

REVIEW ARTICLE

Culturing live foods for fish larviculture using non-microalgal diet: The role of waste-generated bacteria and selected commercial probiotics—A review

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Abstract

Condensed suspension of live microalga cells, for example, *Chlorella vulgaris*, *Nanochloropsis oculata* and *Tetraselmis tetrathele* is often utilized as diets for mass production of live food resources, that is, rotifers, copepods, cladocerans and *Artemia*. These live food resources are essential for fish larviculture in hatcheries. However, the production of sufficient microalgae is costly, laborious and fragile, and thus require cost-effective and stable production technologies, especially for the emerging countries. Studies have shown that locally available biowastes such as fish wastes and chicken manure provide substrates for generating billions of heterotrophic bacterial cells and microparticles as well as growth hormones, which can be used in propagating live food resources. The fish wastes contain essential nutrients that are important for the growth of both live foods and fish larvae. With single feeding of fish wastes, the culture condition of live foods may become unstable, and thus bacterial isolates and selected probiotics, for example, genus *Pseudomonas*, *Moraxella* and *Micrococcus* are needed to stabilize the culture conditions to increase reproduction capacity of the cultured live foods. This article consolidates the results and conclusions of our recent studies on the culture of live food resources, that is, *Proales similis* de Beauchamp, *Brachionus rotundiformis* Tschugunoff, *Tigriopus japonicus* Mori and *Diaphanosoma celebensis* Stingelin, using waste-generated bacteria from fish waste diet (FWD) and selected probiotics. The non-algal materials reviewed in this article are important to ensure constant supply of cheap live foods to improve aquaculture, especially in the developing countries, which lack sophisticated technology for production of high-density microalgae.

KEYWORDS

bacterial biomass, chicken manure extract (CME), fish waste diet (FWD), live food, microalgae, single cell proteins (SCP)

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1 | INTRODUCTION

Live food resources for larviculture are mainly small zooplankton of phyla Rotifera (*Brachionus plicatilis* species complex) and Arthropoda (small crustaceans, e.g., *Artemia*, copepods and cladocerans). These zooplanktons constitute a major component of marine finfish larvae and other large-sized crustacean diet (Hagiwara et al., 2001). However, continuous supply of live food resources has been a major bottleneck in marine hatcheries, leading to limited capacity to produce quality fish seeds for aquaculture development. Overreliance of wild-sourced live food materials is impeded by seasonal quantity variabilities and possible transfer of pathogenic substances into the fish hatcheries (Ogello, 2018). Therefore, continuous mass production of quality live foods under controlled conditions is an indispensable need in the aquaculture industry. The production of zooplanktons under ex situ environment follows a classical marine trophic pyramid that begins with production of fresh microalgae (primary energy source), which are then used as a diet for the zooplanktons (Nagata & Whyte, 1992). Several advances have been made to enhance high-density production of live foods since the mid-19th century when their use was pioneered by Japanese scientists (Ito, 1960; Yoshimatsu & Hossain, 2014). However, this system still suffers from a myriad of challenges notably the instability of culture conditions, which result in decline of zooplankton densities, thus affecting larviculture activities in hatcheries.

Two types of food have been used to culture zooplanktons: (1) live microalga and (2) inert foods, for example, condensed microalga products (Dhont et al., 2013; Navarro, 1999). Cultivation of sufficient live microalgae is a heavy burden to most hatcheries because it is laborious, fragile, time consuming, requires a lot of space and has complications in storage (Duerr et al., 1998; Ogello et al., 2017). Several inert food products, for example, freeze-dried microalga, dried microalga (stored at room temperature) and condensed microalga products such as *Chlorella* product have been developed to enhance portability and increase shelf-life (Navarro, 1999). However, the utilization of these products in most aquaculture system is still a challenge because they are sometimes unstable, require constant aeration to avoid particle sedimentation and clogging of the culture system and high cost which discourages their adoption by emerging markets (Fu et al., 1997; Dhert et al., 2001; Yoshimatsu & Hussain, 2014). It is necessary to establish a more cost-effective and stable live food production systems to enhance aquaculture development, especially for emerging countries. This may involve the use of microbes such as bacteria.

Organic wastes such as fish processing wastes, animal and plant manure have been used for several decades in semi-intensive aquaculture to augment the ponds biological activities (Faid et al., 1997; Elsaidy et al., 2015). The organic wastes influence the carbon sequestration in the aquaculture system, thus leading to increased populations of planktons (Mo et al., 2018). Fish wastes are known for their richness in nutrients especially fatty acids and amino acids (Schneider, Sereti, Eding, et al., 2006). Animal manures such as chicken droppings have been shown to contain hormones that can influence reproduction of zooplankton (Kikuchi et al., 2019; Ogello & Hagiwara, 2015).

These organic wastes also provide a suitable substrate for proliferation of bacteria that play important trophic roles in aquatic ecosystems. The most obvious one being the heterotrophic degradation of organic matter, and they are also grazed upon by zooplankton communities (Arndt, 1993; Kang'ombe et al., 2006). Despite the numerous advantages of organic wastes, limited focus has been given to their exploitation as source of nutrients, bacteria and hormones that can be used to promote nutrient cycling, improve reproduction, and water quality in live food cultures. Our review focuses on utilizing organic biowastes as nutrient source for live foods and the role of probiotics in improving the culture conditions. Specifically, we reviewed some of the recent studies on the effect of fish waste diet (FWD) and probiotic products (PB) on population growth and culture conditions of specific live food cultures. At first, we reviewed literature on conventional methods of live food production using microalgae, yeast and biofloc accentuating the chronology of usage, their unique advantages, and limitations. Second, we reviewed studies focusing on the use of FWD and chicken manure extract (CME) for culturing live foods and the role of probiotics in improving culture stability and reproduction of live foods. Finally, we have consolidated the key conclusions and future perspective for live food culture using organic biowastes and probiotics in the aquaculture sector.

2 | MICROALGAE AS DIET FOR ZOOPLANKTON LIVE PREYS

Phytoplankton occupies the primary level of the aquatic food chain, contributing to the production of tons of aquatic resources (Wikfors & Ohno, 2001). Live food culture, especially of rotifers has been achieved by the use of various forms of microalga (live, dried or frozen microalgae), yeast and bacterial concentrates. Among these food sources, microalga are the most preferred because they constitute proteins with essential amino acids, sterols, pigments and polyunsaturated fatty acids (PUFA) (Ghafoor et al., 2020). Several studies have been done to demonstrate the efficiency of different species of microalgae in the production of live foods. The use of live microalgae is frequently employed in outdoor ponds and out-grower systems to produce zooplanktons (Wikfors & Ohno, 2001). In the early years in Japan, only *Nannochloropsis oculata* was found to be suitable diet for live food culture, especially rotifers. However, the production of *N. oculata* was sometimes insufficient for rotifer especially during winter and the rainy season in summer. Due to the inconsistent supply of *N. oculata*, *Tetraselmis tetraethele* was introduced in 1981 from Singapore, but its production suffered similar constraints as those of *N. oculata*. In addition to seasonal variations, other challenges to consistent microalga production include; the need for large culture space and a lot of fertigation (Maruyama et al., 1997; da Silva & Reis, 2015). Also, the production process is tedious and laborious. In the mid-1980s, the use of condensed *Chlorella* and other dried or frozen microalga products as a diet for live food culture was started to lengthen the shelf-life of microalga-based diets (Yoshimura et al., 1997).

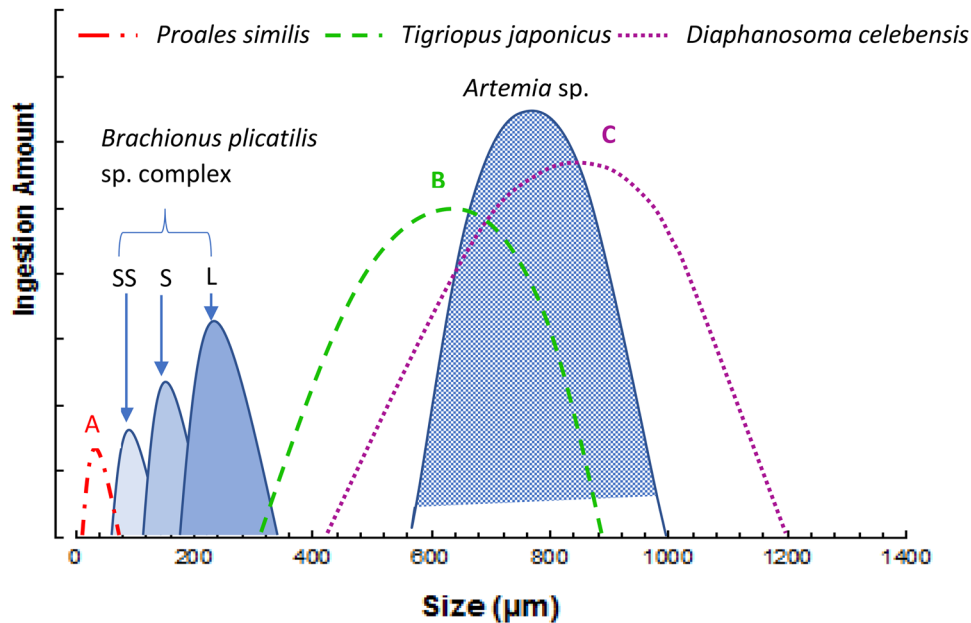


FIGURE 1 Comparative sizes and ingestion amounts of various zooplankton species used in fish larvae culture (L, large; S, small; SS, super small)

Apart from the microalga culture challenges, deficiencies in essential nutrients for the targeted fish larvae such as fatty acids, vitamins and amino acids are a major drawback to utilization of some microalgae (Thépot et al., 2016). The nutritional quality of microalgae is often assayed based on the levels of highly unsaturated fatty acids (HUFAs), especially eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) as well as the concentration of essential amino acids (EAAs). Naturally, the different species of microalgae have varying concentrations of HUFAs. The levels of proteins, amino acids and phospholipids are largely dependent on the metabolism of feed by individual species. *N. oculata* has zero or little amounts of DHA but tends to be rich in EPA with some moderate amount of arachidonic acids (ARA) (Lubzens et al., 1997; Zittelli et al., 1999). On the other hand, DHA enriched *C. vulgaris* is low in EPA and has no ARA (Thépot et al., 2016). Enrichment with HUFAs and vitamins has been used to alleviate nutritional deficiencies for heterotrophically grown microalgae.

The nutritional quality of microalgae is informed by their physical properties. Various microalgal species are characterized by different sizes (Figure 1), and as such, attract corresponding preference by the zooplanktons. Rotifers especially the *Brachionus plicatilis* complex ingest particles of less than 20 µm in diameter, with a preference of particles in the range 2–12 µm (Starkweather & Gilbert, 1977; Vadstein et al., 1993). The foraging behaviour of larger zooplanktons such as copepods and *Artemia* is dependent on the developmental stage and can ingest a wider range of particle sizes microalgae regardless of species. At nauplii stage, the clearance rate of smaller particles (4–8 µm) is high, but at adult stage they show preference for much larger particles (>14.5 µm) (Evjemo et al., 2000; Isari et al., 2013; Jagadeesan et al., 2017; Makridis, 1999).

3 | YEAST AS DIET FOR ZOOPLANKTON LIVE PREYS

The potential of yeast as a suitable replacement for live microalga in larviculture has been studied for years. Yeast cells are small in size (2.5–4.0 µm), high in protein content, relatively low production cost and are not subject to seasonal variation in production (Coutteau et al., 1990; Hirayama, 1987). These attributes made yeast a suitable microalga substitute. Culture experiments of rotifer using yeast as a diet have resulted in high rotifer densities (>1000 ind ml⁻¹). However, fish larvae cultured with yeast fed rotifers had reduced survival rate compared to those produced with rotifers fed on marine *Chlorella*. This has been attributed to low nutritional value of yeast for fish larvae (Nagata & Whyte, 1992; Hamre, 2016). The yeast cells have a thick cell envelope which makes them difficult to digest, hence the low nutrient conversion rate. It has been observed that mechanical disruption of yeast cell envelope using methods such as autolysis and enzyme treatment could improve its digestibility (Coutteau et al., 1990). These treatments facilitate solubility of cytoplasmic contents of yeast cells, thus enhancing their digestibility. However, these treatments lead to the loss of some nutrients and deterioration of water quality due to increased water solubility (Bertolo et al., 2019).

Nutritional deficiencies in yeast have also been assuaged through enrichment processes. Yeast cell can be supplemented with small amounts of HUFAs before being used in live food culture (Øie et al., 1994). Also, supplementing yeast diet with microalga could provide an extra source for the required nutrients. Several authors have observed that mixed diets of microalgae and yeast produce higher population growth of rotifer and other zooplanktons such as cladocerans and *Artemia* (Peña-Aguado et al., 2005; Sarma et al., 2002). The efficiency

of yeast as a diet depends on species, for example, marine yeast (*Candida* sp.), baker's yeast (*Saccharomyces cerevisiae*) and caked yeast (*Rhodotorula* sp.) (Bett et al., 2021).

Microbes play a key role in the utilization of yeast as a diet. Application of yeast to live food cultures induces the growth of microflora which may improve the use of yeast in two ways; (1) providing a supplementary source of nutrients and (2) improving digestibility of yeast cells (Coutteau et al., 1990; Lim et al., 2003). Live food cultures with yeast as diet favour the growth of facultative anaerobic bacteria such as *Pseudomonas* sp., which have been shown to produce vitamin B₁₂ (Hirayama, 1987). Vitamin B₁₂ is responsible for enhancing sexual reproduction in rotifers (Hagiwara et al., 1994; Le et al., 2017). The exoenzymes secreted by the bacteria help in the degradation of the thick yeast cell envelope which increases the digestibility by the live foods (Coutteau et al., 1990).

4 | USE OF BIOFLOC AND INDUSTRIAL SLUDGE IN LIVE FOOD CULTURE

Biofloc technology (BFT) has been used in aquaculture for over 3 decades. BFT combines the removal of excess nutrients from culture media with the production of microbial biomass, which can be used by the culture species as an additional food source (Schryver et al., 2008). This system is based on the knowledge of conventional domestic wastewater treatment system (sludge) which is then applied in aquaculture environments. It involves the variation of carbon and nitrogen ratio that leads to a shift in microbial community within the culture media. When carbon and nitrogen are well balanced, excess ammonia and other nitrogenous wastes in the culture media are converted into bacterial biomass, also called single cell proteins (SCP) (Ahmad et al., 2017; Avnimelech, 1999). Optimum C:N ratio favours the proliferation of heterotrophic bacteria in a fish culture system. The proliferating bacteria consumes organic carbon, that is, it is estimated that 1 g of carbohydrate carbon (C) yields about 0.4 g of bacteria dry weight carbon (C) depending on the C/N ratio, thus immobilizing mineral nitrogen. Avnimelech (1999) estimated that 20 g of carbohydrates is required to immobilize 1 g of nitrogen based on a microbial C/N ratio of 4 and 50 % C in dry carbohydrate. Factors such as dissolved oxygen (DO) concentration, the choice of organic carbon source and the organic loading rate have a significant impact on the floc growth.

Most intensive live food culture systems experience a decline in production due to accumulation of particulate organic matter and other nutrients (Dauda et al., 2019; Yoshimura et al., 1997). These discharged nutrients mainly contain organic carbon, nitrogen and phosphorus, and this could result in significantly elevated concentrations which can eventually lead to the collapse of the culture. In most of these systems, the removal of particulate matter and excess nutrients extends till the harvesting period (Schneider, Sereti, Machiels, et al., 2006). Therefore, a bacterially mediated mechanism for improving the culture condition and efficient utilization of nutrients could be the solution to the constant collapse of live food cultures (De Araujo et al., 2000). Carbon supplementation in such system can restore proper C:N ratio enabling

solid waste conversion into bacteria biomass. BFT technology has been applied in the management of water quality and enhancing growth of zooplankton species in outdoor ponds and out-grower systems. In both BFT and activated sludge systems, the choice of a suitable carbon source to facilitate the proliferation of beneficial bacteria is still a challenge, and supplementation with probiotic products such as yeast, lactic bacteria and bifidobacteria could be a possible solution.

5 | USE OF ORGANIC WASTE AS A DIET FOR LIVE FEEDS

5.1 | Effect of FWD on population growth of rotifers

Organic wastes such as food wastes, fish wastes and plant or animal manures have been employed as a diet in aquaculture systems to enhance zooplankton population (Faid et al., 1997; Mo et al., 2018). The use of fish processing wastes in fishmeal production in aquaculture is also widespread. However, its use in intensive live food production is still limited. It is estimated that more than 40% of the annual global fish captured (about 80 million tons) are discarded as wastes (Kristinsson & Rasco, 2000). Fish wastes are great sources of proteins, amino acids, minerals and fats, with abundant mono-unsaturated oleic acids (Faid et al., 1997; Rebah & Miled, 2013; Schneider, Sereti, Machiels, et al., 2006). Since fish wastes are considered as low value materials, the successful application of these wastes as a diet source for live food could significantly lower the production cost and can be a substitute for microalgae in larviculture.

Some recent studies have demonstrated the suitability of fish processing waste (FWD) as diet of rotifer (Ogello et al., 2018). In one of the studies, *Brachionus rotundiformis* cultured on fish waste with the addition wheat flour as a carbon source showed significantly high densities compared to microalgae-fed rotifers during the second and third harvesting phases but declined at the last harvest (Ogello et al., 2018). Higher growth peaks and specific growth rate (0.342 day⁻¹) were observed in cultures of *Proales similis* with fish waste as a diet (Kagali et al., 2018). The increase in rotifer density with the addition of FWD in these studies can be attributed to (1) direct feeding by the rotifers on fish waste microparticles and bacteria as result of degradation of FWD, (2) production of enzymes and other nutritional factors such as vitamin B₁₂, (Zink et al., 2013) and (3) shift of microbial community composition that favours culture stability (Qi et al., 2009; Planas et al., 2004).

The rapid increase in the density of heterotrophic bacteria biomass because of disintegration of fish waste depends on the carbon (C):nitrogen (N) ratio. The optimal C:N ratio for heterotrophic bacteria production is about 12–15 C:N (Avnimelech, 1999; Schneider, Sereti, Eding, et al., 2006). The C:N ratio in a culture media can be manipulated by using low protein diets or supplying additional carbon sources (Hu et al., 2017). Sodium acetate has been used effectively as a suitable carbon source for experimental purposes. However, due to its high cost, applying it in large scale cultures is not recommended. Optimal C:N ratio aids in the biotransformation of excess NH₃, thus stabilizing the

water quality (Schneider, Sereti, Machiels, et al., 2006). In our studies, we used wheat flour as a carbon source for the outdoor tank cultures (C:N ratio of 16) and low FWD concentration of 0.5 and 0.75 g/L for the glass bottle cultures (Kagali et al., 2018; Ogello et al., 2018).

5.1.1 | Bacterivory among zooplankton species other than rotifers

The suitability of bacteria as a nutrient source for culturing zooplankton can vary depending on the taxa and size. Literature is replete with evidence that effectiveness of bacteria as a diet for live foods decreases with increase in the size of zooplankton (Ogata et al., 2011; Vadstein et al., 1993). Macro-zooplankton especially crustaceans have been shown to feed on bacteria in the wild (Agasild & Nøges, 2005). Also, there are evidence of increase in population of macro-zooplankton in fishponds with addition of bioflocs, which are a source of bacteria (Muthoka et al., 2021). However, there is insufficient studies on the potential of culturing macro-zooplankton using bacteria as a sole diet. To evaluate the effect of FWD on macro-zooplankton in our recent experiments, we cultured copepod: *Tigriopus japonicus* and cladoceran: *Diaphanosoma celebensis* while utilizing microalgae *Tetraselmis tetraathele* and fish waste as diets. Highest density of copepods was observed with cofeeding *T. tetraathele* and FWD. However, FWD alone resulted in unstable growth curve. On the other hand, density of cladocerans was lowest with all FWD compared to microalgae diet.

Macro-zooplanktons play an important role in marine fish culture. Given their wide size range: *T. japonicus* 250–1000 μm and *D. celebensis* 450–1200 μm (Figure 1), they can be used at various stages of fish larva development. Even though monodiets of FWD did not enhance population growth of these macro-zooplanktons, FWD can be co-fed with microalga diets which could improve their nutritional value due to high contents of essential nutrients in FWD. Jung and Hagiwara (2001) reported an increase population of *T. japonicus* in synxenic bacteria cultures. They further postulated that bacteria could modify the interspecific relationship between zooplankton leading to variations in population growth. Other studies also confirm the existence of interspecific relationship in composite culture of various zooplanktons (Han, 2019; Gao et al., 2021). Further exploitation of this mechanism could result in enhanced production. *T. japonicus* nauplii efficiently feed on bacteria, however, may be insufficient as a diet for copepodites and adults (Wang et al., 2015). Supplementation of microalga and bacteria could reduce the amount and cost of high-density microalga used in zooplankton culture.

5.2 | Effects of chicken manure extract on live foods

Organic manure both from animal and plant materials have been used in aquaculture as a source of humic matter to accelerate primary production (Elsaidy et al., 2015). Humic matter influences the lacustrine planktonic food web by supplying the necessary nutrients as well as,

adjusting the physical and chemical environment. Despite their long use, employment of manure in high density zooplankton production systems is still not well developed. Chicken manure has been shown to contain 1–904 $\text{ng}\cdot\text{g}^{-1}$ of 17β -estradiol and 0.05–254 $\text{ng}\cdot\text{g}^{-1}$ of testosterone (Bevacqua et al., 2011; Jenkins et al., 2006). These sex hormones are linked to influencing reproduction and survival of various zooplankton (Hagiwara et al., 2016; Kikuchi et al., 2019; Nakamoto, 2008; Ogello & Hagiwara, 2015). The method of preparing CME was developed by Fukuoka Fisheries and Marine Technology Research Center (Fukuoka city, Japan) and has been in use for over 20 years in the production of *Daphnia* sp. (Nakamoto, 2008). In brief, chicken manure and fossil coral powder are mixed with pond water then boiled for about 1 h. The mixture is left to settle overnight at room temperature and then the supernatant is filtered using a nylon net (100 μm). Ogello and Hagiwara (2015) observed that addition of 2.0 $\text{ml}\cdot\text{L}^{-1}$ of CME on rotifer cultures enhanced their population growth, mixis and body size. They further postulated that, CME acted as hormone that synergistically augmented with rotifer diet, thus increasing both mictic and amictic reproduction.

Another study by Kikuchi et al. (2019) showed that addition of CME to copepod *T. japonicus* cultures induced their reproduction by 1.5–1.7 times and showed 7.4 times higher survival rate. Several studies have shown that sex hormones such as 17β -estradiol, testosterone, oestrogens and androgens can influence reproduction of zooplankton as a result of endocrine disruption (Gallardo et al., 1997; Marcial & Hagiwara, 2007; Preston et al., 2000). Addition of chicken dropping to fishpond also leads to production of heterotrophic bacteria that can be utilized by rotifer and other zooplanktons as a diet (Jenkins et al., 2006). Ogello et al. (2019) blended CME and FWD to culture zooplankton in outdoor culture tanks. In this study, there was significantly higher production of zooplankton in CME + FWD cultures (SGR of 0.42–0.62 day^{-1}) than in control cultures (SGR of 0.35–0.48 day^{-1}). The CME probably facilitated phytoplankton growth in the tanks, thus expanding forage base (i.e. bacteria and phytoplankton) for the zooplankton growth and reproduction. This demonstrated the importance of the synergy of FWD and CME for zooplankton growth and reproduction (Ogello et al., 2019).

5.3 | Limitations of organic waste-based diets

Rotifer culture using organic waste diet is often conducted in agnotobiotic conditions, and therefore it is difficult to regulate the diversity of bacteria that proliferate. Given the specific nature of bacterial action on live foods, it would be important to regulate the bacteria strains used. Also, pre-treatment of waste to eliminate pathogenic microbes involves temperature manipulation either through heat treatment (Esteban et al., 2007) or freezing, which could further denature the nutrients in these wastes (Mo et al., 2018). In our study, FWD was stored at -40°C before being used in the experiments. Freeze treatment could be effective to eliminate majority of pathogenic bacteria, but it might not effectively eliminate psychrophilic bacteria and other cyst forming pathogens. Other limitations on the use of these

ensiled products include quick deterioration of water quality at high concentration (Kagali et al., 2018; Liao et al., 1997) and highly offensive odours, which may be a restrictive factor for indoor utilization (Coello et al., 2000; Faid et al., 1997).

6 | EFFECT OF PROBIOTICS ON LIVE FOOD CULTURES

Bacteria play a key role in nutrient cycling of dissolved organic carbon and transfer of these energy to primary and secondary trophic levels in aquatic food webs (Natrah et al., 2014). Various ecological studies have accentuated the significance of the processes within planktonic-microbial web in the functioning of the marine and limnetic ecosystem (Fermani et al., 2013; Work & Havens, 2003). Under ex situ conditions, bacteria have been shown to play a major role in variability and instability of live food cultures as well as that of marine predator larvae (Verschuere et al., 2000). A lot of focus has been placed on manipulation of the microbial community composition to enhance culture stability and reduce proliferation of harmful bacteria (Bentzon-Tilia et al., 2016). However, the utilization of bacteria as sole nutrient source for live foods is still debatable.

Some of the early studies show that bacteria communities can be used to influence live food culture conditions and population growth. Bdelloidea rotifers such as *Leane inermis* and *Philodina acuticornis* are bacteriophagous and can be cultured successfully with bacteria alone as a diet (Moreira et al., 2016; Ricci, 1984). Monogonont rotifers on the other hand show more specificity in type of bacteria that can be utilized as diet. Much smaller rotifer like *P. similis* and *Keratella cochlearis* can indiscriminately utilize bacteria as a diet source (Bogdan et al., 1980; Lopez et al., 2007). However, for the larger rotifers, the combination of bacteria and microalgae results in much higher population growth unlike bacteria monodiets. For the much larger live foods, single cells of bacteria can be insufficient diet and flocculation can be used to enhance the growth of these species (Hwang & Heath, 1999; Ritala et al., 2017).

Probiotics have been used in terrestrial animals for decades; however, there has been growing interest in their use in aquaculture production in recent years. The expert panel convened by the Food Agricultural Organization (FAO) of the United Nations and supported by the World Health Organization (WHO) described probiotics as live organisms that confer benefits to the host organism when administered in adequate amounts (FAO & WHO, 2002; Hill et al., 2014; Reid et al., 2019). Understanding the effects of various probiotics is complex, and the effective understanding of their working mechanisms is a daunting task (Vdastein et al., 2018). Several authors have attempted to accentuate the significance of probiotics on live food cultures, but it has been limited to the effects on growth, survival, feed conversion and controlling pathogenic microbes (Douillet, 2000; Grotkjaek et al., 2016; Hauville et al., 2016; Lamari et al., 2014); see Table >S1.

From our recent studies, it is evident that probiotics containing lactic acid bacteria (LAB) enhance parthenogenic reproduction which is responsible for population density increase while on the other hand

repressed mictic reproduction that results in resting egg formation (Kagali et al., 2019). The high-amictic reproduction in probiotic-fed rotifers leads to high population growth rate. Bacteria have been shown to influence rotifer population growth in various ways: (1) nutritional effect through direct bacterivory by rotifers, enhancing digestibility of microalgae and secretion of other nutritional factors such as Vitamin B₁₂ and EPA (Le et al., 2017; Nichols et al., 1996) and (2) stabilization of culture environment through shifting microbial community diversity and recycling nitrogenous wastes.

Reproduction of rotifers is internally regulated by factors such as rotifer strain and molecular action or externally by environmental stressors such as temperature, hormones, salinity, food type, food concentration and bacteria (Gilbert, 2016; Kogane et al., 1997; Snell, 2017; Suga et al., 2011). In our recent experiments, we observed that the mictic reproduction of rotifers was repressed by the addition of probiotics (Kagali et al., 2019). Probiotics are live microbial supplement which are intended to benefit the host by improving its microbial balance (Cruz et al., 2012). However, other studies on rotifer mixis induction by bacteria have reported mixed outcomes. For instance, Hagiwara et al. (1994) observed that the effect of bacterial strains on rotifer mixis induction is quite specific. Out of 17 bacteria strains employed in their study, only five strains in the genus *Pseudomonas*, *Moraxella* and *Micrococcus* induced higher sexual reproduction.

Higher mixis can result in decreased rotifer density due to decreased birth rate of amictic females which are responsible for rotifer population growth (Spencer et al., 2001). On the other hand, lower mixis means higher population growth, which is the desired outcome for aquaculturists who are more concerned with intensive rotifer production, but it compromises on the longevity of rotifers. Bacterial effects can be attributed to three factors: (1) utilization of microalgae is increased due to enhanced digestibility by bacteria action. This can be as a result of secretion of extracellular enzymes by bacteria which helps in degrading microalgae (Hauville et al., 2016; Sun et al., 2010). This increases food availability which favours amictic reproduction. (2) Bacteria secreted enzymes and bacteriocins which aggravate the rotifer environment, thus leading to less investment in sexual reproduction. (3) The shift in microbial community structure in the rotifer culture due to the influence probiotics. Several authors have posited that probiotics dominate growth in culture mediums, thus decreasing growth of other microbes (Laloo et al., 2010; Le et al., 2017; Qi et al., 2009).

7 | DIETARY VALUE OF WASTE-FED ROTIFERS TO FISH LARVAE

Rotifers have broad nutritional requirements that must be met to produce stable cultures. Ogello et al. (2019) conducted a preliminary study on the dietary value of FWD-fed rotifer *B. rotundiformis* (SS-type) for larval rearing of marine fish larvae *Silago japonica* under laboratory conditions. Even though the study recorded a low survival rate of 9.7%, which is expected in most marine fish species, the value was comparable to the control experiment in which supplemented microalgae, *C. vulgaris*, was used to culture the rotifers (Ogello et al., 2019).

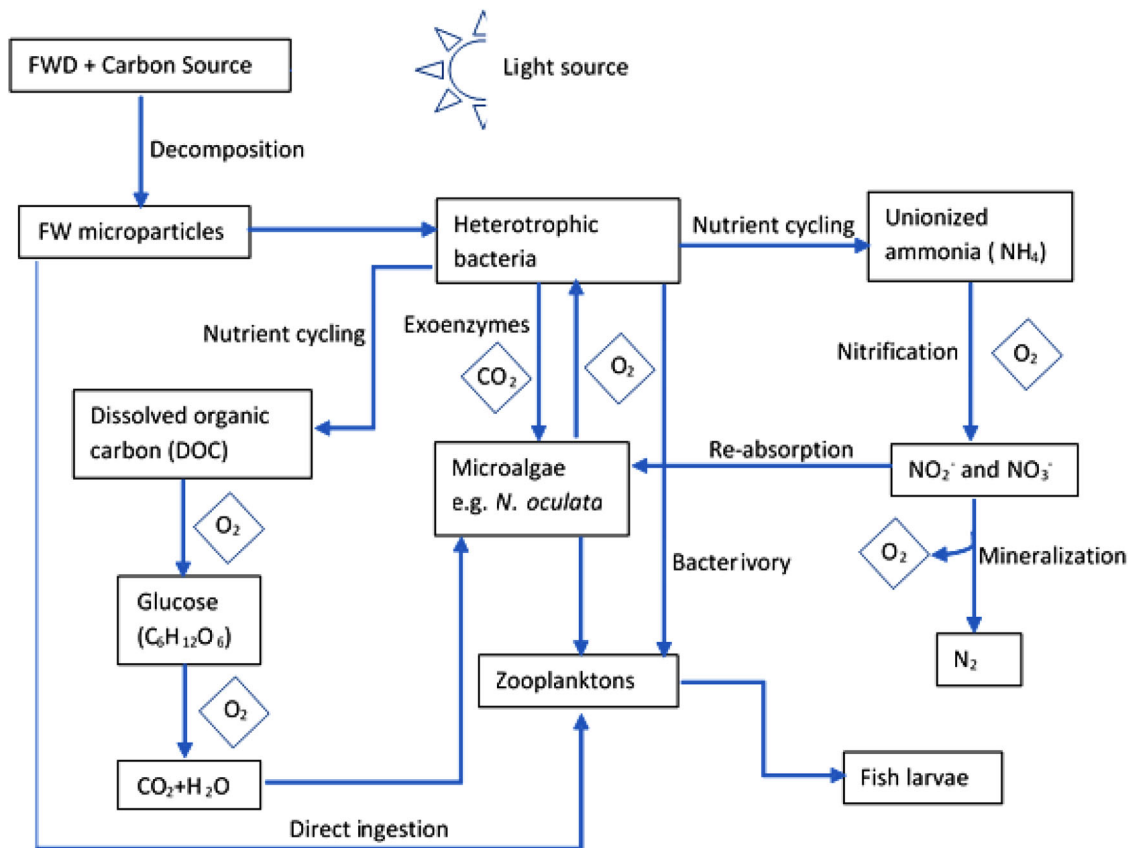


FIGURE 2 A schematic representation of diverse working mechanisms involved during the culture of live foods using fish processing waste (FWD). The arrows indicate the flow of energy in an aquaculture system using FWD

Also, the larval fish development parameters between the test and control diet were similar, thus suggesting a possibility of FWD for the larviculture of *S. japonica*.

The success of larval fish rearing strongly depends on the amount of DHA and ARA present in the diet (Sargent et al., 1999). Since most marine fish larvae cannot synthesize DHA from precursor molecules, for example, EPA or α -linolenic acid (ALA), supplying DHA-rich feeds to fish larvae is important (Masuda et al., 1998). Ogello et al. (2019) obtained DHA/ARA ratio of 2.4 in the waste-fed rotifers, which is a positive step towards culturing marine larval fishes without supplementation with expensive emulsions. However, the optimal DHA/ARA ratio required for proper larval fish development is 10.0 (Sargent et al., 1999).

8 | CONCLUSION AND FUTURE PERSPECTIVES

A suitable diet for live food culture is not only a factor of nutritional content but rather a combined result of the nutritional value of the diet, the cost associated with obtaining and maintaining the diet and the foraging preference of the diet by the live food species. Based on the findings in our studies, FWD offers a suitable alternative to microalgae-based diets for culture of small zooplanktons especially rotifers (Kagali et al., 2018; Ogello et al., 2019). The action of FWD diet can be attributed to its degradation into microparticles and prolifera-

tion of bacteria (Kagali et al., 2018) which are utilized by the zooplanktons as a diet (Figure 2). This could lower the overall cost of production by reducing the investment in microalga production.

Waste-generated bacteria have been produced traditionally in aquaculture ponds through the application of animal manure and other waste products which enhance population of zooplanktons. However, the utilization of these bacteria in high density live food production system has not yet gained prominence. This article provides insights in the potential of fish processing waste as a diet for culturing rotifer and other zooplanktons. The findings in some of our research also demonstrates some of the mechanism of action for FWD. However, there are still some gaps in characterization of bacteria strains involved and various influences on rotifer behaviour. Future research will centre on elucidating these mechanisms.

Probiotic products can be combined with FWD or microalga diets and used in stabilization of the live food cultures. Given the mediation role played by the probiotics on the microbial community as well as the influence on rotifer reproduction, we can therefore infer the important role probiotics can play in enhancing high density rotifer production. Probiotics are prolific and domineering in their growth and therefore can be used to shift the microbial community distribution in axenic rotifer cultures using FWD to favour the growth of beneficial bacteria. Also, the ability of bacteria to enhance degradation of microalgae and other organic particulates could lower the amount of feed used by increasing nutrient efficiency as well as lowering the cost of filtration to

clear the excess sediments. There are a number of probiotic products in the market, and due to the specificity in the action more research should focus on identification of suitable probiotics for live food culture and the elucidation of various mechanisms of action.

Therefore, the non-microalgal diet offers an opportunity to (1) reduce environmental pollution sources by reusing poorly discarded fish wastes; (2) reduce or eliminate direct dependence on the immediately cultured or the expensive on-site microalgae production; (3) to lower the cost of rotifer enrichment, thus making it convenient for aquaculture production, especially in the less developed countries, where malnutrition is prevalent and (4) makes it possible for year round production of live food resources, which are valuable inputs for enhancing aquaculture activities.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The review research was done according to standard ethics requirements. All reviewed literature have been duly cited and acknowledged.

AUTHOR CONTRIBUTIONS

Conceptualization, writing—original draft and writing—review and editing: Robert Nesta Kagali. Writing—review and editing: Catherine Wachera Kiama. Writing—review and editing: Hee-jin Kim. Writing—review and editing: Stenly Wullur. Writing—review and editing: Yoshitaka Sakakura.

DATA AVAILABILITY STATEMENT

The data presented is freely available for the audience.

PEER REVIEW

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