REVIEWS IN Aquaculture

Received: 23 January 2021 | Revised: 8 March 2021 | Accepted: 21 April 2021

DOI: 10.1111/raq.12571

REVIEW

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Genome editing on finfish: Current status and implications for sustainability

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Funding information
Norges Forskningsråd, Grant/Award Number: 295094 and 301401

[Correction added on 31 May 2021, after first online publication: Affiliation 2 has been added for the author Torill Bakkelund Blix and the city for affiliation 3 has been changed from Tromsø to Bergen.]

Abstract

Novel genome editing techniques allow for efficient and targeted improvement of aquaculture stock and might be a solution to solve challenges related to disease and environmental impacts. This review has retrieved the latest research on genome editing on aquacultured finfish species, exploring the technological progress and the scope. Genome editing has most often been used on Nile tilapia (*Oreochromis niloticus* Linnaeus), followed by Atlantic salmon (*Salmo salar* Linnaeus). More than half of the studies have focused on developing solutions for aquaculture challenges, while the rest can be characterized as basic research on fish genetics/physiology or technology development. Main traits researched are reproduction and development, growth, pigmentation, disease resistance, use of trans-GFP and study of the omega-3 metabolism, respectively. There is a certain correlation between the species identified and their commercial relevance, indicating the relevance of most studies for present challenges of aquaculture. Reviewing geographical origin of the research, China has been in the forefront (29 publications), followed by the United States (9) and Norway (7). The research seems not to be dependent on regulative conditions in the respective countries, but merely on the purpose and objectives for the use of genome editing technologies. Some technical barriers identified in the studies are presented together with solutions to overcome these-off-target effects, ancestral genome duplication and mosaicism in F0. One of the objectives for use is the contribution to a more sustainable aquaculture, where the most prominent issues are solutions that contribute to minimizing impact on biodiversity.

KEYWORDS
CRISPR, finfish aquaculture, genome editing, GMO regulations, off-target, sustainability

1 | INTRODUCTION

Aquaculture is the fastest growing food production industry on a world basis. In 2018, the share of aquaculture in total fish production was 46%.[1] Even though the production is growing, the negative effects of this industry often receive much attention. These challenges include diseases, escapees and ecological effects.[2] In Norway, the first-hand value of Atlantic salmon was 68 billion NOK in 2019,[3] and

Norway accounts for over 50% of the world's total production of Atlantic salmon (*Salmo salar* Linnaeus).[4] Despite being highly economically viable and providing working opportunities and export revenues, salmon production is subject to controversies rooted in the challenges the industry faces related to environmental impacts and animal welfare, thus hindering sustainable development.[5,6]

The development of a more efficient aquaculture requires increased utilization of available genetic resources.[7] This includes use of valuable genetic material within selective breeding as for example marker-assisted breeding.[8] Genetic resources are also very useful for introduction, removal or single base exchange using genome editing (GE).[5,9,10] The use of GE demonstrates some promising possibilities for improvement of the aquaculture stocks,[11] with impacts for sustainable and efficient aquaculture.[5] The first approaches using genome editing included techniques as zinc finger nucleases (ZFN) and transcription activator-like endonucleases (TALEN). At present, the most novel method, the clustered regularly interspaced palindromic repeats (CRISPR) system, dominates. This system offers the possibilities of making small changes by fixing alleles and changing trait loci.[9] The CRISPR system is at present considered to be the most efficient, targeted and affordable genome editing technique.[12-14]

Further expansion of the aquaculture production, with the aim to meet future need for food and economic growth, requires contribution to sustainable development. Sustainable development was originally defined by the Brundtland Commission as the '[…] development that meets the needs of the present without compromising the ability of future generations to meet their own needs'.[15] In 2015, the UN set out the 17 common sustainable development goals (SDGs). These were based on the thoughts from the Brundtland Commission – and are common guidelines on how to achieve a sustainable world. The goals are integrated in each other, emphasizing that *everything depends on everything*, and provide a balance where the three dimensions of sustainable development, environmental, economic and social, co-exist.[16] According to Stockholm Resilience Center, food connects all the SDGs.[17] Aquaculture and fisheries are both crucial for future food security, and '[…] offer development pathways to contribute to a more prosperous, peaceful and equitable world'.[1] It is therefore also of crucial importance that new solutions like genome editing can be used in sustainable manners.

Here, we present findings from a systematic review on the current status of genome editing in aquacultured finfish species, hence extending previous reviews.[5,9,18,19,20,21,22] As published in the previous reviews,[5,9,22] there is still a high focus on reproductive traits, but this has recently been expanded to include genes related to other production traits such as disease resistance.

The geographical origin of the research and innovation activities using GE on aquaculture finfish has also been reviewed. In addition, we have compared the number of reports wherein genome editing is used on a specific fish species with the commercial relevance of the species in aquaculture. In the systematic review, several of the identified studies have included some discussion of technical barriers by genome editing including off-target effects, which is highlighted here with the potential solutions. These challenges are also of regulatory relevance and need to be addressed by concrete regulatory approaches.[23,24] Regulatory approaches and concerns have just been briefly discussed in previous studies.[7,21,25] Here, we describe the regulatory approaches in the main countries researching genome editing on aquacultured finfish, and whether the countries have included non-safety factors, as contribution to sustainability, socio-economic and ethical aspects, in assessment of genetically modified organisms (GMOs). Norway is one of the countries which have included non-safety criteria in the regulation of GMOs. Here, we briefly elaborate on how the Norwegian impact assessment regulation can be used for a sustainability assessment of genome edited aquacultured finfish species.

1.1 | Genome editing technologies

Since the discoveries of the DNA structure and function, further research has focused on the ability to modify gene sequences. Enzymes like polymerases, ligases and restriction endonucleases provide the ability to make changes through cutting and ligating, and the polymerase chain reaction (PCR) offers isolation of fragments. Repairing lethal DNA breaks is inherent in cells endogenous machinery. Thus, combining the possibility to both introduce breaks at the desired sequence and cellular self-repair is the foundation for GE.[26]

During the last 20 years, several new techniques for modifying DNA have emerged, both oligonucleotide-directed mutagenesis-based techniques (ODM) and nuclease-mediated site-specific mutagenesis techniques. In this review, we focus on targeted alterations of the fish genome and the site-specific nucleases (SSN), while also recognizing ODM-related activities such as RNA interference (RNAi). There are four categories of site-directed nucleases: meganucleases, ZFN, TALEN and CRISPR.[27]

ZFN is composed of modular DNA recognition proteins.[22] When associated with restriction enzyme FokI, the complex can be designed to recognize specific chromosomal sequences of 9–18 nucleotides, and at dimerization, the FokI enzyme can induce double-strand breaks (DSB).[26] Use of ZFN was established in 1996 and its use within research increased from 2003. The method was hampered by difficulties of design and validation of proteins for specificity in the complex. In addition, ZFN had low efficiency with very few mutations in F0 generation (parent generation), leading to low transmission to F1 generation (first filial generation). These challenges lead to a newer tool emerging in 2010/11, TALEN. As with ZNF, TALEN is using the restriction enzyme FokI and the cleavage requires dimerization. TALEN is, however, easier to design and validate than ZFN and recognizes fewer nucleotides, thus being more efficient than ZFN. The protein design, synthesis and validation are, however, still not efficient enough which hampers widespread use of this tool. All the site-directed nucleases use the organisms repair system to induce either site-specific mutations (insertion or deletion, *indels*) or insertions of new sequences.[27] The most recent technology, CRISPR/Cas nucleases emerged as late as 2012/13[26] and are molecular features of bacteria and archaea for recognition, thus

protection against virus infection.[28] This system is RNA-mediated and performs sequence-specific detection and silencing of foreign nucleic acids. The CRISPR system is organized with the Cas proteins (CRISPR-associated proteins) encoded in operons and 'CRISPR arrays consisting of genome-targeting sequences (called spacers) interspaced with identical repeats'.[29] The repeats are short fragments from foreign nucleic acid that has entered the cell (e.g. by infection of viruses).[26] In the genome editing system, guide RNAs (gRNA) lead the CRISPR system to the target DNA sequence and cleave the target site by the nuclease. The first studies of the CRISPR/Cas system were performed in 1987, while the first publication on CRISPR system for GE was published in 2012.[29]

The nuclease-mediated site-directed techniques ZFN, TALEN and CRISPR induce a DSB at a specific site in DNA. This stimulates natural repair mechanisms. One repair mechanism is non-homologous end-joining (NHEJ), which induces random point mutations, inserting or deleting material (indels). Alternatively, if a donor DNA strand homologous to the sequences bordering the DBS is provided, a homologous directed repair (HDR) will happen. The type of donor determines the type of repair, insertion or replacement of a sequence within the DBS, correction of a base or deletion of a sequence.[9,27,30] The mutations lead to either knockout (KO) or knock-in (KI) of a gene or DNA sequence.

1.2 | Genome editing in aquacultured finfish

As well as being an important research tool, CRISPR could provide an efficient way to expedite genetic improvement of farmed animals. Aquatic animals are easy to work with compared to many terrestrial species due to high fertility rates, short generation time and external fertilization.[9] In 2015, Ye et al.[21] reviewed different fish breeding methods and pinpointed CRISPR system as promising for '[…] efficiency, precision and predictability […]' in fish aquaculture. This was later followed up by Zhu and Ge[22] which published a study on recent advancements in genome editing on finfish, focusing on reproductive traits.[22] Other possibilities were later presented by Gotesman et al.[19] where genome editing and RNAi were pointed out as useful therapy tools for combating pathogens in aquaculture. A concomitant review by Elaswad and Dunham[18] described how different genetic and genomic tools for disease reduction in aquaculture could be achieved by the CRISPR/Cas system. They also highlight the possibility for knock-in (KI) procedures and to the benefits by the combination of genome editing and selective breeding.[18]

The increased speed of technology development within gen(ome) sequencing has aided the rapid development of genome editing technologies. Houston and Macqueen[20] reviewed the exploitation possibilities from sequencing and annotation of the Atlantic salmon genome. They build from Lien et al.[31] which was part of the Salmon Genome Project and had a special focus on the ecology, physiology and evolution of the salmon genome as well as highlighting further possibilities by genome editing. Wargelius[5] focused on sustainability issues related to Atlantic salmon production and other relevant solutions that genome editing may offer. Subsequently, Gratacap et al.[9] published a review on current technical possibilities that genome editing offers for aquaculture species globally. The latter publication listed 21 studies where genome editing was used (successfully) on different aquaculture species (including one oyster species) and categorized the solutions according to traits. To present the current and future status of use of genome editing on aquaculture finfish, we have performed a systematic literature review.

2 | METHOD

The methodological approach used for the systematic literature search is based on relevant items from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[32]

2.1 | Search strategy

For identification of relevant scholarly articles, Google Scholar (GS) and Web of Science (WoS) were used as databases. Search strings included relevant terms as genome editing, aquaculture and aquaculture finfish species (Appendix 1). Only searches that had lower numbers of results (>700) were followed up to collect articles. During the search for articles on use of CRISPR in aquaculture fish species, both publications presenting experimental results and review articles were included. The WoS search included articles from 1995 to 2021 (as of 15.02.21) in order to include work using the ZFN or TALEN technologies. The GS search was restricted to get scholarly articles from the period 2015 to 2021 (as of 15.02.21) to narrow down the result list. Using GS, the retrieved articles were often duplicated since they were from different websites and often composed of newspaper/magazine articles or master theses, while WoS allowed for more precise search (e.g. no newspaper/magazine articles or master theses). Different search strings were also used (see Appendix 1). One search string contained a list of the major aquaculture finfish species given by FAO.[33] These 20 fish species made up 84,2% of total aquaculture production worldwide.[33] An updated list was published in May 2020[1] after the first searches were performed, but it did not contain any significant changes compared to the list of 2018.

The initial identification of articles was mostly based on titles. After identification, each of the abstracts was screened for exclusion records (see Appendix 1). Different exclusion criteria were made because the two databases yielded different types of output lists. This was followed by merging all retrieved scholarly articles having an experimental approach into one list, and any duplicates were removed.

2.2 | Grouping of data

The strategy for grouping the data was done inspired by Catacora-Vargas et al.[34] in order to identify the direction and location of the genome editing field associated with aquaculture finfish species. The

review articles were used as supplements in the current work and were not analysed to the same detail as the experimental articles.

The data in the experimental articles were grouped after: species, objective of the study, trait, gene(s), type of genome editing results (NHEJ/HDR) and institutional affiliation of 1st author. The search for technical barriers in the articles was done through searching for relevant terms in all articles and then coding relevant paragraphs in NVivo 12.6.0 software, followed by analysis of the coding book.

3 | RESULTS

The GS searches dated 06.01.20 and 13.01.20 retrieved 295 and 673 results, with 25/27 and 48/38 relevant empirical/review articles, respectively. The GS search dated 15.02.21 retrieved 170 results with 9/2 relevant empirical/review articles. The searches were performed with different search terms. The two WoS searches dated 12.03.20 and 15.02.21 retrieved 73 and 25 results, with 30/8 and 16/0 relevant empirical/review articles, respectively (see Appendix 1). After comparing the lists with reports based on empirical work, the total number of empirical articles found was 56. Table 1 presents the resulting papers included in this review. The CRISPR/Cas system dominated the field of genome editing on aquacultured finfish (Figure 1). We found two scholarly publications using ZFN,[35,36] one study using TALEN[37] and two studies using both TALEN and CRISPR.[38,14] Use of the CRISPR technology was found in 52 publications. Publications using ZFN were not found after 2016 and TALEN not after 2018. The results from the search showed that publications on GE of aquaculture species emerged from 2012, however, cascade reading has also revealed one paper from 2011. This paper was not included in our study. The number of publications per year increased from 2012 to 2020 (Figure 1). The highest number of reports was published in 2020, and in addition, a publication peak was observed in 2016. The high number of reports using CRISPR compared to other methods supports the increasing interest in CRISPR, which may be due to availability, efficiency and affordability of the technology compared to the other two. This may also reflect the high number of publications in 2016 compared to 2015, considering the development of the CRISPR method from 2012 to 2013. As by the 15th of February 2021, four reports have already been published in 2021, indicating that the number of publications in 2021 might exceed 2020.

4 | DISCUSSION

4.1 | Species and traits

The search included the 20 most exploited aquaculture finfish species globally.[33] Table 1 lists the results according to species and area of interest, while Figure 2 shows the distribution of species. The two most studied species are Nile tilapia (*Oreochromis niloticus* Linnaeus) and Atlantic salmon. Today, the main traits that are selected for in aquaculture in the United States, Europe and China through breeding are growth, disease resistance, processing yields and product quality, reproductive traits, feed conversion efficiency, morphology and tolerance to environmental stressors.[7,87,88] It could therefore be expected that these traits would appear in the studies retrieved in this review. Reproduction (maturity/fecundity) and development were the most studied traits, found in this systematic review, see Figure 3. This also included sex determination and sterility. Then came growth, pigmentation, disease resistance, use of trans-GFP and omega-3 metabolism. The traits studied mirrors the most important traits in modern breeding, where, for example, omega-3 content in fish can be considered important for product quality for human consumption.

In Table 1, we have included categorization of what areas of interest the different papers indicate to have. Considering the CRISPR field of research to be quite young, we acknowledge that areas of interest in each study is/are focused on key issues such as maturity/fecundity – thus being overlapping. However, we have attempted to assign each study the field of interest we consider most prominent – for example being technology development or final product-oriented such as production of sterile fish for aquaculture.

4.2 | Geographical origin of genome editing research compared to major finfish producing countries

In our analysis of the literature, we investigated the institutional affiliation of the 1st author for each study to determine the geographical location of the research, see Figure 4. China is still on the top.[9,22] Others are the United States, Norway, UK, Japan, Egypt, Czech Republic, Republic of Korea, India, France and the Philippines. Some of the papers have been credited two countries because the 1st author had two institutional affiliations at the time of publication. China has produced most publications (29), followed by the United States (9) and Norway (7).

For countries with aquaculture production, the choice to consider genome editing as an approach may depend on the type of challenges the country/region faces, regulative conditions, knowledge about the species and wild relatives and consumers acceptance of GM/GE foods. Moreover, Wargelius[5] argued that a prerequisite for genome editing is that the species genome is fully sequenced and annotated. Considering these proposed criteria, we expect there to be some correlation between the species importance in present aquaculture production, for how long they have been produced, first selective breeding study (history of aquaculture), and to whether genome editing has been approached for this species.

According to FAO,[1] Asia is the major aquaculture producing region according to volume (88.69% of global production), and China is the largest country with a total of almost 58%. America produces 4.63%, Europe 3.75% and Africa 2.67%. It is evident that China as the most producing aquaculture country is also the one doing most research on the use of genome editing on aquacultured finfish.

TABLE 1 Overview of genome editing in aquaculture finfish with respect to fish species, field of interest, specific trait and gene(s), additional remarks, genome editing system and institutional affiliation of 1st author. Abbreviations can be found listed alphabetically below the table. Genes *tyr2* and *slc45a2* when in parentheses are targeted for phenotypic visibility

Species	Interest	Trait	Target genes	Remarks	System	Institutional affiliation 1st author	Reference
Nile tilapia (*Oreochromis niloticus* Linnaeus)	Teleost genetics	Reproduction and development	*dmrt6*		CRISPR	China	Zhang et al.[39]
			aldh1a2, cyp26a1		CRISPR	China	Feng et al.[40]
			Rspo1		TALEN	China	Wu et al.[37]
			sf−1		CRISPR	China	Xie et al.[41]
			gsdf		CRISPR	China	Jiang et al.[42]
			wt1a, wt1b		CRISPR	China	Jiang et al.[43]
			eEF1A1b		CRISPR	China	Chen et al.[44]
			esr1, esr2a, esr2b		CRISPR	China	Yan et al.[45]
			amh homozygous, amhr2 homozygous, amh heterozygous, amhr2 heterozygous		CRISPR	China	Liu et al.[46]
			cyp11c1	Expression rescue	CRISPR	China	Zheng et al.[47]
			rln3a, rln3b		CRISPR	China	Yang et al.[48]
			igf3		CRISPR	China	Li et al.[49]
			tsp1a		CRISPR	China	Jie et al.[50]
			foxh1		CRISPR	China	Tao et al.[51]
	Aquaculture	Reproduction and development	*amh, amhbeta-y, amhy, amhrII*		CRISPR	China	Li et al.[52]
			piwil2		CRISPR	UK	Jin et al.[53]
	CRISPR as tool	Reproduction and development	*nanos2, nanos3, dmrt1, foxl2*		CRISPR	China	Li et al.[54]
			miRNA200a/200b/429a/+ssDNA, miRNA200a/200b, miRNA429a, miRNA125, vasa−3´UTR	HDR	CRISPR	China	Li et al.[55]
Mozambique tilapia (*Oreochromis mossambicus* Peters)	CRISPR as a tool	Reproduction and development	*OmbAct, OmEF1a, TU6*	Cell line	CRISPR	U.S.	Hamar & Kültz[56]
Atlantic salmon (*Salmo salar* Linnaeus)	Aquaculture	Pigmentation	*slc45a2, tyr*		CRISPR	Norway	Edvardsen et al.[57]
				HDR			Straume et al.[58]
		omega−3 metabolism	*Δ 6fads2-a, Δ 6fads2-b, Δ 6fads2-c, Δ 5fads (slc45a2)*		CRISPR	Norway	Datsomor et al.[59]
			elovl2 (slc45a2)		CRISPR	Norway	Datsomor et al.[60]

(Continues)

TABLE 1 (Continued)

		Reproduction and development	*dnd* (*slc45a2*)		CRISPR	Norway	Wargelius et al.[25]
				Expression rescue	CRISPR	Norway/Czech Republic	Güralp et al.[61]
				HDR	CRISPR	Norway	Straume et al.[62]
Chinook salmon (*Oncorhynchus tshawytscha* Walbaum)	Aquaculture	Trans-GFP	*egfp*	Cell line	CRISPR	UK	Gratacap et al.[63]
	CRISPR as tool	Trans-GFP	*megfp*	Cell line	CRISPR	UK	Dehler et al.[64]
	Teleost genetics	Disease resistance	*stat2*	Cell line	CRISPR	UK	Dehler et al.[65]
Rainbow trout (*Oncorhynchus mykiss* Walbaum)	Teleost genetics	Reproduction and development	*sdY*		ZFN	France	Yano et al.[36]
		Growth	*IGFBP−2b1*, *IGFBP−2b2* (*tyr2*[†])	Cell line	CRISPR	U.S.	Cleveland et al.[66]
					CRISPR	U.S.	Cleveland et al.[67†]
Various salmonid cell lines	CRISPR as a tool	Trans-GFP, pigmentation	*gfp*, *slc45a2*	Cell line	CRISPR	UK	Gratacap et al.[68]
Grass carp (*Ctenopharyngdon idella* Valenciennes)	Aquaculture	Disease resistance	*JAM-A*	Cell line	CRISPR	U.S.	Ma et al.[69]
Common carp (*Cyprinus carpio* Linnaeus)	Aquaculture	Growth	*TALEN: sp7*, *runx2*, *spp1a*, *mstn*, *CRISPR: sp7a*, *sp7b*, *mstnba*, *runx2*, *opga*, *bmp2ab*		CRISPR+TALEN	China	Zhong et al.[14]
	Teleost genetics	Pigmentation	*MCIR*		CRISPR	China	Mandal et al.[70]
	Aquaculture	Pigmentation	*ASIP*		CRISPR	China	Chen et al.[71]
Farmed carp (*Labeo rohita* Hamilton)	Aquaculture	Disease resistance	*TLR22*	HDR	CRISPR	India	Chakrapani et al.[72]
White crucian carp (*Carassius auratus civieri* Temminck & Schlegel)	Aquaculture	Pigmentation	*tyr*		CRISPR	China	Liu et al.[73]
Gibel carp (*Carassius gibelio* Bloch)	Teleost genetics	Reproduction and development	*Cgfoxl2a-B*, *Cgfoxl2b-A*, *Cgfoxl2b-B*		CRISPR	China	Gan et al.[74]
Loach (*Paramisgurnus dabryanus* de Thiersant)	CRISPR as tool	Pigmentation	*tyr*		CRISPR	China	Xu et al.[75]
Channel catfish (*Ictalurus punctatus* Rafinesque)	Aquaculture	Reproduction and development	*cgbb*		ZFN	China/U.S.	Qin et al.[35]
		Growth	*mstn*		CRISPR	U.S./Egypt	Khalil et al.[76]
		Disease resistance	*cath (Alligator mississippiensis)*	HDR, transgenic	CRISPR	U.S./Philippines	Simora et al.[77]

(Continues)

TABLE 1 (Continued)

	CRISPR as tool	Disease resistance	*TICAM1*, *RBL*		CRISPR	U.S.	Elaswad et al.[78]
				HDR		U.S./Egypt	Elaswad et al.[79]
Southern catfish (*Silurus meridionali* Chen)	Teleost genetics	Pigmentation	*cyp26a1*		CRISPR	China	Li et al.[80]
Yellow catfish (*Pelteobagrus fulvidraco* Richardson)	Teleost genetics	Reproduction and development	*pfpdz1*		CRISPR	China	Dan et al.[81]
Sterlet (*Acipenser ruthenus* Linnaeus)	Aquaculture	Growth, trans-GFP	*ntl*, *egfp*		CRISPR+ TALEN	China	Chen et al.[38]
		Reproduction and development	*dnd1*		CRISPR	Czech Republic	Baloch et al.[12]
Tiger pufferfish (*Takifugu rubripes* Temminck & Schlegel), Red sea bream (*Pagrus major* Temminck & Schlegel)	Aquaculture	Growth	*mstn*		CRISPR	Japan	Kishimoto et al.[82] Kishimoto et al.[13]
Blunt snout sea bream (*Megalobrama amblycephala* Yih)	Aquaculture	Growth	*mstna*, *mstnb*		CRISPR	China	Sun et al.[83]
Olive flounder (*Paralichthys olivaceus* Temminck & Schlegel)	Aquaculture	Growth	*mstn*		CRISPR	Republic of Korea	Kim et al.[84]
		Growth, reproduction and development	*Myomaker*, *gsdf*		CRISPR	China	Wang et al.[85]
		Disease resistance	*PoMaf1*	Cell line	CRISPR	Republic of Korea	Kim et al.[86]

Abbreviations: *aldh1a2*, retinal dehydrogenase 1a2; *amh*, anti-Müllerian hormone; *ASIP*, agouti signalling protein; *bmp2ab*, bone morphogenetic protein 2; *cath*, cathelicidin; *cgbb*, gonadotropin subunit beta-2, LH gene β-subunit; *Cgfoxl*, Carassius gibelio forkhead box protein L2; CRISPR, clustered regulatory interspaced palindromic repeats; *cyp11c1*, cytochrome P450 11c1; *cyp26a1*, cytochrome P450 26A1; *dmrt1*, doublesex- and mab-3-related transcription factor 1; *dmrt6*, doublesex- and mab-3-related transcription factor 6; *dnd1*, dead end miRNA-mediated repression inhibitor 1; *eEF1A*, Eukaryotic elongation factor 1 alpha; *egfp*, enhanced green fluorescent protein; *elovl2*, fatty acyl elongase 2; *esr1*, *esr2a*, *esr2b*, oestrogen receptor gene 1, 2a and 2b; *fads2*, fatty acyl desaturases; *foxh1*, forkhead box gene h1; *foxl2*, forkhead box protein L2; *HDR*, homologous directed repair; *igf3*, insulin-like growth factor 3; *IGFBP-2b1/2*, insulin-like growth factor-binding protein 2b1/2; *itgb1b*, integrin β-1 b; *JAM-A*, Junctional adhesion molecule-A; *MCIR*, melanocortin 1 receptor; *megfp*, monomeric enhanced green fluorescent protein; *miRNA*, microRNA; non-coding sequence; *mstn*, myostatin; *OmbAct*, Oreochromis mossambicus Beta-Actin promoter; *OmEF1a*, Oreochromis mossambicus elongation factor 1 alpha; *opga*, osteoprotegerin; *nanos2*, *nanos3*, nanos-homologue 2 and 3; *ntl*, no tail; *pfpdz1*, Pelteobagrus fulvidraco PDZ domain-containing protein; *PoMaf1*, Paralichthys olivaceus MAF1; *RBL*, rhamnose binding lectin; *rln3a/b*, Relaxin3; *Rspo1*, furin-domain-containing peptide R-spondin 1; *runx2*, runt-related transcription factor 2; *sdY*, sexually dimorphic on the Y chromosome; *sp7*, specificity protein transcription factor 7; *spp1a*, secreted phosphoprotein 1; *slc45a2*, solute carrier family 45 member 2; *stat2*, signal transducer and activator 2; TALEN, transcription activator-like endonuclease; *TICAM1*, toll-like receptor adaptor molecule gene; *TLR22*, toll-like receptor 22; *tsp1a*, Thrombospondin 1a; *trans-gfp*, trans-green fluorescent protein, an isomer of GFP; *TU6*, Tilapia polymerase III promoter; *tyr*, tyrosinase; vasa-3′UTR, associated with germ cell development; *wt1a* and *wt1b*, Wilms tumour gene 1a and 1b; *ZFN*, zinc finger nucleases.

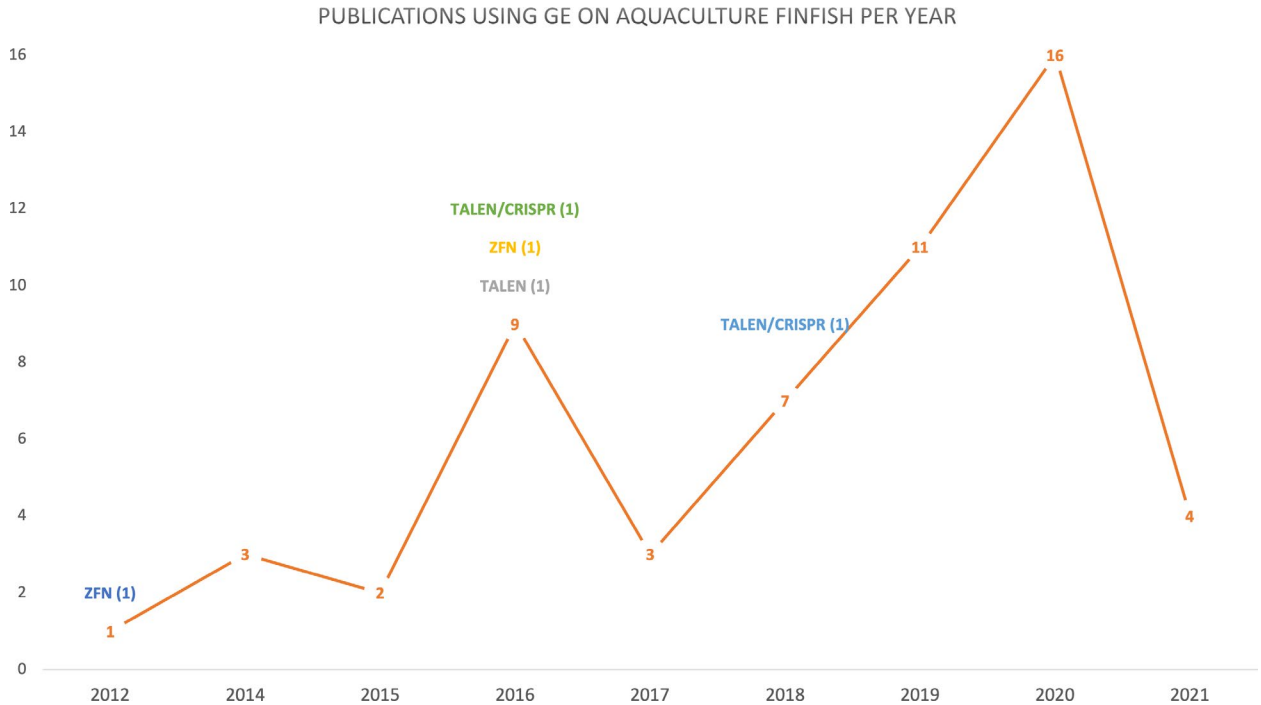

FIGURE 1 Number of articles using GE (genome editing) on aquaculture finfish species retrieved in systematic literature search published per year. Number of publications using other tools than the CRISPR (clustered regulatory interspaced palindromic repeats) system is highlighted with number of TALEN (transcription activator-like effector nuclease), TALEN and CRISPR, and ZFN (zinc finger nuclease)

Norway, the third most important country identified in our study, produces 1,65% of the total volume. Norway does however account for over 50% of the world's total production of Atlantic salmon.[4]

This history of aquaculture could also be compared to the species used in studies of genome editing to see whether there is a correlation between history of farming and the interest in novel tools like genome editing, see Table 2. Nile tilapia is the species which according to the review of Houston et al.[8] has been farmed for longest period, starting about 4000 year ago. The Nile tilapia genome was sequenced in 1998, and subsequent re-sequencing work has improved the coverage and quality of the annotations.[89] This species is also popular for use in research of fish physiology and endocrinology, with specific focus on sex determination and evolution,[55,90] as the results from this review also show. All the studies on this species have a first author associated with China, except one study from the UK (Table 1).

Various carp species show a very old history as aquaculture species, with first farming 2000–1000 years ago.[8] This is also the third most occurring species group in the articles retrieved in this review. All, except two articles on disease resistance in farmed carp (*Labeo rohita* Hamilton)[72] and grass carp (*Ctenopharyngdon idella* Valenciennes),[69] have 1st authors associated with China. Carp species are the most common freshwater aquaculture species in China.[99,100]

The second most studied species with regard to genome editing was through our retrieval, the Atlantic salmon. All these articles had their first author affiliated to a Norwegian institution, except one using Atlantic salmon cell line in the UK.[68] Norway is the third most dominate country in our findings. This might be because of the extensive research on salmon aquaculture in Norway, although showing a short history as a commercial fish species. Norwegian research focuses on breeding together with use of gene technology for marker-assisted breeding etc. facilitated by mapping and sequencing of the salmon genome. The Atlantic salmon has only been bred for about 50 years in Norway, yet it is already the species which globally has the most exploited traits for breeding programmes.[87] The genome of the Atlantic salmon was published as a bacterial artificial chromosome-based map first,[101] and later a high-quality whole genome of the Atlantic salmon was published by31 as part of the Salmon Genome Project.

4.3 | Technical challenges and off-target mutations by using CRISPR technology in finfish

The use of genome editing on finfish, either for commercial use or in research, brings technical challenges that should be considered. Some of these are off-target mutations and mosaicism in the F0 generation.[9]

4.3.1 | Off-target mutations

When genome editing leads to mutations in locations where it was not intended, this is called off-target mutation. These are the result

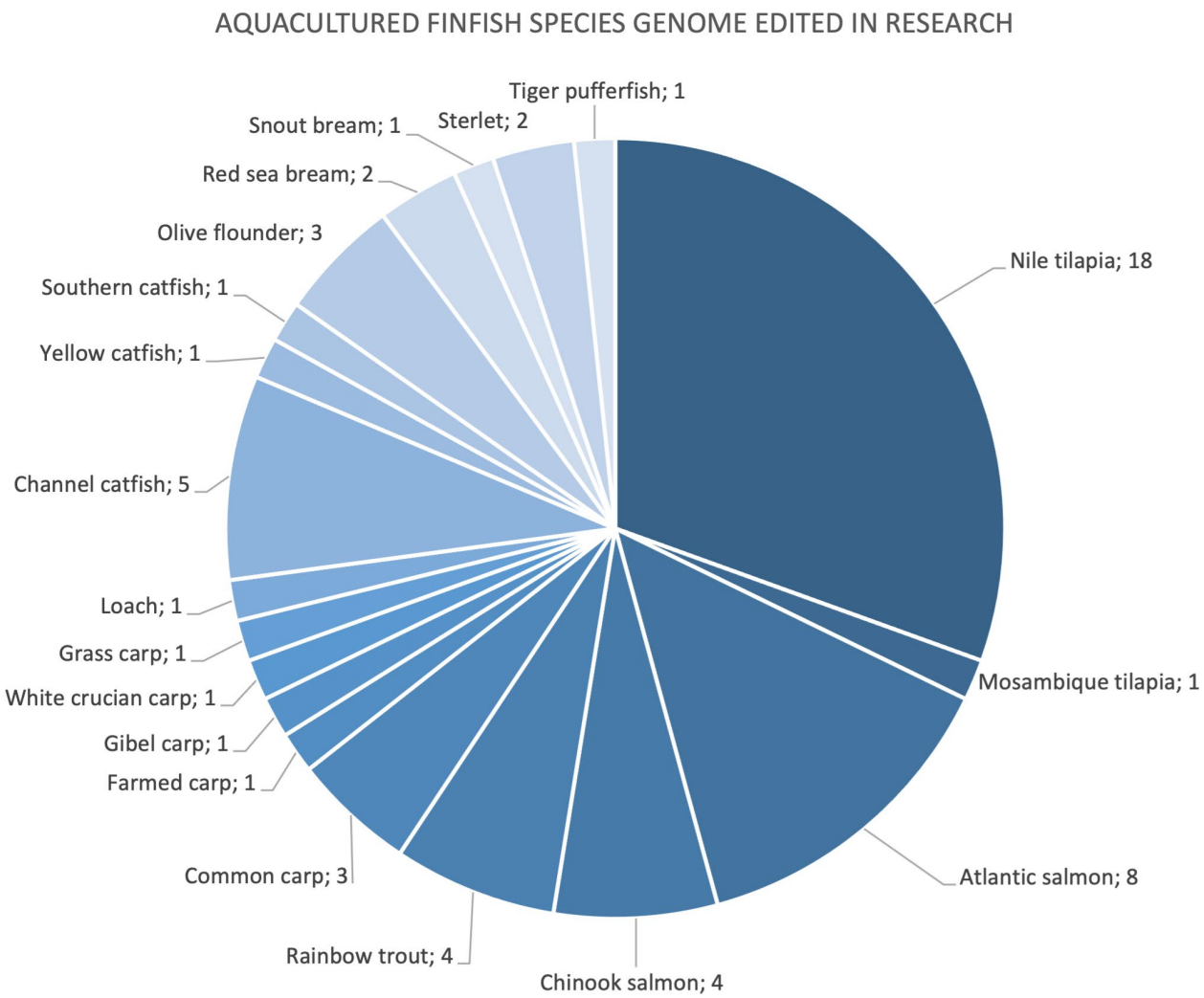

FIGURE 2 Distribution of species used for research on GE (genome editing) in publications found in systematic literature search. Species are sorted according to most used species (groups), and the numbers indicate the number of publications using the species

of the gRNA annealing to unintended or non-target areas of the genome, initiating mutations which might lead to unwanted and/or unknown effects on the organism as change in gene activity, gene silencing or gene knockout.[102] Off-target mutations are difficult to detect since the number and position of nucleotide changes are unknown.[23]

The first approach for avoiding off-target effects may be done by careful design of the gRNA by comparing the planned gRNA(s) to established genome assemblies, which has been done in several of the studies analysed in this review.[13,14,25,49,51,53,57,59,60,61,63,69,72,73,83] Some studies suspect embryo mortality[35,79] and embryo malformation followed by death[12] to be related to off-target effects. Simora et al.[77] experienced that increased mutation rate implied increased embryo mortality after inserting an alligator (*Alligator mississippiensis*) cathelicidin gene for pathogen resistance in Channel catfish (*Ictalurus punctatus* Rafinesque), suspecting this to be either off-target effects or pleiotropic effects. Elaswad et al.[79] argue that the specificity of the CRISPR/Cas9 depends on the protospacer adjacent motif (PAM) and the gRNA. They discuss that an off-target match with 5 mismatching nucleotides could still anneal to the gRNA as a target sequence and that this result could be minimized with better gRNA design. In addition, they suggest that the use of Cas9 nickase mutant with paired gRNAs would reduce the off-target effects. Elaswad et al.[79] do also point to the need for more research on the toxicity in relation to the concentration of gRNA injected into fish embryo, and to what extent this is related to off-target effects. One possible solution to this may be the use of short-life Cas9 variants, however, whether this approach reduces toxicity needs to be further investigated.[58] The second option for controlling off-target mutations is by routine rescreening of the genome for discovery of unintended mutations post-editing. This is, however, difficult since there is natural genetic variation in between strains and families which makes it difficult to find a good comparator to be able to identify potential off-target effects. Khalil et al.[76] report on not having examined the fish genome in edited fish for off-target mutations, only that '[...] no mutations were detected nearby and outside the target site'. Kishimoto et al.[13] found two mismatches for their small guiding RNAs (sgRNAs), however,

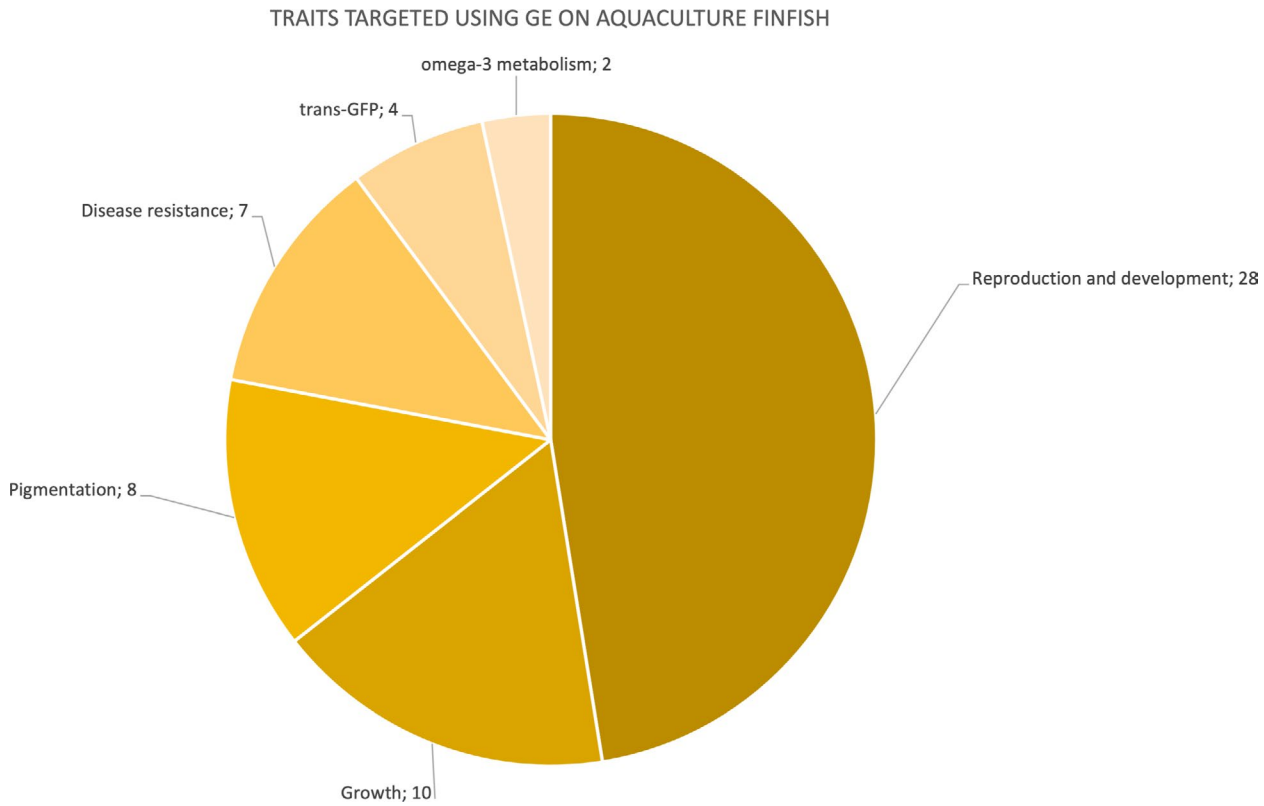

FIGURE 3 Distribution of traits studied using GE (genome editing) in articles retrieved in systematic literature search. Numbers are number of publications targeting the trait. Traits are reproduction and development (including sterility and sex determination), growth, pigmentation, disease resistance, use of trans-GFP (green fluorescent protein) and omega-3 metabolism

a screening post-editing showed that only one target sequence had mutation and thus excluded the possibility for off-target mutations in both F0 and F1 generation. Qin et al.[35] observed mutations within the open reading frame, but not at the ZFN targeted sequence position.

Considering the discussion from the papers identified in this review, there is a further need to identify the presence of off-target and other unintended effects. This may imply to use recent developments as next-generation sequencing and multi-omics approaches, as seen approached in Jin et al.[53] These methods need to be sensitive enough to distinguish between natural variation and mutations introduced by genome editing.

4.3.2 | Effect of ancestral whole-genome duplication

Another challenge relevant when discussing teleosts, and especially salmon, is ancestral whole-genome duplication (WGD) events and particularly the salmonid-specific 4th round (Ss4R). WGD is a duplication of the genome resulting in an extra set of all genes, followed by either sub-functionalization (duplicated gene remains unchanged and shares function of original gene), neofunctionalization (duplicated gene is assigned new function) or non-functionalization (duplicate loses function, e.g. as a pseudogene).[71,103] Because of several rounds of duplication events, different teleost species have different numbers of chromosomes and compositions and functions of paralogues,[45,65] and ploidy levels.[12] Ancestral WGD is a governing aspect when genome editing the teleost genome.[63] At the same time, different authors also emphasize that using genome editing is a convenient method for targeting and mutating genes in such duplicated genomes,[14,66] and Gan et al.[74] specifically used CRISPR/Cas9 to study the role of duplicated genes in Gibel carp (*Carassius gibelio* Bloch). If a group of species has different ploidy level, the one with lowest level should be used as model species for the rest of the group.[38] In the cases where two or more paralogues of a gene are identified, the function and sequence of the paralogues should be determined to consider whether these should be co-targeted or single-targeted, depending on the desired outcome of the mutation. Cleveland et al. emphasize the need for targeting and knocking out both gene duplicates for the protein IGFBP-2b to be able to disrupt the expression of the protein, since the paralogue of one gene may persist the function of the gene and eradicate the effect of the targeted mutation.[66] This was also seen in Datsomor et al.[60] discussing how paralogues can rescue the function of the gene knocked out and co-targeting may be needed to elucidate the function of a gene. In some cases, the duplicated genes might have evolved

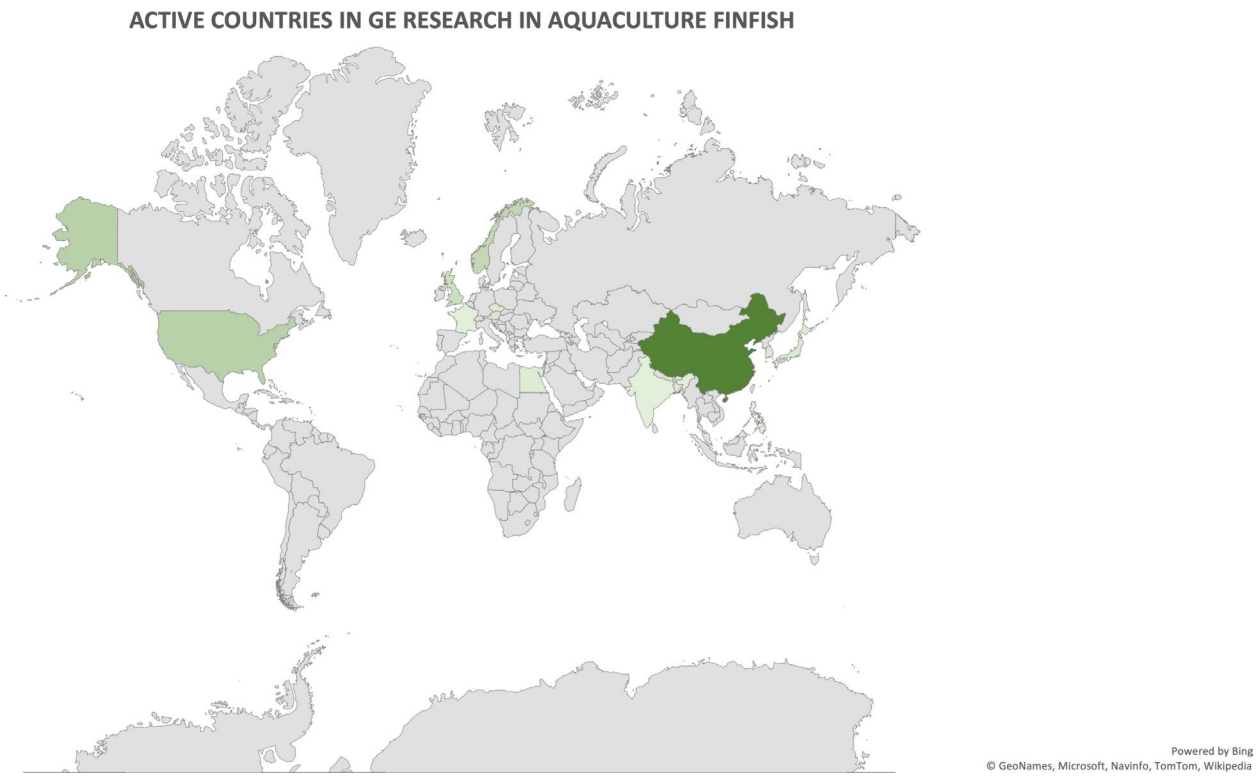

FIGURE 4 Countries involved in studies using genome editing on aquaculture finfish species, based on institutional affiliation(s) of 1st author of all studies retrieved. Darker to lighter colouring indicates the distribution of number of publications, from most to fewer

TABLE 2 Overviews of most used species according to production volume, time of first farming, first selective breeding, number of studies retrieved in this review using the species (56 in total) and genome-wide screening or sequencing of the latter

Production volume (FAO 2020)	Time of first farming (yr. ago) (Houston et al. 2020)	First selective breeding study (yr. ago) (Houston et al. 2020)	GE studies (#/56)	Genome-wide screen/ sequencing reference
Grass carp	Nile tilapia (4000)	Rainbow trout (95)	Nile tilapia (18)	Kocher et al.[91]
Silver carp	Common carp (2000)	Atlantic salmon (50)	Atlantic salmon (8)	Lien et al.[31]
Nile tilapia	Grass carp (1000)	Nile tilapia (40)	Channel catfish (5)	Liu et al.[94]
Common carp	Silver carp (1000)	Common carp (40)	Chinook salmon (4)	Christensen et al.[92]
Bighead carp	Black carp (1000)	Labeo rohita (<40)	Rainbow trout (4)	Berthelot et al.[95]
Catla sp.	Bighead carp (1000)	Silver carp (<20)	Common carp (3)	Xu et al.[93]
Carassius sp.	Milkfish (500)	Grass carp (<20)	Olive flounder (3)	Shao et al.[128]
Osteichthyes	Labeo rohita (100)	Pangasius catfish (<20)	Sterlet (2)	Cheng et al.[97]
Atlantic salmon	Rainbow trout (100)	Wuchang bream (<20)	Red sea bream (2)	Shin et al.[98]
Labeo rohita	Atlantic salmon (50)		Grass carp (1)	Wang et al.[96]

Abbreviations: GE, genome editing; yr. ago, years ago.

new functions, as seen in Cleveland et al.[66] and Chen et al.[44] and then, depending on the desired outcome, single knockout is sufficient and will also reveal the function of each paralogue. Such an operation also depends on the relative difference between the sequences of the functionally different paralogues.[66] If possible, genes that occur only once in the genome can be chosen as a target for the editing to avoid disturbance, and this approach has been done by targeting *slc45a2* and *tyr* in Edvardsen et al.[57] and *dnd* in Wargelius et al.[25]

4.3.3 | Mosaicism

Mosaicism in the F0 generation relates to on what cell stage in the embryo that the editing occurs, as CRISPR system components such as the gRNAs might be degraded, depending on the developmental pace in different species.[104] The most convenient is editing at the one-cell stage. Of the articles retrieved in this review, several reported mosaicisms in their research animals.[12,47,49,51,53,57,59,61,71] Straume et al.[62] reported that mosaicism increased with higher

injection volumes of oligonucleotide donor template. Cleveland et al.[66] emphasize that mosaicism is possible to overcome by generating a F1 generation. Edvardsen et al.[57] found that several individuals in F0 carried the same indel mutations, and a crossing to F1 would generate homozygous non-mosaic fish with the desired mutation. They express that such a result in the F1 generation is a quick process, even though species like salmon has a long generation time of 3–4 years.[57,62] Some studies used knockout of pigmentation as a way of selecting out mosaic individuals before analysis, as complete loss of pigmentation would show the F0 individual not to be a mosaic.[57,58,59,61,62,67] Edvardsen et al.[57] also found that fin clips can be used to identify the knockout phenotype of individuals as it followed the mosaicism to some degree.

4.4 | REGULATIVE FRAMEWORKS IN COUNTRIES DOING GENOME EDITING ON FINFISH – CRUCIAL FOR USE?

How to regulate genome edited organisms as plants and animals has during the recent years been discussed. Regulative issues concern both whether genome edited organisms should be regulated under present regulative frameworks for GMOs or if they should be exempted, and whether the regulation is according to product or process.[105,106] Compared to older GMOs, the newer genome edited organisms can be generated without use of transgene sequences.[105] This is a common topic of discussion, even though insertion of desired sequences is possible using HDR, as shown in four of the retrieved papers of this study.[55,58,72,78] Regulative concerns could affect the use of genome editing in applied research with the goal for commercial use.[9] It has been argued that GMO regulation may hamper research and innovation of genome edited organisms due to the excessive regulatory requirements placed on GMOs.[107]

Ishii and Araki[105] have presented an overview of the different regulative frameworks and made a distinction between those countries that regulate according to product or process. All countries identified in our review, except Norway and the Philippines, were represented in the list of Ishii and Araki. Of the countries identified in our studies, United States, Japan and Republic of Korea have implemented product-based regulations, while India, China and EU (France, Czech Republic) have implemented process-based regulations. Norway has a process-based regulation. UK was also identified as a European country during the research, but at present it is unsure what will be happening from the UK Brexit situation and as such the national legislations. The different ways of formulating the regulations affect whether it is the characteristics of the final organism and its direct effect, or the process and act of changing an organism through gene technology that accept or denies for cultivation and/or release. The latter triggering a specific regulation for GMOs, while in countries who have a product regulation the novel product is regulated under more general food/animal regulative framework. Ishii and Araki did not find any significant differences between countries having product or process-based regulation when it comes to commercial cultivation of GM crops.[105] From our studies, where China and Norway dominate, it seems like the type of regulation do not affect initiative for research, as suggested by Martin-Laffon et al.[107]

When it comes to the newer technologies available through genome editing on crops, Ishii and Araki[105] concluded that countries may be divided on how they will regulate genome edited plants. One example of this is Argentina who developed a new, own regulation for genome edited organisms that do not contain any transgenic DNA (Resolution No. 173/2015), in order to speed up the approval process.[105,108] A regulatory exemption was given for Aquabounty produced genome edited Nile tilapia. This fish is not considered a GMO and has been genome edited for increased filet quality and quantity and for more efficient growth.[109]

In the EU, a genome edited organism was decided by court decision to be a GMO, and so the EU regulation approval process does not divide between the different technologies. However, the European countries doing research on genome edited finfish, the Czech Republic and France has through the Directive (EU) 2015/412, amending Directive 2001/18/EC, a possibility to adopt measures restricting or prohibiting a group of GMOs defined by crop or trait. This can be based on grounds such as those related to socio-economic impacts, avoidance of GMO presence in other products, agricultural policy objectives or public policy (Article 26b[110]). Although this directive is specific on GMO crops, it can be assumed that the same possibilities will be relevant for genome modified and genome edited fish. Norway has through the EEA-agreement harmonized the EU Directive within national legislations.

Besides national regulation, there are also international treaties that regulate GMOs, as the Cartagena Protocol under the Convention on Biological Diversity (CBD). All countries identified and presented in this review, except the United States, have signed the Cartagena Protocol, which regulates import and export of GMOs.[105] Article 26 of this protocol emphasizes '[…] socio-economic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities'.[111] This will favour taking biodiversity into consideration when evaluating new genome modified or edited organisms.

Country members of the EU and countries that have signed the Cartagena Protocol under the CBD have the possibility to consider broader aspects when evaluating genome edited and modified organisms. Such broader aspects can include the socio-economic significance of the production, potential ethical aspect (as animal welfare and consumer autonomy), and how the product contributes to sustainable development.[112] The type of regulative conditions regarding product or process in each country may therefore not be as important when it comes to future commercial use of genome editing,[105] the purpose and goals to be achieved by the genome editing may instead influence the decision and the acceptability of the technology. Ishii and Araki also call upon for more consistent policies, referring to the missing link between the regulation type,

experience with GM crops and relation to the Cartagena Protocol within a country.

In 2018, the growth-enhanced transgenic AquAdvantage (Atlantic salmon) was approved for production in a land-based grow-out facility in Indiana, United States[113] In December 2020, a domestic pig genome edited for removal of galactosyltransferase alpha 1,3 (*GGTA1)* which enables synthesis of alpha-galactose on the cell surfaces was approved by FDA. The major aim was to reduce any hyperacute rejection of pig-to-human xenotransplants. Secondly, the porcine meat could meet food demands of people with allergic reactions caused by alpha-gal syndrome (AGS). As such, the GalSafe pig is intended to be used for both food and medical purposes.[114] These recent approvals of transgenic and GE animals, together with the recently approved GE Nile tilapia in Argentina, could indicate that future approvals of more GM/GE organisms in food production should be expected.

4.5 | CONTRIBUTION TO SUSTAINABLE DEVELOPMENT

Our review outline that some of the challenge's aquaculture is experiencing, like disease and genetic contamination in wild stocks, has possible solutions through genome editing. In Norwegian aquaculture, an expansion of the salmon farming industry requires transition to a more sustainable production. This final section will therefore discuss how the different solutions retrieved in this review can contribute to a more sustainable salmon production, based on how the contribution of genome edited organisms to sustainable development is evaluated under the Norwegian Gene Technology Act (GTA). The GTA is a unique regulation that requires, besides assessment of risk to the environment and health, consideration of the ethics, social utility and contribution to sustainable development of GMOs. This, in addition to the urgent need for innovation and new solutions in aquaculture, is reflected in the focus of the Norwegian studies retrieved in this review, where the aim is to generate a fish more appropriate for a sustainable aquaculture.[25,57,58,59,60,61,62] In support of the research on the germ cell free Atlantic salmon, other studies have looked at growth and maturation,[115] and other sterility candidate genes have later been explored.[116]

The legal document 'Regulations relating to impact assessment pursuant to the Gene Technology Act' describes what should be included in the assessment of sustainable development. This combines 15 control questions related to global impacts, ecological boundaries, human needs (distribution between generations and between rich and poor) and economic growth.[117] According to Rockström et al.[118] the main importance of sustainable development is for it to guide our activities to a safe operating space. This implies that we can produce and consume if it is with respect to the Earth system.[118]

The control questions regarding ecological boundaries and global effects on biodiversity should therefore be taken into wide consideration when evaluating genome edited organisms. All the control questions should also, according to the Norwegian Act, consider both the product and process, to ensure that sustainability is regarded throughout the whole production line/supply chain. The impact of aquaculture on nature environment is also to a large extent the driving force for proposing use of genome editing. However, solving ecological issues cannot have a negative impact on society and/or economy; therefore, all aspects must be evaluated.

The first control questions relevant for aquaculture finfish regarding global impacts and ecological boundaries ask whether the biological diversity is affected globally, whether the ecosystem way of function is affected and whether it will affect energy utilization, climate gases and pollution. Here, the research on reproduction and development is important. Sterile fish will not be able to reproduce with wild stocks after escape, and hence, the impact on environment will be reduced. In Norway, the issue with escaped fish is highly urgent. Güralp et al.[61] have recently published a method using a combination of genetic sterility and rescue, which may allow large scale production of sterile salmon.[61] A sterile fish will not only aid this issue, but it would also be considered a prerequisite for using genome edited fish in ocean pen production. Here, we do, however, want to emphasize the need for more research on how such a sterile salmon would impact wild relatives and surrounding biodiversity when it escapes.[119] Disease resistance could aid any aquaculture sector globally, and it would aid both the economic efficiency of the production, but also animal welfare and the impact on wild stocks, thus both biodiversity and responsible productions aspects of sustainability. Increased welfare is, alongside with sustainability, assumed an important argument for application of genome editing in aquaculture, especially in a country like Norway where ethical responsibility is implemented in the Act.[120] In addition, the Norwegian Animal Welfare Act states that all animals, including fish, have intrinsic value independent of their utility for humans, and shall be treated well and protected from unnecessary pain and strain.[121] Any implementation of genome editing in aquaculture has to consider this and elaborate how animal welfare should be considered for the species in question.

Secondly, the control questions include questions on the distribution of benefits and risks between generations and rich and poor. Anticipation of both the potential beneficial and adverse consequences of using genome editing in aquaculture is difficult because there is no former use to refer and learn from. Regarding GMOs, the standard implication is often that even though we remove an issue, for example disease resistance, some other issue will follow, as for example a new pathogen implying that one need to consider a longer timeframe when assessing potential impacts.

Another important aspect regarding future generations is the preservation of the wild salmon stocks in Norway. Norway holds approximately 25% of the total world population of Atlantic salmon, which has encouraged the preservation of this species.[122,123] In this context, a sterile genome edited fish is not only a solution, but should be a prerequisite for use. Other considerations to be made are whether genome editing allows for intensification or maintenance of the aquaculture production volume. If the former, is that representing a threat or benefit for the opportunities of future generations?

The knowledge earned from studies of genome editing in one species can be used, albeit to a certain degree, in another. The research performed can therefore be useful for other countries with other aquaculture related challenges, including poorer countries with less resources to conduct this kind of expensive research on their own. This transfer of knowledge depends on transparency of the process and the product.

Finally, the control questions are summed up in questions on how the ecological impacts and distribution between generations and rich/poor affect the economic growth. These questions are not directly related to the solutions proposed, but an economic analysis that is outside the scope of this review. We will, however, go briefly through how economic traits could contribute to sustainability.

Pigmentation can be an economic trait, as seen for common carp in various colours, but also a tool in development and use of genome editing like CRISPR/Cas9. Regarded to be a commercial and ornamental trait, this modification will affect goals related to economy through social interest as for example aesthetic value. Both pigmentation and the use of trans-GFP have been applicated in studies aiming at developing CRISPR or TALEN as tools for aquaculture. The sustainability contribution of this use of genome editing will therefore depend on the knowledge generated from the activities. It could, however, also have importance for biosafety as the lacking pigmentation can be used to identify escaped genome edited fish.

In studies looking into growth, eight out of ten studies had aquaculture as main focus (Table 1). Increasing growth for increased production efficiency is valuable for reducing feed costs, but could have implications for welfare, as seen with bone defects after *sp7* and *mstn* KO in common carp.[13,14] In Norwegian salmon production, growth has for long been an important trait in breeding efforts, and here the process is regarded a success. Increased growth can therefore not be regarded as priority in the development of a sustainable production in Norway.

Omega-3 is especially relevant in Norwegian aquaculture, as sufficient amounts of omega-3 fatty acids sustain health benefits for both fish and humans.[59] As described by Datsomor et al.[59,60] LC-PUFAs in the feed is an important contribution to omega-3 synthesis in the salmon. This could lead to less need for live feed and/or fish oil in the feed, which would be of economic and ecological benefit.[124] Efforts within the genome editing field have also been aimed to generate omega-3 producing plants for use in fish feed.[125] This could be an alternative for approaching the issue more directly, alternatively in combination.

Lastly, we want to express the necessity for modifications, additions and changes to be made for the sustainability guidelines to be adapted for evaluation of GE and GM animals, and aquaculture finfish species more specifically, as seen for herbicide tolerant crops in Catacora-Vargas[126] and by the Norwegian Biotechnology Advisory Board.[127] We find it necessary not only to adapt the questions to evaluation of living GM/GE animals, but also to specify the core ideas and evaluation questions. It does, however, give a brief idea of the complexity of addressing genome editing solutions as sustainable because they might (contribute to) solve environmental issues. More study is needed on how to evaluate sustainability in relation to genome editingo fish, in addition to (experimental) study of the effect of genome edited finfish on environment, economy and society.

5 | CONCLUSION

We have found that the main traits researched are reproduction and development, growth, pigmentation, disease resistance, use of trans-GFP and study of the omega-3 metabolism. Compared with previous reviews, we find that there are other genes targeted in more recent studies. Reproduction is still the most targeted trait, but there is also an increase in other traits such as disease resistance, pigmentation and omega 3-metabolism. The knowledge from these studies is relevant both in aquaculture and in more basic research areas like physiology and genetics, and hence not only related to food production animals. At the same time, knowledge about the reproductive cycle, sterility and development is important in the development of an efficient and secure breeding process. Several of the studies mention technical issues such as off-target mutations, the effect of whole-genome duplications and mosaicism. There is a need of more research on the mechanisms and effects by off-target mutations. One identified solution is careful design of the gRNA. Methods used for identification of off-target effects require further elaboration, and these need to be sensitive enough to distinguish between natural variation and mutations introduced by genome editing. There is also a need for more studies on the phenotypic effects of genome editing, and this includes welfare and behavioural studies. Most of the studies retrieved in this review neither discuss implications for welfare, nor ethical considerations related to the activity of modifying the DNA of living organisms.

There is correlation between major producing countries of aquaculture finfish products and the geographical location of research on genome editing in aquaculture finfish. We also saw that a majority (26) of the studies (56) state utilization in aquaculture is the main objective of their research. This implies that there might be interest in the given countries for considering genome editing as a possible solution to aquaculture challenges and development. We have mentioned several regulative factors, like the product/process question, the Cartagena Protocol, the EU Directive 2015/412 amending Directive 2001/18/EC and the Norwegian Gene Technology Act. All these concerns and treaties affect how a country can, and have to, regulate genome modified and/or edited organisms. Based on the research activities in different countries, it seems the question of acceptability is more related to the purpose of the organism and product rather than the regulative conditions in the given country.

All the solutions found in this review can contribute to sustainability in each their own way. We emphasize the importance of

prioritizing environmental sustainability in this regard. Biodiversity is of crucial importance to any food production system, also aquaculture. Its preservation should therefore be of main interest to both breeders, policy-makers and consumers. Evaluating the effect of a GMO on sustainability is required by law in Norway, and description for assessment has been developed for this specific term. These are, however, not fit for a thorough evaluation of live animals and should be revisited.

ACKNOWLEDGEMENTS

T.B.B. and A.I.M. acknowledge financial support from RCN (Grant No. 295094) and Sarah Agapito-Tenfen (scientist, GenØk) and Arinze Okoli (scientist, GenØk) for reading parts of the manuscript. A.I.M acknowledges financial support from GenØk – Center for biosafety. R.A.D acknowledges financial support from RCN (Grant No. 301401) and from UiT – The Arctic University of Norway.

DATA AVAILABILITY STATEMENT

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

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How to cite this article: Blix TB, Dalmo RA, Wargelius A, Myhr AI. Genome editing on finfish: Current status and implications for sustainability. *Rev Aquacult*. 2021;00:1–20. https://doi.org/10.1111/raq.12571

APPENDIX 1

TABLE A1 Systematic literature search details for finding literature on genome editing research in aquacultured finfish species

Search strings	Excluded in search	Database	Date	Period	Results (#)	Selected articles	Review	Empirical
crispr/cas9 farmed atlantic salmon	News articles, ethics-related, conventional breeding, agricultural species, PhD theses, Master theses, basic research fish health, GE feed, patents	Google Scholar	06.01.20	2015–2020	295	52	27	25
salmon aquaculture crispr	Crustaceans, miRNA, interference RNA, sex determination, embryonal development	Google Scholar	13.01.20	2015–2020	673	85	38	47
"TALEN" OR"zinc finger nuclease" OR"CRISPR" OR"CRISPR/Cas9" AND "Grass carp" OR"silver carp" OR"common carp" OR"nile tilapia" OR"bighead carp" OR"carassius" OR"catla" OR"Osteichthyes" OR"atlantic salmon" OR"*roho labeo*" OR"pangasius" OR"milkfish" OR"tilapia" OR"clarias" OR"Wuchang bream" OR"rainbow trout" OR"cyprinidae" OR"black carp" OR"snakehead" OR"*ctenopharyngodon idellus*" OR"*hypophtalmichthys molitrix*" OR"*cyprinus carpio*" OR"*Oreochromis niloticus*" OR"*hypophthalmichthys nobilis*" OR"*catla calta*" OR"*salmo salar*" OR"*labeo rohita*" OR"*chanos chanos*" OR"*Megalobrama amblycephala*" OR"*Oncorhynchus mykiss*" OR"*mylopharyngodon piceus*" OR"*channa argus*"	News articles, ethics-related, conventional breeding, agricultural species, PhD theses, Master thesis, basic research fish health, GE feed, patents	Google Scholar	15.02.21	2020–2021	170	11	2	9
	Research in human physiology, microbiology, environmental DNA, zebrafish, medaka, virology	Web of Science	12.03.20	1995–2020	73	38	8	30
			15.02.21	2020–2021	25	16	0	16