A review of microbiomes in carp polyculture systems

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List of abbreviations

AFLP	amplified fragment length polymorphism
ARISA	automated ribosomal intergenic spacer analysis
CEV	carp edema virus
CyHV	cyprinid herpesvirus
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
DO	dissolved oxygen
FAO	Food and Agriculture Organization
FCR	feed conversion ratio
FRP	fiberglass reinforced plastic
GIFT	Genetically Improved Farmed Tilapia
HCG	human chorionic gonadotropin
HPLC	high-performance liquid chromatography
ICAR	Indian Council of Agricultural Research
IDAAD	International Database on Aquatic Animal Diseases
IMCs	Indian major carps
KHVD	koi herpesvirus disease
KSD	koi sleepy disease
MAEP	Mymensingh Aquaculture Extension Project
MST	microbial source tracking
NGO	nongovernmental organization
NGS	next generation sequencing
PCR	polymerase chain reaction
PET	plasticulture engineering and technology
qPCR	quantitative real-time polymerase chain reaction
RAPD	random amplification of polymorphic DNA
RFLP	restriction fragment length polymorphism
RISA	ribosomal intergenic spacer analysis
rRNA	ribosomal ribonucleic acid
RT-qPCR	reverse transcriptase real-time quantitative PCR
SEM	scanning electron microscopy
SVCV	spring viremia carp virus
TGGE	temperature gradient gel electrophoresis
TRFLP	terminal restriction fragment length polymorphism

Introduction

Aquaculture is one of the fastest growing animal food-producing sectors, with annual growth rates of 9.5% in the 1990s and 5.8% between 2001 and 2016 (FAO 2018). Yet the sector is still constrained by a number of limitations, including inorganic nutrients, fish feces and feed waste, all of which make it difficult to maintain good water quality (Bentzon-Tilia et al. 2016a). In addition, outbreaks of microbial diseases are one of the main impediments to the sustainable growth of the industry (Stentiford et al. 2017; Shinn et al. 2018). Because of this, a greater knowledge of the relationship between microorganisms and aquaculture species would result in improved yields and creative strategies to expand and improve aquaculture (Bentzon-Tilia et al. 2016b; Dittmann et al. 2017).

Worldwide, carp is the fourth-most cultivated fish species in aquaculture, accounting for 11.8% of global farmed fish production (FAO 2022). Cultivating different carp species with either partially or completely different food spectrums and feeding habits in the same pond helps to use available food and water resources in the pond more efficiently (Hao-Ren 1982a; Kestemont 1995).

Carp polyculture began in China during the Tang dynasty (618–907 A.D.) with the co-culture of bighead carp, silver carp, grass carp, common carp and other species with diverse feeding habits (Hao-Ren 1982b). Since then, it has spread all over the world. In Europe, Chinese carp have been introduced into common carp ponds in Poland (Opuszyński 1981), Bulgaria (Dimitrov 1987) and Hungary (Horvath et al. 1984). In India, the three Indian major carps (IMCs) (catla, rohu and mrigal) are mixed with silver carp, grass carp and common carp. In Israel, common carp, silver carp, tilapia hybrids, and mullets are farmed together (Milstein 2005).

All over the world, carp farming practices are being intensified to meet the increasing demand for aquatic products, particularly in countries such as Bangladesh, where there is limited space for developing more ponds. However, maintaining good water quality in intensive cultivation systems is challenging because of the high load of inorganic nutrients, fish feces, overfeeding and the often limited opportunities for water exchange (Bentzon-Tilia et al. 2016a). The shift from extensive to semi-intensive and intensive practices is often accompanied by an increase in infectious diseases, which is one of the main impediments to sustainable growth of the aquaculture industry (Stentiford et al. 2017; Shinn et al. 2018). As such, diseases in aquaculture have been widely studied, particularly in identifying causative agents and preventing disease outbreaks using risk assessments and biosecurity protocols (Bouwmeester et al. 2021).

In farming operations, modifying fish microbial communities has the potential to significantly influence health and disease outcomes (Gilbert et al. 2016; de Bruijn et al. 2018). Aquatic species maintain a closer interaction with their surrounding microbiomes than terrestrial livestock (de Schryver and Vadstein 2014), so these microbiomes have more significant impacts on animal health (Chen et al. 2017). Therefore, a greater understanding of the relationship between microorganisms and fish would help prevent disease, improve yields and create strategies for aquaculture expansion and improvement (Bentzon-Tilia et al. 2016b; Dittmann et al. 2017). Yet, disentangling interactions and identifying keystone species for specific functions in microbial communities have proven difficult because of the complex structure of these communities, especially when environmental impacts on population dynamics and activities are taken into consideration (de Bruijn et al. 2018). Moreover, it is important to understand and establish good microbial connections and balances between the different trophic levels in carp polyculture.

In aquaculture, research has focused on the gut microbiome because of its intricate role in maximizing feed conversion, growth and overall productivity (Perry et al. 2020). Nevertheless, microbiomes on the skin and in the gills and water are expected to be equally important in disease resistance and susceptibility (McMurtrie et al. 2022). To completely comprehend the effect of microbiomes on fish health, we must have a deeper understanding of the interactions between microbial diversity and community variation on organs, including the gills, skin and gut.

To that end, this review describes the current state of research on carp microbial communities. This includes research on bacteria, microalgae and viruses in carp polyculture, as well as the current technology used to detect, monitor and describe microbiomes. In addition, the effects of inputs such as probiotics, antibiotics, fertilizer, feed and sanitizers on microbial communities, water quality and so on are also discussed. Studying microbial composition, and the factors that impact them, can help better understand the microbiome of both carp and carp polyculture systems.

Harvesting food fish.

1. Carp polyculture systems: An overview

Polyculture is the process of growing more than one fish species in the same pond. The underlying principle is that diverse species of fish from various trophic and spatial niches are farmed in the same pond to make optimal use of all of the natural food sources available, including phytoplankton, zooplankton, periphyton, macrophytes, benthos and detritus.

Depending on the level of management in fish production, carp polyculture systems can be classified as extensive, semi-intensive or intensive. Bangladesh, in particular, has had a long tradition of polyculture of three indigenous carps: catla, rohu and mrigal. In the past, extensive culture systems in the country mostly relied on the pond's natural production, as farmers traditionally fertilized ponds using manure and fed their fish seldom or not at all. The result was that the average annual yield was often quite low compared to modern carp polyculture systems, averaging about 1000 kg/ha (Rahman et al. 1992).

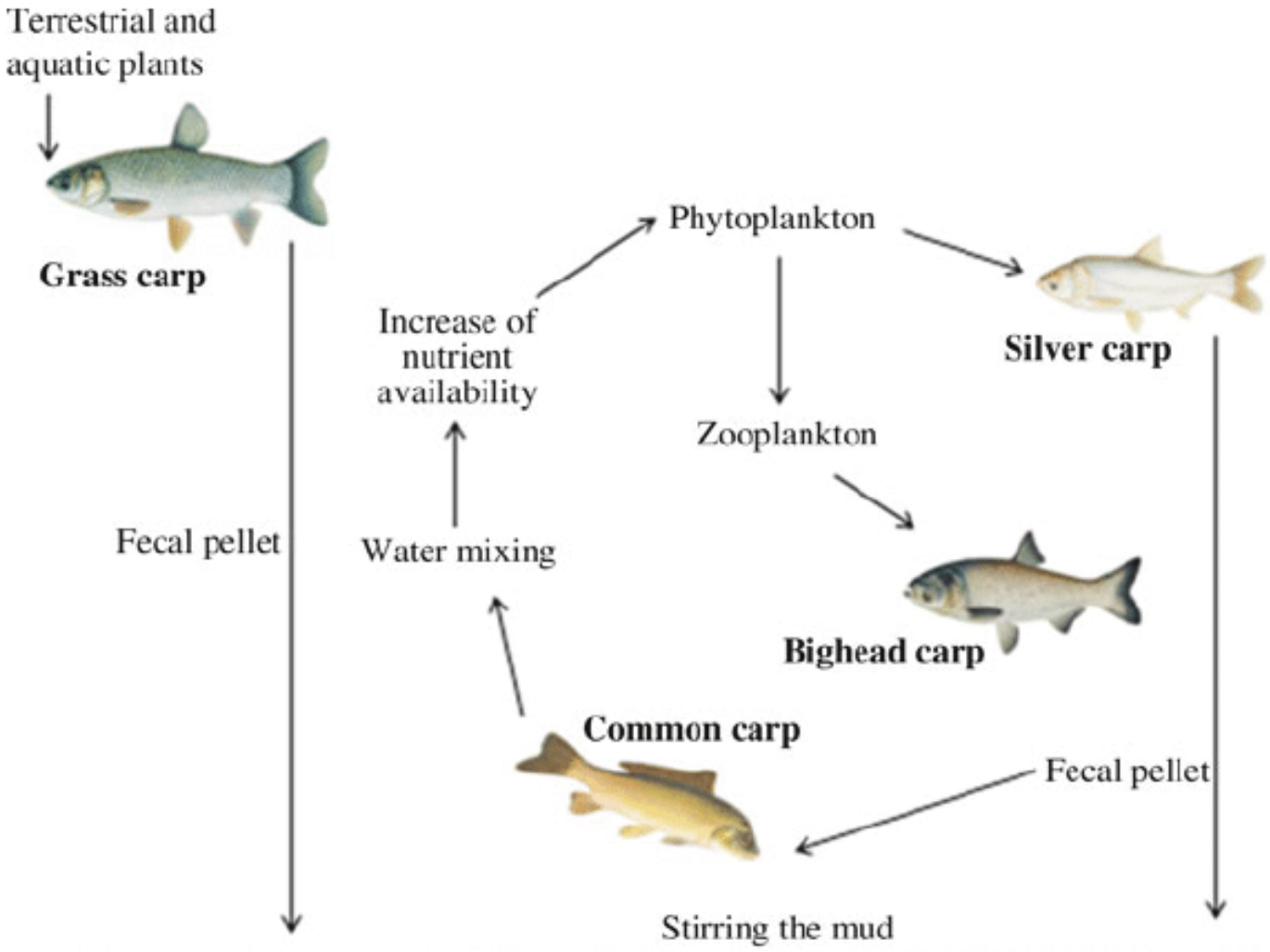
Compared to a monoculture system, polyculture has the potential to greatly improve fish production per unit area. For carps, different combinations of carp species in polyculture systems help enhance the ecosystem of the pond. In Asia, the most popular carp combinations include the three Chinese carps (bighead, silver and grass) and the common carp, though other non-carp species can be cultured as well. Fish can be classified according to their eating habits and ecological niches as surface, column or bottom feeders. Table 1 has descriptions of the respective categories of feeding behavior and representative fish from each.

In most tropical fertilized ponds, stocking phytoplanktophagus silver carp at an appropriate density can suppress algal blooms. Because of its macrophyte-feeding habits, grass carp keep macrophyte under control and contributes an increased amount of partially digested excreta to the pond, which provides food for bottom-dwelling common carp. In disturbing the mud on the bottom in search of food, common and mirror carp in turn help stir up nutrients from the bottom into the water (Figure 1). Bottom dwellers also help aerate sediments (Singh et al. 1991).

Species	Common name	Feeding habits	Ecological niches
Cyprinus carpio	Common carp	Omnivore (benthos + detritus)	Bottom
Ctenopharyngodon idella	Grass carp	Macrophytes	Surface
Hypophthalmichthys molitrix	Silver carp	Phytoplankton	Surface
Aristichthys nobilis	Bighead carp	Zooplankton	Mid-water
Mylopharyngodon piceus	Black carp	Benthos	Bottom
Cirrhina molitorella	Mud carp	Benthos + detritus	Bottom
Cirrhinus mrigala	Mrigal carp	Omnivores	Bottom
Catla catla	Catla	Zooplankton	Surface
Labeo rohita	Rohu	Omnivores	Mid-water

Source: Kestemont 1995.

Table 1. Feeding habits and ecological niches of the different carp species.



Source: Zajdband 2011.

Figure 1. The relationship between the different components of a well-balanced carp polyculture ecosystem.

2. Bacteria in carp polyculture systems

2.1. Bacteria diversity in ponds

One of the most important microorganisms in an aquatic environment is bacteria. In carp ponds, nutrient availability (a bottom-up control) and predatory pressure (a top-down control) (Kalcheva 2008) are what influence the microbiome. First, the organic matter in manure or fertilizers provides bacteria with dissolved and particulate substrates. In turn, the bacteria-laden particles then provide food for filter-feeding and detritus-consuming carp, such as common carp and mud carp, while the mineralized fraction of manure stimulates phytoplankton production, which is food for herbivorous carps, like silver and grass carp (Kalcheva et al. 2010).

Nutrient levels in pond water have been shown to have a significant correlation with microbial populations (Dai et al. 2021). Li et al. (2021) discovered that nutrients, including total nitrogen, total phosphorus, PO_4^{3-}P and NO_3-N, change the microbial population composition in the water column. Similarly, predators such as viruses (Middelboe et al. 2008), nano-flagellates (Jürgens and Matz 2002), ciliates (Sherr and Sherr 1987), nauplii (Havens 1998), rotifers (Bonecker and Aoyagui 2006) and some Cladocera species, like Daphnia (Zöllner et al. 2003), can graze on bacteria in carp production systems. As a result, there is likely a large variation in the bacterial profile among different carp polyculture systems (Table 2).

In silver carp and bighead carp cultivations, *Actinobacteria*, *Flavobacterium*, *Cyanobacteria*, *Limnohabitans* and "rare biosphere" such as *Alcaligenaceae* and *MNG7* are the most abundant genera (phyla) (Meng et al. 2021). In freshwater ecosystems like carp polyculture, *Actinobacteria* can act as a probiotic and play an important role in competition for nutrients and recycling organic matter (Ghai et al. 2014; Shijila Rani et al. 2022). *Flavobacterium*, meanwhile, can be associated with fish disease, particularly in koi, longfin eels and rainbow trout (Loch and Faisal 2015) and can become abundant following the decline of freshwater cyanobacteria blooms (Newton et al. 2011). Cyanobacteria in pond water can fix atmospheric nitrogen and produce dissolved organic compounds that heterotrophic bacteria can then use as a food source (Louati et al. 2015). *Limnohabitans* plays a vital function in transporting carbon from primary producers to higher trophic levels, and "rare biosphere" also has critical ecological roles in bacterial populations (Wang et al. 2017).

In China, Li et al. (2021) investigated dynamic changes in the microbiological composition of water in black carp polyculture ponds using 16S rRNA gene amplicon sequencing. They found that bacterial diversity increased throughout the mid-culture phase and declined during the late culture period because there was less food available, and that *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* were the most abundant phyla in the water column. In aquatic environments, *Proteobacteria* have been shown to participate in a range of biochemical processes, such as carbon and nitrogen cycling (Klase et al. 2019), while *Actinobacteria* are well-known producers of bioactive products, including novonestmycins A, 19-methoxybafilomycin C1 amide and 21-deoxybafilomycin A1,0 (van Keulen and Dyson 2014; Berna et al. 2015; Jose et al. 2021).

In Bulgaria, Kalcheva et al. (2010) directly counted bacteria microscopically to determine plankton communities in carp polyculture ponds that used organic manure. The result showed that bacteria with cocci forms strongly dominated rods in both numbers and biomass.

In a study by Barat and Jana (1991) comparing microbial communities of ammonia and nitrite oxidizers in air-breathing catfish tanks and herbivorous carp polyculture tanks, the results indicated that the quantity of ammonia and nitrite oxidizers was greater in carnivorous air-breathing catfish tanks than in herbivorous carp tanks. This might be because the waste and metabolites from carnivorous air-breathing catfish contributed more to eutrophication than herbivorous carps. Through nitrification and denitrification, bacteria can remove nitrogen from the environment. For example, Nitrosomonas and Nitrobacter can oxidize ammonium (NH_4+-N) ions to nitrite (NO_2-N) and nitrate (NO_3-N) ions, which aquatic plants, algae and bacteria subsequently absorb and eliminate as a source of nitrogen.

Country	Target system	Technology	Dominant bacteria in pond	Reference
India	IMC polyculture	16S rRNA gene amplicon sequencing	**Genera**: *Actinomyces*, *Pseudonocardia*, *Sediminibacterium*, *Bacteroides*, *Exiguobacterium*, *Brochothrix Macrococcus*, *Alkalibacterium*, *Leuconostoc*, *Lactococcus*, *Shewanella*, *Trabulsiella*, *Acinetobacter*, *Psychrobacter*, *Luteolibacter* **Family**: *Coriobacteriaceae*, *Planococcaceae*, *Planococcaceae*, *Halomonadaceae*	Mukherjee et al. 2020
Bulgaria	Carp polyculture using organic manure	Morphological observation	Dominated by free-living cocci bacteria	Kalcheva et al. 2010
China	Black carp polyculture	16S rRNA gene amplicon sequencing	**Phyla**: *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* **Genera**: *Prochlorococcus*, *Bacillus*, *Polynucleobacter*, *Chryseobacterium*, *Novosphingobium*, *Acinetobacter*, *Flavobacterium*, *Oscillospira*, *Ruminococcaceae*, *Agrobacterium*, *Comamonas*, *Jan-thinobacterium*, *Rheinheimera*	Li et al. 2021
	Grass carp, crucian carp (*Carassius carassius*) and bighead carp polyculture	16S rRNA gene amplicon sequencing	**Phyla**: *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria, Verrucomicrobia*	Li et al. 2015
	Silver and bighead carp polyculture	16S rRNA gene amplicon sequencing	**Phyla**: *Betaproteobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*, *Acidobacteria*, *Planctomycetes*	Tang et al. 2021
Saudi Arabia	Common carp	Morphological observation, gram staining, biochemical testing	**Species**: *Aeromonas hydrophila*, *Bacillus* sp., *Corynebacterium urealyticum*, *Edwardsiella* sp., *Micrococcus* sp., *Pseudomonas* sp., *Shewanella putrefaciens*, *Staphylococcus* sp., *Streptococcus* sp., *Vibrio* sp., *Unidentified Gram-negative rods*, *Cellulomonas cellulans*, *Gordona* sp.	Al-Harbi and Uddin 2008
	Common carp and African catfish polyculture	Morphological observation, gram staining, biochemical testing	**Species**: *A. hydrophila*, *Corynebacterium* sp., *C. Urealyticum*, *Edwardsiella* sp., *Micrococcus* sp., *S. putrefaciens*, *Staphylococcus* sp., *Streptococcus* sp., *Vibrio* sp., *Unidentified Gram-negative rods*	Uddin and Al-Harbi 2012

Table 2. Studies on microbiomes in the rearing water of carp polyculture systems.

2.2. Bacteria diversity of carp organs

Bacteria in the digestive tracts of fish serve critical roles in maintaining normal gut functions, including digestion of complex molecules, production of secondary metabolites and defense against pathogens (Bird et al. 2010; Liu et al. 2016; Li et al. 2018). Understanding the composition of these microbial communities is critical for health management. However, the literature on bacteria and pathogens is not univocal, with specific bacterial taxa found in both healthy and diseased fish. Comprehensive examinations of the bacterial load and species in the organs of healthy fish are required to predict disease outbreaks and take preventative measures.

There are several studies on the bacteria composition in the intestine, gills and skin of carp in polyculture systems, as presented in Table 3. Previous research mostly relied on classic culture-based approaches to explore gut microbial diversity in carp species (Ray et al. 2010; Uddin and Al-Harbi 2012; Mandal and Ghosh 2013; Ichthyologica and Piscatoria 2016; Mukherjee et al. 2016). However, recent advances in sequencing technology, computer processing capacity, bioinformatics and statistical tools have made it simpler to examine the microbial diversity in fish (Foysal et al. 2019a).

2.2.1. Bacteria diversity of fish gut

Guts are now the most extensively studied organ in microbial ecology with the advance of high-throughput sequencing (Tyagi and Singh 2017; Foysal et al. 2019a). Mukherjee et al. (2020) indicated that *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* are the main bacterial phyla in the gut of three IMCs and are critical for the host's gut function. In addition, the most prevalent bacterial genera detected in the intestine of healthy grass carp are *Bacteroides*, *Cetobacterium*, *Citrobacter*, *Vibrio*, *Fusobacterium* and *Coprococcus* (Tran et al. 2018). The gut of grass carp could be also a valuable source of potential probiotics based on *Proteobacteria* and *Firmicutes* (Han et al. 2010). This finding deserves further investigation concerning a potential pool of probiotics for carp species.

In addition, Uddin and Al-Harbi (2012) found that *A. hydrophila*, *C. urealyticum*, *S. putrefaciens*, *V. cholerae* and *V. vulnificus*, which were known as opportunistic pathogens, are abundant bacterial species in the intestines of common carp. This finding is consistent with a study by Mahmoud et al. (2004) in which they found diverse bacteria in the intestines of common carp, the most common ones being *Vibrionaceae*, *Enterobacteriaceae* and *Flavobacterium*. It is believed that various sections of the gastrointestinal tract harbor different microbiota (Ni et al. 2014) and that the genotype of the host influences the microbiota of the gastrointestinal tract (Navarrete et al. 2012; Zhao et al. 2013). We believe additional investigation is needed to clarify this.

In carp polyculture systems, different carp species that live in the same pond and eat the same natural diet can harbor different intestinal microbiota (Meng et al. 2021). This might be attributed to host characteristics such genetics, histology and physiology of the intestine (Li et al. 2014). The microbial composition of a fish's gut also develops in response to selection pressures within the gut microenvironment of the host species that occupies a certain ecological niche. IMCs have different ecological niches because of differences in their feeding habits and preferences, so their varied diets influence the composition of the fish's symbiotic gut microbiota.

Using 16S rRNA partial gene sequence analysis, Mukherjee et al. (2020) investigated the critical functions of the gut microbiota in the digestion of IMCs. They found that *Bacteroidetes* and *Actinobacteria* were more abundant in mrigal and rohu, whereas *Proteobacteria* were more abundant in catla. *Bacteroidetes* and *Actinobacteria* might be associated with the existence of complex polysaccharides in their diets because of the consumption of phytoplankton, additional feeds or plant detritus of rohu and mrigal (Thomas et al. 2011). *Proteobacteria* in catla are involved in fermentative peptide, carbohydrate metabolism and vitamin B12 production (Larsen et al. 2014). This reflects their eating habits, which include eating zooplankton and omnivorous feeding. However, there are many bacteria for which specific roles in digestion and immunity remain unclear, requiring further research. Furthermore, Foysal et al. (2019b) demonstrated significant differences in the gut bacterial communities of IMCs when fed the same diet, indicating the presence of species-specific microbial diversity in the gut. Therefore, further studies are required to

identify the influences and parameters that affect the differences in microbial communities found in various IMCs.

In general, the fish gut acts as a reservoir for a variety of opportunistic infections (Wu et al. 2012a; Li et al. 2015). For example, *Aeromonas* is dominant in the intestine of grass and crucian carp in polyculture (Li et al. 2015), while *Klebsiella*, *Acinetobacter*, *Plesiomonas* and *Enterobacter* have all been found in IMCs (Foysal et al. 2019b; Mukherjee et al. 2020). However, *Aeromonas* and *Klebsiella* are both involved in opportunistic infections of tropical freshwater fish (Austin and Austin 2016). In polyculture systems in Bangladesh, *Serratia* was identified in the distal gut of rohu as an opportunistic pathogen, but little is known about its colonization of the gastrointestinal tract and its involvement in digestion and immunity.

2.2.2. Bacteria diversity of fish gills

In contrast to the gut, there is little knowledge about bacterial flora on carp gills. By using counting and traditional isolation techniques, Uddin and Al-Harbi (2012) observed differences in the bacterial load of the water, gut and gills of co-cultured common carp and catfish. Interestingly, bacteria in the carp intestine did not have a higher species diversity than bacteria in the gills, though the reverse was the case for catfish. The dominant bacteria in common carp gills were *A. hydrophila*, S. *putrefaciens* and *V. cholerae*.

Another study in Saudi Arabia identified the bacterial composition in the water and sediment of the pond and in the gills and intestines of common carp (Al-Harbi and Uddin 2008). The authors found that aerobic heterotrophic bacteria (defined by cultivation method) colonized the gills in greater numbers than those found in the surrounding water but in lower numbers than those in the intestine. Bottom-feeding common carp had more significant metabolic activity and a higher feeding rate in warmer temperatures, resulting in higher bacterial loads in the gills and intestines. It should be noted that opportunistic bacteria already on the surface of the gills can lead to disease. Potential disease-causing bacteria in the carp culture systems include *A. hydrophila* and *Pseudomonas* sp. (Austin and Austin 2016), *S. putrefaciens* (Koziñska and Pekala 2004) and *Streptococcus* sp. (Al-Harbi and 1994).

2.2.3. Bacteria diversity of fish skin

In an aquatic environment, fish are directly exposed to the surrounding bacterial microbiome. Yet there is a dearth of interest in investigating the microbiome on the skin, as only a few studies exist on different species (Ringø and Holzapfel 2000; Kapetanović et al. 2006; Liu et al. 2008). To fill this void, it is critical to have a thorough knowledge of the usual microbiomes on these surfaces in order to establish potential prophylactic measures such as probiotics and prebiotics.

Using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), Wang et al. (2010) analyzed the microbial population attached to the gills and skin of gibel carp (*Carassius auratus gibelio*) and bluntnose black bream (*Megalobrama amblycephala Yih*) in polyculture. The study found that the bacterial, actinomycetal and fungal diversities of gibel carp were lower on the gills than those on the skin. Moreover, there was a considerable difference in the bacterial, actinomycetal and fungal communities living on the gills and skin between gibel carp and bluntnose black bream. The genetic makeup of the host was the primary factor in shaping the symbiotic microbial community (Kenny and Valdivia 2009).

The principal interface between a fish and its environment is the mucosal surface of the skin. Mucus performs various functions, including ionic and osmotic control, movement, and defense against microbial diseases (Shephard 1994), and mucus secretion is considered an important factor in the protection against invading pathogens. According to Marel et al. (2010), exposure to water with a high bacterial load did not cause clinical symptoms in carp, but the skin of exposed carp responded quickly with increased mucus production. Hypersecretion of "immature mucins" would wash away adhering bacteria, helping fish defend against pathogens. Furthermore, Chiarello et al. (2015) revealed that the bacterial operational taxonomic units (OTUs) of the skin of European seabass and gilthead seabream had a more diverse bacterial abundance than in the aquatic environment. This might be related to the nutritional conditions on the fish's surface, as the mucus on the skin is made up of a wide range of gel-forming glycoproteins, glycosaminoglycans and proteins that serve as food sources for epibiotic bacteria (Shephard 1994; Bordas et al. 1998).

Carp species	Organ	System	Technology	Bacterial diversity	Reference
Common carp	Gills	Common carp monoculture	Aerobic plate count, traditional isolation techniques	**Species**: *A. hydrophila, C. urealyticum, Micrococcus* sp., *S. putrefaciens, Staphylococcus* sp., *Vibrio* sp., *Unidentified Gram-negative rods*	Al-Harbi and Uddin 2008
	Gut	Common carp monoculture	Aerobic plate count, traditional isolation techniques	**Species**: *A. hydrophila, Bacillus* sp., *C. urealyticum, Edwardsiella* sp., *Micrococcus* sp., *Pseudomonas* sp., *S. putrefaciens, Staphylococcus* sp., *Streptococcus* sp., *Vibrio* sp., *Unidentified Gram-negative rods*	Al-Harbi and Uddin 2008
	Gills	Common carp and African catfish polyculture	Morphology-based technique	**Species**: *A. hydrophila, Corynebacterium* sp., *Micrococcus* sp., *Staphylococcus* sp., *S. putrefaciens., Vibrio alginolyticus, V. cholerae, Vibrio* sp., *V. vulnificus, Unidentified Gram-negative rods*	Uddin and Al-Harbi 2012
	Gut	Common carp and African catfish polyculture	Morphology-based technique	**Species:** *A. hydrophila, Corynebacterium* sp., *Micrococcus* sp., *Bacillus* sp., *Edwardsiella* sp., *Pantoea* sp., *S. putrefaciens, Staphylococcus* sp., *Pseudomonas* sp., *Streptococcus* sp., *V. alginolyticus, V. cholerae, Vibrio* sp., *V. vulnificus, Unidentified Gram-negative rods*	Uddin and Al-Harbi 2012
	Gut	Common carp from floating cages	16 S rRNA gene amplicon sequencing	**Major phyla**: *Proteobacteria, Firmicutes*	Mulyani et al. 2018
Transgenic common carp	Gut	Monoculture	16S rRNA gene amplicon sequencing	**Major phyla**: *Proteobacteria, Fusobacteria, Bacteroidetes, Firmicutes*	Li et al. 2013
IMCs	Gut	IMC polyculture	High-throughput sequencing	**Major phyla**: *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes are the most dominated*	Mukherjee et al. 2020
	Gut	IMC polyculture	16S rRNA gene amplicon sequencing	**25 phyla** (*Proteobacteria* and *Fusobacteria* being the most abundant), 51 classes and 374 genera (*Aeromonas* and *Cetobacterium* being the most abundant)	Foysal et al. 2019b

Carp species	Organ	System	Technology	Bacterial diversity	Reference
Grass carp	Gut	Grass carp, gibel carp and bluntnose black bream polyculture	16S rRNA gene amplicon sequencing	**Major phyla**: *Proteobacteria, Firmicutes, Actinobacteria*	Han et al. 2010
	Gut	Grass carp monoculture	16S rDNA DGGE and T-restriction fragment length polymorphism (RFLP)	**Major phyla**: *Proteobacteria, Firmicutes, Cyanobacteria, Actinobacteria*	Wu et al. 2012b
	Gut	Grass carp, crucian carp and bighead carp polyculture	16S rRNA gene amplicon sequencing	**Major phyla**: *Fusobacteria, Firmicutes, Proteobacteria, Bacteroidetes*	Li et al. 2015
Bighead carp	Gut	Bighead carp and tilapia polyculture, and bighead carp and common carp polyculture	16S rRNA gene amplicon sequencing	**Major phyla**: *Proteobacteria, Firmicutes, Fusobacteria, Cyanobacteria*	Luo et al. 2022
	Gut	Grass carp, crucian carp and bighead carp polyculture	16S rRNA gene amplicon sequencing	**Major phyla**: *Fusobacteria, Firmicutes, Proteobacteria, Bacteroidetes*	Li et al. 2015
Crucian carp	Gut	Grass carp, crucian carp and bighead carp polyculture	16S rRNA gene amplicon sequencing	**Major genera**: *Cetobacterium, Aeromonas*	Li et al. 2015
Gibel carp	Gills and skin	Gibel carp and bluntnose black bream polyculture	16S rDNA or 18S rDNA	**Major phyla**: *Proteobacteria, Firmicutes*	Wang et al. 2010

Table 3. Microbiome studies in the gut, gills and skin of carp in polyculture systems.

2.3. Summary of common bacteria fingerprinting technologies

The need for detecting and identifying microbiomes requires novel methods. Bacteria and other microbes found in water or fish organs used to be identified using traditional detection methods, such as cultivation-based techniques. However, these techniques are unable to extract information on non-cultivable microbes from complicated environmental samples. In recent years, new tools have been transformed and developed in order to make it easier to detect microbiomes. The journey that began with culture-based plate enumeration and has now developed into a number of culture-independent methods.

2.3.1. Cultivation-based methods

Traditional technologies involve a long process of sample collection, serial dilution, plating on selective and appropriate mediums, and waiting for proper incubation time to get visible colonies for further characterization. Identification is based on the physical, biochemical, physiological (phenotype) and/or genetic properties (genotype) of the microorganism using a number of methodologies (morphological observation, gram staining, biochemical testing), depending on the sample or expected objectives (Ferone et al. 2020). This classical counting method is not complex to perform and is low cost, but runs the risk of contamination and is time-consuming because of the required incubation period. Moreover, it can be difficult to count the colony numbers, and the approach can only offer data on the abundance of a few specific target bacteria since fewer than 1% of microbial species are culturable (Lee et al. 2013).

Examining microorganisms directly under a microscope is a simple and economical method. Bacteria are identified according to their shapes, sizes and groupings (e.g. coccus, bacillus, filamentous, in pair or chain formation) or their cell wall characteristics and staining qualities (e.g. Gram positive, negative or acid-fast staining) (Gopinath et al. 2014). Scientists have even developed a method to calculate bacteria colonies fast and simply by using a digital camera and ImageJ software (Sieuwerts et al. 2008). Moreover, there are various staining procedures available, in which BacLight staining provides various benefits over traditional cell counting techniques (Boulos et al. 1999). It is a dependable, quick and simple test that can provide total count and viable cells in a single step (Jassim and Griffiths 2007).

2.3.2. Genetic-based detection methods

Molecular approaches include hybridization-based detection methods such as FISH (Kempf et al. 2000), amplification methods (Fenollar and Raoult 2004), DNA microarrays (Ehrenreich 2006) and whole genome sequencing (Hasman et al. 2014). Genetic sequence-based techniques can identify microbial cells and their toxins by integrating gene analysis while combining PCR with capillary electrophoresis, multiplex PCR-based detection, DGGE and length heterogeneity PCR. Genetic fingerprinting techniques determine the diversity of amplified genetic sequences via differential electrophoretic migration on running gels that depends solely on their size (RFLP), terminal restriction fragment length polymorphism (the use of fluorescently labeled DNA fragments for microbial community profiling, TRFLP), ribosomal intergenic spacer analysis (amplification of the rRNA gene operon region between the small (16S) and large (23S) ribosome subunits called the ribosomal intergenic spacer region (RISA) and (DGGE and temperature gradient gel electrophoresis (TGGE)) (Hameed et al. 2018).

Amplification technologies such as quantitative real-time polymerase chain reaction (qPCR) and reverse transcriptase real-time PCR (RT-qPCR) are used to identify microorganisms based on the 16S rRNA gene or the 26S rRNA gene. Pace (1997) documented the composition of microbial communities for the first time by analyzing the sequence of the 16S rRNA gene using Sanger sequencing. However, this technique requires analyzing individual sequences, meaning that a cloning step was necessary for mixed community research. As a result, a comprehensive study of bacterial diversity using Sanger sequencing is time consuming and so rarely performed.

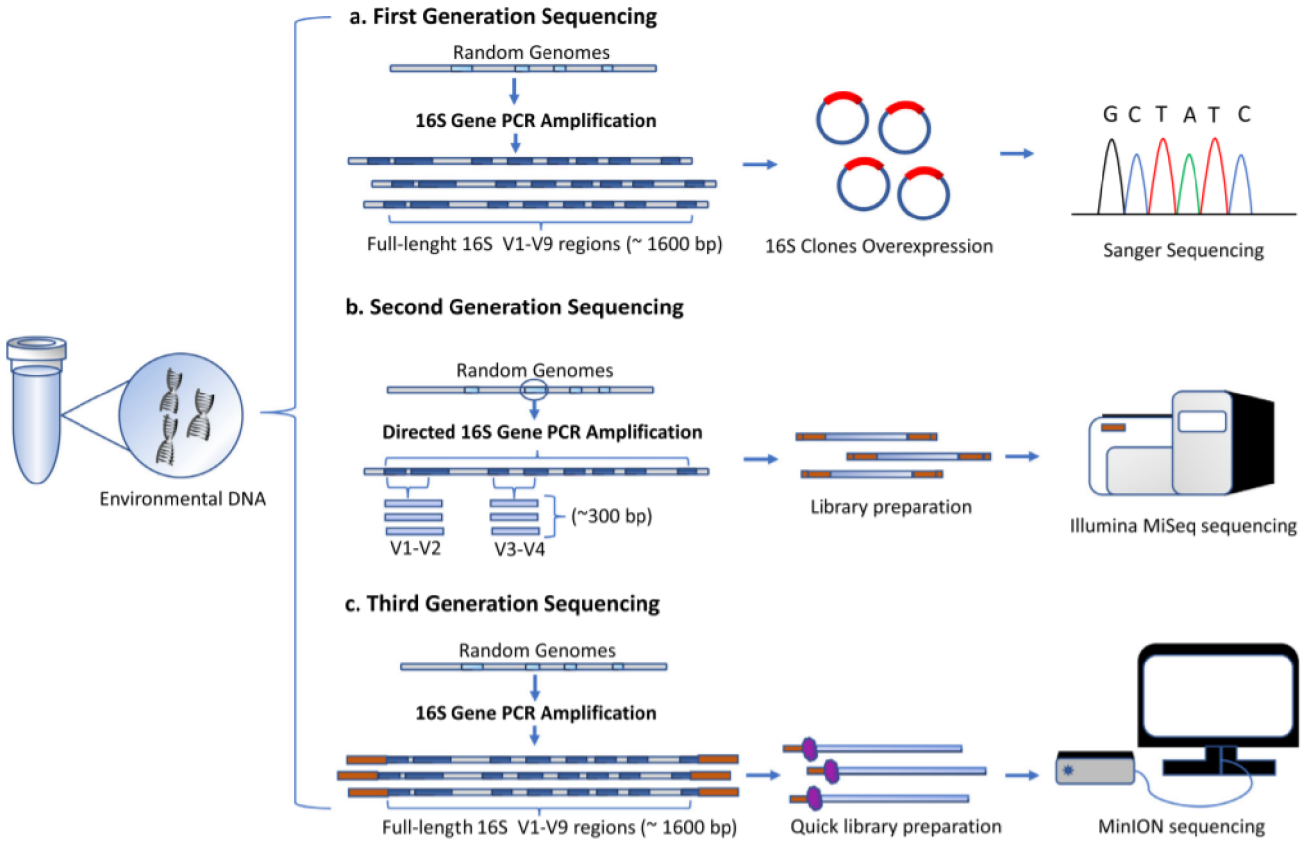
Recently, the development of next generation sequencing (NGS) technologies has allowed the sequencing of hundreds of millions of DNA molecules simultaneously. NGS is primarily used in two ways: (1) identification of whole genome sequencing, which determines the entire genome sequence of a single cultured isolate, such as a bacterial colony, a virus or any other organism, and

(2) "metagenomics," which allows the detection, identification and characterization of a wide range of microorganisms in a single experiment without pre-cultivation within a few days (Grützke et al. 2019). Interestingly, sequencing a genome using NGS is significantly cheaper than using Sanger techniques. According to research by Patel et al. (2016), Sanger sequencing is estimated at about USD 500 per 1000 bases compared to less than USD 0.50 for NGS.

Santos et al. (2020) illustrated the first-, second- and third- generation sequencing techniques shown in Figure 2. Historically, metabarcoding is traditionally performed using first-generation sequencing (Sanger) by amplifying full-length 16S rRNA genes from a DNA sample. Once the amplicon is obtained, the 16S amplicons are cloned, sequences are added into a vector and the vector is then transformed into a host. Finally, plasmid extraction and purification are performed, and the Sanger method is used to sequence the 16S rRNA inserts.

Nowadays, metabarcoding is done using second- and third-generation sequencing platforms. With second-generation sequencing (Illumina), specific regions of the 16S rRNA gene are amplified by PCR. Depending on the scope of the study, it can amplify one or two regions of the 16S gene. By using regions V1-V2 and V3-V4, a paired-end library (the mix of DNA fragments with adapters attached to theirs ends and ready to be sequenced) preparation is often used for this purpose. Adapters (exogenous nucleic acids that are ligated to a nucleic acid molecule to be sequenced) and indexes (unique DNA sequences ligated to fragments within a sequencing library, which allow the posterior sorting and identification of different samples sequenced on a same sequencing run) are then added to the 16S amplicon. Finally, libraries of about 300 bp in length are sequenced on the Illumina MiSeq platform.

Third-generation sequencing (Nanopore) starts with amplifying the full-length 16S rRNA gene from environmental DNA using universal primers. Simultaneously, indexes for multiplexing are



Source: Santos et al. 2020.

Figure 2. The most common metabarcoding sequencing strategies for each sequencing technology generation.

added to the amplicons in the same PCR reaction. Once amplicons have been purified, the library preparation process is performed, consisting of the addition of a protein at a specific tagged region of the 16S amplicons (10 minutes for library preparation). Finally, direct sequencing of the samples is carried out on the MinION sequencer.

2.3.3. Optical-based detection method: Flow cytometry

Although the price of amplicon sequencing is dropping (Sims et al. 2014), these approaches still face financial constraints when high-throughput sampling is required. They are also time-consuming (van Dorst et al. 2014), require large sample volumes and face technical bias problems (Soergel et al. 2012; Klindworth et al. 2013). Flow cytometry is an optical detection-based method for quantifying and assessing microorganisms by guiding individual cells through a laser beam and collecting scattered and fluorescent light. Measurements can be done in minutes. As a result, it is often employed in clinical diagnostics to determine bactericidal activity (Depince-Berger et al. 2016). However, it is still underrepresented in the field of microbial ecology.

Since 2011, flow cytometry techniques have been used with other technologies such as qPCR and fluorescent biosensors to produce more accurate results (Rajapaksha et al. 2019). Flow cytometry is not employed as a stand-alone method because of the numerous dilution steps before analysis. In cases of using antibiotics, it requires minimizing the concentration of debris to make it easier for antibodies to attach to target cells. Unfortunately, this reduces the flow cytometer's potential sensitivity (Rajapaksha et al. 2019). El-Nahas et al. (2013) evaluated the technologies of a direct immunofluorescence test, direct microscopic examination and flow cytometry. The results showed that, although flow cytometry is a fast and sensitive approach for screening large numbers of fecal samples for the presence of protozoan cysts, it did not show the expected sensitivity.

To our knowledge, there is limited use of flow cytometry in investigating microbiomes in aquaculture, especially in carp polyculture systems. Lucas et al. (2010) investigated phytoplankton and bacterioplankton dynamics in an earthen pond during a shrimp grow-out period. They used high-frequency measurements alongside traditional environmental monitoring, specifically oxygen concentrations and a chlorophyll a survey. Heyse et al. (2021) used flow cytometry to monitor bacterial dynamics during shrimp hatchery cultivation.

Flow cytometry is a technique that quickly detects pathogenic bacteria on fish in marine aquaculture. In a study by Endo et al. (2000), flow cytometry was able to discriminate between viable and dead cells for several species of bacteria in seawater. Moreover, there is a good correlation between the results from the flow cytometry method and 16S rRNA amplicon sequencing (Props et al. 2016).

In addition to these approaches, there are other techniques to identify microbiome compositions in aquaculture, such as microbial source tracking (MST), optical biosensors and bioluminescent sensors. Each approach has its own advantages and drawbacks, which are presented in Table 4. To our knowledge, these techniques have not yet been used to identify the microbiome diversity in carp polyculture systems.

Bacteria detection methods	Advantages	Disadvantages
Conventional colony counting and culturing methods	Indicator bacteria could be used to identify the pathogenic contamination levels in the samples. The protocols are not complex, but aseptic conditions should be thoroughly followed to avoid any contamination issues. The bacterial counting techniques are accurate and confirmatory.	Indicator bacteria do not identify the source of contamination, and different indicator bacteria are not with each other. For example, E. coli and enterococci are not well correlated with Salmonella spp. as correlated pathogen indicators. This method identifies only a fraction of bacteria in a given sample. It is exclusive to a specific agar in order to isolate, culture and enumerate the target bacteria. The process is time-consuming (5–7 days) for the targeted results. Environmental contamination issues could be encountered, and aseptic techniques should be thoroughly followed. Some pathogen species might have different cultural aspects to various environmental stressors. It is difficult and tedious to count bacteria manually. It is inconvenient for industrial applications. Non-specialist use is difficult, and personnel should be trained. Some emerging pathogens are not detectable. A lot of instruments are required and cannot be used on site, as some equipment is too large.
MST	This includes both culture-based and culture-independent methods. The microbes and viruses are available in higher densities, making the protocols easier to follow.	The identification of the methods might require fingerprint and host databases that further require genotypic and phenotypic tests.
PCR	This method is not time-intensive and can achieve fast results. Conclusive, robust and unambiguous results are provided with higher and consistent counts. Real-time PCR (qPCR) eliminates the need for laborious post-amplification processes, such as gel electrophoresis, producing fast results. It does not require a calibration curve, so the quantitation is much easier than conventional PCR.	Expensive technology/instrumentation and chemicals are required to follow up once the method has been carried out. Technical expertise and experience is required to handle the protocols. The precision of qPCR drops with decreasing DNA concentrations. PCR has a lack of differentiation between viable and non-viable cells. Contamination of the sample can produce misleading results.

Bacteria detection methods	Advantages	Disadvantages
PCR	Reverse transcriptase PCR (RT-PCR) can distinguish between viable and non-viable cells. RT-PCR detects bacteria in the growth phase. Several organisms can be detected by the introduction of different primers in multiplex PCR. Fluorescence intensity is directly proportional to the amount of amplified product in real-time PCR, providing easy detection even with the naked eye. Multiplex real-time PCR easily detects the target genes. It is an effective, rapid and sensitive technique while altogether reducing the cost and time of screening. Droplet digital PCR (ddPCR) detects each droplet of fluorescence and detects even a small change of fluorescence. The precision is very high.	Prior sequence data is required for designing primers. The techniques can be used to detect only the absence or presence of known pathogens or genes. The primers could be annealed with similar DNA, but also with identical DNA. Incorrect nucleotides could be incorporated by PCR even in low concentrations, which will generate misleading results.
Flow cytometry	This technique can be used to determine the number, size and shape of microorganisms. High sensitivity measurements can be achieved in short times. It is suitable for detecting specific organisms in fluids, scrapes, rinses or whole liquid aliquots. It is able to detect seawater viruses with appropriate fluorescent tags.	Numerous dilution steps are required to be followed before the analysis. There is a lack of tissue and cell architecture to examine in the cells in the suspension. It is difficult to differentiate the cell subpopulations with similar marker expressions. It generates large volumes of data, making excess analyses cumbersome.
Optical biosensors	Optical transducers can detect the smallest changes in a refractive index. This method is convenient when used directly at the interface of solid and liquid. It provides both quantitative and qualitative data. Sample preparation is easy and not tedious. Optical interferometry has a short detection time.	Optical interferometry lacks sensitivity.
Bioluminescent sensors	This method can be used to detect bacterial intracellular adenosine triphosphate (ATP), which is an excellent indicator of microbial contamination.	Detection limits given for Gram-negative bacteria are weak.
Molecular detection methods	Environmental parameters do not influence nucleic acid-based fecal detection assay. These methods provide sensitive and rapid detection. New primers could improve sensitivity. Molecular subtyping methods could be used to identify the different strains within a species.	The complex structure of certain matrices can affect PCR. Some matrices could contain PCR inhibitors that influence amplification efficiency or primer binding.

Source: Rajapaksha et al. 2019.

Table 4. Advantages and disadvantages of bacteria detection methods.

3. Microalgae in carp polyculture systems

3.1. The impact of microalgae on carp polyculture systems

3.1.1. Beneficial impact on carp polyculture systems

Microalgae are important as primary producers in aquatic ecosystems (Nozaki 1999). During the day, microalgae are the main source of oxygen in fishponds. Additionally, they consume nitrogenous waste products. Microalgae blooms can also shade the pond bottom, preventing the growth of aquatic weeds, while the fish fertilize the bloom, which helps keep it alive. Once a bloom is developed, fish feed and fish waste serve as fertilizers to keep the bloom growing. Although limited, research on the diversity of microalgae in carp polyculture ponds is summarized in Table 5.

In addition to bacteria that fish consume, microalgae are nutritious for the different fish species that grow together in polyculture (Hepher 1988; de Silva and Anderson 1994). The filtration physiology of fish is often described as follows: the water containing microalgae particles is pushed into the oropharyngeal cavity by the rhythmic expansion and contraction of the opercula and buccal chamber. Gill rakers filter suitable food particles, with the size of the filtered food particles dependent on the gap of the gill raker (Liu and Haung 2008). While water runs through the gill rakers, the filtered food particles remain and reach the pharynx and are ingested by the coordinated action of the filtering organs (Sun and Meng 1992; Li and Dong 1996; Zhao et al. 2014).

Through this process, it would be expected that the size of filtered food particles would be larger than the gill raker gaps. However, microalgae particles smaller than the gaps were regularly found in the foreguts of silver carp caught in lakes, rivers, reservoirs and ponds (Xie and Liu 2001; Tucker 2006; Ke et al. 2007; Yan et al. 2009), demonstrating that these tiny microalgae particles might also be filtered. As such, silver carp could have evolved additional feeding mechanisms to entrap microscopic microalgae particles. Massive mucus cells were discovered in filter organs and adhered pollen blocks in gill raker ditches and gill raker tubes of silver carp (Li and Dong 1996). This mechanism is known as the "food sinking effect." Therefore, when silver carp filter microalgae particles smaller than their gill raker gaps, this effect could work as an additional feeding mechanism. This is supported by Görgényi et al. (2016), who found nanoplankton less than 10 µm in size in the foregut and hindgut of Asian carp in Lake Balaton, Hungary.

Transporting mola broodfish.

Country	Polyculture system	Microalgae diversity	Notes	References
Bangladesh	Carp and pangasius polyculture ponds	34 genera: Chlorophyceae (15 genera), Cyanophyceae (9 genera), Bacillariophyceae (7 genera), Euglenophyceae (3 genera)	The highest microalgal cell density corresponded with high nutrient concentrations (NO_3-N and PO_4-P). Chlorophyceae was the most dominant group followed by Cyanophyceae, Bacillariophyceae and Euglenophyceae.	Hossain et al. 2008
Pudong New Area, Shanghai	Giant freshwater prawn (*Macrobrachium rosenbergii*), pearl (*H. cumingii*), silver and bighead carp polyculture	Betaproteobacteria (4 genera), Alphaproteobacteria (3 genera), Gammaproteobacteria (2 genera), Acidobacteria (1 genus), Planctomycetes (2 genera), Actinobacteria (2 genera), Bacteroidetes (3 genera), Chloroflexi (1 genera)	Silver and bighead carp polyculture could control cyanobacteria bloom. Water quality parameters significantly influenced the microbial community. Filter feeder polyculture could decrease the total phosphorus level, but the total nitrogen level just the opposite. The proportion of Bacteroidetes and Proteobacteria could evaluate the level of nutrients.	Tang et al. 2021
Plovdiv, Bulgaria	Common carp, hybrid bighead carp and grass carp polyculture	A total of 259 taxa of planktonic algae were identified during a 2-year study (2018–2019)	High stocking density of grass carp can seriously affect the functioning of the aquatic ecosystem. Cyanoprokaryotes from the genera Aphanizomenon, Dolichospermum and Microcystis, which are potent cyanotoxin producers affecting the ecosystem and human health, were found in the system.	Dochin 2020
Lake Balaton, Hungary	Filter-feeding Asian carps (hybrids of silver carp and bighead carp) polyculture	In the water, there were 100 phytoplankton species, with Cyclotella ocellata having the highest relative abundance. In the foregut of fish, there were 138 phytoplankton species, the most frequent being Cyclotella ocellate. In the hindgut of fish, there were 149 viable phytoplankton species, while 60 taxa were detected neither in the foregut nor in Lake Balaton.	There are viable cells of several phytoplankton taxa (e.g. diatoms, blue-greens, desmids, volvocalean and chlorococcalean green algae), which managed to survive the physical and chemical digestion. Cryptophytes, dinoflagellates and euglenophytes were observed in both the lake water and foregut samples but were absent in the hindgut samples.	Görgényi et al. 2016

Table 5. Diversity of microalgae in carp polyculture systems in literature.

3.1.2. Harmful microalgae blooms

It is widely known that the ecological balance of several physicochemical and biological elements is what determines the productivity of microalgae. As shown in Table 6, the occurrence and abundance of microalgae in polyculture ponds are influenced by a variety of environmental parameters, including temperature, light, dissolved oxygen, pH, nutrient composition and soil condition.

Parameters	Values	Note	References
Temperature	26.6°C–31.97 °C	High temperature is the most important factor in regulating the growth of microalgae, especially Chlorophyceae. Higher temperature is favorable for the growth and development of Cyanophyceae.	Pearsall 1932; Hossain et al. 2008; Grover et al. 2020
pH	7.02–8.11	Higher pH limits the availability of CO_2, inhibiting cell growth.	Chen and Durbin 1994; Hossain et al. 2008
Dissolved oxygen (DO)	4.2–9.6 mg/L	Hight DO causes a decrease in the photosynthetic efficiency, which is a result of photorespiration activity.	Hossain et al. 2008; Raso et al. 2012
NO_3-N	0.46–1.38 mg/L	The microalgal population was more positively correlated with NO_3-N than with PO_4-P.	Khan et al. 1998; Hossain et al. 2008.
PO_4-P	0.83–1.73 mg/L	The requirement of NO_3-N concentration was more than PO_4-P concentration for the growth of a diatom, Skeletonema costatum.	

Table 6. Optimal environmental parameters for microalgae in fishponds.

In aquaculture ponds, certain microalgal blooms (cyanobacterial and buglenophycean) have been linked to water quality problems. When cyanobacteria blooms occur, they can kill animals and wildlife (Carbis et al. 1995; Negri et al. 1995). Larger cyanobacteria, such as Anabaena, Aphanizomenon, Microcystis and Oscillatoria, can create an off-flavor in fish and form surface scum, which often causes algae to die-off and water quality to deteriorate (Kim et al. 2018).

A study investigated the effect of blue-green algae blooms (*Microcystis*, *Oscillatoria* and *Anabaena*) on water nutrition, plankton diversity and density, and fish production of three carp polyculture ponds located in West Godavari District, Andhra Pradesh, India (Padmavathi et al. 2017). The research showed that *M. aeruginosa* is the most harmful to fish, causing the highest mortalities in carp, followed by *Anabaena* and *Oscillatoria*. In addition, the presence of blue-green algae had an effect on the diversity and density of other plankton. Plankton diversity was lowest in the ponds with *Microcystis* bloom, followed by the ponds of *Anabaena* sp. and *Oscillatoria* sp. This indicates that *Microcystis* has the more inhibitory effect on others. Blue-green algal species grow out to become dominant, eliminating most other species in the ecosystem through excretions until they are only found sporadically (hetero-antagonism). As a result, the phytoplankton during blue-green algal blooms is abundant but not diverse (Lefèvre et al. 1952). In the study, nitrogen-deficient waters were more favorable for *Anabaena* bloom formation than *Microcystis* and *Oscillatoria* blooms. This could be one of the reasons that *Anabaena* blooms are uncommon in fishponds fertilized with inorganic nitrogen.

Similarily, a survey conducted in many fishponds in coastal areas of Andhra Pradesh reported that *Microcystis* blooms are widespread, followed in frequency by blooms of *Oscillatoria*, *Arthrospira* and *Spirulina*, while *Anabaena* blooms are rare. As such, an inorganic nitrogen shortage in the surface layer of the pond could stimulate the growth of blue-green algae. Furthermore, Rhee and Gotham

(1980) suggested that an Nitrogen: Phosphorus ratio (N:P) of less than 5 is one of the factors that can initiate the development of cyanobacteria over other algae in pond waters.

Cyanobacteria blooms are more common in nutrient-depleted environments. This might be because they store previously available nitrogen, which they use when nitrogen levels in the pond are low. After algal blooms, there is a rise in nutrient levels, which could be attributed to the release of stored nitrogen and phosphate during algal breakdown (Fogg et al. 1973). According to Hammer (1964), the buildup of orthophosphates by blue-green algae contributed to their bloom formation and the release of phosphates into the water after decomposition.

3.2. The impact of carp species on microalgae abundance

Many fish species, each with its own eating habits, are able to control the algal and microbial composition of green water. For example, silver carp efficiently minimize the development of harmful algae as well as excessive blooms of other species (Zhang et al. 2006). In Shanghai, Tang et al. (2021) used DNA extraction and sequencing to assess the ability of carps to control cyanobacterial blooms in polyculture of silver and bighead carps with triangle sail mussel (*Hyriopsis cumingii*). They found that the abundance of cyanobacteria was greater in the polyculture systems with silver and bighead in the carp than those without them. However, *Microcystis*, which was the dominant cyanobacteria in the groups without the two carps, could form cyanobacterial blooms. In the silver and bighead carp polyculture groups, the dominant cyanobacteria were Subsection_Family_unclassified, *Synechococcus* and *Cyanobacteria_norank*. Cyanobacteria from these genera could be microscopic and so unable to produce algal blooms.

This result proved that silver and bighead carp polyculture could increase diversity of microbial species. The introduction of silver and bighead carps was related to the abundance of microorganisms such as *Betaproteobacteria* (4 genera), *Alphaproteobacteria* (3 genera), *Gammaproteobacteria* (2 genera), *Acidobacteria* (1 genus), *Planctomycetes* (2 genera), *Actinobacteria* (2 genera), *Bacteroidetes* (3 genera) and *Chloroflexi* (1 genera). Therefore, silver and bighead carps can help control cyanobacterial blooms and improve microbiome diversity.

An enclosure experiment carried out in the Three Gorges Reservoir in China discovered that reducing certain zooplankton species, such as rotifers and copepods, results in a trophic cascade, releasing phytoplankton from herbivory and enabling it to develop (Zhou et al. 2011). Similarly, Ke et al. (2008) conducted an experiment in a Chinese lake by stocking bighead carp and silver carp. They concluded that phytoplankton density decreased only when bighead carp concentrations were very high and zooplankton resources were declining. Wang et al. (2008) conducted research on 45 shallow lakes in China and found that lakes with greater bighead carp and silver carp yields had a higher chlorophyll a concentration but poorer visibility. This could be because smaller phytoplankton species that bighead carps do not consume had increased in abundance or because bighead carp feeding and excretion boosted nutrient cycling and the development of phytoplankton.

Furthermore, bighead carp have an impact on the size of the phytoplankton in ponds. Freshwater pearl cultivation research in China discovered that stocking bighead carps caused a decrease in the size of phytoplankton (Yan et al. 2009). This is because bighead carp consumed larger phytoplankton and/or fed on zooplankton. Normally, zooplankton feeds on smaller algae, so smaller algae could become more abundant if zooplankton numbers decrease.

3.3. Techniques for identifying microalgae

3.3.1. Traditional microscopy

Morphological features are used as a fundamental unit for classifying phytoplankton. Picoplankton (0.2–2 m), nanoplankton (2–20 m) and microplankton (20–200 m) are the three size classes of phytoplankton. Traditionally, visual microscopy is used to determine phytoplankton morphology (Godhe et al. 2007), though this approach is successful only for nanoplankton and microplankton. Microscopy allows for both morphological identification and a look at the inside features of microorganisms (5–200 m), as well as the presence of photosynthetic pigments, which are required for human-based taxonomic recognition and classification (Barsanti et al. 2021).

The first low-resolution microscopy, produced by Robert Hooke and Antonie van Leeuwenhoek, allowed for a more precise identification of morphology, giving an initial characterization of plankton taxonomic diversity (Johnson and Martiny 2015). With the development of scanning electron microscopy (SEM), it has become possible to obtain more precise visual information at the species, genus or higher level of phytoplankton, for those with distinct features. Many ultra-structures of phytoplankton that are used to identify species can only be viewed using SEM methods (diatoms) (Erickson et al. 2011). Additionally, this approach helps identify a specific harmful genus of microalga without definitive species identification, such as potentially hazardous organisms of the genus *Pseudonitzia* (Erickson et al. 2011).

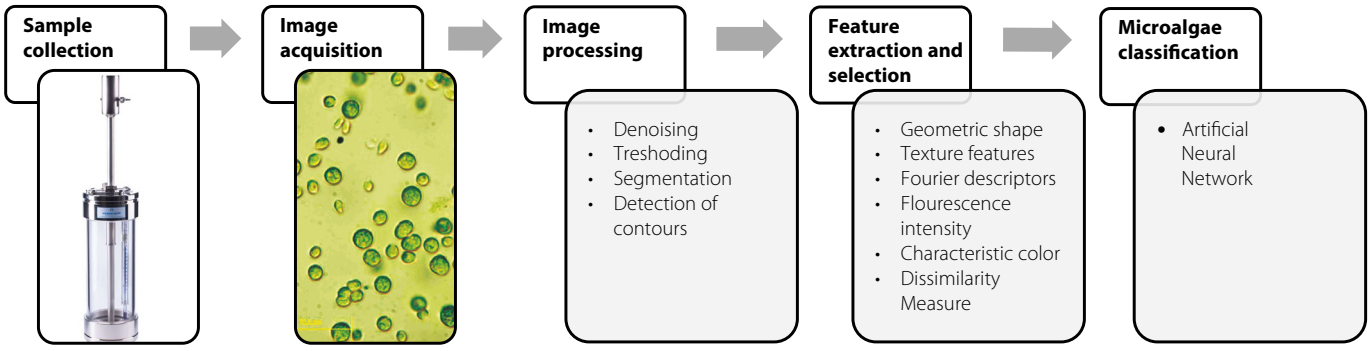
However, because phytoplankton species have a limited range of physical traits and show phenotypic plasticity, classification based on these observable characteristics can be unreliable and could lead to wrong conclusions when examining samples from two distinct environments. According to Erickson et al. (2011), the morphological approach necessitates the use of costly laboratory equipment, such as SEM, as well as time-consuming analysis. Additionally, failures in collecting or preserving microalgae make it difficult to determine the morphology of a given species after a sample is stored. For example, most picoplankton and many soft-bodied dinoflagellates lyse upon storage. Furthermore, it requires highly skilled specialists trained to recognize and distinguish one taxon from another on the basis of often subtle morphological differences.

3.3.2. Digital microscopy

Given the limitations of visual microscopy, scientists have attempted to automate different steps of the sampling and classification processes using digital microscopy. This technology requires a hardware platform comprised of an optical microscope coupled with a color CCD (Charge-Coupled Devices) digital camera, for capturing images, and a powerful personal computer (Barsanti et al. 2021). The computer should be equipped with custom-designed software capable of combining robust image segmentation, shape feature extraction, in-focus algae identification and classification, and successive taxonomy categorization (Barsanti et al. 2021).

Some systems have been developed for classifying microalgal images, which depends on identifying the morphology of phytoplankton (Grosjean et al. 2004; Remsen et al. 2004; Gorsky et al. 2010; Mosleh et al. 2012), absorption spectroscopy (Coltelli et al. 2013 and 2014; Xu et al. 2020) or fluorescence spectroscopy (Schulze et al. 2013). These technologies help generate image data of previously unknown plankton species and obtain an essential database for analyzing the composition of plankton assemblages (Barsanti et al. 2021).

As shown in Figure 3, there are five steps for automatically categorizing images of microalgae using digital microscopy: sample collection, image acquisition, image processing, feature extraction and selection, and microalgae classification.



Source: Barsanti et al. 2021.

Figure 3. The five steps of the automatic microalgae classification process by means of digital microscopy.

The digital microscopy is commonly used to identify and classify phytoplankton because it has several advantages. First, the process of acquiring and reproducing images is straightforward, time-efficient and has few errors. Second, digital photos can be easily edited by annotating, automatically adding scale bars, adjusting brightness and contrast, and eliminating background contamination. Third, digital images can be kept in searchable databases, which allows people to retrieve information rapidly and efficiently while also storing large amounts of data. This opens up great opportunity for phycological study and enables the development of dynamic, up-to-date taxonomic tools (Droop et al. 1993). Storing, archiving and retrieving digital pictures are simplified for both individuals and institutions since images can be accessible from a single central location. Image analysis can also be used to do morphometric analysis on microalgae (Sheath 1989; Droop 1994; Stoermer 1996). This process is quicker, more efficient and more accurate than other methods, including the now-outdated use of digitizing tablets in combination with projected pictures or printed photos (Theriot and Ladewski 1986; Steinman and Ladewski 1987; Goldman et al. 1990; Mou and Stoermer 1992).

3.3.2.1. Holography

In situ imaging provides a direct way to record particle characteristics in their natural environment. There are different imaging techniques used to characterize phytoplankton *in situ*. These include lidar imaging (Churnside and Wilson 2004; Busck 2005; McKenzie et al. 2020), bulk and planar laser-induced fluorescence (Prairie et al. 2011; Jaffe et al. 2013), laser sheet reflective imaging, such as the Laser Optical Plankton Recorder (Checkley et al. 2008), underwater light microscopy (Mullen et al. 2016), imaging-in-flow cytometry (Olson and Sosik 2007), silhouette photography (Milligan 1996), and holography (Watson 2011).

Holography is a new technology with promising potential for *in situ* morphological research. It can offer 3-D spatial distributions of particles in the micron to centimeter range inside a freestream sample volume, allowing for high-resolution mapping of particle properties without fragmenting them (Nayak et al. 2021). It has a sample volume that is two to three orders of magnitude larger than normal light microscopy. Holography can deliver non-invasive, non-destructive, high-resolution 3-D imaging of objects in their natural surroundings at a resolution and sample volume size that no other device can match.

Because of the capacity to optically split the hologram into separate image planes during reconstruction, all individual particle properties, 3-D spatial distribution, and motion can be extracted (Erickson et al. 2011). In general, the holography approach can be used to get data on the following (Nayak et al. 2021):

- particle counts and size distributions
- particle shape metrics, including cross-sectional area, major and minor axis lengths, and aspect ratio
- the three-dimensional spatial structure of the particle field, such as nearest neighbor distances and particle orientation
- particle identification and classification
- particle interactions, such as aggregation, settling speed, predator-prey behavior and swimming trajectories of organisms
- three-dimensional velocity distributions obtained by merging particle image velocimetry with holography.

Moore et al. (2017 and 2019) employed holography to describe the composition of phytoplankton communities during a toxic algal bloom in western Lake Erie, US. The profiling device could characterize vertical distributions of colony and cell counts of dominated cyanobacterial *Microcystis aeruginosa* and *Planktothrix aghardii*. Most *Microcystis* colonies were discovered at the surface, whereas *Planktothrix* colonies were found in greater numbers deeper in the water column, and the vertical variability was connected to the light availability and tolerance of either species.

Additionally, *in situ* holography can also be used to measure predator–prey behavioral interactions, micro- and fine-scale biophysical interactions, as well as the geographic distributions, behaviors and identification of species (Erickson et al. 2011). For example, Sheng et al. (2007) used holographic microscopy to demonstrate that prey alters the swimming behaviors of heterotrophic dinoflagellates.

3.3.3. Pigment color technology

One of the limiting factors of phytoplankton development is competition for sunlight (Kirk 2010). Because of this competition, various species of phytoplankton have evolved distinct accessory pigments, enabling them to absorb complementary portions of the light spectrum, and, therefore, to coexist. As such, the types and ratios of accessory pigments present can be used to classify phytoplankton families through colorimetric techniques (Johnson and Martiny 2015).

Chlorophyll a is the primary pigment found in phytoplankton and is essential to absorb light. However, numerous accessory pigments, such as carotenoids or other forms of chlorophyll, like b or c, are taxon-specific and are useful for classifying phytoplankton into functional groups (Johnson and Martiny 2015).

Modern high-performance liquid chromatography (HPLC) allows for quick examination of phytoplankton group composition, as more than 40 samples can be studied per day using an autosampler attached to the HPLC. As shown in Table 7, this enables a more in-depth investigation of the structure and dynamics of phytoplankton communities than was previously achievable using microscopic enumeration of phytoplankton (Schlüter et al. 2000).

Gieskes and Kraay (1986) used multiple linear regression to quantify the contribution of the individual phytoplankton groups to the total concentration of chlorophyll a. Using linear regression in this manner requires that the ratios of the different diagnostic pigments to chlorophyll a are constant and that there is a large dataset for statistical validity. However, this strategy cannot be used when the ratios change, for example, because of light adaptation (Ecol et al. 1997). Mackey et al. (1996) introduced another method, the CHEMTAX program, in which factor analysis and the steepest descent algorithm are used to find the best fit to the data based on the suggested ratio of pigment and chlorophyll a of both diagnostic pigments and pigments present in several phytoplankton groups to be determined.

Light, nutrition availability and other environmental variables can cause shifts in pigment ratios. So even though these ratios can give information on phytoplankton physiological diversity, they are not precise enough to quantify specific taxa. Moreover, this approach only determines the contributions of pigment-related groups to the population. For example, HPLC found that fucoxanthin-containing species supplied 50% of a given sample but could not distinguish between the diatoms, chrysophytes or prymnesiophytes that could have contributed this fucoxanthin. However, because of their high throughput and low cost, chromatography-based techniques are still used for characterizing a phytoplankton community (Johnson and Martiny 2015).

Pigment	Associated taxa
19″-Butanoyloxyfucoxanthin	Chrysophytes and haptophytes
19″-Hexanoyloxyfucoxanthin	Chrysophytes and haptophytes
Neoxanthin	Prasinophytes, euglenophytes, and chlorophytes
Chlorophyll b	Prasinophytes, euglenophytes, Prochlorococcus and chlorophytes
Violaxanthin	Prasinophytes and chlorophytes
Diadinoxanthin	Dinoflagellates, chrysophytes, haptophytes, euglenophytes, and diatoms
Lutein	Prasinophytes and cyanobacteria
Zeaxanthin	Prasinophytes, cyanobacteria, and chlorophytes
Divinyl chlorophyll a	Prochlorococcus

Source: Johnson and Martiny 2015.

Table 7. Taxon-diagnostic pigments as quantified by high-performance liquid chromatography.

An alternative method by Everitt et al. (1990) involved classifying plankton based on pigment types, and then estimating the relative abundance of each class' pigments and their relative contribution to the total chlorophyll a in the sample from data in the literature. A difference was used to determine the abundance of classes that did not have distinct marker pigments. The difference between the calculated and observed chlorophyll a concentration was used to measure how well the model's predictions matched experimental data, and an iterative approach was employed to reduce this difference by adjusting the chlorophyll a/ marker pigment ratios. The disadvantage of this technique was that the calculation by a difference process for those classes that do not have clear marker pigments often resulted in predictions of unrealistic or even negative concentrations for these classes (Mackey et al. 1996).

Spectrophotometry is another approach used to identify phytoplankton pigment. The principle is to illuminate a water sample with broadband radiation (the sun in the case of satellite-based studies) and measure the quantity of light it absorbs as a function of wavelength (Erickson et al. 2011). Spectrophotometric analyses require the solution of simultaneous equations in which the unknown pigment concentrations are modeled as a function of the measured absorbance at pigment-specific peak wavelengths (Porra 2006). These techniques are capable of quantifying chlorophyll a, b and c (Jeffrey and Humphrey 1975), total carotenoids, and pheophytins after acidification (Lichtenthaler 1987). Although the procedure is simple, the outcomes are highly dependent on the empirical equation used. The greatest accuracy is obtained when equations derived for pigment standards are used in conjunction with unknown samples evaluated on the same equipment (Wellburn 1994).

3.3.4. Cells and colonies technology

Since the mid-1980s, flow cytometry has been a critical tool in plankton research for its quick quantitative measurements of individual cells. Flow cytometry is a technique that uses a laminar flow stream to pass single cells through a focused laser beam (or beams). After that, scattered laser light is used to quantify and characterize cells since it is proportional to cell size and the refractive index (Johnson and Martiny 2015). Additionally, fluorescence can be used to diagnose some types of pigments, such as chlorophyll and phycoerthythrin. The combination of approximate size/refractive index and pigment composition can also be used to identify numerous important phytoplankton species, including *Prochlorococcus, Synechococcus* and pennate diatoms, as well as to count additional apparent populations that are not taxonomically resolved (Olson et al. 1985).

Flow cytometry works for phytoplankton that is smaller than the diameter of the nozzle (particles should be less than 30–50 m in diameter for a standard nozzle). It is the most robust approach for counting major picoplankton populations (Shapiro 2004; Lomas et al. 2011). Because of the relatively low sample volumetric flow rate (usually 10–50 µL per minute), it is most efficient when particle concentrations are very high, on the scale of 10^4–10^7 objects/ml (Erickson et al. 2011). For bigger cells (more than 10 µm), imaging cytometry is preferable (Johnson and Martiny 2015). This approach combines optical characterization by microscope with sample stream characterization by flow cytometry. The result is much better throughput than traditional microscopic examination (Sieracki et al. 1998; See et al. 2005). Images of each cell are taken as it passes over the imaging point, and the cells are afterward recognized either manually or automatically using training data sets.

Flow cytometry is a useful approach for assessing regular environmental water samples in order to avoid algal blooms (Dennis et al. 2011). As cyanobacteria include phycobillisomes, which are made up of autofluorescent pigments such as phycoerythrin, phycocyanin and allophycocyanin (MacColl 1998; Telford et al. 2001), flow cytometry can used to identify these natural pigments (Telford et al. 2001; Dennis et al. 2011).

Patel et al. (2019) performed a study case to determine the cyanobacterial profiles of freshwater bodies from 36 water bodies throughout 14 New Jersey counties by flow cytometry. Cyanobacteria were found in all 36 water samples tested, and the majority of cyanobacteria and phytoplankton species were abundant in phycoerythrin. The authors concluded that flow cytometry is an effective and quick approach to test water samples regularly, allowing for greater volume sampling

of freshwater bodies. Additionally, portable flow cytometers are available to analyze samples onsite for more precise analysis.

Furthermore, data on the genome size of microalgal groups with sexual reproduction, such as *Zygnematophyceae* and *Bacillariophyceae*, can be used to investigate the function of polyploidy in algal evolution and speciation (Mann and Poulíčková 2010). The release of the nuclei from the protoplast by the lysis buffer enables precise estimation of the nuclear genome size. Flow cytometry is the optimal approach for estimating the size of the nuclear genome. In comparison to previous technologies, such as Feulgen microdensitometry, scanning microspectrophotometry or DNA image cytometry, flow cytometry allows for the measurement of a high number of cells in a short period of time (Doležel and Bartoš 2005).

3.3.5. Molecular technologies for microalgae detection

The application of molecular technology in phycology started in 1970, when phycologists established molecular methods as an indirect detection approach to identify carbohydrates, nucleic acids, proteins and toxins from microalgae. Since then, DNA-based techniques have been explored to enhance understanding of genetic diversity, molecular systematics, evolution and even adaptive responses for all species, not only microalgae (Bott et al. 2010; Kudela et al. 2010; Medlin and Kooistra 2010). According to Ebenezer et al. (2011), technology to detect microalgae can be categorized into five groups as per the target molecule: toxins, proteins, carbohydrates, RNA and DNA. These techniques are often used for detecting harmful bloom-forming species. Each technique has its own advantages and disadvantages, which are presented in Table 8.

Ebenezer et al. (2011) synthesized published papers regarding indirect methods for the detection, quantification and diversity of microalgae from 1985 to 2011 (Table 9). Most detection tools used for microalgae rely on a DNA-based method since toxin, RNA and protein methodologies are unstable (Ebenezer et al. 2011). DNA-based detection methods for microalgae are generally conducted by PCR amplification of a target gene or DNA markers. DNA-based assays have largely been used for identifying and characterizing harmful algae bloom species with the use of species-specific PCR primers and genetic markers (Godhe et al. 2008; Penna and Galluzzi 2008; Wang et al. 2008).

Johnson and Martiny (2015) summarized some of the major technological advances in microalgal diversity characterization (Table 10). The most recent approach of DNA metabarcoding by NGS can identify species quickly in environmental samples. This approach is rapid and cost-effective (Bourlat et al. 2013; Ji et al. 2013), making it easier to track and assess biodiversity over large areas and time periods (Janzen et al. 2005; De Vargas et al. 2015). Additionally, it has a higher sensitivity for detecting uncommon species (Zhan et al. 2013) and identifying species that cannot be distinguished based on morphological traits.

Besides amplification/detection, further procedures must be taken into account to make these tools useful for environmental monitoring activities and also for field applications. They are sample pre-concentration, nucleic acid extraction, applicability and miniaturization/ portability. Moreover, different parameters need to be carefully controlled and defined during the development of DNA-based biotechnological tools (Toldrà et al. 2020).

Techniques	Advantages	Limitations
Toxin profile	It detects toxic species.	There is a toxin difference among strains, making it difficult to analyze.
Fluorescent-conjugated lectin probe	It characterizes closely related species.	Lectins bind non-covalently with polysaccharides on cell surfaces.
Allozyme (or isozyme) electrophoresis	It is easy to develop and cost-effective.	It underestimates the level of genetic variation.
Antibody probe	Quantification and identification are rapid.	Variation in cell protein is caused by external factors, cross-reactivity with antigens.
Sandwich hybridization assay	Variation in surface protein can be characterized, and false negative signals are minimzed.	The homogenization of cells, specificity and sensitivity of the probe in field studies are in question.
Fluorescence in situ hybridization (FISH)	It provides highly specific, rapid identification and analysis of natural samples.	It is tedious, time consuming, expensive and requires technical expertise to manufacture a species-specific probe.
Oligonucleotide array	Detection is quick, sensitive and simultaneous, and allows the enumeration of species.	It has expensive equipment and a longer hybridization incubation time, and the platform is not flexible.
Restriction fragment length polymorphism (RFLP)	It can discriminate between closely related species.	A large volume of pure, high molecular weight genomic DNA is required.
Random amplification of polymorphic DNA (RAPD)	It can discriminate between closely related species.	Reproducibility rate is low in genotyping, and it is sensitive to reaction conditions.
Amplified fragment length polymorphism (AFLP)	It can tell genetic variation between species and is highly reproducible.	It is expensive and requires more technical expertise.
Single-strand conformation polymorphism	It can identify species in complex assemblages.	It is time consuming.
DNA sequencing	It provides accurate comparisons and is easy to use.	Comparable data is required.
Massively parallel signature sequencing	It can discriminate between closely related species.	There is a species-specific sequence bias and a loss of specific sequences.
Automated ribosomal intergenic spacer analysis (ARISA)	It can determine the presence of an organism from environmental samples.	Comparable data is required and it has intraspecific variation Intraspecific variation: comparing individuals of differing species.
Isothermal nucleic acid sequence-based amplification	Identification of species in samples containing low concentration of cells is rapid and reliable.	–
Loop-mediated isothermal amplification method	There is a simple operation with a rapid reaction and ease of detection.	–

Techniques	Advantages	Limitations
Single cell PCR	It can detect non-culturable species.	It is labor-intensive in single cell isolation.
Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE)	It can differentiate between similar strains and compare the composition of natural populations.	Detection is non-quantitative and the equipment is expensive.
Melting curve analysis	It is easy to handle and cost-effective.	Resolution is low for discrimination.
Inter simple sequence repeat	It is simple to use and inexpensive.	There are few available markers; marker developments are difficult from a large number of isolates.
Microsatellites/single sequence repeats	This is largely used to study population structure and intraspecific genetic diversity.	There are few available markers; marker developments are difficult from a large number of isolates.
Multiplex/semi-multiplex PCR	It is highly specific, sensitive and can determine multiple species.	It decreases the sensitivity.
PCR coupled dotblot/low density microarray hybridization	It is rapid and highly specific and requires less quantity of genome.	It is unsuitable for field applications.
High throughput microarray	It is accurate, inexpensive to maintain, has good sensitivity and specificity, and is rapid and easy to use; configuration of assay platform is easy.	It is unsuitable for field applications.
Terminal restriction fragment length polymorphism (TRFLP)	Results are highly reproducible for repeated samples.	False (pseudo) T-RFs can appear.
PCR assay	It is simple to use and cost-effective.	Specific primers are required.
Real-time PCR	It is highly specific, sensitive, cost-effective, quantitative and is applicable to preserved environmental samples.	It can provide information on one species or a few target species.

Source: Ebenezer et al. 2011.

Table 8. Molecular techniques involved in the detection of microalgae, their advantages and limitations.

Year	Biomolecules														
	Carbohydrate/protein/toxin				RNA	DNA									
1985				Ab											
1986															
1987	TP														
1988		IP					RFLP				SB				
1989															
1990															
1991															
1992															
1993			LB												
1994															
1995															
1996					OP	SH				PCR					
1997									RAPD						
1998															
1999															
2000															
2001								AFLP		RT		SC			
2002											ST		MA		
2003															
2004															
2005															
2006															
2007															
2008															
2009														NGS	
2010															
2011															

Abbreviations: TP toxin profile, IP isozyme pattern, LB lectin binding, Ab antibody, OP oligonucleotide probe, FISH, SH sandwich hybridization, RFLP restriction fragment length polymorphism, AFLP amplified fragment length polymorphism, RAPD random amplification of polymorphic DNA, PCR polymerase chain reaction, RT real-time PCR, SB sequence-based.

Note: Colored cells in each column represent individual technologies based on published papers (different time scale) and individual technologies based on corresponding years of publications between the first and the latest.

Source: Johnson and Martiny 2015.

Table 9. Indirect methods for detecting and quantifying the diversity of microalgae based on published papers (different time scale).

Technique	Application	(Phylo) genetic resolution	Throughput	Dynamic range	Limitations	Example references
Phylogenetic diversity						
Microscopy (light, fluorescence, electron)	Large distinct cells, few samples, direct counts	Morphologically distinct	Low	Limited	Large cells	Malfatti and Azam 2009; Thompson et al. 2012
Pigments (HPLC)	Many samples	Coarse	Medium	High	Influenced by physiology	Mackey et al. 1996; Bidigare et al. 2005
Flow cytometry	Many samples, direct counts	Coarse	Medium	High	Small cells (<~20 µm)	Olson et al. 1985
Imaging cytometry, video plankton recorder	Many samples, direct counts	Coarse	Medium	High	Large cells (>~10 µm)	Sieracki et al. 1998; See et al. 2005
Marker genes: ARISA, DGGE, TRFLP	Many samples, approximate counts	Medium	High	Medium	DNA/PCR based	Moeseneder et al. 1999; Zeidner and Béjà 2004
Marker genes: clone library	Few samples	Medium (depending on the product and length)	Low	Medium	DNA/PCR based	Pichard et al. 1997; Moon-Van Der Staay et al. 2001
Marker genes: amplicon library	Many samples, approximate counts	Medium (depending on the product and length)	High	Medium	DNA/PCR based; significant data analysis	Sogin et al. 2006; Hunt et al. 2013
Marker genes: qPCR	Many samples, direct counts, specific targets	Variable (depending on the PCR primers)	High for samples, low for groups	High	DNA/PCR based	Johnson et al. 2006
Marker genes: hybridization	Many samples, approximate/ direct counts, specific targets	Variable resolution (depending on the oligonucleotides)	High for samples, low for groups	High	Predefined targets	Amann and Fuchs 2008
Multiple marker genes	MLST	High	Low	NA	Requires culture or a large proportion of the genome sequence	Maiden et al. 1998

Technique	Application	(Phylo) genetic resolution	Throughput	Dynamic range	Limitations	Example references
Genomic diversity						
Genomics	Domesticated population (or sorted cells)	High	Low	NA	Domestication of cells (or missing parts of genome if sorted cells), significant data analysis	Rocap et al. 2003; Armbrust et al. 2004
Metagenomics	Few samples	High	Low	NA	Significant data analysis	Venter et al. 2004; DeLong et al. 2006
Physiological diversity						
HPLC pigment labeling	Few samples	Coarse	Low	High	Incubation-based	Goericke and Welschmeyer 1993
RT-qPCR (single target)	Many samples, direct quantification, known targets	Variable (depending on the PCR primers)	High for samples, low for groups	High	RNA/PCR based, intermediate step to physiology	Paul et al. 1999; Church et al. 2005
RT-amplicon library	Many samples, approximate quantification, known targets	Variable (depending on the PCR primers)	High	Medium	RNA/PCR based, intermediate step to physiology, significant data analysis	Campbell et al. 2011; Hunt et al. 2013
Microarray	Few samples, quantification, many/known targets	High	High for targets, low for samples	High	Intermediate step to physiology	Martiny et al. 2006; Zinser et al. 2006
Transcriptomics	Few samples, quantification	High	High for targets, low for samples	Medium	Intermediate step to physiology	Allen et al. 2008
Metatranscriptomics	Few samples, approximate quantification, unknown targets	High	High for targets, low for samples	Low	Significant data analysis, intermediate step to physiology	Poretsky et al. 2009
Proteomics (single or multiple proteins)	Few samples, approximate quantification, known targets	Medium	High for targets, low for samples	Low	Significant data analysis (if multiple proteins), intermediate step to physiology	Dyhrman et al. 2012

Technique	Application	(Phylo) genetic resolution	Throughput	Dynamic range	Limitations	Example references
Metaproteomics	Few samples, approximate quantification, known targets	Medium	High for targets, low for samples	Low	Significant data analysis, intermediate step to physiology	Morris et al. 2010
Physiology (growth, behavior, etc.)	Intermediate samples	Low	Low	Variable	Domestication of cells	Falkowski and Owens 1980; Franks 1992
Biochemistry (elemental composition, fatty acids)	Variable samples	Low	Low	Variable	Variable, often requires domestication of cells	Bertilsson et al. 2003; Van Mooy et al. 2006
Tracer uptake (e.g. BrdU, isotopes)	Many samples, quantification	Low	Low	Variable	Incubation-based	Casey et al. 2007
Metabolites	Few samples, quantification	Low	Low	Medium	Incubation-based, significant data analysis	Osanai et al. 2014
Other						
Cell sorting (flow cytometry, microfluidics)	Any downstream single-cell-based (or population-based) approach	Variable	Low	Low	Ability to optically define cells (with or without stained probes)	Malmstrom et al. 2013
Single-cell DNA amplification (e.g. MDA)	Any downstream DNA-based approach	Variable	Low	Low	Partial genome coverage	Zhang et al. 2006

Abbreviations: ARISA automated ribosomal intergenic spacer analysis, BrdU bromodeoxyridine, DGGE denaturing gradient gel electrophoresis, HPLC high-performance liquid chromatography, MDA multiple displacement amplification, MLST multilocus sequence typing, NA not applicable, PCR polymerase chain reaction, qPCR quantitative polymerase chain reaction, RT reverse transcription, TRFLP terminal restriction fragment length polymorphism.

Source: Johnson and Martiny 2015.

Table 10. Phytoplankton biodiversity techniques: applications and properties.

4. Viruses in carp culture

Viral infections are one of the major reasons for economic loss in aquaculture, either by significantly reducing fish growth rate or by directly killing the host fish. Among the several viral infections that affect carps, cyprinid herpesvirus 3 (CyHV-3), spring viremia virus and carp edema virus (CEV) are the major ones that infect koi or common carp. Other infections also cause extensive damage to carp aquaculture (Table 13). In carp polyculture systems, different fish species can spread the virus further. Radosavljević et al. (2012) conducted an experiment by co-culturing goldfish, silver carp, grass carp, prussian carp and tench with CyHV-3 infected carp. After that, the authors cohabitated them with native carp for 2 weeks and examined them for CyHV-3 by PCR. The results showed that CyHV-3 was present in the organs of all fish species, suggesting that CyHV-3 can cause latent infection and has the potential to infect a broader host range. Moreover, goldfish, silver carp, grass carp, prussian carp and tench can serve as vector species for CyHV-3, without showing any clinical signs.

4.1. Common viral diseases in carp

4.1.1. Koi herpesvirus disease

Koi herpesvirus disease (KHVD) is a lethal disease in koi and common carp, with CyHV-3 as the causative agent (Waltzek et al. 2005 and 2009). Since the first description of KHVD by Hedrick et al. (2000), the virus has rapidly emerged on a global scale (Haenen et al. 2004) (Table 12). In 1996, KHVD was reported in England by analysis of preserved tissue samples. Since then, outbreaks of KHVD have been recorded regularly in Europe, South Africa, the US and Asia. In Asia, it has been reported in Israel, Indonesia, Taiwan, China, Thailand, Japan and Malaysia.

The seasonal occurrence of KHVD outbreaks in farmed and wild populations seems to be linked to water temperature, with the majority of outbreaks occurring between 18°C and 28°C (Gilad et al. 2003; Yuasa et al. 2008; Rakus et al. 2013). Experimentally, temperatures of 16°C –18°C have also been shown to be favorable for the occurrence of KHVD in carp (Gilad et al. 2003; Yuasa et al. 2008). Skin sores, sloughing off the epithelium and a lack of mucus are among the most common symptoms of CyHV-3 infection. The fish lose their appetite, are lethargic and lie on the bottom of the tank with their dorsal fin curled. The fish's gills are often necrotic and hyper-secrete mucus, resulting in suffocation. Furthermore, fish seem to be more vulnerable to secondary infections by bacteria, parasites or fungi, which can result in increased mortality in the population (Michel et al. 2010; Rathore et al. 2012).

4.1.1.1. Susceptibility

CyHV-3 causes KHVD in carps of all ages, but younger fish (1–3 months, 2.5–6 g) seem to be more vulnerable to infection than mature fish (1 year, 230 g) (Oh et al. 2001a). When young carps were exposed to CyHV-3 infection, the majority of infected juveniles (>13 days after hatching) died, whereas larvae (3 days after hatching) did not (Ito et al. 2007). Table 11 shows the susceptible host range of CyHV-3 and the nature of infection in different fish species.

According to Costes et al. (2009), the skin covering the fin and the body is the major portal of entry for CyHV-3 into carp. CyHV-3 causes an infection in the skin of common carp, which is linked with lower levels of mRNA expression for genes encoding various mucosal barrier components. It disrupts the skin's barrier function during CyHV-3 infection, making the skin more susceptible to secondary infections in the circumstances of the disease (Adamek et al. 2013).

4.1.1.2. Transmission

CyHV-3 is not only associated with carps, but it is also asymptomatically carried by other fish species, which could contribute to the transmission of CyHV-3 between or among farmed and wild populations (Bergmann et al. 2010; El-Matbouli and Soliman 2011; Fabian et al. 2013). CyHV-3 is easily spread through the movement of infected carp because of the delayed onset of disease and the possibility of persistent or latent carriers (St-Hilaire et al. 2005; Bergmann and Kempter 2011; Uchii et al. 2014). Early viral replication at the entry point (skin) cannot only transmit the virus inside

Host fish species	Nature of Infection	Reference
Common carp	Symptomatic	Hedrick et al. 2000; Perelberg et al. 2003; Haenen et al. 2004
Koi carp	Symptomatic	Hedrick et al. 2000; Perelberg et al. 2003; Haenen et al. 2004
Goldfish (*Carassius auratus*)	Asymptomatic/carrier	El-Matbouli et al. 2007; Sadler et al. 2008; El-Matbouli and Soliman 2011
Goldfish × koi carp hybrid	Symptomatic	Bergmann et al. 2010
Crucian carp × koi carp hybrid	Symptomatic	Bergmann et al. 2010
Goldfish × common carp	Symptomatic	Hedrick et al. 2006
Grass carp	Asymptomatic/carrier	Kempter and Bergmann 2007
Ide (*Leuciscus idus*)	Asymptomatic/carrier	Kempter and Bergmann 2007

Source: Rathore et al. 2012.

Table 11. The nature of infection of CyHV-3 in different fish species.

infected fish, but also across the fish populations. About 2–3 days after infection, fish rub against other fish or objects, leading to skin-to-skin transmission (Michel et al. 2010). Tolo et al. (2021) demonstrated that physical contact between fish was the primary mode of CyHV-3 transmission, rather than through contaminated water.

Additionally, transmission occurred primarily during the virus' incubation stage, before the onset of symptoms and a related decline in contact rate. Therefore, skin to skin contact between infected and native carp during breeding, social feeding or necrophagous behaviors could result in direct transmission of CyHV-3 (Raj et al. 2011; Ilouze et al. 2012). The rapid transmission of CyHV-3 has also been related to the cohabitation of koi, the transportation of live fish, the release of infected koi, and transmissions through waterfowl and piscivorous birds in carp cultivation (Tu et al. 2004b; Taylor et al. 2011; Torres-Meza et al. 2020). Specifically, viral particles can be linked to inorganic and organic particulate matter and accumulate in plankton, allowing CyHV-3 to survive in the aquatic environment (Minamoto et al. 2011).

Several factors could influence the transmission of the virus, including the viral load of infected conspecifics, the behavior of infectious and healthy fish, the demography and density of the population, the climate, and the existence of disease vectors and reservoirs (Hepher 1988). Moreover, the effect of temperature on the transmission rate of CyHV-3 has been well described by laboratory studies (Gilad et al. 2003; Yuasa et al. 2008) and mathematical modeling (Omori and Adams 2011). Temperature influences the time of seasonal spawning, carp metabolic and immunological function and the transmissibility of CyHV-3, both within and across hosts (Perelberg et al. 2003; Uchii et al. 2014).

4.1.2. Spring viremia of carp

Spring viremia of carp disease is caused by a single-stranded RNA virus, *Rhabdovirus carpio*, and has been reported in common carp (Ahne et al. 2002), goldfish, koi, silver carp, crucian carp, bighead carp, grass carp and other non-carp species (Fijan 1984; Shchelkunov and Shchelkunova 1989; Ahne et al. 2002)

In 1971, spring viremia carp virus (SVCV) was initially found in Yugoslavia (Fijan 1972), and was subsequently detected in the Americas (Warg et al. 2007) and Asia (Teng et al. 2007). Currently, SVCV infection has been reported in many European countries, including England, the Netherlands, France, Spain, Germany, Italy, Czech Republic, Austria, Croatia, Bosnia and Herzegovina, Hungary, Poland, Serbia, Romania, Moldova, Slovakia, Bulgaria and the former Soviet Union (Ahne et al. 2002). SVCV has also

Country	Year of reporting	Disease status	Reference
Belgium	2010	Reported present or known to be present	International Database on Aquatic Animal Diseases (IDAAD)
Canada	2010	First detection of KHV by PCR	Garver et al. 2010
China	2002	First detection of KHV by PCR	Liu et al. 2002
China (Hong Kong)	2006	Reported present or known to be present	NACA and FAO 2017
Chinese Taipei	2004	Reported in the country for the first time	Tu et al. 2004a
Czech Republic	2010	First clinically apparent KHV infection	Novotny et al. 2010
Denmark	2008	Reported present or known to be present	IDAAD
Germany	1999	Reported in the country for the first time	Neukirch and Kunz 2001
Indonesia	2005	Reported present or known to be present	Sunarto et al. n.d.
Ireland	2011	First detection of KHV in imported koi by PCR	IDAAD
Israel	1999	Reported in the country for the first time	Hedrick et al. 2000
Japan	2004	Reported in the country for the first time	Sano et al. 2004
Korea	2001	First report of KHV	Oh et al. 2001a
Luxembourg	2008	Reported present or known to be present	IDAAD
Malaysia	2008	Reported present or known to be present	NACA and FAO 2017
Netherlands	2008	Reported present or known to be present	IDAAD
Philippines	2006	KHV associated mortalities in koi carp	Somga et al. 2010
Poland	2006	Reported in country for the first time	Bergmann et al. 2006
Romania	2010	First reported occurrence of KHV	IDAAD
Singapore	2006	Reported present or known to be present	NACA and FAO 2017
Slovenia	2008	First reported occurrence of KHV	IDAAD
Spain	2011	First reported occurrence of KHV	IDAAD
Sweden	2011	Reported present or known to be present	IDAAD
Thailand	2009	Report of KHVD outbreak	Pikulkaew et al. 2009
UK	1999	First reported occurrence of KHV	Walster 1999
US	2000	First reported occurrence of KHV	Hedrick et al. 2000

Source: Rathore et al. 2012.

Table 12. Status of KHVD worldwide.

been reported in China (Liu et al. 2004), Korea (Oh et al. 2001b), the Middle East (Perelberg et al. 2003), the US (Dikkeboom et al. 2011) and most recently in Canada (Garver et al. 2010).

SVCV outbreaks often occur in the spring, when the water starts to warm up (Ahne 1986). Adult carp are affected when the water exceeds 17°C and juveniles at 22°C–23°C (Ahne 1986). Additionally, morbidity and mortality are influenced by fish density, geographical location, fish species and the immunological status of fish exposed to the virus (Ahne 1986). SVCV has an incubation period of 7–15 days in experimental conditions. Infected fish can exhibit a number of clinical symptoms, including lethargy, ascites, exophthalmia, pale gills, generalized darkening of the body surface, persistent fecal casts, skin and branchial hemorrhages, and vent distention/protrusion (Ahne et al. 2002).

4.1.2.1. Susceptibility

Hill (1977) observed that wild carp were more sensitive to SVCV than cultivated carp. SVCV can infect fish of all ages, but since the disease is seasonal, fish at 9–12 and 21–24 months old are the most vulnerable. According to Shchelkunov and Shchelkunova (1989), young fish were more susceptible to SVCV than 1.5-year-old fish. Farmed carp are more susceptible than grass carp, and grass carp are more vulnerable than the bighead × silver carp hybrid (Shchelkunov and Shchelkunova 1989).

Gills served as an entry point and a major location for SVCV replication. Between 6 and 11 days after infection, SVCV had spread through the bloodstream. The virus had also been found in the kidney, liver, spleen, heart and alimentary tract, at which time clinical signs appear. At 11 days after infection, viral shedding occurred through mucous casts and feces, where the virus remained alive for many days (Ahne 1978).

4.1.2.2. Transmission

Infected fish and asymptomatic carriers can both carry SVCV. This virus is shed in the feces and urine of infected fish, as well as their gills and skin mucus. At temperatures ranging from 4°C to 10°C, the excreted virus can remain pathogenic in the water for more than 4 weeks and in the mud for approximately 6 weeks (Ahne et al. 2002). Although waterborne transmission is thought to be the major mode of infection, bloodsucking parasites like leeches and carp louses can act as mechanical vectors for SVCV (Ahne et al. 2002), though contaminated equipment could also be a cause of infection (Ahne 1977). Other aquatic arthropods and fish-eating birds are potential vectors as well.

4.1.3. Carp edema virus disease

Carp edema virus (CEV) disease, commonly known as koi sleepy disease (KSD), is a serious acute infection caused by a poxvirus (family *Poxviridae*) in common carp and ornamental koi. This disease has a mortality rate of up to 80%–100% and has spread rapidly around the world. Since the first case of KSD was reported in Japan in the 1970s, the illness has spread to a number of countries, including France, the Netherlands, Germany, Italy, Austria, Poland and India (Haenen et al. 2014; Jung-Schroers et al. 2015; Pretto et al. 2015; Swaminathan et al. 2016; Matras et al. 2017). In the US, KSD outbreaks occurred in the following states because of imported and domestic koi: California (1996 and 2010), Washington (2005), North Carolina (2005), Georgia (2005 and 2010) and Florida (2014 and 2015) (Hedrick et al. 1997; Waltzek et al. 2014)

KSD epidemics are most common when water temperatures range from 15°C to 25°C. During the spring and fall, the majority of fish display clinical signs. The disease also has the potential to spread extensively at low temperatures, with a prolonged disease duration and a low mortality rate (Rehman et al. 2020). KSD is distinguished by sluggish behavior, fish laying on the bottom of ponds, skin sores around the mouth and at the base of fins, anus inflammation, enophthalmos, and enlarged and necrotic gills that cause hypoxia and respiratory distress (Pikula et al. 2021).

4.1.3.1. Susceptibility

Previous research found that more than 80% of the virus load was found in the gills of infected fish (Adamek et al. 2019), so the gills are thought to be the pre-dilation location for CEV reproduction. The disease is commonly detected at water temperatures ranging from 15°C to 25°C in koi to 6°C–10°C in common carp and

can kill up to 75%–100% of juvenile koi during an outbreak (Hedrick et al. 1997; Miyazaki et al. 2005). In Japan, KSD is most common among juvenile koi during the rainy season, after they have been stressed by being transported from green-water earthen nursery ponds to clear-water concrete-lined ponds for grading.

4.1.3.2. Transmission

Fish play a significant role in the spread of KSD as carriers (Lewisch et al. 2015). The virus is shed from an infected fish's gills and contaminates the water in which infected and healthy fish coexist (Oyamatsu et al. 1997; Adamek et al. 2017). For example, the addition of infected fish without quarantine into an established pond of koi can cause disease. The optimal cohabitation period is 12 hours for the transmission of the CEV virus from infected koi and common carp to other fish species.

There are still significant information gaps about KSD transmission pathways (Matras et al. 2019). Way et al. (2017) state that some of the gaps include (i) understanding the virus' survival mechanism outside of the host, (ii) identifying other susceptible aquatic species (carriers) other than carp, (ii) whether the virus exists as a low-level persistent infection and whether there are aquatic vectors, (iv) what the prevalence is of KSD infection in carp populations, and (v) identifying additional important environmental factors responsible for triggering KSD. The other question is whether KSD can be transferred vertically from an infected parent fish to its offspring through eggs or sperm.

Disease	Cyprinid herpesvirus 3 disease	Spring viremia of Carp	Koi sleepy disease	Carp pox	Hemorrhagic disease of grass carp
Disease synonyms	Koi herpesvirus, carp nephritis and gill necrosis virus	Infectious dropsy of carp	CEV	Cyprinid herpesvirus 1 (CyHV-1), koi pox, carp herpes virus, herpesviral epidermal proliferation in carp, herpesvirus septicemia in carp	Grass carp reovirus, grass carp hemorrhagic virus, fish reovirus
Pathogen common name and synonyms	Herpesvirus (DNA virus)	Rhabdovirus Rhabdovirus carpio (RNA virus)	Poxvirus	Herpesvirus Herpesvirus cyprini (DNA virus)	Reovirus
Species affected	Common carp, koi, other species	Common carp, koi, goldfish, grass carp, bighead carp, silver carp, crucian carp	Common carp, koi	Common carp, koi	Grass carp, black carp
Affected life stage	Juveniles and adults, though young fish are more susceptible than adults	Mostly at 9–12 and 21–24 months old because of seasonality, though young fish are more susceptible than adults	All age groups, though young fish are more susceptible than adults	Fish over a year of age, though young fish are more susceptible than adults	Fry and year-old fingerlings usually infected, though infections occasionally occur in older fish (2-3 years old)
Transmission	Direct contact, fecal matter, infected water/mud, equipment, vectors	Direct contact, fecal matter, infected water/mud, equipment, vectors	Direct contact, fecal matter, infected water/mud, equipment, vectors	Direct contact, fecal matter, infected water/mud, equipment, vectors	Direct contact, fecal matter, infected water/mud, equipment, vectors
Optimal temperature	18°C–27°C	5°C–18°C	15°C–25°C in koi 6°C–10°C in common carp	<20°C	25°C–28°C

Disease	Cyprinid herpesvirus 3 disease	Spring viremia of Carp	Koi sleepy disease	Carp pox	Hemorrhagic disease of grass carp
External observation	Gill necrosis, sunken eyes, notched nose, secondary bacterial and parasitic infections	Exophthalmia, pinpoint skin hemorrhage, abdominal distention, mucus from vent	Extensive erosions or hemorrhages of the skin, skin sores around the mouth and at the base of fins, anus inflammation, enophthalmos, enlarged and necrotic gills	Smooth raised wart-like skin lesions	Exophthalmia, dark body color, hemorrhages at the base of fins, gill covers and mouth cavity, high mortalities at 25°C–28°C
Internal observation	A few variable signs	Edema, inflammation, pinpoint hemorrhages of many organs including swim bladder	None	None	Hemorrhages throughout the musculature, enteritis (red intestine), hemorrhagic gills or pale gills, internal hemorrhages in organs such as liver, spleen, kidney and intestine
Behavior observation	Lethargy, swimming close to the surface, respiratory distress, erratic behavior	Lethargy, swimming low on tank or pond bottom, awkward swimming	Unresponsiveness and lethargy, sluggish behavior, fish laying on the bottom of ponds	None	None
Diagnostic methods	Clinical signs, behavior changes Histological observation Direct methods (virus isolation and PCR) (most sensitive) Indirect methods (Enzyme-linked immunosorbent assay (ELISA)) Immunofluorescence				
Treatment	None	None	None	None	None
Vaccination	Inactivated vaccine developed in Japan, DNA vaccine, live attenuated virus, recombinant vaccines	Killed or attenuated virus, DNA vaccines containing a glycoprotein of virus	Information not found	Information not found	Tissue-culture based vaccine used in China since 1970
Prevention/ Control	Vaccination Depopulate infected stocks Practice good biosecurity including quarantine Purchase fish from known reputable source Restrictions on movements and transportation of fish Keep susceptible species separated Treat water with chlorine prior to draining to eliminate pathogens				

Table 13. Comparison of common viral diseases in carp: general information.

4.2. Current status of viruses in carp worldwide

The first reported outbreak of KSD occurred in Japan in the 1970s (Murakami et al. 1976), and a second outbreak was documented in the US about two decades later (Hedrick et al. 1997). Recently, outbreaks have occurred in different countries, including India (Swaminathan et al. 2016), China (Zhang et al. 2017), Hungary (Adamek et al. 2018), Poland (Matras et al. 2017), the US (Padhi et al. 2019), Korea (Kim et al. 2018), Iraq (Toffan et al. 2020), the Czech Republic, Slovakia (Matějíčková et al. 2020) and Croatia (Zrnčić et al. 2020). In Japan, the first outbreak of KHVD occurred in October 2003 in Kasumigaura Lake (Sano et al. 2004). Subsequently, a number of outbreaks were documented in almost every region of Japan, perhaps related to the spread of infected carp from Kasumigaura Lake (Kimiya 2004).

Water temperature is a significant factor in the outbreak of viral diseases, so the occurrence varies between seasons and geographical regions. For example, KSD in juvenile koi occurred at temperatures of 15°C–25°C (Murakami 1976), so the disease normally appeared in summer in European countries or during the rainy season in tropical countries. In Germany, KSD occurred in koi populations in early summer at water temperatures of 18°C–22°C (Adamek et al. 2021), and a KSD outbreak in Thailand happened during the rainy season (July–October) when the temperature was between 24°C and 25 °C (Pikulkaew et al. 2020). During winter or early spring, at low water temperatures of 6°C–9°C, the disease appeared among wild common carp in the UK (Rehman et al. 2020).

The rapid and widespread spread of viral diseases is likely a result of the global trade in ornamental and aquarium fish, shortcomings in diagnostic methods and a lack of legislation to control and prevent the incursion of viruses (Zhang et al. 2017). According to Mikolaj Adamek et al. (2021), the intense trading of common carp and koi without necessary risk mitigating measures causes viruses to spread. The phylogenetic diversity of virus variants found in Hungarian carp shows that KSD has evolved and spread over a long period of time in European carp populations (Adamek et al. 2018). Effective methods for preventing viral infection in carp culture are urgently needed to minimize chances for outbreaks, such as avoiding the introduction of susceptible fish into farming ponds when the water temperature becomes suitable for virus outbreaks and using virus-free water and disinfecting water and tools when outbreaks occur on farms. Moreover, disease diagnostics should be included in health surveillance and disease monitoring programs to prevent further spread of the disease. In most of the studies (Table 14), endpoint PCRs, nested PCRs and real-time PCR assays have been developed and validated for detection owing to their high sensitivity. The disease can also be controlled by a vaccine, which can be administered by injection, immersion or orally.

Country	Disease	Analyzed target	Detection technology	Highlight	Reference
Asia					
Japan	CyHV-3 disease	Water in the Tamagawa River	TaqMan PCR	KHV-DNA were found in the water of the Tamagawa River several months before KHVD outbreaks in 2004.	Haramoto et al. 2007
	CyHV-3 disease	Gills and brains of wild common carp in Lake Biwa	Detection of virus with PCR Measurement of serum anti-CyHV-3 antibodies using ELISA	The authors investigated the distribution of CyHV-3 in a population of wild common carp in Lake Biwa 2 years after the initial outbreak of CyHV-3 disease in 2006: • 31% of fish larger than 300 mm tested positive (6% for fish <300mm) • 54% of fish larger than 300 mm contained anti-antibodies (0% for fish <300 mm) • wild common carp that survived after infection became carriers.	Uchii et al. 2009
Iran	CyHV-3 disease	Common carp from two local farms in Tehran and Alborz provinces	Clinical characterization, histopathological examination, PCR	CyHV-3 can be transmitted from imported ornamental koi to farmed cyprinids in Iran.	Ahmadivand et al. 2020
Thailand	KSD	Dead juvenile koi in a koi pond at San Kamphaeng, Chiang Mai	Histopathological examination, PCR and DNA sequencing	CEV was confirmed in imported ornamental koi. There was co-infection by opportunistic pathogens including *Dactylogyrus* sp., *Gyrodactylus* sp. and *Saprolegnia* sp. on the skin and gills.	Pikulkaew et al. 2020
China	KSD	12 moribund fish from the area of Chengdu experiencing an infectious disease in December 2016	Nested PCR, sequencing and phylogenetic analysis	The pathogen involved in the outbreak was CEV. CEV should be monitored in the cold season and at low temperatures.	Ouyang et al. 2020

Country	Disease	Analyzed target	Detection technology	Highlight	Reference
Europe					
Croatia	CyHV-3 disease KSD	30 common carp and the crossbreed *C. carpio* × *Carassius auratus* from Croatian carp farms (including samples from disease outbreaks) from 2015 to 2019	Clinical characterization, histopathological examination, real-time and conventional PCR for detecting and sequencing KHV, nested PCR for detecting CEV	During 2016, there was an outbreak of koi herpesvirus disease in an isolated area, after which a KHV eradication program was successfully performed. During 2018 and 2019, an outbreak of CEV occurred by the introduction of carps from an infected farm to one of the lakes.	Zrnčić et al. 2020
Germany	KSD	Gill samples of healthy and diseased carp and koi from 2007 to 2016 all over Germany	End-point PCR and sequencing and phylogeny	CEV was found in archived carp samples dating back to 2007, as well as koi samples dating back to 2009. From 2015 to 2016, CEV was found in 69% of cases in carp populations and 41% of koi populations from all over Germany. Clinical KSD occurred in carp populations at water temperatures of 8°C–12°C and in koi populations at water temperatures of 18°C–22°C.	Adamek et al. 2021
Slovakia and the Czech Republic	KSD	Gill, spleen and kidney of diseased fish from five outbreak cases in the Czech Republic between 2013 and 2014 and in Slovakia in 2019	Real-time PCR and sequencing and phylogeny	CEV detection was only obtained from samples of gill tissue. CEV was detected from four cases belonging to genogroup I and one case belonging to genogroup II.	Matějíčková et al. 2020
Hungary	KSD	Moribund carp from 17 fish farms and angling ponds in different regions of Hungary in winter 2016 and early spring 2017	Histopathological examination, endpoint PCR, sequencing and phylogeny	CEV DNA was detected by qPCR in 13 of 17 of these carp. In seven fish, there were more than 1 × 104 copies of virus-specific DNA sequences per 250 ng of DNA. Genogroups I, IIa, IIb of CEV were present, with genogroup I being most abundant.	Adamek et al. 2018
Italy	KSD	Common carp from an outbreak at Camazzole Lake in June 2020	Real-time PCR for detection and nested PCR for confirmative analysis, histopathological examination, sequencing and phylogeny	There were severe cutaneous hyperemia and increased mucus production on the skin and gills of fish. Gills, kidney and brains from all the fish analyzed tested positive for CEV.	Marsella et al. 2021

Country	Disease	Analyzed target	Detection technology	Highlight	Reference
North America					
US	CyHV-3 disease	67 koi tissues samples that were submitted for diagnosis during KHVD outbreaks from 10 different states in the US from 1999 to 2019	Endpoint PCR and sequencing	The study identified the genetic diversity of the different isolates: 31 isolates belonged to the Asian genotype branch and 36 to the European genotype branch. From 1999 to 2013, the main KHVD genotype was European. The Asian KHVD genotype emerged in the US in 2008, increasing in incidence until 2019.	Shahin et al. 2020
	Koi sleepy disease	526 dead common carp from an outbreak at Mill Pond and Woodcliff Lake Reservoir in 2017	Histology and transmission electron microscopy Endpoint PCR, sequencing, and phylogenetic analysis	The presence of CEV was confirmed by endpoint PCR, qPCR and transmission electron microscopy. The CEV genogroup I strain was detected, making this the first report of this genogroup outside of Europe. CEV is not detectable in gills after abatement of clinical signs.	Lovy et al. 2018
Mexico	SVCV	10 wild common carp from Tecocomulco, a natural freshwater lagoon in the state of Hidalgo (no disease outbreaks and/ or disease-related mortalities)	Macroscopic, histological and microbiological analyses, electron microscopy, RT-PCR, sequencing and phylogenetic analyses	Although no epidemic or fish mortalities were reported, the collected samples exhibited clinical signs, histological results and RT-PCR results related to SVCV.	Ortega et al. 2019

Table 14. Current status of viruses in carp worldwide.

5. Factors affecting microbiomes

5.1. Substrate

Several unique strategies have been developed to increase the efficiency of nutrient conversion in aquaculture systems. One of them is based on the formation of microbial biofilms that adhere to a substratum inside the culture unit and is referred to as the periphyton (Azim and Little 2006). Several studies have shown that fish production in ponds with periphyton substrates (particles with a large surface to volume ratio where the microorganisms can form a biofilm on) is greater than in ponds without them (Keshavanath et al. 2001; Azim et al. 2002a; Azim et al. 2002b; Shafi et al. 2021). Periphyton increases the overall nutrient efficiency of a fishpond. Periphytic algae also helps enhance water quality by their higher rates of nitrification and ammonia absorption. Heterotrophs present in biofilm can get organic nutrients from the dead or uneaten periphyton cells that stay attached to the substrates. Algae can reuse the inorganic nutrients that are produced as a result of the decomposition of organic nutrients (Azim et al. 2004).

In a polyculture system of three IMCs, even though catla do not feed on periphyton, their net yields were three times larger in treatments with periphyton than in the control treatment. This is because catla has less competition for planktonic food since rohu mostly eats periphyton, which leaves planktonic food for catla to feed on (Azim et al. 2002b). Polyculture of rohu and catla was superior to monoculture of either species in periphyton-based aquaculture systems (Azim et al. 2001). The periphyton system is a flexible package that can be adapted to the requirements, capabilities and resources of the farmers (Azim et al. 2004).

5.2. Fertilization

Using fertilization to stimulate the trophic chain components from the bottom up is a cost-effective and ecologically friendly approach to enhance fish production (Woynarovich et al. 2010). In a carp polyculture system, precise application of inorganic and organic fertilizers can increase fish production. Inorganic fertilizers can supply additional carbon sources (Cole 1999) as well as boost the availability of mineral nutrients in the rearing environment (Jana et al. 2001). Dissolved organic carbon is an essential source of nutrients for bacteria (Kosolapov et al. 2017). Mineral nutrients, on the other hand, are the growth limiting factors for bacteria (Matz and Jürgens 2003). Fresh manure (a type of organic fertilizer) can provide fishponds with some of the necessary critical mineral nutrients and carbon for heterotrophic bacteria growth. The biochemical oxygen demand, total nitrogen and phosphorus content, and freshness of manure are important for feeding the fish, and they act as a source of nutrients for phytoplankton production, including autotrophic and heterotrophic microorganisms (Wohlfarth and Schroeder 1979). The increased bacterial biomass produces carbon dioxide through respiration, which increases the amount of dissolved inorganic carbon available to phytoplankton (Green 2015).

Numerous types of manure have been used in carp polyculture, in which cattle, poultry and semi-liquid pig manure are of the highest interest (Wohlfarth and Schroeder 1979). Chicken manure can enhances the growth of numerous zooplankton species, outperforming cow and pig manure (Kang'ombe et al. 2006). Moreover, bacterial loads (total bacterial count, total coliform count, *Escherichia coli* and *Salmonella*) in fishponds receiving fermented chicken manure were much lower than those in ponds receiving raw chicken manure. Fermented chicken manure was mainly used to convert waste to energy and was more bacteriologically safe than using fresh chicken manure (Elsaidy et al. 2015). Jha et al. (2008) compared four management regimens in ornamental fishponds: poultry manure, live zooplankton, cow dung and a commercial pellet diet. They discovered that the average numbers of heterotrophic bacteria in pond water fertilized with poultry manure and cow dung were substantially greater than in other treatments. In addition, manure, together with the bacteria that grow on it, is a great microscopic food source for zooplankton because of its high protein content (Woynarovich et al. 2010).

The composition of the livestock manure microbiome varied depending on the producing species and had a significant impact on the water body and carp gut microbiome. Fertilization with livestock manure has a prebiotic impact on carp polyculture systems by providing organic matter, macronutrients and micronutrients to the pond microorganisms (Minich et al. 2018). However, the presence of pathogens in manure is considered one of the most important factors in the transmission of disease. Pathogens in manure have been reported to survive for up to 4 months in aquatic environments, depending on the type of manure, temperature, pH, oxygen level, ammonia concentration and the presence of competing organisms (Guan and Holley 2003).

In the case of inorganic fertilizer, excessive nutrient loading can result in a decrease in gross primary productivity as a result of algae shading the pond surface. On the contrary, nutrients in organic manure would be released more gradually and consistently. The more regulated the nutrition release rate, the more efficiently phytoplankton consume nutrients and the greater the fish output. Furthermore, the excreta of grass carp can be used to fertilize the water and produce plankton for filter-feeding fish to consume (Kumar et al. 2005). Therefore, the use of a combination of grass carp and organic fertilizer resulted in the highest net fish yield, followed by the use of only organic fertilizers and then inorganic fertilizers (Kumar et al. 2005).

5.3. Feeding

In carp polyculture, rohu, catla, common carp and grass carp are fed directly. Silver and bighead carps are not fed. Instead, they filter small floating particles from carp feeds. Grass carp are fed with fresh green terrestrial plants. Supplementary feeds such as grains, by-products and compounded farm-made or commercial feeds are used to complement the natural food. With the shift toward intensive fish culture, the use of balanced feed ingredients of plant and animal origin, like pelletized feeds, becomes necessary. The amount of feed should be proportional to the stocking concentration of carp. The relative daily amount of feed should not exceed 1%–3% of the biomass of growing one-summer-old carp and 3%–5% of the biomass of older groups of carp. This will cause fish to seek natural food (Woynarovich et al. 2011).

In semi-intensive systems, supplemental feeding benefits the fish directly as feed and indirectly as fertilizer (Milstein 1992). When fish are fed only cereals, the proportion of nutritional supply driving the bacterial–detrital food chain can reach over 95%, with just 5% being used directly for fish growth (Olah 1986). Natural foods can still contribute up to 40%–68% of the output in ponds when supplemental feeding is used (Cam et al. 1991; Burford et al. 2002 and 2004; Porchas-Cornejo et al. 2012).

Energy for natural food production can be boosted by increasing the Carbon: Nitrogen ratio (C:N) of nutrient inputs into the pond. Energy is often provided by administering carbohydrates in dry feed to increase the C:N ratio to 15–20 (Crab et al. 2007; Asaduzzaman et al. 2008; Avnimelech and Kochba 2009). When the C:N ratio of nutrient input exceeds 10, significant quantities of bacterial biomass are found in the food web, in which heterotrophic bacteria become dominant (Lancelot and Billen 1985; Boyd 1990). Organic and inorganic nitrogen are taken up by heterotrophic bacteria, which keeps ammonia and nitrite levels in the pond low (Avnimelech 1999; Hari et al. 2004 and 2006). Heterotrophic bacteria serve as a protein source, promoting nutrient flow through the food web and the production of fish that graze on natural foods (Asaduzzaman et al. 2008).

However, the increase in stocking density and dry feed use is believed to reduce the relative contribution of natural food to fish production (Kabir et al. 2019). Moreover, adding large amounts of dry feed has an negative effect on water quality, particularly when the feed is unbalanced or of low nutritional value (Poxton and Lloyd 1898; Poxton and Allouse 1987). According to Schlott et al. (2011), the intensity of supplemental feeding in semi-intensive carp ponds should be adjusted based on quantitative and qualitative measures of zooplankton growth, which is ultimately regulated by fish predation.

Although cereal feed is protein-poor and is high in carbohydrate, zooplankton is rich in protein and low in carbohydrate (Ruttkay 1975). The major zooplankton groups found in earthen carp ponds are protozoans, rotifers and two crustacean groups, copepods and cladocerans (Anton-Pardo and Adámek 2015). As a live feed, zooplankton is a critical nutrition and protein source for many

aquaculture species and plays an important role as a natural food for juveniles, adults and marketable-sized fish. However, live feed consumed by fish can contain diverse bacteria, often including pathogens like *Flavobacterium* (Skjermo and Vadstein 1993). Therefore, live feed ingestion is one of the ways fish can become infected with disease (Snoussi et al. 2006). Until now, there have been limited studies on the effects of dry feed and live feed on the microbiome of carp polyculture systems. In our opinion, more studies need to be done to answer the following questions: How do dry feed and live feed shift the microbiome in water environments and in the guts of carp? How does the introduction of dry feed affect live feed production in the pond?

5.4. Water quality

Water quality parameters, such as temperature, pH, oxygen, carbon dioxide, nitrogen compounds, organic materials, inorganic pollutants and pesticides, should be evaluated either on a regular basis or when there are problems. The following paragraphs discuss the importance of water quality parameters and seasonal changes on microbiomes in the water body.

5.4.1. Dissolved oxygen (DO)

DO levels are mainly determined by the relative magnitudes of photosynthetic oxygen production and total plankton respiration (Steel 1980). Phytoplankton is the primary supplier of DO in fishponds, as well as the primary sink for oxygen, both directly as consumers and indirectly as the source of detritus upon which the majority of bacterial respiration depends (Boyd 1973; Dupree and Huner 1984). If algal biomass could be maintained at intermediate levels, DO levels would improve considerably. Net primary production is poor in dense algal growth, because phytoplankton are limited by nutrients or light by their density (Javornicky 1980; Laws and Malecha 1981).

Fishponds with low levels of DO can promote the growth of waterborne microorganisms by encouraging nutrient regeneration from anoxic sediments (Testa and Kemp 2012). However, low DO inhibits nitrification and the coupled nitrification–denitrification process (Kemp et al. 1990), lowering inorganic nitrogen (N_2) removal and increasing NH_4 buildup. Nitrifier abundance was highest during the rainy season, followed by winter, and was lowest during the summer, when oxygen levels are low (Kumari et al. 2011). In other words, during the summer heterotrophic bacteria have more competition for oxygen with other microorganisms (zooplankton, protozoa, etc.) than they do during the rainy and winter seasons (Donderski and Kalwasinska 2003).

5.4.2. pH

In general, the ideal pH range for pond fish cultivation is between 6.5 and 8.5. Any value outside this range will reduce fish productivity and can result in death. Acidic water (pH <5) leaches metals from rocks and sediments, resulting in decreased fish population development. Autotrophic photosynthetic activity may cause high pH, which can kill fish, especially in the late spring when NH_4 concentrations are high. The strong inorganic carbon absorption by phytoplankton results in a high pH (Pokorny et al. 1994), and high pH levels in fishponds during summer might be a reason for the low abundance of nitrifiers (Kumari et al. 2011).

5.4.3. Temperature

Several papers have shown that temperature has a significant impact on the composition of the microbial community of carp ponds (Tas et al. 2009; Tian et al. 2009; Hu et al. 2022). As certain microalgae blooms, such as cyanobacterial bloom, are associated with high temperatures, there is a positive association between temperature and chlorophyl a (Bouvy et al. 1999; Havens 2008). In addition, the abundance of species in the core microbiome of carp pond sediments was highly correlated with water temperature, which adversely affected the efficiency of sulfur cycling in sediment-based microbial communities (Zhao et al. 2020).

Several studies have shown that seasonal fluctuations and temperature variations influence fish-gut microbial composition (Nayak 2010; Ringø et al. 2016). Seasonal shifts in the skin microbiome were more significant, whereas the gut microbiota were more stable regardless of seasonal effects (Tarnecki et al. 2017). Changes in the overall bacterial abundance of gut microbiota have been reported between the summer and fall seasons

(Al-Harbi and Uddin 2004). During the summer months, the nitrification rate in the pond drops because of an increase in heterotrophic bacteria respiration in soil and water (Kumari et al. 2011). This could be the explanation for a decrease in the nitrifying bacteria population in sediment and water throughout the summer season (Gundersen and Mountain 1973).

5.4.4. Nitrogen compounds

In general, nitrogenous organic matter decomposes mostly near the water-mud interface. The NH_4^+-N formed during organic nitrogen decomposition adheres to the mud's surface before being released into the water, where it repeatedly rises to the surface and escapes into the air (Mei et al. 1995). Ammonifying and denitrifying bacteria are found mostly in pond mud, but nitrobacteria and nitrifying bacteria are only found in the superficial mud.

An increase in supplementary feed and unconsumed feed can increase the concentration of ammonia in a pond. Ammonia is expected to be critical in determining the structure of the ammonia-oxidizer community (Koops et al. 2003). Carlucci and Strickland (1968) discovered that when the ammonia concentration rose, the activity of the ammonia-oxidizing bacteria increased. During the summer, the nitrification potential rate and number of nitrifiers were lowest in the top and bottom layers of the fishpond because heterotrophic bacteria and autotrophic algae are more successful at competing for ammonia than nitrifiers. During the rainy and winter seasons, nitrifying bacteria might be able to consume ammonia even at low concentrations because of decreased competition (Yoshifumi et al. 2009).

5.5. Sanitizers

Stresses, including heating, freezing, drying, radiation, starvation, and chemicals such as acids, sanitizers and preservatives can cause sub-lethal injury to bacteria (Ray 1979; Wesche et al. 2009). Quick lime is often used to disinfect ponds in carp polyculture systems (Mukesh and Brahmane 2019). In grass carp–prawn polyculture ponds, a mixture of potassium monopersulfate, malic acid, sodium chloride and coptis extract are used to kill main pathogenic microbes (Liu et al. 2015). Zaske et al. (1980) discovered that when *E. coli* was exposed to oligotrophic natural water and reagent grade water, the cell membrane was damaged. This damage can cause further structural failure by causing lesions or eroding critical envelope components.

In addition, sanitizers such as chlorine, fungicides and other chemicals can harm indicator and pathogenic coliforms (Izumi et al. 2016). Formalin protects fish and fish eggs against bacterial, fungal, parasitic and viral infections (Nelson Herwig et al. 1979; Piper et al. 1982; Meyer and Schnick 1989; Hakimoglu 2001), while iodine is most efficient against bacteria and viruses (Alderman 1984; Snow and Wright 1985) as well as fungi (Schreck 1991). There is limited study on the effect of sanitizers on the microbial community in fish culture, so more research is required to explain how sanitizers can impact the microbiome in both carp polyculture ponds and in fish guts.

5.6. Microbial products

In aquaculture, interest in the application of microbial products to improve the survival, growth and feed use of stocked animals, as well as increase water quality, has been growing (Gatesoupe 1999; Farzanfar 2006; Qi et al. 2009). Table 15 summarizes studies on the effects of microbial products on carp microbiomes. Probiotics have been associated with the benefits of reduced intestinal pathogenic microbiology (Abid et al. 2013) by colonizing the gastrointestinal mucosal epithelium in the digestive tracts of several fish species (Merrifield et al. 2010; Sharifuzzaman and Austin 2010; Nakandakare et al. 2013) and can prevent the activity of prospective pathogens by producing inhibitory molecules and/or directly competing for space, nutrients and oxygen (Chen et al. 2010; Addo et al. 2017; Nandi et al. 2017a and 2017b). To our knowledge, however, little research has been conducted on the impact of supplemental microbial products on the microbiome community in freshwater polyculture systems.

The addition of microbial products might not have a significant impact on water quality and the microbial community of polyculture systems, because fish with various feeding behaviors can increase the stability of the system to prevent bacterial colonization (Zhou et al. 2017). One study has shown that adding probiotics to carp polyculture systems had no significant impact on

the bacterial community in water (Zhou et al. 2017). Recent interest has increased in the production of live microalgae with probiotics because interactions between species can increase the value of the end product. Several studies have demonstrated that introducing algae, together with probiotics, can impact the microbiota and increase gut health and total production in fish, shrimp and mussel aquaculture (Perković et al. 2022). Therefore, more research is needed to develop a probiotic, including both a bacterial and an algal species that can enhance microbiomes in carp polyculture systems.

There are two main factors that determine the influence of microbial products on water quality: (1) the extent of exogenous bacterial colonization and (2) their relative dominance in the existing bacteria in the pond under cultivation (Zhou et al. 2017). The availability of ecological niches also plays an important role in the adaptation of exotic microorganisms to their environment (Kassen and Rainey 2004). The evolution of microbial communities can be impacted by genetic diversity, population dynamics and habitat partition (Dutilleul 1993) and by a nutrient gradient, which can be formed at small spatial scales owing to the lack of turbulence (Fenchel 2002). As such, it is important to understand how these factors influence the impacts of microbial products on microbiomes in carp polyculture.

Tang et al. (2016) examined the effects of three commercial microbial products (Novozymes pond plus, Zhongshui BIO-AQUA, and Effective Microorganisms) on production performance and water quality in a polyculture system of grass carp, gibel carp and silver carp with low nutrient loading reveals. The findings revealed that the addition of the three commercial microbial products did not substantially enhance production or change water quality. Therefore, long-term experiments should be conducted to investigate the function of microbial products in freshwater polyculture systems with different nutrient loadings and species compositions.

Besides probiotics, prebiotics are another type of dietary supplement that could help with growth, digestive enzyme activity, immune response, stress resistance and improved water quality (Dawood and Koshio 2015). Common prebiotics used in carp culture are Mannanoligosaccharide, β-Glucan, Xylooligosaccharide, Inulin, Chitosan, etc. (Dawood and Koshio 2015). Prebiotics are non-digestible materials for fish that can be metabolized by gut microbiota (Ringø et al. 2014). Dietary prebiotic supplementation can change or alter gut morphology and enhance commensal microbiota growth in both diversity and count (Kühlwein et al. 2013; Hoseinifar et al. 2016a; Jung-Schroers et al. 2016a). However, to our knowledge, no study has been done on the impact of prebiotics on carp polyculture systems.

5.7. Climate change

Global and local environmental changes influence the structure and dynamics of ecosystems through the networks of species that interact with each other in a community. Increased temperatures result in a decrease in the biomass of benthic and pelagic producers, with a similar impact on the biomass of zooplankton, zoobenthos and pelagic bacteria, as well as producing an increase in the number of pelagic viruses (Shurin et al. 2012).

Climate warming has led to a strong increase in the occurrence of toxic cyanobacteria blooms in lakes, ponds and reservoirs worldwide (Macke et al. 2015). Temperature, pH and DO were shown to be strongly linked with changes in microbial community composition, and they are also claimed to have an impact on bacterial development and contribute to ecosystem processes (Guan et al. 2020).

Li et al. (2021) demonstrated that temperature is the major environmental factor shaping the dominant microbial genera in the water column, including *Prochlorococcus, Chryseobacterium, Acinetobacter, Rheinheimera, Polynucleobacter* and *Janthinobacterium*. Jana et al. (2019) discovered that a 5°C increase in water temperature during winter caused a 36% increase in the yield of fish in polyculture systems (rohu, mrigal, bata, Japanese punti, grass carp, common carp, magur and freshwater prawn) through microbial-driven augmented manure mineralization, resulting in increased primary productivity, zooplankton abundance and ecological integrity. Furthermore, Chopyk et al. (2020) observed that a storm event enhanced total bacterial diversity, the relative abundance of Bacteroidetes and the amount of antibiotic resistance genes in a mid-Atlantic agricultural pond in the US. As such, significant work will need to be undertaken to better understand the impact of climate change on the microbiomes of carp polyculture systems.

Species	Microbial products	Effects	Ref.
Common carp	*Paenibacillus polymyxa, Lactobacillus fermentum, ferulic acid, Lactobacillus, Saccharomyces cerevisiae, Bacillus amyloliquefaciens*	Improved fish survival after *A. hydrophila* challenge	Harikrishnan et al. 2010; Huang et al. 2015; Gupta et al. 2016; Ahmadifar et al. 2019
	β-1,3/1,6-glucan	Higher amount of bacterial OTUs in carp gut Decreased abundance of *S. putrefaciens* and *Vibrio* sp. in gut	Jung-Schroers et al. 2016b
	Short chain fructo-oligosaccharide	No effect on total viable counts of heterotrophic aerobic bacteria in gut Increased lactic acid bacteria	Hoseinifar et al. 2016b
	Fructo-oligosaccharide	Increased abundance of total heterotrophic bacterial population and lactic acid bacteria	Hoseinifar et al. 2014
Grass carp	*Bacillus amyloliquefaciens* BaX030	Increased abundance of intestinal probiotics (*Fusobacterium, Proteobacteria, Gemmobacter*) Decreased abundance of potential pathogenic bacteria (*Planctomycetes, Aeromonas*)	Zhou et al. 2022
	Bacillus subtilis Ch9	Increased abundance of total aerobic and facultative anaerobic bacteria Increased abundance of *Bifidobacterium* and *Lactobacillus*	Wu et al. 2012
	Streptomyces amritsarensis N1-32	Improved fish survival after *Aeromonas veronii* challenge	Li et al. 2020
Javanese carp	*Enterococcus faecalis*	Improved fish survival after *A. hydrophila* challenge	Allameh et al. 2017
	Enterococcus faecalis, Lactobacillus fermentum and *Leuconostoc mesenteroides*	Increased abundance of lactic acid bacteria in gut Decreased abundance of Gram-negative bacteria in gut	Allameh et al. 2016
Catla	*Bacillus amyloliquefaciens*	Inhibited *A. hydrophila, Edwardsiella tarda, Vibrio harveyi* and *V. parahaemolyticus*	Das et al. 2013
Rohu	Lactic acid bacteria	Increased survival of challenged fish with *A. hydrophila*	Maji et al. 2017
	B. subtilis, Lactococcus lactis, S. cerevisiae	Increased abundance of total heterotrophic bacterial population	Mohapatra et al. 2012
Crucian carp	Lactic acid bacteria	Increased abundance of *Firmicutes* and *Proteobacteria* in gut Decreased abundance of *Actinobacteria* in gut	Liu et al. 2022
Koi carp	*Lactobacillus plantarum* C20015	Increased survival of challenged fish with *A. veronii*	Zhang et al. 2020
Gibel carp	Chitosan	Suppressed pathogen bacteria *A. veronii* Improved *Cellulomonas hominis, Bacillus oceanisediminis* and two uncultured bacterium species	Chen et al. 2014

Table 15. Summary of studies investigating the effects of microbial products on carp microbiomes.

6. Conclusion and suggestions

Studies on bacterial and microalgae communities in carp polyculture systems have shown a link between the host microbiome and the rearing environment. This illustrates the importance of steering this rearing water microbiome to reduce the emergence of diseases and improve carp health.

Overall, the bacterial microbiome associated with water is often more diverse than the microbiome associated with fish. In carp polyculture systems, the dominant microbial taxa are formed by the composition of the microbial community in the rearing environment and by selecting different carp species in establishing the internal and external microbiome.

At the organ level, microbiome niche differentiation has been recorded. Moreover, many species in carp polyculture systems, each with their own eating habits, can control the size and composition of microalgae in the pond. The variation in microalgal populations might be caused by variations in mixing conditions, carp species composition, nutrition availability, etc. The farming of carp polyculture might be hampered by the formation of enormous microalgal blooms owing to water quality problems and the enrichment of nutrients caused by the microbial decomposition of unused feed and fish metabolic wastes.

The differences in the microbiome between studies with the same fish species could be a result of the animal life stages, sample size, pond diversity and several technical differences, such as genome region and sequencing platform (Foysal et al. 2019a). It also depends on diets, fish populations, rearing conditions and geographical environments (Tran et al. 2018). A significant problem with the available data on the carp microbiome is the wide variety of findings from various laboratories, using different experimental designs and methodologies. Additionally, there is already strong evidence suggesting that factors such as substrate, feeds, fertilizers, sanitizers, microbial products and the manipulation of environmental parameters can strongly influence carp microbiomes. We have identified three areas that show promise to impact carp polyculture management:

1. **Technological improvements**: A significant problem with the available carp microbiome data is the wide variety of findings from different laboratories, using different experimental designs and methodologies. It is important to note that most of the studies have relied on results from older, less-accurate methodologies, such as DGGE and agar plating, or more recently semi-quantitative NGS. In recent years, combining data from these technologies with quantitative methods has yielded surprisingly different insights into the compositional dynamics occurring in microbial ecosystems (Props et al. 2016). In the next few years, using new tools, such as single-cell technologies, to undertake detailed high-resolution sampling campaigns will produce a greater understanding of critical processes and interactions between important taxa in these environments.

2. **Microbiome health baselines**: Getting insights into the microbiomes of broodstock, eggs, larvae and water in both hatchery and nursery systems will be extremely useful. This knowledge is needed to shape the microbiome of the rearing water in a system and to understand its influence on the host (fish) microbiome. For example, fish egg microbiota shape larval microbiota (Olafsen 2001) and can influence subsequent larval performance (Vadstein et al. 2013). The fish egg microbiome can be influenced by the holding tank, genetics (family effects) and egg disinfection procedures. Clearly, a better understanding of the factors influencing the egg microbiota of fish could contribute information relevant to improving management and help identify the role of the microbiota in determining egg quality.

3. **Product management**: Little research has been done on the impact of supplemental microbial products on the microbiome community in carp polyculture systems.

In these systems, fish with diverse feeding methods can increase the stability of the system to prevent bacterial colonization (Zhou et al. 2017). As such, research is required to investigate the effectiveness of microbial products on improving the microbiomes. Although there could on occasion be a limited role for antibiotics (under veterinary advice), their effect on the general physiology of the microbiome, through the selection of a specific collection of clones and genes (Martínez 2017), needs to be better understood. Studies to determine how antibiotics alter the microbiomes of culture systems will be useful.

Rearing G3 rohu broodfish.

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Appendix. Current farming practices

Bangladesh

The traditional polyculture of indigenous IMCs in Bangladesh has become more diverse over the past several decades because of the introduction of exotic carps, tilapia and catfish. More than 60% of the country's freshwater aquaculture production capacity originates from polyculture systems that combine IMCs such as rohu, catla and mrigal as well as other carps like silver, grass, common and bighead (DOF 2018).

Carp hatchery

Prior to 1990, the majority of carp-seed production came from harvesting natural spawn from rivers. Since then, 963 hatcheries have been established in Bangladesh, with 97.3% of production coming from private hatcheries, 2.31% from public sector hatcheries and 0.39% from the wild (WorldFish 2021). To induce artificial spawning, the pituitary gland was used in all fish species except silver and bighead carp, which were treated with human chorionic gonadotropin (HCG) (Abdul Halim et al. 2018). Carp species used in hatcheries in Bangladesh are presented in Table 16.

According to Khatun et al. (2017), the most intense hatchery and nursery operations in Bangladesh run between June and December, though they sometimes extend into February. Rearing carp fry and fingerlings in nurseries is commonly divided into three stages: (1) rearing fry early (2) rearing fry as normal and (3) rearing fingerlings. The majority of hatcheries and nurseries have their own broodstock and nursery ponds. The hatcheries obtain broodstock mostly from other farmers' ponds (41.3%), their own ponds (16.3%), the wild (16.3%), traders (11.5%), government and private hatcheries, and other sources. Nowadays, farmers prefer larger fingerlings for stocking for several reasons (WorldFish 2021):

- They can use seasonal water bodies for culture.
- They can produce large foodfish in a culture period that lasts less than a year.
- Larger fingerlings have a lower mortality rate.
- They can use a large number of ponds for nursing.
- Nursery production is more profitable than grow-out production, with a quick return on investment.
- Nurseries are a year-round business.

Major groups	Common name	Scientific name
Indian major carps	Rohu carp	*Labeo rohita*
	Catla carp	*Catla catla*
	Mrigal carp	*Cirrhinus cirrhosus*
	Kalibaush	*Labeo calbasu*
Exotic carps	Silver carp	*Hypophthalmichthys molitrix*
	Grass carp	*Ctenoparyngodon idella*
	Common carp	*Cyprinus carpio var. commonis*
	Mirror carp	*Cyprinus carpio var. specularis*
	Bighead carp	*Aristicthhys nobolis*
	Black carp	*Mylopharyngodon piceus*

Source: Abdul Halim et al. 2018.

Table 16. Carp species used in hatcheries for seed production in Bangladesh.

Khatun et al. (2017) recently highlighted major problems with carp hatcheries in Bangladesh:

- Some hatcheries do not have broodstock ponds.
- They focus on seed quantity rather than quality.
- There is a lack of knowledge about the genetic status of hatchery stocks.
- There is a lack of knowledge about the effects of open water stocking on wild stocks.
- Broodstock are replaced with leftover fish.
- Inbreeding, negative selection and hybrid introgression degrade the genetic quality of seeds.
- A limited number of parent stocks leads to genetic drift.
- The genetics of domesticated stocks have been degraded.

To produce high-quality seeds, farmers must know about the pedigree of broodstock, use parents from two distinct sources, treat fish properly to avoid inbreeding and reject fish with poor health or anatomical abnormalities (Abdul Halim et al. 2018).

Carp grow-out

Aquaculture production systems in Bangladesh are mostly improved extensive and semi-intensive systems, usually by smallholder farmers. In intensive systems, fish are stocked at a low density of 2500–3000/ha, with a culture period of 8–10 months (WorldFish 2021). Because the majority of farmers are poor and live in rural areas, they are unable to adopt intensive fish production, which requires a greater supply of inputs. As a result, semi-intensive culture techniques that require moderate inputs, stocking rates and adequate manure, with or without supplementary feeding, are suitable for them (Roy 2016).

In recent years, the shift from extensive to semi-intensive or intensive systems has resulted in an extraordinary increase in fish output, as well as an increase in demand for fish feeds, fingerlings and fertilizers (Hossain et al. 2022). Manufactured feed is also gradually replacing farm-made aquafeed because it allows fish to grow faster (Ahmed 2007; Sarker et al. 2016).

In Bangladesh, fish also can be cultured in seasonal culture waterbodies, such as floodplains, rice fields or pits, or in shrimp/prawn farms in polyculture with carp during the wet months when water salinity decreases (WorldFish 2021). Additionally, inorganic fertilizer is widely used in carp polyculture systems, though the optimal dose for good natural feed production has yet to be determined. Many farmers also lack the financial resources to invest in inorganic fertilizer. This is one reason why the production of carp per unit area in Bangladesh is lower than that of neighboring countries (Sayeed et al. 2007). On top of this, small farms are more productive than larger farms, but the cost per unit production is high because of their considerable input use. To maximize productivity, small farmers devote more time caring for their ponds than large farmers, who often adopt a hands-off approach (Hossain et al. 2022).

A study conducted in Sylhet District in Bangladesh found that most small farms use monoculture, while large farms practice both monoculture and polyculture. Pond preparation includes the use of lime, followed by other traditional chemicals such as potash, fertilizers and sulfites. Others pond inputs can include local feeds such as rice bran, meat bone, wheat bran, mustard oil cake with vitamin premix, or commercial feed.

The possibility of disease decreases with increased farm size. Several factors can cause disease outbreaks: poor management by inexperienced farmers, inadequate inputs, poor understanding of fish health management and a lack of water quality. Numerous farms do not monitor water quality even when it deteriorates. This is most likely a result of a lack of knowledge, facilities and equipment for determining water quality parameters. Additionally, farmers get little support from either fish health specialists or diagnostic laboratories, and there is a shortage of preventative measures and treatments for diseases reported. Disease is also related to the season, with outbreaks in winter (27.78%), probably linked to the low water level of ponds and related poor water quality, followed by the summer (21.3%) and rainy (14.81%) seasons (Deb et al. 2021).

Fishery health and aquaculture management training has expanded, but at a lower rate than the rapid intensification of aquaculture (Abdul Halim et al. 2018). Farmers and farm managers in Sylhet District reported that they received little support from fish extension services, fish health specialists, governmental organizations and nongovernmental organizations (NGOs). Approximately 64% of farmers who engaged in raising animals have received training in aquaculture management from governmental organizations and NGOs at least once. However, only 30.5% of farm managers who engaged in managing the many aspects of making a farm run smoothly reported having received such training experience (Deb et al. 2021).

Many development projects have helped promote improved small-scale carp polyculture in Bangladesh. For example, WorldFish developed a 3-year training and extension program called the Development of Sustainable Aquaculture Project. This initiative had a substantial impact on increasing production productivity, profitability and efficiency. Farmers gained a better understanding of the recommended types and mixes of inputs to use. In the long term, technical suggestions should be revised to maximize output per unit input (Murshed-E-Jahan et al. 2008).

WorldFish also established an aquatic animal disease detection laboratory in Bangladesh that uses RT-PCR technology to support initial outbreaks and mitigate the losses at small-scale farms. The laboratory offers its testing services to hatchery operators and fish farmers at minimum cost (WorldFish 2021).

Another project, the Danish-funded Mymensingh Aquaculture Extension Project (MAEP), had a great impact on improving the well-being of poor farming families through carp polyculture. The MAEP trained almost 100,000 families, and the average annual extrapolated fish output increased from 1 to 3.3 t/ha over the duration of the project. Poor farming families sold around 80% of their fish harvests and consumed about 20%, providing economic advantages as well as family nutrition (Edwards 2007).

Mohsin et al. (2012) highlighted the problems that now exist in Bangladesh's carp culture. He noted that the majority of farmers do not get technical support from relevant government groups or NGOs and must solve their difficulties through discussion with neighboring farms. Other issues include a lack of quality fish seeds, impure fertilizers that result in poor production and the inability to obtain desired fertilizers, high prices for supplementary feeds, lack of sufficient credit, water quality issues, fish diseases, fish poaching, market price fluctuations and a labor shortage. In addition, multiple ownership of water bodies and the high lease value of a pond are significant constraints.

Disease management practices

In Bangladesh, small-scale farms had a higher frequency of fish diseases (13.8%) than medium- (12.4%) and large-scale farms (9.3%). This was related to an increased stocking density of small fry, inexperienced fish farmers, insufficient inputs and a lack of knowledge on fish health management (Faruk et al. 2004).

There are just a few diagnostic laboratories or support services in the country, and they are all located in universities or government research centers that are far away from rural fish farms. Field surveys are the most practical way to collect disease data from a large region, with a large number of farmers, but many farmers do not understand disease symptoms and consequently do not often report outbreaks. Farmers used the following therapies to treat diseases: lime and potash, liming, lime and salt, salt and potash, antibiotics, different chemicals, high dosages of vitamin C, complete water exchange and transportation to another pond (Aftabuddin et al. 2016).

India

Aquaculture production in India has been increasing steadily over the years, with hatcheries and grow-out production being the two main types of activities. The country's carp polyculture system is based on combinations of three to six species, including the three IMCs (catla, rohu and mrigal) as well as combinations of the three IMCs and three exotic carps (silver, grass and common carp) (Jayasankar 2018). Other species such as giant freshwater prawn (*Macrobrachium rosenbergii*) and native catfish (*Clarias*

batrachus) are also becoming popular. The state of West Bengal is the largest producer and consumer of carp, accounting for over 32% of total production, followed by Andhra Pradesh (15.6%), Uttar Pradesh (8.6%), Maharashtra (more than 8%) and others (Kumar 2011).

Carp hatcheries

Farmers in India have acknowledged the economic viability of rearing seed, which includes raising spawn to fry in a nursery and then growing them to fingerlings in rearing ponds (Laxmappa 2015). More than 90% of seeds are produced through induced spawning in hatcheries. Farmers use high-quality brooders to produce superior progeny throughout the breeding season, which is done early in the monsoon season to optimize the amount of time available for raised fry to develop at a high density. Fingerlings are reared at a lower density in a healthy culture environment to enhance fast growth and a high survival rate (Sankalp Se Sidhi 2017).

The Indian Council of Agricultural Research (ICAR)-All India Coordinated Research Project on plasticulture engineering and technology (PET) is based at the ICAR-Central Institute of Freshwater Aquaculture in Bhubaneswar. The project designed and developed a complete set of hatchery systems made of fiberglass reinforced plastic (FRP) for fish breeding and hatchery rearing of seeds (Mohapatra et al. 2016). Approximately 1–1.2 million carp can be produced in a single run using FRP tanks, which is enough to produce fingerlings for stocking in grow-out ponds with a stocking density of 5000 fingerlings/ha (Mohapatra et al. 2017). Because of the minimal investment required and the ease of operation, even small farmers have been able to develop and effectively manage such hatchery units in various regions of India. Additionally, since these units are smaller, they are well-suited for initiating seed production programs for a variety of small and medium-sized carps (Mohapatra et al. 2017).

Apart from domestic use, fish seed is exported to neighboring countries such as Bhutan, Nepal and Bangladesh, not only by states with a surplus of fish seed, but also those with a deficit, taking advantage of seasonal availability and the accessibility of seed production centers to sell seed to other states or regions (Sankalp Se Sidhi 2017).

Carp grow-out

Carp polyculture procedures include pond preparation, liming, fertilization, stocking management, supplemental feeding, water quality control and health management. Farmers in Andhra Pradesh have developed numerous innovative carp culture techniques, which have significantly increased carp yield:

- Fingerlings are stunted in the first year and then used in grow-out the following year.
- Stunted fingerlings are stocked at a higher initial weight.
- Farm-made rice bran combined with various kinds of oil cakes and mineral mixes is fed to livestock on a regular basis at the farm.
- Ponds are fertilized using a combination of organic manures and inorganic fertilizers.
- Proper tank/pond management is followed, from stocking to harvest.
- Carp spawn during the monsoon season, and fingerlings are usually ready for stocking when the winter season begins.
- Overwintered fingerlings are cultivated with maintenance feeding at concentrations of over 10 animals per square meter.

When they are refilled in grow-out ponds at reduced densities the next year, the carp show remarkable compensatory growth and reach market size within a year (Shivananda Murthy 2002; Laxmappa 2015).

Singh et al. (2009) assessed the level of technical efficiency and its determinants for small-scale fish production in West Tripura District of the state of Tripura. They reported that 98% of these fish farmers practiced carp polyculture. Rohu was the most dominant fish species, constituting more than 25% of the total fish production, followed by mrigal (23%), common carp (18%), catla (17%) and silver carp (16%)

(Singh 2006). However, not all farmers culture the same combination of species. In addition, seed quality is important to the success of production, and the majority of farmers rely on middlemen to get fingerlings of a mix of carp species, but the quality of these fish is uncertain (Singh 2008). Although government hatcheries offered fingerlings with a greater variety of species and higher quality, farmers had a limited or insufficient supply from them.

Over 65% of farming households relied on lime, cattle dung, rice bran and oil cake as inputs. While the majority of farmers cultivated fish for personal use, others sold their product to supplement their financial requirements. Furthermore, the more experience a farmer had, the greater their productivity and technical efficiency (Revilla-Molina et al. 2008). According to Temu (1999), farmers' ages were directly or indirectly related to their farming practices through labor, management and knowledge. Young and middle-aged farmers were more likely to accept new technologies, while older farmers were more cautious and risk averse and so were less willing to experiment with new technologies (Temu 1999).

To improve the production of carp polyculture, WorldFish conducted a carp intensification program (2016–2022) in Odisha State and got remarkable results. Fish farmers in Odisha have been able to double their production from 2.5 to 5 t/ha per year. The program focused on promoting aquaculture output using advancements in seed, technology and farming practices. Farmers began stocking "zero size" fingerlings weighing 100–200 g instead of the smaller fingerlings they were used to. They implemented better management practices, such as liming their ponds regularly, assessing water quality parameters, conducting a plankton density analysis and monitoring their fish for disease. Some farmers were given commercial feed as part of a government farm input subsidy (WorldFish 2019).

However, there are still a lot of difficulties in carp polyculture that farmers need to overcome. Vignesh et al. (2017) listed many significant constraints in freshwater aquaculture in India: poor seed quality, short water supply, high cost of supplementary feed, high cost of electricity, lack of skilled labor, disease outbreaks, poaching, high mortality during culture periods, low farmgate prices, problem of direct selling to buyers, low productivity, low net returns and inadequate availability of quality seeds. Climatic change and financing facilities are two other key limitations to freshwater aquaculture development in India (de Silva and Soto 2009; Roy 2015).

Pakistan

In Pakistan, research was conducted at 778 carp farms in three provinces: 400 in Punjab, 220 in Sindh and 158 in North-West Frontier Province. The research showed that semi-intensive/intensive systems had stocking rates of 0.47 fish/m^2, whereas extensive systems had rates of 0.44 fish/m^2. In most semi-intensive and intensive farms, the pond water was enriched with chemical and organic fertilizers, and the water level was regularly monitored. To boost water productivity, more than 50% of extensive farms used organic manure. Supplementary feed was used in 95% of semi-intensive/intensive farms and only 38% of extensive farms. Overall, semi-intensive/intensive farms are technically more efficient compared to extensive farms (Sharma 1999).

In Shorkot (District Jhang), Pakistan, semi-intensive carp polyculture relies mostly on fertilization and supplemental feeding to increase the production of the fishpond. Inorganic fertilizers include urea, diammonium phosphate and nitrophos (a fertilizer containing equal amounts of phosphorus and nitrogen), while organic fertilizers include cattle dung, chicken waste and goat droppings. Even within the same region, the types and amounts of fertilizer used varies. While fishmeal is a high-quality component of fish feed, it is too expensive for the majority of fish farmers. Therefore, farmers use low-protein inputs like polished rice, gluten and dry bread pieces coupled with salt-tolerant plants like kallar grass, barseem and sorghum to lower the production cost of their feeds. These farming practices result in significant differences in the growth parameters of different species at different sites (Chughtai and Mahmood 2012).

Vietnam

The carp polyculture system in Vietnam is a combination of the macro-herbivorous grass carp with three to five secondary species. Grass carp make up 50%–60% of fish biomass in the pond; the rest is made up of other species, including the omnivorous common carp, mud carp (*Cirrhinus cirrhosis*), mrigal and Nile tilapia, as well as filter feeders like silver carp and bighead carp (Steinbronn 2009).

The culture systems include using ponds and cages, with a stocking density of 1–2.5 fish/m^2. Productivity of pond polyculture is 6–20 t/ha.

There are usually three combinations of stocked species that are cultured (Vo Van Binh 2021):

1. rohu and mrigal (65%–70%), silver carp (5%–10%), grass carp (5%), black carp (5%) and common carp (5%)
2. grass carp (35%), rohu (30%), common carp (20%), silver carp (15%), black carp (5%)
3. tilapia (65%), common carp (20%), silver carp (15%).

In broodstock management, tags should be used for identification. Proper stocking density of broodstock is about 1 fish/2 m^2 with a sex ratio of 1:1. The duration of broodstock maturation is usually about 4 months. They are fed a high protein and lipid diet, with supplementation of probiotics to support digestion and minimize water pollution. Fish reproduction is induced artificially by using hormone (pituitary grand, Luteinizing hormone-releasing hormone (LHRH), gonadotropin-releasing hormone (GnRH), Human Chorionic Gonadotropin (HCG), Ovaprim), after which the fish are squeezed to retrieve eggs and sperm. Alternatively, reproduction is done semi-artificially when, after a second hormone injection, fish are released back into the tank spawn naturally. There are two methods used to incubate eggs: (1) the Chinese method, which requires large amounts of water, mass production and is suitable for floating eggs, and (2) the Russian method, which is suitable for sticky and floating eggs and is usually used for common carp. During larvae and fingerling culture, green water is important to maintain proper water quality. Proper formulated feed at different fish stages, together with green water can reduce the FCR and disease outbreaks.

However, fish production is low because of poor feed quality and a scarcity of natural food resources (Thai et al. 2007; Steinbronn 2009), mass mortality of grass carp from species-specific diseases (Van et al. 2002; Steinbronn 2009), water scarcity, high labor requirements, poor water quality and pesticide pollution (Steinbronn 2009). Research in Yen Chau District demonstrated that farmers have historically used organic fertilizer, such as buffalo manure, in carp polyculture ponds. However, this manure is a direct feed source for the fish rather than a way of maintaining natural food in the pond. In addition, farmers have a limited understanding of the biology and nutritional requirements of the cultivated fish species. Natural food resources like phytoplankton, zooplankton and zoobenthos, and their relevance for each specific fish species, are poorly understood. As such, farmers were not keen on using chemical fertilizer for their pond as a way of providing food for fish (Pucher et al. 2013).

Other Asian countries

Japan has historically cultivated koi carp, which are descendants of the common carp. There are more than 20 different varieties of koi, each with a different color, pattern and type of scale. Successful koi rearing requires establishing an artificial habitat that closely resembles the natural environment. A variety of parameters affect growth rates in koi, including water temperature, water quality, stocking density and genetic background. Koi eat most actively at temperatures over 15°C, so sexually immature fish will develop quickly during the summer months when the temperature is higher (Nica et al. 2019). Koi farms are located in many areas of Japan: 48% of the farms are found in Niigata Prefecture, followed by Gifu (8%) and Hiroshima (6.5%) (Ikuta and Yamaguchi 2005), but there is limited information in the country about best management practices of koi farming.

Sharma and Leung (2000) examined differences in technical efficiencies of semi-intensive/intensive and extensive carp pond culture systems among the major carp producing countries in South Asia, specifically Nepal, India, Bangladesh and Pakistan. They collected data about the output and input variables involved in

the stochastic meta-production frontier for carp pond culture in the region as well as the production frontiers for individual countries. The technical efficiency was then calculated by measuring all output and input variables per hectare. The results showed that carp cultivation in India is the most efficient among semi-intensive and intensive farms. Carp farming in Nepal is the least effective, while Bangladesh and Pakistan are in the middle. Among extensive systems, farms in Pakistan and Nepal tended to be more technically efficient than farms in the other two countries. In addition, common, silver and grass carp were the most dominant species in Nepal, whereas rohu, mrigal and catla were more dominant in India, Bangladesh and Pakistan. The stocking density varied from about 0.5 fish/m^2 in Pakistan to 1.7 fish/m^2 in Bangladesh.

Europe (Hungary)

The majority of EU pond aquaculture is polyculture of warm-water species, particularly carps. Cyprinid species such as common, silver and bighead carp, as well as other coarse fish (for angling), account for about 80% of fishpond production. Annually, the EU produces about 70,000 t of common carp. In 2018, its top three producers were Czech Republic, Poland and Hungary, accounting for 71% of common carp production. Herbivorous carp production is estimated at 5000 t, with grass carp accounting for 40%, bighead carp 30% and silver carp 30% (European Commission 2021). Throughout Central Europe, numerous fish farms specialize in raising and rearing carp. For example, in Germany over 100 farms with a total of 133 carp strains were reported in 2007 (Hartman et al. 2015). In Europe, the duration of fish production ranges between 2 and 4 years, depending on the final size of fish that customers demand (0.3–3 kg). A 3-year production cycle maximizes the use of pond resources in Central Eastern Europe, so it is the most widely practiced option (Woynarovich et al. 2011).

The traditional Hungarian carp farming system consists of three sequential culture periods, which run from April to October. After harvesting in autumn, fishstocks cultured over one production season and over two production seasons are put in overwintering ponds and restocked for continued growth the following spring, while fish cultured over three production seasons are sold for human consumption. Although the majority of farms operate throughout all three culture periods, some farms focus only on the first two seasons and sell fish cultured over three production seasons to other farms.

Gross yields per unit area do not differ significantly between different rearing stages (Gyalog et al. 2017). In Europe, most farmers practice extensive polyculture system because it is the cheapest way to produce carp. The natural food that develops in ponds is essential to fish growth. Although farmers use manure and fertilizer, they are still far from optimal in terms of using physical, financial and human resources. Even though fish farmers could generate profit from fish culture, many of them need to enhance their management practices to achieve better, safer and more profitable outcomes (Woynarovich et al. 2011).

According to Cornia (1985), small farms normally have higher yields than larger ones because they use more intensive production technologies and more land. Gyalog et al. (2017) analyzed the pre-harvest technology of Hungarian carp pond aquaculture. They indicated that, in comparison to large farms, numerous small and medium-sized farms optimize their production technologies by adopting a more extensive or intensive style of management. Although the stocking rate of common carp is similar across smaller and bigger farms, the weight increase ratio of biomass on small farms is almost twice that of larger farms. In addition, because of the variability of farmers' production technology, pond farming operations cannot be easily standardized and repeated across several production units. There is no technical driver for farm concentration, since small farms can be managed more intensively than medium- and large-scale farms. In Hungary, the main limitation in aquaculture is a lack of a skilled workforce capable of maintaining water quality and controlling feeding efficiency (European Commission 2015).

Country	Common carp species	Technological characteristics	Problems
Bangladesh	IMCs, silver carp, grass carp, common carp, bighead carp	• The culture period is 8–10 months. • Carp are mostly cultured in improved extensive and semi-intensive systems. • Species are stocked at a very low density of 2500–3000/ha • There are 963 private hatcheries and 103 government hatcheries. • Farmers prefer to produce larger fingerlings for stocking. • Culture systems include grow-out in seasonal cultured waterbodies (floodplains, rice fields, borrow pits), shrimp/prawn farms (45% shrimp/prawn, 55% fish) and culturing fingerlings in rice fields. • Small farms use monoculture while large farms practice both monoculture and polyculture. • Small-scale farms had a higher frequency of fish diseases (13.8%) than medium- (12.4%) and large-scale (9.3%) farms. • Small-scale farmers devote more time to pond care in order to maximize productivity than large farmers, who often adopt a hands-off approach.	• There is a lack of knowledge, facilities and equipment for determining water quality parameters. • There is a shortage of preventative measures and treatments for diseases. • There is little technical support from relevant government groups or NGOs. • There is a lack of quality fish seeds, and fertilizers are impure. • Problems include high prices for supplementary feeds, lack of sufficient credit, water quality issues, fish diseases, fish poaching, market price fluctuations and a labor shortage. • Only a few diagnostic laboratories or support services exist, and these are located far away from rural fish farms. • Some hatcheries do not have broodstock ponds. • The primary goal is to produce more seed rather than quality seed. • There is a lack of knowledge about the genetic status of hatchery stocks. • There is a lack of knowledge about the effects of open-water stocking on wild stocks. • Broodstock are replaced with leftover fish. • Inbreeding, negative selection and hybrid introgression have degraded the genetic quality of seeds. • A limited number of parent stocks has resulted in genetic drift. • The genetic quality of domesticated stocks is low.
India	IMCs, silver carp, grass carp, common carp, or other non-carp species such as giant freshwater prawns, native catfish	Innovative carp culture techniques have been developed: • Fingerlings are stunted in the first year and used in grow-out the following year. • Stunted fingerlings are stocked with a higher initial weight. • Farm-made rice bran combined with various kinds of oil cakes and mineral mixes is fed to the livestock on a regular basis at the farm. • Farmers use a combination of organic manures and inorganic fertilizers. • Proper tank/pond management is followed, from stocking to harvest. • Carp spawn during the monsoon season, and fingerlings are usually ready for stocking when the winter season begins. • Overwintering fingerlings are cultivated with maintenance feeding at concentrations of over 10 /m^2.	• Seed quality is poor. • Water is in short supply. • The cost of supplementary feed is high. • The cost of electricity is high. • There is a lack of skilled labor. • Disease outbreaks occur. • Fish are poached. • Culture periods have high mortality rates. • Farmgate prices are low. • Selling directly to buyers is a problem. • Productivity is low. • Net returns are low. • The availability of quality seeds is inadequate. • Climate change is a problem, and financing facilities are lacking.

Country	Common carp species	Technological characteristics	Problems
Pakistan	IMCs, silver carp, grass carp, common carp, bighead carp	• Semi-intensive/intensive farms are technically more efficient than extensive farms.	• Fishmeal is a high-quality fish feed that is too expensive. • Farmers often used low-protein diets, including rice polish, choker, gluten and dry bread pieces coupled with salt-tolerant plants like kallar grass, barseem and sorghum • The genetic quality is low.
Vietnam	Common carp, mud carp, mrigal, silver carp, bighead carp, Nile tilapia	• Broodstock density is about 1 fish per 2 m^2. • The sex ratio is 1:1. • Broodstock are fed a high protein and lipid diet, with supplementation of probiotics. • Fish reproduction is induced artificially by using hormones. • Green water is very important to maintain proper water quality. • Proper formulated feed at different fish stages together with green water can lower the FCR and disease outbreaks.	• Feed quality is poor. • There is a scarcity of natural food resources . • Species-specific diseases occur. • Water is scarce. • The labor requirements are high. • Water quality is poor. • Pesticides pollute the water.
Europe	Common carp, silver carp, bighead carp, silver carp, grass carp	• The carp farming system consists of three sequential culture periods, which run from April to October. • Broodstock density is about 10–20 carp brooders/ha. • Fish reproduction is induced artificially by using hormone. • Carp propagation takes place once a year, during late spring (around May). The period lasts about 4–6 weeks. • In nursery ponds, zooplankton is the main source of essential protein in the diet of developing fry. • Carp grow-out production can be extensive, semi-intensive or intensive. • Small farms use more intensive production technologies than large farms. • With similar stocking, the weight increase ratio of biomass on small farms is almost twice that of larger farms.	• Because of the variability of farmers' production technology, pond farming operations cannot be easily standardized and repeated across several production units. • There is a lack of a skilled workforce capable of maintaining water quality and controlling feeding efficiency.

Table 17. Current farming practices of carp polyculture in Asian and European countries.

About WorldFish

WorldFish is an international, not-for-profit research organization that works to reduce hunger and poverty by improving aquatic food systems, including fisheries and aquaculture. It collaborates with numerous international, regional and national partners to deliver transformational impacts to millions of people who depend on fish for food, nutrition and income in the developing world.

The WorldFish headquarters is in Penang, Malaysia, with regional offices across Africa, Asia and the Pacific. The organization is a member of CGIAR, the world's largest research partnership for a food secure future dedicated to reducing poverty, enhancing food and nutrition security and improving natural resources.

For more information, please visit www.worldfishcenter.org