



Review

Broodstock and seed production in biofloc technology (BFT): An updated review focused on fish and penaeid shrimp

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ABSTRACT

Among aquaculture rearing systems, Biofloc Technology (BFT) has great potential for ecological intensification as it produces natural food sources for selected aquatic species by cycling nutrients in a balanced food chain and through complex biopathways. In this system, nitrogenous wastes in the culture media are converted into edible microbial flocs, reducing feed conversion ratios (FCRs), and costs by up to 30% in grow-out conditions. In hatcheries, BFT has an integral role in providing high-quality live foods that are essential for successful reproductive performance and further larviculture. In addition, genetic (fish and shrimp) improvement programs in a closed-life cycle and biosecure conditions is becoming a priority. BFT can maintain biosecurity in a zero or limited water exchange system and ensure stabilized water quality parameters in indoor facilities. Moreover, BFT can provide a wide range of in-situ nutrients, such as fatty acids, vitamins, and phospholipids, that promote broodstock gonadogenesis and gametogenesis to mass-produce high-quality offspring. Additionally, BFT microbial-rich community act as a natural probiotics and contains immunostimulants, such as peptidoglycan derived from bacterial cell walls, which can help against negligent antibiotics utilization. In this review, the quality and reproductive performance of biofloc-based broodstock of selected aquaculture species are comprehensively discussed, as well as the further impact on egg and larval quality.

1. Introduction

As the global population continues to grow, the demand for food is increasing rapidly. In this context, aquaculture can play a critical role in meeting this demand and ensuring food security for millions of people worldwide. If properly managed, aquaculture can provide a sustainable way to produce aquatic protein to meet the growing demand for nutrient-rich food (Sharifinia et al., 2023). However, system selection is key to achieve this goal. Biofloc technology (BFT) has been successfully applied to aquaculture in recent years (Emerenciano et al., 2017; Khanjani and Alizadeh, 2023). In this system, animal wastes, mainly nitrogenous metabolites, along with unfed feed and mucus, are converted into microbial biomass that not only preserves water quality but

can also be consumed as food by cultured animals (Hargreaves, 2013; Crab et al., 2012; Avnimelech, 2015; Debbarma et al., 2022; Mi et al., 2023; Khanjani et al., 2023b). Mainly in indoor, but also in outdoor conditions, in different salinities and water depths, BFT has been operated in tanks or lined ponds, with strong aeration and water movement (Lim et al., 2021). The suspended microbial aggregates recycle and reduce toxic wastes, such as ammonia, nitrite, and nitrate, which enhances feed efficiency and decreases feed costs (Khanjani et al., 2022d; Li et al., 2023). BFT also has beneficial effects on the physiological function of farmed aquatic animals by boosting their immunocompetence (Ekasari et al., 2014; Cardona et al., 2016; Khanjani et al., 2021b; Xuan et al., 2022) and promoting reproductive performance (Emerenciano et al., 2012b; Braga et al., 2015, 2018; Cardona et al., 2016;

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Magaña-Gallegos et al., 2021; Khanjani, 2022). Tilapia and shrimp reproduction have been positively affected by BFT, as biofloc contains various essential nutrients (Emerenciano et al., 2015; Ekasari et al., 2015a). In fact, biofloc contains essential amino and fatty acids, antioxidants, and vitamins that can fortify reproduction in brooders (Ju et al., 2008; Kuhn et al., 2009; Colombo et al., 2023). Furthermore, some microbial-derived components in biofloc, such as glucan 1,3, lipopolysaccharides, and peptidoglycans, can act as natural immunostimulants and improve animal health (Ekasari et al., 2014; Kim et al., 2015; Kumar et al., 2015; Cardona et al., 2016; Zhang et al., 2017; Khanjani et al., 2022b; Azimi et al., 2022; Gullian-Klanian et al., 2023).

BFT has been successfully applied to a variety of shellfish and fish species during nursery and grow-out phases. For example, during nursery phase of various shrimp species such as Indian white prawn (*Penaeus indicus*, Anand et al., 2023), Pacific white shrimp (*Litopenaeus vannamei*, Samochoa et al., 2007; Irani et al., 2023), *Farfantepenaeus* sp. (Emerenciano et al., 2011), Banana shrimp (*Fenneropenaeus merguensis*, Khanjani and Sharifinia, 2022; Khanjani et al., 2022a), and Freshwater prawn (*Macrobrachium rosenbergii*, Pérez-Velasco et al., 2023) or fish species such as Nile tilapia (*Oreochromis niloticus*, Ekasari et al., 2015a, 2015b; García-Ríos et al., 2019; Vicente et al., 2020; Khanjani et al., 2021a; Gullian-Klanian et al., 2023), African catfish (*Clarias gariepinus*, Popoola and Miracle, 2022), and Common carp (*Cyprinus carpio*, Minabi et al., 2020) were successfully cultured. In grow-out phase, shrimp species such as Black tiger shrimp (*Penaeus monodon*, Arnold et al., 2009), *L. vannamei* (Samochoa et al., 2007; Huang et al., 2022) were cultured and fish species such as *O. niloticus* (Manduca et al., 2020), *Oreochromis* sp. (Ekasari et al., 2023), and *Huso huso* (Aghabarari et al., 2021) were successfully tested in this system. However, the potential of BFT for broodstock production and mass production of high quality seeds in farmed aquatic species are less well understood. Furthermore, the knowledge regarding selected cultured aquatic species to produce broodstock under BFT condition is still limited, especially compared to nursery and grow-out phases. Therefore, this review discuss key topics related to BFT tailored to reproduction, including biosecurity, biofloc consumption, broodstock production, egg and larval quality, and the use of biofloc as a nutritional source to enhance the reproductive performance.

2. Ensuring biosecurity in BFT-based broodstock production

Biosecurity is a top priority in aquaculture, and pathogen surveillance is critical to prevent disease outbreaks, especially in super-intensive systems where stress and economic risks are heightened (Khanjani et al., 2022c; Emerenciano et al., 2022). In recent decades, shrimp farms have been greatly affected by disease outbreaks worldwide, mainly through the penetration of pathogens via infected post-larvae and water (Wasielky Jr et al., 2006; Da Silveira et al., 2022). As a result, farmers were forced to develop and adopt more biosecure practices to minimize the risks associated with pathogen exposure (Browdy et al., 2001). Thus, closed-life cycle broodstock production has become a priority in the shrimp industry to ensure biosecurity and prevent vertical transmission, e.g., viruses and other key pathogens. Breeding programs in closed facilities are often performed to optimize production through successive generations and improve relevant traits such as growth, disease tolerance, and reproductive performance (Preston et al., 1999; Regunathan, 2008). Currently, traditional ways to produce 'biosecure' shrimp/fish broodstock include remote inland areas, remote islands and coastal zones, using flow-through, recirculation aquaculture system (RAS), BFT and/or hybrid techniques (e.g. integrated multitrophic aquaculture system, Khanjani et al., 2022e, BioRAS, Zimmermann et al., 2023). In this sense, BFT is considered a strategic tool for broodstock production as it can recycle in situ nutrients and organic matter while minimizing water consumption and discharge in a closed loop, providing better biosecurity conditions and reducing the prevalence of contagious pathogen outbreaks (Pimentel et al., 2023).

Other advantages of BFT such as 'natural probiotic effect' and production of microbial-derived components acting as natural immunostimulants has been comprehensively described by Aguilera-Rivera et al., 2014; Ferreira et al., 2015; Khanjani et al., 2023a, and will be further discussed below.

The literature has demonstrated the advantages of closed BFT systems for broodstock production (including specific pathogen-free or SPF) and pre-maturation phases (Emerenciano et al., 2012b, 2014; Braga et al., 2015; Magana-Gallegos et al., 2018a, 2018c; Magaña-Gallegos et al., 2021; Tahoun, 2022). It is also possible to produce breeders in small areas near or within hatchery facilities to minimize risks of diseases spread caused by transportation. For instance, in penaeid shrimp and some fish species, breeders traditionally are cultured in large ponds at low density in conventional water exchange systems. In outdoor facilities; however, there remains a high risk associated with the accumulation of organic matter, the blooming of pathogenic bacteria (e.g. cyanobacteria), and fluctuations in water physicochemical factors (e.g., temperature, dissolved oxygen, pH, and NH₃-N and NO₂-N), which could adversely affect animal health (Panigrahi et al., 2019; Pimentel et al., 2023).

In Table 1 reported range of water quality factors in reared brooders in BFT system compare to other rearing systems. When performed in indoor facilities such as greenhouses, BFT can stabilize culture condition, especially water quality parameters to ensure environmental consistency (Xu et al., 2016; Oliveira et al., 2022). Additionally, the presence of heterotrophic microorganisms and related extra-cellular compounds with natural probiotic characteristics can restrict the propagation of pathogens, for instance, pathogenic bacteria through the quorum quenching process, or trigger immune responses in breeders (Emerenciano et al., 2022; Khanjani et al., 2023c; He et al., 2023). A review by Kumar et al. (2021) highlighted that dominant microbiota in BFT systems are mainly represented by *Lactobacillus*, *Bacillus*, and *Vibrio*, along with other bacterial groups that may have health-promoting effects on hosts and water bioremediation. Also, the microorganisms in BFT can maintain microbial balance in the host gut by inhibiting the propagation of pathogenic bacteria and promoting the colonization of beneficial bacteria, which can provide various health-promoting effects in broodstock (Kumar et al., 2021). Moreover, BFT can reduce microbial pathogenesis by downregulating virulent gene expression, disrupting quorum sensing processes in pathogen colonies, and producing antimicrobial compounds (e.g., bacteriocins, siderophores, lysozymes, proteases, etc.) that inhibit their growth (Kumar et al., 2021). Thus, BFT can simultaneously provide biosecurity and improve the health status of broodstock, which could be used for long-term genomic selection to increase specific trait gains in breeding programs for shrimp/fish.

3. Bioflocs, complementary food source for broodstock

Proper nutrition is essential for the successful reproductive performance of broodstock in aquaculture. Inadequate nutrition can negatively affect gametogenesis, fertilization, hatching rates, and spawning activity (Harrison, 1990; Wouters et al., 2001; Goodall et al., 2016; Hernandez de-Dios et al., 2022). Broodstock diets generally fall into three categories: (i) pre-spawning diets, which aid the development of sexual organs and enhance nutritional reserves for final maturation; (ii) spawning diets, which provide essential nutrients and energy for the production of nutrient-rich eggs; and (iii) post-spawning diets, which help broodstock recover energy and nutrients and promote immunocompetence and survivability (Izquierdo et al., 2001; Hernandez de-Dios et al., 2022). Therefore, it is crucial to provide broodstock with nutritionally balanced diets to ensure the high quality and quantity of seeds in aquaculture operations (Browdy, 1998; Coman et al., 2007, 2013; Arnold et al., 2013; Emerenciano et al., 2013b; Ekasari et al., 2016; Nascimento et al., 2023). For more than two decades, the application of BFT to shrimp broodstock has been started in Tahiti (Emerenciano et al., 2012a). They found that reproductive performances of *L. stylirostris* in

Table 1
The range of water quality factors in reared brooders in BFT system compare to other rearing systems.

Rearing system	Cultivated species	IW (g)	RP (days)	T (°C)	pH	DO mg/L	TAN mg/L	NO ₂ mg/L	NO ₃ mg/L	TSS mg/L	Highlights	Reference
BFT	<i>L. stylirostris</i>	40.6	70	24.70	7.78	6.80	0.00	0.01		97.10	Optimum water quality factors for <i>L. stylirostris</i> brooders.	Cardona et al., 2016
CW				25.54	8.20	7.09	0.00	0.00		3.20		
BFT	<i>C. gariepinus</i>	657	122	25.4–29.6	5.72–7.94	2.76–7.66	0.01–0.59	0.07–0.42	0.07–0.22	188–1250	Optimum water quality factors for <i>C. gariepinus</i> brooders.	Ekasari et al., 2016
CW				25.3–29.2	4.52–8.22	3.74–8.30	0.01–0.53	0.03–0.49	0.20–0.38	160–1150		
BFT	<i>F. duorarum</i>	7.4	210	25.8	8.3	6.2	0.4	0.2	12.7		Optimum water quality factors for most penaeid species.	Emerenciano et al., 2013c
CW				26.2	8.4	6.1	0.3	0.3	7.3			
BFT	<i>F. brasiliensis</i>	0.025	30	23.7	6.5	5.9	0.4	0.9			BFT had higher levels of nitrogenous metabolites.	Emerenciano et al., 2012c
CW				24.3	8.3	7.1	0.1	0.5				
BFT	<i>O. niloticus</i>	163.7	21	29.08	6.42	6.14	0.26		0.27	549.5	Despite significant differences, water quality values in both systems were within the optimum range for tilapia reproduction.	Alvarenga et al., 2017
CW				29.29	7.19	6.44	0.44	1.19	77.5			
BFT + FF	<i>L. vannamei</i>	36.40	30	29.79	7.57	6.48	0.15	0.04	30.46		High levels of NO ₃ -N in BFT treatments were related to the zero-exchange water system, which led to the accumulation of inorganic compounds through evaporation and culture processes.	Braga et al., 2015
BFT + BF				29.49	7.65	6.48	0.12	0.06	28.04			
BFT + JF				29.59	7.71	6.59	0.13	0.06	28.18			
CW				29.50	7.76	6.56	0.11	0.05	1.36			
BFT, maturation phase	<i>L. vannamei</i>	35.0	40	28.1	7.9	6.18	0.6	0.5	11.0		The water quality parameters for <i>L. vannamei</i> were all within acceptable ranges.	Emerenciano et al., 2013b
BFT + FF, maturation phase				36.2	27.8	7.8	6.14	0.6	0.5	11.5		
Non-BFT	<i>F. indicus</i>	37.35	300	27.8–28.8	7.93–8.35	4.94–5.70					All water quality parameters were in acceptable range for <i>F. indicus</i> .	Nur et al., 2022
BFT		38.05	330	27.2–29.1	7.83–8.16	4.64–5.85	0.00–4.34	0.03–2.92	0.02–2.82			
CW without probiotic	Red tilapia	127.5 female, 148 male	60	27.00	7.93	5.12	0.32	0.50	33.67	132.67	Despite significant differences, water quality values were within the optimum range for tilapia reproduction in both systems.	Tahoun, 2022
CW + probiotic				27.00	7.93	5.03	0.27	0.45	31.00	130.67		
BFT without probiotic				26.93	7.87	5.00	0.24	0.35	28.00	267.33		
BFT + probiotic	<i>L. setiferus</i>		30	26.90	7.67	5.02	0.19	0.29	26.00	306.00	The water quality parameters for <i>L. setiferus</i> rearing were all within acceptable ranges.	Barral-Pintos and Gaxiola, 2022
BFT				27.66–27.58	7.96–8.0	4.54–4.62	0.11–0.14		0.11–0.19	18.8–23.8		
CW				27.29	7.92	4.58						

Abbreviations: Initial weight = IW, Rearing period = RP, Temperature = T, Dissolved oxygen = DO, Total suspended solids = TSS, Total ammonia nitrogen = TAN, Nitrite = NO₂, Nitrate = NO₃, Fresh food = FF, Broodstock feed = BF, Juvenile feed = JF.

BFT was notably enhanced compared to the breeders reared in earthen ponds. Nutrients such as “native protein” (Emerenciano et al., 2012a), lipid (Wasielesky Jr et al., 2006), amino acids (Ju et al., 2008) and fatty acids (Izquierdo et al., 2006; Ekasari et al., 2010) in a form of microbial aggregates (or ‘bioflocs’) are available 24/7 as in situ nutrients. In this regard, biofloc is a multicomplex of organic and inorganic nutrients, physical substrate, and a variety of aquatic microorganisms (e.g., zoo and phytoplankton, heterotrophic/chemoautotrophic bacteria, filamentous cyanobacteria, dinoflagellates, and nematodes; Khanjani et al., 2023d). In addition, biofloc water contains a wide range of bioactive compounds (dissolved or suspended in microbial particles) such as carotenoids, chlorophylls, phytosterols, bromophenols, amino sugars, vitamins, and minerals (Ju et al., 2008; Khanjani et al., 2023a). In this sense, an enhanced reproductive performance has been observed due to the continuous nutrient-rich biofloc intake, especially during pre-spawning phase (Cardona et al., 2016; Khanjani, 2022), helping to (i) meet the nutritional requirements of broodstock (Magaña-Gallegos et al., 2021; Khanjani, 2022), and (ii) reduce FCRs. In lab-scale systems observed a ~ 30% FCR reduction of those fish raised in BFT compared to clear-water condition in Nile tilapia (~100 g) juveniles (Azim and Little, 2008). Khanjani et al. (2021a) evaluated Nile tilapia fingerlings and observed ~20% less FCR compared to the conventional water-exchange system.

In brooders, Cardona et al. (2016) highlighted that biofloc may represent an important food source, particularly for phospholipids (PL) and polyunsaturated fatty acids (PUFA). However, proximal composition or nutritional value of biofloc can vary depending on its culture conditions and is closely related to its microbiota (Ju et al., 2008; Emerenciano et al., 2012c; Khanjani et al., 2023a). Biological and biochemical profiles of biofloc are mainly determined by the source of water, the microbial inoculum, the carbon/nitrogen ratio, the substrate, temperature, salinity, light intensity, the concentration of dissolved oxygen, and turbulence in the water column, among other factors (Martinez-Cordova et al., 2015; Sharawy et al., 2022). Table 2 shows the biochemical composition of biofloc in broodstock cultivation tanks of different species. In different culture conditions and phases, biofloc is composed of 12 to 50% protein, 0.5 to 41% lipids, 14 to 59% carbohydrates, and 3 to 61% ash in a dry weight basis (Azim and Little, 2008; El-Sayed, 2021). Biofloc consisted of 34.69% protein, 4.83% lipid, 8.22% fiber, and 19.65% ash in African catfish breeders rearing tanks (Ekasari et al., 2016). The same study demonstrated linoleic acid (LA, 3.58 mg/g dry weight), α -linolenic acid (ALA, 0.46), arachidonic acid (ARA, 0.40), eicosapentaenoic acid (EPA, 1.67), docosahexaenoic acid (DHA, 0.29 mg/g dry weight) indicating low levels of long chain polyunsaturated fatty acids (LC-PUFA) in biofloc biomass (Ekasari et al., 2016).

In penaeid shrimp broodstock, fresh food items (e.g. polychaetas, squid, mussels, oysters, among others) are believed to be an essential nutrient source for proper gonadal development, improved gonadal maturity, eggs fecundity and hatching rates, as well as enhanced larvae quality (Tacon, 2017). However, there are biosecurity risks regarding transferring viable pathogens into shrimp aquaculture systems (Tacon, 2017). Once solution could be complete formulated feeds, however comparable reproductive performance was not achieved as yet (Wouters et al., 2001). In this sense, BFT can be used during broodstock production aiming to improve their nutritional status, storage and supplement key essential nutrients for later reproduction period (Emerenciano et al., 2015).

Dietary lipids are vital source of energy, sterols, phospholipids, essential fatty acids and fat-soluble vitamins which all important to the sexual maturation, the egg production (i.e., oogenesis and vitellogenesis), hormones/eicosanoids substrates and embryonic development in fish (Izquierdo et al., 2001) and crustaceans (Wouters et al., 2001). For instance, the reproductive process, egg hatching rate, and larval survival of penaeid shrimps are believed to be highly influenced by lipids that provide energy and essential nutrients, such as phospholipids (PL) and

essential fatty acids (Teshima et al., 1989; Cahu et al., 1994; Xu et al., 1994; Cardona et al., 2016). Moreover, LC-PUFA have important role in successful reproduction of crustaceans, directly involved in synthesis of steroid hormones, yolk components, and development of biomembranes and neuronal systems (Harrison, 1990). Crustaceans have limited capacity to synthesize LC-PUFA through de novo biosynthesis (Mourete, 1996), thus these essential nutrients should be included in their diets (Wouters et al., 2001; Racotta et al., 2003). As mentioned above, the amount of these essential nutrients in biofloc is scarce and should be provided through the diet of broodstock in BFT conditions (Cardona et al., 2016; Magaña-Gallegos et al., 2021). Emerenciano et al. (2013a) in a review paper demonstrated that lipid levels in biofloc biomasses can vary, and high levels are normally found in photoautotrophic (green-biofloc) diatom-based systems (De Schryver et al., 2010) where diatoms can contain lipid levels of up to 25% (Salehizadeh and Van Loosdrecht, 2004).

Regarding the importance of specific fatty acids (FA), LA, ALA, ARA, EPA and DHA can be found in high concentrations in maturing gonads of various crustacean and fish species suggesting vital role of this on reproductive performance, quality of gametes, spawning success and larval development (Izquierdo et al., 2001; Ravid et al., 1999; Wouters et al., 2001; Meunpol et al., 2005; Huang et al., 2008; Coman et al., 2011; Leelatanawit et al., 2014; Kumar et al., 2018). These FA can have regulatory effects on the steroidogenesis in the early maturation stages (Xu et al., 2017), acting as precursors of prostaglandins (PG), e.g., PGE2 and PGF2 α in the cyclooxygenase pathway, key role in oocyte maturation (Ravid et al., 1999; Wimuttisuk et al., 2013; Sumpownon et al., 2015), vitellogenesis (Sumpownon et al., 2015) and spawning (Spaziani et al., 1993, 1995) in various marine and freshwater crustacean species. For instance, Huang et al. (2008) reported that fecundity and egg production in *P. monodon* is highly correlated with ARA content of eggs. Tilapia breeders fed crude palm oil based-feed (rich in n-6 fatty acids) displayed higher concentrations of ARA in gonads, eggs, and larvae than those fed fish oil or linseed oil (Ng and Wang, 2011). Even though LC-PUFA content in biofloc biomasses is relatively low, some cases indicated high levels of some specific FA in certain conditions, e.g. high ARA content in tilapia BFT-based culture (Azim and Little, 2008) or freshwater bioreactors (Ekasari et al., 2010). Regardless the condition, BFT can positively impact gonadal development in earlier stages and further penaeid reproduction outputs (Emerenciano et al., 2015).

4. Positive effects of BFT on the reproductive performance and physiology of brooders

Recent studies have investigated the effects of BFT on the reproductive performance and physiology of various cultured aquatic species (Ekasari et al., 2013, 2016; Alvarenga et al., 2017; Emerenciano et al., 2014; Khanjani, 2022) (Tables 3, 4 and 5). A study by Ekasari et al. (2013) examined the reproductive performance of Nile tilapia brooders in a BFT system with a control group. The results showed that brooders in the BFT system produced significantly more fries than the control group that was associated with the increased blood glucose and total cholesterol levels. These findings suggested that BFT can positively influence the reproductive performance of brooders by increasing the availability of energy and essential nutrients such as cholesterol as the main precursor of the steroid hormones and potentially leading to higher yields and quality of offspring. In addition, Ekasari et al. (2015a, 2015b) found that maintaining broodstock in a BFT increased tilapia GSI, larval survival and tolerance to salinity stress and *Streptococcus agalactiae* infection due to the natural food availability in the form of microbial protein in BFT for gonad development. Similarly, in another study, Ekasari et al. (2016) found that rearing African catfish brooders in a BFT system showed comparable fecundity and eggs quality to those in the control system. Furthermore, BFT increased the quality of the eggs and embryonic development rate, resulting in lower larval mortality (Ekasari et al., 2016). Poli et al. (2015) reported that the survival of

Table 2
Biochemical composition of bioflocs collected from broodstock rearing tanks.

Rearing system	Cultivated species	IW (g)	RP (days)	SD	CSU	C/N	CP	CL	CF	CAR	Ash	AA	EPA	DHA (% of TFA)	Reference
BFT	<i>F. duorarum</i>	21.9	45	4.75 shrimp/m ²	Molasses and wheat bran	20:1	28.00	0.5	3.2	22.7	35.8	0.3	0.5	0.3	Emerenciano et al., 2014
BFT + FF		23.5				20:1	30.40	0.6	3.1	18.1	39.6	0.4	0.5	0.4	Ekasari et al., 2013 Goodall et al., 2016
BFT	<i>O. niloticus</i>	84.56	84	20 fish/m ³	Molasses	15:1	37.4	11.9	16		17.7	0.49	0.2	0.11	
CW diets with MB	<i>P. monodon</i>						47.4	8.4	3.0		12.6				Braga et al., 2015
BFT + FF	<i>L. vannamei</i>	36.40	30	14.56 g/L			30.7	8.3	2.1		9.9				
BFT + BF							18.64	0.64	1.46	24.97	54.29				Cardona et al., 2016
BFT + JF							23.17	1.35	1.88	25.68	47.92				
BFT	<i>L. stylirostris</i>	40.6	70	12 shrimps/m ²	Molasses	20:1	22.96	0.95	2.72	24.78	48.59	0.06–0.65	0.42–4.41	0.09–0.7	
BFT	<i>L. vannamei</i>				Molasses	15:1	28.5–31.4	0.65–1.4			35.5–38.6				Khanjani, 2022
BFT with 4% lipid	<i>C. carpio</i>	22	120	48 fish/m ²	Tapioca flour	20:1	32.20	4.0	9.0						Manzoor et al., 2020
BFT with 6% lipid							31.85	6.0	8.8						
BFT with 8% lipid							32.07	8.0	9.2						
BFT with 10% lipid							32.25	10.0	9.07						
BFT with size 10 µm	<i>F. brasiliensis</i>	21.9	45	4 shrimps/m ²	Molasses	20:1	1.18	1.61				0.33	0.75	0.16	Magana-Gallegos et al., 2018a
BFT with size 50 µm							30.8	7.7				0.82	2.40	0.06	
BFT with size 100 µm							33.0	7.6				0.00	1.79	0.00	
BFT with size 250 µm							31.9	6.1				0.90	2.93	0.10	
BFT with size 500 µm							29.1	8.1				1.04	3.63	0.14	
BFT with size 50 µm	<i>L. vannamei</i>						34.9	10.5				0.79	2.70	0.22	Magana-Gallegos et al., 2018c
BFT with size 100 µm							33.0	7.7				1.06	3.49	0.32	
BFT with size 250 µm							34.9	7.4				0.69	2.04	0.25	
BFT with size 500 µm							35.0	8.1				0.34	1.06	0.35	
BFT	<i>L. vannamei</i>	35.0	40	4 shrimp/m ²	Molasses	20:1	18.4	0.3	2.1	35.7	34.5	0.4	0.3	0.2	Emerenciano et al., 2013b
BFT + FF		36.2					26.3	0.7	3.4	20.2	41.5	0.3	0.5	0.4	

Abbreviations: Biofloc technology = BFT, Clear water = CW, Initial weight = IW, Rearing period = RP, Stocking densities = SD, Carbon source used = CSU, Carbon to nitrogen ratio = C/N, Crude protein = CP, Dry weight = DW, Crude lipid (% DW) = CL, Crude fiber (% DW) = CF, Carbohydrate (% DW) = CAR., Microbial biomass = MB, Fresh food = FF, Broodstock feed = BF, Juvenile feed = JF, Arachidonic acid = AA, Eicosapentaenoic acid = EPA, Docosahexaenoic acid = DHA, Total fatty acids = TFA.

Table 3
Reproductive performance of fish broodstock under BFT and other rearing systems.

Rearing system	Cultivated species	IW (g)	RP (days)	SD	CSU	C:N	HSI %	GSI %	ED	Number of eggs	Survival of the larvae (%)	Highlight	Reference
BFT	<i>O. niloticus</i>	84.56	84	20 fish/m ³	Molasses	15:1	1.9	2.64	1.55 mm	1243 (egg/ind)		Total fry production of broodstocks in BFT tanks was significantly higher than that in the control tanks (8491 vs. 5154). reproductive performance of tilapia broodstocks in floc-based system was better than in the control.	Ekasari et al., 2013
CW							1.84	3.08	1.45 mm	890 (egg/ind)			
BFT	<i>O. niloticus</i>	163.7	21	6.5 kg/m ³	Molasses	6:1	1.98–3.07	2.1–3.58		1065 (egg/ind)	98.4	BFT can alter the energy mobilization in the post-spawning period. There was no evidence of the negative effects of BFT on Nile tilapia reproduction. %62.5 ready-to-spawn females in BFT, 58.5% ready-to-spawn females in CW.	Alvarenga et al., 2017
CW							1.68–2.3	1.3–3.36		1137 (egg/ind)	95.3		
BFT	<i>C. gariepinus</i>	657 Female	122	5 fish/m ²	Molasses	10:1	1.19	6.15		RF = about 100,000	70–80	Housing African catfish broodstock in BFT significantly affected the embryonic development rate and the larval quality. Improvements in survival and growth were observed in the larvae housed in BFT. Relative fecundity of biofloc broodstock was 26% higher than that of the CW.	Ekasari et al., 2016
CW		543 Male		4 fish/m ²			1.11	6.38		RF = about 74,000	30–40		
BFT	<i>O. niloticus</i>		14	8.3 fish/m ³	Molasses	10:1					90–98%	The application of biofloc technology for tilapia brood fish maintenance and larval production can improve Nile tilapia fry quality and production performance.	Ekasari et al., 2015a
CW											67–75%		
BFT with 4% lipid	<i>C. carpio</i>	22	120	48 fish/m ²	Tapioca flour	20:1	1.44	15.04	69.32 µm	AF = 7856		Biofloc improves gonadal maturation of common carp broodstock at a dietary supplementation with 8% lipid compared with conventional system of broodstock management.	Manzoor et al., 2020
BFT with 6% lipid							1.70	15.45	83.89 µm	AF = 8700			
BFT with 8% lipid							1.97	24.47	73.55 µm	AF = 9913			
BFT with 10% lipid							1.44	19.73	75.58 µm	AF = 8450			
CW with 10% lipid							1.67	17.97	53.85 µm	AF = 6484			
CW without probiotic	Red tilapia	127.5 female,	84	12 fish/m ³						AF = 829.42	51.67		
CW + probiotic		148 male								AF = 828.31	65.00		
BFT without probiotic					Corn starch	10:1				AF = 919.69	68.33		
BFT + probiotic										AF = 966.31	73.00		

Abbreviations: Biofloc technology = BFT, Clear water = CW, Initial weight = IW, Rearing period = RP, Stocking densities = SD, Carbon source used = CSU, Carbon to nitrogen ratio = C/N, Hepatosomatic index (HSI), Gonadosomatic index (GSI), Relative fecundity (Eggs/kg female) = RF, Fecundity = F, Absolute fecundity = AF, Egg diameter = ED.

Table 4
Reproductive performance of shrimp broodstock under BFT and other rearing systems.

Rearing system	Cultivated species	IW (g)	RP (days)	SD	CSU	C:N	LP (days)	Percentage daily spawner (%/d)	Eggs or nauplii production (N/spawner)	Survival (%)	Highlight	Reference
CW+ Domesticated	<i>Farfantepenaeus duorarum</i>		30	4.3 shrimp/m ²	Molasses	20:1	17	89	33.5 × 10 ³ Eggs 25.6 × 10 ³ Nauplii	88 Female broodstock	Wild population of <i>F. duorarum</i> presented significantly better reproductive outcomes as compared to domesticated ones	Emerenciano et al., 2012b
CW+ Wild							11	76	71.9 × 10 ³ Eggs 51.9 × 10 ³ Nauplii	94 Female broodstock		
BFT	<i>L. vannamei</i>	35.0	40	4 shrimp/m ²	Molasses	20:1	22	88.9	94.1 × 10 ³ Eggs 50.3 × 10 ³ Nauplii	88.9	The better outcomes obtained in females that received short-term FF supplementation justified the FF use in <i>L. vannamei</i> broodstock	Emerenciano et al., 2013b
BFT + FF		36.2					23	94.4	111.2 × 10 ³ Eggs 62.1 × 10 ³ Nauplii	91.1		
CW + FF	<i>F. duorarum</i>	21.1	45	4.75 shrimp/m ²	Molasses and wheat bran	20:1	25	25	23.3 × 10 ³ Eggs	82.5 Female broodstock	The better reproductive performance demonstrated by females raised in biofloc justified the application of this technology in <i>F. duorarum</i> broodstock.	Emerenciano et al., 2014
BFT		21.9					29	80	31.6 × 10 ³ Eggs	88.2 Female broodstock		
BFT + FF		23.5					23	81.7	48.7 × 10 ³ Eggs	87 Female broodstock		
BFT+ Domesticated	<i>F. brasiliensis</i>	21.9	45	4 shrimps/m ²	Molasses	20:1	16		123.3 × 10 ³ Eggs	93 Female broodstock	Wild shrimp broodstock have better reproductive performance than domesticated shrimp	Magana-Gallegos et al., 2018a
BFT+ Wild		39.5					11		175.8 × 10 ³ Eggs	96 Female broodstock		
BFT	<i>F. indicus</i>	38.05	330		Molasses	15:1		11.4	33.832 Nauplii	30.70 post larvae	<i>F. indicus</i> can successfully produce healthy post larvae without prior broodstocks ablation.	Nur et al., 2022
CW		37.35	300					8.0	51.246 Nauplii	23.26 post larvae	daily spawners, Nauplii production and postlarvae survival shows any significant differences, between BFT and CW.	
BFT	<i>P. vannamei</i>	38.6	30	0.27 g/l	Molasses	15:1			60.8 × 10 ³ Eggs	97.57 Female broodstock	The reproductive performance of Pacific white shrimp broodstock in the system with limited exchange of water is better than the conventional system.	Khanjani, 2022
CW									47.5 × 10 ³ Eggs	95.21		

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Table 4 (continued)

Rearing system	Cultivated species	IW (g)	RP (days)	SD	CSU	C:N	LP (days)	Percentage daily spawner (% /d)	Eggs or nauplii production (N/spawner)	Survival (%)	Highlight	Reference	
CW diets with MB	<i>Penaeus monodon</i>	142.8–14.1	40	2–3 shrimp/m ²			12.2	76.3	230,644 Egg production, 80,533 Nauplii production	89.7–92.3 Female broodstock	Inclusion of microbial biomass within broodstock grow-out and maturation diets (at the rates presented in their study) did not enhance reproductive performance of domesticated broodstock.	Goodall et al., 2016	
		150.9–158.0					12.4	71.9	225,541 Egg production, 65,261 Nauplii production	93.9–94.9			
		163.1 – 170.7					12.3	68.4	255,535 Egg production, 70,092 Nauplii production	86.8–89.5 Female broodstock			
CW+ ablated	<i>F. brasiliensis</i>	40.8	45					83.3	241.9 × 10 ³ Eggs	66.7 Female broodstock	Unilateral eyestalk ablation affects not only the broodstock survival, but the offspring quality as well	Magaña-Gallegos et al., 2018b	
CW+ non-ablated		40.2						20	269.21 × 10 ³ Eggs	90 Female broodstock			
BFT+ ablated	<i>L. vannamei</i>	40.5	30	3.2 shrimp/m ²	Molasses	20:1		60	149.3 × 10 ³ Eggs	90 Female broodstock	Indicating that both eyes talk ablation and pre-maturation culture conditions (i. e., either biofloc or clear-water) affected the quality of Eggs and egg production numbers in <i>L. vannamei</i> .	Magaña-Gallegos et al., 2021	
BFT+ non-ablated		40.2		5.8 shrimp/m ²					37.8	128.5 × 10 ³ Eggs			100 Female broodstock
CW+ ablated		43.9		3.2 shrimp/m ²					60	99.9 × 10 ³ Eggs			95 Female broodstock
CW+ non-ablated		42.3		5.8 shrimp/m ²					54.1	127.1 × 10 ³ Eggs	97.3 Female broodstock		
BFT	<i>L. stylirostris</i>	40.6	70	12 shrimp/m ²	Molasses	20:1		52	1.05 × 10 ⁷ Eggs number	68.73 post larvae	Biofloc is a source of further dietary lipids that can act as energetic substrates. Improving the reproduction of the brood stock also leads to an improvement in the quality of the larvae.	Cardona et al., 2016	
CW									35	0.61 × 10 ⁷ Eggs			45.48 post larvae
BFT	<i>L. stylirostris</i>	40.5	30	2 shrimp/m ²		8:1		77.1	3.9 × 10 ³ Per g of spawner's body weight	32 Female broodstock	Results evidenced better overall spawning performance for <i>L. stylirostris</i> broodstock produced under floc condition than in earthen ponds.	Emerenciano et al., 2012a	
Earthen pond		53.6		15 shrimp/m ²					53.8	3.3 × 10 ³ Per g of spawner's body weight			30.1 Female broodstock

Abbreviations: Biofloc technology = BFT, Clear water = CW, Initial weight = IW, Rearing period = RP, Stocking densities = SD, Carbon source used = CSU, Carbon to nitrogen ratio = C/N, Diets with microbial biomass = MB, Fresh food supplementation = FF, Latency period (days) = LP.

Table 5
Physiology performance of different species broodstock under BFT and other rearing systems.

Rearing system	Cultivated species	Experimental design	CAT	SOD	GSH	Gl.	Cho.	AG.	Highlights	Reference
BFT	<i>L. stylirostris</i>	Mean weight 40.6, each tank was stocked with 12 animals/m ² , experiment during 10 weeks.	1.24 μmol./ min./ mg protein	6.36 U./mg protein	2.56 mmol./ mg protein				BFT compared to CW breeding improved the antioxidant status of shrimps.	Cardona et al., 2016
CW			0.61 μmol./ min./ mg protein	3.31 U./mg protein	1.20 mmol./ mg protein					
CC, in HP	<i>Litopenaeus setiferus</i>	Shrimp were randomly placed in twenty 100L indoor tanks at a rate of 3 shrimp per tank. Two types of culture systems were used during the prematuration phase: CW and BFT. The CW system consisted of 10 tanks connected to a recirculating system with three 1000L reservoirs, and 100% water exchange was performed three times a week. In contrast, the BFT system consisted of 10 isolated tanks filled with 20L of biofloc.	1.33 U./mg protein	16.57 U./mg protein	5.89 μM/ min./ mg protein	1.66 mg/g	1.21 mg/g	27.43 mg/g	The HM cholesterol analysis revealed an interaction between factors, with higher concentrations found in the CW treatments and lower concentrations found in the BFT treatment. In contrast, the antioxidant status analysis showed that the culture system was not a determinant factor of these indicators. The oxidative stress status of the hepatopancreas was found to be related to diet.	Barral-Pintos and Gaxiola, 2022
CF, in HP			1.12 U./mg protein	18.32 U./mg protein	7.98 μM/ min./ mg protein	0.69 mg/g	0.9 mg/g	10.36 mg/g		
BC, in HP			1.63 U./mg protein	14.3 U./mg protein	5.42 μM/ min./ mg protein	1.74 mg/g	0.93 mg/g	25.93 mg/g		
BF, in HP			1.07 U./mg protein	21.78 U./mg protein	7.01 μM/ min./ mg protein	1.18 mg/g	1.25 mg/g	12.34 mg/g		
CC, in HM						0.22 mg/ mL	0.26 mg/ mL	0.49 mg/ mL		
CF, in HM						0.21 mg/ mL	0.26 mg/ mL	0.3 mg/ mL		
BC, in HM						0.22 mg/ mL	0.21 mg/ mL	0.5 mg/ mL		
BF, in HM						0.19 mg/ mL	0.14 mg/ mL	0.38 mg/ mL		
CW	<i>O. niloticus</i>	Nile tilapia at respective average 85 g, and stocked at a density of 20 fish/m ³ at a male:female ratio of 1:4. Molasses (44% C) was added in BFT treatment at an estimated C:N ratio of 15. 84 days of culture				51 mg/ dL	157 mg/dL		Blood glucose and Cholesterol levels in the fish in BFT treatment were constantly higher than those of the control	Ekasari et al., 2013
BFT						55 mg/ dL	168 mg/dL			
BFT with 4% lipid	<i>C. carpio</i>	120 days to evaluate optimum dietary lipid requirements for gonadal maturation of <i>C. carpio</i> fed with varying dietary lipid levels under biofloc-based systems. 180 fingerlings 22 g, randomly distributed in 15 tanks (300L) at the rate of 48 no./m ² . The C/N ratio of 20:1 was maintained using tapioca flour.				0.13 mg/ mL	157.63 mg/dL		Biofloc has been shown to contribute to serum cholesterol levels, which has a positive correlation with reproductive parameters. This may be a reason for the enhanced maturation parameters observed in BFT systems.	Manzoor et al., 2020
BFT with 6% lipid						0.08 mg/ mL	162.76 mg/dL			
BFT with 8% lipid						0.24 mg/ mL	240.35 mg/dL			
BFT with 10% lipid						0.10 mg/ mL	270.90 mg/dL			
CW						0.14 mg/ mL	143.81 mg/dL			

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Table 5 (continued)

Rearing system	Cultivated species	Experimental design	CAT	SOD	GSH	Gl.	Cho.	AG.	Highlights	Reference			
BFT+ ablated HP	<i>L. vannamei</i>	Shrimp were selected based on size (females ~39–41 g, males ~27–29 g), and there was stocking at a density of 5.8 shrimp/m ² in one tank with a BFT and one tank with a CW at a 1:1 female to male ratio. For 30-day experimental period. The BFT was maintained at a carbon/nitrogen (C/N) ratio of 20:1 by daily addition of sugarcane molasses				2.6 mg/g	6.9 mg/g	38.6 mg/g	Females cultured in BFT without eyestalk ablation had the most nutrient reserves and highest egg production. Eyestalk ablation reduced egg quality in BFT. CW-cultured females with eyestalk ablation had similar nutrient reserves but lower egg production.	Magaña-Gallegos et al., 2021			
BFT+ ablated HM						27.9 mg/dL	49.9 mg/dL	116.0 mg/dL					
BFT+ ablated OV							6.6 mg/g	7.5 mg/g			11.0 mg/g		
BFT+ ablated Eggs							0.6 mg/g	8.4 mg/g			21.3 mg/g		
BFT+ non-ablated HP							3.2 mg/g	10.4 mg/g			57.0 mg/g		
BFT+ non-ablated HM							19.3 mg/dL	31.0 mg/dL			50.0 mg/dL		
BFT+ non-ablated OV							1.2 mg/g	6.6 mg/g			10.7 mg/g		
BFT+ non-ablated Eggs							1.0 mg/g	9.3 mg/g			23.9 mg/g		
CW+ ablated HP							3.0 mg/g	8.0 mg/g			35.4 mg/g		
CW+ ablated HM							22.6 mg/dL	45.3 mg/dL			73.7 mg/dL		
CW+ ablated OV							4.6 mg/g	6.2 mg/g			11.4 mg/g		
CW+ ablated Eggs							0.7 mg/g	9.3 mg/g			22.8 mg/g		
CW+ non-ablated HP							2.5 mg/g	6.7 mg/g			27.3 mg/g		
CW+ non-ablated HM							30.7 mg/dL	47.0 mg/dL			82.5 mg/dL		
CW+ non-ablated OV							2.6 mg/g	6.2 mg/g			11.1 mg/g		
CW+ non-ablated Eggs							0.6 mg/g	8.0 mg/g			17.7 mg/g		
CW+ Domesticated, HP, 1st spawn			<i>F. duorarum</i>	During 30 days, reproductive performance trial was performed using four 12,000 L round lined maturation tank with recirculation system. In each tank, groups of 17 females and 35 males from each source were stocked (female to male ratio 1:2 and stocking density of 4.3 shrimp/m ²).				2.2 mg/g			66.5 mg/g	Wild population of <i>F. duorarum</i> presented better reproductive performance as compared to domesticated ones. Multiple spawning was reflected in some biochemical variables such as AG and cholesterol content in HP, and OV. The higher spawning activity was reflected in lower levels of AG and cholesterol content in wild HP and OV.	Emerenciano et al., 2012b
CW+ Domesticated, OV, 1st spawn								0.6 mg/g			15.8 mg/g		
CW+ Wild, HP, 1st spawn								1.6 mg/g			9.1 mg/g		
CW+ Wild, OV, 1st spawn								1.0 mg/g			26.5 mg/g		
BFT, HP	<i>L. vannamei</i>	During 40 days, reproductive performance trial was performed using four 12,000-L round lined maturation tank with recirculation system. In each tank, groups of 18 females and 27 males from each source were stocked.				3.22 mg/g	62.8 mg/g	BFT + FF females had a slower reduction in biochemical composition than BFT females due to prior supplementation. FA levels in eggs remained stable or slightly increased, indicating a transfer of nutrients from HP and OV to the eggs.	Emerenciano et al., 2013b				
BFT, OV						4.31 mg/g	14.5 mg/g						
BFT + FF, HP						3.29 mg/g	59.1 mg/g						
BFT + FF, OV						4.02 mg/g	12.8 mg/g						

Abbreviations: Catalase = CAT superoxide dismutase = SOD, glutathione peroxidase = GSH-PX, Glucose = Gl. Cholesterol = Cho. Acylglycerides = AG, Ovary = OV, Hepatopancreas = HP, Hemolymph = HM, Clear water commercial pellet = CC, Clear water fresh food = CF, Biofloc commercial pellet = BC, Biofloc fresh food = BF.

Table 6
The composition of essential fatty acids in eggs from BFT and other rearing systems.

Rearing system	Cultivated species	LA % of TFA	ALA	AA	EPA	DHA	Reference
BFT	<i>C. gariepinus</i>	12.05	0.63	8.57	1.90	25.40	Ekasari et al., 2016
CW		23.23	1.82	9.18	1.77	23.23	
CW+ FF	<i>F. duorarum</i>	2.9	1.6	2.8	6.60	8.80	Emerenciano et al., 2014
BFT		3.6	1.3	2.3	5.8	8.90	
BFT + FF		3.2	2.3	2.3	5.3	6.3	
BFT + Neutral lipids	<i>L. stylirostris</i>	1.88	0.25	0.72	3.75	1.21	Cardona et al., 2016
BFT + Phospholipids		1.92	0.44	1.31	9.77	10.49	
CW + Neutral lipids		2.05	0.35	0.72	5.68	10.68	
CW + Phospholipids		0.57	0.11	0.53	3.48	3.45	
CW + Domesticated	<i>F. duorarum</i>	2.9	0.8	2.2	7.5	10.1	Emerenciano et al., 2012b
CW + Wild		2.6	0.6	1.9	6.6	8.5	
BFT	<i>L. vannamei</i>	3.4	2.7	2.3	7.1	8.1	Emerenciano et al., 2013b
BFT + FF		3.9	2.9	2.2	8.3	9.0	

Abbreviations: Total fatty acids = TFA, Linoleic acid = LA, Alpha-linolenic acid = ALA, Arachidonic acid = AA, Eicosapentaenoic acid = EPA, Docosahexaenoic acid = DHA.

South American catfish larvae was significantly improved in a BFT system, which was strongly correlated with their higher resistance to a parasite infestation. Moreover, Manzoor et al. (2020) confirmed the beneficial effects of bioflocs on common carp gonadal development using a BFT system and dietary lipid supplementation. Females in the BFT treatment showed advanced ovary maturity stages, and 8% dietary lipid supplementation in BFT led to the highest absolute fecundity, HSI (1.97% vs. 1.67%), and GSI (24.47% vs. 17.97%) in brooders that was associated with higher blood cholesterol level in this species. Cholesterol is known as a precursor of steroid hormones such as testosterone, estrogens and progesterone (Lubzens et al., 2010) and the increment of blood cholesterol level suggesting better sexual maturation in brooders as also reported in Nile tilapia (Ekasari et al., 2013). However, in study by Barral-Pintos and Gaxiola (2022) on *Litopenaeus setiferus*, hemolymph cholesterol showed higher concentrations in brooders reared in CW compared to those reared in BFT that could be related to the 18h starvation before sampling.

It is believed that a number of factors contribute to fish gonad maturation and spawning, including (1) the nutrition of females (food availability, amino acids, fats, ascorbic acids, vitamin E); (2) physiological factors (hormones, morphological changes, mobilization of energy reserves); and (3) ecological factors (food availability, water quality, exposure to toxins, and environment) (Rocha, 2008). Compared to CW, aquatic animals reared in the BFT treatment might have performed better in terms of reproduction. As a potential readily available food source, bioflocs in the BFT treatment may support not only brooders reproduction but also larval survival and development (Ekasari et al., 2013).

Previous studies conducted on various Penaeid shrimp species have indicated that BFT has the potential to improve their antioxidant status and spawning performance (Emerenciano et al., 2012a, 2012b; Emerenciano et al., 2013b, 2014; Cardona et al., 2016; Khanjani, 2022; Nur et al., 2022). It has been reported that imbalances in the antioxidant defense system can impair reproductive performance (Mansour et al., 2006; Sabeti et al., 2016; Shi et al., 2018). For instance, Montalvo et al. (2022) demonstrated that when *L. vannamei* was fed with an optimal amount of vitamin E, sperm quality increased while the expression and activities of prophenoloxidase, glutathione peroxidase, and superoxide dismutase genes in the reproductive tissue decreased. Furthermore, Barral-Pintos and Gaxiola (2022) evaluated the effects of different culture systems and food items, including CW with commercial pellets (CC), CW with fresh food (CF), BFT with commercial pellets (BC), and BFT with fresh food (BF) on the pre-mature *L. setiferus* males F0 generation. Results showed that the CC and BF treatments had higher values of sperm quantity and a higher proportion of normal spermatid cells in males. In addition, the GSI values were also higher in the BF treatment compared to other treatments. However, authors of the above-

mentioned study did not show a link between the oxidative status of the hepatopancreas and sperm quality. In another study, Cardona et al. (2016) reported that *L. stylirostris* brooders reared in BFT exhibited a higher health status compared to those reared in CW by increasing survival rate (52.6% in CW vs. 79.8% in BFT), and antioxidant capacity leading to higher resistance of brooders to handling stress during reproduction process (Wabete et al., 2004). Furthermore, reproduction performance indices, including spawning rate and frequency, GSI and number of eggs spawned were increased in brooders reared in BFT. Finally, larvae from brooders from BFT exhibited higher survival rates at the Zoe 2 (+ 37%) and postlarvae 1 (+ 51%) stages mainly due to the extra nutrients that shrimp obtained from natural productivity during BFT rearing (Cardona et al., 2016).

BFT can be used as an alternative technique for establishing biosecure condition and supplying nutrients requirements during pre-maturation of domesticated shrimp. For instance, Braga et al. (2015) applied BFT system to spare dietary protein and exclude fresh foods in pre-mature *L. vannamei* males. There were four experimental groups, including: control (CW): in which animals were reared in a CW system (90% daily water exchange rate) and fed with a mixture of fresh foods (fish, crab and squid), BFT + FF: animals reared in BFT and were fed with the same fresh foods; BFT + BF: animals reared in BFT and fed with a formulated brooder diet containing 52.51% protein and BFT + JF: animals reared in BFT and fed with a formulated juvenile diet containing 39.91% protein. Results showed that male brooders in BFT + FF group had the lowest normal sperm rate (65.97 ± 8.53) than other groups (86.08–89.29%). Other sperm quality parameters such as spermato-phore weight, melanization, sperm count and dead sperm rate did not affect by treatments. The findings of this study showed that the use of BFT allowed dietary protein sparing and exclusion of fresh foods in pre-maturation of *L. vannamei* males without drastic negative effects on their sperm quality. Moreover, Nur et al. (2022) reported that *F. indicus* can mature, spawn, and produce seeds without ablation in BFT system. The non-BFT treated brooders produced more nauplii than the BFT treated one, but post-larvae survival was low. BFT-treated brooders performed slightly better and tended to increase from the beginning of the spawning period, but the percentage of daily spawners did not differ significantly. Moreover, survival rate in the BFT pre-maturation brooders (30.70%) was higher than non-BFT brooders (23.26%) (Nur et al., 2022).

The effects of BFT on the reproductive performance of various shrimp species have been investigated in several studies. For instance, Emerenciano et al. (2014) found that the number of eggs per spawn increased, and the latency period between spawning decreased in *F. duorarum* brooders reared in BFT. Brooders reared in BFT and fed with complementary food also had better performance than those without any supplementary foods. Similarly, Khanjani (2022) reported that

P. vannamei brooders reared in BFT produced more eggs per spawn than those in CW, and the average density of nauplii was also higher in BFT. Emerenciano et al. (2013b) reported that female brooders of *L. vannamei* in BFT or those reared in BFT with fresh food supplementation produced more eggs per gram of body weight than those in CW. Moreover, Cavalli et al. (1997) reported that *Farfantepenaeus paulensis* brooders raised in a BFT system had a higher spawning rate (80–82%) than those in CW (25%). The number of eggs per spawning was also higher in *F. paulensis* broodstock reared in BFT (86,800 eggs per spawning) compared to those in CW (86,400 eggs per spawning) (Peixoto et al., 2004). Similarly, Emerenciano et al. (2014) reported that *F. duorarum* broodstock reared in BFT produced more eggs per spawning (23,300 to 48,700) than those in CW (14%).

The spawning performance (Including: incubation period, the number of spawners ready to spawn each time the eyestalk is cut, the frequency of pial maturation in female spawners, the number of eggs per spawning, the number of eggs per unit of body weight, and the cumulative spawning rate) of *L. stylirostris* blue shrimp reared in the system with limited water exchange is significantly higher than that of broodstock raised in the normal system (Emerenciano et al., 2012a).

It is likely that the number of eggs in each spawning stage varies according to the rearing conditions, the feeding type, the rearing system, the species type, and environmental factors such as photoperiod, temperature, and salinity (Emerenciano et al., 2013a; Khanjani, 2022). There is a higher survival rate, GSI, number of fertilized eggs and larval survival in broods reared in a limited water exchange system than in those reared in CW (El-Sayed, 2021). Likewise, other studies have shown that natural foods associated with microbial populations improve reproductive performance and egg quality in *Farfantepenaeus brasiliensis* (Magana-Gallegos et al., 2018b) and *L. vannamei* (Magaña-Gallegos et al., 2021).

In comparison with those specimens that had been cultured in a CW system, unilaterally ablated eyestalk broodstock developed with biofloc had increased spawning frequency, female survival, and offspring quality (Emerenciano et al., 2014; Cardona et al., 2016). According to Magana-Gallegos et al. (2018b), eggs spawned from *F. brasiliensis* females without eyestalk ablation contained more nutritional reserves than those from females with eyestalk ablation. Due to these advantages, broodstock developed in BFT can be used without unilateral eye ablation procedures, as increased nutritional status could improve reproductive performance by improving egg quality (Wouters et al., 2001; Emerenciano et al., 2014; Zacarias et al., 2019; Magaña-Gallegos et al., 2021). Such superior performance might be the result of better control of water quality parameters and the continuous availability of biofloc as a natural food source, as opposed to conventional systems where immature broodstock are often limited to pelletized feeds. In young breeders, these nutrients are necessary for gonad formation and ovary development. As nutrients are continuously available, they can be stored in the hepatopancreas, transferred to hemolymph, and then directed to the ovary, resulting in better sexual tissue development and reproductive activity (Emerenciano et al., 2013b; Cardona et al., 2016).

With biofloc, nutrient absorption, assimilation, and cellular nutrition are improved (Tacon et al., 2002). Regarding rearing shrimp brooders in BFT system, Emerenciano et al. (2013b) reported that using fresh foods (frozen mussel + squid at 3% biomass) and formulated feed (2% of biomass) for 20 days in *L. vannamei* brooders resulted in better eggs production (23%), greater nauplii production (18%), shorter latency period, higher spawning frequencies, higher hatching rate (10%) and more eggs LC-PUFA content under BFT conditions. Using fresh foods in BFT system resulted in higher crude protein and lipid levels in flock as well as a higher concentration of filamentous cyanobacteria and nematodes. In addition, using fresh foods (frozen mussel + squid, 1:1 ratio, at a rate of 2% biomass) and commercial diet (3% of the biomass) for 45 days in BFT system in the pink shrimp (*Farfantepenaeus duorarum*) significantly enhanced spawning performance such as eggs count per spawn, eggs count per g of female, egg size and higher spawning activity

as compared with those reared in CW system and fed with fresh foods (Emerenciano et al., 2013c). BFT system also increased 15% nauplii production/female in *P. stylirostris* that coincided with increased antioxidant capacity in female brooders (Cardona et al., 2016). The better spawning performance of females reared in biofloc are probably because of the better nutritional condition during pre-mature period as evident by the availability of natural microbiota (biofloc) and fresh foods supplementation that resulted in high degree of gonad development.

5. Positive effects of BFT on egg quality and egg nutritional quality

Egg quality, especially regarding its nutritional composition has key role in viability of progeny. As it was mentioned, egg's quality is primarily determined by physiological condition, health status, and nutritional history of brooders (Wouters et al., 2001; Racotta et al., 2003).

Racotta et al. (2003) found that the diameter of eggs and their size distribution also indicate the quality of the egg and its nutrient reserves. Various factors may affect egg size, such as its biochemical composition, broodstock weight, individual variation not caused by genetics, season, and energy utilization for reproduction (Anger, 2001; Emerenciano et al., 2014). According to Emerenciano et al. (2014), the size of eggs in *F. duorarum* broodstock was determined for three treatments: CW system (268 μm), biofloc technology (BFT) (276 μm), and BFT with fresh food supplementation (278 μm). In broodstock raised in biofloc media, nutrient concentration in the hepatopancreas affects ovarian development and nutrient transfer to eggs. Besides feed (fresh and dry pellets), nutrients also come from floc formation (Emerenciano et al., 2014).

The quality of eggs in African catfish was evaluated in BFT and CW systems (Ekasari et al., 2016). About 63% of eggs in brooders reared in BFT had over 1.0 mm of diameter, compared to those in CW system that about 29% of eggs had diameter more than 1 mm. Both treatments had similar size distributions on day 84 and day 122, but the size ranges tended to narrow at 0.80 mm–1.4 mm. It is possible that the maturation process was accelerated in brooders reared in BFT than those in CW system based on their egg size distribution. In addition, at the end of the trial (day 122), brooders in BFT had a 26% higher relative fecundity than those in CW system (Ekasari et al., 2016). Finally, fertilization and hatching rates of brooders reared in BFT were higher than control. Moreover, eggs from brooders reared in BFT were more developed and needed less time to hatch. Rearing brooders in BFT might improve the maternal transfer of nutrients to their offspring that enhance embryonic development rate (Ekasari et al., 2016). In another research, Magaña-Gallegos et al. (2021) reported that during the pre-maturation period of *L. vannamei*, the eggs from females reared in BFT were significantly smaller mainly due to higher egg count (absolute fecundity) in ovaries than those raised in the CW system. According to Emerenciano et al. (2012b), *F. duorarum* produce more eggs per spawning period in their natural habitat. During the pre-maturation period, *F. duorarum* in which the eyestalks were removed were cultured in the BFT and release more eggs than those in the CW system. The eggs of females cultured in the BFT were larger than those from females cultured in a CW system, even when fresh food (squid and mussel) was provided during the pre-maturation period. According to Emerenciano et al. (2014), *F. duorarum* raised in BFT exhibited better spawning performance, including the number of spawns per ablated female, eggs per spawn, and relative fecundity and compared to specimens cultured in a CW system. Also, Cardona et al. (2016) reported that *L. stylirostris* cultured in the BFT produced a greater number of eggs.

Nutritional quality and quantity of the egg yolk mainly depends on the maternal nutritional history, especially during maturation (Racotta et al., 2003; Emerenciano et al., 2014). This issue is vital for those nutrients that can't be synthesized through de novo, such as LC-PUFA, that may be depleted during embryogenesis (Teshima et al., 1989). Table 6 illustrates the amounts of EFA in eggs of female brooders in different species raised under different conditions. As a result of high spawning

activity performed by females in FLOC+FF treatment, egg fatty acid profiles showed lower levels of EPA, DHA, and PUFAs (n-3), and (n-6) (Emerenciano et al., 2014). The LA level was also higher in biofloc treatments, probably because biofloc particles contain a high amount of LA (Azim and Little, 2008; Ekasari et al., 2010). ALA levels were higher in treatments that received fresh food supply, including squid and mussels, which contributed to ALA supplementation (Cahu et al., 1994; Hoa et al., 2009). LC-PUFA play a crucial role in penaeid shrimp reproduction (Xu et al., 1994; Coman et al., 2011). According to Xu et al. (1994), EPA plays a specific role in ovarian development, while DHA may play a role in early embryogenesis related to egg hatchability in larval Chinese prawn (*Penaeus chinensis*). Cahu et al. (1995) conducted a similar study with domesticated *F. indicus* and found that hatching percentage was correlated with dietary LC-PUFA level. A correlation has also been found between egg AA content and fecundity and egg production in black tiger shrimp (Huang et al., 2008; Coman et al., 2011). The study of Cardona et al. (2016) suggests that the enrichment of eggs with PL, n-3 LC-PUFA, and AA may also explain the better survival of larvae from females treated with BFT.

A study using BFT during the pre-maturation period of *L. vannamei* showed that females with no eyestalk ablation had a greater amount of nutrient reserves in the hepatopancreas, such as acylglycerides and cholesterol, which can increase their ability to produce high-quality eggs (Magaña-Gallegos et al., 2021). During the pre-maturation period, eyestalk-ablated females cultured in both BFT and CW systems had the same nutrient reserves in the hepatopancreas. However, there was a reduction in nutrient reserves (i.e., glucose, cholesterol, and acylglycerides) in eyestalk-ablated females compared to females reared in BFT without eyestalk ablation (Magaña-Gallegos et al., 2021). As a result of eyestalk ablation, females in the BFT system with no eyestalk ablation had the highest number of consecutive spawning periods per brooder and the highest number of eggs per spawning.

6. Positive effects of BFT on larvae quality

The use of high-quality seed could increase the survival rate of fingerlings and reduce the cost of production. This purpose can be accomplished by improving broodstock management, standardizing production procedures in hatcheries and nurseries, and conducting research on high quality seed and fingerling production (Rurangwa et al., 2016). There is a direct relationship between brooders quality and their offspring (Cardona et al., 2016; Harrison, 1990; Zakeri et al., 2011; Hamre, 2011; Izquierdo et al., 2001). In African catfish brooders reared in BFT the quality of larvae significantly improved might be due to the promotion of embryonic development rate (Ekasari et al., 2016). In addition, the survivability and growth rate of larvae increased in BFT regardless of their brooder origin (Ekasari et al., 2016). A possible explanation for this phenomenon is either the improved water quality in BFT that supports a better survival and growth of larvae, or the increment of larval tolerance originated from brooders that reared in BFT.

Live microbial materials in biofloc may act as immunostimulator and enhance the animal's protection against environmental stress by increasing the production of proteins with chaperone functions (e.g., heat shock proteins) that involve in cell protection from protein damage (Rollo et al., 2006) and/or increasing antioxidant capacity (Cardona et al., 2015; De Souza et al., 2016).

In a study, Ekasari et al. (2015a) reported that Nile tilapia larvae originating from the BFT brooders have higher survivability than those originating from the CW tank. BFT larvae were more resistant to *Streptococcus agalactiae* infection and osmotic stress than CW larvae (Ekasari et al., 2015a). Two mechanisms were proposed by the authors of the study regarding the increment of larval tolerance originated from female brooders reared in BFT: first, there is a possibility of offspring inheriting maternal immune protection, including innate and adaptive immune factors such as serine protease-like molecules, lectins, immunoglobins, antibodies, complement factors, and macroglobulins (Seppola et al.,

2009; Swain and Nayak, 2009; Zhang et al., 2013; Padeniya et al., 2022). Also, various parameters may affect maternal immunity, including inherent parental factors (e.g., age, size, nutritional history and maturation level) and environmental factors (e.g., culture condition, water quality, and the biosecurity status) (Zhang et al., 2013). According to Zhang et al. (2013), when brooders are exposed to certain pathogens, they synthesize more immune factors that can be transferred to their offspring that increase their immunocompetence by rapid and efficient immune responses against pathogens. In BFT system, brooders and larvae are exposed to a wide array of microbe-associated molecular patterns derived from heterotrophic bacteria that can be consumed by cultured animals (Ekasari et al., 2015a, 2015b) that activate their innate immunity (Padeniya et al., 2022). The BFT can provide continuous source of live foods that easily accessible for larvae. As a result, larvae in the BFT system have access to food without social interaction such as intracohort competition during feeding that reduce size heterogeneity and may also reduce cannibalism on other potential negative consequences (Bossier and Ekasari, 2017).

7. Perspectives and conclusion

BFT as a system and the microbial aggregates (bioflocs) serve multiple purposes in broodstock production, including maintaining water quality, functioning as a food supplement, and contributing to biosecurity. As reviewed in this manuscript, with limited water changes in BFT, pathogen entry is reduced, and the probiotic properties of bioflocs can limit the activity of pathogens. BFT systems also allow for higher stocking densities while minimal water and space are required. In addition to the biosecurity benefits, bioflocs provide essential nutrients that support reproduction and embryonic and larval development, which can lead to improved larval quality. BFT also allows breeders to intake suspended nutrient-rich particles (bioflocs) in the culture media, which act as a supplementary food source with positive physiologic and immunostimulatory effects, boosting maturation, reproductive performance, and seed quality. Further research is required to exactly examine the influence of BFT on the reproductive performance, e.g. applying molecular methods to properly understand the microbial mechanisms and its impact on the reproductive physiology and later seed quality.

Authorship statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Aquaculture.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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