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REVIEW

REVIEWS IN Aquaculture

A review of using duckweed (Lemnaceae) in fish feeds

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Abstract

One of the primary sustainability challenges in aquaculture is replacing fish meal with plant-based ingredients in aquafeeds. Plants are not optimal due to low protein content and antinutritional factors which can cause gut dysbiosis. Duckweed (*Lemnaceae*) is a family of aquatic plants with high protein content and has been used successfully for various types of animal feeds. In this systematic review and meta-analysis of 58 papers, we summarize the extent by which duckweed has been used in fish production including the species of fish tested, the grow-out stage of fish, and method of application. Duckweed studies spanned a total of 18 species of fish (16 freshwater and two marine) that collectively are valued at 263 billion USD annually, and comprise 28% of total aquaculture production by mass. The average experiment length was 72 days (SD 42), primarily at the fingerling life stage. Duckweed was fed to the fish through live grazing, dried, and pelleted forms with 20% inclusion as the most common formulation. The *Lemna* spp., dominated by *L. minor*, *L. gibba*, and unknown *Lemna* species, were the most commonly used for feeds. *Spirodela polyrhiza* was the second most common. Duckweed inclusion levels between 15% and 30% were associated with positive outcomes on fish growth and feed conversion ratio without any negative impact on survival rates. Most duckweed species, especially from *Wollfiella* have not been tested as a fish feed but should be explored whereas most studies focused on freshwater fishes rather than marine.

KEYWORDS
aquaculture, duckweed, fish feeds, Lemnaceae, plant based, sustainable

1 | INTRODUCTION

1.1 | Aquaculture challenges with sustainable feeds

Aquaculture or aquatic farming is the fastest-growing livestock production method.[1] The majority of finfish production currently occurs in freshwater systems, but the greatest opportunity for expansion is in marine culturing systems.[2] Despite double-digit annual growth over the past 30 years, finfish production still has many environmental challenges including the development of sustainable feeds. Finfish production (especially marine finfish) has relied heavily on inclusion of fish meal in the feeds. This is not a sustainable practice because it has caused increased pressure on wild fish populations.[3] Historically, fish feed sustainability has primarily been focused on reducing and replacing fish meals with alternative inputs including plants, animal by-products, insects, and single-cell proteins that include bacteria, yeasts, and microalgae.[4] Pressure from environmental NGOs, seafood sustainability councils, and ultimately, consumer preferences have successfully influenced the industry in the past 20 years to reduce fishmeal in fish feeds by replacing it with plant-based protein such as soy.[5]

1.2 | Fish meal replacement in aquaculture

While fishmeal replacement with plant-based proteins has been overall beneficial for the conservation of wild fish stocks, new challenges have emerged with regard to efficient consumption and digestion. Most fish species are not herbivores and have not evolved the same metabolic machinery to enable the successful digestion of plants. The most commonly farmed groups of fishes, according to ISSCAAP (International standard statistical classification of aquatic animals and plants) standards, are the carps, barbels, and other cyprinids which include approximately 35 species.[6] Grass carp, *Ctenopharyngodon idella*, are the most commonly farmed fish by volume worldwide and are primarily herbivores, but can also grow on a carnivorous diet. Most of the other commercially important carps including the 'Chinese carps' (silver carp, bighead carp, and common carp) and the Indian Major Carps (rohu, catla, and mrigal) are generally considered omnivores with very few being carnivorous.[7,8] While many farmed species of fish are not herbivores, the ones that primarily consume aquatic plants or phytoplankton in nature rather than large-scale commercial crops such as maize, rice, soy, etc. Many traditional crop plants that are commonly used in agriculture contain a variety of antinutritional factors (ANFs) including but not limited to non-starch polysaccharides, proteinase inhibitors, lectins, saponins, oligosaccharides, oestrogenic compounds, phytic acid, β-glucans, glucosinolates, erucic acid, pigments, gossypol, quercitin, and quinolizidine alkaloids.[9–12] These ANFs generally impact palatability and can cause gut distress leading to inflammation, lower feeding rates, and subsequently poor growth.[13] Soybean meal was first shown to cause gastrointestinal enteritis in Atlantic salmon in 1996.[14] As finfish aquaculture in both marine and freshwater systems continues to grow globally, using sustainable fish feeds will be critical to long-term viability.

1.3 | Macrophytes as fish feeds

Not all alternative fish feed ingredients are equivalent in terms of sustainability. Use of endangered or protected plants should be prohibited. Incorporation of plants which would otherwise be used for human consumption should be limited. Finally, attention needs to be given to the way in which plants are cultivated to ensure that only sustainable farming practices are used.[15] Various crops including soy, oil palm, maize, and rice have been commonly used in fishmeal replacement, but can be associated with unsustainable farming practices such as deforestation.[16] Aquatic plants, including duckweeds, are an attractive alternative because they generally do not require terrestrial land use change, are efficient in phytoremediation including wastewater treatment processes, have extremely fast growth rates, and are easy to harvest.[17] One of the first observations and applications of aquatic plant consumption by fish was demonstrated by Opuszynski et al. where they showed how grass carp can be used to control excess aquatic vegetation growth in ponds exposed to warm water effluent from thermal power plants.[18] Since then, various macrophytes including water fern (*Azolla* spp.), water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), water spinach (*Ipomoea aquatica*), water lilies (*Nymphaea* spp.), and duckweed (*Lemnaceae*) have been widely explored and used as ingredients in fish feeds.[19] Macrophytes are an advantageous fish feed ingredient due to their high crude protein content and rapid growth. Duckweed is of interest due to its large number of species and global distribution.

1.4 | Duckweed and its use in fish feeds

There are currently 36 different species of duckweeds delineated across five monophyletic genera: *Spirodela*, *Landoltia*, *Lemna*, *Wolffiela*, and *Wolffia* within the family *Lemnaceae*. Species identification is very difficult and often requires molecular tools such as amplified fragment length polymorphism (AFLP) or genotyping by sequencing of plastid sequences.[20] Despite advances in molecular biology and sequencing-based approaches, seven species are still not able to be reliably identified using genomics but instead rely on morphological measures.[21] The most significant morphological distinction amongst genera is the presence of basic roots in the subfamily Lemnoideae, which includes the genera *Lemna*, *Landoltia*, and *Spirodela*. The subfamily Wolffiodeae does not have roots and includes the genera *Wolffia* and *Wolffiella*.[22] There are several key advantages duckweeds possess when considering their use in biotechnological applications. First, duckweeds are globally distributed with several species present across multiple continents. These include *S. polyrhiza*, *Landoltia punctata*, *Lemna aequinoctialis*, *L. minor*, and *L. trisulca*.[23] Duckweeds have one of the fastest growth rates among higher plants with a relative growth rate ranging from 0.153 to 0.519 relative growth per day and doubling times reported between 1.34–4.54 days[24] and some as fast as 16–48 h.[25] The nutritional composition of duckweeds is generally well suited for use in both livestock (poultry, cattle, and fish) and human diets.[26–28] Specifically, duckweed has crude protein content ranging between 20% and 35%, lipid content between 4% and 7% with 48%–71% of the fats being polyunsaturated fatty acids (PUFAs), and a good balance of amino acid profiles[29] with *Wolffia microscopica* and *Wolffiella hyalina* identified as ideal for human consumption.[30]

Here we review the current literature investigating the use of duckweed in fish feed. We include papers that look at various methods of consumption of duckweed ranging from direct grazing to inclusion into a feed pellet. Along with investigating the traditional growth endpoints of growth rate, food conversion ratio, and survival rate, we also include short studies that look at palatability and digestibility. We perform a more thorough meta-analysis or cross comparison of duckweed as a fish feed for the most prominent and well-studied fish species, including grass carp and Nile tilapia.

2 | MATERIALS AND METHODS

2.1 | Overview of analysed studies and exclusion criteria

We performed a systematic literature review searching the title or abstract for the keywords 'duckweed' and 'fish.' We searched across three prominent databases: web of science, proquest, and EBSCO up until Feb 22 2023. Duplicate or repetitive manuscripts found across multiple databases were removed so as to not double count. Only research articles written in English were included. A total of 58 articles were included and are listed in Table S1. We were able to access the full article for 55 of the 58 studies and subsequently downloaded and included it in the supplement data. The full article for three of the studies were not accessible thus we only derived information from the abstract. The studies that were not accessible are indicated in the metadata (PDF < column>: FALSE) and included the following: Peters 2009, Hlope 2011, and Zhang 2017 (Table S1).

2.2 | Duckweed cultivation, processing, and fish-rearing metadata, curated from studies

A common set of metadata was collected for each of the 58 studies and is indicated across the columns. This includes <year> 'publication date', <country> 'location where the study took place', <fish species> 'the species of fish used in the study'. If a study used multiple species of fish, then there is an additional row in the datasheet for each unique species. Fish are indicated by scientific names followed by common names. All studies include at least one fish species. The next columns <fish age class> indicate the approximate age or size class of the fish used in the study. 'Fry' typically refers to fish less than 30 days post hatch or ~less than 1 g, whereas fingerlings could be a few months post hatch or between 1 g and 10–20 g. The size class can differ across species however and we generally list the size class based on what was reported by the authors in the manuscript. For instance, where the size class is not stated by the authors, we approximate this based on the actual starting size of the fish. Three of the 58 studies did not report this information. The next column <starting fish mass (g) > refers to the average mass of the fish used at the start of the experiment. Three of the studies did not report this value and an additional study we did not have access thus four total studies had missing data here. Next, <experiment length (days) > the total time of the experiment was recorded in days. Two of the 58 studies did not report the values.

Next, we indicated the type of duckweed used in the experiment. If the duckweed type was not listed, we simply indicated this as <Duckweed spp>. One study referred to the duckweed used as *Lemna minima*. *Lemna minima* in the past has been synonymous with multiple species of duckweed including *L. minor*, *L. aequinoctialis*, and *L. minuta*.[31] We recorded this as a '*Lemna* sp.'[32] Two other studies referenced an incorrect name for duckweed *Lemna polyrhiza*. Since we do not know if they meant *S. polyrhiza* or another species of *Lemna*, we have left the name as such *Lemna polyrhiza*.[33,34] In the past, *L. polyrhiza* was incorrectly used to indicate *S. polyrhiza*.[31] The method of preparation of the duckweed was recorded: <natural grazing>, <raw/fresh>, <dried>, <dried and pelleted>, <fertilized>, and <fermented>. Natural grazing is indicative of allowing the duckweed to grow naturally in the pond or experimental tank whereas raw/fresh indicates when the duckweed was harvested from another pond and then presented to the fish. Dried indicates when the duckweed was harvested from a given pond and allowed to dry for some period of time before being given to the fish to consume. Dried and pelleted indicates duckweed that was dried, ground into a powder, and then incorporated into a pellet 'fish feed'. Fertilized is indicative of the duckweed being harvested from one pond and then placed into another pond with fish. Although this would technically fall under the <raw/fresh> category, we include it as fertilization because that is how the authors describe it in the paper. Fermented indicates where duckweed was dried, ground into a powder, and then processed through a fermentation reaction lasting more than 1 week.

The next series of columns indicates the inclusion level of duckweed used in the experiment. For any experiment that had fish naturally graze on duckweed or presented raw or crudely processed duckweed, this would be indicative of 100% inclusion. The inclusion level primarily changed or varied in experiments where duckweed was dried and incorporated into a pelleted feed. Here we simply indicate the various inclusion levels tested within a given experiment. Our next series of columns indicates the general types of endpoint measurements taken for the fish. The feed conversion ratio <FCR> is a measure of how efficiently feed is incorporated into fish biomass. <Growth> can be measured in several ways including overall growth or specific growth rate (SGR). The <survival> is indicative of survival rate. The <apparent digestibility> is a measure of how much of the feed is utilized by the fish and generally requires the measure of faecal pellets of the fish and/or uneaten feed. The <palatability> column indicates studies that looked at if the extent fish accepted or rejected some form of duckweed. The <taste-humans> indicates a study that measured the extent humans like the taste of fish growth on duckweed as feed. Finally, the <other> column indicates studies that did uncommon endpoints not directly related to the health of the fish. This includes microbiome sampling, transcriptomics, or other physiological measures of the blood or liver. The column <TEST> indicates the general conclusion of the study with regard to duckweed performance as a feed. Because every study is different, it is difficult to compare holistically. Despite this challenge, we indicated the overall sentiment of the study as duckweed versus control: worse, same, or better than control. For the <fish endpoint measures> column, we list the measures as either −1 (worse than control), 0 (same as control), or 1 (better than control) to give a general sense of how duckweed broadly performs across studies. Finally, we include general notes from the studies. We generate figures using Prism 9.4.1.

3 | RESULTS AND DISCUSSION

A total of 58 articles published from 1977 to 2022 were included in this review (Table S1; Table 1). The number of studies evaluating the use of duckweed as a fish feed has been steadily increasing since the 1980s (Figure 1a). Research has been conducted across 31 countries with the majority occurring in China (9 studies) and India (6 studies; Figure 1b). China and India are both leaders in freshwater fish aquaculture production.[35]

Within these 58 articles, a total of 18 species of fish along with an additional four hybrid species were included (Figure 2). While most studies focused on one species of fish, several studies analysed various species simultaneously often in polyculture. The majority of experiments focused on freshwater aquaculture fishes. Overall, only three of the fishes are typically reared in marine environments. This

TABLE 1 Research articles evaluating use of duckweed (Lemnaceae) as a fish feed.

Year	CTY	Fish_species	age	mass	days	DW_species	DW_prep	DW_inclusion	Outcome	References
1977	USA	*C. idellus*	adt	320	41	*L. minor*, *L. gibba*	nat, raw	100	AD	36
1978	USA	*C. idellus*	fry	3	68	*Lemna* sp.	raw	100	FCR,G,SR	32
1978	USA	*C. idellus*	juv	63	68	*Lemna* sp.	raw	100	FCR,G,SR	32
1982	USA	*C. idella* × *H. nobilis*	juv	40	60	*W. columbiana*, *L. gibba*	raw	100	FCR,G	37
1984	ISR	*O. niloticus* × *O. aureus*	adt	139[a]	89	*L. gibba*	raw	50, 100	G,SR	38
1986	IND	*C. idellus*	fng	5.5	133	*W. arrhiza*	nat, raw	100	FCR,G,AD	39
1986	IND	*H. molitrix*	fng	15.5	133	*W. arrhiza*	nat, raw	100	FCR,G,AD	39
1986	IND	*C. carpio*	fng	15	155	*W. arrhiza*	nat, raw	100	FCR,G,AD	39
1986	IND	*B. gonionotus*	fng	9.5	120	*W. arrhiza*	nat, raw	100	FCR,G,AD	39
1986	IND	*C. mrigala*	fng	5	155	*W. arrhiza*	nat, raw	100	FCR,G,AD	39
1986	IND	*L. rohita*	fng	6	155	*W. arrhiza*	nat, raw	100	FCR,G,AD	39
1991	GBR	*C. idellus*	fng	11.5[a]	33	*Lemna* sp.	pellet	100	AD	40
1992	CHN	*C. idellus*	fng	3.78	21	*L. minor*, *S. polyrhiza*	raw	100	G,SR	41
1992	NLD	*C. idellus*	adt	131[b]	2	*L. minor*	raw	100	PAL	42
1992	THA	*O. niloticus*	juv	27.5	70	*L. perpusilla*, *S. polyrhiza*	raw	100	FCR,G,SR	43
1992	THA	*O. niloticus*	juv	42.5	70	*L. perpusilla*, *S. polyrhiza*	raw	100	FCR,G,SR	43
1993	CHN	*C. idellus*	fng	8.7	1	*L. minor*	raw	100	PAL	44
1994	PHL	*C. chanos*	juv	50	90	*Lemna* sp.	fert	100	G,SR	45
1999	NGA	*O. niloticus*	fng	13.9	56	*S. polyrhiza*	pellet	5, 10, 20, 30, 100	FCR,G,SR	46
2001	NGA	*O. niloticus*	fng	13.9	56	*S. polyrhiza*	pellet	5, 10, 20, 30, 100	FCR,G	47
2002	IND	*L. rohita*	fng	6.4	80	*L. polyrhiza*	raw, ferment	10, 20, 30, 40	FCR,G,AD	33
2003	BGD	*B. gonionotus*	NR	NR	NR	*Lemna* sp.	raw	100	FCR,G,SR	48
2003	BGD	*C. carpio*	NR	NR	NR	*Lemna* sp.	raw	100	FCR,G,SR	48
2003	BGD	*C. catla*	NR	NR	NR	*Lemna* sp.	raw	100	FCR,G,SR	48
2003	BGD	*L. rohita*	NR	NR	NR	*Lemna* sp.	raw	100	FCR,G,SR	48
2003	CZE	*C. idellus*	fng	19.6	14	*S. polyrhiza*	nat, raw	100	FCR,G	49
2004	NLD	*O. niloticus*	juv	56.1	50	*L. minor*	raw, pellet	15	G,AD	50
2004	NLD	*O. niloticus*	juv	90	49	*L. minor*	nat, raw	20, 40	G,AD	51
2004	BGD	*C. idellus*	NR	NR	NR	*Duckweed spp*	nat	100	OTH	52
2004	BGD	*H. molitrix*	NR	NR	NR	*Duckweed spp*	nat	100	OTH	52
2004	BGD	*O. niloticus*	NR	NR	NR	*Duckweed spp*	nat	100	OTH	52
2004	TUR	*C. carpio*	fry	0.29	90	*L. minor*	pellet	5, 10, 15, 20	G	53
2005	NLD	*O. niloticus*	juv	90	35	*L. minor*	raw, pellet	20, 40	AD	54
2007	EGY	*O. niloticus*	fng	20	112	*L. gibba*	nat, raw	100	FCR,G	55
2008	KHM	*O. niloticus*	fng	21.5	120	*Duckweed spp*	nat	100	G,SR	56
2008	KHM	*C. carpio*	fng	18.9	120	*Duckweed spp*	nat	100	G,SR	56
2008	KHM	*C. mrigala*	fng	19.5	120	*Duckweed spp*	nat	100	G,SR	56
2009	VEN	*O. mossambicus*	fng	NA	84	*L. obscura*	pellet	15, 25, 35	FCR,G	57
2011	ZAF	*C. rendalli*	juv	64	224	*L. minor*	nat, raw	100	G	58
2011	JPN	*C. complex*	juv	19	99	*L. aoukikusa*, *L. gibba*, *S. polyrhiza*	nat	100	PAL	59
2011	NGA	*C. gariepinus*	juv	50	14	*L. pauciscostata*	pellet	30	AD	60
2012	CAN	*O. niloticus*	juv	48	20	*L. gibba*	raw	100	G	61
2013	SAU	*O. niloticus*	juv	33.4	90	*L. gibba*	nat, raw	100	FCR,G,SR	62
2013	CHN	*C. idellus*	fng	1.8	60	*L. minor*	raw	100	G,OTH	63
2013	SWE	*P. hypophthalmus*	fng	8.5	30	*L. polyrhiza*	pellet	30	AD	34
2013	CHN	*C. idellus*	juv	99.6	60	*L. minor*	dried	100	G	64

(Continues)

TABLE 1 (Continued)

Year	CTY	Fish_species	age	mass	days	DW_species	DW_prep	DW_inclusion	Outcome	References
2013	RUS	*R. rutilus*	juv	4.8	1	*L. minor*	pellet	30	AD	65
2014	BRA	*O. mossambicus* × *O. niloticus*	fng	0.8	83	*L. valdiviana*	pellet	100	FCR,G	66
2015	CHN	*C. idellus*	fry	0.39	70	*L. minor*	nat	100	OTH	67
2015	CHN	*C. idellus*	fry	0.39	70	*L. minor*	nat	100	OTH	68
2016	India	*C. idellus*	fng	16.3	120	*L. minor*	pellet	20, 40, 60, 80, 100	FCR,G,SR	69
2017	PAK	*C. idellus*	fng	5.54	90	*L. minor*	pellet	20	FCR,G	70
2017	PAK	*H. molitrix*	fng	5.54	90	*L. minor*	pellet	20	FCR,G	70
2017	CHN	*C. carpio*	juv	53	56	*Duckweed spp*	pellet	3, 6, 9, 13, 14	FCR,G	71
2017	MYS	*E. fuscoguttatus* × *E. lanceolatus*	juv	10.3	70	*L. minor*, *S. polyrhiza*	pellet	5	FCR,G,AD	72
2018	BGR	*C. carpio*	fng	12	40	*L. minor*	pellet	10, 30	FCR,G,SR	73
2018	NLD	*O. mossambicus*	fng	20	7	*L. minor*	dried	100	OTH	74
2018	EGY	*C. idellus*	fng	10.3	70	*S. polyrhiza*	raw	100	G,SR,PAL	75
2019	CHN	*C. carpio*	adt	492	56	*Duckweed spp*	nat, raw	50, 100	OTH	76
2019	CHE	*O. mykiss*	fry	0.28	28	*L. punctata*, *S. polyrhiza*	pellet	6, 12	FCR,G	77
2020	IND	*L. rohita*	fng	10.7	90	*L. minor*	pellet	30	FCR,G	78
2020	CHN	*C. carpio*	adt	493	56	*Duckweed spp*	NR	50, 100	OTH	79
2020	BGR	*C. carpio*	fng	41	60	*L. minor*	pellet	50, 100	FCR,G	80
2021	ECU	*C. gariepinus*	fng	1.27	48	*L. perpusilla*	pellet	6, 12, 18	G,SR	81
2021	BRA	*O. niloticus*	juv	22	28	*L. minor*	raw	100	FCR,G	82
2021	IDN	*O. niloticus*	NR	NR	90	*L. perpusilla*	raw	50, 100	TAS	83
2021	MYS	*C. auratus*	fng	2.3	49	*Lemna* sp.	nat	100	FCR,G,SR	84
2022	KEN	*O. niloticus*	fng	2	84	*L. minor*	pellet	5, 10, 15, 20, 30	FCR,G,SR	85
2022	IND	*C. carpio*	fry	0.48	60	*L. minor*	pellet	5, 10, 15, 20	FCR,G	86
2022	IND	*C. carpio*	fry	0.47	60	*S. polyrhiza*	pellet	5, 10, 15, 20	FCR,G	87
2022	ITA	*O. mykiss*	juv	125	90	*L. minor*	pellet	10, 20, 28	G	88
2022	IDN	*L. calcarifer*	fng	7.53	63	*L. minor*	ferment	15, 25, 35, 45	FCR,G,OTH	89
2022	DZA	*O. niloticus*	fry	0.87	56	*L. minor*	pellet	5, 10, 20	FCR,G,SR	90

Note: CTY code, country code; USA, United States; ISR, Israel; IND, India; GBR, England; CHN, China; NLD, Netherlands; THA, Thailand; PHL, Philippines; NGA, Nigeria; BGD, Bangladesh; CZE, Czech Republic; TUR, Turkey; EGY, Egypt; KHM, Cambodia; VEN, Venezuela; ZAF, South Africa; JPN, Japan; CAN, Canada; SAU, Saudi Arabia; SWE, Sweden; RUS, Russia; BRA, Brazil; PAK, Pakistan; MYS, Malaysia; BGR, Bulgaria; CHE, Switzerland; ECU, Ecuador; IDN, Indonesia; KEN, Kenya; ITA, Italy; DZA Algeria. Fish species: species of fish used in the experiment: *C. idellus*: *Ctenopharyngodon idellus* (grass carp); *C. idella* × *H. nobilis*: *Ctenopharyngodon idella* × *Hypophthalmichthys nobilis* (hybrid grass carp); *O. niloticus* × *O. aureus*: *Oreochromis niloticus* × *Oreochromis aureus* (hybrid Nile × blue tilapia); *H. molitrix*: *Hypophthalmichthys molitrix* (silver carp); *C.carpio*: *Cyprinus carpio* (common carp); *B. gonionotus*: *Barbodes gonionotus* (silver barb); *C. mrigala*: *Cirrhinus mrigala* (Indian major carp); *L. rohita:Labeo rohita* (roho); *O. niloticus*: *Oreochromis niloticus* (Nile tilapia); *C. chanos*: *Chanos chanos* (milkfish); *C. catla*: *Catla catla* (catla); *O. mossambicus*: *Oreochromis mossambicus* (Mozambique tilapia); *C. rendalli*: *Tilapia Coptodon rendalli* (redbreast tilapia); *C. complex*: *Carassius complex* (crucian carp); *C. gariepinus*: *Clarias gariepinus* (African catfish); *P. hypophthalmus*: *Pangasianodon hypophthalmus* (striped catfish); *R. rutilus*: *Rutilus rutilus* (common roach); *O. mossambicus* × *O. niloticus*: *Oreochromis mossambicus* × *Oreochromis niloticus* (Nile × Mozambique); *E. fuscoguttatus* × *E. lanceolatus*: *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus* (hybrid grouper); *O. mykiss*: *Oncorhynchus mykiss* (Rainbow trout); *C. auratus*: *Carassius auratus* (goldfish); *L. calcarifer*: *Lates calcarifer* (Barramundi); age (fish age class): fry = fry, fng = fingerling, juv = juvenile, adt = adult, NR = unknown, not reported; mass: the reported average mass of the fish at the beginning of the experiment (grams); DW_prep: preparation of duckweed used in the fish feed: nat = natural grazing where the duckweed grows in same water as the fish; raw = raw or fresh where dw is harvested from a separate pond and fed to fish with minimal processing; dried = harvested dw is dried (typically solar) before presented to fish; pellet = harvested dw is dried, ground into a powder or meal, incorporated into a pellet with other ingredients; fert = harvested dw is added to a pond as a 'fertilizer' to promote growth of natural feed; ferment = harvested dw is dried and then fermented followed by incorporation into a pelleted feed; <> if multiple methods used, separated by comma. DW_inclusion: refers to the per cent of the diet which was derived from duckweed; if controls were used such as a commercial diet, they are not included here; outcome: indicates the primary outcome(s) measured in the experiment as it relates to the fish.

Abbreviations: <>, if multiple outcomes measured, separated by comma; AD, apparent digestibility; FCR, food conversion ratio; G, growth rates including but not limited to SGR (specific growth rate); NA, data unknown, data access is restricted; NR, data unknown, not reported in paper; OTH, other which can include outcomes such as fish transcriptome, microbiome etc.; PAL, palatability for the fish; SR, survival rate; TAS, taste or palatability for the human consuming the fish; REF, references.

[a]Average inferred from length (23–25 cm).

[b]Inferred from length (23–25 cm).

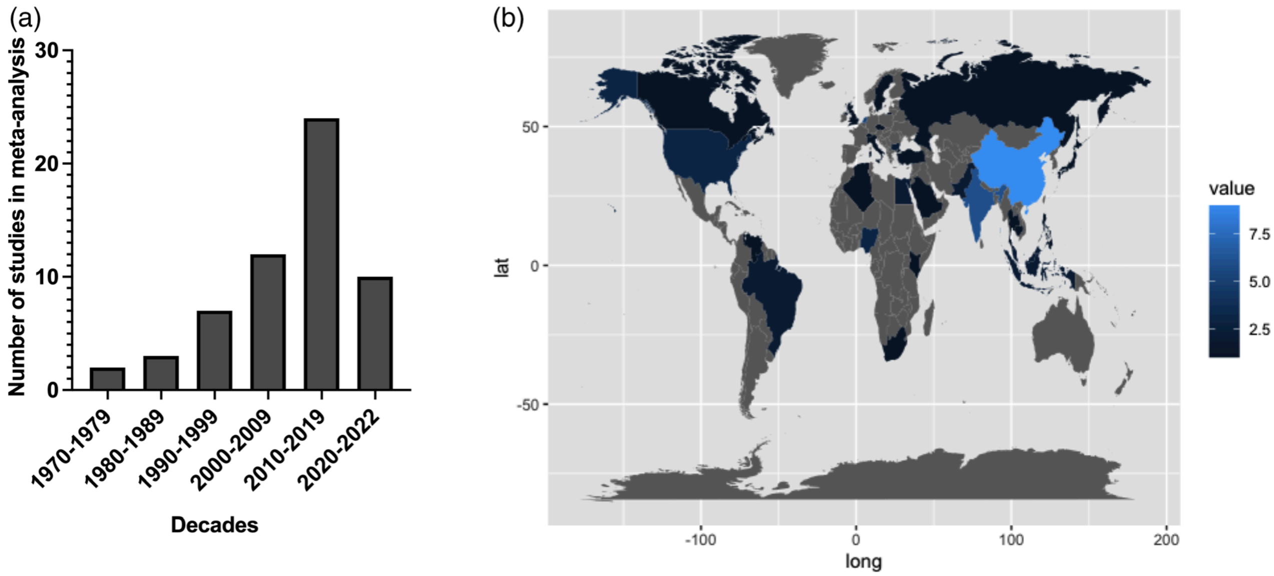

FIGURE 1 Distribution of duckweed studies. (a) Number of studies evaluating duckweed as a fish feed summed per decade. (b) Geographical histogram of duckweed studies.

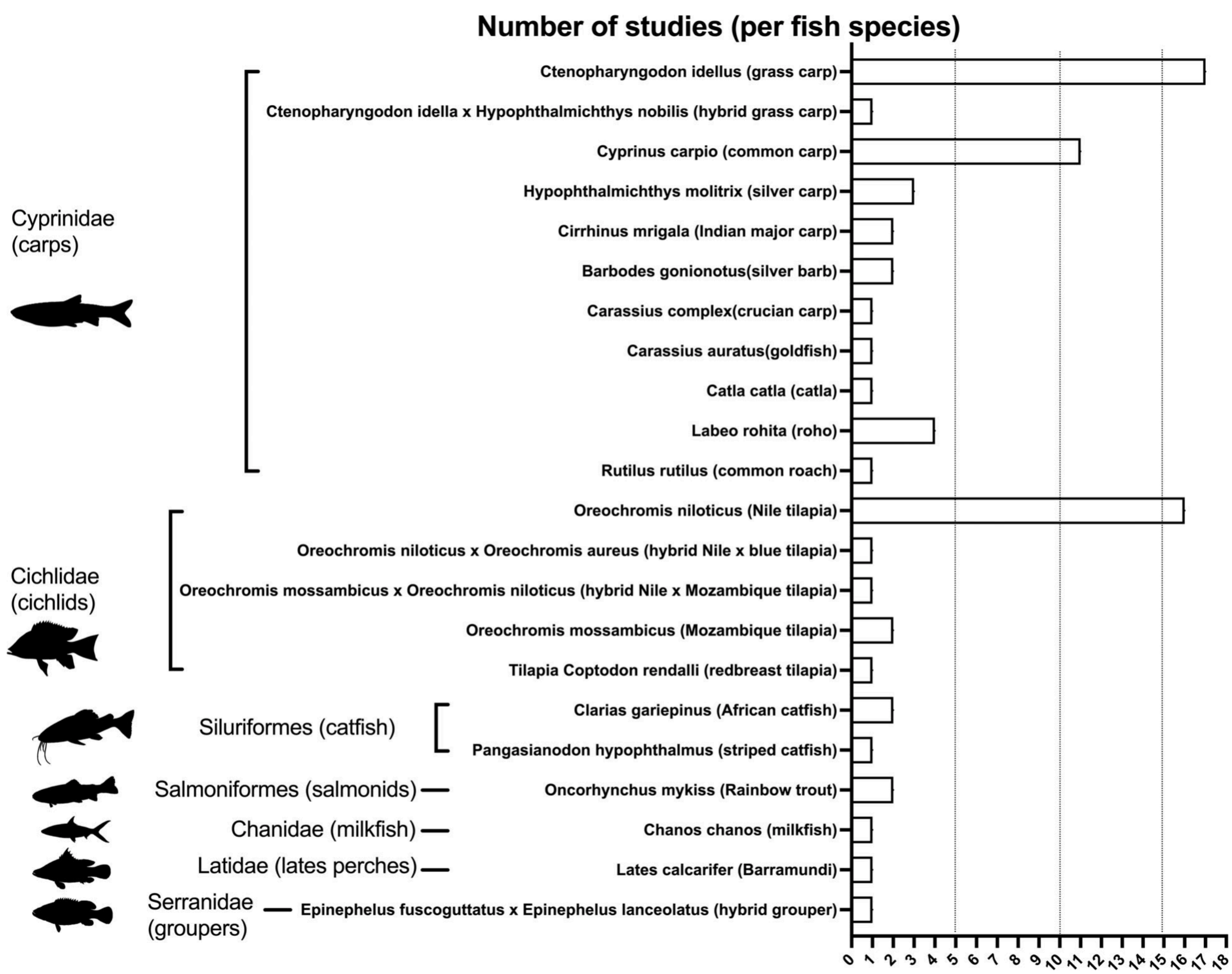

FIGURE 2 Duckweed fish feed experiments conducted across 18 species and 4 hybrid species of fish. Silhouettes adapted from phylopic.org.

includes the hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) and milkfish (*Chanos chanos*), which are both exclusively marine. The third species, barramundi (*Lates calcarifer*), is often grown in marine cages, but can also be reared in freshwater or brackish water. The only other fish that can be reared in brackish waters is Mozambique tilapia, *Oreochromis mossambicus*. All of the other fishes are grown in freshwater habitats. Of the families of fishes evaluated, the most common were the Cyprinidae (carps) and Cichlidae (cichlids). The grass carp, *Ctenopharyngodon idellus*, had the most studies at 17, while the common carp, *Cyprinus carpio*, had the second highest amongst carp at 11 studies. Nile tilapia, *Oreochromis niloticus*, had the second most studies overall at 16. A total of three studies were conducted on catfishes including two on African catfish, *Clarias gariepinus* and one study on striped catfish, *Pangasianodon hypophthalmus*. (Figure 2).

3.1 | Variation in experimental design

The majority of experiments lasted between 50 and 100 days in total length. The average experimental time was 71.7 days in length. Four experiments were very short lasting less than 10 days. Three of these were focused on measuring palatability of feeds in grass carp[42] and common roach while one focused on heavy metal bioaccumulation.[74] The longest experiment was 224 days[58] (Figure 3a). The majority of experiments used fish at the fingerling stage, followed by juveniles (Figure 3b). Fish grow the fastest when they are small thus changes in size or growth rates are easiest detected when fish are naturally growing at fast rates. The average starting mass of the fish from all experiments was 45.7 g (Figure 3c). For experiments starting at the fry stage, the average starting mass was 0.77 g. At the fingerling stage, the average starting mass was 11.2 g. The starting mass at the juvenile stage was 51.99 g and at adult was 314 g (Figure 3c). Based on these analyses, it is clear that there is still a great opportunity to understand how duckweed inclusion impacts the growth stage of fish aquaculture. As fish age, they grow less and this is when the most feed is consumed. Understanding how and if duckweed inclusion can enable continued growth in larger fish will have the most economic impact on farmers.

3.2 | Duckweed species and incorporation into fish feeds

Currently, there are 36 known species of duckweed represented across five genera including (*Wollfia*, *Wollfiela*, *Landolita*, *Lemna*, and *Spirodela*).[20] The authors of the reviewed papers reported a total of 13 known species across four genera (*Wollfia*, *Landolita*, *Lemna*, and *Spirodela*) of duckweed that were used across the studies. Many studies or experiments acknowledged they did not know the species of duckweed used and simply labelled it as duckweed (nine studies) or *Lemna* species (nine studies; Figure 4). Duckweed can be very challenging to correctly identify and we suspect that many of the labels from these experiments may indeed be incorrect. Since genomic resources are now available for most species of duckweed, we advise that future studies should take advantage of molecular barcoding approaches to validate the duckweed species they are using.[23] Of the four genera, *Lemna* had the most experiments (54) followed by *Spirodela* (11), *Wollfia* (7), and *Landolita* (1) (Figure 4). Here an experiment is defined by the feeding of a unique species of fish with a unique duckweed. The overall number is higher than the total number of reviewed studies because some studies evaluated multiple species of duckweed or multiple species of fish. It is unclear why no species of *Wollfiela* has been evaluated as a fish feed. Overall, there appears to be a great opportunity to evaluate these other duckweed species in fish feeds.

Duckweed has a great potential to be used as an animal feed due to rapid growth, high crude protein content (35%–45%), a high lipid content, and low fibre content.[26,91] Duckweed has been incorporated

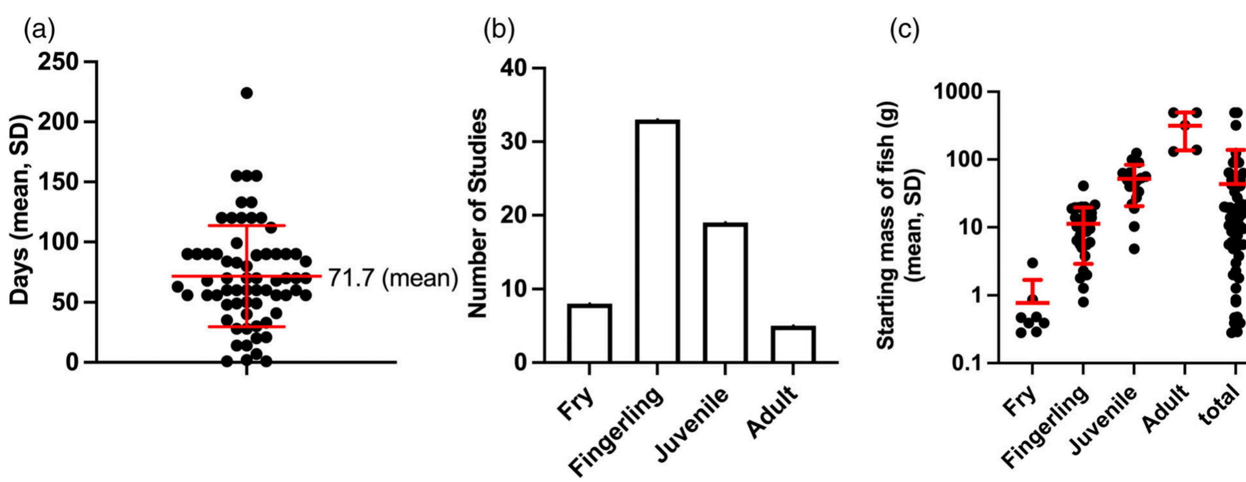

FIGURE 3 Fish grow out experiments. (a) The experiment length in days for all articles. (b) The summed total of studies per fish age class as described in the study or derived from the metadata of starting mass. (c) The distribution of starting mass of fish by age class and then all together.

into fish feed in a variety of methods including natural grazing (22 studies), raw or fresh (32 studies), dried (2 studies), dried and incorporated into a pellet (27 studies), application to the pond as a fertilizer (1 study), and fermented (2 studies; Figure 5a). In some instances, fish would graze on the duckweed as a natural feed while in other experiments fish were fed supplemental feeds in addition to the natural duckweed in the pond. The raw/fresh classification includes studies where the duckweed was grown on some other pond system, harvested and often drained followed by presentation to the fish in a separate system. In many low and middle-income countries (LMIC) duckweed aquaculture is commonly used in human wastewater systems to treat and purify wastewater, because it removes nutrients and prevents pathogens from growing.[92,93] In the 1970s and through the 1990s, the majority of experiments were focused on feeding fish unprocessed duckweed in fresh forms. The first study using a dried form of duckweed was in 1991, where they were calculating the bioenergetics of grass carp fed different diets using a respirometer.[40] Fasakin et al. was the first to incorporate dried duckweed into a pellet form, fed to Nile tilapia, in 1999. In the past two decades, the majority of new research on duckweed as a fish feed has relied on incorporating dried duckweed meal into pelleted fish feed (Figure 5b). To date, only two papers have explored the process of fermenting duckweed as a means to increase digestibility by the fish through breaking down indigestible fibres like cellulose. In the first example, Ray et al. isolated a *Bacillus* spp. from the gut of a common carp which had extracellular amylolytic, cellulolytic, proteolytic, and lipolytic properties. The authors declare that the duckweed, *Lemna polyrhiza*, was then fermented using this *Bacillus* isolate for 15 days at 37°C and then incorporated into the feed. A 30% inclusion of the fermented *L. polyrhiza* resulted in the best growth performance of the rohu fingerlings from the 80-day experiment.[33] Twenty years later, Mustofa et al. performed a similar experiment where they isolated a *Bacillus* spp. from

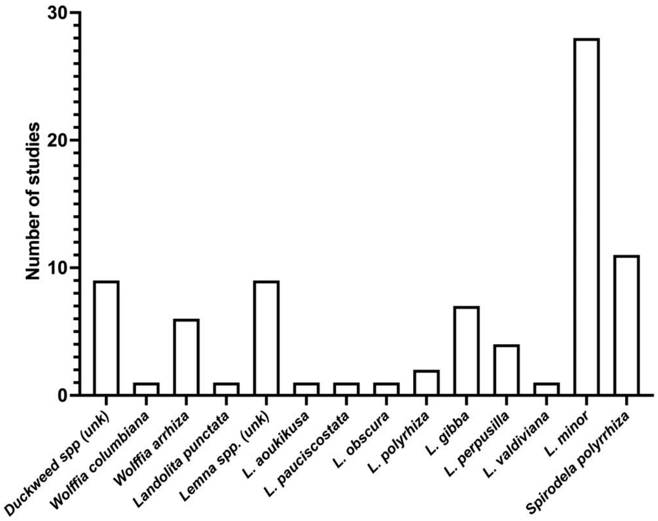

FIGURE 4 The types of duckweed used in experiments. Total sum of studies per duckweed species across duckweeds. If an unknown or unclassified species of duckweed was used, it was grouped into 'Duckweed spp (unk)'. *Two experiments incorrectly refer to the use of 'Lemna polyrhiza' as the duckweed used. While it is likely the authors meant *S. polyrhiza*, we cannot know for certain thus we have retained it as *L. polyrhiza*.

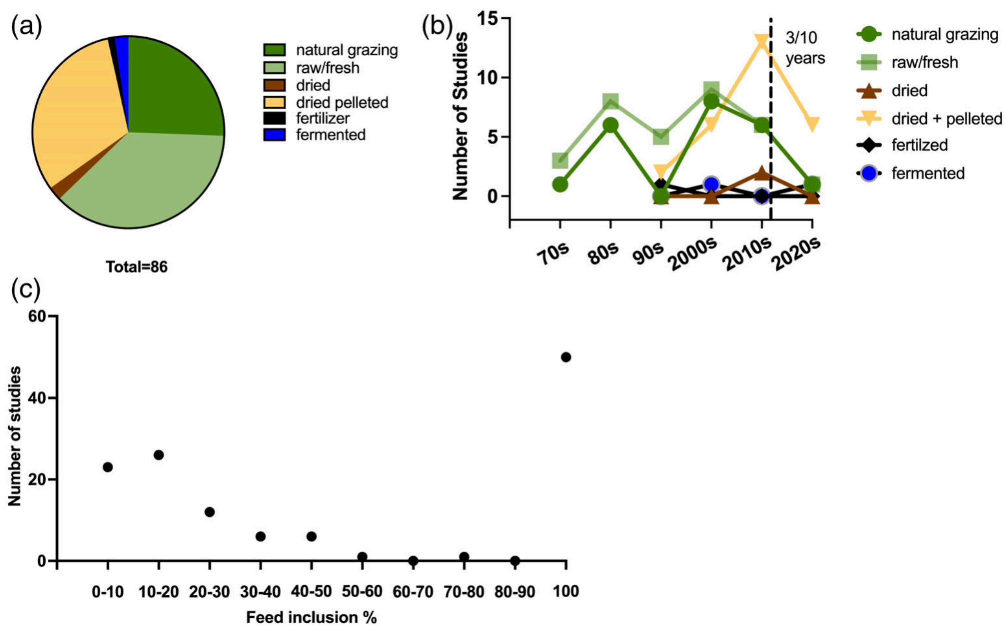

FIGURE 5 Method of duckweed inclusion as fish feed. (a) Overall number of studies utilizing duckweed in various forms including natural grazing, raw/fresh, dried, dried and then included into pelleted feed, fertilizer, and fermented. (b) The number of studies using processing methodologies over time from 1977 to 2022. (c) The summed number of studies for each inclusion rate. A 100% inclusion rate includes studies where fish were presented with whole fresh duckweed. Studies with partial inclusions will be indicative of use in a pelleted feed where some percentage of the feed is dry duckweed powder or meal.

the gut of the crayfish *Cherax canii*. Dried *Lemna minor* duckweed was fermented for 16 days at 37°C and then included in pellet feeds that were fed to the marine aquaculture fish, Barramundi. The 25% inclusion of fermented duckweed had the best results for growth whereas an inclusion of up to 35% was still similar to control diets.[89] Duckweed fermentation by means of animal gut isolates may enable much broader utilization across marine aquaculture fishes. Aside from studies feeding fish only duckweed, the most common inclusion rates of pelleted feeds were between 0% and 30% with the peak being 10%–20% (Figure 5c).

3.3 | Endpoints of fish experiments

A variety of endpoint measures were assessed across the studies including feed conversion ratio (FCR), growth rate (includes overall growth rate and specific growth rate SGR), survival rate, digestibility of the feed, palatability of the feed, palatability of the fish as consumed by humans, and 'other'. Other includes non-typical endpoint measures including molecular assays of the fish (e.g., transcriptomics) or microbiome analysis of the water. Growth rate and FCR were the most common endpoint measures across all experiments (Figure 6a). We assessed the general results from these studies to understand the general impact duckweed has on fish aquaculture. Overall, both growth and FCR were positively impacted by consumption of duckweed suggesting duckweed utility as a component in fish feed. Several studies did report negative impacts on survival rate and lower performance of digestibility as noted (Figure 6b). This suggests that additional research will be needed to tease out the impacts.

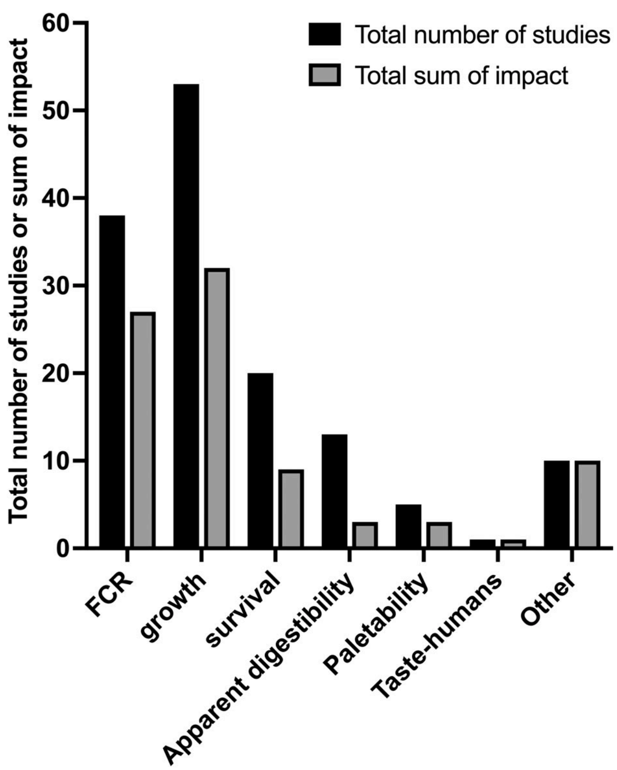

FIGURE 6 Endpoints of fish feed experiments. Total number of studies and total sum of impacts where the following endpoints of the fish were used. The 'sum of impact' captures the extent the endpoint measures had positive or negative outcomes. For each outcome in a study, the overall impact of consuming duckweed was rated as 'worse than control = −1', no difference from control = 0, or better than control = +1′. The sum of these values were tabulated.

3.4 | Concerns with use of Duckweed in fish feeds

Certain aspects of duckweed aquaculture and biology may pose a threat to future use as fish feeds. Duckweeds proliferate in nutrient rich, low-flow bodies of water. This can include stagnant water ponds including crude wastewater lagoons. Because of this feature, duckweed aquaculture is often applied as a wastewater treatment method in LMIC to reduce human pathogen loads while creating biomass to use for fish feed.[52] Duckweed grown on human wastewater can accumulate human pathogens such as *Aeromonas*.[94] Thus, it is critical to design systems to decontaminate or sterilize duckweed from human pathogens when used as fish feed. Duckweed grown in wastewater systems will often accumulate heavy metals such as Cadmium and Zinc which if then used as a feed may result in bioaccumulation in the muscle.[74,95,96] Heavy metal bioaccumulation in duckweeds varies across species.[97] However, aquatic plants may also accumulate organic contaminants including pesticides from field runoff.[98] Thus future work should aim to use comparative genomics, genetics, and gene editing to understand these mechanisms to develop contaminant free, duckweed lines for use exclusively in animal feeds.

Another long-term concern for using plant-based feeds, including duckweed, in fish feeds is the presence of antinutrients that include many molecules such as tannins and phytic acid. These antinutrients may result in intestinal inflammation and slowed growth in the fish.[11] Duckweeds can contain high levels of oxalate and phytates that impact calcium and general mineral uptake respectively in humans.[99] *Spirodela* and *Lemna* spp. also contain components including oxalic acid that negatively influence the taste or palatability to the fish.[100,101] Palatability problems are further amplified when developing plant-based feeds for carnivorous rather than herbivorous fishes.[102] However, post-harvest processing methods such as drying, washing, heating, or enzymatic digests facilitate anti-nutrient removal.[99] Two studies in this review showed how duckweed fermentation further had a beneficial impact on fish growth.[33,89] Although they did not evaluate changes or reductions in anti-nutrient factors, they did look at digestibility. Anaerobic fermentation of macrophytes including duckweed (*Lemna minor*, *Spirodela polyrhiza*) and water fern (*Azolla filiculoides* and *Eichhornia crassipes*) was shown to reduce anti-nutrient factors and also fibre content.[103] Anaerobic digestion is a common tool to remove organic and inorganic contaminants from wastewater systems[104] thus should be further explored in improving duckweed safety for use in fish feed.

3.5 | Duckweed feeding experiments with Grass carp, *Ctenopharyngodon idella*

Grass carp, *Ctenopharyngodon idella*, is a herbivorous, fast-growing fish native to Asia.[105] It was originally cultured and used for aquatic vegetation control, but due to its fast growth quickly became a staple food source.[106] Grass carp use pharyngeal teeth and a horny pad in its mouth to masticate and chew plant material, damaging plant cell walls and enabling digestion of approximately 60%–70% of plant material.[107] Aquaculture of grass carp has grown to become the number one fish worldwide with an estimated 5.79 million tonnes of production in 2020, making up 11.8% of total inland farmed fish and 6.6% of total farmed fish production.[35] We focus only on a subset of studies focused on the primary aquaculture outcomes of feed conversion ratio, specific growth rate, average grams per day, survival rate, or apparent digestibility (AD). Table 2 summarizes the results of studies wherein duckweed was fed to grass carp, but excludes studies that analysed palatability or other endpoints, which typically have very short experiment times of 1 day or less. FCR ranged from 1.23 to 6 for all experiments with an average of 2.42. Most of the growth studies used *L. minor*. Specific growth rates (SGR) of fish-fed natural or raw *Lemna* ranged from 1.62 to 6.91. Two studies used *S. polyrhiza* and each had a lower SGR of 0.55 and 0.7. The overall average SGR for all experiments was 1.85, while the average growth rate in grams per day was 0.84. Survival rate was very high in all experiments ranging from 93% to 100% with an average of 98.6%.

AD of energy was measured in three different studies and estimated to be 64.9% for *L. minor* and *S. polyrhiza*-fed fish, 65% *L. minor* and *L. gibba*-fed fish, and 65% for *Lemna* sp. fed fish suggesting that different duckweed species may have an overall similar digestibility (Table 2). Grass carp growth is sufficiently supported by an all plant diet, but may benefit from an optimized inclusion into pelleted feed. One study looked at the impact of adding a cellulase to the pelleted feed and showed an increase in growth likely due to an increased digestibility.[64] Most of these studies look at a very narrow window of the fish growth cycle, focusing on the fingerling to juvenile stage. The average experiment length was only 79 days whereas the typical production cycle for grass carp is 8–10 months. Future studies need to look at consumption of duckweed over the full production cycle. In addition, most of these studies were conducted in small tanks or concrete ponds whereas the majority of grass carp production occurs in large earthen ponds. Experiments should be scaled to mimic typical production systems.

3.6 | Duckweed feeding experiments with Nile tilapia, *Oreochromis niloticus*

Nile tilapia is a globally important fish for aquaculture production. In 2020, Nile tilapia had the third highest production of all farmed fish at 4.41 million tonnes making up 9% of volume for inland production and 5% of all animal aquaculture.[35] Similar to the analysis of carp, here we focus on a subset of the tilapia studies that focus on the primary aquaculture outcomes of feed conversion ratio, specific growth rate, average grams per day, survival rate, or AD. Table 3 summarizes the results of studies that examine feeding duckweed to tilapia, but excludes studies that analysed human taste preferences or other endpoints. Nile tilapia is primarily grown in the tropics and the average water temperature across experiments was 27.5°C. The average experiment length was 68 days and the average starting mass of fish was 22 g. Only one study looked at impacts of fish growth during the fry stage[90] whereas no study evaluated growth performance during the entire typical grow-out cycle. Around 40% of the studies were conducted in relatively small aquaria settings (<100 L; mean = 7860 L, median = 252 L, range:50–25,000 L). The feeding rate across studies ranged from once per day to four times per day while the amount of feed given was on average 7.5% body weight per day. The first study in this analysis showed that a duckweed feeding rate of 3%–5% per body weight per day was optimal for growth. Higher feeding rates were associated with fish mortality.[108]

Broadly, there were five studies evaluating tilapia-fed natural or fresh duckweed and seven that evaluated duckweed incorporated as a pelleted feed including one study that had both (Table 3). The species of duckweeds used in the experiments were *L. minor* ($n = 4$), *S. polyrhiza* ($n = 3$), *L. gibba* ($n = 2$) and *L. perpusilla* ($n = 1$) with one study declaring simply 'duckweed species'. The FCR of duckweed incorporated pelleted feed was generally lower than that of fresh duckweed as feed (FCR: 3.42 ± 2.14, 1.74 ± 1.04; natural, pelleted; mean, SD). The SGR and average daily growth rate (g/day) were generally higher in studies using duckweed pellets as compared to natural or fresh grazing (SGR: 0.93 ± 0.85, 1.93 ± 0.43; g/day: 0.76 ± 1.14, 0.41 ± 0.45; natural, pelleted; Table 3). Finally, although most studies reported excellent survival rates of greater than 95%, some studies or conditions within studies had abnormally low survival rates. The mean survival rates from natural or fresh grazing experiments were generally lower than from experiments using pelleted feed (SR: 75.5 ± 31.43, 95.3 ± 5.57; natural, pelleted). Together, we conclude that across all major aquaculture metrics, tilapia performance is highest when using duckweed incorporated into pelleted feeds rather than fresh grazing. This is consistent with the carp meta-analysis and the overall progression of the research community using pelleted feeds. The incorporation and use of pelleted feeds is optimal as it allows for production and storage. It may also facilitate feed presentation to the fish and it allows for better intake as you can change the size of the feed to match the age class of the fish. Pelleted feeds may have a benefit over fresh or natural materials simply because they are optimized for the nutritional constraints of a given species of fish like tilapia in this instance. Specifically, pelleted feeds enable the researcher to ensure they are balanced with vitamins, amino acids, carbohydrates and fats. However, tilapia still grew decently well on fresh duckweed or by natural grazing. Duckweed polyculture with fish is feasible and useful for smallholder low intensity farmers. Only one study looked at feeding fish standard pelleted feeds along with fresh duckweed and found that Nile tilapia consumed approximately 0.5% of their body weight in fresh duckweed, which could help reduce feed costs.[82]

TABLE 2 Duckweed feed experiments measuring FCR, growth, SR, or AD for grass carp, *C. idellus*.

Duckweed			Tank conditions			Fish conditions				FCR	Growth		SR	AD	
%	prep	spp.	Size (L)	SD (fish/L)	°C	Mass (g)	Feed rate	Feed amount	Exp (days)	(dm)	SGR	GR (g/day)	%	%	References
100	nat	Lmin	NR	NR	25	0.39	cont	cont	70	–	4.19	0.099	–	–	67
100	nat	Lmin	NR	NR	25	0.39	cont	cont	70	–	4.19	0.099	–	–	68
100	raw	Lmin	6000	0.0333	25	1.8	cont	cont	60	–	4.35	0.377	–	–	63
100	raw	Lem	NR	0.5300	25	3	2×	sat	68	1.60	5.36	0.536	99	–	32
100	nat/raw	Warr	180,000	0.0011	29	5.5	NR	1000%	133	6.00	3.50	4.000	96	–	39
100	raw	Spol	10,000	0.0030	19	10.3	2×	sat	70	5.18	0.55	0.075	93		75
100	nat/raw	Spol	1000	0.002, 0.006	21	19.6	cont	cont	14	2.00	0.70	0.143	–	–	49
100	raw	Lem	NR	0.5300	25	63	2×	sat	68	2.70	6.91	1.150	99	–	32
100	dried	Lmin	1000	0.0300	24	99.6	1×	sat	60	–	1.62	2.741	–	–	64
100c	dried	Lmin	1000	0.0300	24	99.6	1×	sat	60	–	1.70	2.935	–	–	64
20	pellet	Lmin	60	0.4167	27	16.3	1×	5%	120	1.23	0.56	0.131	100		69
40	pellet	Lmin	60	0.4167	27	16.3	1×	5%	120	1.28	0.56	0.132	100		69
60	pellet	Lmin	60	0.4167	27	16.3	1×	5%	120	1.26	0.59	0.140	100		69
80	pellet	Lmin	60	0.4167	27	16.3	1×	5%	120	1.25	0.58	0.136	100		69
100	pellet	Lmin	60	0.4167	27	16.3	1×	5%	120	2.27	0.59	0.138	100		69
21	pellet	Lmin	54	0.5000	26	5.54	1×	4%	90	1.58	1.56	0.185	–	–	70
21	pellet	Lmin	182.5	0.5000	26	5.54	1×	4%	90	2.75	1.15	0.113	–	–	70
100	raw	Lmin Spol	12	1.2500	27	3.78	NR	sat	21	–	1.77	0.081	–	64.9	41
100	nat/raw	Lmin Lgib	55	NR	21	320	NR	NR	41	–	–	–	–	65	36
100	pellet	Lem	60	<1	22	11.5[a]	NR	0.50%	33	–	–	–	–	65.0	40

Note: Duckweed %: per cent of the feed which is comprised of duckweed; 100c: refers to a study where cellulase was included in the feed; Duckweed prep: preparation method used (nat = natural grazing, raw = raw or fresh, pellet = made into pellet); Duckweed spp.: Lmin = L. minor, Lgib = L. gibba, Lem = unknown Lemna spp., Warr = W. arrhiza, Spol = S. polyrhiza; Feed rate: number of times per day the fish is fed - if continuously fed = cont; Feed amount: sat = to satiation; cont = continuously fed; % = % body weight.
Abbreviations: AD: apparent digestibility of energy (%); FCR, food conversion ratio; Growth GR, growth rate (g/day); Growth SGR, specific growth rate; SR: survival rate.

TABLE 3 Duckweed feed experiments measuring FCR, growth, SR, or AD for Nile tilapia, *O. niloticus*.

Duckweed			Tank conditions			Fish conditions				FCR	Growth		SR	AD	
%	prep	spp.	Size (L)	SD (fish/L)	°C	Mass (g)	Feed rate	Feed amt	Exp (days)	(dm)	SGR	GR (g/day)	%	%	References
100	raw	Lper	25,000	0.0004	26.2	26.3	NR	2.5	70	2.20	0.97	0.37	100	–	108
100	raw	Lper	25,000	0.0004	26.2	27.4	NR	5	70	3.70	1.09	0.45	83	–	108
100	raw	Lper	25,000	0.0004	26.2	25	NR	7.5	70	6.00	1.04	0.38	20	–	108
100	raw	Lper	25,000	0.0004	30.2	39.6	NR	1	70	1.90	0.41	0.19	97	–	108
100	raw	Lper	25,000	0.0004	30.2	41.1	NR	2	70	1.90	0.80	0.44	100	–	108
100	raw	Lper	25,000	0.0004	30.2	43.7	NR	3	70	1.60	1.34	0.97	100	–	108
100	raw	Lper	25,000	0.0004	30.2	40.4	NR	4	70	2.30	1.40	0.96	60	–	108
100	raw	Lper	25,000	0.0004	30.2	43.6	NR	5	70	3.30	1.09	0.71	27	–	108
100	raw	Lper	25,000	0.0004	30.2	40.4	NR	6	70	3.30	1.03	0.61	17	–	108
100	raw	Spol	25,000	0.0004	26.2	25.6	NR	2.5	70	3.10	0.59	0.19	97	–	108
100	raw	Spol	25,000	0.0004	26.2	27.9	NR	5	70	5.90	0.63	0.22	90	–	108
100	raw	Spol	25,000	0.0004	26.2	25.5	NR	7.5	70	9.40	0.68	0.22	87	–	108
100	nat/raw	Lgib	480	0.0208	29	21.41	NR	NR	140	2.20	0.58	1.910	-	–	55
100	nat/raw	Lgib	480	0.0208	30.5	23.27	NR	NR	112	2.10	1.04	4.619	-	–	55
0	nat	ctl	20,000	0.0015	30.7	21.5	2×	4	120	–	0.69	0.228	94	–	56
100	nat	DW	20,000	0.0015	30.7	21.5	2×	4	120	–	1.24	0.653	90	–	56
100	nat/raw	Lgib	480	0.0313	27.3	33.4	NR	70	90	2.33	0.85	0.064	80	–	62
100	raw	Lgib	54	NR	29.4	48	NR	NR	20	–	−1.09	−0.678	-	–	61
0	pellet	ctl	70	0.1429	21.1	21.95	3×	sat	28	–	3.34	1.214	100	–	82
100	raw	Lmin	70	0.1429	21.1	21.95	3×	sat	28	–	3.44	1.275	100	–	82
0	pellet	ctl	80	0.1875	27.6	0.87	4×	11	56	1.25	2.10	0.036	99	–	90
5	pellet	Lmin	80	0.1875	27.6	0.87	4×	11	56	1.04	1.87	0.029	99	–	90
10	pellet	Lmin	80	0.1875	27.6	0.87	4×	11	56	1.19	1.87	0.029	99	–	90
20	pellet	Lmin	80	0.1875	27.6	0.87	4×	11	56	1.32	2.28	0.039	98	–	90
0	pellet	ctl	50	0.5000	25.9	2	2×	5	84	0.68	2.24	0.131	96	–	85
5	pellet	Lmin	50	0.5000	25.8	2	2×	5	84	0.77	1.89	0.093	81	–	85
10	pellet	Lmin	50	0.5000	25.8	2	2×	5	84	0.79	1.91	0.095	83	–	85
15	pellet	Lmin	50	0.5000	25.9	2	2×	5	84	0.67	2.03	0.108	89	–	85
20	pellet	Lmin	50	0.5000	25.9	2	2×	5	84	0.80	1.83	0.087	95	–	85
0	pellet	ctl	252	0.0595	26.5	13.5	2×	5	56	1.60	2.43	0.700	98	–	46
5	pellet	Spol	252	0.0595	26.5	13.4	2×	5	56	1.80	2.33	0.643	96	–	46
10	pellet	Spol	252	0.0595	26.5	13.9	2×	5	56	1.80	2.25	0.625	96	–	46

(Continues)

TABLE 3 (Continued)

Duckweed			Tank conditions			Fish conditions				FCR	Growth		SR	AD	
%	prep	spp.	Size (L)	SD (fish/L)	°C	Mass (g)	Feed rate	Feed amt	Exp (days)	(dm)	SGR	GR (g/day)	%	%	References
20	pellet	Spol	252	0.0595	26.5	14.4	2×	5	56	1.90	2.15	0.598	100	–	46
30	pellet	Spol	252	0.0595	26.5	13.5	2×	5	56	2.00	2.07	0.527	98	–	46
100	pellet	Spol	252	0.0595	26.5	14.3	2×	5	56	4.30	0.81	0.146	96	–	46
0	pellet	ctl	252	0.0595	27.7	13.9	2×	5	56	1.60	2.38	0.693	98	–	47
5	pellet	Spol	252	0.0595	27.7	13.9	2×	5	56	1.80	2.26	0.634	100	–	47
10	pellet	Spol	252	0.0595	27.7	13.9	2×	5	56	1.80	2.25	0.625	96	–	47
20	pellet	Spol	252	0.0595	27.7	13.9	2×	5	56	1.90	2.21	0.607	100	–	47
30	pellet	Spol	252	0.0595	27.7	13.9	2×	5	56	2.00	2.02	0.520	97	–	47
100	pellet	Spol	252	0.0595	27.7	13.9	2×	5	56	4.30	0.86	0.154	96	–	47
0	pellet	ctl	480	0.0313	27.3	33.4	NR	4	90	2.26	1.44	0.145	93	–	62
0	pellet	ctl	70	0.5714		56.4	4×	15	50	1.00	2.09	2.053	100	85	50
15	pellet	Lmin	70	0.5714		60.2	4×	15	50	1.20	1.91	1.908	100	84	50
0	control	Lmin	70	0.5714	28	90	NA	NA	–	–	–	–	–	85	54
20	raw	Lmin	70	0.5714	28	90	NA	NA	–	–	–	–	–	79	54
40	raw	Lmin	70	0.5714	28	90	NA	NA	–	–	–	–	–	72	54
20	dried	Lmin	70	0.5714	28	90	NA	NA	–	–	–	–	–	81	54
40	dried	Lmin	70	0.5714	28	90	NA	NA	–	–	–	–	–	74	54

Note: Duckweed %: per cent of the feed which is comprised of duckweed. 100c: refers to a study where cellulase was included in the feed; Duckweed prep: preparation method used (nat = natural grazing, raw = raw or fresh, pellet = made into pellet); Duckweed spp.: Lper = L. perpusilla, Lmin = L. minor, Spol = S. polyrhiza, Lgib = L. gibba; DW = duckweed spp, ctl = control pellet; Feed rate: number of times per day the fish is fed - if continuously fed = cont; Feed amt 'feed amount': sat = to satiation, cont = continuously fed, % = % body weight of the fish.

Abbreviations: AD: apparent digestibility of energy (%); FCR, food conversion ratio; Growth GR, growth rate (g/day); Growth SGR, specific growth rate; SR, survival rate.

Identifying the optimal inclusion rate of duckweed into pelleted feed based on a meta-analysis of these studies is not straightforward. The studies incorporating duckweed into a pelleted feed generally took the approach to evaluate some percentage of fishmeal replacement rather than a complete replacement. The exact feed formulations differed across all experiments making it challenging to hone in on best practices. Finally, different species of duckweed were used across experiments, which is known to impact growth differently.[108] In general, however, several studies identified that inclusion of duckweed or replacement of fishmeal by 10%–30% is generally feasible without having negative impacts on the growth of the fish. Fasakin et al. found no differences in tilapia growth when feeding fish a pellet with a fishmeal replacement of up to 20% *S. polyrhiza* (26% of total diet), which was an estimated 30% cost reduction in feed.[46] In a comparison of macrophytes, *S. polyrhiza* at 10% inclusion rate gave similar results to water fern, *Azolla africana*, at a 5% inclusion rate, which was no different than the control pellet. Economically, the optimal diet was a duckweed inclusion at 30%.[47] Opiyo et al. found that pellet inclusion of up to 15% *L. minor* had no significant differences in FCR or SGR in tilapia as compared to control.[85] Furthermore, fish that consumed duckweed actually had higher amounts of long-chain polyunsaturated fatty acids (LC-PUFA) with 10 times more EPA and DHA as compared to control fish.[85] Feeding tilapia 15%–20% *L. minor* can have a significant impact on improving the nutritional value of tilapia for human consumption. Future research should aim to look at other species of duckweed from both temperate and tropical regions.

Two studies evaluated AD. Schneider et al. compared a traditional commercial feed to various plant-based fishmeal replacements at 15% and found that a duckweed partial replacement resulted in more faecal production (excretion) and lower growth of the fish but with lower overall nitrogenous waste in the system. The AD was estimated to be 84%.[50] In the other study, they evaluated the AD across various duckweed preparation methods including 20% raw, 40% raw, 20% dried, and 40% dried. They found that the apparent digestibility was highest when tilapia were fed dried duckweed at a lower inclusion level (20% dried ∼81% AD, 20% raw ∼79% AD, 40% dried ∼74%, 40% raw ∼72%, control ∼87%).[54]

4 | CONCLUSIONS

Duckweed is an attractive, alternative fish feed with numerous benefits over terrestrial crops. However, one of the major limitations of commercial incorporation of duckweed into fish feeds is the challenge of scaling duckweed cultivation. Mechanization of harvesting, drying, milling, and general processing into feeds is also underdeveloped. Duckweed may be best suited for an alternative feed in resource-limited countries where its utilization is circular. An example would be using duckweed as a means for wastewater remediation followed by processing for animal feed. Future duckweed-incorporated feed experiments should focus on evaluating impacts of the full growout cycle of the fish (especially grass carp and tilapia), which can be between 6 and 12 months depending on the temperature and species. The majority of existing experiments focus on the larval or fingerling stage but the biggest value opportunity is during the growout stage. Very few species of duckweed overall have been evaluated as a feed source with no representatives of *Wolffia* or *Wolffiela* for tilapia and only one species of *Wolffia* evaluated for grass carp. Most of the studies looked at growth in small tank systems whereas the majority of freshwater carp and tilapia culture occurs in medium to large-sized earthen ponds (400–10,000 m^3 or 400,000–10,000,000 L). Thus, expanding to larger grow-out experiments is needed to properly evaluate practicality of using duckweed either in supplementation to feeds or as a fishmeal replacement. No studies have evaluated fermentation of duckweed and incorporation into the pellet for grass carp or tilapia although fermented duckweed showed promising results on a marine fish. There remains a great opportunity to expand duckweed diet incorporation studies to the hundreds of fish species cultured globally. Very few marine fish studies have been performed thus this is an opportunity for future trials, particularly with high value, marine species like *Atlantic salmon*. Finally, at least 11 other species of tilapia besides *O. niloticus* are cultured as 'orphan' or 'Indigenous' crops in Sub-Saharan Africa meaning they are cultured primarily for National consumption and are not exported.[109] Some of these species (e.g., *O. aureus*, *O. mossambicus*), are more tolerant of colder, temperate climates.[110] Duckweed, particularly those used in wastewater treatment systems, can accumulate heavy metals which can potentially bioaccumulate in fish and humans which consume fish. Thus, future research should aim to develop methods of removing or mitigating heavy metal toxicity and antinutrient factors (ANFs) from duckweed. Biotechnological innovations may enable exploration of duckweed detoxification of ANFs or heavy metals and include genome editing or selective breeding of duckweed, duckweed fermentation using fish gut-derived microbes, and fish gut probiotics to enable fish to detoxify compounds in-vivo.

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PATIENT CONSENT

Not applicable (no human subjects).

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

There are no conflicts.

CLINICAL TRIAL REGISTRATION

Not applicable.

AUTHOR CONTRIBUTIONS

Jeremiah Minich J: Conceptualization; investigation; funding acquisition; writing – original draft; writing – review and editing; visualization; methodology; formal analysis; validation; data curation. **Todd Michael P:** Writing – review and editing; methodology; validation; project administration; supervision; resources; investigation; conceptualization; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

Neither authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available completely in the Table S1 along with Table 1. The actual research papers used in the review are all cited and available.

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SUPPORTING INFORMATION

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

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