater increase the risk of eutrophication and stressed the surrounding aquatic ecosystem.

3.2.3. Bacteria resistant

Pharmaceutical compounds found in aquaculture wastewater, such as antibiotics, steroids, and non-steroidal anti-inflammatory drugs (NSAIDs), may also degrade the quality of the surrounding water. Common antibiotics in aquaculture wastewater include cyclines, quinolones, macrolides, and sulfonamides. Zou et al. [47] discovered that low concentrations (up to 7722 ngL^{-1}) of sulfadiazine (SDZ), OTC, norfloxacin (NOR) and ofloxacin (OFL) were detected in rivers near fish pond. Frequent use of antibiotics causes bacteria in the surrounding environment to develop resistance. According to Tendencia et al. [48], OTC is the most reported case due to the presence of OTC-resistant bacteria. Based on the analysis of Le et al. [49], *Bacillus* and *Vibrio* are examples of bacteria that resisted to trimethoprim (TMP) and sulfamethoxazole (SMX) antibiotics. These antibiotics are at low concentration of 0.1 mg/mL. Although the concentration detected was low in a short period of time, the situation will deteriorate, and it will accumulate in the water body and cause a greater impact on the surrounding environment.

4. Aquaculture effluent treatment

As the adverse impact caused by aquaculture wastewater on the environment is alarming, there is an urgent need to find effective treatment methods to separate or degrade undesired substances from the wastewater, and to recover nutrients, sludge, and water from the effluent. The aquaculture industry has identified several treatment methods, such as RAS, biological, and physiochemical. Fig. 3 depicts the classification of aquaculture wastewater treatment methods and their existing technologies.

4.1. Recirculation aquaculture system farming model

RAS is a closed system that recirculates clean water into the culturing environment and has been used widely by the culturist as a farming model. The RAS processes includes several unit operations, such as culture tank, solid waste removal, anaerobic digestion and disinfection. Several authors mentioned the advantages of RAS, including reduced water consumption [50], improved sludge management [51] and reduced pollution [52]. Fig. 4 depicts a general commercial RAS configuration widely designed by culturists [53].

4.1.1. Physical filtration

Physical filtration is the first entering unit to remove the solid waste consisted of biofilter floc, feces and excessive waste feed [54]. The unit operation involved in the filtration can be classified into sedimentation, mechanical filtration or centrifugation stages. According to Badiola et al., centrifugation is not recommended to be used in the recirculating

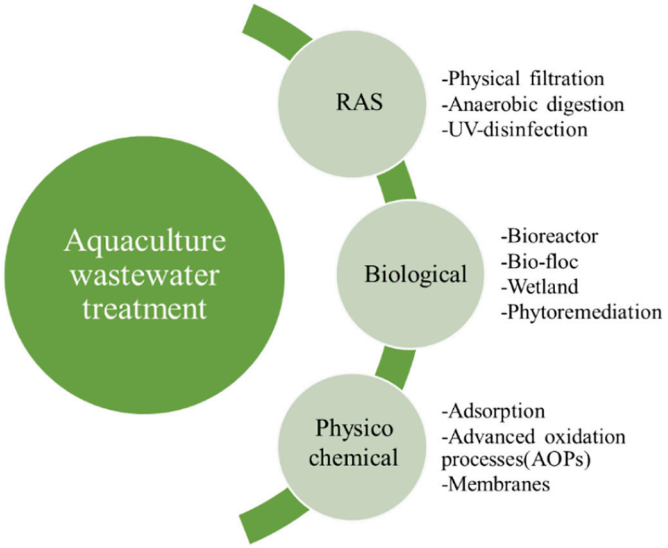

Fig. 3. Classification of aquaculture treatment technology.

aquaculture system since it required high energy and provide less efficiency of solid removal [55]. In addition, sedimentation considered less effective due to the insufficient residence time for the particulates to settle down and thus result low solid removal and time consuming [56]. Franco-Nava et al. reported that microscreen filter such as drum filter is the most common unit employed to remove the particulates and suspended solids [57]. Several factors affecting the effectiveness of the drum filter such as the particles size distribution of the suspended solids and the quality of the treated RAS water [58]. The mechanism of the drum filter is based on the aperture size in which only the clean water will pass through and the solid will be remained inside the screen. [59].

4.1.2. Anaerobic digestion

Anaerobic digestion (AD) is a process involving the facultative bacteria under anaerobic conditions to degrade the organic matter [60]. This process is a subsequent step after physical filtration in order to minimize the generated organic sludge. There common type of digester has been employed by the culturist in treating the sludge including continuously stirred tank reactor (CSTR), upflow anaerobic sludge blanket (UASB) and anaerobic sequencing batch reactor (ASBR). Table 2 summarizes the digestion efficiency of the sludge for CSTR, UASB and ASBR digester. Zhang et al. reviewed that AD offers several advantages such as high loading rates, compatible with wastewater treatment unit of RAS, minimal risk of contamination caused by sludge and high survival rate of anaerobic bacteria without having any feed for an extended period of time.

4.1.3. UV disinfection

The RAS consists of complex microbial communities, which impacts both fish health and the surrounding environment. Disinfection of aquaculture water hence becomes vital to decrease the risk of entry and spreading of pathogens into the fish rearing system, and to ensure the biosecurity of land-based security. UV treatment is effective to disinfect the aquaculture water by destabilizing the microbial composition and inactivate the pathogenic bacteria in the RAS [67]. Dahle et al. [67] successfully decreased the number of live bacteria to an extent of 89% reduction of colony forming units, without compromising microbial water quality surrounding the fish in the RAS. In another study, Qi et al. [68] introduced mercury-free UV-LED, combined with peroxymonosulfate (PMS) as a potential strategy to disinfect aquaculture water in RAS. The proposed strategy not only improved the inactivation efficiency of UV-LED, but also lowered the energy consumption. In the research, the UV-LED/PMS combination attained 0.57 log total bacteria

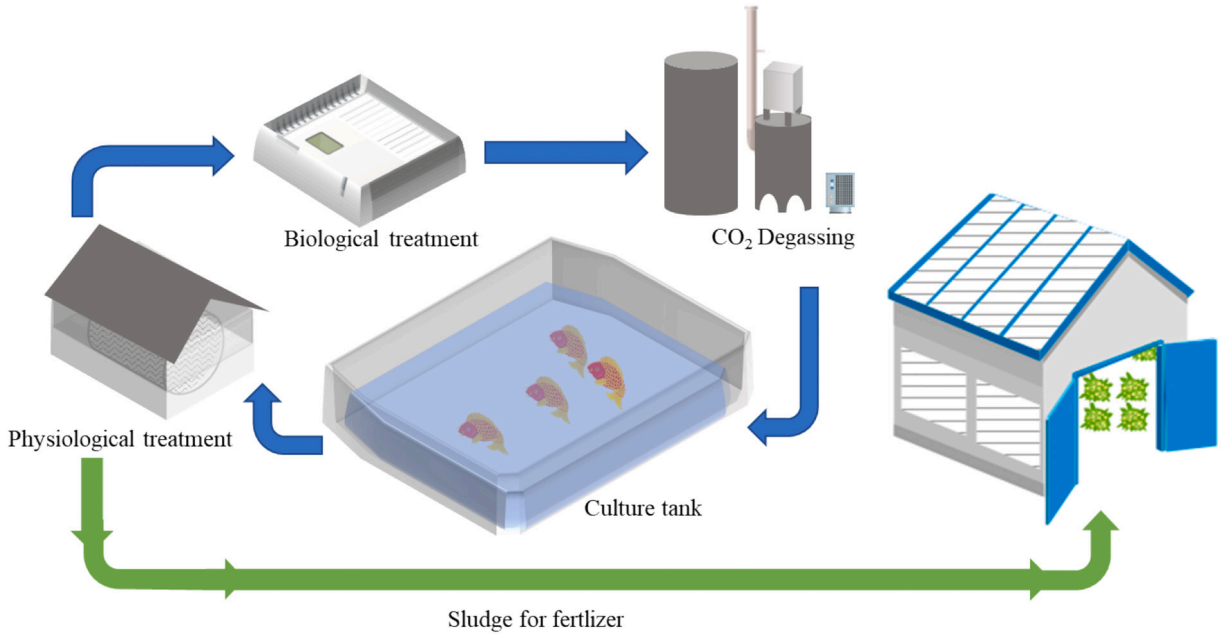

Fig. 4. Schematic diagram of recirculating aquaculture system.

Table 2
Anaerobic digester efficiency in different culture environment.

Type of digester	Culture	Digestion efficiency (%)	Ref.
CSTR	Salmon	58	[61]
UASB	Striped bass	92	[62]
UASB	Prawn	100	[63]
UASB	Seabream	80	[64]
ASBR	Scortum bacoo	91	[65]
CSTR	Salmon Smolt	74	[66]

reduction of RAS within 60 min, using only single UVA-LED chip. The disinfection capacity is expected to be enhanced by exposing the water to higher irradiance intensity in the UVA-LED/PMS system. A higher irradiance intensity could be achieved by coupling dozens of UVA-LED chips in the system.

The current unit operation in RAS has several limitations due to present of low concentration pharmaceutical residual in the feed additives used in the processing. Not to mention, excessive feeding activity to meet high production demand may also lead to excessive nutrient accumulation composed nitrates, phosphates and organic matter [69]. Freitag et al. [70] found that the presence of nitrate from recovered RAS significantly inhibited the growth of cultured species for a long period of time. The remaining fine solids classified as organic matter will remain in the circulating water, and promote the growth of heterotrophic bacteria to a certain extent [71]. As a result, several technologies, including physicochemical and extensive biological treatment, have been investigated to improve the current RAS.

4.2. Biological method

The biological method, also known as bioremediation, is a low-cost traditional technique that uses microorganisms to restore environmental quality. Algae is a commonly used microorganism in bioremediation because it can be used in a variety of environmental conditions. According to a number of studies, the efficiency of bioremediation largely depends on the availability of nutrients, biosorption mechanisms, microbial activities, operating conditions, and the use of different types of algae [72,73]. The main nutrients used as substrates for biomass production in aquaculture wastewater are carbon, nitrogen, and phosphorus. The commonly used biotechnology in aquaculture is bioreactor, biofloc technology, wetland and phytoremediation.

4.2.1. Bioreactor

Bioreactor is a treatment method that uses biologically active organisms such as algae to perform biological reactions to achieve the purpose of product separation and purification. There are two types of bioreactors: moving bed bioreactor and fixed bed bioreactor. A trickling filter (TF), for example, is a fixed media bed bioreactor in which aerobic bacteria are embedded in a film and form a biofilm. The substrate on which the microbial activity occurs determines the performance of the bioreactor. Tsukuda et al. [74] found that the removal rate of nitrogenous compound in the fluidized sand bed (FSB) is related to the carbon source represented by the substrates. Carolyne et al. [75] investigated the influence of different beds in the TF reactor and discovered that woodchips improved the contaminant removal efficiency by 94%. Like the type of bed used in bioreactors, algae play a vital role in increasing biological activity, so it can decompose organic matter in aquaculture wastewater. *Tetraslemis suecica* is a kind of microalgae used in tubular photobioreactors, which can remove 49.4% of nitrate and 99% of phosphate from fish farm wastewater [76]. Andreotti et al. [77] obtained a high removal efficiency of 94.4% dissolve inorganic nitrogen (DIN) and 96.06% dissolved inorganic phosphorus (DIP).

Researchers have begun to investigate the potential of integrated bioreactors with various technologies to overcome the limitations of single-unit bioreactor. Shitu et al. [78] reviewed the development of a hybrid moving bed bioreactor (MBBR) as a better process for creating a sustainable culture environment for farmed fish. According to Li et al. [79], at most of 98.7% of E2 has been removed through MBBR after 68 days' process. In addition, the combination of bioreactors and nanoparticles (NPs) technology may increase processing efficiency. Hesni et al. [35] reported that in the designed bioreactor, *chlorella vulgaris* microalgae and iron oxide NPs were used, and the results showed high removal of NO^{-}_{3} (92.2%), NO^{-}_{2} (89.3%), NH_4 (93.67%), and PO^{3-}_{4} (89.25%). However, some integrated systems seem to be in the development stage, and they were not entirely suitable for real aquaculture wastewater. Since some parameters must be considered, it is expected that the implementation of a denitrification reactor in RAS will incur additional operating costs. For example, the RAS requirements for denitrification unit may only be applicable to nitrate concentrations

above 50 ppm [80]. Pungrasmi et al. [81], on the other hand, reported that combining nitrification and denitrification processes in a single unit operation can increase the removal rate of ammonia and nitrate without affecting the growth of culture species and may reduce costs. Table 3 shows the literatures of existing bioreactors for treatment of aquaculture wastewater.

4.2.2. Biofloc-technology

Biofloc technology (BFT), also known as symbiotic process, employs accumulated biolfloc particles derived from bacteria, algae, and organic matter to improve water quality in aquaculture [82]. The BFT system is considered to be more cost effective than RAS due to fewer facilities requirement in the process. It is classified into two configurations of batch reactor and continuous reactor. Fig. 5 illustrates two configuration systems of biofloc technology, including non-circulation system and recirculation system [83]. There is no need for a filtration unit in BFT, such as the biological treatment stage, because the microbial activity of toxic nitrogen conversion occurs in the water column [84]. In super-intensive culture systems, feeding more frequently produces more culture species. The increased microbial activity due to nutrient availability is the result of increased culture species diversity. However, large amounts of feces and uneaten nutrients will remain in the system, negatively impacting water quality. Therefore, feed is vital in controlling aquaculture water quality.

4.2.3. Wetlands and phytoremediation

Wetland is an economical system to help the culturist to reduce the pollution towards the surrounding environment by performing several processes including nitrification, denitrification, demineralization in the present of living (plants, bacteria, fungus) and non-living organism (soil, water, light, air, minerals) [85]. The performance of the wetland is depending on the design and configuration of the wetland, nutrient availability, water salinity and root characteristics [86]. Lin et al. [87] have reported at least 98% nitrogen removal from the aquaculture wastewater after a few months operation. However, the increased hydraulic loading rate leads to a decrease in wetland performance, which results to a decrease in the removal efficiency of suspended solids in aquaculture wastewater [88].

Phytoremediation is an emerging technology that uses living plants to reduce the toxicity of the aquaculture environment. This method largely depends on the selectivity of plants that contribute to the main function of the treatment. Ghaly et al. [89] have shown that plants such as barley, oat, and rye have different pollutant removal percentages due to differences in growth rate and disease resistance. According to Chavan et al. [90], the implementation of phytoremediation technique in the wetland system can provide better contaminants removal, and the operation is simple and cost-effective. Like the wetland system, several factors affect the performance of phytoremediation, such as a good rooting system, high biomass source and high growth rate [91,92].

Table 3
Literatures of the bioreactor treatment technology for aquaculture wastewater.

Type of reactor	Microbial species	Removal efficiency (%)		Ref.
		TN	TP	
Tubular photobioreactor	*Tetraslemis Suecica*	49	99	[76]
Mixed bubble column photobioreactor	*T. suecica* *D. tertiolecta*	90	90	[77]
Mixed bubble column photobioreactor	*Galbana*	32	79	[48]
Hybrid MBBR	*Chaetomorpha maxima*	43	84	[79]
FSB	–	27	–	[74]
Trickling filter	–	95	50	[75]
FeO-Bioreactor	–	92	89	[35]

Table 4 summarizes the research on the removal of pollutants through phytoremediation based on the types of plants used. In addition, Liu et al. [93] made some improvements by isolating several types of microalgae to achieve high pollutant removal from actual aquaculture wastewater.

4.3. Physicochemical

Physicochemical treatment of aquaculture wastewater involves the separation and degradation of wastes from the wastewater. The removals of undesired substances are predominantly achieved by refining the properties of the treatment technologies to deal with the different characteristics of the substances, to treat them physically, chemically or the combination of both. Examples of the physicochemical treatment methods deployed in the aquaculture industry include adsorption, AOPs and membranes.

4.3.1. Adsorption

Adsorption is a potential treatment technology employed to purify aquaculture wastewater. In the adsorption process, the substances to be treated (adsorbate) are captured by the adsorbent (Fig. 6), thereby separating the undesired substances from the bulk fluid. The adsorbent usually consists of a highly porous surface, at which the adsorbate could accumulate and retain. The bonding between the adsorbate and the adsorbent are generally governed by Van der Waals forces, covalent bonding or electrostatic attraction. Adsorption has several well-known advantages, for instance low cost, handy operation, and resistance to toxic chemicals [99]. An effective absorbent must be inert and have a large superficial area to boost the adsorption efficiency [100]. Thus, a huge variation of absorbents has been explored to achieve the desired separation efficiency. Ammonia and phosphorus are common constituent in aquaculture wastewater, and they must be eliminated to minimize harm to fish farms and the surrounding environment. Several researchers have studied adsorption technology to separate ammonia and phosphorus from aquaculture wastewater. In an investigation conducted by Zadinelo et al. [100] with real fish farming water containing 0.84 mg/L of ammonium ions as the target substance, the smectite clays with various chemical compositions are used to adsorb ammonium ions. By using 0.5% (*w*/w) clay, 93% of ammonium ions were successfully removed. Chitosan was deployed to adsorb ammonia from several fish farms in the city of Palotina-PR in Bernardi et al.'s [8] study. For fish farm water with an ammonia content of 0.14, 0.27 and 0.50 mg/L, the removal efficiency of ammonia was 100%, showing the great potential of chitosan as an adsorbent to treat aquaculture wastewater. In order to treat another common contaminant, Kumararaja et al. [101] harnessed aluminium pillared bentonite as an adsorbent to remove the phosphate in aquaculture wastewater. It was found that the removal efficiency varied with the salinity of the discharged water. Several samples of aquaculture wastewater were collected for the study, including the waters of shrimp farm and fish larval ponds. The resulting phosphate removal rate reached 85.3% to 99.6%.

Adsorption is more commonly used to treat therapeutic substances in aquaculture wastewater. Aitcheson et al. [102] studied the adsorption behavior of four therapeutants: oxytetracycline, malachite green, formaldehyde and chloramine-T on coal-based activated carbon 207EA. The criticality to remove these substances was highlighted when oxytetracycline was found to persist in the sediment for several months and it encourages the growth of oxytetracycline-resistant bacteria. Malachite green is known to promote liver tumor and is toxic to mammalian cells, while formaldehyde is a probable human carcinogen. Chloramine-T's organic trihalomethane byproducts may also be carcinogenic. By altering the pH, temperature and ionic strength, the highest adsorption efficiency of oxytetracycline, malachite green, formaldehyde and chloramine-T were 88, 100, 99 and 99%, respectively. Another antibiotic under the tetracycline family found in aquaculture wastewater, namely the chlortetracycline (CTC) was adsorbed onto

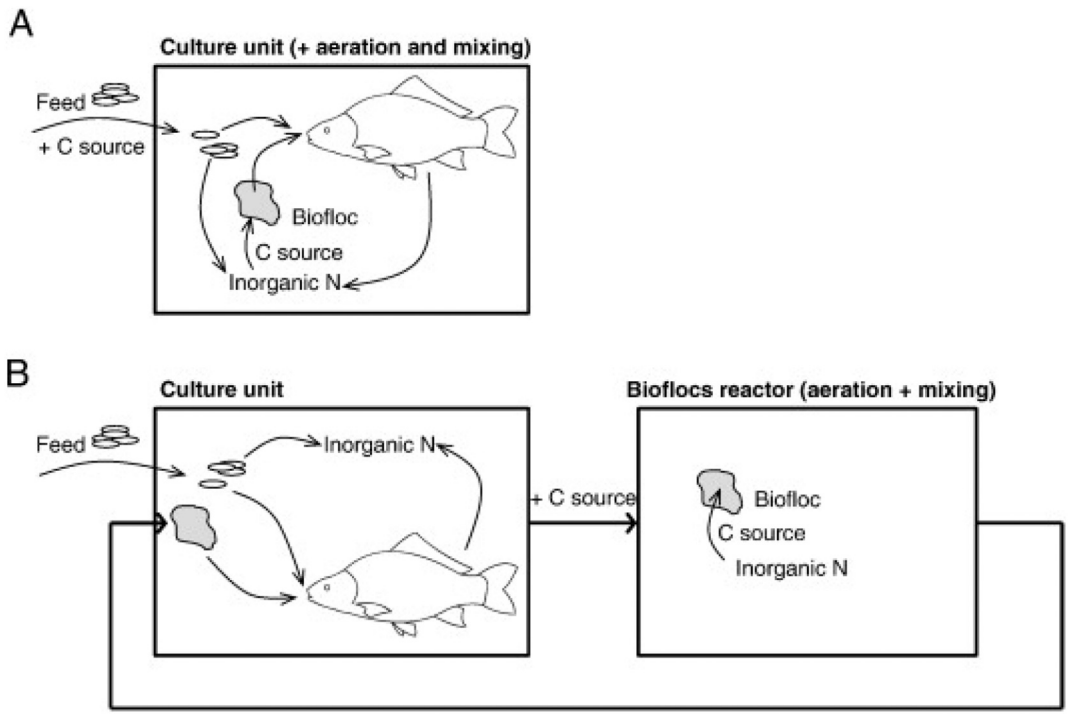

Fig. 5. Classification of bio-floc system. (A) Batch in which the bioflocs is introduced in the culture tank. (B) Continuous system in which the biofloc reactor is separated from the culture tank. Reprinted with permission from Crab et al. [83] Copyright 2012 Elsevier.

Table 4
Efficiency of phytoremediation on treatment of aquaculture wastewater.

Type of plants	Efficiency (%)			Ref.
	NH_3-N	TSS	Phosphate	
C. asiatica	98	90	64	[94]
I. aquatica	73	73	50	[94]
E. crassipes	74	73	98	[94]
P. stratiotes	78	98	89	[94]
S. molesta	64	89	89	[94]
Entodon obtusatus-*P. kessleri* TY microalgae	96	–	96	[93]
Azolla Pinnata	78	–	79	[95]
Water Hyacinth	85	78	88	[96]
Morning glory (Ipomea asarifolia)	85	73	53	[97]
Water lettuce	72	–	75	[98]

lanthanum modified zeolite (La-Z) by Yu et al. [103]. According to reports, both internal and external diffusion in a multi-step process are the controlling factors of the La-Z adsorption rate. In that experiment, 98.4% of removal rate was attained with the initial CTC concentration of 5 mg/L, 20 min adsorption time and pH 7.

Apart from being a standalone technology, adsorption can also be combined with RAS to maximize the separation efficiency of undesired components. In an effort to separate tricaine methanesulfonate (MS-222) from a real aquaculture wastewater, Ferreira et al. [104] incorporated an adsorption process to RAS, using pyrolyzed biological paper mill sludge as an adsorbent. The aquaculture effluent was under the conditions of 18–19 °C, 20–21% salinity and pH 7–7.5. During the experiment, the dissolved organic carbon and inorganic carbon content in the effluent did not compete with MS-222 to occupy adsorption sites on the adsorbent. The examined adsorbent proved to be effective when the adsorption efficiency of the aquaculture wastewater was similar to that evaluated using ultrapure water. Adsorption can also be integrated with photocatalysis process. Zeolite was coupled with TiO_2 by Nomura et al. [105] to remove sulfamonomethoxine (SMM) and its intermediates in freshwater aquaculture wastewater. When TiO_2 was used alone, the degradation of SMM was inhibited, while the usage of composite zeolite/TiO_2 alleviates the issue. The composite zeolite/TiO_2 also

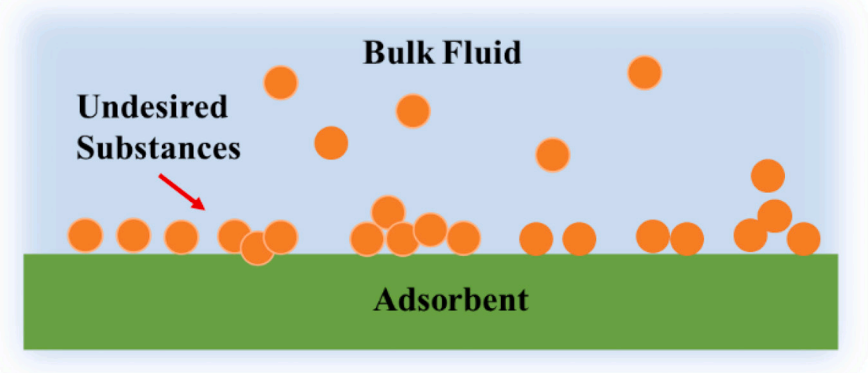

Fig. 6. Illustration of capturing of undesired substances on an adsorbent.

demonstrated their efficacy when the evaluated SMM was decomposed completely within 30 min.

4.3.2. Advanced oxidation process

The advanced oxidation process (AOP) mechanism is initiated by highly reactive oxidants, mainly hydroxyl radicals (•OH) which accelerate the oxidation and degradation of a large number of pollutants in wastewater, as described by Eq. (1).

$$\text{Organic species} + \cdot OH \rightarrow CO_2 + H_2O + \textit{inorganic ions} \quad (1)$$

The reactive oxidants applied in the wastewater are capable to oxidize the undesired compounds in the water unselectively. The contaminants in the water would be effectively disintegrated from the original form once reacted with the reactive species. Besides •OH, superoxide radicals (•O2-), hydroperoyl radicals (•HO2-), sulfate radicals (•SO4-) and organic peroxyl radicals (•ROO), generated from hydrogen peroxide (H_2O_2) or ozone (O_3) are formed in AOP. The formation of these radicals can be attained by several methods, such as ozonation, ultraviolet (UV) irradiation, Fenton oxidation, photocatalysis or combination of these technologies. In most cases, the applications of AOP are to treat organic compounds. As shown in Table 5, the different types of AOP processes used are capable to degrade various organic constituents in aquaculture effluent.

In addition to the organic compounds listed in Table 5, AOP is mainly use to degrade antibiotics in aquaculture wastewater. The research of Liu et al. [112] used green synthesized rGO@nFe/Pd nanocomposite to successfully remove 77.9% rifampicin antibiotics from aquaculture wastewater during the Fenton oxidation process. Another common antibiotic found in aquatic farming, sulfamonomethoxine (SMM), was treated by Nomura et al. [113] using a rotating advanced oxidation contactor equipped with high-silica zeolite/TiO_2. An almost perfect degradation was achieved after 3 h of AOP application. Besides SMM, the treatment process developed in the study can also remove the conversion by-products of SMM from fresh aquaculture wastewater (FAWW), without being affected by coexisting substances in FAWW. In another study, Pereira et al. [114] initiated the photocatalysis of oxalinic acid and oxytetracycline. Oxalinic acid and oxytetracycline are the two most extensively found antibiotics in aquaculture water. In that study, TiO_2 was suspended on a pilot plant scale under natural solar radiation. With an initial concentration of 20 mg/L of separate OXA and OTC, as well as a mixture of OXA/OTC, the photocatalytic treatment successfully removed antibiotics with 100% efficiency. The cumulative UV energy required per liter of solution was very low, at approximately 1 kJ_{UV}/L, demonstrating that the process is sustainable. In that study, it was reported that although several inorganic ions including Cl^-, SO_4^{2-}, NO_3^-, NH_4^+ and HCO_3^- did not interfere with the degradation process of OXA and OTC, the presence of PO_4^{3-} affected the efficiency of photocatalysis employing TiO_2. Hence, when using TiO_2 photocatalyst to treat wastewater with complex constituent, the presence of PO_4^{3-} must be considered.

The efficacy of AOP, which was the ultimate goal of the treatment process, was focused in the narration above. In a more thorough view, the entire technical operation process involves many other components and mechanisms, revealing the overall advantages and disadvantages of the AOP, as listed in Table 6. It is explicit that AOP offers superior treatment efficiency for organic compounds, yet there are some limitations that may jeopardize the sustainability of the process.

4.3.3. Membranes separation technology

The application of membrane aquaculture wastewater treatment is rising, as the experimental results of using membrane in both the laboratory and on-site are promising. Membrane technologies has shown great potential in eliminating fine contaminants found in aquaculture effluent, including organic compounds, viruses and pathogenic bacteria [116,117]. The operation mechanism of membranes varied based on the types and configurations. In general, membranes act as a separation unit that filter undesired substances from the water. The membrane, which serves as a selective barrier would allow certain molecules to pass through while refraining other substances from penetrating through the membrane. Separation of the contaminants from the wastewater are hence achieved. Nonetheless, membranes technology is often plagued by fouling issues, in which the flux declines over time, due to the deposition of undesired substance on the membrane surface that clogged the membrane pores [118,119]. A research by Widiasa et al. [118] reported that the fouling behavior depends on the membrane pore size and the type of foulant. The constituents in an aquaculture system are complex and their characteristics are also varied. Thus, the specifications of the membranes used in the treatment such as molecular weight cut off, surface characteristics and materials are tuned to achieve the best separation performance and minimize fouling in the respective researches. Studies employing membrane technologies in treating

Table 5
Different types of AOPs treating various organic constituents in real aquaculture wastewater and their respective treatment efficiencies.

Types of AOPs	Wastewater sources	Treated organic constituents	Treatment efficacies	Ref.
Ultrasonic cavitation coupling with H_2O_2 and Fenton reagent	Tilapia fish farm	Total ammonia nitrogen	100% removal	[106]
Fenton process coupled with coagulation	Nile Tilapia farm	COD, turbidity, phosphorus, nitrite, suspended solids, BOD	>99% removal of COD, turbidity, phosphorus and nitrite, 88% removal of BOD	[107]
Electrochemical oxidation	Seafood breeding factory	NH_4^+-N, NO_2^-N, P, COD	98% removal of NH_4^+-N, 96% removal of NO_2^--N, 72% removal of P and 48% removal of COD	[108]
Electro-Fenton	Real aquaculture system	Total organic carbon (TOC), nitrate	97.3% removal of TOC, 94.8% removal of nitrate	[109]
Ozonation	Atlantic salmon, *Salmo salar* farm	Waterborne hormones: estradiol (17β-estradiol), 11-ketotestosterone (11-KT), and testosterone	~70% removal of estradiol, ~20% removal of 11-ketotestosterone and ~ 20% removal of testosterone	[110]
UV/H_2O_2	American eels, *Anguilla rostrata* farm	Natural estrogen (17β-estradiol; E2)	~90% removal of estrogen	[111]

Table 6
Advantages and disadvantages of advanced oxidation process [115].

Advantages	Disadvantages
AOPs are capable to address limitations of conventional wastewater treatment systems.	H_2O_2 utilized in AOP systems might be harmful to human.
Potential reduction in treatment steps as AOPs can transform organic compounds directly into simpler inorganic compounds (water, carbon dioxide, and salts) with minimum sludge production.	Large consumptions of acid and base as AOPs, especially those that involved Fenton oxidation are conducted under acid conditions.
AOPs can serve as a pretreatment for biological treatment process, and also as a posttreatment prior to effluent discharge into the environment.	Relatively high-cost processes, possible formation of recalcitrant byproducts that may be more toxic than the parent compounds.
AOPs offer quick reaction rate for wastewater treatment.	Ineffective towards certain types of toxic compounds that are resistant to •OH attack.

aquaculture wastewater are summarized in Table 7.

In addition to its own efficiency, membrane also involves in technology integration to further improve the efficacy of aquaculture wastewater treatment [125]. Some studies have associated membrane to recirculating aquaculture system. Holan et al. [126] pointed out that the conventional systems in RAS faces the challenge of removing fine solid particles (<35 μm) and colloidal particles (<1 μm). The challenge can lead to the accumulation of particles and harm the cultured species. A biofilm membrane bioreactor (BF-MBR) was then introduced in the RAS water treatment system to ease the challenge. The results showed that the installed ultrafiltration membrane was capable to remove colloidal particles and reduce the turbidity to 44% and 77% respectively, which was typically much lower than the turbidity obtained in conventional systems. Besides lowering the bacteria concentrations up to 80%, the ammonia content was also reduced by 56% compared to conventional system. In the experiment, healthier cod larvae with higher survival rate and growth rate were obtained. Other than integrating RAS and membrane systems, Sharrer et al. [127] also investigated the function of MBR in the treatment of wastewater from RAS cultured rainbow trout. In the study, the MBR can remove up to 99.98% and 99.99% of total suspended solids and total volatile solids, respectively. Excellent removal efficiencies of up to 95.5% and 96.1% have also been achieved in the removal of total nitrogen and phosphorus in wastewater. Widiasa et al. [118] included fouling studies in their effort to use polysulfone ultrafiltration (UF) membranes (10, 50 and 100 kDa) in the RAS system to remove contaminants from aquaculture wastewater. According to their investigation, 100 kDa membrane was the most promising UF membrane for RAS owing to its highest flux without compromising the rejection of contaminants in aquaculture wastewater. The 100 kDa UF membrane successfully removed humic acid and shrimp feed at 94.5% and 99.3% respectively, and simultaneously removed microalgae, pathogenic bacteria (*Vibrio harveyi*) and viruses (IHHNV, Infection hypodermal and hematopoietic necrosis virus) from the effluent.

5. Sustainable development of aquaculture

As summarized, aquaculture is booming globally to bridge the gap between the supply and demand of fishery products, which has aroused the attention to the treatment of wastewater in order to minimize the impact of aquaculture on the environment. It is projected that the world population will exceed approximately 9 billion by 2050 [128]. As micronutrient deficiency is still an urgent problem for a growing population, the rapid growth of aquaculture is anticipated to provide people with sufficient essential proteins and micronutrients [129]. Nevertheless, the efforts to ensure food source for the population should not be tied to sacrificing the well-being of the surrounding environment. Although a plenty of aquaculture treatment technologies were introduced in Section 4, the regulation on the discharge of for aquaculture wastewater is vague in many countries, and there is no clear standard for the safety level of constituents, especially the CEC content in the wastewater [7]. In coherence with the rapid development of the aquaculture industry, it is utterly important for academia, government and industry to establish partnerships to critically plan and implement sustainable development strategies for the aquaculture industry [128]. The aquaculture industry regulations must be drafted and executed as a comprehensive guideline for the entire life cycle of aquaculture.

5.1. Aquaculture regulations

Aquaculture is usually operated by several departments, so it is necessary to implement regulations to supervise each division to ensure the efficiency and safety of aquatic production [130]. Some non-government organizations, such as Global Aquaculture Alliance (GAA) and International Finance Corporation (IFC), have established wastewater standards for aquaculture. GAA recommends aquaculture farmers to adopt environmentally responsible production methods, to ensure the discharged effluent complies with standards. IFC provides low-interest loans to developing countries to encourage the development of aquaculture projects that comply to water quality standards. On the government side, many countries, such as the United States, have established aquaculture regulations based on sewage standards. The US Environmental Protection Agency has made rule-making procedures for aquaculture [131]. Other countries such as Thailand and Taiwan imposed aquaculture effluent standards which involved several

Table 7
Aquaculture wastewater treatment employing membrane technologies.

Membrane technologies	Membrane characteristics	Aquaculture constituent (mg/L)	Operating conditions	Efficacies	Ref.
Membrane photobioreactor with cultured microalgae	PVDF hollow-fiber microfiltration membrane	N: 6.81 P: 0.42	T: 25 °C pH: 6.8–7.2 HRT: 1 day	Removal of: TN: 86.1% TP: 82.7%	[120]
Forward osmosis	Thin film composite membrane surface modified with SiO_2	NH_3: 2.10 NO_2^-: 0.14 BOD_5: 7.3	Flowrate: 200 mL/min T: 25 °C	Removal of: NH_3: 99% NO_2^-: 35% BOD_5: 93%	[121]
Dead end permeation cell	PSF nanofiltration membrane	P: 71.7 TAN: 75	P: 6 bar pH 6	Removal of: P: 95% TA: 85%	[122]
Dead end permeation cell	PES membrane	P: 1.074 TA: 0.43	P: 4–8 bar	Removal of: TP: 96% Ammonium: 86%	[123]
Membrane distillation	Polypropylene electrospun membrane	NH_3: 4.20 NO_2^-: 0.12 PO_4: 1.42	T_f: 60 °C T_p: 20 °C Flowrate: 0.3 L/min	Removal of: NH_3: 97% NO_2^-: >99% PO_4: >99%	[9]
Membrane distillation	PVDF templated membrane	TA: 23.18 TP: 1.84 Ca: 10.28 Na: 12.64 Mg: 1.90 TOC: 354.80	T_f: 60 °C T_p: 20 °C Flowrate: 0.5 L/min	Removal of: TA: 93.8% TP: 99.6% Ca: 98.9% Na: 97.6% Mg: 100% TOC: 96.3%	[124]

HRT: long hydraulic retention time, PES: polyethersulfone, PSF: polysulfone, PVDF: polyvinylidene fluoride, N: nitrogen, P: phosphorus, TA: total ammonium TAN: total ammonia nitrogen, TOC: total organic carbon, TP: total phosphorus, T_f: feed temperature, T_p: permeate temperature.

parameters, including total suspended solids (TSS), pH, biochemical oxygen demand (BOD), and COD. For Thailand, the effluent discharged from the aquaculture farm shall meet specifications of pH 6.5 to 9.0, BOD not exceeding 20 mg/L, TSS lesser than 70 mg/L, and total nitrogen not more than 4.0 mgN/L. For Taiwan, the aquaculture effluent should have pH between 6.0 and 9.0, TSS not more than 30 mg/L, BOD and COD lesser than 30 and 100 mg/L respectively [132]. In China, there are three different wastewater discharge standards, which can be further categorized into five grades of sub standards. For aquaculture, the standard applied is the Environmental Quality Standards for Surface Water (GB3838–2002) [133]. It is recommended that countries without clear regulations for aquaculture can follow the effluent standard drafted by GAA and IFC. Some countries like Malaysia that has not enforce regulations for aquaculture embraced alternatives by using guidelines of Environmental Quality (Industrial Effluent) Regulation 2009 as the main reference for aquaculture wastewater discharge standards [3].

6. Conclusions

Aquaculture has become the world's main fresh food production sector, forcing culturists to compete in the market. Because of this demand, improper feeding practices and the use of pharmaceutical products have posed a risk to the environment. This article reviews the current aquaculture treatment technology, classified as RAS, biological, AOPs, absorption, and membrane. To achieve the goal of zero discharge of aquaculture wastewater, traditional RAS is a commonly used closed system technology in the aquaculture field. However, due to some limitations of RAS, researchers have been motivated to upgrade the current RAS to improve system efficiency by introducing additional treatment technology. The major challenges of biological and adsorption are the selection of specific macrophytes and adsorbents, respectively. It has been found that some macrophytes and adsorbents are highly dependent on the source of nutrients in aquaculture wastewater. AOP, on the other hand, is considered to be one of the potential solutions for the degradation of CEC. However, major drawbacks of AOP have been identified, such as the generation of toxic by-products. Membrane treatment can simultaneously remove CEC and nutrient recovery. Nevertheless, due to the accumulation of suspended solids in wastewater, membrane fouling and instability would occur. Furthermore, membrane fouling leads to increased operating costs due to frequent replacement of membrane. Since multiple functions can be used in one process, hybrid technology offers better treatment than independent methods. Nonetheless, the technology appears to be in its early stages, and additional research on its effectiveness and system optimization is needed. As a result, when designing treatments for the pilot scale, researchers must consider a variety of factors. Through appropriate treatment methods, the ultimate goal of reducing environmental pollution can be achieved.

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Declaration of competing interest

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 paper.

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Harnessing genomics to fast-track genetic improvement in aquaculture

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Abstract | Aquaculture is the fastest-growing farmed food sector and will soon become the primary source of fish and shellfish for human diets. In contrast to crop and livestock production, aquaculture production is derived from numerous, exceptionally diverse species that are typically in the early stages of domestication. Genetic improvement of production traits via well-designed, managed breeding programmes has great potential to help meet the rising seafood demand driven by human population growth. Supported by continuous advances in sequencing and bioinformatics, genomics is increasingly being applied across the broad range of aquaculture species and at all stages of the domestication process to optimize selective breeding. In the future, combining genomic selection with biotechnological innovations, such as genome editing and surrogate broodstock technologies, may further expedite genetic improvement in aquaculture.

Aquaculture has a crucial and rapidly increasing role in food security and economic stability worldwide. More than 90% of global aquaculture occurs in low- and middle-income countries, where it provides major contributions to the Sustainable Development Goals of the United Nations, either directly through human consumption or indirectly through economic growth[1]. Global production of finfish and shellfish reached 172.6 million tons in 2017, approximately half of which is currently derived from aquaculture[2]. Capture fisheries, which harvest organisms in naturally occurring marine and freshwater environments for commercial purposes, are placing serious pressures on wild stocks, with minimal scope for sustainable expansion[3]. By contrast, aquaculture is the fastest-growing food production sector globally[1]. With major limitations on wild capture and terrestrial farmland exploitation[4], its future importance as a source of affordable and nutritious animal protein for human diets is evident. However, intensification of aquaculture production poses environmental concerns, such as habitat destruction[5] and infectious disease outbreaks, which have a negative impact on the health and welfare of farmed (and potentially wild) populations[6] and may be exacerbated by climate change[7].

Selective breeding for genetic improvement of production traits has great potential to increase the efficiency and reduce the environmental footprint of aquaculture. However, in contrast to the terrestrial livestock and crop sectors, aquaculture is based on a hugely diverse group of finfish and shellfish species (FIG. 1), comprising an estimated 543 different animal species, including 362 finfish, 104 molluscs, 62 crustaceans, 9 other aquatic invertebrates and 6 frogs and reptiles[2] (although aquatic plants and algae are also cultured for human use and consumption, the aquaculture of these organisms is beyond the scope of this Review and is covered elsewhere[8,9]). Farming of approximately 70 of these species underpins 80% of the global aquaculture production volume, compared with just three livestock species (pig, chicken and cow), which make up 80% of global meat production (FIG. 1b; Supplementary Tables 1,2), and four plant species (rice, wheat, maize and potatoes), which underlie two thirds of worldwide crop production[10]. Despite their diversity, aquaculture species tend to share two key features that enhance their potential for genetic improvement. Firstly, they remain in the early stages of the domestication process[11] (FIG. 1), which is linked to higher within-species genetic diversity. Secondly, they are highly fecund, with typically external fertilization. This feature of their reproductive biology allows for flexibility in breeding programme design and widespread dissemination of selectively bred strains to producers, often without the need for several tiers to multiply and disseminate sufficient numbers of genetically improved animals for production[12]. Therefore, there is a pressing opportunity to use domestication and selective breeding programmes to harness the as-yet largely untapped genetic potential of farmed aquatic species, as highlighted in a recent landmark report by the FAO[13]. This potential for cumulative and permanent improvement of production traits is evident from the typically high genetic gains in aquaculture breeding programmes;

Aquaculture
The farming of fish, crustaceans, molluscs, aquatic plants and algae in freshwater or saltwater environments, typically for human food.

Genetic gains
Improvement in average genetic value, and therefore improved phenotypes, in a population due to selection over cycles of selective breeding.

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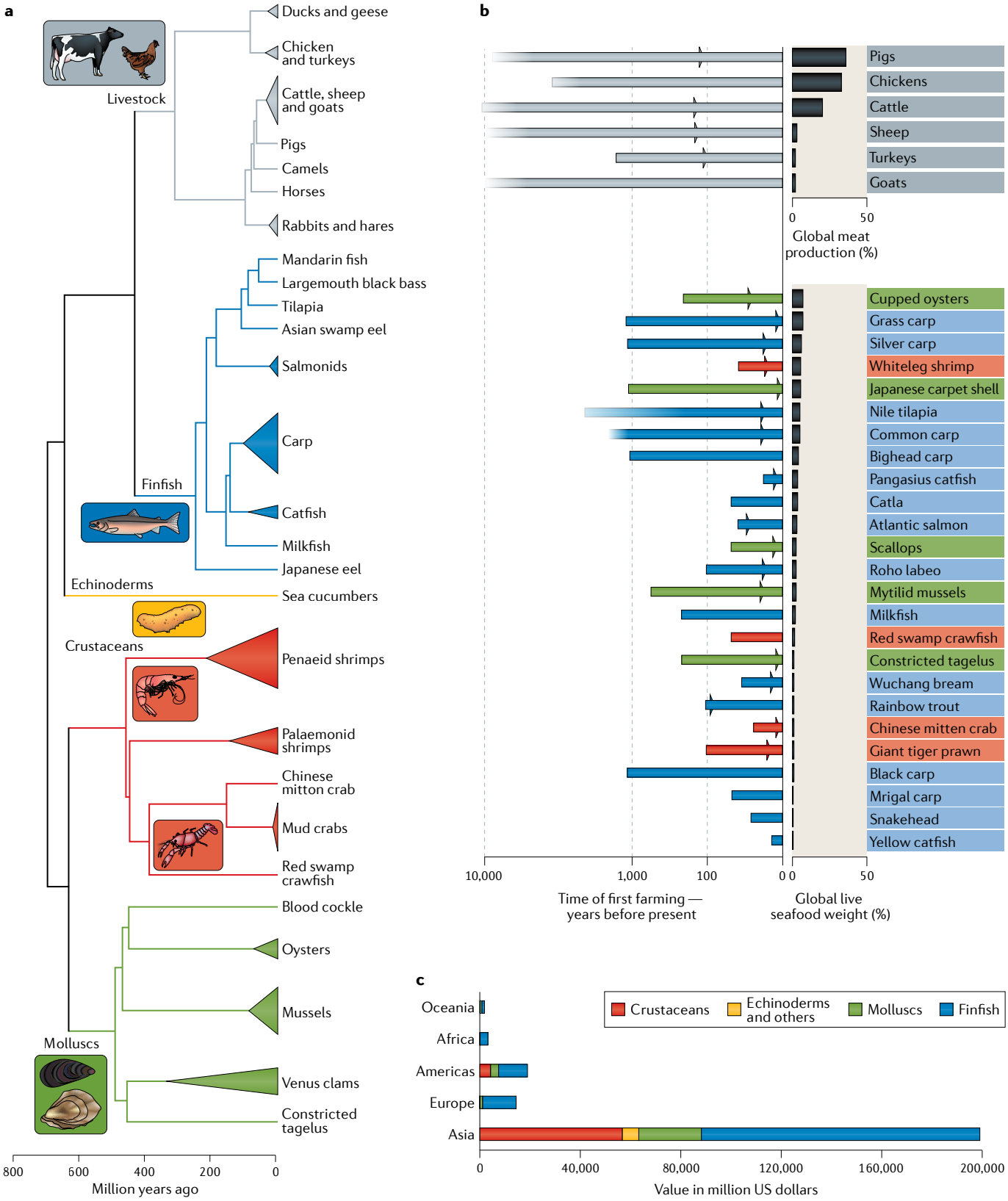


Fig. 1 | **Summary of global aquaculture diversity and production.** **a** | Phylogenetic tree showing farmed species with an annual production value higher than US$1 billion per annum (Supplementary Table 6). Estimated divergence times are from REFS[194–200]. **b** | The time at which species were first farmed or domesticated, including species which account for 80% of all farmed seafood production and 95% of all meat globally. The arrow in the bar denotes the point at which the first scientifically driven selective breeding studies were undertaken for each species (note, this could not be identified precisely for chickens or goats). Fading of timelines denotes uncertainty (Supplementary Tables 1,2,4). **c** | Seafood production globally by sector and continent[2] (Supplementary Table 7).

Base populations
Populations of animals used to start a selective breeding programme.

Genomic selection
The selection of breeding individuals for genetic improvement of a trait of interest based on the use of genome-wide genetic markers to estimate genomic breeding values. Genetic marker genotypes and phenotypes are measured in a reference population to predict breeding values of selection candidates that have genotypes only.

for example, an average 13% growth increase per generation in Atlantic salmon (*Salmo salar*)[14], which is substantially higher than the growth observed in breeding programmes for terrestrial livestock species[12,15].

Genomic tools are hugely valuable to inform sustainable genetic improvement[16], and their affordability and accessibility mean they can now be applied at all stages of the domestication and genetic improvement continuum, from informing the choice of base populations through to advanced genomic selection in closed commercial breeding nuclei (BOX 1). Furthermore, they can be applied to characterize, utilize and conserve wild aquatic genetic resources, and inform the management of interaction between farmed and wild aquatic animals throughout this continuum.

This Review provides an overview of the status of domestication and selective breeding in aquaculture species, highlights how tailored application of genomic tools can expedite sustainable genetic improvement in diverse species and environments, and explores the potential of emerging genomic and biotechnology techniques, such as genome editing or surrogate broodstock technologies, to promote step improvements in aquaculture breeding and production.

The domestication of aquaculture species

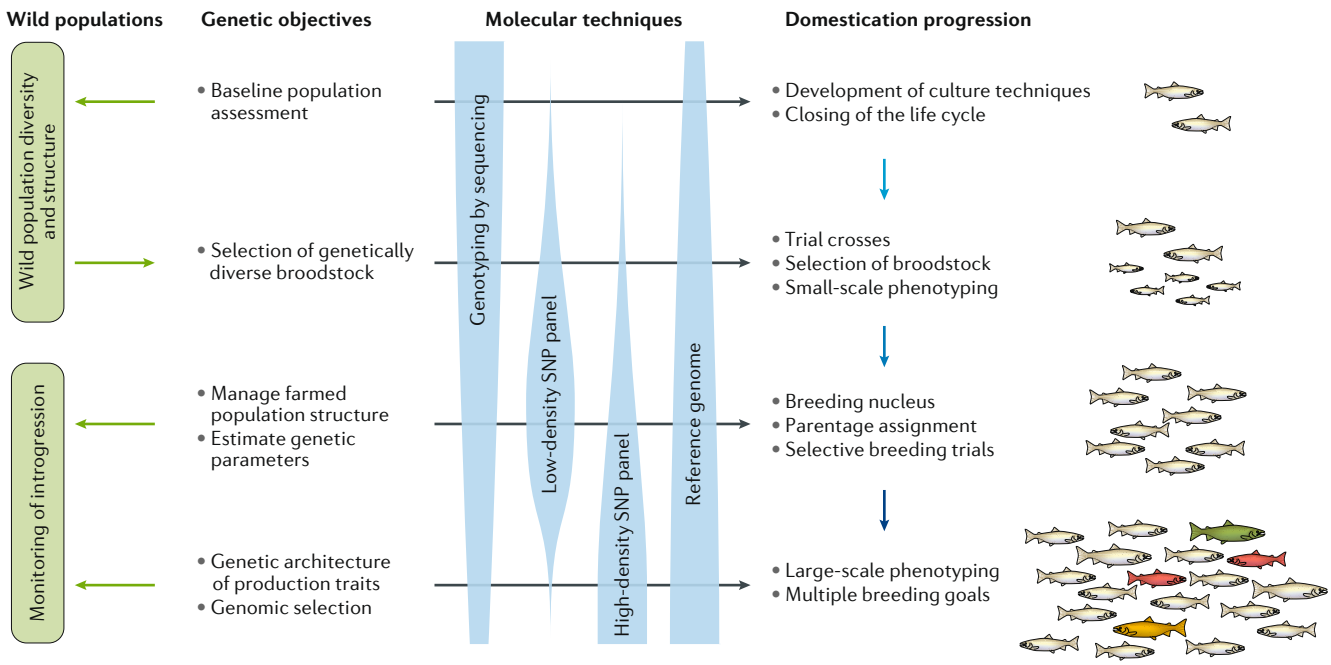
Domestication in the context of this Review is considered to be the process of moving from an exclusive reliance on wild broodstock to completion of the full life cycle in captivity, and use of modern selective breeding

Box 1 | A road map for genomic tools matched to different stages of the domestication process

Historically, the mismanagement of genetic resources and diversity during the domestication process has led to reduced genetic resilience[39] and the subsequent emergence of 'crowd' diseases in farmed populations[201], which can be catastrophic for emerging industries. Targeted use of appropriate genomic tools throughout the domestication process can help to retain genetic diversity in both wild and farmed populations, which is likely to contribute to mitigation or prevention of these issues.

Genomic tools have already made substantial contributions to the optimization of scientific breeding programmes and to proactive species conservation strategies for both farmed and wild populations of target species[202,203]. Given recent and rapid technological developments, together with improved accessibility and increased cost-efficiency, optimal genomic tools can be applied at each stage of the progression along the domestication and selective breeding continuum (see the figure). For example, cleaner fish, such as ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*), are used in commercial salmon production to eat sea lice from the skin of the salmon and are a key aspect of integrated pest management. Wrasse and lumpfish[204] production began in 2009 and 2011 (REF.[205]), respectively, with closure of the life cycles in captivity in 2018 and 2016 (REF.[206]) and reference genomes released by 2016 (REF.[207]) and 2018 (REF.[208]). Both domestication processes have combined animal biology, health management and nutritional requirements with the development of genomic tools for genetic management and enhancement[206]. The aforementioned trial crosses, which are crucial when establishing base populations for breeding, can be performed in combination with the cost-effective genotyping by sequencing (GBS), and both phenotype and genomic information can be used to optimize broodstock selection. This process should run concurrently with evaluation of wild stock population structure, using the same genomic tools to inform management strategies for species conservation and rapid diagnostics of genetic introgression[202] (see the figure).

When moving towards more advanced selective breeding programmes, bespoke tools such as single-nucleotide polymorphism (SNP) arrays can be applied, but their cost-effectiveness needs to be considered and contrasted with that of GBS. Both of these tools can then be applied to understand the genetic architecture of production traits, and to support genomic selection to maximize genetic gain and minimize inbreeding. SNP discovery and high-density genotyping also pave the way for the generation of targeted low-density SNP panels, which can have concurrent uses to support parentage assignment, stock management, traceability and low-cost genomic selection. Finally, due to the relative ease of generating reference genome assemblies, they should be created at the outset of the domestication of a new species for aquaculture, as they inform the choice of marker panels for genotyping and subsequent studies to understand the biology of production traits.



for genetic improvement of production traits, such as growth and disease resistance. Historically, the selection of species amenable to reproduction in farmed environments was pivotal to defining which livestock and aquaculture species were farmed. For example, domesticated species tend to display behavioural plasticity that enables them to adapt to a range of captive environments[17,18]. A key difference between livestock and aquaculture species is that domestication of terrestrial livestock occurred in tandem with global human migration several millennia before the informed management of breeding populations, and modern livestock lines have typically undergone multiple major genetic bottlenecks[11]. By contrast, the time lag between domestication and selective breeding is considerably shorter in aquaculture species, with both occurring in tandem in many cases. Consequently, genomic tools can be used from the outset to inform, optimize and expedite the two processes (BOX 1), providing a more detailed understanding of their impact on species' genomes and physiology.

For certain major aquaculture species, such as carp (members of the family Cyprinidae) and tilapia (members of the family Cichlidae), aquaculture and domestication have been ongoing in some form for millennia[19], but selective breeding programmes to enable genetic improvement are much more recent[20] (FIG. 1b). Currently, only a minority of aquaculture production is derived from selectively bred stocks, estimated at approximately 10% in 2012 (REF.[21]). However, this proportion is increasing rapidly, particularly for species with high production volume and value, with approximately 75% of the top 10 finfish, crustacean and mollusc species (by production volume) benefitting from some form of modern selective breeding programme (Supplementary Tables 3,4). The use of genetic technologies also varies dramatically by continent, with more than 80% of European aquaculture production derived from selective breeding programmes[22]. The availability and application of selective breeding depends on the local environmental, social, political and economic landscapes, all of which can present major hurdles, especially in low- and middle-income countries[23]. These programmes enable cumulative, permanent and sustainable genetic gain for target production traits[15,24], and are fundamental to scale up aquaculture production in the context of finite resources[13].

Moving towards genetic improvement via selective breeding requires progression along the 'levels of domestication' scale[25], which reflects our ability to control the life cycle of the farmed species in captivity. While the number and diversity of aquaculture species present challenges for this process, new husbandry techniques linked to improved understanding of reproductive biology and larval rearing will help overcome these challenges.

The burgeoning genomic toolbox

Genomic resources for aquaculture generally lag behind those for terrestrial livestock, in particular for sequencing and assembly of reference genomes (TABLE 1). Several high-value species remain without a publicly available high-quality reference genome and have limited genomic resources. In part, this reflects the traditionally challenging nature of genome assembly in non-mammalian and non-avian species, particularly for aquatic species with complex genomic features. These include the widespread presence of duplicated loci due to genome duplication events, for example, in salmonids[26], cyprinids[27] and sturgeons[28], and the exceptionally high heterozygosity observed, for example, in bivalve species[29,30] and crustaceans[31]. Such features seriously hinder assembly algorithms using short-read sequence data; as a result, many existing assemblies are very fragmented. However, these genomic features can underlie adaptive capacity and phenotypic plasticity in production environments[26,32], and might contribute to the genetic regulation of production-relevant traits[26,32].

The latest sequencing technologies, including platforms that generate long reads, for example, single-molecule real-time sequencing (Pacific Biosciences) and nanopore sequencing (Oxford Nanopore), and linked reads (10x Genomics), are increasingly being applied to aquaculture species to improve assemblies (Supplementary Table 5). When combined with long-range scaffolding technologies such as high-throughput chromatin conformation capture approaches (Hi-C; for example, Dovetail Genomics) and/or optical mapping (for example, Bionano Genomics), high-quality contiguous assemblies are possible even for challenging genomes[33]. For example, a recent genome assembly of the yellow perch (*Perca flavescens*) resulted in 24 ($2n = 24$) chromosome-size scaffolds covering 99% of the complete assembly, with N50 of 37.4 Mb[34]. All major aquaculture species are likely to benefit from such high-quality assemblies soon.

With genome sequencing of a target species coming within reach of individual laboratories, it no longer requires the degree of coordinated effort and funding that led to the first farmed animal species' reference genome assemblies, including Atlantic salmon[26]. However, standardization and coordination of multiple assemblies, including population- or 'breed'-specific assemblies, and their functional annotation remain a challenge for which international coordination and community-led initiatives are required.

A key component of the genomic toolbox to inform domestication and selective breeding is genotyping. Single-nucleotide polymorphism (SNP) array platforms have been created for many high-value aquaculture species (TABLE 1), and genotyping by sequencing (GBS) techniques, including restriction site-associated DNA sequencing (RAD-seq)[35] and derivatives, have been applied in many species to obtain population-level SNP data without major prior investment or the immediate need for a reference genome[36,37].

Genomics applied to domestication

The establishment and management of genetically diverse base populations is essential to domestication and the formation of breeding programmes, as it underlies the future genetic potential to be exploited via selective breeding[38]. Poor broodstock management and hatchery practices that lead to inbreeding depression have been hypothesized to result in reduced population fitness, increased susceptibility to stress and

Breeding nuclei
The elite broodstock animals that are maintained only for breeding, which is followed by multiplication and dissemination of the genetically improved animals for production.

Surrogate broodstock
Sterile animals used for the production of gametes of another individual, strain or species.

Broodstock
A group of sexually mature individuals used in aquaculture for breeding purposes.

Behavioural plasticity
The ability of an organism to change its behaviour following exposure to stimuli, such as changing environmental conditions.

Genetic bottlenecks
Sharp reductions in genetic diversity, typically due to large reductions in population size caused by environmental events or human activities.

Linked reads
Linking together of short sequence reads to provide long-range orientation, based on the addition of a unique DNA barcode to each read generated from an individual molecule.

Scaffolding
An approach during genome assembly where contigs (that is, continuous assembled sequences) are linked into larger contiguous sequences including gaps of known length.

Genotyping by sequencing
(GBS). A method using high-throughput sequencing to discover and genotype genome-wide single-nucleotide polymorphisms within a population.

Inbreeding depression
The reduced biological fitness in a given population as a result of inbreeding, typically due to deleterious recessive alleles.

Table 1 | **Genomic resources for aquaculture species with the highest production value**

Species	Production value (US$ billion)	Genome size (Gb)	Scaffold N50 (Mb)	Coding genes	Published SNP arrays (number of SNPs)	Resequenced genomes
Finfish						
Atlantic salmon (*Salmo salar*)	16.69	2.96	1.36	48,775	7 (15,000–286,000)	165
Grass carp (*Ctenopharyngodon idella*)	12.64	0.90	6.45	27,263	–	1
Silver carp (*Hypophthalmichthys molitrix*)	10.26	1.10	0.31	–	–	–
Nile tilapia (*Oreochromis niloticus*)	7.61	1.00	38.8	29,550	2 (50,000–58,000)	65
Bighead carp (*Hypophthalmichthys nobilis*)	7.31	1.01	0.08	–	–	–
Crustaceans						
Whiteleg shrimp (*Litopenaeus vannamei*)	26.74	1.63	0.6	24,987	1 (6,000)	–
Red swamp crawfish (*Procambarus clarkii*)	10.00	2.07	0.001	136,962	–	–
Chinese mitten crab (*Eriocheir sinensis*)	9.54	1.54	0.49	–	–	–
Giant tiger prawn (*Penaeus monodon*)	5.59	1.44	0.007	18,115	1 (6,000)	2
Oriental river prawn (*Macrobrachium nipponense*)	2.09	–	–	–	–	–
Molluscs						
Japanese carpet shell (*Ruditapes philippinarum*)	6.95	2.56	0.048	108,034	–	15
Chilean mussel (*Mytilus platensis*)	2.50	–	–	–	–	–
Constricted tagelus (*Sinonovacula constricta*)	1.41	–	–	–	–	–
Pacific cupped oyster (*Crassostrea gigas*)	1.24	0.55	0.4	28,398	2 (27,000–190,000)	516
Blood cockle (*Tegillarca granosa*)	1.02	–	–	–	–	–
Echinoderms						
Japanese sea cucumber (*Apostichopus japonicus*)	1.40	0.8	0.48	30,350	–	1

Full data are provided for the top 20 species per taxonomic group in Supplementary Table 5. Gb, gigabase; Mb, megabase; SNP, single-nucleotide polymorphism.

disease and, ultimately, 'boom-and-bust' production cycles[39,40]. Tailored use of genomic tools can be applied at each stage of the domestication and selective breeding continuum to inform and optimize the process (BOX 1).

An example of genomics-enabled domestication of a new target species is the Australasian snapper (*Pagrus auratus*) in New Zealand. Rapid generation of de novo genome maps[41], transcriptomes[42], GBS methods[41,43] and estimation of genetic diversity and genetic parameters[43] were applied to inform the selection of base populations, retention of genetic diversity during domestication and investigations into the biology of production traits. Similarly, the recent widespread use of cleaner fish (for example, Ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*)) for co-culture with Atlantic salmon to help tackle sea lice (*Lepeophtheirus salmonis* and *Caligus rogercresseyi*) has led to expedited genomics-enabled domestication and breeding of lumpfish (BOX 1). These cases are early examples of how genomics technology has rapidly become accessible and should be applied from the outset to inform domestication and subsequent genetic improvement.

Moreover, genomic tools are valuable to tackle species-specific breeding and production issues related to the highly diverse biology of aquaculture species. For example, a key component of the domestication–genetic

improvement continuum in aquaculture species is an early understanding of sex determination, where a diverse array of genetic and non-genetic systems have been described[44]. These can vary within a genus and even within a species, and sequential hermaphroditism presents an additional challenge in several commercially important aquaculture species[45]. GBS techniques have been widely applied to assess the genetic basis of sex determination[46], for example, in Nile tilapia (*Oreochromis niloticus*)[47], Atlantic halibut (*Hippoglossus hippoglossus*)[48], European seabass (*Dicentrarchus labrax*)[49] and mud crabs (*Scylla* sp.)[50]. The genetic markers identified in these studies can be applied to predict the sex of juveniles and to control the sex ratio in both broodstock and production animals. An additional species-specific reproductive challenge is mass spawning, which is a feature of several marine aquaculture species, such as gilthead sea bream and barramundi. Mass spawning causes practical challenges such as uneven parental contribution and difficulty in tracking individual pedigrees, which can result in inbreeding[51]. Although multiple interventions are possible to enable pedigree tracking (for example, pair spawning or stripping using hormonal induction)[52], genetic markers are frequently applied to track stock relatedness to minimize loss of genetic diversity within a closed breeding nucleus[51].

Of note, the reliability of genomic data alone to predict adaptive potential of populations is questionable[53], and genomic tools should be used as a complement to phenotypic evaluations of stocks. These evaluations may include trial diallelic crosses between strains in multiple environments, which can inform on additive genetic and heterotic effects on traits of interest, in addition to genotype and environment (G × E) interactions[54] (discussed in more detail below). Such information can be used to optimize selection of the base population, ensuring it has substantial genetic variation to be utilized for effective directional selection[38]. However, while hybrid vigour resulting from strain crosses can result in notable one-off gains in production, and genomic tools can provide insight into the underlying molecular mechanisms of this heterosis[55], exploiting additive genetic variation via within-strain breeding programmes is likely to result in superior performance after a small number of generations of selection[54].

Genomics applied to selective breeding

The establishment of well-managed selective breeding programmes for aquaculture based on recording of pedigree and routine measurements of traits has been successful in increasing the production of several species[14]. Just as genomic tools are applied to inform and optimize domestication, they can improve selective breeding in several ways, including by maximizing genetic gain and minimizing inbreeding[16].

Major-effect loci in recently domesticated populations. A key factor in defining the optimal use of genomic tools is the genetic architecture of production traits in the breeding goal; that is, whether genetic variation in target traits is underpinned by few major-effect loci or (as is typically the case in farmed animal populations)[12] many loci of minor effect. Farmed aquatic populations face selection pressures that are vastly different from those faced by their wild counterparts. Due to the recent and ongoing domestication process, previously neutral alleles in wild populations may be beneficial for production phenotypes, and these will remain among the standing genetic variation in aquaculture populations. During the millennia of domestication of terrestrial livestock, such loci are likely to already be fixed via soft sweeps. However, in aquaculture species, they may present a one-off opportunity for rapid genetic improvement via marker-assisted selection (MAS) based on the use of targeted quantitative trait locus (QTL)-linked markers to augment breeding decisions.

A well-known example is the major QTL affecting resistance to infectious pancreatic necrosis (IPN) virus in Atlantic salmon, for which rapid uptake of MAS by the industry had a major role in preventing outbreaks of IPN (BOX 2). Other applications of QTLs for disease resistance include breeding of a Japanese flounder (*Paralichthys olivaceus*) strain with resistance to the viral disease lymphocystis[56] based on a major QTL for lymphocystis resistance[57], and use of MAS based on a QTL affecting resistance to bacterial cold water disease in rainbow trout (*Oncorhynchus mykiss*)[58]. Other noteworthy examples of major effect loci in salmon include *vgll3*, which controls the timing of sexual maturation and explains 30–40% of the phenotypic variation in age at maturity[59,60], as well as loci for resistance to pancreas disease[61] and cardiomyopathy syndrome[62,63]. Similarly, in Nile tilapia, a locus explaining 79% of the phenotypic variation in salinity tolerance was detected[64], although validation of the size of the effect in independent populations is required to make generalized conclusions about this trait.

As genomics is increasingly used to study traits of interest to aquaculture in additional species and populations, the number of loci of major effect will presumably rise. While MAS has had limited success in terrestrial livestock, its use within aquaculture populations at the early stages of domestication can provide rare but striking examples that highlight the value of genetic improvement to the industry.

Genomic selection to accelerate trait improvement. Genome-wide association studies in aquaculture species have highlighted that most traits of relevance to production are polygenic in nature[65,66] (that is, under the control of many loci, typically of small effect). For genetic improvement of such traits, routine trait measurement and tracking of relationships between individual animals in a breeding population is required[67]. The availability of large full-sibling families gives both power and flexibility to a breeding programme design, for example allowing the routine testing of full siblings of the selection candidates (sib testing) for traits that are practically challenging or impossible to measure on the selection candidates themselves, such as disease resistance (BOX 2; FIG. 2). However, for these sib-testing traits, selection candidates from a given family have the same estimated breeding value, placing limitations on the genetic gain that can be achieved while maintaining genetic diversity. Genetic marker data are required to accurately capture

Sequential hermaphroditism
Where an individual in a species is born as one sex but can later change to the opposite sex.

Mass spawning
Release of high numbers of eggs and sperm into the water, where fertilization occurs externally. Also known as broadcast spawning.

Soft sweeps
Increases in frequency and/or fixation of a favourable allele at an existing polymorphic locus due to strong positive selection pressure.

Marker-assisted selection
(MAS). The selection of breeding individuals for genetic improvement of a trait of interest based on genetic markers linked to a quantitative trait locus affecting that trait.

Quantitative trait locus
(QTL). A region of the genome that explains a significant component of variation in a trait of interest.

Box 2 | Genetic solutions to major diseases in aquaculture

Infectious disease outbreaks are a major and ongoing threat to the economic and environmental sustainability of aquaculture[209]. Most farming occurs in open-water environments, providing frequent contact with pathogens (including wild reservoirs of infection), and at high stocking densities conducive to the rapid spread of infection. Outbreaks of single pathogens can destroy national aquaculture industries, as highlighted by outbreaks of infectious salmon anaemia virus in Chile in 2007–2010 (REF.[210]), and annual losses of shrimp equating to ~10% of the global industry due to white spot syndrome virus[211]. Options to fully mitigate such diseases via vaccination (in finfish only), biosecurity and pharmaceutical interventions are limited in aquaculture systems for several reasons. Firstly, physical handling is logistically and financially challenging; secondly, the open-water nature of many farming systems makes outbreaks difficult to contain; and thirdly, the early stage of research in many species means there is a paucity of vaccination and/or treatment options for diseases.

The power of genetic and breeding technologies to prevent or mitigate infectious diseases is increasingly recognized. Encouragingly, host resistance to most aquaculture diseases is heritable[212–214], and sib-testing schemes together with genomic selection provide an effective route to breeding more resistant stocks without compromising the biosecurity of the breeding nucleus (FIG. 2). Indeed, disease resistance has become a major component of advanced aquaculture breeding programmes[22], whereas in terrestrial livestock this is limited by logistical and financial challenges relating to routine measurement of disease resistance traits[215].

Refining and optimizing the collection of disease resistance data in both experimental and production environments is an important goal. Disease resistance is typically measured using laboratory-based pathogen challenges of pedigreed populations of animals, using outcomes such as survival or pathogen burden to quantify the resistance traits[212]. However, disease outcomes in an outbreak depend on several epidemiological factors, and new traits such as the propensity of an infected individual to transmit disease have been suggested to have a genetic basis in farmed fish[216]. Benchmarking disease resistance traits measured in experimental settings with respect to outcomes in production environments is key to achieving disease prevention and control via improved genetics.

The example of IPN in salmon

Infectious pancreatic necrosis (IPN) is a viral disease that was one of the primary concerns for salmon farming, particularly around the turn of the 21st century, with frequent outbreaks causing high levels of mortality (up to 90%) in stocks both in freshwater hatcheries and following transfer to sea cages. Resistance to IPN was shown to be moderately to highly heritable[217], and breeding companies began to implement family-based selection. In parallel, teams from the UK and Norway identified a single major quantitative trait locus on chromosome 26 that could explain 80–100% of genetic variation in resistance to IPN virus in seawater field trials[218] and experimental freshwater trials[219–221]. High-throughput sequencing subsequently enabled the development of SNP-based genetic tests to predict IPN resistance of salmon without the need for regular disease challenge experiments[222,223]. The practical outcome of these experiments was extensive use of marker-assisted selection for the favourable allele in all major salmon breeding programmes, assisted by the fact that the resistance allele is dominant[220,223]. The results were striking, with a sustained decrease in the incidence of IPN outbreaks to near zero[72] (see the figure). Follow-up functional studies highlighted marked differences in gene expression response to infection between resistant and susceptible salmon fry[224] and suggested that epithelial cadherin may be part of the mechanism underlying the quantitative trait locus[223]. However, the exact causative mutations and the nature of their effect remain at least partly elusive.

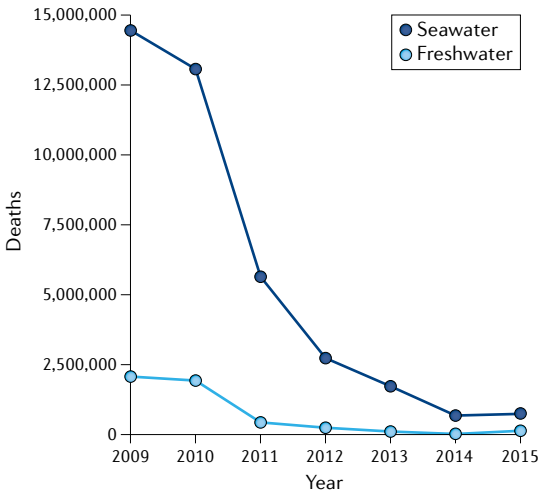


Figure adapted from REF.[72], Elsevier.

Mendelian sampling
The chance factor in the process of distributing half the genetic material from each parent to the offspring, which is the source of within-family genetic variation.

SNP arrays
A type of DNA microarrays that are used to genotype genome-wide polymorphisms within a population.

Reference population
In genomic selection, the population of animals that have both genotypes and phenotypes. These data are used to estimate genetic marker effects, which are then applied to predict breeding values for genotyped selection candidates.

Accuracy
In the context of genomic selection, accuracy is the correlation between the estimated genomic breeding values and the true breeding values.

Phenotyping
Collection of measurements relating to traits of interest in the goals of a breeding programme.

the within-family (or Mendelian sampling) component of genetic variation for such traits.

Genomic selection[68] was first tested in Atlantic salmon breeding, made possible by development of the first high-density SNP arrays[69,70] and demonstration of their utility to accurately predict breeding values in a typical salmon breeding programme setting[70,71]. Genomic selection in aquaculture breeding is based on the same concept as for terrestrial livestock, with genome-wide genotype and phenotype measurements taken on a reference population used to train a prediction model, which is then applied to genotyped selection candidates to predict genomic estimated breeding values[12,68]. Importantly, the high fecundity and large family sizes in aquaculture species offer two major advantages. Firstly, the close relationship between the reference population and the selection candidates results in high selection accuracy, even at low marker density, which is likely to be due to long genomic segments shared between close relatives. Secondly, routine phenotyping can be performed on these close relatives for different traits and in diverse environments, including 'field' testing in commercial farm settings (FIG. 2). In the past 5 years, most advanced breeding programmes for major aquaculture species have routinely used genomic selection[66,72], and developments in low-cost genotyping technologies are enabling technology transfer to smaller and more fragmented sectors.

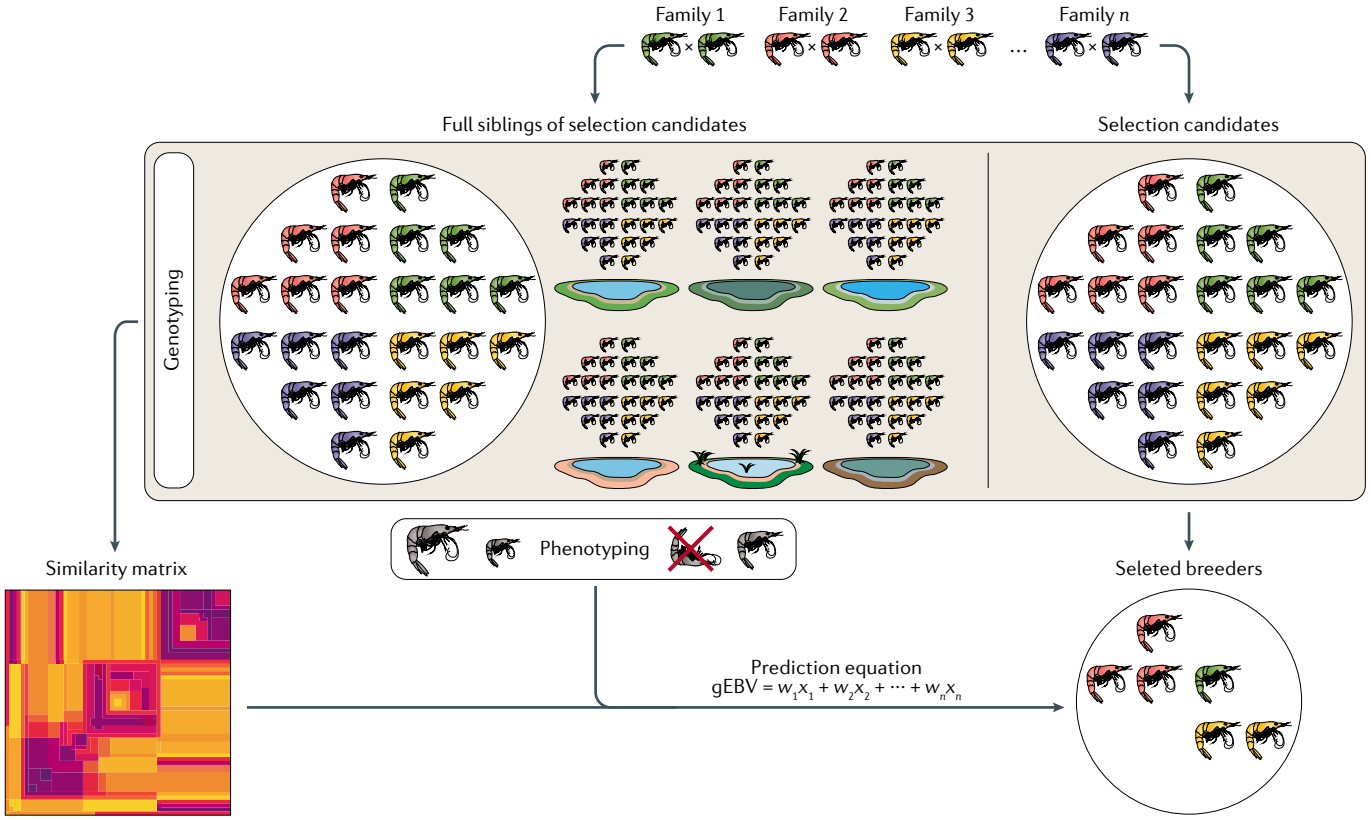


Fig. 2 | **Genomic selection within an aquaculture breeding programme.** Full siblings from a number of families are split into selection candidates and animals for phenotypic evaluation. These full siblings of the selection candidates can be grown in different environmental conditions and phenotyped for different traits, for example, using pathogen challenges to estimate resistance to different diseases or measuring performance traits in diverse production environments. The selection candidates and their phenotyped full siblings are all genotyped, and a genomic relationship matrix reflecting the genetic similarity between each pair of animals is built. This relationship matrix and the collected phenotypes enable the estimation of breeding values for the selection candidates through the use of genomic selection models such as GBLUP (genomic best linear unbiased prediction) or Bayesian models[12]. gEBV, genomic estimated breeding value.

The availability of large full-sibling families can be exploited with use of within-family genomic selection, with very low-density markers used to estimate genomic breeding values within families with known pedigree-based estimated breeding values[73]. The increased accuracy of genomic prediction compared with pedigree prediction is evident in a range of aquaculture species, with a median increase in prediction accuracy of 24% for growth-related traits and 22% for disease resistance traits (TABLE 2). These increases in prediction accuracy are fairly consistent across species and genotyping platforms, with SNP arrays primarily used in high-value species, but GBS giving equivalent findings in several other finfish, crustacean and shellfish species (TABLE 2). Most studies of genomic selection in aquaculture species use genomic best linear unbiased prediction (GBLUP) approaches, which harness genomic relationships to estimate the genetic merit of individuals[66]. A range of Bayesian models have been tested in several species but without consistent differences in prediction accuracy compared with the simpler GBLUP approach[66]. Adequate sample size for the genotyped and phenotyped population is key to fully assess the efficacy of genomic selection (for example, more than 1,000 individuals), but the population structure is equally important, as prediction accuracy is very dependent on the proximity of relationships between animals in the training and validation sets[74]. While several thousand genome-wide markers are also required, it is noteworthy that a reduction in SNP density to only 1,000 or 2,000 SNPs tends to be sufficient to achieve the asymptote of prediction accuracy where these close relationships exist[66,75]. However, the accuracy drops drastically as the relationship between the reference and test populations becomes more distant, as demonstrated in Atlantic salmon[76] and common carp (*Cyprinus carpio*)[77]; therefore, routine trait measurement and genotyping are required each generation to retrain the genomic prediction models.

Low-cost solutions for democratizing genomic selection. Capitalizing on the advantages offered by high fecundity in aquaculture breeding programmes requires genotyping of thousands of animals per generation, which can be prohibitively expensive. While genomic selection has become commonplace in a few highly developed aquaculture sectors (for example, aquaculture of salmonids, tilapia and shrimp), genomic tools are yet to be routinely incorporated into breeding programmes for

Genomic best linear unbiased prediction
(GBLUP). A modification of the pedigree-based best linear unbiased prediction method that incorporates SNP information in the form of a genomic relationship matrix and defines the additive genetic covariance among individuals to predict breeding values.

Bayesian models
In the context of genomic selection, the use of multiple-regression methods incorporating prior information on marker effects, which are used widely for genomic prediction of breeding values.

many species (TABLE 1; Supplementary Table 5). Hence, to translate the benefits of genomic selection to most aquaculture species, there is a clear need to develop cost-effective and species-specific tools, together with effective knowledge transfer to help democratize the technologies. Lower-density SNP panels, potentially typed using targeted GBS techniques (for example, GT-seq)[78] or fluorescence-based assays, tend to be cheaper than SNP arrays. Low-density genotyping can be integrated with genotype imputation to increase the accuracy of genomic selection to levels approaching those obtained with high-density genotyping[79–81]. Imputation relies on genotyping only a subset of the animals at high density (in an aquaculture breeding scheme, typically the parents of the reference population and selection candidates), defining the set of haplotypes in this subset, genotyping offspring at low density and imputing genotypes to high density on the basis of those haplotypes[79]. Considering that breeding programmes for many aquaculture species routinely use low-density SNP panels for parentage assignment[51], combined-purpose low-density panels can offer the benefit of genomic selection at little added cost (and may reduce the need for physical tagging). The addition of selected functional markers linked to major QTL would add further value to combined-purpose panels to enable concurrent parentage assignment, MAS and imputation-based genomic selection. Further research to develop cost-effective and pragmatic genomic selection approaches is essential to translate its benefits to aquaculture sectors with smaller margins, including in many low- and middle-income countries.

From sequence to consequence: identifying causative variants for target traits. Mapping and understanding the causative variants or functional variants that have an impact on complex traits is a fundamental goal of biology but also has potential additional benefits for increasing rates of genetic gain in breeding either through improved selection accuracy or as targets for genome editing (FIG. 3). The reduction in prediction accuracy with more distant relationships between reference and validation sets[74] is partly because QTL are captured via linked markers rather than causative genetic variants. Research from terrestrial livestock breeding hints at the potential of harnessing whole-genome sequencing data[82], and incorporating weighting of putative functional genomic variants (for example, BayesRC)[83] into genomic selection models to increase accuracy, although improvements in prediction accuracy have been rather minor in most cases. The use of whole-genome sequencing of key selected individuals (for example, parents) combined with imputation to whole-genome sequences based on genome-wide SNP genotypes will result in population-scale sequence data for aquaculture species and allow testing of such approaches soon. However, the cost of whole-genome sequencing and the effectiveness of low-density SNP panels described earlier mean that substantial increases in selection accuracy would be necessary to justify its routine use in breeding programmes.

The high fecundity harnessed for sib testing is also advantageous for high-resolution genetic mapping experiments, and genome-wide association studies are used to highlight genomic regions associated with traits of interest. However, such regions often contain hundreds to thousands of candidate causative variants and dozens of genes, and most of these variants are in non-coding regions, potentially affecting transcriptional regulation. Shortlisting those variants and genes that are more likely to be causal can be facilitated by using a pipeline of functional genomics techniques, together with knowledge of the biology of the trait in question (FIG. 3).

Improvements to the annotation of reference genomes of aquaculture species is integral to the process of identification of causative genetic variants. RNA sequencing (RNA-seq) combined with advances in software for read alignment and quantification have facilitated genome-wide prediction of coding and non-coding genes in many aquaculture species[32], replacing microarrays as the standard for global quantification of gene expression. Single-cell RNA-seq is yet to be widely applied to aquaculture species, but offers opportunities to understand complex and rare cell populations and uncover regulatory relationships between genes, thereby improving genome annotation and detection of putative causative variants[84].

Discovery and exploitation of epigenetic marks in aquaculture species also represents a crucial step to help bridge the genotype–phenotype gap[85] and prioritize variants for downstream functional testing. Emerging genomic technologies are enabling the elucidation of genome-scale patterns of cytosine methylation, chromatin accessibility, histone modifications, transcriptional start sites and transcript variants[86]. These tools enhance the scope to identify putative causative variants within regulatory sequences (for example, enhancers) that are active under specific environmental conditions (for example, during disease outbreaks). In addition, aquaculture species also benefit from the existence of extant and recently diverged wild counterparts, and use of comparative genomics and orthology analysis can help predict functional variants on the basis of sequence conservation[87]. The Functional Annotation of Animal Genomes (FAANG) initiative[88] is a concerted effort to map such features in livestock, with the Functional Annotation of All Salmonid Genomes (FAASG) being an equivalent community initiative for salmonid fish[32], and comparable initiatives are likely to follow for other major aquaculture species.

Ultimately, the identification of functional variants will require functional studies such as genome editing of a specific allele to assess consequences for the trait of interest in cell culture and/or whole-animal systems (see the section 'Genome editing to accelerate genetic improvement').

Towards accurate high-throughput phenotyping. Obtaining accurate phenotypes en masse is critical for any breeding programme since the accuracy of trait measurement directly affects genetic gain per generation. Phenotype measurements can be particularly challenging for aquaculture species because manual measurements before harvest typically require the handling of large numbers of animals outside the water, presenting a logistical and financial challenge. Therefore,

Genotype imputation
The statistical inference of unobserved genotypes based on knowledge of haplotypes in a population, typically used to predict high-density marker genotypes when most individuals are genotyped for low-density marker genotypes.

Causative variants
Polymorphisms within the genome of a population that have a direct effect on a trait of interest, as opposed to simply being a genetic marker associated with the trait.

Genotype–phenotype gap
The gap in knowledge of how variation at the level of the genome causes an effect on a phenotype of interest.

Table 2 | **Summary of studies testing genomic prediction for production traits in aquaculture species**

Species	Trait	Measurement	Heritability (pedigree)	Accuracy (pedigree)	Relative increase (%)	Genotyping technology (number of SNPs)	Ref.
Atlantic salmon (*Salmo salar*)	Growth	Weight	0.60 (0.48)	0.70 (0.58)	21	SNP array (132,000, 112,000 postfiltering)	159
		Length	0.61 (0.51)	0.66 (0.56)	18		159
	Resistance to sea lice	Lice count	0.33 (0.27)	0.60 (0.48)	25	SNP array (132,000, 33,000 postfiltering)	160
		Lice count	0.22(0.27)	0.46 (0.43)	7		160
		Lice count	0.11 (0.10)	0.50 (0.41)	22	SNP array (50,000, 37,000 postfiltering)	161
		Log lice density	(0.14)	0.52 (0.34)	52	SNP array (220,000)	70
	Resistance to amoebic gill disease	Gill score	0.24 (0.25)	0.62 (0.51)	22	Two-species SNP array (17,000, 7,000 postfiltering)	162
		Amoebic load	0.25 (0.36)	0.70 (0.60)	17		162
		Gill score	0.28 (0.32)	0.72 (0.61)	18	SNP array (55,000, 53,000 postfiltering)	163
	Resistance to salmon rickettsial syndrome	Time to death	0.27 (0.18)	0.41[a] (0.34)	21	SNP array (50,000, 50,000 postfiltering)	164
		Binary survival	0.39 (0.26)	0.26 (0.20)	30		164
	Fillet pigmentation	–	(0.43)	0.44 (0.36)	22	SNP array (220,000)	70
	Muscle fat	–	0.25 (0.36)	0.56 (0.60)	–7	SNP array (57,000, 50,000 postfiltering)	165
	Omega-3 fatty acid content	DHA	0.20 (0.21)	0.41 (0.33)	24		165
		EPA	0.04 (0.06)	0.32 (0.37)	–14		165
Rainbow trout (*Oncorhynchus mykiss*)	Resistance to bacterial cold water disease	Binary survival	–	0.68[a] (0.36)	89	SNP array (57,000, 45,000 postfiltering)	166
		Time to death	0.33 (0.37)	0.67[a] (0.34)	97	SNP array (57,000, 36,000 postfiltering)	167
		Binary survival	0.35 (0.35)	0.70[a] (0.36)	94		167
		Time to death	0.29 (0.31)	0.49 (0.50)	–2	SNP array (57,000, 41,000 postfiltering)	168
		Binary survival	0.45 (0.48)	0.46 (0.41)	12		168
	Resistance to infectious pancreatic necrosis virus	Time to death	0.25 (0.40)	0.53 (0.49)	8	SNP array (57,000, 38,000 postfiltering)	169
		Binary survival	0.24 (0.35)	0.56 (0.50)	12		169
	Resistance to salmon rickettsial syndrome	Time to death	0.45 (0.38)	0.78[a] (0.61)	28	SNP array (57,000, 27,000 postfiltering)	170
		Binary survival	0.55 (0.54)	0.60[a] (0.47)	28		170
	Resistance to infectious haematopoietic necrosis virus	Time to death	0.23 (0.33)	0.33 (0.13)	154	SNP array (57,000, 35,000 postfiltering)	171
		Binary survival	0.25 (0.28)	0.39 (0.24)	63		171
	Resistance to columnaris disease	Binary survival	0.32 (–)	0.11 (–0.02)	–650	SNP array (57,000, 36,000 postfiltering)	172
		Binary survival	0.51 (–)	0.22 (0.06)	267	SNP array (57,000, 34,000 postfiltering)	172
Coho salmon (*Oncorhynchus kisutch*)	Resistance to salmon rickettsial syndrome	Time to death	– (0.14)	0.52 (0.27)	93	ddRAD (9,000)	173
		Binary survival	– (0.27)	0.81 (0.31)	161		173
Carp (*Cyprinus carpio*)	Growth	Length	0.33 (0.33)	0.71 (0.60)	18	RAD-seq (20,000)	174
	Resistance to koi herpesvirus	Binary survival	0.50 (0.61)	0.53[a] (0.49)	8	RAD-seq (16,000)	77
Nile tilapia (*Oreochromis niloticus*)	Growth	Harvest weight	0.36 (0.31)	0.60 (0.48)	25	SNP array (43,000, 32,000 postfiltering)	175
		Fillet yield	0.21 (0.21)	0.62 (0.54)	15		175
		Harvest weight	0.17 (0.22)	0.29 (0.19)	53	SNP array (59,000, 48,000 postfiltering)	176
		Fillet weight	0.16 (0.24)	0.34 (0.18)	89		176
		Fillet yield	0.23 (0.33)	0.54 (0.46)	17		176
European sea bass (*Dicentrarchus labrax*)	Resistance to viral nervous necrosis	Binary survival	0.43 (0.27)	0.62[a] (0.67)	–7	RAD-seq (9,000)	177

Table 2 (cont.) | **Summary of studies testing genomic prediction for production traits in aquaculture species**

Species	Trait	Measurement	Heritability (pedigree)	Accuracy (pedigree)	Relative increase (%)	Genotyping technology (number of SNPs)	Ref.
Gilthead sea bream (*Sparus aurata*)	Resistance to pasteurellosis	Time to death	0.28 (0.22)	0.44[a] (0.30)	47	2b-RAD (22,000)	178
	Resistance to pasteurellosis	Time to death	0.32 (0.32)	0.54[a] (0.45)	20	2b-RAD (28,000)	179
		Binary survival	0.33 (0.31)	0.56[a] (0.46)	22		179
Turbot (*Scophthalmus maximus*)	Resistance to scuticociliatosis	Resilience	0.15 (–)	0.46 (0.41)	12	2b-RAD (18,000)	180
		Resistance	0.26 (–)	–	–		180
		Endurance	0.12 (–)	–	–		180
Japanese flounder (*Paralichthys olivaceus*)	Resistance to *Edwardsiella tarda*	Binary survival	– (–)	0.603 (–)	–	WGS (1.9×10^6)	181
Channel catfish (*Ictalurus punctatus*)	Growth	Harvest weight	0.27 (–)	0.37 (0.29)	28	SNP array (660,000, 55,000 postfiltering)	182
		Residual carcass weight	0.34 (–)	0.31 (0.24)	29		182
Large yellow croaker (*Larimichthys crocea*)	Growth	Body weight	0.60 (–)	0.41 (–)	–	ddRAD (30,000)	183
		Body length	0.59 (–)	0.40 (–)	–		183
	Omega-3 HUFA	–	0.44 (–)	0.30 (–)	–	ddRAD (32,000)	183
Yellowtail kingfish (*Seriola lalandi*)	Growth	Weight	0.47 (0.42)	0.69 (–)	–	DArT-seq (14,000)	184
		Length	0.43 (0.42)	0.67 (–)	–		184
		Condition index	0.21 (0.11)	0.44 (–)	–		184
Yellow drum (*Nibea albiflora*)	Growth	Body length	– (–)	0.38[a] (–)	–	GBS (54,000)	185
		Swimming bladder index	– (–)	0.17[a] (–)	–		185
		Swimming bladder weight	– (–)	0.22[a] (–)	–		185
		Body thickness	– (–)	0.24[a] (–)	–		185
		Body height	– (–)	0.30[a] (–)	–		185
		Body length/body height ratio	– (–)	0.36[a] (–)	–		185
		Gonad weight index	– (–)	0.37[a] (–)	–		185
Pacific oyster (*Crassostrea gigas*)	Growth	Shell length	0.26 (0.23)	0.54 (0.44)	23	Two-species SNP array (38,000, 23,000 postfiltering)	186
		Shell height	0.23 (0.20)	0.60 (0.47)	28		186
		Wet weight	0.35 (0.31)	0.67 (0.54)	24		186
	Resistance to ostreid herpesvirus	Binary survival	0.37 (0.25)	0.76 (0.64)	19		187
Yesso scallop (*Patinopecten yessoensis*)	Growth	Shell height	0.48 (–)	0.53 (–)	–	2b-RAD (2,000)	188
		Shell length	0.48 (–)	0.46 (–)	–		188
		Shell width	0.36 (–)	0.55 (–)	–		188
Zhikong scallop (*Chlamys farreri*)	Growth	Shell length	0.42 (–)	0.65[a] (–)	–	2b-RAD (31,000)	189
		Shell height	0.47 (–)	0.70[a] (–)	–		189
		Shell width	0.54 (–)	0.63[a] (–)	–		189
		Whole weight	0.28 (–)	0.64[a] (–)	–		189
Whiteleg shrimp (*Litopenaeus vannamei*)	Growth	Body weight	0.32 (–)	0.62 (–)	–	2b-RAD (23,000)	190
		Body length	0.45 (–)	0.61 (–)	–		190
		Body length	– (–)	0.30[a] (–)	–	SLAF-seq (6,000)	191
		Body weight	– (–)	0.41[a] (–)	–		191
	Resistance to AHPND	Time to death	0.26 (0.24)	0.50 (0.47)	6	2b-RAD (23,000)	192
		Binary survival	0.16 (0.15)	0.21 (0.20)	5		192

Table 2 (cont.) | **Summary of studies testing genomic prediction for production traits in aquaculture species**

Species	Trait	Measurement	Heritability (pedigree)	Accuracy (pedigree)	Relative increase (%)	Genotyping technology (number of SNPs)	Ref.
Banana shrimp (*Fenneropenaeus merguiensis*)	Growth	Body weight	0.55	0.76 (0.65)	17	DArT-seq (9,000)	193
		Body length	0.49	0.73 (0.60)	22		193
		Head length	0.39	0.42 (0.32)	31		193
		Body width	0.61	0.72 (0.60)	20		193
		Tail weight	0.45	0.77 (0.66)	17		193
		Meat yield	0.10	–	–		193
	Colour	Dark (raw shrimp)	0.18	0.59 (0.53)	11		193
		Red (cooked shrimp)	0	NA	–		193
	'Flesh streaks'	–	0	NA	–		193
	Yellow hepatopancreas	–	0.03	NA	–		193
	Resistance to HPV	Viral load	0.35	0.60 (0.09)	567		193

AHPND, acute hepatopancreatic necrosis disease; 2b-RAD, restriction site-associated DNA sequencing using type IIB restriction endonucleases; DArT-seq, diversity array technology sequencing; ddRAD, double digest restriction site-associated DNA sequencing; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GBS, genotyping by sequencing; HUFA, highly unsaturated fatty acid; NA, not available; RAD-seq, restriction site-associated DNA sequencing; SLAF-seq, specific locus amplified fragment sequencing; SNP, single-nucleotide polymorphism; WGS, whole-genome sequencing. [a]Alternative statistical models to genomic best linear unbiased prediction were used, (for example, Bayesian models or ridge regression best linear unbiased predictor).

the ability to collect such data both directly on the selection candidates in the breeding nucleus and on relatives of those candidates in test or production environments can present a limitation to genetic progress in breeding programmes. Computer vision technologies are being widely applied to automate plant and terrestrial livestock phenotyping, and its utility to accurately predict traits of interest has been demonstrated in several aquaculture species[66,89]. Optical sensors and machine vision systems can be used to monitor behavioural and health traits in tank or cage environments, while hyperspectral imaging approaches can inform on fillet content and characteristics[89]. For instance, underwater cameras for real-time in situ data collection are being used for tasks such as sea lice monitoring in Atlantic salmon farms[90], and their use is likely to expand for more widespread data collection and phenotyping[89]. Connected mobile devices for affordable on-farm monitoring and automation of aquaculture environments (that is, sensor technologies and the Internet of things) have major potential for monitoring individual traits such as behaviour and feed intake, while enabling the collection of huge volumes of environmental data. Transforming such data into meaningful phenotypes for breeding is a substantial challenge, and consequently data interpretation and decision tools such as machine learning and artificial intelligence will assume greater importance in aquaculture[91]. Together with routine genomic evaluations, the effective combination of increasingly high-resolution and high-volume phenotyping in breeding nuclei, in production environments and after harvest will lead to more precise and effective genetic improvement of aquaculture species.

Internet of things
A network of physical objects that use sensors and application program interfaces to connect and exchange data over the Internet.

Genetics and the environment

Tackling genotype by environment interactions in aquaculture breeding. The performance and robustness of a farmed animal is dependent on the interaction between its genotype and the environment, which can vary substantially in aquaculture both within and across farms. For example, water quality presents a key challenge with limited environmental control, resulting in substantial within-farm and across-farm variation in partial pressure of CO_2, temperature and other parameters. The transition to on-land recirculating aquaculture systems or floating closed-containment systems with close control of environmental conditions is plausible for certain species such as Atlantic salmon[92], but the level of investment required to establish and maintain these systems is substantial and is unlikely to be feasible for most situations. As such, genetic improvement in a breeding programme is intrinsically linked to the environment in which traits are recorded, and G × E interactions commonly result in genotype reranking such that the best-performing genotypes in one environment may not be the best in another, placing a limitation on realizing genetic gains in breeding programmes[20,93]. The extent and nature of G × E interactions depend on the trait in question and can be quantified by measuring the genetic correlation between the trait in different environments. Studies across multiple aquaculture species have highlighted that such correlations tend to be positive, albeit only moderate in magnitude for growth and survival traits[93], highlighting the need to account for G × E interactions in aquaculture breeding programmes.

The domestication and genetic improvement of local strains and species, which may be better adapted to the local environment, is one route to reducing the impact of G × E interactions. However, well-managed breeding programmes are expensive, and as such the current trend is consolidation into large and high-technology programmes that harness high fecundity (often including multiplication layers) to disseminate single lines into production facilities worldwide. In this scenario, breeding programmes need to account for G × E interactions to maximize the benefits of genetic improvement[94]. The possibility of disseminating many closely related animals to diverse geographical locations and environmental conditions (FIG. 2) can be coupled with

phenotyping technologies for routine data collection to feed back information on performance under diverse settings. This may facilitate production of differentiated strains tailored to specific environments, or inclusion of robustness as a target trait such that a single strain has phenotypic plasticity within and across diverse environments[95]. An example of a robust strain that performs well in multiple environments is the genetically improved farmed tilapia (GIFT) strain. In the late 1970s, inadequate tilapia stocks were hampering the development of aquaculture in Asia. To develop a strain with robust performance in high- and low-input systems across diverse environments, a base population including wild and farmed strains from eight African and Asian countries was established. The breeding programme focused primarily on improving growth rate, but involved multiple farmers in different countries in evaluations to account for G × E interactions. The GIFT strain is now farmed in 16 countries across Asia, Africa and Latin America and grows 85% faster than the base population[96].

Genomic selection can facilitate the breeding of more robust strains in aquaculture species where reference populations (including close relatives of selection candidates) are tested in diverse environments[93,97]. The performance of a genotype along an environmental gradient for any measurable trait can be used to calculate the response curve, or reaction norm, of that genotype[93]. This reaction norm can be used as a target trait for genomic selection to reduce sensitivity to environmental variation, with notably superior results to sib-testing schemes alone[97]. The variation within and between production environments is typically larger for aquaculture in low- and middle-income countries; as breeding programmes in such settings increase in sophistication, low-cost genomic selection methods should be applied to help improve resilience of stock performance within and across environments to maximize the benefits of genetic gain for producers.

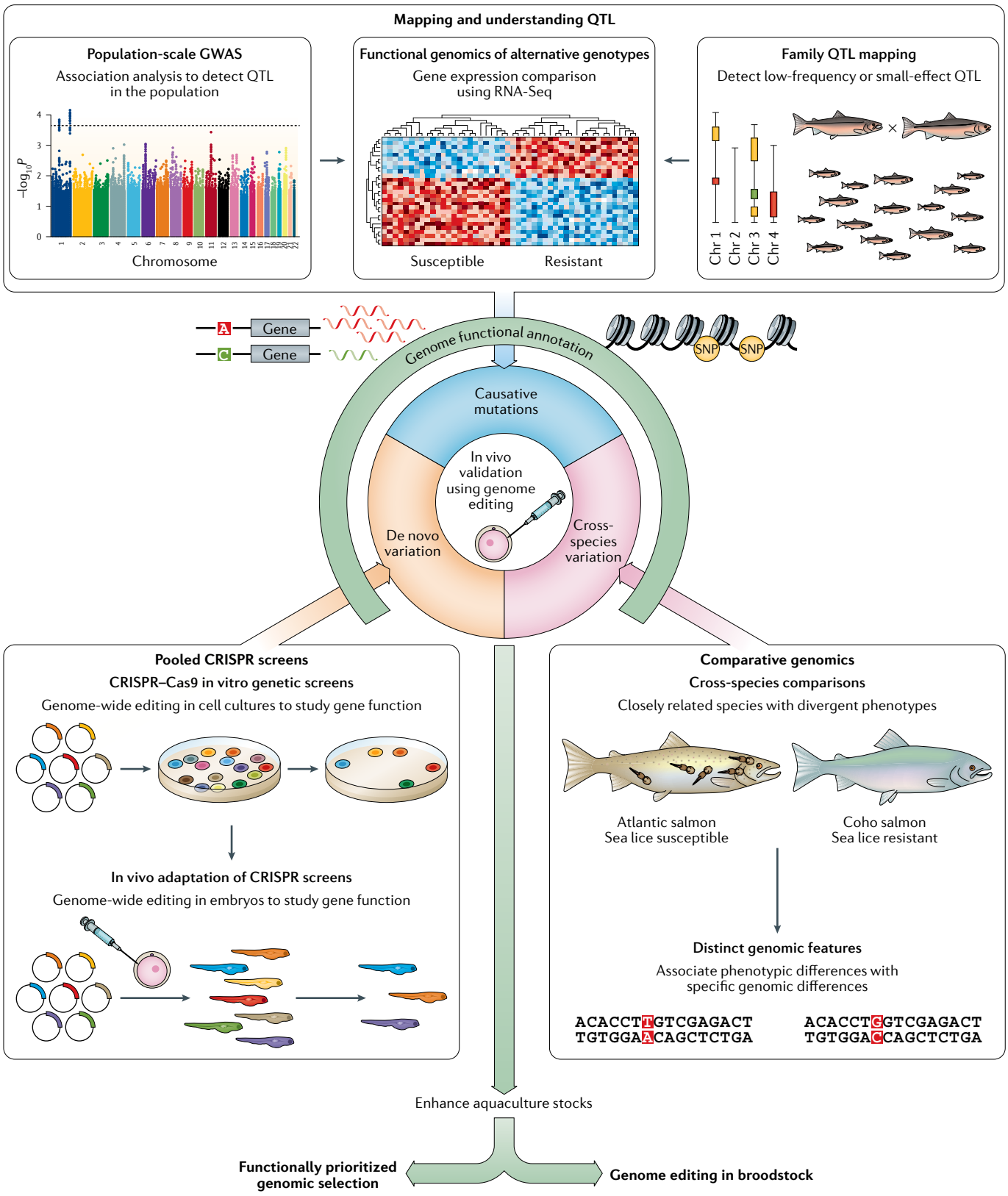
Epigenetic programming to improve performance and environmental adaptation. Epigenetic mechanisms or 'marks' (for example, cytosine methylation, histone modifications, chromatin accessibility state) can be influenced by the environment and result in substantial phenotypic variation from the same genomic DNA blueprint[85]. Recent domestication can profoundly alter the epigenome of hatchery-reared animals via alterations to the DNA methylation profile[98], highlighting the potential for rapid epigenetic reprogramming. This potential can be harnessed by intentional environmental manipulation during crucial life stages, in particular larvae and broodstock, to improve production traits later in life and/or in subsequent generations[85,99,100]. For example, early-life use of plant-based diets increased the acceptance and use of these diets in later life in rainbow trout[101], and early-life stress can modulate future stress or immune responses in Atlantic salmon, which may have implications for robustness in adult stages[102,103]. Multigenerational epigenetic effects are of most relevance to selective breeding, and have been proposed to play a role in the fitness of the Manila clam (*Ruditapes philippinarum*), where adults exposed to low pH levels during gonadal maturation had faster-growing offspring compared with controls[104], and in the Sydney rock oyster (*Saccostrea glomerata*), where larvae of parents incubated under low-pH conditions grew and developed faster in low-pH conditions and had higher fitness as adults[105]. The development of assays to assess genome-wide cytosine modification, chromatin structure and accessibility across multiple aquaculture species will help elucidate the mechanisms underpinning these epigenetic phenomena, and the availability of isogenic finfish lines is a useful resource to help distinguish genetic and epigenetic effects[106].

For heritable epigenetic marks that affect production traits, it is highly likely that their impact will be directly captured and utilized by conventional sib testing and genomic selection, which are both based on phenotypic similarity between relatives[107]. However, distinguishing additive genetic and epigenetic components of phenotypic variation may facilitate improvement in genetic parameter estimation and prediction of response to selection[100]. Furthermore, an interesting intersection between epigenetic programming and genetic improvement via selective breeding may be related to optimizing of robust performance of improved stocks in multiple environments. The use of genomics to support breeding of 'robust' strains for multiple environments described earlier can be augmented with tailored epigenetic programming to improve the performance of these strains in specific farmed environments. Furthermore, there is likely to be genetic variation in the response to targeted environmental manipulation, and genomic prediction using large full-sibling families each split into groups tested with targeted environmental treatments can be used to assess this (FIG. 2). Therefore, selection for improved response to epigenetic programming could be a route to realizing genetic improvement for impact across diverse production environments.

The microbiome as a predictor of performance. The microbiome is a critical component of the interaction between animals and their environment, and contributes to the health and performance of farmed animals[108,109]. Colonization and development of bacterial communities are essential for immune function and are influenced by host physiology and immune response. Host microbial composition is heritable to some extent in marine species[110,111], and differences have also been observed between farmed and wild strains of Atlantic salmon[102] and Pacific whiteleg shrimp (*Litopenaeus vannamei*)[112]. Microbiome research in aquaculture species is currently primarily focused on gaining an understanding of its composition in various species[109,113]. Developments in DNA sequencing technologies have provided drastic improvements in microbiome analyses, in particular metagenomics approaches to sequencing all genomes within a sample. Microbiome sequencing may have potential when paired with host genotyping for the prediction of production traits, with a potential example trait being the ability of salmonids to tolerate increasingly vegetarian diets[114]. In terrestrial livestock, microbiome similarity matrices have been used to replace or complement the host genomic relationship matrix, with an improved predictive ability for feed conversion

Genomic relationship matrix
A matrix containing the estimation of the proportion of total genomic DNA shared by any two individuals based on genome-wide genetic marker data.

efficiency in Holstein Friesian dairy cattle[12]. In this context, microbiome composition can be considered as an 'intermediate phenotype' resulting from both host genetic and environmental influences, and has potential value in prediction of trait performance in later life, rather than prediction of offspring performance. The latter may depend in part on the heritable component of the microbiome, but is likely to be captured within additive genetic variation and breeding values for production traits.



Introgression
The deliberate movement of a target locus from one species or strain (donor) into another (recipient) by the creation and repeated backcrossing of a hybrid with one of the donor species or strains.

Effective population size
The size of an idealized population that would give rise to the rate of inbreeding and the rate of change in variance of allele frequencies actually observed in the population under consideration. It is approximate to the number of individuals that contribute gametes to the next generation.

Germplasm
In the context of animal breeding, the genetic material of a breeding programme.

Primordial germ cells
The stem cells specified during early development that will differentiate to form male and female gametes, therefore representing the precursors of the germline.

Interaction between farmed and wild animals. The recent domestication of aquaculture species means that farmed species often coexist in close proximity to wild counterparts, with frequent interaction and interbreeding possible between the two groups. As species move along the domestication scale towards closed selective breeding populations, the genetic divergence between farmed and wild populations widens. The genomes of farmed species are significantly altered by domestication and genetic improvement programmes, which exert intense selection pressures[115]. As domestication progresses, high-density genotyping or sequencing of multiple populations of farmed and wild populations and comparison of genetic diversity across the genome to identify common signatures of selection can be applied to gauge these effects[116,117].

Divergence of wild and farmed populations results in notable differences in growth, morphology, life history, behaviour and physiology[118]. The impact of domestication on animal physiology has been demonstrated by studies of gene expression and genome methylation, which show marked differences after a few generations of hatchery breeding in salmonids[119]. Introgression of potentially maladapted alleles into wild populations can lead to undesirable changes in life history traits, reduced population productivity and decreased resilience[120]. Many species of marine fish and invertebrates are characterized by high connectivity, with associated high gene flow, and high effective population size[121], such that the effects of introgression from farm-reared animals is rapidly diluted. Such introgression may even be beneficial in some species (for example, bivalve shellfish) by contributing to natural recruitment and adding genetic variation to wild populations[122,123]. By contrast, freshwater and anadromous species are characterized by fairly small effective population sizes[124], and gene flow can be heavily modified (or blocked)[125,126]. Consequently, inflow of genes from farmed animals can result in rapid and substantial alterations to the gene pool in populations of these species[124]. Therefore, methods of preventing escapees and interbreeding of farmed and wild animals are important for the sustainability of aquaculture and its long-term coexistence with extant wild populations[124,127,128]. Engineering and management solutions are unlikely to completely prevent escapes, and genetic technologies to prevent such introgression include triploidy, currently used in a range of species, including salmonids and oysters[129,130], or other means of inducing sterility in production stocks such as germ cell ablation via genome editing[131] (see the section Genome editing to accelerate genetic improvement).

In addition to protecting wild stocks, it is important to maintain genetic resources for farmed strains as they undergo domestication. Biobanking is applied for conservation of germplasm of aquatic animals, both for vulnerable wild species and for farmed strains, to avoid losing genetic diversity. There are established repositories and gene banks for finfish and shellfish, and technologies for preservation of gametes, tissues and cell lines are developing rapidly, with detailed reviews available[132,133]. However, the field remains at a fairly early stage compared with equivalent efforts in crops and terrestrial livestock. Whereas cryopreservation of sperm is routine for several fish and shellfish species, the cryopreservation of oocytes is much more challenging. Cryopreservation of ovarian tissues is a promising alternative but would require research into the in vitro culture of these tissues[133]. Surrogate broodstock (discussed later) hold promise to preserve genetic resources through transplant of primordial germ cells[134]. As these methods develop, preservation of aquatic genetic resources will benefit from more centralized efforts, akin to seedbanks for crops, together with associated FAO standards and procedures for biobanking[135].

Biotechnology in aquaculture breeding

Biotechnological innovations hold promise to tackle production barriers in aquaculture. These advances include the use of genome editing technologies to make targeted changes to the genomes of aquaculture species, resulting in improved health and performance, use of reproductive biotechnologies such as surrogate broodstock to expedite genetic gain, and combinations of both approaches.

Genome editing to accelerate genetic gain. Genome editing tools such as engineered CRISPR–Cas9 systems[136,137] are invaluable for understanding genetic regulation of economically important traits and have potential to accelerate genetic gain in aquaculture breeding programmes (FIG. 3). The enzyme Cas9 makes a DNA double-strand cut at a genomic site corresponding to a guide RNA, which results in either small insertions or deletions that can lead to loss-of-function mutations (non-homologous end joining) or user-defined edits to the genome based on a provided DNA template (homology-directed repair). Since the first demonstration of effective genome editing in Atlantic salmon[138], CRISPR–Cas9 has been successfully applied in various farmed finfish and mollusc species, primarily for gene knockout and as proof of principle[139]. Microinjection into early-stage embryos is the most commonly used delivery method but can be inefficient, and alternative delivery methods,

◀ Fig. 3 | **Discovering functional variants using genomics and genome editing.** Three complementary strategies to discover causative variants affecting traits of interest for aquaculture breeding are represented. The first is 'mapping and understanding quantitative trait loci (QTLs)', which harnesses genome-wide association studies (GWAS) and within-family QTL mapping approaches to detect genomic regions associated with these traits, followed by functional genomic comparison of animals carrying alternative genotypes at the identified QTL. Identified single-nucleotide polymorphisms (SNPs) within the region of candidate genes are then annotated according to their position in the genome to prioritize them as targets for validation using CRISPR–Cas9 genome editing. The second is 'comparative genomics', where two closely related species that differ for a high-priority trait (for example, resistance to sea lice) are compared using comparative and functional genomics, again leading to potential genome editing targets for validation. The third is 'reverse genetics', where pooled, genome-wide CRISPR screens can be applied in cell culture, followed by screening based on markers of infection or resistance to infection to identify key genes involved in disease resistance. The high fecundity of aquaculture species may allow analogous approaches in vivo using Cas9 transgenic broodstock followed by screening of embryos or juveniles. The three categories of functional variants identified in the inner circle all have potential for genetic improvement, either via functionally enriched genomic selection or directly via genome editing of broodstock after a further testing and validation phase of research. Chr, chromosome.

such as electroporation of sperm, hold promise[140]. Genome editing can be used as a component of pipelines to identify putative causative genes and variants, for example, by assessing the effect of gene knockouts on traits of interest. Use of genome-wide loss-of-function CRISPR screens such as genome-scale CRISPR knockout (GeCKO)[141] in aquaculture species offers a powerful tool to explore the genetic basis for resistance to certain pathogens; successful editing of a salmonid fish cell line using a lentivirus delivery system suggests that this approach is technically viable[142]. However, cell line resources for many aquaculture species, in particular invertebrate species, are limited, and targeted development of suitable cell lines for important aquaculture species is required. As an alternative, in vivo GeCKO may be plausible in species with external fertilization, abundance of embryos and feasible early-life screens[139]. This approach is likely to require the development of Cas9-stable broodstock and a method of delivering guide RNA libraries en masse to early-stage embryos. Combining such genome-wide screening approaches with mapping, and shortlisting causative functional variants in QTL regions, will create opportunities for targeted experiments testing candidate causative alleles, followed by assessment of the consequences on the trait (FIG. 3).

Several potential applications of genome editing could expedite genetic improvement. Firstly, it could enable the rapid fixation of favourable alleles at QTL segregating within breeding populations[143]. Secondly, genome editing could facilitate introgression-by-editing of favourable alleles from other populations, strains or species, potentially including wild stocks, into a breeding population[139]. Finally, it is possible to create de novo alleles on the basis of knowledge of the biology of the trait in question or using targets from GeCKO screens. For example, removal of an exon of the *CD163* gene in pigs (*Sus scrofa*) resulted in complete resistance to porcine reproductive and respiratory syndrome virus[144].

Although disease resistance is likely to be the primary focus for genome editing in aquaculture, other traits, such as adaptation of stocks to plant-based diets or sterility to prevent introgression and unwanted effects of precocious maturity[145,146], are additional key objectives. For example, knockout of the germline-specific genes *dnd1* in Atlantic salmon[131] and *nanos2* or *nanos3* in Nile tilapia[147] resulted in sterility. For practical applications, genome editing needs to be integrated into well-managed breeding programmes to ensure maintenance of genetic diversity. Genome editing en masse in production animals is unlikely to be feasible and, therefore, editing of the germline of broodstock animals is highly likely to be the most effective approach. Sterility requires special consideration because it is by definition not heritable, and inducible transgenic targets may be required. However, sterility may be a useful trait to include with other genome editing targets to negate the risk of edited alleles being transferred to wild stocks (for example, via escapees).

Refinement of genome editing methods is occurring constantly, and use of modified CRISPR–Cas systems such as CRISPR activation or CRISPR interference can induce differences in expression levels of target genes instead of complete knockout[148–150]. Such tools will be valuable in elucidating the functional genetic basis of production traits, for fundamental understanding of genome function and for future application in aquaculture breeding programmes. However, it is critical that edited stocks are carefully studied to detect and avoid off-target editing and are rigorously monitored to discount deleterious pleiotropic effects; aquaculture can follow procedures used for terrestrial livestock to achieve these goals[151]. Furthermore, any practical application for aquaculture depends entirely on an acceptable regulatory and public approval landscape[152], and the approval of the genetically modified AquaAdvantage salmon (Aquabounty) as fit for human consumption by the US Food and Drug Administration and the Canadian Food Inspection Agency was a recent landmark[153]. Target traits that have concurrent production and animal welfare or environmental benefits should be a focus for genome editing in aquaculture, and public and policymaker engagement with the technology, its benefits and its risks is absolutely vital.

Surrogate broodstock to reduce generation intervals. A key factor in the rate of genetic gain in a breeding programme is the length of the generation interval. Consider the breeder's equation:

$$\Delta G = \frac{ir\sigma_A}{y},$$

where ΔG is the genetic gain over time, i is the selection intensity, r is the selection accuracy, σ_A is the additive genetic variance and y is the generation time. Genomic selection has resulted in a step increase in selection accuracy, and much research is now devoted to achieving further minor increases[66]. However, decreasing generation time has potential for more drastic changes to genetic gain, especially considering that many of the major aquaculture species have relatively long generation intervals (for example, up to 20 years in sturgeon, family Acipenseridae). Surrogate broodstock technologies are based on the concept of isolation of the primordial germ cells of selected broodstock animals at an early life stage and transplantation of these cells into the surrogate; that is, a germ cell-ablated specimen of a species with a shorter generation time (FIG. 4). When combined with genomic selection to predict breeding values of embryos or juveniles, surrogate broodstock technology could potentially reduce the generation interval without substantial loss of selection accuracy. Germ cell isolation, transplantation and successful gamete production in surrogate broodstock have been demonstrated across species within a genus, and even across genera[154]; for example, rainbow trout offspring were produced when spermatogonia from rainbow trout were injected into newly hatched sterile masu salmon (*Oncorhynchus masou*)[155]. The same technology has other potential applications; for example, to produce offspring from a species which is challenging to rear in captivity using surrogates, such as production of Atlantic bluefin tuna (*Thunnus thynnus*) gametes from chub mackerel (*Scomber japonicus*) as

Pleiotropic effects
In the context of genome editing, the unintended impacts on traits other than the target trait due to a specific edit.

Selection intensity
The number of phenotypic standard deviation units that selected parents are superior to the mean of a population.

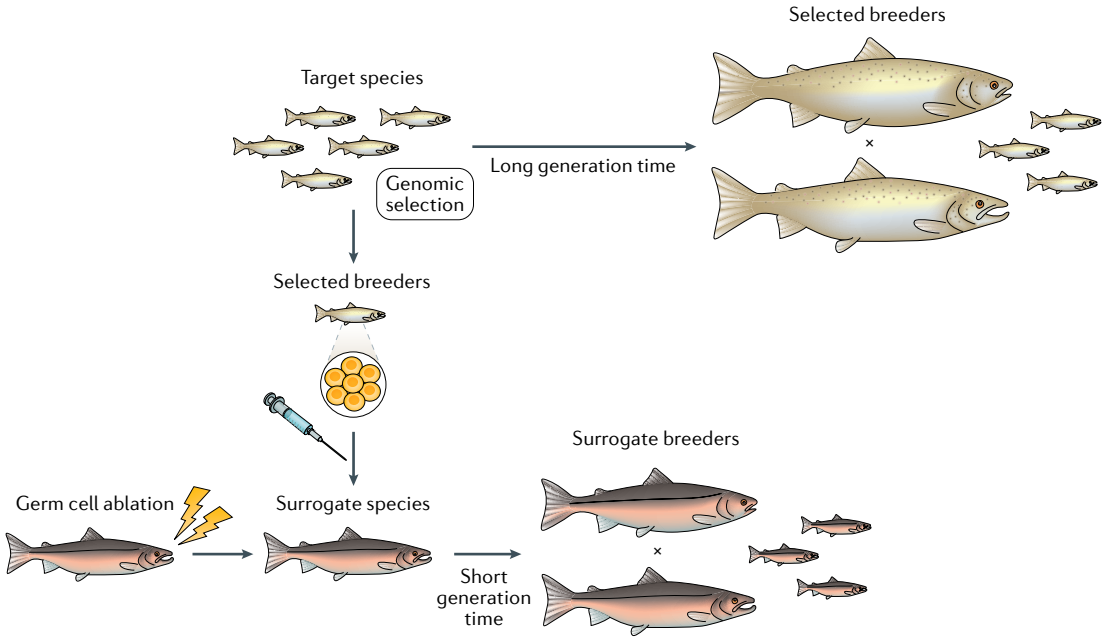


Fig. 4 | **Potential application of surrogate broodstock technology to accelerate genetic gain.** This approach involves the transplantation of germ cells from a donor species (target) to a recipient species (surrogate), which then produces gametes of the donor. The main interest for aquaculture is to transfer the germ cells of the selected breeders of the farmed species to a surrogate that is easier to maintain in captivity and has a shorter generation time, reducing the time between two successive rounds of selection. This approach ensures the success of production and accelerates the rate of genetic gain of the breeding programme. The germ cells of the surrogate must be ablated before transplantation. In this respect, germ cell-free animals can be obtained through chromosome set manipulation (that is, triploidy)[155] or the functional manipulation of genes fundamental for germ cell survival (for example, through genome editing)[131].

a surrogate[154]. In addition, surrogate technology can be coupled with genome editing of primordial germ cells to create germline-edited animals, as successfully demonstrated in chickens[156]. This approach is a route to genome editing for aquaculture species where access to the newly fertilized embryos is challenging, such as in certain crustaceans[157] or ovoviviparous species such as rockfish (*Sebastes* spp.)[158]. While clearly a long-term and high-risk research goal, the combination of surrogate broodstock, genome editing and genomic selection has potential to drastically increase the rate of genetic gain in breeding programmes via the reduction of the generation interval. Extensive effort and resources have been put into the use of functional genomic data to increase selection accuracy in breeding, and reproductive technologies require equivalent attention.

Conclusions

In contrast to terrestrial livestock and crop production, most aquaculture production derives from species for which domestication and breeding is at an early stage. Genetic improvement and dissemination of germplasm originating from a well-managed breeding programme makes possible cumulative increases in production traits, and facilitates adaptation to emerging challenges, such as climate change or infectious disease outbreaks. Due to recent growth and increased availability, genomics should be used from the outset of domestication and breeding programme design to inform base population composition, maintain genetic diversity and understand sex determination and differentiation. Genomic selection has revolutionized terrestrial livestock breeding and is commonplace in advanced aquaculture sectors such as the salmon sector, but judicious application of multipurpose cost-effective marker panels may be necessary to translate those benefits to most aquaculture species, for which the industries are smaller and more fragmented.

The ability to disseminate closely related individuals to diverse testing and production environments, combined with genomic selection, should be applied to tackle G × E interactions and improve robustness. Genomic tools can also inform on the potential of the microbiome and epigenome as useful intermediate phenotypes, and as conduits to increase capacity for adaptation of stocks to environmental challenges. For the more advanced aquaculture sectors, the immediate future will include mapping and understanding functional genomic variants, and harnessing the species' high fecundity to perform high-resolution genetics and genomics experiments paired with highly contiguous and well-annotated genome assemblies. Genome editing is key to this process and as such requires species-specific optimization both in vivo and in cell culture, with the development of suitable cell lines for aquaculture species being an important focus, for example, to assist with genome-wide CRISPR screens for disease resistance. The widespread commercial application of genome editing in aquaculture seems to be several years away, but it has clear potential for step changes in trait improvement to help

Ovoviviparous
Producing offspring by means of eggs that are hatched within the body of the parent.

address production barriers. In the longer term, developments in surrogate broodstock technology combined with genomic selection have the potential for shortening generation intervals to expedite genetic gain.

Underpinning many of these advances is improved knowledge of the genetics and biology of key production traits, which is particularly pertinent for the many aquaculture species from understudied taxa with major knowledge gaps relating to fundamental inheritance and genome biology. Overall, there is now an unprecedented opportunity to harness genomics to fast-track the domestication and genetic improvement of farmed aquatic species, which will be necessary to secure the sustainable growth of aquaculture as one of the most promising solutions to the current global food security challenge.

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Progress in valorisation of agriculture, aquaculture and shellfish biomass into biochemicals and biomaterials towards sustainable bioeconomy

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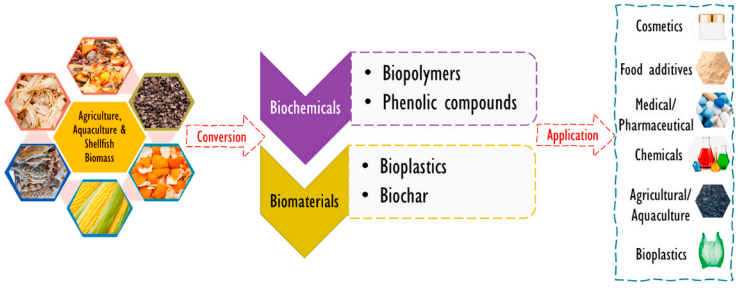
HIGHLIGHTS

- Valorisation of agriculture, aquaculture, shellfish biomass wastes is reviewed.
- Pectin, furfural, vanillin and bioactive compounds can be extracted from biomass.
- The industrial application of phenolic compounds from biomass is discussed.
- Bioplastic from biomass and bacteria is desirable to replace conventional plastic.
- Pyrolysis combined with modification techniques improve biochar properties.

GRAPHICAL ABSTRACT



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ABSTRACT

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The recurrent environmental and economic issues associated with the diminution of fossil fuels are the main impetus towards the conversion of agriculture, aquaculture and shellfish biomass and the wastes into alternative commodities in a sustainable approach. In this review, the recent progress on recovering and processing these biomass and waste feedstocks to produce a variety of value-added products via various valorisation technologies, including hydrolysis, extraction, pyrolysis, and chemical modifications are presented, analysed, and discussed. These technologies have gained widespread attention among researchers, industrialists and decision makers alike to provide markets with bio-based chemicals and materials at viable prices, leading to less emissions of CO_2 and sustainable management of these resources. In order to echo the thriving research, development and innovation, bioresources and biomass from various origins were reviewed including agro-industrial, herbaceous, aquaculture, shellfish bioresources and microorganisms that possess a high content of starch, cellulose, lignin, lipid and chitin. Additionally, a variety of technologies and processes enabling the conversion of such highly available bioresources is thoroughly analysed, with a special focus on recent studies on designing, optimising and even innovating new processes to produce biochemicals and biomaterials. Despite all these efforts, there is still a need to determine the more cost-effective and efficient technologies to produce bio-based commodities.

1. Introduction

For around a century and a half, fossil resources were the indisputable feedstock to produce fuels, chemicals and materials. In the energy sector for instance, over 80% of the energy consumption around the globe are based on fossil fuels (i.e., natural gas, coal and petroleum), and around 10% of the fossil resources are applied in the non-energy sector, such as the chemical industry (IEA, 2020). With the increasing world population and the simultaneous rise of the living standards in many countries worldwide, the shortcomings and unsustainability of the fossil-based linear economic model started to be clearly revealed with the emergence of highly complicated economic, societal, ecological, geopolitical issues around the world (i.e., recurrence economic crises, accentuated disparities and mass migration, climate change and greenhouse gas emissions, armed conflicts around resources, transgressed planetary boundaries, etc.) (Steffen et al., 2015; Sillanpää et al., 2017; Johnsson et al., 2019).

It was not until a few decades ago that governments and large companies, mainly in developed countries, started paying attention to the adverse impact of such issues on sustaining and promoting economic growth, social welfare and environmental protection. As a quick response to such an alarming global context, national and international policies and plans were drafted (Fourie, 2018; Tsani et al., 2020), and more responsible actions were taken by the private sector (Topple et al., 2017; Florini and Pauli, 2018), but with limited impact considering the wide amplitude and complexity of such issue, conflicting objectives between the public and private sectors, and overall the need for a systemic paradigm shift to set course for a genuine sustainable development worldwide.

More recently, sustainable development has become a priority for policymakers and influential stakeholders around the world, with the necessity to gradually shift away from fossil resources as a key endeavour. Such delicate and lengthy enterprise comes with its own set of challenges that need to be faced to make a smooth transition towards low-carbon, resource-efficient societies, and sustainable economic systems. In this context, bioeconomy emerges as an alternative paradigm providing bio-based resources as viable replacements to the fossil-based counterparts to produce biofuels, biochemicals and biomaterials.

Despite numerous studies on the valorisation of biomass, there is a lack of inclusive review on designing and innovating new processes to extract useful compounds for the production of sustainable biochemicals and biomaterials. Thus, in the present review, the focus is on exploring the recent progress, achievements, and multitude of opportunities to valorise biowastes from agriculture, aquaculture, and shellfish processing sectors into value-added bio-based chemicals and materials for various applications in different economic sectors. Further, the ongoing and future challenges towards a sustainable bioeconomy are discussed, as well as the anticipated impacts of its implementation, from both social and environmental perspectives.

2. Conversion of biomass into biochemicals

Table 1 summarises the conversion of biomass into biochemicals via various valorisation techniques. Biochemicals can be produced from biopolymers derived from biomass materials. For example, pectin can be extracted from fruit waste for use in the pharmaceutical and cosmetic industries. Agricultural residues are made of hemicellulose, cellulose and lignin. Hemicellulose can be synthesised into furfural, cellulose can be converted into sugar alcohols or sorbitol, whilst lignin can be converted into vanillin. Shellfish and aquaculture wastes consist of bioactive compounds such as chitin, chitosan and protein that can be used in many applications.

2.1. Biochemicals from biopolymers

2.1.1. Food wastes

The agro-industry generates large quantities of food waste such as skin, damaged fruits and vegetables (e.g., citrus peel, banana peel, potato, apple pomace), seed wastes (e.g., grape, carob, pumpkin, date, mango), coffee waste, husk and nuts shell. These food wastes contain a considerable amount of organic matter, such as proteins, fatty acids, phenolic compounds, polysaccharides and dietary fibers (Di Donato et al., 2014). In general, global per capita food waste increased from 287 kcal/cap/day in 1992 to 473 kcal/cap/day in 2013 and is projected to increase to 812 kcal/cap/day in 2050 (Barrera and Hertel, 2021). This translates to approximately 1.6 billion tonnes of food wastage, of which the edible portion accounted for 1.3 billion tonnes (FAO, 2013). Several million metric tons of citrus (3.4 Million tons) and apple (89.3 million tons) are produced each year (Wang et al., 2014). Approximately 10–20% of these fruits are used to produce juice at industrial-scale, hence generating approximately 25–50% of waste containing 10–25% of pectin (Wang et al., 2014). Likewise, banana peel waste (10.9 million tons) and coffee husk compose 21.7 and 12.4% of pectin, respectively (Sanchez-Vazquez et al., 2013). Pectin has a high economic value with 1 billion USD of the market size in 2019 and is projected to reach 1.5 billion USD in 2025 (Petkowicz and Williams, 2020).

Pectin is a natural polysaccharide constituted by repeated homogalacturonans region (HGs) of α-(1–4)-D-GalA*p* and heteropolymeric region of rhamnogalacturonans (RGs) and arabinogalactans. Depending on the degree of methyl esterification (DM), HGs can be divided into two groups low methyl-esterified HGs (DM <50%) or high methyl-esterified HGs (DM >50%) (Müller-Maatsch et al., 2016). Pectin has several industrial applications in the pharmaceutical, food and cosmetic field (Minzanova et al., 2018). It is an important ingredient with interesting functional proprieties such as being a stabilizer, emulsifier, thickener and gelling agent (Mellinas et al., 2020).

Pectin modification could be an interesting way to obtain derivatives with new functional properties. Several methods have been used for obtaining pectin derivatives such as chemical substitution (e.g., oxidation, thiolation, quaternization, amidation, sulfation, alkylation, etc.),

grafting and cross-linking (Chen et al., 2015). These modifications are able to enhance the water solubility gel strength, emulsifying proprieties, or antibacterial activity of pectin (Liu et al., 2017; Ciriminna et al., 2020). In addition, the depolymerisation of pectin using chemical, physical, or enzymatic degradation has also been used to obtain pectin oligosaccharides with enhanced biological activities (Ogutu and Mu, 2017). In fact, pectic-oligosaccharides degraded with free radical depolymerisation or ultrasonication showed enhanced antioxidant, antiglycation and prebiotic proprieties, which can be used as a biomaterial for tissue and bone engineering (Chaouch et al., 2015; Gómez et al., 2016).

2.1.2. Agricultural residues

The valorisation of agricultural residues becomes an interesting trend owing to the existence of bioactive compounds that allow the development of several industrial sectors (Ullah et al., 2015; Bhuyan et al., 2020). Agricultural residues generated during cultivation, harvesting, and post-harvesting from different crops such as maize, rice, and wheat contain a high amount of lignocellulosic polymers *viz.* cellulose, hemicellulose, and lignin. An estimated 3287 Mt of agricultural residue (fresh weight) of the primary global crops was produced annually in several countries or regions, including Argentina, Canada, Brazil, China, India, United States of America, and EU27 (Tripathi et al., 2019). Among the various crops, cereals remain the primary contributor to global agricultural residue production (Centore et al., 2014).

Cellulose is the most plentiful polymer worldwide. It consists of glucan chains linked by β-1,4-glycosidic bonds with different degrees of polymerization (DP). Cellulose possesses a high economic value with a global market size of 211.6 billion USD in 2019 and is projected to reach 235 billion USD in 2026 (Trache et al., 2017). Cellulose and its derivatives have a large, wide spectrum of functions in various fields (Li et al., 2018). This biopolymer demonstrates versatility in many industrial applications such as textile, cosmetic, medical, and pharmaceutical. For instance, cellulose nanocrystalline mixed with other polymers have been used to prepare edible films for food application (Trache et al., 2017), whilst cellulose nanofibers have been used to prepare aerogel for biomedical applications (Shaghaleh et al., 2018; Abdul Khalil et al., 2020). Likewise, sorbitol or sugar alcohols could be produced via chemo-catalytic transformations of glucose obtained from cellulose (Li et al., 2013).

Lignin is the second most abundant polymer in the world, which contains phenolic polymer with randomly cross-linked C_9 units (Wang et al., 2018). It has been reported that lignin from biomass such as wood and sugar beet pulp can be synthesised into vanillin (Aarabi et al., 2017). Vanillin is usually extracted from vanilla beans or petrochemical materials (e.g., guaiacol) to be applied as a flavouring additive in the cosmetics and food industries (Wang et al., 2018). Nevertheless, the demand for vanillin has surpassed the supply of vanilla beans, whilst the cost of petrochemical products has been increasing due to high competition with other products (e.g., plastics, solvents, drugs). Lignin can be extracted from various types of biomasses and converted into vanillin via various recovery methods such as chemical oxidation and enzymatic hydrolysis, thus making it a sustainable resource to produce vanillin.

In contrast to cellulose, mainly composed of glucose, hemicellulose is a heteropolysaccharide that contains xylose, glucose, galactose, mannose, arabinose, and galacturonic acid (Machmudah et al., 2017). Hemicellulose can be synthesised into furfural that can be used as chemicals in the agrochemicals and pharmaceutical industries (Luo et al., 2019). It has been reported that global production of furfural in 2019 was 551 million USD, and it is estimated to value more than 700 million USD by 2024 (Montaña et al., 2020). There are various valorisation technologies such as pyrolysis, solvolysis and hydrolysis to convert hemicellulose into furfural. Hui et al. (2019) reported that hydrolysis of hemicellulose (derived from corn cob) using superacid SO_4H-functionalized ionic liquids improved the yield of furfural up to 95%, as compared to conventional solvents such as toluene (67%) and acetone (44%). In another study, Fan et al. (2019) investigated the pyrolysis of corn cob using sulphuric acid as a catalyst. Nevertheless, the yield of furfural (19 wt%) was lower than the hydrolysis of corn cob, as reported by Hui et al. (2019).

2.1.3. Shellfish and aquaculture wastes

In 2018, total global capture fisheries and aquaculture productions were 96.4 and 114.5 million tons, respectively, with a total farm gate sale value of 263.6 billion USD for aquaculture production (FAO, 2018).

Table 1
Conversion of biomasses into biochemicals via various valorisation technologies.

Source of biomass	Valorisation technologies	Product	Yield of product	Remarks	Refences
Citrus	Alkylation	Pectin	–	Alkylation improves the molecular characterization, conformation and gel properties of pectin.	Liu et al. (2017)
Sweet potato	Ultrasonic treatment	Pectin	–	Sonication improves galacturonic acid content and the antioxidant activity of pectin.	Ogutu and Mu (2017)
Cellulose	Simultaneous hydrolysis and hydrogenation	Sorbitol	95%	The yield of sorbitol is influenced by the reaction condition such as temperature, catalyst, the composition of molten salt hydrate and hydrogen partial pressure.	Li et al. (2013)
Tomato peels	Acid hydrolysis	Cellulose	10–13%	Acidified sodium chlorite/potassium hydroxide removed lignin and hemicellulose from tomato peels to produce a higher yield of cellulose (13%) as compared to chlorine-free sodium hydroxide/hydrogen peroxide, which produce 10–11% of cellulose.	Jiang and Hsieh (2015)
Wood	Alkaline nitrobenzene oxidation	Vanillin	12–13%	The interunit linkages (β-*O*-4) in lignin highly influence the yield of vanillin.	Wang et al. (2018)
Sugar beet pulp	Lignin oxidation	Vanillin	–	Vanillin yield is greatly influenced by oxygen pressure, temperature, reaction time, and concentration of catalyst.	Aarabi et al. (2017)
Corn cob	Hydrolysis using superacid SO_4H-functionalized ionic liquids	Furfural	95%	Strong acid strength improves the conversion of hemicellulose into furfural.	Hui et al. (2019)
Corn cob	Pyrolysis using sulphuric acid as catalyst	Furfural	19 wt%	Sulphuric acid can be used as a catalyst to enhance dehydration of hemicellulose to increase the yield of furfural.	Fan et al. (2019)
Shrimp shell	Classical deacetylated chitosan (CDC) and ultrasound-assisted deacetylated chitosan (UDC)	Chitosan	17%	UDC is more effective in producing chitosan with a high degree of deacetylation and better antioxidant activity compared to CDC. The chitosan shows potential to be used as an antimicrobial agent.	Hafsa et al. (2016)
Litchi fruit pericarp (LFP)	Extraction	Phenolic	0.17 mg/g	LFP contains high total phenolic content, which is comparable to synthetic antioxidants such as butylated hydroxyl toluene (BHT). LFP extract shows the potential to be used as antioxidants and food additives.	Das et al. (2016)

(–) data are not available.

Approximately 25% of shellfish part is edible, while the remaining inedible parts (i.e., heads, shells, skeletons) lead to the inevitable accumulation of shellfish waste (Özogul et al., 2019). Interestingly, many studies have reported the useful bioactive compounds (e.g., chitin, astaxanthin, protein) that can be extracted from shellfish waste (Vázquez et al., 2013; Özogul et al., 2019). Instead of discarding the shellfish waste in the ocean, landfill or incinerator, several techniques are developed to transform shellfish waste into biologically active polysaccharides that can be utilised in many applications.

Chitosan is a linear polysaccharide and the main component of shellfish shells. It consists of D-glucosamine chains linked by β-1,4-glycosidic bonds and also a natural biopolymer produced by deacetylating chitin in an alkali solution (Younes and Rinaudo, 2015). Chitosan and its derivatives have been exploited for many years by the industry due to their abundance, renewable sources, biodegradability and non-toxicity (Özogul et al., 2019). Likewise, chitosan is widely used in food to make edible and biodegradable films and in the preservation of food against microbial deterioration due to its antimicrobial property (Hafsa et al., 2016, 2021). In the recent decade, chitosan derivatives obtained through chemical modification (i.e., acylation, carboxylation, thiolation, phosphorylation, quaternization) and cross-linking have been widely used in several fields such as pharmaceuticals and wastewater treatment (Negm et al., 2020). In fact, chitosan derivatives exhibited enhanced new features such as reactivity and water solubility. Furthermore, they are investigated in several fields such as food quality, wound healing, drug delivery and tissue engineering (Zhou et al., 2021). In addition, the depolymerisation of chitosan using chemical, physical, or enzymatic degradation have been used to obtain chitosan oligosaccharides with enhanced antimicrobial activity, immunostimulant and anticancer properties (Zou et al., 2016).

2.2. Biomass-derived phenolic compounds

Phenolic compounds are a class of secondary metabolites implicated in several biological processes during all plant growth and development (Ben Mrid et al., 2021). Phenolic compounds comprise phenolic acids, flavonoids, coumarins, lignans and tannins, that are naturally found in the different parts of the plants and fruits. The wide range of these chemical compounds confers to their different activities and potential applications in the pharmaceutical and food processing industries (Albuquerque et al., 2021).

2.2.1. Application of phenolic compounds in food processing industry

In the food processing sector, phenolic compounds are mainly used for food preservation as a food additive and/or active packaging (Das et al., 2016; Zeng et al., 2019). In fact, the potential benefit of phenolic compounds as bio-preservatives lies in their ability to extend the shelf life of perishable products with the ability to delay or prevent oxidation and microorganism's growth (Bouarab Chibane et al., 2019). These phenolic compounds could be generated by the food industry itself. Indeed, in the food industry, the fruit processing industry generates an enormous quantity of wastes (e.g., peels, seeds, pulp); thus, the large amounts of phenolic compounds that are present in these wastes could constitute natural sources of antioxidants and could therefore be used for food preservation.

Indeed, different hydroxybenzoic and hydroxycinnamic acids, flavonoids, and hydrolysable tannins were reported to be highly present in various fruit wastes such as citrus, apple, mango, and pomegranates (Kessy et al., 2018). The antioxidant ability of these groups of natural molecules has been proven in multiple studies and attempts to apply these molecules have already been started. In this regard, phenolic compounds can be extracted from Litchi pericarp (*Litchi chinensis* Sonn), which showed potent inhibition of the lipid peroxidation in sheep meat nuggets. This inhibition was similar to the synthetic antioxidant butylated hydroxyl toluene (BHT) (Kessy et al., 2018).

In another study, Caleja et al. (2016) measured the antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay in yogurt fortified by phenolic compounds from *Foeniculum vulgare* Mill. In fact, the plant extract showed an EC50 of 94 mg mL^{-1} compared to the potassium sorbate that showed an EC50 of 111 mg mL^{-1} (Caleja et al., 2016). Phenolic compounds have also been used to increase the functional properties of food. For instance, *C. sinensis* phenolic compounds have enhanced the bioactive profile of bread and cheese (Rashidinejad et al., 2014; Pasrija et al., 2015).

2.2.2. Application of phenolic compounds in pharmaceutical field

Due to their antioxidant capacities, phenolic compounds from agricultural food wastes have generated a lot of interest in the pharmaceutical field as well. For instance, phenolic compounds from citrus wastes were evaluated for their antiglycation activity as well as their ability to inhibit digestive enzymes such as lipase, α-glucosidase, and α-amylase. The polyphenol fraction showed high inhibition of the AGEs and potent inhibitory activity against pancreatic lipase and α-glucosidase (Fernandes et al., 2020). In another study, consumption of 800 mg/day of resveratrol increased the blood antioxidant capacity and decreased blood pressure in diabetic subjects (Seyyedebrahimi et al., 2018).

In a recent study, phenolic compounds have been found to minimise the risk of aging-related diseases, including metabolic syndromes (e.g., diabetes, obesity) and neurodegenerative diseases (e.g., Huntington's disease) (Arruda et al., 2020). Phenolic compounds such as rutin and curcumin can improve motor and memory performance by modulating signalling pathways engaged in oxidative damage, inflammation and autophagy, such as reducing neurons degeneration and oxidative stress. Ferulic acid, gallic acid and curcumin can be used to prevent obesity by reducing adipogenesis and adipose inflammation, which results in the reduction of fat accumulation and body weight (Luna-Vital et al., 2020). Nevertheless, the antagonistic and synergistic effects of both phenolic compounds and other food components (e.g., carbohydrates, proteins) should be further investigated to improve the understanding and stability of phenolic compounds as a dietary supplement.

3. Conversion of biomass into biomaterials

Other than biochemicals, biomass can be treated via various biological, chemical and thermochemical techniques to produce biomaterials. Biomass can be used to replace fossil-based materials in manufacturing various types of products. This includes the production of bioplastic from biopolymers derived from biomass sources to replace conventional plastics. The production of biochar from biomass using emerging thermochemical technologies has gained significant attention in order to replace the constant use of fossil-based products such as coal and tar, which is discussed in the subsequent section.

3.1. Bioplastics

Plastics are essentially organic materials made up of polymers. Their plasticity and other desirable physical properties such as lightweight, durable, low density and inexpensive make plastics the preferred material for use in a wide range of applications across various industries (Bagheri et al., 2017; Narancic et al., 2020). However, the recycling process of plastics, especially the conventional petroleum-based plastics, is difficult due to the complications with various mixtures of plastic types (e.g., differences in processing conditions, not compatible due to immiscibility with different types of plastics) and the reduction in quality after the recycling process of reheating (Hopewell et al., 2009; Gutowski et al., 2013). To date, approximately 90% of plastics are still produced from fossil feedstocks, and their production accounts for 4–8% of global oil consumption (Narancic et al., 2020). Their prevalent usage in almost every industry, coupled with the extremely slow degradation rate (e.g., 10–20 years or 500–1000 years) of conventional plastics in environmental conditions (Ward et al., 2019; Chamas et al., 2020), have led to serious environmental problems, including the formation of

meso-, micro-and nanoplastics that could accumulate in all ecosystems and are biologically hazardous to almost all trophic levels of the food chain (Mattsson et al., 2015; Botterell et al., 2019; Wang et al., 2021).

Unlike conventional plastics, bioplastics are bio-based plastics, i.e., made up of renewable sources such as biomass by the action of living organisms, with or without biodegradable characteristics (Batori et al., 2018). Similar to their petroleum-based counterparts, bioplastics are recyclable or incinerable. Although bioplastics can potentially serve as better options due to their lesser greenhouse gas emission, reduction in the reliance on fossil fuels, reclamation of by-products, and the diversification of local resources, the production of bioplastics remain low (approximately 1% of the global plastic production) (Narancic et al., 2020; Coppola et al., 2021). This is due to the high production cost of bioplastic, such as the expensive energy source used for microbial fermentation (Wan Mahari et al., 2022a). In addition, a large amount of raw materials (e.g., carbon sources, chemicals, microorganisms) are needed to generate a high yield of bioplastics. Contrary to conventional plastic production that depends solely on the chemical processing of fossil fuel, the source and production of bioplastics are diverse.

3.1.1. Bioplastics production from biomass extraction

3.1.1.1. Polysaccharide-based. Bioplastics produced from biological treatments are made up of agro-polymers, either plant-, animal- or

Table 2
Sources and method of bioplastic synthesis.

Source of bioplastics	Type of bioplastics	Method of bioplastic synthesis	Main findings	References
1. Polysaccharide-based	Starch-based Thermoplastic starch Starch-based film	- Cross-linking, esterification, pregelatinization - Nano-SiO_2 combined with potato starch film	- Starch-based polymer is abundantly available and cheap but needs a plasticiser and water to be used as a deformable thermoplastic material.	(Khan et al., 2017; Zhang et al., 2018)
	Cellulose-based Cellulose aleuritate Cellulose acetate Cellulose-based films	- Acylation via a mixed anhydride system - Acetylation process - Delignified banana stem fibers via an ionic liquid	- Cellulose-based polymer is less affected by acids compared to polystyrene and polypropylene. - Suitable to be applied in the pharmaceutical and food industries.	(Heredia-Guerrero et al., 2017; Mostafa et al., 2018; Ai et al., 2021)
	Carrageenan-based Carrageenan-based film	- Polymer casting - Plasticiser	- Carrageenan-based film derived from seaweed exhibits excellent physical and mechanical properties that are desirable to be used as bioplastic film, especially in non-food and food packaging. - Further studies are needed to evaluate the commercial potential and economic feasibility of this bioplastic film.	(Sudhakar et al., 2020)
	Alginate-based Alginate-based film	- Solvent casting or extrusion technique	- Alginate-based film can inhibit the growth of microorganisms, reduce the evaporation of water, and improve the shelf life of food products. - Further studies are needed to upscale and commercialise the bioplastic film.	(Senturk Parreidt et al., 2018)
	Chitin and Chitosan Crab Shells (*Portunus pelagicus*)	- Solvent casting	- Chitin-based film shows greater ultimate tensile strength compared to the commercial plastic film. - Further studies are needed to optimise the extraction and synthesis process for commercialisation.	(Fernando et al., 2016)
2. Protein- and lipid-based	Protein-based Fish gelatin (animal) Zein (plant) Kafirin (plant) Wheat gluten (plant)	- Plasticised, casting, mixing, extrusion. - Zein-based films blended with oleic acid and xanthan gum - Extraction, physical and chemical modification	- Nano-curcumin fish gelatin film has better mechanical properties and shelf life compared to fish gelatin film. - Zein-based films blended with oleic acid and xanthan gum have higher water solubility, opacity and tensile strength compared to zein-oleic acid film. - Kafirin-derived film possesses desirable bioplastic properties but needs to compete with the other products produced from sorghum, especially food.	(Serna and Filho, 2015; Taylor and Taylor, 2018; Matche et al., 2020)
	Lipid-based Castor oil Soybean oil	- Chemical treatment - Organoclay nanocomposites, epoxidation	- Maleated castor oil foams are cost-effective, smoother and stronger with comparable compressive stress at 25% strain as commercial polyurethane foams. - Incorporation of organoclay nanocomposite on the epoxidised soybean oil has improved the mechanical strength properties (e,g. tensile toughness, tensile strength) of the bioplastics.	(Wang et al., 2008; Tanrattanakul and Saithai, 2009)
3. Microbe-origin	Pullulan (fungus) FucoPol (bacteria) Polyhydroxyalkanoate (PHA)	- Enzymatic hydrolysis and fermentation	- Pullulan has distinctive functional features (e.g., inhibit bacteria growth, extend shelf-life) and is proclaimed as safe for use in food packaging. Future studies are needed to apply pullulan on the food market and industrial scale. - FucoPol possesses flocculating and emulsion stabilising capacity and membrane forming capacity, which can be applied in a multilayer packaging material. - PHA has tissue biocompatibility in animals and humans, thus can be used in the medical industry. PHA can replace conventional plastic due to its biodegradability, but it requires a high cost for raw materials during microbial fermentation.	(Ferreira et al., 2014; Singh et al., 2019)
4. Petrochemical-based	Polybutyrate adipate terephthalate (PBAT) Polybutylene succinate (PBS)	- Polyester manufacturing technology - Copolymerisation	- PBAT is produced from polycondensation of adipic acid, butanediol and terephthalic acid. It is biodegradable and can be used to replace fossil-based plastic. - PBS has good thermo-mechanical properties and is biodegradable. The application of PBS in the biomedicine industry is attracting attention.	(Gigli et al., 2016; Jian et al., 2020)

microbe-based (Table 2). Being regarded as the most abundant macromolecules in the biosphere, polysaccharides are complex carbohydrates that support the structural constituent of plants and animals or serve as energy storage material (Ferreira et al., 2016). In addition to polysaccharides, proteins and lipids of plants such as soy and gluten and animals such as casein, whey and collagen are also being harnessed as raw materials for bioplastic production (Felix et al., 2017).

Starch-based bioplastics derived from plants are preferred and consist of 20% of the global bioplastic production due to their abundance and stable thermoplastic behaviour (Ferreira et al., 2016; Jiang et al., 2020). They are easily available as starch can be obtained easily from common agricultural crops and various plant parts, including cereals (Xu et al., 2010; Marichelvam et al., 2019), grains and nuts (Santana et al., 2018; Tsang et al., 2019). Starch granules are made up of amylose (linear microstructure) and amylopectin (branched microstructure), both of which depend on amylose/amylopectin ratio, granule size, plant source and other physicochemical properties such as pH (van Soest and Essers, 1997; Seung, 2020). These influential properties, in turn, determine the quality of the bioplastics produced.

The general concept of converting starch into bioplastic film involves two essential steps of thermal treatment: starch gelatinisation to destroy the starch granules' crystalline structures via heated water and solution casting or annealing to allow the re-crystallisation or retrogradation of the gelatinised starch (Seung, 2020). One of the most common techniques used during gelatinisation is extrusion. The extrusion process involves melting and solidification. When water temperature above the gelatinisation temperature is applied, hydrogen bonding within starch granules would be disrupted, allowing the integration of water molecules with starch molecules and consequently resulting in the swelling and dissolution of crystallites (Lim et al., 2000). The amylose/amylopectin ratio influences the mechanical properties of the bioplastics produced, with the amylose content positively correlating with tensile strength, whereas higher amylopectin content corresponds to higher strain (Tanetrungroj and Prachayawarakorn, 2015). As most starch-based biofilms have inadequate mechanical properties (e.g., hard and brittle), glycerol is often added to retard the swelling and gelatinisation of starch to achieve bioplastic with high mechanical stability (Santana et al., 2018).

Being the main constituent of plant cell walls, cellulose is earth's most abundant organic polymer and is composed of linear β-D-glucose units. The regular structure and abundance of hydroxyl groups in cellulose result in strong hydrogen-bonded crystalline fibers with high mechanical strength. Coupled with other characteristics such as low cost, durability, biocompatibility, chemical stability and renewability, cellulose is a prime candidate for producing bioplastic (Wang et al., 2013). Commonly used raw material source for bioplastic production includes pulps, sugarcane bagasse fibre, cocoa pod husk and wood fibre (Wang et al., 2013; Azmin et al., 2020; Kamau-Devers and Miller, 2020). Although there are various commercialised derivatives, common industrial cellulosic materials include cellulose acetate, cellulose esters and regenerated cellulose. Cellulose-based bioplastic is commonly produced using thermo-chemical treatment methods. Plant ingredient needs to be delignified using sodium hydroxide and purified using sodium hypochlorite during cellulose extraction. Subsequent gelatinisation could involve the addition and heating of starch to allow thorough mixing before air dying on casts. Often, in addition to chemical modification to improve the thermoplastic properties of cellulose, the inclusion of plasticisers and blending with other polymers are incorporated to alter and enhance the mechanical and chemical properties of the final cellulose product (Ferreira et al., 2016). By incorporating hot-pressing to the cellulose hydrogel, Wang et al. (2013) developed a new class of cellulose bioplastic with superior tensile and flexural characteristics, good thermal stability and exhibited low thermal expansion in comparison with conventional plastics and regenerated cellulose biofilms (Wang et al., 2013).

Carrageenan is a linear polysaccharide obtained from red seaweeds (family Rhodophyceae). It is widely used as a food thickener and emulsifier and is often found in meat products and yogurt (Mena-Casanova and Totosaus, 2011). Due to their good mechanical properties and edible characteristic, carrageenan is commonly used to produce edible biofilms and coatings (Bico et al., 2009). The addition of plasticiser polyethylene glycol (PEG) was shown to enhance the tensile strength of carrageenan-based biofilm (Sudhakar et al., 2020). Alginate is another natural polysaccharide derived from algae, specifically brown seaweeds (e.g., genura *Laminaria* and *Ascophyllum*). In addition, alginate can be produced by two bacterial genera, i.e., *Pseudomonas* and *Azotobacter* (Hay et al., 2013). Alginate is soluble in water and suitable for the production of biofilm due to characteristics such as non-toxic, biodegradable, biocompatible and low cost. The linear structure found in alginate entails a strong membrane structure of alginate-based biofilms. On top of that, as a polyuronide, alginate is a natural ion exchanger and, in its charged state, could lead to gel formation (Senturk Parreidt et al., 2018). The process of converting carrageenan into bioplastic is straightforward; carrageenan and alginate are subjected to thermal treatment for proper mixing, followed by the solvent casting method. Various additives, such as plasticisers (El Miri et al., 2018), surfactants (Albadran et al., 2018), antimicrobials (Raybaudi-Massilia et al., 2008), antioxidant and antibrowning agents (Robles-Sánchez et al., 2013), and nutritional additives (Bazargani-Gilani, 2018) have been tested and incorporated onto alginate-based coatings or biofilms to enhance their functionality.

Being the primary element that provides structural support in the exoskeleton of arthropod crustaceans, chitin is now being harvested from shell waste of crustaceans (e.g., shrimps, prawns and crabs), aquaculture, and fishery. Although chitin only makes up approximately 20% of the exoskeletal wastes, the global annual production of chitin was estimated to be at 362,000,000 MT (Fernando et al., 2016). In general, chitin is easily extracted using either biological or chemical extraction. Both extraction methods isolate, demineralise and deproteinise chitin. However, the chemical extraction method is known to produce chitin of higher purity (Younes and Rinaudo, 2015; Fernando et al., 2016). Following chitin extraction, polymer film formation is done by mixing chitin with dissolution solvent and moulded into film via cold-pressing (Fernando et al., 2016). An important derivative of chitin that is also used in the production of bioplastic is chitosan; a polysaccharide derived from the partial deacetylation (about 50%) of chitin (Shamshina et al., 2020). Similar to chitin, chitosan biofilms are also biodegradable, edible and renewable (Fernandez and Ingber, 2014). Owing to its soluble properties, chitosan is used in various forms, including solutions, gels, films and fibres. Chitosan film production is relatively straightforward – purification (dissolve in acid and filter through membranes), chemical adjustment (alter pH to about 7.5), washing and drying (Rinaudo, 2006).

3.1.1.2. Protein- and lipid-based. In addition to plant-based sources, another potential source of bioplastic production is protein and lipid obtainable from livestock and biomass (e.g., agricultural residue). One such example is the extraction of casein and whey proteins from cheese production and expired dairy products (Wagh et al., 2014). Casein and whey proteins exhibit superior film-forming characteristics, including flavourless, elastic, transparent, high nutritional value, and have been used to produce food packaging material (Wagh et al., 2014; Chalermthai et al., 2019). The process of biofilm production from casein and whey involves denaturation in heated aqueous solution (above 75 °C), adjusting pH to 5.6 using NaOH, adding plasticizer (*e.g.,* glycerol and sorbitol) and final casting (Wagh et al., 2014).

Another potential renewable and easily available biopolymer source is collagen and gelatin. The main source of collagen and gelatin is from the leather industry and with minimal contribution from the animal slaughtering and processing industry (Matche et al., 2020). Leather solid wastes, specifically during fleshing and shaving phases, contain raw

collagen that could be synthesised into collagen hydrolysate (CH) after enzymatic hydrolysis (Haroun, 2010). Haroun (2010) successfully developed a biodegradable thermoplastic film by blending modified polyethylene (MPE) with CH (Haroun, 2010). The author also reported the production of biopolymer with 20% of CH that can achieve 63% biodegradation in 24 days. Gelatin is produced from chemically treated collagen, and its properties are influenced by the features of input collagen and the extraction parameters (Nur Hanani et al., 2014; Ramos et al., 2016). Although gelatin is widely used in food, packaging, pharmaceutical and photographic industries, it is still limited by the low thermal stability and mechanical properties (Ramos et al., 2016). Matche et al. (2020) recently developed biodegradable films from fish gelatin for the packaging of fish fillets (Matche et al., 2020). The addition of carrageenan and laminarin improved the properties, whereas the incorporation of curcumin added antimicrobial features to the developed film (Matche et al., 2020). Compared to other biopolymers, gelatin is superior in terms of exhibiting excellent oxygen barrier and heat sealability (Nur Hanani et al., 2014).

Zein, kafirin and gluten are proteins found in maize, sorghum and wheat, respectively. Gluten has high thermoplastic and viscoelastic properties and is capable of withstanding various chemical modifications. However, its gliadin and glutenin proteins may confer celiac toxicity and wheat allergies to some people, thus rendering it unsuitable to be used in food-related and biomedical sectors (Taylor et al., 2013). There are limited records of allergic cases with zein, but kafirin is an excellent nontoxic choice for celiac sufferers (Pontieri et al., 2013). Chemical treatment or thermo-mechanical treatments can be used in the production of gluten films. Wheat gluten can be either mixed with acetic acid, Na_2SO_4 and glycerol before spreading and drying at 60 °C, or mechanically mixed with glycerol using a mortar and subsequently subjected the mixture to heating press at 150 bar (Domenek et al., 2004). Zein is normally extracted using chemicals such as 70% ethanol, whereas kafirin is derived using thermal (warm water) and the addition of a reducing agent (Schober et al., 2011). Serna and Filho (2015) developed a zein-oleic acid blend film with greater water solubility and opacity by adding xanthan gum during the mechanical mixing process (Serna and Filho, 2015).

Apart from being protein-based, bioplastic could also be made from lipids (triglycerides). Due to their economic values, soybean oil, castor oil and linseed oil are readily available. Soybean oil was epoxidised with acetic acid and hydrogen peroxide before transforming into bioplastic via curing process using methyl-tetrahydrophthalic anhydride and 1-methylimidazole (Tanrattanakul and Saithai, 2009). Castor oil needs to be maleated using maleic anhydride before being synthesised into plastic sheets via copolymerisation reaction that is initiated by styrene (Wang et al., 2008). Vaicekauskaite et al. (2019) developed cross-linked polymer composites using epoxidised linseed oil and 1-hydroxyethane-1,1-diphosphonic acid, with organic industrial wastes (e.g., pine bark, grain and weeds) as fillers (Vaicekauskaite et al., 2019). Low-temperature curing (20–25 °C) is sufficient in forming the composite films.

3.1.2. Bioplastics synthesised from microorganisms

Several types of microorganisms, including yeast, fungus and bacteria, are able to produce polysaccharides that can be synthesised into biofilms (Ferreira et al., 2016) (Table 2). Pullulan is synthesised via the fermentation process of liquefied starch under specific conditions using non-genetically modified, non-toxigenic and non-pathogenic *Aureobasidium pullulans*, a type of black yeast-like fungus (Prajapati et al., 2013). In addition to being biodegradable and impermeable to oxygen, pullulan has great mechanical strength, is highly-water soluble and is not easily digested by our guts' digestive enzymes, making it a preferred choice in the pharmaceutical and food industries (Singh et al., 2019). Various carbon sources, such as starch, soybean oil, beet molasses and other agro-industrial waste, could be used as the fermentation target for the production of pullulan (Cheng et al., 2011; Prajapati et al., 2013). Pullulan has been commercially produced via fermentation, and optimal yield (more than 70%) of pullulan is expected within 100 h. The recovery process of pullulan includes removal of *A. pullulans* via filtration, removal of melanin via activated charcoal treatment, and subsequent precipitation and purification using organic solvents (Singh et al., 2019).

Xanthan gum is produced similarly to pullulan via fermentation process by bacteria such as *Xanthomonas campestris* (Palaniraj and Jayaraman, 2011). On the industrial scale, xanthan can be produced via batch or continuous operation using a wide variety of substrates and nutrients. The production of xanthan is relatively easy to manipulate and carried out as *X. campestris* grows optimally under 28–30 °C and neutral pH (Gumus et al., 2010; Palaniraj and Jayaraman, 2011). FucoPol is a microbial polysaccharide produced by *Enterobacter* A47. Unlike pullulan and xanthan, the production of FucoPol relies on using glycerol as its carbon source, and a yield of 7.8 g/l was reported after 4-day production using bioreactor (Ferreira et al., 2014)

Another important biopolymer produced by microorganisms is polyhydroxyalkanoates (PHAs) that can be synthesised by a wide variety of bacteria (Anjum et al., 2016). PHAs are gaining attention due to their resemblance to commonly used petrochemical polymers, i.e., polypropylene and polystyrene (Sudesh et al., 2000). PHAs can be synthesised via the fermentation process, and their characteristics are dependent on carbon source, choice of bacteria, and fermentation conditions (Khatami et al., 2021). Bacteria hosts involved in PHA synthesis can be divided into two types – those that require excess carbon source and stress conditions (e.g., *Pseudomonas oleovorans*), and those that do not need to be subjected to nutrient starvation (e.g., *Azotobacter vinelandii* and *Escherichia coli*).

3.1.3. Bioplastics synthesised from petrochemicals

To address the issue of biodegradability, researchers have also dwelled on the potential of producing biodegradable products of petrochemical origin. Among them, aliphatic-aromatic co-polyesters, particularly the Polybutyrate adipate terephthalate (PBAT), show great biodegradable ability due to their soft-chain ester bonds that are sensitive to hydrolysis (Mochizuki and Hirami, 1997). PBAT is not only biodegradable but, owing to the presence of aromatic unit in its molecule chain, also exhibits strong mechanical properties such as flexibility, good thermal stability and moderate crystallinity (Cranston et al., 2003; Jian et al., 2020). PBAT is produced via the poly-condensation process (pre-mixing, pre-polymerisation, and final-polymerisation) of three essential ingredients, namely butanediol (BDO), adipic acid (AA) and terephthalic acid (PTA). During the poly-condensation process, which often involves high vacuum and temperature (>190 °C), and a long reaction period, zinc-, tin-, and titanium-based organometallic elements may be used as catalysts. An in-depth review of the commercially available PBAT was reported by Jian et al. (2020) (Jian et al., 2020). In an attempt to further reduce the reliance on fossil fuel, bio-based BDO produced via biological fermentation is being tested as a replacement to petrochemical-derived BDO (De Bari et al., 2020). Similarly, sebacic acid derived from Castrol oil is also in the trial as a potential substitute for AA (Jian et al., 2020).

Similar to PBAT, Polybutylene succinate (PBS) is an aliphatic polyester and is well known for its thermal stability, great mechanical properties, biodegradability and acceptable production costs. Since 1993, PBS has been commercially available in the form of biodegradable mulching films, bags, textiles and foams (Xu and Guo, 2010; Gigli et al., 2016). The production of PBS involves two fossil-based monomers, succinic acid (SA) and BDO. The production process of PBS is similar to that of PBAT, involving polycondensation that can be further detailed out in two stages, esterification process and the removal of either water or methanol, and subsequent removal of BDO at high temperature and reduced pressure to yield PBS of high molecular weight (Gigli et al., 2012). Currently, increasing studies have shown that SA and BDO can be produced via fermentation, and an acceptable yield has been obtained (Bechthold et al., 2008; Forte et al., 2016). This implies that full

bio-based PBAT and PBS are achievable and would reduce dependency on petrochemical (fossil fuel) in the near future.

3.2. Biochar

Biochar is a carbonaceous material produced from biomass *via* thermochemical conversion technologies (e.g., pyrolysis, gasification, torrefaction). The biochar is categorised as biomaterials due to its origin from biomass and desirable properties that shows potential to be used in multi applications such as absorbent, catalyst, solid fuel, and fertiliser (Wan Mahari et al., 2020b; Ren et al., 2021; Yan et al., 2022).

Table 3 summarises the recent studies on biochar recovery via thermochemical conversion technologies of biomass materials. The thermochemical technologies are distinguished based on the operating temperatures, heating source, reaction condition (purged by N_2, O_2, or CO_2). Pyrolysis is an emerging thermochemical technology that decomposes biomass under an inert environment in a temperature range of 300–900 °C to convert biomass into value-added biochar (Parvez et al., 2019; Wan Mahari et al., 2020a). Pyrolysis can be classified into conventional and advanced pyrolysis techniques. In conventional pyrolysis, the furnace is commonly used as the heating source, while the heating rate of the pyrolysis process determines the type of pyrolysis process, which can be slow, fast or flash pyrolysis (Azwar et al., 2022). In advanced pyrolysis techniques, modifications are performed to the pyrolysis system to improve the thermal cracking performance and quality of the biochar. For example, pyrolysis is incorporated with microwave

Table 3
Previous studies on biochar production via various valorisation technologies.

Source of biomass	Valorisation technologies	Yield of biochar	Properties of biochar	Main findings	References
Coffee husk briquettes	Slow pyrolysis	35–40%	C: 70–74%, H: 2–4%, N: 3–4%, O: 20–23%, S: 0.1–0.2% Calorific value: 27 MJ/kg	- Low heating rates allowed better heat transfer between the particles, thus increasing the yield of biochar. - However, the high temperature was needed to improve the yield and quality of bio-oil.	Setter et al. (2020)
Reed	Fast pyrolysis	–	C: 89%, H: 3% Surface area: 1545 m^2/g Adsorption capacity: 1019 mg/g	- Fast pyrolysis improved the porosity properties of carbon products, leading to their high adsorption capacity. - Carbon products can be used as gas storage material or adsorbent.	Rahbar-Shamskar et al. (2020)
Spruce wood chips	Pyrolysis and gasification	–	C: 87 wt%, H: 0.6 wt%, S: <0.1 wt% Surface area: 1253 m^2/g Adsorption capacity: 67 mg/g	- Lower temperature increased the adsorption capacity of biochar. - Require high carbon conversion efficiency to produce biochar with high surface area and adsorption capacity.	Ravenni et al. (2019)
Peat	Hydrothermal carbonisation and torrefaction	70–80%	C: 59–65 wt%, H: 5 wt%, O: 23–28 wt% Calorific value: 23–26 MJ/kg Surface area: 2 m^2/g	- Carbonisation increased the surface area of biochar in contrast to torrefaction. - Torrefied biochar possessed higher energy yield but lower calorific value compared to carbonised biochar.	Krysanova et al. (2019)
Microalgae	Torrefaction and chemical treatment	55–75%	C: 54–68 wt% H: 7–13 wt % O: 17–32 wt% Calorific value: 21–31 MJ/kg	- Wet torrefaction increased the calorific value of biochar for use as solid fuel. - Acid hydrolysis pretreatment improved the active sites and sorption capacity of biochar that can potentially be used as adsorbent.	Yu et al. (2020)
Orange peel waste	Microwave pyrolysis and gas activation	31–44 wt %	C: 63–78 wt%, H: 2–5 wt %, O: 19–32 wt% Surface area: 159–305 m^2/g Adsorption capacity: 96–159 mg/g	- CO_2 activation developed more micropores in contrast to steam activation that developed more mesopores. - Microwave pyrolysis combined with gas activation is a desirable approach to produce activated carbon with high adsorption capacity for the removal of dye from wastewater.	Yek et al. (2020)
Switchgrass	Microwave activation and catalytic pyrolysis	–	C: 26–36 wt%, H: 1–2 wt %, O: 11–17 wt% Surface areas: 38–76 m^2/g Micropore specific surface area: 329–402 m^2/g	- Microwave activation and catalytic pyrolysis increased surface area and cation exchange capacity of biochar. - Biochar boosted plant growth and lowered the concentration of heavy metals in contaminated soil. - Field studies should include the influence of abiotic and from the environment.	Mohamed et al. (2021)
Waste palm shell	Pyrolysis and microwave activation	45 wt%	C: 82%, H: 4%, O: 14% Surface area: 540 m^2/g Micropore surface area: 679.22 m^2/g Adsorption capacity: 595 mg/g	- Pyrolysis combined with microwave activation increased heating rate, reduced operating time, and improved the yield of biochar. - The biochar possessed desirable quality as an adsorbent to treat landfill leachate.	Lam et al. (2020a)
Coconut shell	Pyrolysis and chemical modification	–	C: 72 wt%, O: 21 wt% Surface area: 304 m^2/g Specific electrosorption capacity: 33.9–68.4 mg/g	- Incorporation of MnO_2 nanocomposites into activated biochar has improved the properties of biochar as an electrochemical material for desalination or energy storage. - This technology is energy-efficient, high recovery of effluent, ecologically friendly, and could prevent fouling problems.	Adorna et al. (2020)
Wakame (Seaweed)	Pyrolysis and chemical modification	–	C: 42%, H: 1%, O: 16% Surface area: 744.15 m^2/g Adsorption capacity: 480 mg/g	- Biochar impregnated with magnetic nickel exhibited high adsorption capacity for methylene blue, thus showing excellent potential to be used in wastewater treatment.	Yao et al. (2020)

C: carbon N: nitrogen H: hydrogen S: sulphur O: oxygen.
(–) data are not available.

activation or chemical coating to enhance the heating rate, biochar yield and biochar quality (Adorna et al., 2020; Wan Mahari et al., 2022b) (see Table 3).

3.2.1. Conventional pyrolysis

Setter et al. (2020) investigated the influence of slow pyrolysis temperature (350–450 °C at a heating rate of 0.5 °C/min) of coffee husk briquettes on the pyrolysis product distributions and quality (Setter et al., 2020). It was reported that the biochar yield decreased from 40 wt % to 34 wt%, whereas the fixed carbon content and the distribution of the pores increased over increasing temperature. These findings can be explained by the increase of the devolatilisation of the organic material at higher temperature ranges (Parvez et al., 2019). To date, slow pyrolysis has been incorporated with microwave heating (termed microwave pyrolysis) as an alternative to conventional heating. Through volumetric and internal heating, microwave radiation could increase the chemical reaction rate of biomass at lower temperatures, and in turn provide shorter residence time and high energy consumption efficiency (Mahari et al., 2021a). Parvez et al. (2019) applied pyrolysis of gumwood under different pyrolysis temperatures (600–800 °C) using conventional (e.g., heated by the furnace) and microwave heating (Parvez et al., 2019). They reported that microwave pyrolysis has better performance owing to the 13.5% higher energy efficiency and more biochar and gas yield (about 4 wt%) than conventional pyrolysis. This can be explained by the enhanced heterogeneous reactions between the gases, char and secondary cracking of oil vapours into incondensable gaseous fractions.

On the contrary, fast pyrolysis is performed at higher operating temperature ranges from 550 to 1000 °C and heating rate (≥200 °C/min) but shorter residence time (several seconds) than the slow pyrolysis. Rahbar-Shamskar et al. (2020) carried out fast pyrolysis of reed followed by activation of the produced biochar to improve the biochar properties and its application feasibility, especially for gasoline vapor recovery application (Rahbar-Shamskar et al., 2020). The study revealed that the biochar produced by fast pyrolysis followed by the zinc chloride or ammonium phosphate activation possessed micro/mesoporous structures while the phosphoric acid activation had produced microporous structures with high surface areas (497–1545 m^2/g) compared to the biochar (4 m^2/g) derived from activation-free fast pyrolysis. The micro/mesoporous structures of $ZnCl_2$ activated biochar resulted in a higher adsorption capacity of 1019 mg/g, which was 10 times higher than that shown by commercially activated carbon.

Flash pyrolysis involves heating and pyrolysis of biomass at a high-temperature range of 600–1200 °C, extremely high heating rate (>1000 °C/s), and short vapour residence time (<0.5 s), producing a small amount of biochar product (5–15 wt%) (Chen et al., 2020; Foong et al., 2020). Theoretically, the high temperature achieved within a short time allows the promotion of volatile production and secondary cracking of volatiles while hindering the volatiles re-condensation/combination with biochar, which subsequently results in the production of a high amount of gaseous product compared to biochar (Foong et al., 2020). Nonetheless, it is difficult to govern and optimise the reaction processes as flash pyrolysis is more likely to experience limited mass transfer due to inhomogeneous heat transfers (Jiang and Wei, 2019; Palumbo et al., 2019). Previous studies had reported that the particle temperatures and heating rates during flash pyrolysis were uneven and not well operated in a wire mesh reactor, leading to the discrepancy between the experimental data and model predictions for the reactor (Dufour et al., 2011), thus would bring inaccurate estimations of the simplified solid-state kinetic model of flash pyrolysis.

3.2.2. Pyrolysis combined with gas activation

Biochar could be activated by gaseous agents (e.g., air, carbon dioxide, water vapor and steam) at 700–1100 °C to improve its properties. This, in turn, removes the incomplete combustion products and other impurities from biochar while improving its porosity, surface area, surface reactivity and nutrient retention (Kazemi Shariat Panahi et al., 2020). Despite the various benefits, gas activation has difficulty in reaction temperature control along with nonuniform activation and local overheating (Kumar et al., 2020). Moreover, the gas activation reduces certain functional groups' abundance like carboxyl and phenolic groups on biochar surface. The carboxyl group (-COOH) that functions as a binding site for heavy metals could be washed out during gas activation, resulting in less oxidised biochar and ineffective metal remediation application. Similarly, the phenolic group is also prone to this process, resulting in the formation of less polar biochar (Kazemi Shariat Panahi et al., 2020).

Yek et al. (2020) produced activated orange peel biochar using CO_2 and steam activation at 700 °C (Yek et al., 2020). The steam-activated biochar showed higher surface area (305.1 m^2/g) and adsorption efficiency (136 mg/g) for Congo Red compared to CO_2-activated biochar (158.5 m^2/g, 91 mg/g) and pristine biochar (95.6 m^2/g, 0 mg/g). In another study, Kwak et al. (2019) determined the effects of feedstock type (wheat straw, canola, sawdust, and manure pellet) and steam activation on lead (II) adsorption capacity to demonstrate the potential use of biochar for heavy metal removal in water treatment (Kwak et al., 2019). The steam activation was reported to have increased the surface area (up to 1–356 m^2/g) and lead (II) adsorption capacity (41–195 mg/g) compared to pristine biochar (0.8–302 m2/g, 43–109 mg/g). The canola straw biochar was found to be the most efficient biochar for lead (II) adsorption (195 mg/g), while both steam activated sawdust biochar (41 mg/g) and non-activated sawdust biochar were less efficient for metal adsorption due to their low lead (II) adsorption capacity attributed to the low pH of the biochar.

3.2.3. Pyrolysis combined with microwave activation

Biochar can be activated using microwave irradiation operated at frequencies of 0.03–300 GHz and wavelength of 0.01–1 m and under low process temperature between 200 and 300 °C. During microwave activation, the biochar particles experience a polarization such as a dipole orientation, where the electrons that surround the atoms are displaced trillion times per second. The friction between the rotating molecules will produce thermal energy during microwave radiation (Kazemi Shariat Panahi et al., 2020). Uniform thermal energy could be internally transferred within the biomass, activating biochar and in turn providing larger surface area and more functional groups compared to non-activated biochar. These features give benefits for its application in environmental management since microwave-activated biochar could improve contaminated soil and cation exchange capacity (Mohamed et al., 2021). Mohamed et al. (2021) reported that biochar produced from microwave pyrolysis of switchgrass possessed high surface area and cation exchange capacity (Mohamed et al., 2021). As a result, the biochar not only reduced heavy metals in soil but also supplied nutrients that boosted the growth of the wheat plant. Although microwave activation is regarded as a fast and efficient heating approach (Jung et al., 2019), it is limited by the ability of biomass to absorb microwave radiation (Mahari et al., 2021b). Therefore, microwave absorbent is needed to rectify the limitation of certain biomass to absorb microwave radiation.

Lam et al. (2020a,b) developed a single-step microwave steam activation for producing biochar from waste palm shell (WPS) for application as biosorbent in hazardous landfill leachate treatment (Lam et al., 2020b). This method exhibited a high heating rate (70 °C/min), producing 45 wt% of highly microporous biochar with a surface area of 679 m^2/g. In contrast, the conventional heating approach only produced ≤12–17 wt% of biochar. They also reported that the activated biochar showed high adsorption capacity (595 mg/g), which led to 65% removal of chemical oxygen demand from landfill leachate. The finding was nearly comparable with commercial coconut shell activated carbon which has an adsorption capacity of 663 mg/g and 70% removal of chemical oxygen demand.

3.2.4. Pyrolysis combined with chemical modification

Chemical modification is a common method of chemical treatment to improve the properties and porous structure of biochar. Adorna et al. (2020) synthesised an activated biochar nanocomposite using coconut shell-derived biochar and α-MnO_2 nanocomposite via indirect co-precipitation methods (Adorna et al., 2020). During indirect co-precipitation, the biochar was firstly mixed with HNO_3 and Mn $(NO_3)_2{\cdot}4H_2O$ for 24 h, followed by mixing with $KMnO_4$ for another 24 h. The α-MnO_2 is commonly known for its excellent ion intercalation ability, thus improving the properties of as-prepared composite with a high specific surface area of 304 m^2/g, mesopore volume ratio, capacitance retention, good hydrophilicity and making it an excellent electrode material for capacitive deionization application. It was also reported that the specific capacitance (410–523 F/g) of the MnO_2--biochar nanocomposite at 5 mV s^{-1} was higher than pristine biochar (42 F/g), activated biochar (146 F/g), commercial MnO_2 (57 F/g) and lab-prepared MnO_2 (342 F/g), leading to the higher specific electrosorption capacity of 33.9–68.4 mg/g compared to MoS_2/g-C_3N_4 (24.16 mg/g), 3-D graphene (21.58 mg/g), and MnO_2/activated carbon (9.26 mg/g) (Adorna et al., 2020).

In addition, the biochar can be coated chemically with functional nanoparticles to introduce additional features to the biochar surface, improving the feasibility of biochar for various applications. For example, Hu et al. (2019) prepared a functional chitosan/biochar-nanosilver composite for improving the antibacterial purposes in drinking water purification via coating with $AgNO_3$ solution and carbonization (Hu et al., 2019). Firstly, a carbon-silver complex was prepared by dipping the corn straw in $AgNO_3$ solution for 24 h, followed by carbonization over 300–1000 °C for 1 h. Then, it was mixed with a chitosan-polyvinyl pyrrolidone solution to produce a chitosan/biochar-nanosilver composite. The introduction of chitosan could strengthen the weak bond between carbon and silver while having the ability to adsorb metal ions and inhibit the growth and reproduction of fungi, bacteria, and viruses during water treatment.

3.2.5. Gasification

Biochar can also be produced through gasification performed at high temperatures (700–900 °C) in the presence of various gaseous media, including nitrogen, air, oxygen, steam, or carbon dioxide (Kim et al., 2020). However, the gasified biochar is usually discarded from the gasification plants considering that the syngas is always the main product of interest from gasification (Ravenni et al., 2019). During gasification, the feedstock undergoes several operating steps; starting from drying, pyrolysis (e.g. char production), heterogeneous char gasification followed by homogeneous reactions (e.g. pyrolysis volatiles are subjected to reforming, cracking, and Water Gas Shift reactions) (Cortazar et al., 2020). The char gasification reactivity can be improved by increasing the heating rate and lowering the char production temperature (e.g., during the pyrolysis step). Specifically, the low temperature of the pyrolysis step at around 400 °C and char gasification of less than 1000 °C may result in optimal gasification reactivity (Tian et al., 2020).

The gasified biochar contains inorganics (e.g. alkali and alkaline earth metals originating from the feedstock) and has a higher energy yield than pyrolysed biochar, depending on the feedstock (e.g. feedstock with low O/C ratio) and operating conditions of the gasifier (Ravenni et al., 2019; Kim et al., 2020). The carbon atoms of biochar endured during the whole gasification reactions are arranged in the most stable structures and physically activated into a microporous surface with a high specific surface area. Ravenni et al. (2019) compared the properties and adsorption capacity for naphthalene between gasified biochar and steam-activated pyrolysed biochar (Ravenni et al., 2019). They found that gasified biochar had a better surface area (1253 m^2/g) and naphthalene adsorption capacity (66.7 mg/g) compared to steam-activated pyrolysed biochar (553 m^2/g, 60.5 g/g).

Nevertheless, most of the industrialized gasification technology is operated at harsh conditions (at elevated temperatures of up to 1400 °C) using entrained bed gasifier that requires high capital and operation cost (Prajitno et al., 2020). Catalytic gasification is thus opted to improve the process efficiency of conventional gasification (Kim et al., 2020). Furthermore, gasification of biomass with steam is also another attraction as it is capable of converting low-grade solid fuels into high economic value and cleaner fuel products at a higher reaction rate (2–5 times) than using conventional CO_2 while effectively removing the condensable volatiles (tar) during the pyrolysis stage, preventing tar slagging from the reactor (Tian et al., 2020).

3.2.6. Torrefaction

Torrefaction is a pyrolysis method to improve the fuel characteristics of biomass at mild temperatures ranging between 200 and 300 °C (Krysanova et al., 2019; Chen et al., 2020). During torrefaction, the biomass would undergo several chemical reactions such as dehydration, condensation, de-carbonylation, de-methoxylation, decarboxylation, aromatization and intermolecular re-arrangement (Krysanova et al., 2019). Compared to pyrolyzed biochar, torrefied biochar is richer in oxygen-containing functional groups due to the use of lower operating temperatures (Li et al., 2019). It has also been reported to have a higher energy yield (90%) compared to hydrochar (80%) (Krysanova et al., 2019).

Nonetheless, the surface morphology of torrefied biochar still needs further improvements. Krysanova et al. (2019) studied the surface morphology between torrefied biochar and carbonised biochar (Krysanova et al., 2019). It was reported that the torrefied biochar was lack of dispersed structure and tended to agglomerate with other particles compared to carbonised biochar, which contained a highly dispersed structure with microspheres. Such a structure is desirable to prevent agglomeration. This may be explained by the intensification of dehydration and decarboxylation reactions of torrefaction which strongly destroys the structural parts of biomass, hence triggering a more disassembled material structure. In addition, the carbonised biochar possessed a more dispersed structure due to the aromatisation and polymerisation of material caused by the increase in temperature and duration of hydrothermal carbonisation (Krysanova et al., 2019).

Several modifications on torrefaction have been investigated to improve the features of torrified biochar, such as the incorporation of acid hydrolysis with torrefaction. Yu et al. (2020) successfully improved the features of microalgae biochar via torrefaction incorporating with sulphuric acid (Yu et al., 2020). They found that the addition of sulphuric acid could initiate hydrolysis that could facilitate the carbohydrate and protein decomposition of microalgae at relatively low torrefaction operating temperature (160–180 °C). The obtained biochar surface has higher porosity and loopholes that could serve as binding sites for bio-adsorbent applications. In addition to porosity features, Li et al. (2019) focused on the modification of the surface complexion of biochar using oxidative torrefaction (Li et al., 2019). They investigated the corn stover-derived biochar properties produced at different thermochemical conversion technologies between conventional torrefaction (performed at 250 °C under inert condition), oxidative torrefaction (performed at 250 °C under air environment) and pyrolysis (performed at 500 °C under inert condition). It was revealed that biochar produced from oxidative torrefaction had higher oxygen content and oxygen-containing group compared to conventional torrefaction and pyrolysis. This led to the improvement of biochar properties such as surface complexion, chemisorption and uranium adsorption capacity (111.52 mg/g) compared to biochar obtained from conventional torrefaction (101.57 mg/g) and pyrolysis (56.21 mg/g).

4. Techno-economic and environmental perspectives of agriculture, aquaculture and shellfish biomass recovery

Techno-economic analysis is conducted to evaluate the economic growth and bioeconomy of the biochemicals and biomaterials production from biomasses. Capital cost, plant capacity, operational cost and

raw materials are the main factors that influence the production cost of biomaterials and biochemicals. Economic indicators such as net present value (NPV), internal rate of return (IRR) and payback period are crucial to determine the economic performance and feasibility of the valorisation process to produce biochemicals and biomaterials. Table 4 summarises the techno-economic analysis of biochemicals and biomaterials production from the biomasses.

Arora et al. (2018) evaluated the techno-economic assessment of mango processing waste biorefinery. There are four stages of the pectin extraction process from mango waste, which are dissolution of proto-pectin, purification of the extract, separation of pectin from the liquid *via* precipitation, and drying of the pectin extract. It was revealed that the NPV for recovery of pectin and seed oil (41 million USD) is higher than the recovery of pectin only (14.2 million USD). NPV represents the difference between the current value of cash inflows and the present value of cash outflow (Viganó et al., 2022). The sensitivity analysis revealed that the capacity of the plant, operation time, and composition of raw materials (e.g. mango seed, mango peel) are the key aspects that influence the production cost and feasibility of the biorefinery approach to producing value-added products.

Khwanjaisakun et al. (2020) performed techno-economic assessment of vanillin production from Kraft lignin via the oxidation process. The energy consumption to produce lignin-based vanillin is higher than petroleum-based vanillin due to the high quantity of raw materials (e.g., feedstocks, solvents) needed to extract and generate lignin-based vanillin, which requires high energy to process the raw materials and remove the impurities of lignin. Hence, further studies should investigate the improved separation and purification techniques that consume a lower amount of raw materials (e.g., solvents) and energy during lignin-based vanillin production. Despite the high energy consumption to produce lignin-based vanillin, the cost of Kraft lignin is significantly cheaper than raw materials to produce petroleum-based vanillin, such as glyoxylic acid and guaiacol. Therefore, the production of lignin-based vanillin is more economical as compared to petroleum-based vanillin.

Thompson et al. (2021) compared the techno-economic assessment of furfural production from sugar beet pulp using pyrolysis and hydrolysis techniques. It was found that the production cost of furfural using pyrolysis (846 USD/metric ton) is lower than that obtained using hydrolysis (980 USD/metric ton). This is due to the high operational cost of hydrolysis, which consumes a large volume of water and high energy/electricity to heat the water. The production of furfural using pyrolysis has significantly lower environmental impacts by releasing lower greenhouse gas (267 kg CO_2 eq./metric ton) compared to hydrolysis (1095 kg CO_2 eq./metric ton). This study also suggests that portable pyrolysis operations close to biomass collection sites can significantly reduce the operating and variable costs as well as greenhouse gas emissions, which lead to sustainable furfural production.

In the shellfish and aquaculture industries, Gómez-Ríos et al. (2017) investigated the techno-economic assessment of chitosan production from the shrimp shell. There were two approaches used in the study, which are the physical-chemical method combined with chemical deacetylation (PC-CDA) and the fermentative physical-chemical method combined with chemical deacetylation (FPC-CDA). It was found that FPC-CDA requires lower energy consumption, water usage and chemicals (e.g., sodium hydroxide) compared to the PC-CDA process. Nevertheless, FPC-CDA demands bigger space which contributes to the increase of investment in fixed assets up to 15% compared to the PC-CDA process. Interestingly, the NPV and IRR values for chitosan

Table 4
Techno-economic assessment for biochemicals and biomaterials production from biomasses using various valorisation technologies.

Source of biomass	Valorisation technologies	Product	Capacity	Remarks	References
Mango	Extraction	Pectin	10 tons/h	Capital cost: 23.2 USD Operational cost: 6.99 million USD Net present value: 14.2 USD Internal rate of return: 20% Payback period: 4.2 years	Arora et al. (2018)
Kraft lignin	Oxidation, Extraction	Vanillin	30–120 g/L	Highest yield of vanillin: 9.25% Payback period: 6.19 years Internal rate of return: 22.6%. Greenhouse gas emission: 134–155 kg CO_2/hr	Khwanjaisakun et al. (2020)
Sugar beet pulp	Pyrolysis	Furfural	4592 ton/year	Production cost: 846 USD/ton Greenhouse gas emission: 267 kg CO_2 eq.	Thompson et al. (2021)
Sugar beet pulp	Hydrolysis	Furfural	6560 ton/year	Production cost: 980 USD/ton Greenhouse gas emission: 1095 kg CO_2 eq.	Thompson et al. (2021)
Shrimp waste	PC-CDA	Chitosan	–	Capital cost: 0.7865 million USD Net present value: 0.4977 million USD Internal rate of return: 26.6% Payback period: 5 years Gross margin: 68%	Gómez-Ríos et al. (2017)
Shrimp waste	FPC-CDA	Chitosan	–	Capital cost: 0.9166 million USD Net present value: 0.4789 million USD Internal rate of return: 24.4% Payback period: 6 years Gross margin: 71%	Gómez-Ríos et al. (2017)
Acai by-products	Pressurised liquid extraction	Phenolic	500 L	Gross margin: 84% Return of investment: 145% Net present value at 7% interest: 175 USD x 10^6 Internal rate of return: 325% Revenues: 41 USD/year x 10^6	Viganó et al. (2022)
Orchard waste	Pyrolysis	Biochar	–	Total fixed and variable costs: 1542.16 USD Production cost: 449 to 1845 USD/Mg of biochar	Nematian et al. (2021)
Oil palm empty fruit bunch	Pyrolysis	Biochar	4800 ton/year	Greenhouse gas emission: 0.046 kg CO_2 eq./year Production cost: 524 USD/year Net present value: 123 USD Payback period: 10 years Internal rate of return: 8.96%	Harsono et al. (2013)

PC-CDA: Physical-chemical method and chemical deacetylation.
FPC-CDA: Fermentative physical-chemical method and chemical deacetylation.
(–) data are not available.

production by the PC-CDA process are higher than the FPC-CDA process, thus more economically feasible. This study also revealed that the cost of raw materials, processing time and investments for assets greatly influence the quality and production cost of chitosan. There are several factors that affect the quality of chitosan, such as water solubility, deacetylation degree, and mineral and protein content.

Andreasi Bassi et al. (2021) studied the economic feasibility and environmental impacts of PHA production from food waste and sewage sludge. It was found that PHA produced from urban biowaste has lower environmental impacts and production costs compared to the PHA produced from first-generation biomass (e.g., maize, sugarcane) and polyurethane. Nevertheless, the production cost of PHA is significantly higher than petroleum-based polymers (conventional plastics) due to the use of expensive raw materials as carbon substrate and chemicals during the extraction process. Recently, Wan Mahari et al. (2022a) reported that liquid oil derived from microwave co-pyrolysis of plastic waste and used cooking oil can be used as carbon substrate to generate bioplastics. The use of wastes during microbial fermentation could replace the use of expensive raw materials as carbon substrate, which may reduce the production cost of PHA. However, more research on optimisation and techno-economic assessment should be done to validate the feasibility of this approach.

In Brazil, Viganó et al. (2022) evaluated the techno-economic analysis of phenolic compounds extraction from acai (*Euterpe oleracea*) by-products (i.e., seed and fibers). Extraction vessels with different capacities such as 50 L, 200 L and 500 L were used in the assessment. Long extraction time increased the cost of manufacturing due to the high consumption of solvents and raw materials. Interestingly, the use of a larger capacity extraction vessel (500 L) shows a higher value of gross margin, return of investment, net present value, internal rate of return and revenue compared to a smaller capacity extraction vessel (50 L), thus showing the profitability and potential of this technique to be upscaled. The payback period of the 500 L extraction vessel is also shorter compared to the 50 L extraction vessel, which indicates faster recovery of the initial investment cost. This techno-economic evaluation from this study suggests that large-scale pressurised liquid extraction of acai seed and fibers could reduce the cost of manufacturing and produce high profit, thus can be applied in biorefinery plants to produce phenolic compounds with antioxidant properties.

In the United States of America, there are several financial incentives to encourage the production of biochar, such as non-financial policy support, loans, as well as research and innovation fund. Nematian et al. (2021) reported techno-economic assessment of biochar production from orchard waste using pyrolysis in California. The total fixed and variable costs are approximately 1542 USD, which includes the cost of processing equipment, machinery, storage facility, raw materials (e.g., lubricants, fuels), labour and miscellaneous (e.g., disposal of waste). The estimated production cost to produce biochar ranges from 449 to 1845 USD/Mg of biochar, which is economically feasible due to the low cost of biomass waste. In another study, Harsono et al. (2013) reported a high and positive NPV, which indicates biochar production from oil palm waste is economically profitable. The techno-economic assessment from this study provides essential information to minimise risks associated with biochar production from agricultural biomass. This finding also encourages the circular bioeconomy concept that recovers useful biomaterials from agricultural waste.

5. Conclusion and outlooks

Biomass is an abundant source of renewable energy and sustainable material production. This review reveals that agriculture, aquaculture, and shellfish biomass possess unique and desirable properties which make them suitable to be converted into value-added products (e.g., biochemicals and biomaterials) *via* various valorisation techniques. The following conclusions and outlooks could be drawn from this review:

1. Biorefinery of biomass and biowaste is a reliable approach to reduce the volume of waste while sustaining the production of new products with high added value.
2. Polysaccharides are the main compound present in biomass and biowaste, including food wastes, agricultural residues, and marine aquaculture by-products. Biomasses contain polysaccharides that can be converted into biopolymer (e.g., pectin, furfural, vanillin) for use in many applications (e.g., pharmaceuticals, cosmetics, agricultural).
3. Bioactive compounds such as phenolic can be extracted from food waste and agricultural residues for use in the pharmaceutical and food processing industries.
4. Biomasses can be converted into bioplastics *via* chemical treatment (e.g., chemical coating) and biological treatment (e.g., fermentation).
5. Bioplastics synthesised by biomass is cheaper than that synthesised by microorganisms. The bioplastics synthesised from biomass could reduce dependency on petrochemicals as plastic sources.
6. Pyrolysis can be mixed with various modification techniques such as gas activation, microwave activation and chemical coating to enhance the pyrolysis performance and properties of biochar.
7. Newly developed microwave steam activation and gasification show great promise to produce biochar with high surface area and adsorption capacity compared to other valorisation techniques.
8. The production of biochemicals and biomaterials from biomass sources is economically feasible due to the low cost of raw materials.
9. The challenge is to maintain or improve the performance of the valorisation technologies to ensure a better quality of biochemicals and biomaterials production as compared to fossil-based products.
10. The biomass sources must be adequate and sustainable to manage the demand to produce bioproducts to be applied in many sectors.

Credit author statement

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Declaration of competing interest

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 paper.

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Research article

Subtopic: Advances in water and wastewater treatment harvesting of *Chlorella* sp. microalgae using *Aspergillus niger* as bio-flocculant for aquaculture wastewater treatment

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Aquaculture wastewater

ABSTRACT

Microalgae have been increasingly used to generate biofuel, thus a sustainable technique should be implemented to harvest the biomass to ensure its existence in the environment. *Aspergillus niger* was used as bio-flocculant to harvest microalgae from aquaculture wastewater via flocculation technique over a range of pH and mixing rate. The bio-flocculant showed ability to adapt at a wide range of pH from 3.0 to 9.0 and at a mixing rate of 100–150 rpm, producing a harvesting efficiency of higher than 90%. The treated water possessed low concentration of chlorophyll-a (0.3–0.6 mg L^{-1}) and cell density (2×10^6–3×10^6 cell mL^{-1}). These indicate that *Aspergillus niger* is a promising bio-flocculant to be used in harvesting microalgae, thus promoting the use of flocculation as a green technology in aquaculture wastewater treatment.

1. Introduction

Microalgae with a wide range of commercial applications have attracted a lot of attention from many researchers. Recently, various solid-liquid separation techniques are available for microalgae harvesting, including centrifugation, filtration, flotation and coagulation-flocculation. Centrifugation and filtration are mainly used as microalgae harvesting technique in commercial system. Nevertheless, these techniques are yet to be economically sustainable due to high energy consumption (Granados et al., 2012).

Coagulation-flocculation is widely used in removing particles and organic matter present in water and wastewater treatment due to the accessibility and cost-effectiveness of this process (Liu et al., 2019; Renault et al., 2009). In this process, flocculants are useful agents for agglomeration of colloids, cells and suspended particles. It is commonly utilized in drinking water production, wastewater treatment, fermentation processes and food production. Flocculants can be categorized into three groups: synthetic organic flocculants such as polyethyleneimine and polyacrylamide, inorganic flocculants such as aluminium sulphate and polyaluminium chloride, and natural flocculants (bio-flocculants) such as chitosan (Gao et al., 2006). Chemical flocculants including synthetic organic and inorganic flocculants are used in industrial fields due to its low cost and high flocculating performance (Hailong et al., 2009).

Bio-flocculant such as chitosan and extracellular biopolymeric biodegradable substances secreted by microorganisms (bacteria, fungi, algae) are also available for use in coagulation-flocculation (Lama et al., 2016; Riaño et al., 2012). These types of flocculants have great potential for use in industrial applications, but there are still limitations such as low flocculation efficiency and large dosage requirement that need to be dealt with (Czemierska et al., 2017). Currently, the use of chemical flocculants is undesirable because the major components in chemical flocculants could incur some environmental and health problems such as Alzheimer disease and Parkinson disease (Ahmad et al., 2011; Campbell, 2002). Hence, sustainable harvesting technique needs to be developed to control biomass density of the microalgae using bio-flocculant derived from naturally available materials. The bio-flocculant derived from fungi has been used in harvesting microalgae biomass

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(Al-Hothaly, 2018). Several filamentous fungi such as *Aspergillus, Penicillium, Trichoderma, Spicaria* and *Hyaloflorea* were reported to have the ability to entrap sludge solids to form bio-aggregation and strengthen the flocculation structure due to the unique filamentous properties (Bala Subramanian et al., 2010; Fakhru'l-Razi et al., 2002). Filamentous fungus composes of molecularly sticky hyphae which are postulated to be able to attach and entrap microalgae cells and remove it from the surrounding water (Nasir et al., 2015).

This study was performed to investigate the feasibility of filamentous fungus, *Aspergillus niger (A. niger)* as bio-flocculant in harvesting microalgae biomass. Prior to harvesting process, bio-flocculant formation under different conditions in terms of pH and mixing rate was evaluated in order to determine the optimum conditions for cultivation. The aim was to develop a novel method for harvesting microalgae biomass in an effort to cater for increasing microalgae production and utilization in near future.

2. Materials and methods

2.1. Cultivation of microalgae, chlorella sp. and bio-flocculant, Aspergillus niger

Green microalgae, *Chlorella* sp., was isolated from African catfish aquaculture wastewater and cultivated using an artificial growth media, Bold Basal Medium (BBM). Microalgae were incubated at a constant temperature of 20 ± 2 °C and maintained at pH of 6.9 ± 2 under continuous illumination. The microalgae samples were continuously aerated with sterile-filtered air. For monitoring of microalgae cell growth, cells were measured at 686 nm of optical density (OD686) using UV–Vis spectrophotometer (Shimadzu UV-1800, Japan) and BBM (Bold Basal Medium) growth media was used as control.

Bio-flocculant from filamentous fungus, *A. niger* was isolated from the same place as for microalgae. The mother plate of *A. niger* was allowed to propagate on the potato dextrose agar (PDA) at a constant temperature of 27 ± 2 °C in an incubator (Memmert INE 200, Germany). The *A. niger* spore from plate was inoculated into a 250 mL flask containing 150 mL Potato Dextrose Broth (PDB) growth medium and cultivated for 72 h in an incubator shaker (Lab Companion SI-600, Korea). Then, the *A. niger* was formed in pellet shape known as bio-flocculant by shaking the flask in the incubator shaker at 37 °C and 125 rpm; the speed was remained constant throughout the cultivations.

2.2. Cultivation condition for bio-flocculant formation

In this study, the production of bioflocculant, produced by *Aspergilus niger* was investigated to determine the optimal cultivation conditions. Shahadat et al. (2017) stated that the growth of microorganisms generally depends on the pH of the medium where it is being cultivated. Thus, the influence of pH on bio-flocculant formation was investigated and adjusted using portable pH Meter (Thermo Scientific™ Orion Star™ A121, US) in this study. The pH values on bio-flocculant formation in submerged culture was adjusted to 11 pH values (5.45 (control), 2.0, 3.0, 4.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0) with NaOH (1 M) and HCl (1 M), respectively. Bio-flocculant in pellet formed were grown in the medium composition (PDB) and 125 rpm of mixing rate.

Mixing rate is one of the most influential factors for pellet formation accordingly Ibrahim et al. (2015). The formation was carried out at pH 5.5 which served as a control with varying mixing rate between 0, 25, 50, 100, 125 and 150 rpm. Each sample from different mixing rate was collected and evaluated after three days cultivation period.

2.3. Harvesting microalgae using bio-flocculant

2.3.1. Determination of pH

pH is one of the factors that play a crucial role in the harvesting process (Laamanen et al., 2016). In order to determine the suitable range of pH for the harvesting process, eight different initial pH values (control, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) were adjusted at specific mixing rate. The control sample was prepared with the initial pH of microalgae medium set at 6.5. These initial pH values were kept constant during the whole cultivation period by regulating the pH for every 12 h using 1 M NaOH solution to increase the pH and 1 M HCl solution to lower the pH.

2.3.2. Determination of mixing rate

Another factor that can affect the harvesting process is mixing rate. According to BinAhmed et al. (2015), mixing rate could affect the flocs distribution and consequently the efficiency of the coagulation-flocculation process. In determining the effect of mixing rate in harvesting process, six different mixing mode were varied: 0 (Control), 25, 50, 75, 100, 125 and 150 rpm. The 0 rpm was indicated as control and as a comparison with the effect of mixing rate towards harvesting efficiency.

2.4. Statistical analysis

All the data were recorded in Microsoft Office Excel™ (Version 15.0.4727.1000, Microsoft Corporation, United States of America). OriginLab™ Pro (Version 9.0, OriginLab Corporation, United States of America) was used as the main software for graphical analysis. The statistical analysis was performed via Minitab™ 16 and SPSS Statistics 20. Two-way ANOVA was selected for investigation of two factors simultaneously such as the effect of various pH and mixing rate towards the harvesting efficiency. One-way ANOVA was then employed to determine the significance within each factor based on the result of two-way ANOVA. This statistical tool distinguished the significance within each factor and interaction between the factors. All the statistical analysis was performed at confidence interval.

3. Results and discussion

3.1. Cultivation condition on bio-flocculant formation

Numerous factors are considered to have influence on the formation of *A. niger* in pellet form, namely; nutritional requirements, culture medium, temperature, pH, mechanical force, aeration and morphology of the fungi (Wang et al., 2013). The combinations of electrostatic interaction, hydrophobicity and specific interactions from spore wall components also attributes to *A. niger* pellet formation (Zhang and Zhang, 2016). According to Abubakar et al. (2013), pH value of culture medium has been shown to have more decisive influence towards the mycelial and spore coagulation. Therefore, the effect of medium of different pH and mixing rates were important to be investigated since these parameters dominate the cell growth and bio-flocculant production.

3.1.1. Effect of pH on bio-flocculant formation

Based on observations under different pH values of PDB medium, pellet growth was observed in all conditions after 3 days of cultivation, except for pH 10.0 and pH 11.0 (Fig. 1). Similar findings have been reported by Abubakar et al. (2013), showing that the fungus was tolerant of acidic and neutral conditions but not favorable in alkaline condition for pellet development. The results of the effect of pH on pellet size are tabulated in Table 1. Non-uniform pellet size was observed at pH 3.0 with an average diameter of 5–12 mm, resulting in formation of 40 pellets per 10 mL of medium. However, the average diameter size for most soft pellet in this pH range was closed to 5 mm. At pH 2.0, small pellets were observed, having an average diameter of 3 mm and producing 620 pellets per 10 mL of PDB medium.

No significant difference ($P > 0.05$) in the growth was recorded between the control pH (pH 5.0) with pH 4 and pH 6 where pellets had formed well and at about similar size. Uniform pellets were formed at pH ranging from 4.0 to 6.0 with a bigger average pellet size of 7.0 mm.

Fig. 1. Formation of soft pellet in different pH condition after 3-day cultivation.

Table 1
Number and size of pellets at different pH of the culture medium after 3-day cultivation.

	pH									
	2	3	4	5	6	7	8	9	10	11
Number of pellet/10 mL PDB	620	40	15	13	18	78	27	10	N/A	N/A
Average diameter of pellet ± 0.5 (mm)	3	5–12	7	6	7	4	5	9	N/A	N/A

Note: N/A represents pellet not available in this pH.

Van Leeuwen et al. (2012) also stated that formation of compact pellet mainly occurred at this pH range (4.0–6.0). At pH 7.0, the pellet size was the smallest compared to pH 4.0, pH 5.0 and pH 6 which an average diameter of 5 mm. Followed by pH 8.0 and pH 9.0, the pellet size had increased at both pH to 5 mm and 9 mm, respectively. No fungal pellets growth was observed at pH 10.0 and pH 11.0. This shows that, although certain alkaline medium (pH 8.0 and 9.0) might favour the pellet formation of *A. niger,* higher pH values from 10.0 tend to hinder its pelletization.

The results obtained confirm the earlier findings from experiments carried out by Nair et al. (2016) and Grimm et al. (2005), which reported that the biomass of filamentous fungus is activated at slightly acidic pH conditions and this leads to higher rates of growth at lower pH. Fungal spore generally exhibits negative surface charges that are affected by pH and ionic strength. On the other hand, higher pH values are considered to be a factor causing negative charges that can decrease spore aggregation (Akiba et al., 1994; Zhang and Zhang, 2016).

The effect of pH medium on soft pellet formation could be related to conidial aggregation during submerged cultivation and considered to originate from the electrostatic surface properties of the spores. These properties are significantly influenced by the presence of melanin that contained surface coating covering the outer spore wall layer (Wargenau et al., 2013). This signified pH value as the most influential factor for *A. niger* pellet formation towards number and size of pellets. The results showed that pH was the key factor affecting the formation of soft pellet and also could be controlled by adjusting the glucose concentration and the number of fungal spores added (Zhou et al., 2012).

3.1.2. Effect of mixing rate on bio-flocculant formation

Fig. 2 shows the significant influence of the mixing rates on the bio-flocculant obtained from *A. niger* in soft pellet formation. Mixing rate was found to have a great impact on the formation of soft pellet fungus since the soft pellet would not form at lower mixing rate between 0 and 50 rpm (Serra et al., 2008). There are several factors that influence the formation of soft pellets in non-agitated culture and slow mixing. This is possibly due to the inability of conidia or spore to aggregate in forming clumps without introducing the shaking rate; this is supported by Porcel et al. (2005) who reported that the pellet diameter and compactness were affected by the agitation intensity. Presence of oxygen provided by the mixing process is essential in pellet formation since *A. niger* grows in heterogenous and aerobic condition (Veiter et al., 2018).

Mixing rate at 125 rpm was chosen as optimal mixing rate since it produced pellet with ideal characteristics - well rounded and in regular size (Fig. 2). However, Zhang and Hu (2012) and Liu et al. (2010) reported that 150 rpm and 170–180 rpm were the most optimum mixing rate for formation of pellets and acceptable for most cultures. On the

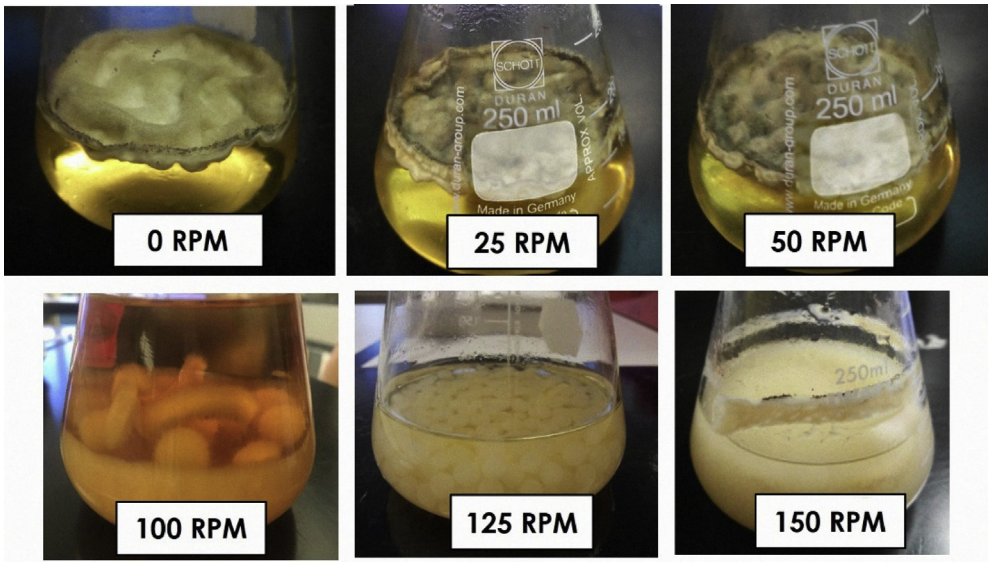

Fig. 2. Macroscopic morphology of pellet formed after 72 h at six different mixing rates (0, 25, 50, 100, 125 and 150 rpm).

contrary, this study demonstrates that strong mixing rate found to be unfavourable for pellet formation since the pellet was incompletely formed and begun to break down at 150 rpm of mixing rate. It also proved that an inverse relationship was observed between mixing rate and pellet formation due to the observation that increasing mixing rate did not result in formation of good pellets.

The excessive mixing rate could prevent the formation of pellets exhibiting lesser branching mycelia and a suitable mixing rate is helpful to disperse conidia and nutritive particles after inoculation as reported by Ibrahim et al. (2015). Mixing rate is related to providing oxygen, so the difference in results with other researchers could also be attributed to the different oxygen demands of different species of microorganisms. It was observed from this study that the formation of soft pellet was influenced by the changes of mixing rate. In addition, mixing rate is important to maintain adequate aeration which can affect nutrient absorption and enzymatic reaction (Abu Tawila et al., 2018).

3.2. Harvesting of microalgae biomass by fungus as bio-flocculant

The effects of the key factors, including pH and mixing rate on the harvesting process were investigated to identify the optimal conditions for the harvesting of microalgae biomass. These factors give effect towards particles and flocculating agents during flocculation process. In general, the role of pH enhances the secretion of enzyme while the role of mixing rate improves the uniformity and influences the attachment process. For instance, high mixing rate causes damage on microalgae pellet attachment, as well as low mixing rate reduces the potential of attachment. Therefore, the influence of both factors on the harvesting needs to be understood.

3.2.1. Effect of pH on the harvesting process

pH refers to the degree of alkalinity or acidity in the water. Efficiency of the microalgae biomass harvesting as influenced by variations of pH were examined. It was previously obtained that the optimum dosage of harvesting was 30 g L^{-1} (pellet per volume of microalgae culture) with 4.8×10^7 cell mL^{-1} (microalgae biomass) and could be applied in determining the effects of pH (Nasir et al., 2015). The harvesting efficiency did not show a clear difference in cell density and chlorophyll-a for all different pH, respectively (Fig. 3). According to one-way ANOVA analysis there is a significant difference ($P < 0.05$) between pH level and harvesting period in terms of harvesting efficiencies.

The use of various pH (3.0–9.0) throughout this study had achieved high harvesting efficiency (88.0–98.4%) of *Chlorella* sp. cell density and chlorophyll-a (chl-a) throughout the three days of harvesting period (Table 2). This study is in accordance with the findings of Nam et al. (1996) which reported that the harvesting efficiency of *A. niger* and the kaolin clay was appropriate at a wide range of pH (pH 3.0 to pH 8.0). It was observed that all harvesting flasks had produced clear water after harvesting period. Bio-flocculant was observed to entrap almost all microalgae biomass after the 3-day harvesting period. This shows that the bio-flocculant derived from *A. niger* is well-suited for harvesting microalgae biomass because it shows ability to endure various pH conditions. Therefore, it allows harvesting with uncontrolled pH, which can be implemented to keep low operational costs.

3.2.2. Effect of mixing rate on the harvesting process

The evaluation of mixing rate was succeed based on the optimum dosage at 30 g L^{-1} (weight of pellet per volume of microalgae culture) and the optimum pH of microalgae culture at pH 5.0–6.0 previously obtained. The harvesting efficiency of microalgae biomass in terms of cell density and chl-a throughout the three days harvesting period is depicted in Table 3. After 3 days of harvesting period, the final remaining microalgae biomass for all different mixing rate were in the range of 0.3–4.9 mg L^{-1} of chl-a concentration and 1.3×10^6–11.1×10^5 cell mL^{-1} of cell density.

The microalgae cells began to attach to the bio-flocculant on Day 1 for all different mixing rates, from 25 rpm to 150 rpm except at no mixing rate (0 rpm). At slow mixing rate (25 rpm), the harvesting of microalgae biomass minimally occurs but the trends of harvesting efficiency had shown that the process is less effective with the percentage removal less than 65% until Day 3. This might because low mixing rate can cause non-uniform distribution of flocculants. As a result, the flocs formed were relatively weak and become destabilized after some time, possibly due to the decrease in separation efficiency (Choi, 2015).

The harvesting of microalgae biomass could be observed as the mixing rate was further increased to 50 rpm. Microalgae biomass started to attach by adsorption at soft pellet on Day 1 for both 50 and 75 rpm. Nevertheless, the microalgae biomass was found separated again from the soft pellets at these mixing rates after another 24 h. The releasing biomass remained suspended in the culture water for the remaining experimental period.

It can be concluded from the results in Table 2 that the percentage removal was increased with the increasing of mixing rate. However, when the mixing rate was increased further which exceeded the limit, the percentage removal was decreased slightly, reaching 91.5% for cell

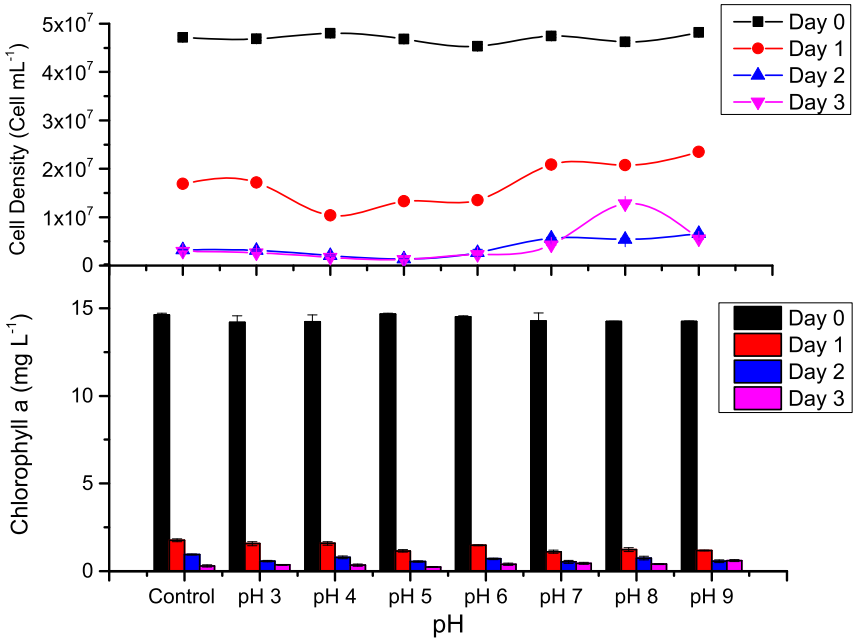

Fig. 3. Cell density and chlorophyll-a of *Chlorella* sp. biomass removal at various pH of culture namely; 6.45 (control), 3, 4, 5, 6, 7, 8 and 9. throughout 3-day of harvesting period.

Table 2
Percentage removal of *Chlorella* sp. cell density and chlorophyll-a at various pH by bio-flocculant (*Aspergillus niger*) throughout a 3-day of harvesting period.

pH	Harvesting Period (Day)					
	Cell Density (Cell mL^{-1})			Chlorophyll-a (mg L^{-1})		
	1	2	3	1	2	3
Control	$87.9^{b,c}$	$93.5^{a,b,c}$	98.0^{c}	$63.8^{b,c}$	93.1^{b}	93.2^{a}
3	$87.2^{b,c}$	93.7^{c}	94.3^{c}	$64.4^{b,c}$	80.7^{b}	92.9^{b}
4	88.9^{a}	94.5^{a}	$96.7^{a,b}$	$77.9^{a,b}$	$95.6^{a,b}$	92.9^{a}
5	$92.1^{a,b}$	96.2^{a}	98.4^{a}	71.6^{a}	97.1^{a}	92.2^{a}
6	$89.8^{a,b}$	$95.2^{a,b}$	$97.3^{b,c}$	$73.0^{a,b}$	$94.2^{a,b}$	90.9^{a}
7	$92.2^{c,d}$	$96.3^{b,c,d}$	96.9^{d}	56.1^{a}	88.1^{a}	89.5^{a}
8	88.9^{d}	$90.6^{c,d,e}$	$93.0^{d,e}$	$53.9^{a,b}$	86.4^{c}	88.8^{b}
9	84.4^{d}	$87.4^{d,e}$	90.5^{a}	50.0^{c}	85.8^{d}	88.2^{c}

Note: Different superscripted within the same row represents significant different group based on 95% confidence level.

Table 3
Percentage removal of *Chlorella* sp. cell density and chlorophyll-a by bio-flocculant (*Aspergillus niger*) at various mixing rates throughout a 3-day harvesting period.

Mixing Rate (rpm)	Harvesting Period (Day)					
	Cell Density (Cell mL^{-1})			Chlorophyll-a (mg L^{-1})		
	1	2	3	1	2	3
0 (Control)	2.7^{d}	7.7^{e}	9.6^{e}	2.11^{e}	2.55^{e}	3.16^{e}
25	37.3^{c}	45.2^{d}	48.6^{c}	41.7^{d}	46.7^{e}	54.9^{c}
50	74.8^{b}	71.5^{c}	56.1^{c}	75.2^{c}	61.1^{d}	54.1^{d}
75	79.0^{b}	72.8^{b}	70.2^{c}	82.8^{d}	78.7^{c}	72.9^{b}
100	$82.1^{a,b}$	88.8^{b}	94.3^{b}	$84.8^{a,b}$	90.1^{b}	94.4^{a}
125	$80.2^{a,b}$	96.2^{a}	97.3^{a}	81.2^{b}	96.3^{a}	97.6^{a}
150	85.3^{a}	86.1^{b}	91.5^{b}	86.5^{a}	87.0^{b}	93.1^{a}

Note: Different superscripted within the same row represents significant different group based on 95% confidence level.

density and 93.1% for chl-a at rate of 150 rpm. This was probably because an excessive mixing rate would disturb the attachment process of microalgae biomass to the soft pellet. Nevertheless, the hairy filaments of soft pellets cannot properly grip the microalgae biomass because of the clumping ability based on mixing rates. This was probably due to re-stabilization of the cells at fast mixing rate, as a result, the microalgae biomass tended re-dispersed again and suspended in growth medium (Chen et al., 1998).

This statement can be proven by the result that the cell density concentration and chl-a obtained at 150 rpm were higher compared to 100 rpm. This result also agrees with the findings by Lananan et al. (2016), which studied about the mechanism of flocculation where the stronger mixing rate that exceeded the best mixing range would cause the breakage of formed flocs and released aggregated microalgae biomass back into suspended forms.

Even though the increasing of mixing rate from 100 rpm to 150 rpm did not clearly show significant difference in microalgae biomass harvesting efficiency, the results do indicate that the mixing rate between 100 rpm and 125 rpm were the favored rate for the harvesting process. It was shown in Fig. 4 that the harvesting had successfully achieved more than 95% of efficiency at this mixing rate (125 rpm). Therefore, mixing rate is an important factor in enhancing the harvesting efficiency. Thus, the agitation rate needs to be controlled even though the operating cost is not too costly.

3.2.3. Harvesting mechanism

As stated by Aljuboori et al. (2015), understanding the harvesting behavior and identifying the harvesting mechanism of bio-flocculant would improve the harvesting efficiency. Generally, flocculants may influence the flocculation process by several mechanisms namely sweeping, charge neutralization and bridging (Vandamme et al., 2014; Verma et al., 2012). According to Acharya et al. (2010), pH 5.0 may increase the enzyme production which β-glucosidase and pectinase. Hosseini Koupaie et al. (2019) also reported that this enzyme enhances the harvesting efficiency since substrate type for *A. niger* is macroalgae. However, from this study pH are not significant due harvesting process occurred in all tested pH. Furthermore, the mixing rate was likely to have a major influenced in microalgae harvesting since the mixing rate also had a significant impact on the charge neutralization and sweep

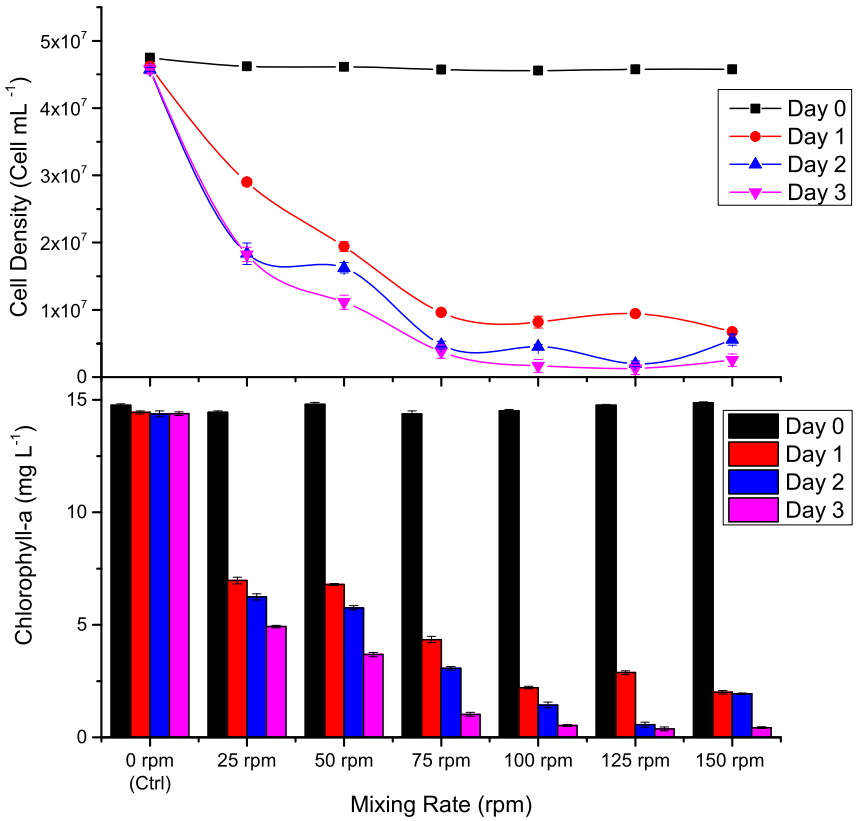

Fig. 4. Microalgae *Chlorella* sp. cell density and chlorophyll-a at various mixing rates throughout a 3-day harvesting period.

coagulation (Ahmad et al., 2011). This was likely because, higher mixing rate could develops shear forces and re-stabilization among the suspended microbial cells in the culture medium and the production could drops due to cell damages resulted from cell collision (Ibrahim et al., 2015).

On top of that, Sandri and Silveira (2018) reported that fungi belonging to the genus *Aspergillus* can be efficiently produced pectinolytic enzymes, which referred to as pectinases in submerged cultivation. This enzyme would promote the sweeping mechanism of coagulation-flocculation process (Vandamme, 2013). Based on macroscopic observations, no evidence of particle clusters supporting the hypothesis that sweep flocculation was the main removal mechanism. Sweeping flocculation for this study occurred when the microalgae biomass gets trapped in the soft pellets and settles down as shown in Fig. 5.

4. Practical applications and future research perspectives

The output of this study could provide a detailed description on the potential bio-flocculant, *A. niger* for harvesting microalgae biomass in natural and environmentally-friendly approach. Apart from that, by-product of the harvesting process also could market as high-value products. The harvesting microalgae using *A. niger* provides a strong foundation for the development of sustainable microalgae technology, zero-discharge green aquaculture wastewater treatment and

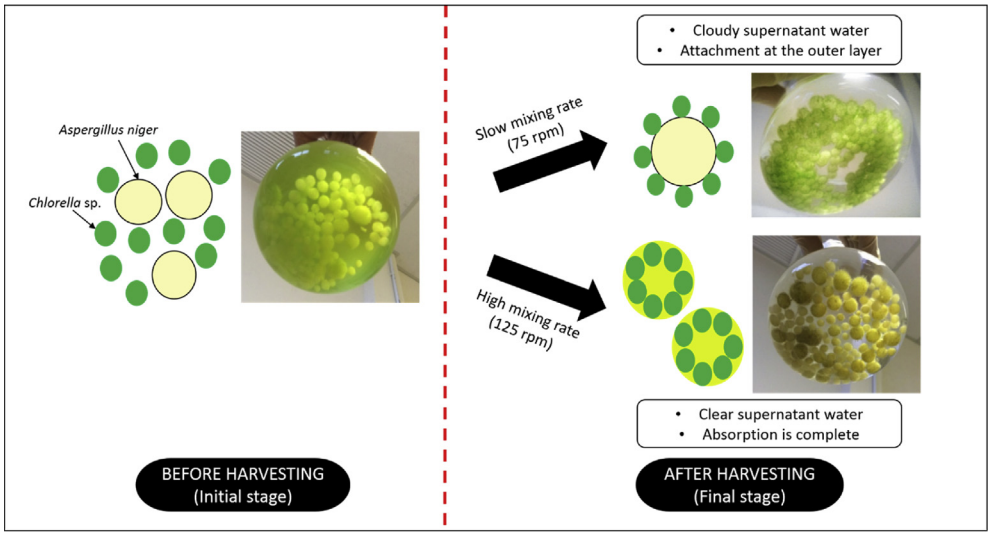

Fig. 5. Harvesting mechanism.

identification of suitable biological flocculant for microalgae biomass utilization.

Besides that, harvesting of microalgae biomass utilizing natural bio-flocculant will reduce the negative impact of aquaculture on the environment. In addition, this will also help in the production of valuable biomass which is highly potential to be used as biofuel feedstock, pharmaceutical products and bio-fertilizer that may reduce the cost of the related industry in Malaysia. The biological approach gives several advantages. Integration of microalgae and fungus harvesting would help in transforming the expensive conventional harvesting technique into green, low-cost and efficient harvesting approach.

5. Conclusion

This study reveals the capability of environmental friendly bio-flocculant, *A. niger* for harvesting *Chlorella* sp. through a harvesting process using *A. niger* in soft pellet form. The presents study indicated that the pH and mixing rate plays an important role in bio-flocculant production. Additionally, the optimal conditions for microalgae harvesting in terms of pH and mixing rates resulted in harvesting efficiency with > 85% and close to 100%, respectively. Hence, *A. niger* could be regarded as a novel bio-flocculant for harvesting microalgae. Successful application of *A. niger* as bio-flocculant would develop low-energy and chemical-free 298 sustainable microalgae harvesting.

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THE ROYAL SOCIETY PUBLISHING

The role of the gut microbiome in sustainable teleost aquaculture

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As the most diverse vertebrate group and a major component of a growing global aquaculture industry, teleosts continue to attract significant scientific attention. The growth in global aquaculture, driven by declines in wild stocks, has provided additional empirical demand, and thus opportunities, to explore teleost diversity. Among key developments is the recent growth in microbiome exploration, facilitated by advances in high-throughput sequencing technologies. Here, we consider studies on teleost gut microbiomes in the context of sustainable aquaculture, which we have discussed in four themes: diet, immunity, artificial selection and closed-loop systems. We demonstrate the influence aquaculture has had on gut microbiome research, while also providing a road map for the main deterministic forces that influence the gut microbiome, with topical applications to aquaculture. Functional significance is considered within an aquaculture context with reference to impacts on nutrition and immunity. Finally, we identify key knowledge gaps, both methodological and conceptual, and propose promising applications of gut microbiome manipulation to aquaculture, and future priorities in microbiome research. These include insect-based feeds, vaccination, mechanism of pro- and prebiotics, artificial selection on the holobiome, in-water bacteriophages in recirculating aquaculture systems (RAS), physiochemical properties of water and dysbiosis as a biomarker.

1. Introduction

Since its conception in the 1980s describing soil ecology [1], the term microbiome has evolved into an intensely studied area of research. In recent decades, this area has begun expanding from an anthropocentric and medically dominated field, into a taxonomically broad field, examining research questions in non-model species, from trees [2] to frogs [3], and increasingly, fish. The diversification in microbiome studies has been driven by increased access to next generation sequencing (NGS), a tool that is not reliant upon culture-based techniques, which often require previous knowledge of target microbes.

Currently, gut bacterial communities have been assessed in over 145 species of teleosts from 111 genera, representing a diverse range of physiology and ecology (figure 1*a*), often with similarities in bacterial phyla composition between fish species, dominated by Bacteroidetes and Firmicutes [5,6]. Non-model taxa from an array of aquatic ecosystems have had their gut microbiomes sequenced using NGS, with studies extending beyond species identification, into hypothesis testing which was once only feasible in model systems. Examples of studies on non-model teleost gut microbiomes range from those demonstrating rapid gut microbiome restructuring after feeding in clownfish (*Premnas biaculeatus*) [7] to the effect of differing environmental conditions, such as dissolved oxygen content, on the gut microbial diversity of blind cave fish (*Astyanax mexicanus*) [8].

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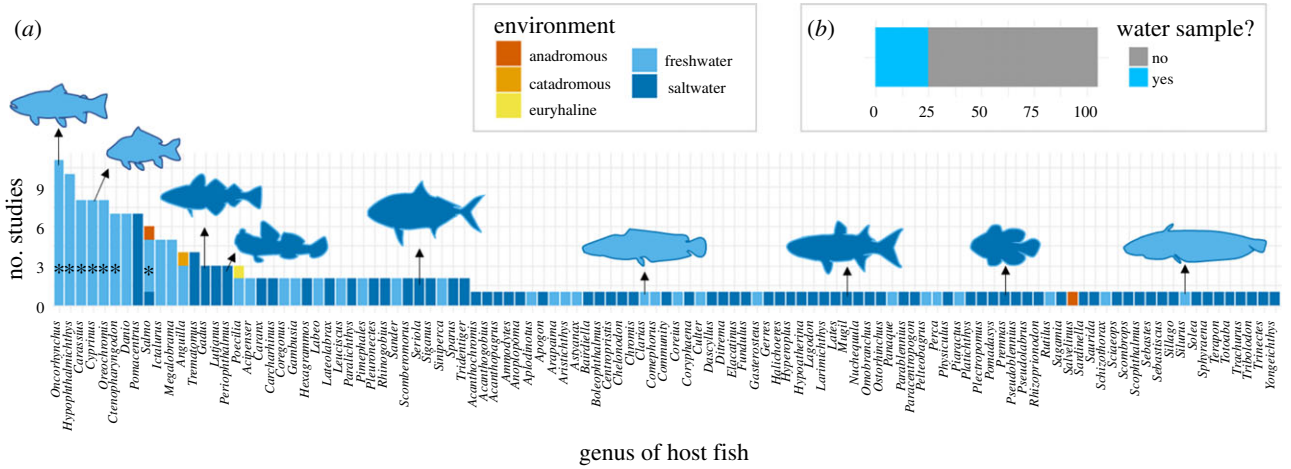

Figure 1. (*a*) Number of studies on the gut microbiome using NGS broken down by the genus of fish that the study was conducted on, as well as the environment those fish same from. Asterisk represents salmonid, carp and talapia. (*b*) The number of studies that assessed the water microbial communities. Gut microbiome studies were compiled using Web of Science [4] and only include studies that implemented NGS. It is acknowledged that total microbiome research extends further than this. Further information on search terms and filtering can be found in the electronic supplementary material. (Online version in colour.)

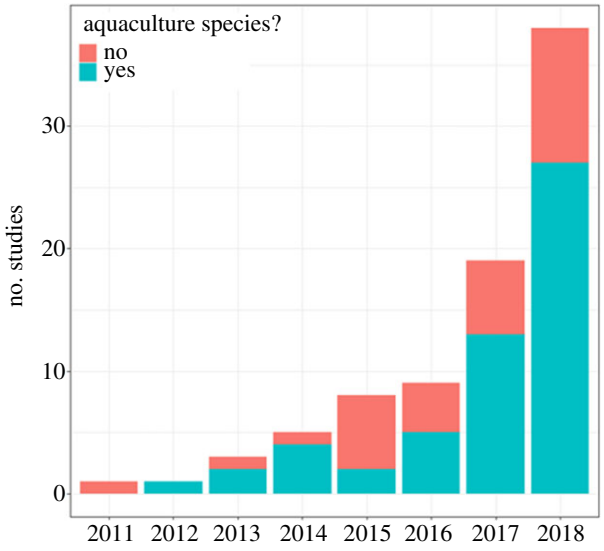

Figure 2. Growth in the studies using NGS on fish gut microbiomes, including food aquaculture species (aquaculture status taken from FishBase [12]). Further information on search terms and filtering can be found in the electronic supplementary material. (Online version in colour.)

Interest in the gut microbiome of fish has accelerated for many reasons, as not only do teleosts represent the most diverse vertebrate group [9], they are also of significant economic importance, including in aquaculture [10]. Aquaculture now provides over 45% of fish-based food products globally [11], and influence of the aquaculture industry on teleost gut microbiome research is demonstrated by the research questions tackled, with a clear bias towards salmonids (genera: *Oncorhynchus* and *Salmo*), carp (genera: *Hypophthalmichthys*, *Carassius*, *Cyprinus* and *Ctenopharyngodon*) and tilapia (genus: *Oreochromis*) (figure 2).

Rapid growth of the aquaculture industry has led to mounting pressure to make it more sustainable [13], and here we discuss four key components relevant to its sustainability in the context of the teleost gut microbiome: diet, immunity, artificial selection and closed-loop systems. We highlight some key deterministic factors important to aquaculture, although as shown in figure 3, there are numerous interacting ecological processes. More in-depth reviews focusing on these specific interactions are available, for example, interactions between the gut microbiome and the immune system [14], energy homeostasis [15] and physiology [16]. Understanding and manipulating microbial–host–environmental interactions (figure 3*a*) and associated functional capacity in these areas could contribute substantially towards achieving a more sustainable aquaculture industry. We identify potential for future research, both methodological and conceptual. Other microbiomes are known to impact host function, in particular, the skin microbiome and its relationship to immunity [17], however, due to their differing ecology [18] and aquaculture applications [19], the gut microbiome will remain our focus here.

2. Diet

The gut microbiome has long been linked with diet, yielding insights into the commensal relationship between certain microbes and host. It has been shown that the teleost gut microbiome produces a range of enzymes (carbohydrases, cellulases, phosphatases, esterases, lipases and proteases) which contribute to digestion [10,20]. More intimate relationships also exist, for example, anaerobic bacteria in the teleost gut have a role in supplying the host with volatile fatty acids [21], an end product of anaerobic fermentation that provides energy for intestinal epithelial cells [22]. Gut microbes also synthesize vitamins and amino acids in the gut of aquatic vertebrates [23,24]. For example, the amount of vitamin B_{12} positively correlated with the abundance of anaerobic bacteria belonging to the genera *Bacteroides* and *Clostridium*, in Nile tilapia (*Oreochromis niloticus*) [25]. Here, we discuss this host–microbe relationship in the context of contemporary aquaculture, with a focus on two timely issues: fishmeal and starvation.

(a) Fishmeal

Fishmeal is an efficient energy source containing high-quality protein, as well as highly digestible essential amino and fatty acids [26], which is included in feed for a range of teleost species. Fish used in fishmeal production is, however,

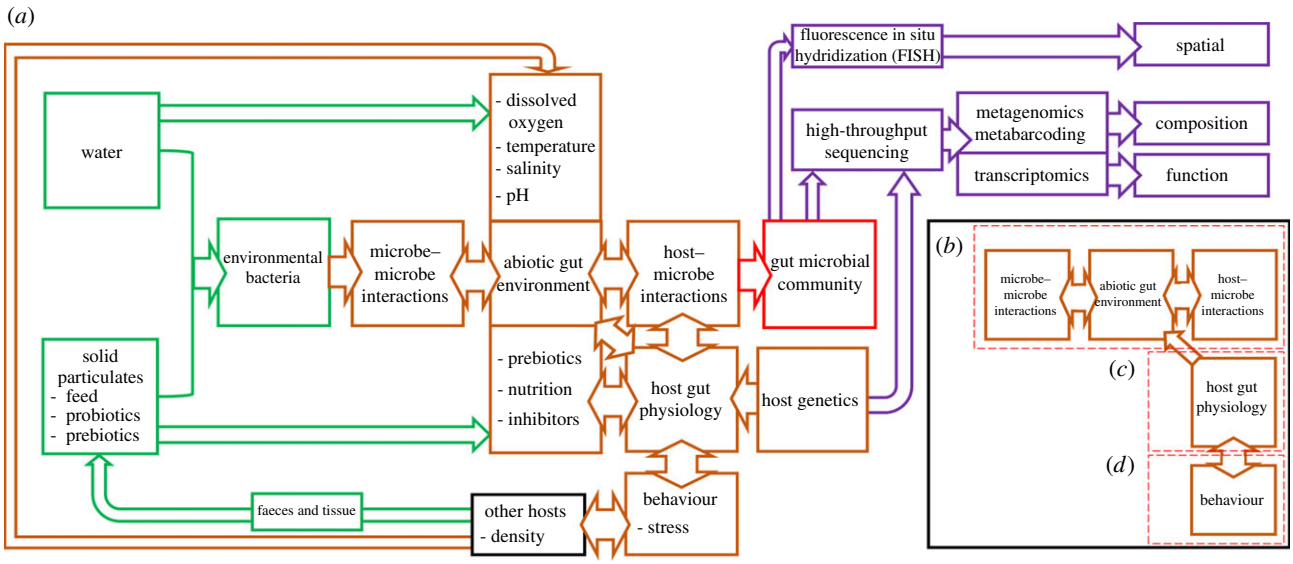

Figure 3. (*a*) Schematic view of the deterministic processes that influence gut microbial communities in fish. Community assemblage of bacteria in the gut starts with inputs from the environment (green), such as the bacteria within the water column, or in solid particulates of biofilm, sediment and feed. Once ingested, these bacteria are influenced by interacting deterministic processes (brown) such as the host's abiotic gut environment, interaction with the hosts' physiology through the gut lining and its secretions, as well as interactions between other microbiomes. The outcome (red) is final community assembly, which can be characterized using an array of cutting-edge molecular techniques (purple). A subset of the boarder interactions is provided, with focus on (*b*) microbe–environment–host interactions, (*c*) host gut physiology and (*d*) behaviour. (Online version in colour.)

predominantly sourced from capture fisheries, putting pressure on already overfished stocks [13]. Despite a global decrease in fishmeal production, from an average of 6.0 million tonnes between 2001 and 2005 to 4.9 million tonnes between 2006 and 2010 [27], and growth in plant-based substitutes (e.g. wheat gluten, soya bean protein and pea protein), some aquaculture species still require a proportion of fish-sourced amino acids and proteins [28].

As dietary changes can alter the fish gut microbiome [29], there has been a considerable rise in the number of studies investigating the influence of alternative plant-protein sources on host–microbe interactions. Plant-protein sources have been shown to disturb the gut microbiota of some fish, with the production of antinutritional factors (factors that reduce the availability of nutrients) and antigens, impeding host resilience to stress [30], metabolism [31] and immune functioning [32]. Fish fed plant-protein-based diets can exhibit alterations in their intestinal morphology including disruption to the lamina propria and mucosal folds [33], which may modify attachment sites for commensal bacteria [34], and can therefore impact microbial composition [32,35].

Insect meal is increasingly used in aquafeed as a protein source with a high nutritional value [36], and several studies have demonstrated its potential use in manipulating the gut microbiome in fish [37,38]. As insects are chitin rich, these diets have been associated with prebiotic effects, through increased representation of beneficial commensal bacteria such as *Pseudomonas* sp. and *Lactobacillus* sp., which in turn improves performance and health in some fish [37]. Despite this, however, the beneficial effects of chitin are species specific, with Atlantic cod (*Gadus morhua*) and several cyprinid species demonstrating increased growth rates on diets with varying levels of chitin, whereas tilapia hybrids (*O. niloticus* × *O. aureus*) and rainbow trout (*Oncorhynchus mykiss*) both display decreased growth rates [39]. Chitin can therefore not be described as a probiotic for all species. The influence of insect meal on microbial-mediated functions also remains underexplored, with little known about the extent to which species-specific responses to a chitin-rich diet are microbially mediated [40], offering scope for future research.

(b) Starvation

Starvation is common in the production of valuable species such as salmon [41], sea bream [42], halibut [43] and cod [44], prior to handling, transportation and harvest, but is also used as a method to improve fillet quality. However, starvation is likely to have a substantial impact on host–microbe interactions (figure 3*b*). Gut microbial communities of the Asian seabass (*Lates calcarifer*), for example, shifted markedly in response to an 8-day starvation period, causing enrichment of the phylum Bacteroidetes, but a reduction of Betaproteobacteria, resulting in transcriptional changes in both host and microbial genes [45]. Perturbation to the gut microbiome could lead to the opening of niches for other commensal or even pathogenic bacteria [46], especially if this is combined with the compromised immune system of a stressed host [47] (figure 3*d*). Even if all fish are terminated shortly after starvation, gut microbial community changes before termination could cause long-term impacts to the microbial composition of water and biofilters in closed recirculating aquaculture systems (RAS). RAS systems will be discussed in greater detail later in this review.

3. Immunity

Gut microbial communities have strong links to immunity [48], which is pertinent in fish as they are in constant contact with water, a source of pathogenic and opportunistic commensal microbes [49]. In addition to this, fish cultured intensively are often stocked at high densities, allowing for easier transmission of microbes. Therefore, a microbially diverse gut microbiome in aquaculture is important to prevent unfavourable microbial colonization [50], and although the mechanisms are not fully

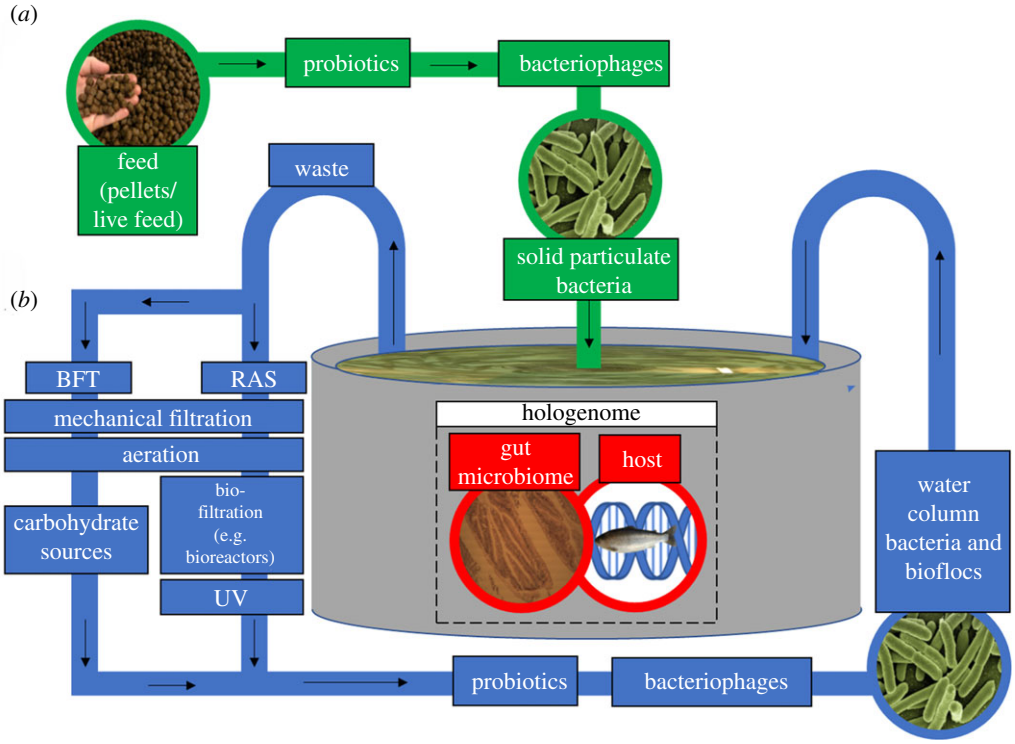

Figure 4. Schematic diagram of (*a*) feed inputs (green), (*b*) water processing (both RAS and BFT) (blue) and the (*c*) species being cultivated, along with its gut microbiome (red). (Online version in colour.)

understood, some key processes have been identified. For example, *Bacillus* and *Lactobacillus*, two common probiotic genera of bacteria used in aquaculture, are able to stimulate expression of inflammatory cytokines in the fish gut [51], increase the number of mucus layer producing goblet cells [52] and increase phagocytic activity [53]. Furthermore, comparison in gene expression between gnotobiotic zebrafish (*Danio rerio*) and conventionally reared zebrafish has shown bacteria induced expression of myeloperoxidase, an enzyme that allows neutrophil granulocytes to carry out antimicrobial activity [54]. Colonizing microbes can also modulate host gene expression to create favourable gut environments, thereby constraining invasion by pathogens [23], while also promoting expression of proinflammatory and antiviral mediators genes, leading to higher viral resistance [55]. Reducing viral and bacterial pathogens, such as *Vibrio* sp. and *Aeromonas* sp., is important for fish health in aquaculture, and will be discussed further in the context of closed-loop systems later in the review.

The interaction between the gut microbiome and the immune system is bilateral, for example, secretory immunoglobulins in fish recognize and coat intestinal bacteria to prevent them from invading the gut epithelium [56]. Similarly, in wild three-spined stickleback (*Gasterosteus aculeatus*), a causal chain (diet → immunity → microbiome) was discovered, demonstrating the impact of diet on fish immunity and thus the microbial composition of the gut [57]. Understanding microbial–host–environmental interactions like this are crucial for aquaculture, where, as previously discussed, diet is often manipulated.

(a) Antibiotics

As most antibiotics used in aquaculture display broad-spectrum activity, they can affect both pathogens and non-target commensal microbes [58]. Oxytetracycline is one of the most widely used veterinary antibiotics, with 1500 metric tonnes applied between 2000 and 2008 to salmon aquaculture in Chile [59]. However, oxytetracycline was seen to reduce gut microbial diversity in Atlantic salmon (*Salmo salar*), while enriching possible opportunistic pathogens belonging to the genus *Aeromonas*, and leading to a high prevalence of multiple tetracycline resistance-encoding bacterial genes [60]. Long-term exposure to oxytetracycline has also been reported to negatively affect growth, immunity and nutrient digestion/metabolism in Nile tilapia (*O. niloticus*) through antibiotic-induced disruption to the microbiota [61], causing considerable changes in the representation of Bacteroidetes and Firmicutes.

Vaccination has become a widespread prophylactic measure applied in aquaculture to improve immune functioning and disease resilience in farmed fish [62]. One study attempted to identify potential alterations in the microbiota structure and localized immune responses caused by a novel recombinant vaccine against *Aeromonas hydrophila* in grass carp (*Ctenopharyngodon idella*) [63]. Results from their study suggest that oral vaccines can target *Aeromonas* sp. through activation of innate and adaptive immune defences within the intestine without causing large disturbances in non-target microbiota populations. Given the importance of the immune response in regulating the gut microbiome [64], only a small number of studies have investigated the influence of vaccines on the resident microbiota composition and function in fish, providing grounds for future study.

(b) Pro- and prebiotic supplementation

In view of the challenges associated with antibiotics, studies have examined the impact of alternative, prophylactic measures such as pro- and prebiotics (figure 4*a*). As literature on the types of pro- and prebiotics used in aquaculture have been reviewed elsewhere [65,66], as well as their effectiveness [67,68], we focus here on the ability of these compounds to induce changes in host physiology and function through shifts in the gut

microbiome. As has already been discussed, *Bacillus* sp. and *Lactobacillus* sp. have a beneficial effect on immunity and are suggested to provide an alternative approach to controlling disease in aquaculture. Targeted microbiota manipulation using these same bacteria have also been reported to exert beneficial effects on fish growth through (i) alterations in gut morphology [69], leading to improved digestion and metabolism [70] and (ii) microbial-mediated regulation of the genetic components involved in growth and appetite control [71,72]. Recently, the establishment of *Lactobacillus* probiotic bacteria within the gut microbiota was also associated with improved learning/memory capacity and changes in shoaling of zebrafish [73,74], indicating a potential gut–brain interaction pathway similar to what is described in higher vertebrates [75].

Research into the modulation of gut microbial communities using prebiotic compounds has expanded also. Certain dietary components have been reported to induce changes in gut morphology within the fish host, including vacuolation of enterocytes [76] and enhancing mucosal barrier integrity [77]. Improved mucosal protection and disease resilience are thought to be driven by microbes and associated microbial metabolites. Several prebiotics have been reported to manipulate the resident microbiota community of a host in favour of Firmicutes and short-chain fatty acid producing communities [78]. Mechanistic pathways remain elusive, however, with additional research required.

4. Artificial selection

Within aquaculture, selection has been applied routinely to increase production by enhancing desirable traits such as growth and disease resilience [79,80]. Recent evidence suggests, however, that host genetics plays a fundamental role in determining the gut microbiota in fish [81]. The 'hologenome' concept proposes that the host organism, along with their commensal microbial community, form one unit of selection [82]. Host physiology, for example, is determined in part by the host's genome and has the ability to shift gut microbiome composition, as demonstrated in zebrafish, whereby host neural activity and subsequent gut motility is able to destabilize microbial communities [46] (figure 3*c*). Although not described in teleosts, the reverse has also been seen, whereby microbial communities are able to regulate the host's gut through: (i) serotonin signalling [83,84], (ii) macrophages and enteric neurons interactions [85], (iii) metabolism of bile salts [86] and possibly, (iv) metabolism of short-chain fatty acids such as butyrate [87]. The host–microbe relationship means that traits selected during breeding programmes may be traits from the hologenome. Pyrosequencing studies have also shown significant changes in the microbial community composition of genetically improved fish compared with domesticated individuals [88,89]. Artificial selection has also been demonstrated on single species of bacteria, with *Aeromonas veronii* selected to exhibit greater colonization success in gnotobiotic zebrafish [90]. Environmental filtering of the reservoir of bacteria surrounding the fish generates the potential for improving colonization success of commensal bacteria. Currently, bacterial communities selected by breeding programmes could be neutral, sympathetic or antagonistic to the goals of artificial selection, and understanding this relationship will be vital in manipulating the hologenome.

5. Closed aquaculture systems

Many environmental problems plague current aquaculture practices. In addition to those already discussed, there are also issues with parasite transmission to wild fish [91], interactions between wild and escaped farmed fish [92], and release of faeces and excess feed into the environment [93]. One way to better control these problems is to remove aquaculture from ecosystems and bring it into a land-based setting [94].

(a) Manipulating environmental microbiota

RAS and biofloc technology (BFT) are forms of aquaculture which use microbial communities to minimize excess nutrients and pathogens in rearing water (figure 4). In these systems, microbial reconditioning of the rearing water is vital as fish are stocked at high densities, resulting in elevated levels of organic material, which can promote microbial growth [95]. Selection of competitive, slow-growing K-strategist bacteria shifts the community from autotrophy to heterotrophy activity. Such shifts allow for a microbial community which maintains both water quality, through nutrient recycling, and inhibits the growth of fast-growing, opportunistic r-strategists, which include many bacterial pathogens such as *Aeromonas* sp. [96,97]. RAS and BFT could therefore be combined with vaccination against bacterial pathogens such as *Aeromonas* sp., as previously discussed, to reduce infections. The selection of K-strategist microbial communities differ between RAS and BFT. In RAS; K-selection is achieved by passing rearing water through heterotrophic biofilters [98], whereas in BFT, a high carbon to nitrogen ratio within rearing water is conditioned by the addition of carbohydrate sources, favouring heterotrophic K-strategist bacteria [99]. High-carbon conditions in BFT systems also promote nitrogen uptake into microbial biomass, which forms protein-rich bacterial 'flocs' that supplement feed [100].

Manipulation of microbes associated with live feed cultures is critical to the production of fish larvae as live feeds often contain opportunistic pathogens (figure 4*a*), resulting in stochastic mortality [64]. While traditional approaches involve non-selective, temporary methods (i.e. physical/chemical disinfection [101]), more recent efforts have shifted towards targeted manipulation through probiotics, for example, the successful use of *Phenylobacterium* sp., *Gluconobacter* sp. and *Paracoccus denitrificans* in rotifer (*Brachionus plicatilis*) production [102]. Lytic bacteriophages have also proven somewhat successful in reducing the prevalence of opportunistic pathogens, such as *Vibrio* sp. [103–105]. Live feed also appears to play a critical role in the delivery and establishment of colonizing gut microbiota in fish larvae upon first feeding [106]. Supplementation of live feed cultures with beneficial microbes, such as the previously mentioned *Lactobacillus* spp. and *Pediococcus* sp., has become common practice in hatcheries, with beneficial effects on growth, mucosal immunity and stress tolerance of larvae [17,107,108]. Bacteriophages and probiotics have also been applied directly to tank water (figure 4*b*); probiotics such as *Bacillus* spp. preventing fish mortality from *Vibrio* spp. infections [109] and *Flavobacterium columnare*-infecting phages have been shown to persist in RAS for up to 21 days [110]. Far less is known about the application of probiotics directly to tank water when compared with feed application [111]; however, and the use of bacteriophages is still in its infancy, providing potential for future research.

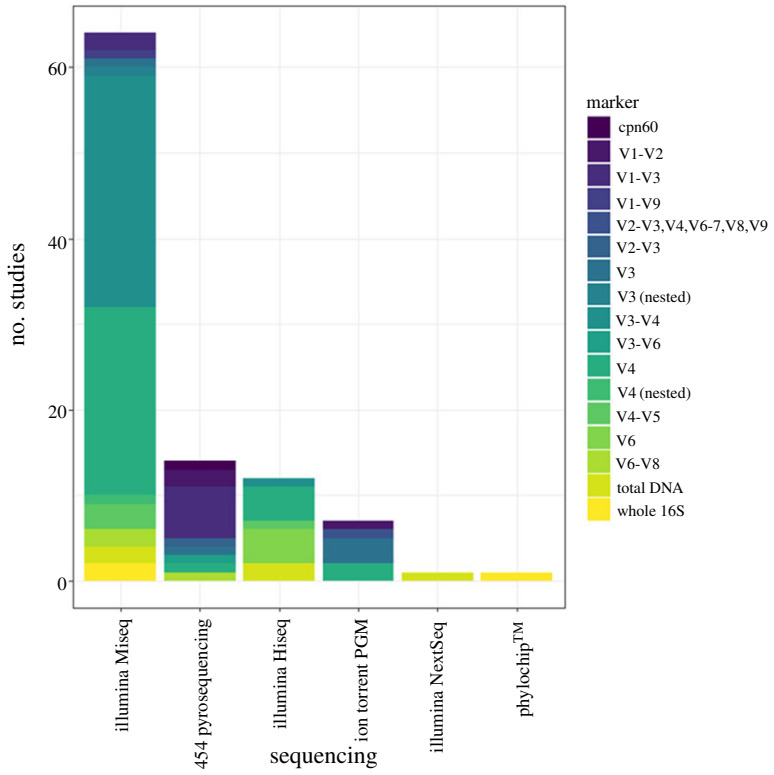

Figure 5. Methodological approaches used in high-throughput sequencing of fish gut microbiomes, broken down by the type of sequencing platform and genetic marker. Marker types are predominantly variable regions (V) within the 16S ribosomal RNA gene. Further information on search terms and filtering can be found in the electronic supplementary material. (Online version in colour.)

(b) Controlling environmental variables

Changes in abiotic conditions in the water column propagate into the gut, as seen with dissolved oxygen concentration [8]. Such parameters are hard to control within the natural environment, but closed-loop systems provide consistent abiotic conditions, and allow for other variables, such as hologenome (figure 4*c*), to be manipulated with greater ease. The effect of many important physiochemical water properties (e.g. nitrate, ammonia and phosphate) on the teleost gut microbiome has not been studied, however, let alone how these properties interact [112]. Salinity is another important physiochemical property for the gut microbiome in many aquaculture species. When Atlantic salmon transition from freshwater to saltwater, individuals can experience a 100-fold increase in gut bacteria, combined with a shift in dominant microbial taxa [113]. Increasing salinity in RAS systems can, however, negatively impact nitrate removal in bioreactors [114], highlighting the importance of understanding interacting physiochemical properties.

(c) Dysbiosis as a stress biomarker

The use of closed-loop systems is a progression to a more intensive method of aquaculture, mirroring the progression seen in animal agriculture, and a crucial element to sustainable intensification is welfare. It is possible to measure fish welfare through physiological and behavioural indicators, with a current focus on identifying stress. The microbiome has been identified as another potential biomarker [64] due to its interaction with the host immune system, and its responsive nature to stressors [115,116]. Therefore, identifying imbalances in the gut microbiome, or dysbiosis, could be a useful predictor of stress-related syndromes, which could ultimately lead to mortality. Using non-invasive faecal samples could complement other non-invasive stress biomarkers, such as water cortisol [117], allowing for the optimization of husbandry, alerting operators to chemical (e.g. poor water quality, diet composition imbalance, accumulation of wastes), biological (e.g. overcrowding, social dominance, pathogens), physical (e.g. temperature, light, sounds, dissolved gases) or procedural (e.g. handling, transportation, grading, disease treatment) stressors [118]. More research is needed, however, in assessing the reliability and accuracy of faecal microbiome sampling in identifying stress.

6. Conclusion and future applications

The teleost gut microbiome has a clear role in the future of aquaculture, and although research has come a long way in recent decades, there are still many areas of gut microbiome research that require further development. As highlighted in figure 1*b*, there are still key elements lacking from many studies, particularly those assessing metacommunity composition, with the lack of water samples being particularly glaring. The ability to sample the environmental metacommunity with ease is one of the strengths of using a teleost model. Another methodological problem that will hinder comparability, reproducibility and metanalysis of fish gut microbiome datasets is the varying degree of sequencing platforms and markers (figure 5). A solution to this problem would be to focus on one marker, and one sequencing platform, with many metabarcoding microbiome studies adopting the V3 and V4 regions, sequenced on Illumina platforms. It is noted, however, that different markers and sequencing platforms work better in some systems with no simple fit-all approach. Therefore, tools that incorporate differences in taxonomic

identification that arise through using different methodological approaches will be vital in comparing datasets.

Current findings, as summarized here, show that the teleost gut microbiome plays an important role in aquaculture, however, the literature is dominated with studies performed on mammals, leading to limited data on functional capacity of fish gut microbiomes [64]. Furthermore, a knowledge gap exists between ascertaining the composition of the microbiome and understanding its function, partly due to the complexity and variability in the ecology of teleost gastrointestinal tracts [119] and unknown bacterial taxa. More specifically, however, it has been caused by the lack of synthesis between multiple cutting-edge molecular techniques. Progression in teleost gut microbiome research will depend on combining function (RNA sequencing), composition (metabarcoding and metagenomics) and spatial distribution (fluorescence *in situ* hybridization). Understanding host genetic diversity (population genomics) and expression (RNA sequencing) of that diversity, all while incorporating environmental variation, will also be vital.

Finally, there are many areas in which synergies between gut microbiomes and aquaculture can be made. These have been highlighted through the review, but, in summary, include a better understanding of the gut microbiome with respect to insect-based feeds, vaccination, mechanism of pro- and prebiotics, artificial selection on the hologenome, in-water bacteriophages in RAS/BFT, physiochemical properties of water and dysbiosis as a biomarker.

Data accessibility. All data collected in the systematic review can be found in the electronic supplementary material.

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