DOI: 10.1111/are.13707

ORIGINAL ARTICLE

WILEY Aquaculture Research

Fish-processing wastes as an alternative diet for culturing the minute rotifer *Proales similis* de Beauchamp

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Funding information
Japanese Society for Promotion of Sciences (JSPS) KAKENHI, Grant/Award Number: JP17H038662; Japan International Cooperation Agency (JICA); JKUAT/PAUSTI/AU Network project_Nagasaki University, Grant/Award Number: J1610573

Abstract

We evaluated the suitability of fish waste diet (FWD) for culturing the minute rotifer *Proales similis* through the observation of their population growth and particle size selective feeding. A total of five treatments either with *Nannochloropsis oculata* or FWD (0.75 and 0.50 g/L) or the combination of *N. oculata* and FWD were set up in triplicates. *P. similis* were cultured in diluted natural seawater (8 g/L salinity) and 26 ± 1°C with the diet treatments being applied randomly. We daily monitored the rotifer density, the number of bacteria and the water quality in all the cultures. The population density of *P. similis* increased exponentially in all treatments, while the mean growth in FWD 0.75 g/L was significantly higher ($p < .05$) than that in the control. Addition of *N. oculata* to FWD resulted to lowered *P. similis* population growth. Bacterial colony count was high in FWD and introduction of *P. similis* to the diet decreased their density. The estimated bacteria ingestion rates were generally in the range of 6.03×10^2–1.24×10^4 bacteria/rotifer/hr and there was a positive linear relationship between bacterivory and rotifer population growth. We also observed a shift in the particle size distribution with a reduction in the frequency and concentration of small-sized particles (<2.5 μm) at day 6. These results accentuate the potential of fish-processing waste as diet for culturing *P. similis* which feeds on bacteria and small particles (≤2.5 μm) that are by-products of degradation of this diet.

KEYWORDS
bacteria, fish waste diet, minute rotifer, particle size, *Proales similis*

1 | INTRODUCTION

Rotifers are among the most important aquatic organisms due to their ubiquitous nature in most aquatic ecosystems. They are also very diverse with about 2,220 species known so far (Le et al., 2017). Among these, species in the genus *Brachionus* have been exploited for decades as live food for the initial stages of marine fish larviculture (Hagiwara, Gallardo, Assavaaree, Kotani, & De Araujo, 2001). The most exploited species being *Brachionus plicatilis* which is a species complex comprising 15 morphotypes described based on the size of lorica as large (L-type), small (S-type) and super small (SS-type) (Hagiwara, Suga, Akazawa, Kotani, & Sakakura, 2007; Mills et al., 2017). The lorica length of *B. plicatilis* ranges between 130 and 340 μm while that of *Brachionus rotundiformis* Tschugunoff ranges between 100 and 210 μm (Hagiwara et al., 2007). This poses a challenge to hatcheries where they culture fish larvae with mouth gapes <100 μm after hatching such as groupers.

Proales similis de Beauchamp is a minute rotifer and is a promising live food for rearing fish larvae with small mouth gape (Wullur, Sakakura, & Hagiwara, 2011). *P. similis* species belongs to the genus *Proales* Gosse 1886 which comprises of 44 species currently (Segers, 2007; Segers & Wallace, 2001; Wilts, Bruns, Fontaneto, & Ahlrichs,

2012). The total body length and body width ranged between 40 and 110 μm and 40 ± 6 μm, respectively (Wullur, Sakakura, & Hagiwara, 2009). The small size nature compounded with its lack of lorica results to better ingestion and digestion by fish larvae. Literature abounds with evidence of *P. similis* as a suitable initial food for a number of marine fish larvae. Hirai et al. (2012) in their study showed that *P. similis* was suitable for the rearing of humphead wrasse (*Cheilinus undulatus*) larvae at the start of feeding. Other research on seven band grouper (*Epinephelus septemfasciatus*) larvae (Wullur et al., 2011) and Japanese eel (*Anguilla japonica*) larvae (Hagiwara, Wullur, Marcial, Hirai, & Sakakura, 2014; Wullur et al., 2013) has resulted in similar conclusion.

Rotifer production in majority of hatcheries follows a classical food chain in aquatic system that involves rotifers feeding on microalgae and being used as live feed for fish larviculture. Most common is the use of enriched "Chlorella" product (Hirayama, 1987; Maruyama & Hirayama, 1993). However, the production of quality and sufficient microalgae for rotifer production is costly, laborious and fragile, and thus, requiring cost-effective and stable technologies. The discovery of "microbial loop" concept by Azam et al. (1983) showed that bacteria can act as a primary source of energy that can be transferred to higher trophic levels in this case, protists and metazoans including rotifer (Agasild & Nõges, 2005; Hagiwara, Hamada, Hori, & Hirayama, 1994; Planas et al., 2004; Villamil, Figueras, Planas, & Novoa, 2003). Initially, baker's yeast was used as a diet for *Brachionus* sp. as well as decomposed to propagate bacteria thus supporting rotifer growth (Hirayama & Funamoto, 1983). However, the baker's yeast cultures were unstable and low in nutrients. Waste-generated bacteria can be exploited for culturing live feeds, especially *P. similis* due to its minute size.

Fish-processing wastes provide an excellent source for microbial growth, especially bacteria (Faid, Zouiten, Elmarrakchi, & Achkari-Begdouri, 1997; Liao et al., 1997; Martone, Borla, & Sánchez, 2005; Rebah & Miled, 2013). Twenty-five per cent of the total fish catch worldwide is considered as waste (FAO, 2016; Rebah & Miled, 2013) and they lead to environmental pollution if they are not utilized or disposed well. To avoid this, fish wastes can be incorporated into rotifer production cultures as an alternative to the microalgal diets. Fish is a rich diet and it has been shown to be an excellent source of protein, amino acids, oils, especially omega 3 and 6 (Ghaly, Ramakrishnan, Brooks, Budge, & Dave, 2013), as well as enzymes (Coello, Brito, & Nonus, 2000; Rebah & Miled, 2013; Vázquez, Docasal, Mirón, González, & Murado, 2006). Studies have reported successful production of rotifer species, for example, *B. plicatilis* (Hino & Hirano, 1984; Hirata, Murata, Yamada, Ishitani, & Wachi, 1998; Watanabe, Sezaki, Yazawa, & Hino, 1992) and *B. rotundiformis* (Loo, Chong, Vikineswary, & Ibrahim, 2016; Ogello, Wullur, Sakakura, & Hagiwara, 2017) using waste-generated bacteria; however, such studies are not yet reported for *P. similis* which is an emerging larval fish food in marine hatcheries.

The protocol for incorporating fish waste diet (FWD) into the culture media has initially been developed in our laboratory (Ogello et al., 2017) and successfully used in culturing *B. rotundiformis*. In this research, we evaluated the effect of FWD on the population growth of *P. similis*. We also observed variations in population density of bacteria during the culture period and the bacterivory by *P. similis*. Size selective feeding by *P. similis* on bacteria, fish waste particles and *Nannochloropsis oculata* was also investigated. Based on this study, it was observed that fish-processing waste can be a potential alternative diet for culturing minute rotifer *P. similis*. These findings have significance in the design of appropriate culture conditions, diet and feeding regime for *P. similis*. The use of fish-processing waste as a diet for culturing rotifer will provide a cheaper alternative to more costly microalgae diet, especially for emerging economies, and will encourage its adoption.

2 | MATERIALS AND METHODS

2.1 | Rotifer

Stock cultures of *P. similis* were obtained from Aquaculture Biology Laboratory at Nagasaki University. The *P. similis* was initially collected from an estuary on Ishigaki Island in Okinawa, Japan, and clones have been maintained under our laboratory conditions (at 25°C and 22 g/L salinity) (Wullur et al., 2009) for over 10 years. The rotifer stock cultures were optimized to experimental conditions with preliminary experiments with different salinities and temperatures and then acclimatized to the FWD to prepare for the observations.

2.2 | Preparation of FWD

Fish wastes (heads and flesh) of the chub mackerel (*Scomber japonicus* Houttuyn, 1782) were collected from various fish markets in Nagasaki prefecture, Japan, and were frozen at −80°C. The fish wastes were weighed to desirable weight for each treatment (Table 1) using a digital balance and then wrapped with plankton net 200 μm of mesh size. Ogello et al. (2017) established that 0.5 g/L fish waste diet was optimum for culturing rotifer *Brachionus rotundiformis*; therefore, in range-finding test, we used a lower and upper weight of FWD.

2.3 | Experimental design

The minute rotifer *P. similis* was cultured in 600 ml glass bottles containing GF/C filtered, diluted natural sea water (8 g/L salinity) and sterilized by autoclaving at 121°C for 15 min. The temperature was adjusted at 26 ± 1°C during the culture period. The initial stocking density was 53 individuals (ind)/ml in each treatment. In this experiment, we considered five treatments as follows:

Daily monitoring was conducted by taking 1 ml triplicate samples from each treatment and counting the number of individuals fixed by Lugol's iodine on a Sedgewick Rafter cell chamber under a dissecting microscope. The averages of the samples were used to estimate the population per millilitre in each treatment.

TABLE 1 Description of the treatments used in the experiment. The culture labels were randomly generated and were used as a reference to treatments in this study only

Culture label	Diet	Rotifer	Aim
CON	*Nannochloropsis oculata* 2.5×10^6 cells per ml	Yes	Control
FWD1C	0.75 g/L FWD only no rotifer	No	Evaluate bacteria growth in FWD
FWD1A	0.75 g/L FWD only	Yes	To test the effect of higher diet concentration
FWD1B	0.75 g/L FWD + 2.5×10^5 *N. oculata* only at the start of the experiment	Yes	To test the effect of cofeeding at high FWD concentration
FWD2A	0.5 g/L FWD only	Yes	To test the effect of lower diet concentration
FWD2B	0.5 g/L FWD + 2.5×10^5 *N. oculata* only at the start of the experiment	Yes	To test the effect of cofeeding at low FWD concentration

FWD, fish waste diet.

Growth rate was calculated using the formula $\mathrm{gr} = \left[\frac{\ln N_t - \ln N_0}{t}\right]$ where gr = specific growth rate; N_t = total population at time (t); N_0 the population at the start of the experiment; and t = time (Lotka, 1913). Population doubling time (T_d) was calculated using the formula, T_d = ln (2)/gr where gr = specific growth rate (Lotka, 1913).

2.4 | Effect on water quality parameters

Water quality parameters (temperature, dissolved oxygen (DO), salinity and pH) were assessed daily to establish the health of the culture. The water temperature and DO levels were taken using Micro TX3-Micro fibre-optic oxygen transmitter (PreSens – Precision Sensing GmbH, Germany); the water salinity was measured with a refractometer and the pH by pH meter (HM-30G DKK TOA, Japan). For the particle size density measurements, the culture media were sampled every 2 days from each treatment and the density was then assayed using a Sysmex PDA-500AD particle density counter (Sysmex, USA). Ammonia concentration in the treatments was monitored using a photometer system for water analysis (Palintest ® 8000 Ltd, USA) according to the company's protocol.

2.5 | Isolation and characterization of bacterial flora in the cultures

The culture media of 10 ml were sampled from each treatment and serially diluted three times (10^0, 10^{-1}, 10^{-2} and 10^{-3}) before inoculating onto Marine agar 2216 Difco™ plates (90 × 15 mm petridish) (Devaraja, Yusoff, & Shariff, 2002; Rodina, 1972) and incubated for 30 hr at 28 ± 1°C under 12:12 hr light regime. The number of colony-forming units (CFUs) of bacteria was counted under stereomicroscope and used to estimate the abundance of bacteria in each treatment.

2.6 | Estimation of bacterivory by *P. similis*

Grazing rate of *P. similis* was predicted using an empirical model (Vaque, Gasol, & Marrase, 1994) that estimates community grazing rate (GT, in bacteria/ml/hr) from independent variables zooplankton abundance (MR, individuals/ml), temperature (T, in °C) and bacterial abundance (BAC, in number of colony-forming units, CFUs/ml).

$$\log \mathrm{GT} = -3.21 + 0.99 \log \mathrm{MR} + 0.28T + 0.55 \log \mathrm{BAC}$$

2.7 | Data analysis

All analyses were performed with the aid of R-software (R 3.4.2). First, all the data were subjected to a Bartlett's test to test for homoscedasticity. Then, the mean population growth rate and water quality parameters in all the treatments were compared by two-way ANOVA. All significantly different means were separated using the Tukey–Kramer test at 0.05 level of significance (Lander, 2017). The growth and population data were log transformed to normalize the data and fitted in linear regression models to compare relationships within various variables.

3 | RESULTS

All the treatments showed an increasing population from day 0 to day 10. The highest individual density (1,605.3 ± 45.0 ind/ml) was observed in FWD1A treatment where we had the highest amount of fish waste diet (0.75 g/L). Addition of phytoplankton in the same diet quantity (FWD1B) recorded lower population density (1,248.1 ± 26.1 ind/ml) (Figure 1). There was significant difference in rotifer density in all the five treatments ($p < .01$, $F = 95.83$, $n = 3$), time ($p < .001$, $F = 878.01$, $n = 3$) and their interactions ($p < .01$, $F = 5.98$, $n = 3$) with FWD1A yielding higher density compared to control on day 7 (Tukey–Kramer test, $p < .01$). The amount of fish waste diet also influenced the population growth of *P. similis* as observed in FWD1A which had the highest amount of diet and the highest rotifer density compared to FWD2A which had the lowest amount and lower rotifer density (1,297.04 ± 7.35 ind/ml) ($p < .01$, $F = 19.41$, $n = 3$).

There was a significant difference in the growth rate among all treatments ($p < .05$, $F = 17.55$, $n = 3$). The highest growth rate (0.342 ± 0.002 per day) was observed with FWD1A treatment. FWD1A and CON1A had the lowest doubling time 2.02 ± 0.016 and 2.06 ± 0.007 days, respectively. This was significantly lower compared to the other three treatments ($p < .05$, $F = 15.98$, $n = 3$) (Table 2). It took 2 days to double the population of *Proales similis* regardless of the diet used.

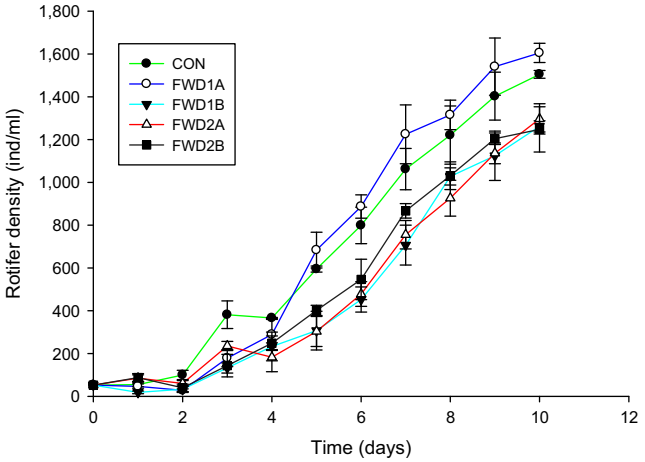

FIGURE 1 Growth performance of *Proales similis* under five different culture conditions (mean ± *SD*, *n* = 3). CON: *N. oculate* 2.5 × 10^6 cells per ml; FWD1A: 0.75 g/L fish waste diet; FWD1B: 0.75 g/L fish waste diet + *N. oculate* 2.5 × 10^5 cells per ml; FWD2A: 0.5 g/L fish waste diet; FWD2B: 0.5 g/L fish waste diet + *N. oculate* 2.5 × 10^5 cells per ml

TABLE 2 Growth rate (gr), doubling time (T_d) and highest density at day 10 of *Proales similis* in all treatments

Treatment	Growth rate (gr)	Doubling time (T_d)	Highest density (ind/ml)
CON	0.336 ± 0.001^b	2.066 ± 0.007^b	1,505.0 ± 18.33^b
FDW1A	0.342 ± 0.002^a	2.027 ± 0.016^b	1,605.3 ± 44.97^a
FWD1B	0.318 ± 0.002^c	2.180 ± 0.014^a	1,262.0 ± 26.05^d
FWD2A	0.321 ± 0.005^{bc}	2.161 ± 0.037^a	1,297.0 ± 71.21^c
FWD2B	0.317 ± 0.008^c	2.187 ± 0.057^a	1,248.0 ± 105.83^{cd}

The letters represent the differences in means where a > b > c > d (one-way ANOVA, Tukey–Kramer test).

Dissolved oxygen in all treatments reduced gradually from the initiation of the experiment and stabilized slightly towards the end of the experiment (Figure 2a). As fish waste decomposed, the oxygen demand increased. The introduction of rotifer compounded this demand and the dissolved oxygen in the culture media rapidly reduced from day 0 to day 6. There was a significant difference in all treatments ($p < .05$, $F = 6.55$, $n = 3$) with control treatment having higher DO concentration (5.46 ± 0.07–8.02 ± 0.12 mg/L) than all other diet treatments in all days ($p < .001$, Tukey–Kramer test). The DO concentration in FWD1B (0.75 g/L FWD + *N. oculata*) was lower (3.60 ± 0.05–5.70 ± 0.20 mg/L) than in FWD1A (0.75 g/L FWD) (4.85 ± 0.15–6.05 ± 0.10 mg/L). Ammonia (NH_3) concentration increased from the first day in all treatments (Figure 2b). In control treatment with *N. oculata* alone, the increase in the ammonia concentration (0.02 ± 0.01–0.40 ± 0.03 mg/L) was lower compared to other treatments ($p < .05$, Tukey–Kramer test). The increase was significantly higher in FWD1A, especially at day 2 where we recorded 0.58 ± 0.16 mg/L compared to other treatments ($p < .05$, $F = 15.36$, $n = 3$). The concentration reduces from

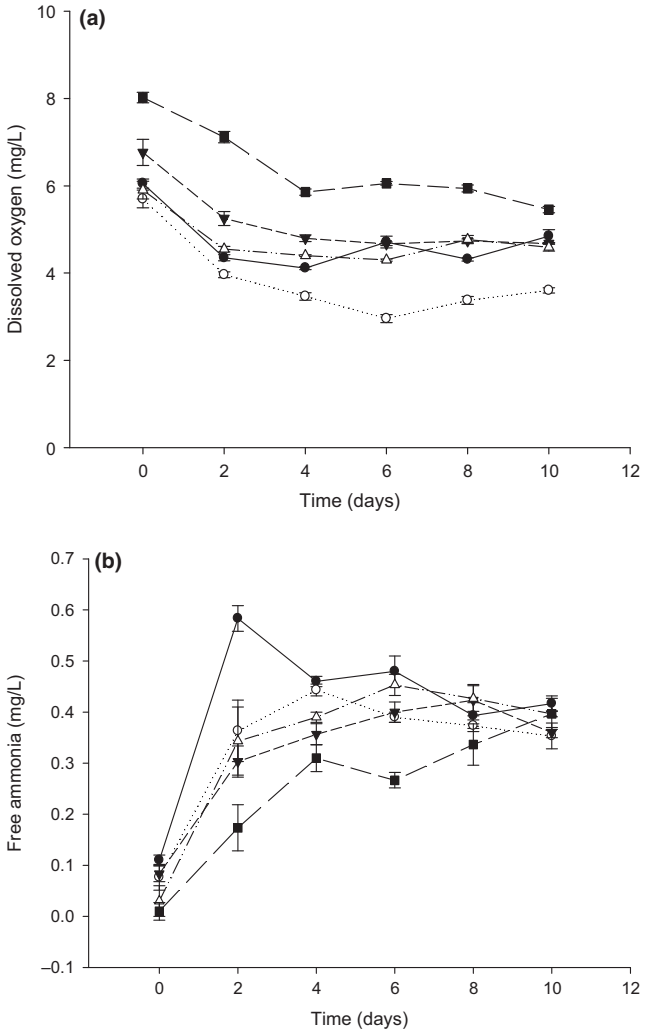

FIGURE 2 Temporal fluctuations of (a): values of dissolved oxygen (DO) and (b): values of ammonia (NH3) concentrations in all culture treatments (Mean ± *SD*). CON1A: ■ and long dashed lines; FWD1A: ● and full line, FWD1B: ○ and dotted line, FWD2A: ▼ and medium dashed line and FWD2B: Δ and medium dashed line and two dots

day 4, and on day 10, there was no significant difference between the treatments. FWD1A (0.75 g/L FWD) had highest ammonia concentration compared to FWD2A (0.50 g/L FWD) on day 1 to day 5.

The total bacterial count ranged between 5.0 × 10^2 and 8.47 × 10^5 CFUs/ml in different treatments taken from day 2 to day 10 at intervals of 2 days. There was a significant effect of FWD ($p < .01$, $F = 89.46$), time ($p < .01$, $F = 6.80$) and their interactions ($p < .05$, $F = 4.55$) on the bacteria density. The bacterial count in FWD1C (only FWD without rotifer or *N. oculata*) was higher (1.07 × 10^5–8.47 × 10^5 CFUs/ml) than that in all other groups in all sampling days. FWD1A had higher bacterial density growth compared to FWD1B (Figure 3). The phytoplankton in the FWD1B lowered the proliferation of bacteria in the culture with maximum bacteria density of 7.80 × 10^4 CFUs/ml recorded on day 6. The lowest bacteria colony count was recorded in the control experiment, where the

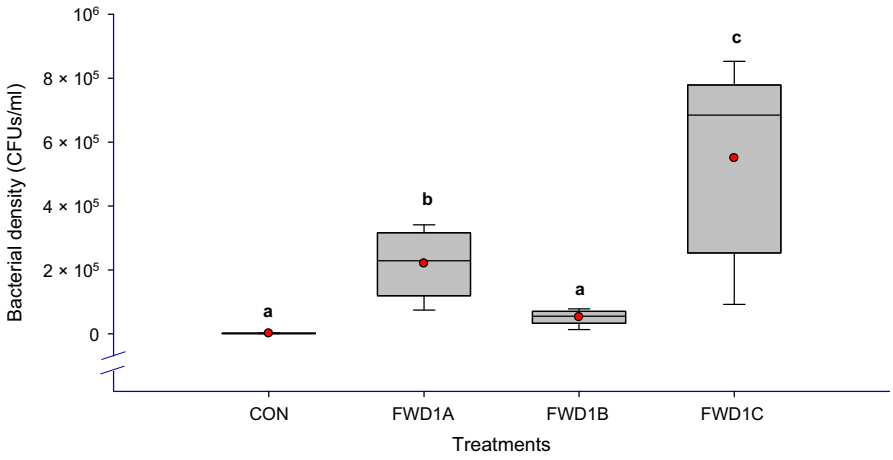

FIGURE 3 Bacterial density (number of CFUs/ml) in all treatment with *P. similis*. CON: *N. oculata* + *P. similis*; FWD1A: 0.75 g/L fish waste diet + *P. similis*; FWD1B: 0.75 g/L fish waste diet + *N. oculate* 2.5 × 10^5 cells per ml + *P. similis*; FWD1C: 0.75 g/L fish waste diet without *P. similis*. The error bars represent 5 and 95 percentiles while the red dots show the mean. (Tukey–Kramer's test, a< b < c at 0.05 significance level)

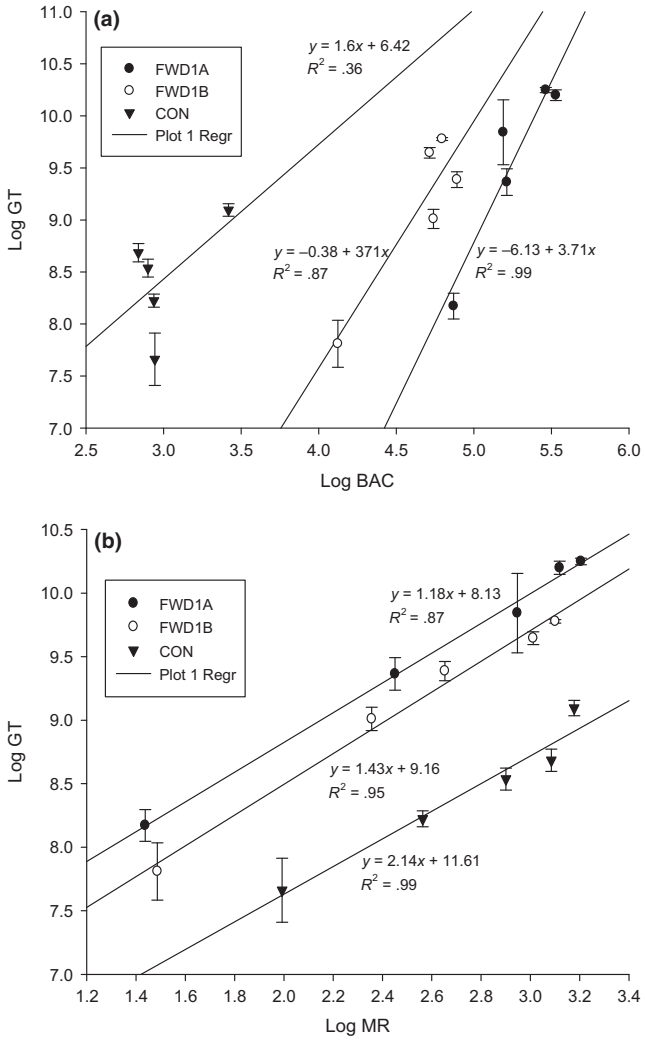

FIGURE 4 Minute rotifer *P. similis* bacterivory in all treatments. (a) relationship between grazing rate (GT) and bacteria population (BAC); (b) relationship between grazing rate (GT) and rotifer population growth (MR). Analysis conducted using linear regression model

population decreased from the start (1.0 × 10^3 CFUs/ml) to day 8 (7.16 × 10^2 CFUs/ml) followed by a slight increase towards the end of the experiment (2.67 × 10^3 CFUs/ml).

The estimated bacteria ingestion rates were generally in the range of 6.03 × 10^2–1.24 × 10^4 bacteria/rotifer/hr. *P. similis* in FWD1A showed higher grazing rate compared to all other treatments ($p < .05$, $F = 6.25$, $n = 5$). We observed a positive relationship between bacterivory and bacterial population (Figure 4a) with the relation being significantly higher in FWD1A ($R^2 = .99$, $p < .05$, $n = 45$) compared to control ($R^2 = .36$). Grazing also increased with an increase in *P. similis* population density (Figure 4b). The relationship between rotifer growth and bacteria grazing rate is slightly weaker ($R^2 = .87$) compared to the other treatments.

The particle size distribution in culture medium also showed differences among the diet (Figure 5). A higher particle concentration (1.36 ± 0.24 × 10^6/ml) was observed in FWD only compared to other treatments. There was a shift in particle distribution as the culture period progressed and the concentration of the smaller particles reduced while that of the larger particles increased as observed in Figure 5 (a vs. b) and (c vs. d). This was the case in FWD1A and FWD1B only. As the population of *P. similis* increased the concentration of smaller particles reduces, we observed a reduction in the mean concentration of small particles (0.5–2.5 μm) from day 3 (2.19 ± 0.34 × 10^6/ml) to day 6 (7.96 ± 0.48 × 10^5/ml) (FWD1A). A higher concentration of larger particles (>2.5 μm) was observed with the addition of *N. oculata* in FWD1B. In FWD1B, the frequency of small particles reduced from >90% at day 3 to <15% at day 6. There was no difference at the concentration and frequency of small particle between day 3 and day 6 in FWD1C; however, there was a significant increase in large particles on day 6 (Figure 5b,d).

4 | DISCUSSION

This study observed a significant population growth of *P. similis* when cultured with FWD. These results showed the potential of using FWD as an alternative to microalgae diet which is generally used for the rotifer production. FWD1A which had the highest concentration of FWD (0.75 g/L) resulted in significantly higher population of *P. similis* compared to the control fed on single diet of *N. oculata*. The previous study with FWD also found the positive effects on the population growth of *B. rotundiformis* (Ogello et al.,

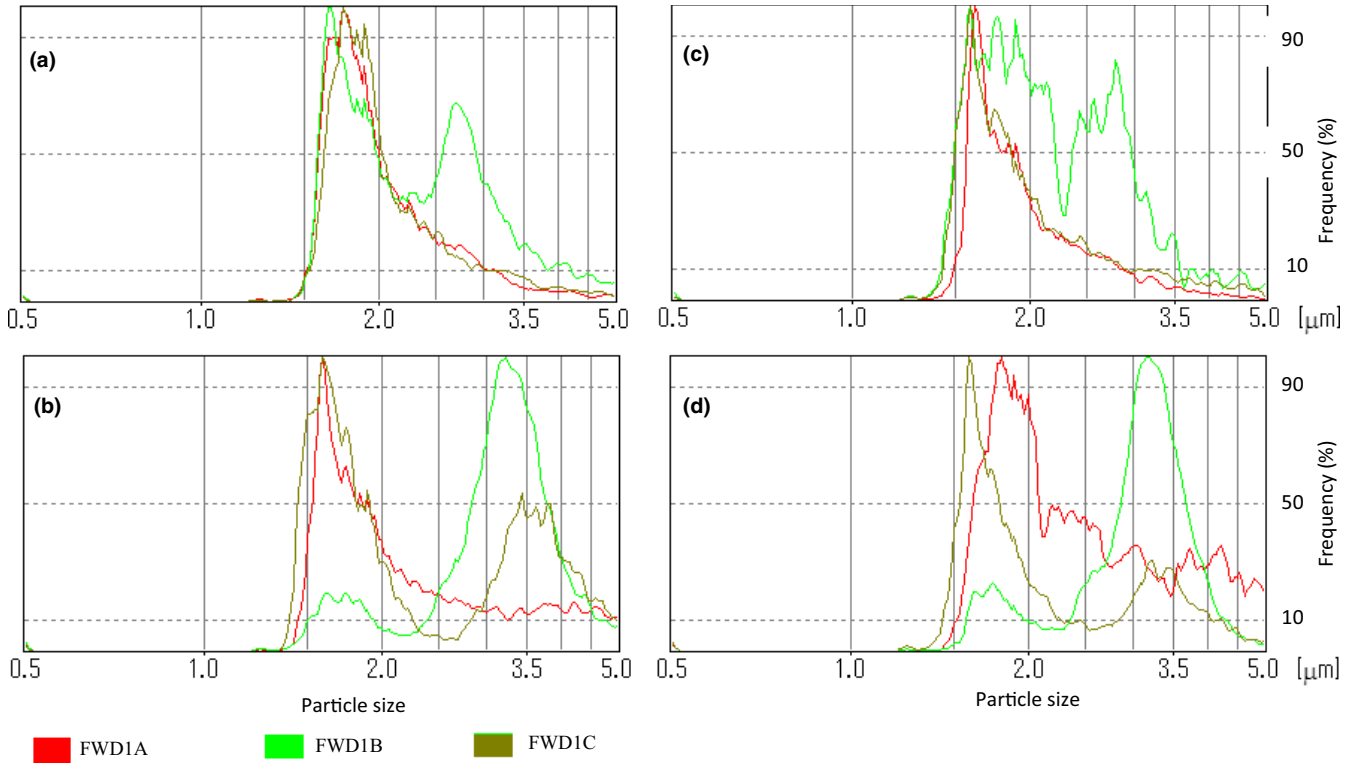

FIGURE 5 Particle size distribution within culture medium of different treatments; (a) and (b) FWD 0.75 g/L on day 3 and 6; (c) and (d) FWD 0.50 g/L on day 3 and 6, respectively. FWD1A: 0.75 g/L fish waste diet + *P. similis*; FWD1B: 0.75 g/L fish waste diet + *N. oculate* 2.5×10^5 cells per ml + *P. similis*; FWD1C: 0.75 g/L fish waste diet without *P. similis*

2017), whereas its functional factors are still undefined. This study revealed two expected effects of FWD as follows: (1) the decomposition of FWD resulted in the proliferation of bacterial flora in the culture medium which enhanced *P. similis* population growth, and (2) the *P. similis* fed directly to on small-sized particles of FWD.

The estimated number of bacteria colonies in the culture medium actively increased with FWD. Bioflocs technology which was first developed by Avnimelech (2012) has become a very popular technology in aquaculture. The working mechanism in culturing *P. similis* using FWD is somewhat similar to Biofloc technique. Biofloc technique utilizes a micro-aggregate of organic material to enhance probiotic bacteria bloom (Aguilera-Rivera et al., 2014; Avnimelech, 2012; Ferreira et al., 2015) which are then used to confer growth benefits to the culture species. Fish waste has previously been used as a source of microbial organisms, amino acids and enzymes (Faid et al., 1997; Ghaly et al., 2013; Liao et al., 1997; Rebah & Miled, 2013). The results in our study show an increase in rotifer growth rate with FWD which agrees with these findings. Bacteria play a key role in nutrient cycling by ingesting the dissolved organic matter (carbon) in this case, decomposing FWD and converting it into particulate state making it readily available and can be utilized by *P. similis* as it feeds on the bacteria cells. Therefore, this mechanism can be applied to enhance population growth of *P. similis*.

The population of bacteria was reduced by the addition of *N. oculata* in FWD1B (Figure 3). Phytoplankton are closely associated with bacteria growth (Lochte, BjØrnsen, Giesenhagen, & Weber, 1997; Reinthaler & Herndl, 2005; Robarts, Zohary, Waiser, & Yacobi, 1996). However, very few studies have been conducted to elucidate this association under laboratory condition. The relationship between phytoplankton and bacteria is sometimes negative (Tada, Nakaya, Goto, Yamashita, & Suzuki, 2017), hence the reduction in bacteria population. Lehman, Abella, Litt, and Edmondson (2004) in their study concluded that there was a mix of correlations which was more dependent on specific species. Other studies have shown that phytoplankton acted as a competitor for resources, especially nutrients (Pearman, Casas, Merle, Michell, & Irigoien, 2016) as well as secreted algicidal compounds which were negative forces for bacteria growth (Natrah, Bossier, Sorgeloos, Yusoff, & Defoirdt, 2014). *Nannochloropsis* sp. contain terpenes and glycosides which are active compounds and have been shown to occlude the growth of some bacteria, for example, *Vibrio anguillarium* (Sharifah & Eguchi, 2011). These mechanisms could explain the reduced bacteria population in our treatments.

Quantification of bacterivory is critical for the understanding of the nutrient pathways in aquatic systems as well as to accentuate the bacteria–rotifer relationships. The bacteria-grazing rate had a positive relationship with the increase in the *P. similis* density in the FWD. The number of bacteria in the FWD with *P. similis* was lower compared to that of FWD without *P. similis*. This variation in bacteria density could be linked to *P. similis* bacterivory. Metazooplankton have been shown to graze on bacteria in other studies (Hagiwara et al., 1994; Hwang & Heath, 1999; Starkweather, Gilbert, & Frost, 1979; Work & Havens, 2003). Agasild and Nõges (2005) demonstrated that rotifers were able to feed on fluorescent-labelled microspheres which were used to mimic the size of bacteria. Le et al. (2017) also showed that addition

of probiotics to the culture of *P. similis* increased its growth performance. Apart from nutrient cycling, bacteria also enhance the growth of rotifer (Jung & Hagiwara, 2001; Makridis, Fjellheim, Skjermo, & Vadstein, 2000; Planas et al., 2004; Selmi, 2001) by enhancing their sexual reproduction (Hagiwara et al., 1994) by promoting production of vitamin B12 (Hirayama & Funamoto, 1983; Yu, Hino, Ushiro, & Maeda, 1989). The findings in our study concur with these studies and we can infer that *P. similis* utilized bacteria cells for nutrients resulting to the increase in population.

Proales similis also fed on small-sized particles of FWD. There was a significant reduction in the small-sized particles (0.5–2.5 μm) in our culture (Figure 5) indicating the probability that *P. similis* fed on these particles. Rotifers are filter feeders (Xiang et al., 2017) and feed on particles less than their body size (Hino & Hirano, 1984; Ogata, Tokue, Yoshikawa, Hagiwara, & Kurokura, 2011; Starkweather & Gilbert, 1977). They also show particle size-dependent feeding (Vadstein, Øie, & Olsen, 1993). These findings support the possibility that *P. similis* ingested the small-sized fish waste particles (≤2.5 μm). The difference in the population growth of *P. similis* in FWD and CON can be explained as the preference of *P. similis* to bacteria and other smaller particles as diet compared to *N. oculata*. *N. oculata* is 1–2 μm in size and average bacteria size is 0.2–1 μm; this makes bacteria cells more preferable due to their smaller size as opposed to the microalgae. The preference of smaller particles (≤2.5 μm) shows that *P. similis* fed on individual cells or small colonies of bacteria; hence, bacteria that form larger colonies will not be preferred.

A number of researchers have demonstrated the effect of environmental conditions on growth and reproduction of rotifer and other live feeds. Hagiwara, Hino, and Hirano (1985) in their study observed that external conditions affected both hatching rate and incubation period of eggs though the conditions examined were temperature, chlorinity and lighting. Reports from other studies have shown that rotifer could tolerate higher pH but increased concentration of un-ionized ammonia decreased age-specific survivorship and the population growth also declined (de Araujo, Snell, & Hagiwara, 2000; Shu, Niu, & Yin, 2008; Yang et al., 2017; Yu & Hirayama, 1986). In this study, higher fish waste diet concentration resulted to higher ammonia concentration (Figure 2a) hence resulting to cultures crushing at any concentration above 0.75 g/L of FWD. Addition of *N. oculata* to FWD lowered the DO concentration in FWD1B and FWD2B (Figure 2b) hence slower increase in rotifer density which is similar to observations by other authors.

Addition of phytoplankton to the fish waste aggravated the water quality, that is why *P. similis* showed lower population growth in this treatment. The reduced amount of dissolved oxygen became a limiting factor to *P. similis* growth leading to a decline in its density. Field studies have shown that excessive proliferation of phytoplankton increases oxygen demand due to the photosynthetic activity (Arauzo & Valladolid, 2003; Liang, Lu, Min, Liu, & Yang, 2018; Yang et al., 2017) which in turn increases the pH and subsequently increases the production of unionized ammonia (Shu et al., 2008), thus leading to a reduction in zooplankton biomass. In our experiment, the decomposition of FWD requires oxygen and combined with the oxygen demand of *P. similis* metabolic activity as well us phytoplankton activities; it is reasonable to assume that the low population growth in FWD1B and FWD2B can be attributed to low DO and high NH_3 concentration. These factors combined with a reduction in bacteria population as aforementioned are considered as the key reasons of slow growth experienced in this treatment.

5 | CONCLUSION

This study demonstrated that FWD could be a suitable diet in culturing minute rotifer *P. similis*. It also elucidated the synergistic relationship between particle size and bacteria growth to population increase in *P. similis*. The abundance of bacteria reduced with addition of microalgae (*N. oculata*). This association between microalgae and bacteria could favour more pathogenic bacteria and thus responsible for culture instability in most live feed production systems.

ACKNOWLEDGMENTS

This research was partly supported by Japanese Society for Promotion of Sciences (JSPS) KAKENHI Grant number JP17H038662 to AH as well as Japan International Cooperation Agency (JICA) which provided Ph.D. fellowship to RN under the JKUAT/PAUSTI/AU Network project_Nagasaki University (J1610573). The authors also wish to acknowledge Dr. Hee-Jin Kim for her editorial help and other anonymous reviews for improving the quality of the manuscript.

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How to cite this article: Kagali RN, Ogello EO, Sakakura Y, Hagiwara A. Fish-processing wastes as an alternative diet for culturing the minute rotifer *Proales similis* de Beauchamp. *Aquac Res*. 2018;00:1–9. https://doi.org/10.1111/are.13707