Aquaculture 435 (2015) 424–430

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

A comparison among different *Pavlova* sp. products for cultivation of *Brachionus plicatilis*

S. Rehberg-Haas [a,c,*], S. Meyer [a], S. Lippemeier [b], C. Schulz [a,c]

[a] GMA — Gesellschaft für Marine Aquakultur mbH, Büsum, Germany
[b] BlueBioTech GmbH, Büsum, Germany
[c] Institute of Animal Breeding and Husbandry, Christian-Albrechts-University, Kiel, Germany

ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form 14 October 2014
Accepted 15 October 2014
Available online 24 October 2014

Keywords:
Brachionus plicatilis
Pavlova sp.
Live feed cultivation
Microalgae

ABSTRACT

In the present study the potential of different products of the marine microalga *Pavlova* sp. for the cultivation of rotifers (*Brachionus plicatilis*) was tested. Two growth performance trials were conducted: In a first laboratory scale experiment rotifers were cultivated for 14 days with *Pavlova viridis* concentrate, *P. viridis* fresh culture, *Pavlova* sp. fresh culture, baker's yeast and *Nannochloropsis* sp. concentrate. The *P. viridis* fresh culture fed groups resulted in significantly the highest rotifer density (109.2 rotifers mL^{-1}) and instantaneous growth rate ($G = 0.14 \pm 0.02\ d^{-1}$). There were no significant differences found of the G between the *P. viridis* concentrate group and the *Pavlova* sp. fresh culture group. The baker's yeast fed group showed significantly the lowest rotifer numbers and growth rate. Based on the high growth rate of the *P. viridis* fresh culture group in the first experiment, different *P. viridis* products (concentrate, fresh culture, frozen concentrate, and freeze-dried powder) were examined in the second (larger scale) experiment and compared to *Nannochloropsis* sp. concentrate. The highest rotifer growth rate G in experiment 2 was determined for the frozen *P. viridis* group ($G = 0.09 \pm 0.03\ d^{-1}$), although it was not significantly different in comparison to the G of the rotifers fed with *Nannochloropsis* sp. and the *Pavlova* concentrate and fresh culture. The frozen *Pavlova* product seems the most suitable *Pavlova* product for the cultivation of live feed and it provides advantages of storability and application.

1. Introduction

Despite the intensive effort in form of research on the development of larval microdiets so far no relevant success has been achieved (Conceição et al., 2010). Hence, the utilization of rotifers (*Brachionus plicatilis*) as starter feed for the rearing of marine fish larvae and crustaceans is still essential for commercial marine hatchery procedures (Bengston, 2003). However, the stable rotifer cultivation is difficult and time consuming. The provision of sufficient amounts of rotifers during the crucial phase of first feeding is still challenging in the rearing of marine fish larvae, as the use of rotifers involves biomass production and the boosting with essential nutrients, too. The successful rotifer cultivation depends on a range of complex interacting factors, such as water quality and nutrition. Moreover, a stable body composition is necessary to obtain a steady rotifer culture (Dhert et al., 2001). Hirayama et al. (1979) and Okauchi and Fukusho (1984) displayed positive nutritional effects of microalgae in rotifer cultivation. Further important factors describing the overall rotifer culture performance like growth rate (Yúfera and Pascual, 1980) and filtration and ingestion rates (Savas and Guclu, 2006) are affected by the diet, too. Renaud et al. (2002) reported positive effects of diets containing microalgae rich in proteins and essential fatty acids on growth rates of live feed species.

The expensive and time-consuming biomass production of rotifers is tried to be achieved by the use of cheap sources like baker's yeast. Although the cultivation of rotifers on baker's yeast only can be successful over several weeks (Lubzens et al., 1995), these cultures are often instable and can crush spontaneously. Additionally the yeast fed rotifers have a lack of the essential nutrients (Hirayama, 1987). Thus the use of microalgae is an alternative to provide a wider range of needed nutrients, especially fatty acids (Ben-Amotz et al., 1987), and to ensure a more stable cultivation. In the past a few microalgae species have been used to cultivate and enrich rotifers. Still these species do not feature the optimal essential fatty acid composition. *Tetraselmis* sp. and *Nannochloropsis* sp. contain considerable amounts of eicosapentaenoic acid (EPA, 20:5n − 3) and nearly no docosahexaenoic acid (DHA, 22:6n − 3) (Hu and Gao, 2003; Koven et al., 1990; Patil et al., 2007; Watanabe et al., 1983), *Isochrysis* sp. contains substantial amounts of DHA and only little amounts of EPA (Ben-Amotz et al., 1987; Lubzens et al., 1985; Patil et al., 2007). Hence, a great potential can be attributed to the microalga *Pavlova* sp., as it contains both EPA (18.0 mg g DW^{-1}; Patil et al., 2007) and DHA (13.2 mg g DW^{-1}; Patil et al., 2007) in distinct amounts. However, so far it has not been possible to produce

* Corresponding author at: GMA — Gesellschaft für Marine Aquakultur mbH, Büsum, Germany. Tel.: +49 4834 965399 14.
E-mail address: rehberg@gma-buesum.de (S. Rehberg-Haas).

http://dx.doi.org/10.1016/j.aquaculture.2014.10.029
0044-8486/

Pavlova sp. in a larger scale of industrial relevance. The most common strains of *Pavlova* are known to be sensitive against shear force and high temperature (>28 °C) and they are very sensitive to downstreaming processes and have a very short shelf-life, limiting their applicability for the industrial purpose. For this project new production techniques for cultivation, harvest and preservation of chosen *Pavlova* microalgae strains have been developed and the large-scale production has been successfully implemented, to provide different *Pavlova* sp. products. In order to establish "off the shelf algae products" the aim of the present study was to evaluate the effects of different *Pavlova* strains (*Pavlova* sp. and *Pavlova viridis*) and of the different *Pavlova* products (fresh algae culture, concentrate, frozen concentrate, freeze-dried powder) on the culture performance by investigating the culture growth, filtration and ingestion rates of the rotifers and the culture water quality (total organic carbon — TOC; dissolved organic carbon — DOC).

2. Material and methods

2.1. Experiment 1

2.1.1. Rotifer culture

In the pre-experimental period rotifers (*Brachionus plicatilis*, L-strain, mean lorica length 199.8 μm) were cultivated regularly in 30 L circular tanks on a combination of baker's yeast (*Saccharomyces cerevisiae*; common grocery store, stored at 4 °C) and *Nannochloropsis* sp. concentrate (BlueBioTech, Büsum, Germany; 12×10^9 cells mL^{-1}). At the beginning of the first laboratory scale cultivation experiment 1 L flasks were stocked with rotifers of about 30 ind mL^{-1}. Rotifers were cultivated in semi-continuous cultures. Over the experimental period of 14 days the salinity (20 PSU) and the temperature (22.4 ± 0.7 °C) were maintained at a constant level. Light was set on a 24 h photoperiod. Water quality parameters were maintained in a safe range and were measured regularly (7.0–7.3 pH; GMH 3530, Digital pH-/mV-/Thermometer, GREISINGER electronic, Germany; 9.7 ± 0.1 mg L^{-1} O2; Handy Polaris; Oxy-Guard International A/S, Birkerod, Denmark).

2.1.2. Experimental treatments

In the first experiment the potential of two different *Pavlova* strains – *P. viridis* and *Pavlova* sp. (CCMP 1228) – as cultivation products was evaluated. The experimental treatments for the first trial were 1) Baker's yeast, 2) *Nannochloropsis* sp. concentrate (BlueBioTech, Büsum, Germany; 12×10^9 cells mL^{-1}; 144 μg DW μl^{-1}), 3) *P. viridis* concentrate (35 μg DW μl^{-1}), 4) *P. viridis* fresh culture (0.8 μg DW μl^{-1}) and 5) *Pavlova* sp. fresh culture (1.0 μg DW μl^{-1}). Each product was tested in a four time replication and was applied at a daily ration of 0.8 g DW per 1×10^6 rotifers. Algae cultures (*Pavlova* sp.; *P. viridis*, *Nannochloropsis* sp.) were cultivated by BlueBioTech (BBT, Büsum, Germany) under standard greenhouse conditions (modified F-media, 32 PSU, 23–25 °C, pH 8.5). Fresh culture treatments were taken from running cultures and concentrates were obtained by flow-through centrifugation.

2.1.3. Sampling

A volume of 100 mL water was removed from the culture on a daily basis and five 1 mL sub-samples were preserved with Lugol's solution for further counting of population and eggs under a stereo-microscope. The egg ratio (ER; eggs ind^{-1}) was calculated as:

$$ER = \text{eggs}\,ml^{-1}/\text{female rotifers}\,ml^{-1}. \quad (1)$$

The instantaneous growth rate (G; d^{-1}) of rotifers was calculated according to Theilacker and McMaster (1971):

$$G = (\ln N_t - \ln N_0)/t; \quad (2)$$

where N_t = number of rotifers at time t [d] and N_0 = number of rotifers at the start of the experiment.

After sampling the culture volume was filled up to 1 L including the daily feed ration.

2.2. Experiment 2

Concerning the findings from experiment 1 *P. viridis* was chosen for the next rotifer trial. The second trial was extended to a larger scale of rotifer cultivation.

2.2.1. Rotifer culture

In the second experiment 10 L tubular containers were stocked with rotifers (pre-treatment like experiment 1, see above) at a density of about 50–100 ind mL^{-1}. Rotifers were cultivated in semi-continuous cultures. The salinity (20 PSU) and the temperature (21.5 ± 0.2 °C) were maintained at a constant level during the experimental period of 14 days. Lighting was set on a 24 h photoperiod. Water quality parameters were maintained in a safe range for the rotifers and measured regularly (7.0–7.4 pH; GMH 3530, Digital pH/mV-/Thermometer, GREISINGER electronic, Germany; 9.6 ± 0.1 mg L^{-1} O2; Handy Polaris; Oxy-Guard International A/S, Birkerod, Denmark).

2.2.2. Experimental treatments

The algae products examined in the second experiment were: 1) *Nannochloropsis* sp. concentrate (BlueBioTech, Büsum, Germany; 12×10^9 cells mL^{-1}; 144 μg DW μl^{-1}), 2) *P. viridis* concentrate (35 μg DW μl^{-1}), 3) *P. viridis* fresh culture (0.8 μg DW μl^{-1}), 4) *P. viridis* frozen concentrate (35 μg DW μl^{-1}), 5) *P. viridis* freeze-dried powder. Each product was tested in a five time replication and was applied at a daily ration of 0.75 g DW per 1×10^6 rotifers. Algae cultures (*P. viridis*, *Nannochloropsis* sp.) were cultivated by BlueBioTech (BBT, Büsum, Germany) under standard greenhouse conditions (modified F-media, 32 PSU, 23–25 °C, pH 8.5). Fresh culture treatment was taken from running culture and the other products were obtained by flow-through centrifugation. The *P. viridis* concentrate was stored at 4 °C, the frozen *P. viridis* product was stored at −20 °C and certain amounts were thawed right before use. The dried *P. viridis* product was lyophilized and stored dry at 4 °C. The nutritional content of algal raw material is shown in Table 1.

2.2.3. Growth performance

A volume of 1.5 L was removed daily from each container. Five 1 mL samples were preserved with Lugol's solution for further counting of population and eggs under a stereo-microscope. The ER and G were calculated as shown in Eqs. (1) and (2).

After sampling the culture volume was filled up to 10 L including the daily microalgae ration.

Table 1
Nutritional content of microalgae *Nannochloropsis* sp. and *Pavlova viridis*.

	Nannochloropsis sp.	*Pavlova viridis*
Nutrient composition [g kg^{-1}DM]		
Crude protein	230	398
Crude lipid	395	196
Crude ash	61	100
NfE[a]	314	306
Gross energy *[MJ kg^{-1} DM]*	26.63	23.26
PUFA[b] *[mg g^{-1} DM]*		
EPA	25–35	35–45
DHA	–	7–11

[a] NfE: Nitrogen free extract = 1000 – (crude protein + crude fat + crude ash) [b] Polyunsaturated fatty acids EPA and DHA (Lippemeier, unpublished results).

2.2.4. Filtration and ingestion

Additionally the product concentration in the cultures was measured every hour for 7 h and again 20 h after feeding at days 5 and 12. Product concentration was measured as optical density (750 nm) by means of USB200 spectrometer and attached light source DT-Mini-2-GS (Ocean Optics, Inc., Dunedin, FL, USA). The absorbance of light was related to product concentration (μg DW mL^{-1}) using standard curves for each product.

Filtration and ingestion rates were calculated as follows (Yúfera and Pascual, 1985):

$$F = (\ln C_0 - \ln C_t)/N \times t, \quad (3)$$

where F = filtration rate in mL ind^{-1} min^{-1}, C_0 = initial cultivation product concentration in μg DW mL^{-1}, C_t = final cultivation product concentration in μg DW mL^{-1}, N = rotifer density in ind mL^{-1}, and t = duration of the treatment in min, and

$$I = F \times \sqrt{C_0 \times C_t}, \quad (4)$$

I = ingestion rate in consumed cultivation product in μg DW ind^{-1} min^{-1}.

In order to account for sedimentation of cultivation products, additional concentration measurements of the diluted products were recorded in a separate setup without rotifers. The filtration and ingestion values were corrected for sedimentation.

2.2.5. TOC/DOC analysis

At days 1, 7 and 14 samples for total and dissolved organic carbon (TOC and DOC) analyses were taken. Culture water samples were filtered (30 μm mesh size) in order to remove rotifers. The analyses were conducted by means of the Shimadzu TOC-L Total Organic Carbon Analyzer (Shimadzu, Kyōto, Japan) using the 680 °C combustion catalytic oxidation method.

2.2.6. Statistical analysis

The statistical analyses for both experiments were performed using SPSS 18.0 for Windows (SPSS Inc., Chicago, U.S.). Data are presented as mean ± standard deviation (SD) for each treatment and compared between treatments. The Kolmogorov–Smirnov test was used to check data for normal distribution. Data was analyzed for variance homogeneity by Levene (confirmed if $p < 0.05$) and post-hoc multiple comparison was carried out by parametric Tukey-HSD (if test for homogeneity was confirmed) ($p < 0.05$) or non-parametric Dunnett-T3 test (if test for homogeneity failed).

3. Results

3.1. Experiment 1

All *Pavlova* cultivated groups in the first experiment showed reasonable culture performances (Fig. 1 and Table 2). The *P. viridis* fresh culture fed group showed the highest final rotifer density (160.7 ± 8.5 rotifers mL^{-1}) and the significantly highest G (G = 0.14 ± 0.02 d^{-1}) followed by the *Pavlova* sp. fresh culture group (G = 0.12 ± 0.01 d^{-1}) and the *P. viridis* concentrate group (0.12 ± 0.03 d^{-1}). All groups resulted in significant higher values than the baker's yeast group, which showed a negative instantaneous growth rate (G = −0.12 ± 0.03 d^{-1}). A sharp decline of the egg density was recorded at day 6 in all experimental groups, which is also included in the calculation of the ER.

3.2. Experiment 2

3.2.1. Growth performance

The highest rotifer density at the end of experiment 2 was determined in the *Nannochloropsis* sp. group, although it was not significantly different from the results of the *Pavlova* frozen concentrate and fresh culture. The density of the *Pavlova* concentrate was lower, though not significantly different to the two aforementioned products. The lowest rotifer numbers were found for the group that was fed the freeze-dried *Pavlova* meal at the final sampling day (Fig. 2). The instantaneous growth rate exhibited no significant differences between the *Nannochloropsis* sp. group and the *P. viridis* groups, except for the freeze-dried *P. viridis* group (Table 2).

3.2.2. Filtration and ingestion

The significantly highest filtration (day 5: $5.615 \times 10^{-6} \pm 3.562 \times 10^{-7}$mL ind^{-1} min^{-1}; day 12: $3.289 \times 10^{-6} \pm 2.051 \times 10^{-7}$ mL ind^{-1} min^{-1}) and ingestion (day 5: $4.188 \times 10^{-4} \pm 2.755 \times 10^{-5}$ μg DW ind^{-1} min^{-1}; day 12: $1.917 \times 10^{-4} \pm 2.680 \times 10^{-5}$ μg DW ind^{-1} min^{-1}) values were observed in the *Pavlova* fresh culture group. The *Pavlova* freeze-dried group resulted in negative values for both parameters at both sampling days. This is due to lower concentration values measured at starting point 0 (Fig. 3)

3.2.3. TOC/DOC analysis

The total organic carbon (TOC) content ranged from 42.5 mg L^{-1} (group *Nannochloropsis*, day 1) to 141.2 mg L^{-1} (group *Pavlova* fresh culture, day 14). For dissolved organic carbon (DOC) the values ranged from 30.9 mg L^{-1} (group *Nannochloropsis*, day 1) to 126.8 mg L^{-1} (group *Pavlova* fresh culture, day 14). Both parameters measured in the *Nannochloropsis* group and the *Pavlova* freeze-dried group ranked at low levels at all sampling days, whereas the values increased continuously in the other experimental groups (*P. vir.* conc., *P. vir.* fresh and *P. vir.* frozen). The highest values were found at all sampling days for the *Pavlova* fresh culture group (Fig. 4).

4. Discussion

The present work shows that the cultivation of rotifers on various *Pavlova* sp. products is possible and reveals reasonable growth performances in comparison to the commonly used microalga *Nannochloropsis* sp. In the first experiment the *P. viridis* fresh culture resulted in the best growth performance of rotifers. The baker's yeast group showed a negative growth performance in the experimental period, which can be attributed to the lack of important nutrients, when rotifers are fed on baker's yeast alone (Brown, 2002; Hirayama, 1987). Based on the findings of experiment 1 *P. viridis* was used for the subsequent experiment. In the second experiment the best result for the final rotifer number was found in the rotifer group fed the *Nannochloropsis* sp. concentrate. Although this is not in agreement with experiment 1, it has to be noticed that trends in both experiments show good culture growth rates of rotifers, when fed with *Nannochloropsis* sp. concentrate, *P. viridis* fresh culture, frozen concentrate or concentrate. Differences of exact numbers can occur, as it is known that one of the major issues regarding rotifer cultivation is the unpredictability in culture growth development (Lubzens and Zmora, 2003).

However, the good growth performance of rotifers fed with *Nannochloropsis* sp. might be caused by the favorable size of these microalgal cells (2–4 μm), which can be ingested more easily by the rotifers than *Pavlova* sp. cells (4–6 μm). The smaller cell size might also be more suitable for the processing in the mastax of the rotifers. It was shown by Baer et al. (2008) and Rothhaupt (1990) that rotifers ingest particles selectively. Still the highest instantaneous growth rates in experiment 1 were calculated for all three *Pavlova* treatments (Table 2; highest value 0.14 ± 0.019 for *P. viridis* fresh culture) and in experiment 2 the highest growth rate was found in the frozen

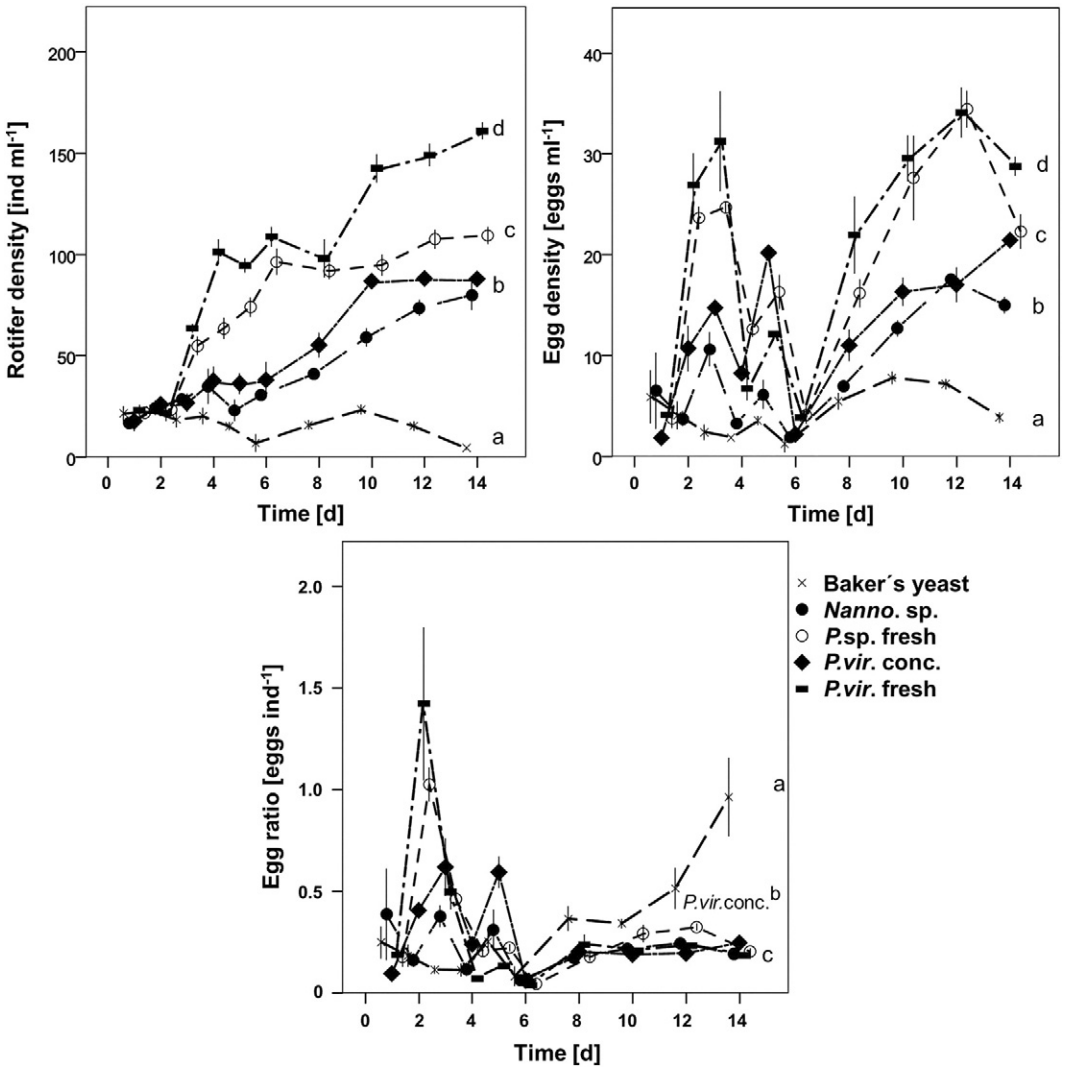

Fig. 1. Culture performance of the first experiment shown as rotifer density, egg density and egg ratio during the experimental period (mean ± SD, n = 4). Values with the same superscript are not significantly different ($p < 0.05$).

P. viridis group, although not significantly different from the other groups except for the freeze-dried *Pavlova* group (Table 2). A comparison of the observed growth rates with results of other studies is difficult, as the growth rate depends strongly on the specific rotifer strain, food type and food concentration (Stemberger and Gilbert, 1985). Certainly the different starting densities must be considered, as it was not possible to adjust the same numbers in all experimental vessels. Hence, especially in experiment 2 the lower starting density of the *P. viridis* frozen group, allowed a greater potential to grow than in the other groups.

The ERs in both experiments are in agreement with results of other studies (Kostopoulou and Vadstein, 2007; Yúfera, 1987). In the first experiment the *P. viridis* fresh culture group showed the highest ER value (1.1 ± 0.3, day 2). The highest value in the second experiment was found for the *P. viridis* fresh culture group at day 2, too, though it was not significantly higher than the other groups except for the freeze-dried *Pavlova* group. Furthermore, all groups displayed a peak at day 2 except for the *Pavlova* freeze-dried group in experiment 2, which showed the peak at day 4 indicating a delay in the process of events, as well as a slower and lower culture growth. The decline of the ER values derived by the decline of egg density at day 6 of experiment 1 is an example of the unpredictability of rotifer culture development. Often changes or unfavorable environmental conditions (temperature, water quality) are the reasons for culture growth declines (Støttrup and McEvoy, 2003). However, in this case no clear indications like changes of environmental conditions were recorded. It might be referred to a population dynamical compensation of the increasing rotifer density.

The filtration values were the highest for the fresh culture *Pavlova* group. The live cells of this product probably feature the best distribution qualities in the culture water and can therefore be easily ingested by the rotifers. Overall the rotifers accepted the *Pavlova* products as good as the *Nannochloropsis* concentrate. The negative filtration and ingestion rate values of the *Pavlova* freeze-dried group derive from the lowest concentration values measured at the starting point 0. This product was difficult to distribute in the water column. Although the other

Table 2
Instantaneous growth rate G (mean ± SD) of rotifer cultures fed the experimental diets in experiments 1 (n = 4) and 2 (n = 5). Values with the same superscript are not significantly different ($p < 0.05$).

Experiment 1		Experiment 2	
Feed types	Culture growth G [d^{-1}]	Feed types	Culture growth G [d^{-1}]
Baker's yeast	-0.12 ± 0.03^{a}	*Nanno.* sp. conc.	0.07 ± 0.01^{a}
Nanno. sp. conc.	0.11 ± 0.02^{b}	*P. vir.* conc.	$0.06 \pm 0.03^{a,b}$
P. vir. conc.	0.12 ± 0.03^{c}	*P. vir.* fresh	$0.04 \pm 0.03^{a,b}$
P. vir. fresh	0.14 ± 0.02^{d}	*P. vir.* frozen	0.09 ± 0.03^{a}
P. sp. fresh	0.12 ± 0.01^{c}	*P. vir.* freeze-dried	0.01 ± 0.02^{b}

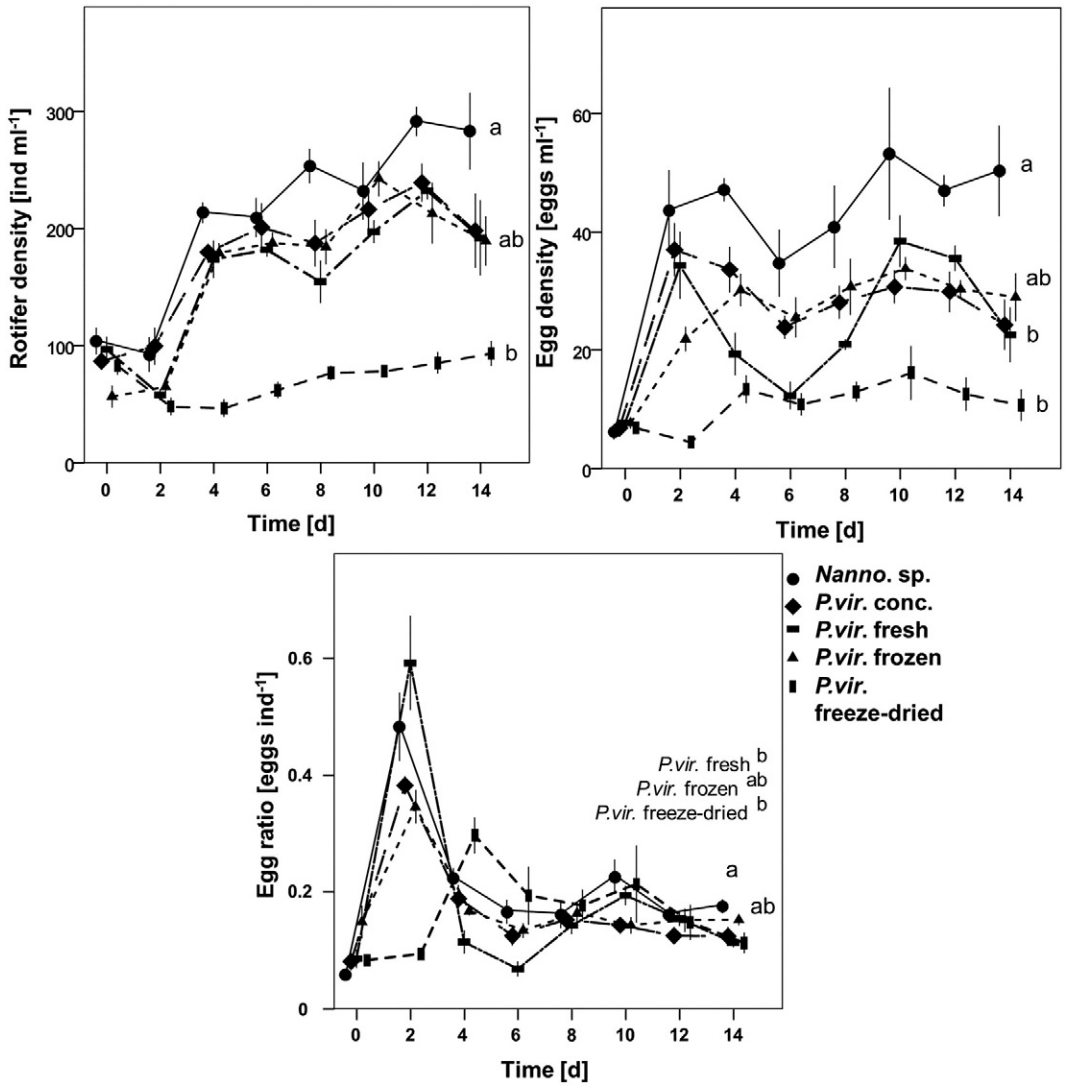

Fig. 2. Culture performance of the second experiment shown as rotifer and egg density and egg ratio during the experimental period (mean ± SD, n = 5). Values with the same superscript are not significantly different ($p < 0.05$).

products were homogenously mixed at the time of concentration measurement, this might not have been the case for the freeze-dried *Pavlova* product.

The TOC and DOC contents are defined as the measure of the total (TOC) and dissolved (DOC) organic carbon load derived from metabolism and decomposition of organisms and bacterial growth in water

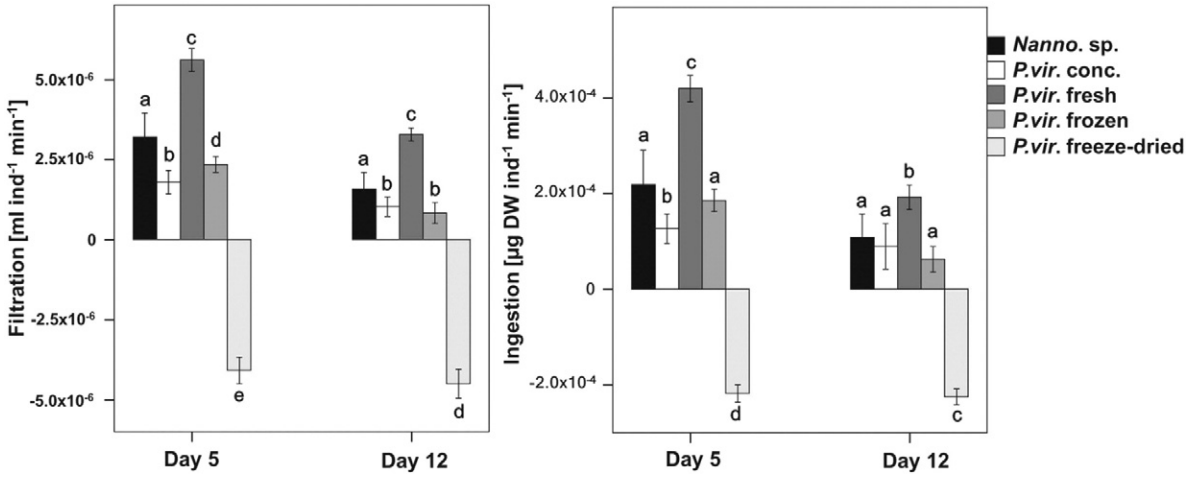

Fig. 3. Filtration and ingestion rate (mean ± SD, n = 5) at day 5 and day 12 over a period of 20 h. Values with the same superscript are not significantly different ($p < 0.05$).

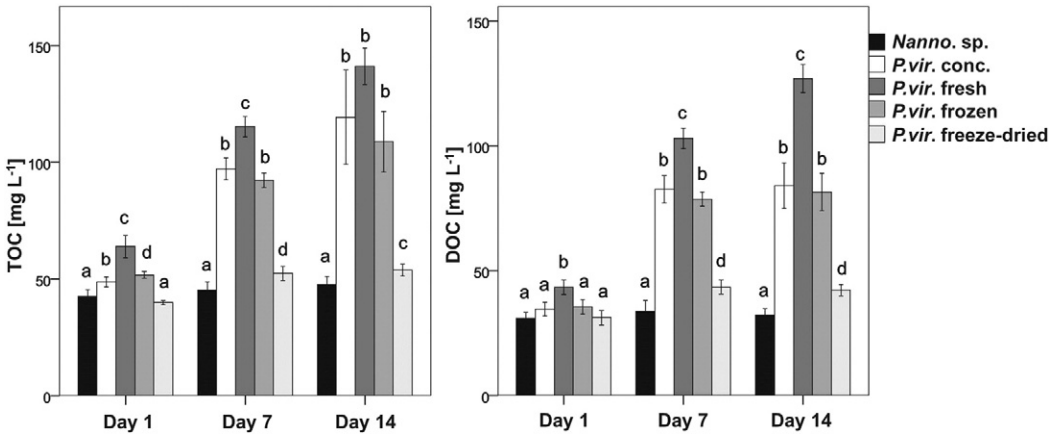

Fig. 4. Total organic carbon (TOC) content and dissolved organic carbon (DOC) content in culture water samples at days 1, 7 and 14 (mean ± SD, n = 5). Values with the same superscript are not significantly different ($p < 0.05$).

samples. The low TOC and DOC values of the freeze-dried *P. viridis* fed cultures can be explained by the fast sinking of the particles. On that account the major amount of the organic material sedimented quickly and therefore the time of decomposition in the water column was shortened. Furthermore, the freeze-dried *P. viridis* group remained at very low rotifer densities throughout the experiment. Hence, only low metabolism and decomposition of organisms occurred leading to low TOC and DOC values. Similarly in the *Nannochloropsis* groups low TOC and DOC values were measured at all sampling days. However, in contrast to the freeze-dried *P. viridis* the *Nannochloropsis* sp. cells did not feature fast sinking or increased sedimentation. This algal product seems to be ingested by rotifers quickly and it maintained a stable culture, therefore there is no increased bacteria growth or increased rotifer mortality and degradation.

The highest and also increasing values of TOC and DOC determined at days 1, 7 and 14 were recorded for the *P. viridis* fresh culture. This is probably due to the high load of organic carbon that is brought with the algae culture medium. Also the bacterial growth might have been stimulated by this additional nutrient load coming from the culture medium. A high microfloral growth might also have been possible for the *P. viridis* concentrate and frozen concentrate groups, which also revealed high TOC and DOC values. Furthermore, an increase of these values was recorded for these 3 experimental groups, whereas the TOC and DOC concentrations of the other two groups (*Nannochloropsis* sp. and *P. viridis* freeze-dried) stayed at equal levels at all sampling dates. The increase of TOC and DOC values for the *P. viridis* fresh culture, concentrate and frozen concentrate group, is probably due to increasing microbial growth rather than to accumulation processes, which otherwise might have occurred in the other two remaining groups, as well. Although the most common bacterial species do not necessarily have a negative effect on rotifer cultures, the microflora needs to be controlled because of possible introduction into the fish larvae system by the rotifers (Støttrup and McEvoy, 2003). For example *Vibrionaceae*, which have been identified in rotifer cultures (Nicolas et al., 1989), can be harmful to fish larvae by hampering intestinal passage and assimilation. However, not only harmful microbiota, but also probiotic bacteria strains can occur in rotifer cultures. These probiotic strains can also enhance rotifer cultures (Douillet, 2000). Therefore, further research on the impact of *P. viridis* products on the microbial flora is needed.

The results of the present work show differences in the suitability of the tested algae products. First of all the *Pavlova* fresh culture products (experiments 1 and 2) seem to be qualified for the cultivation of rotifers, mainly because the algae cells were completely intact and the cells stayed in the water column and were therefore available for the rotifers. However, as mentioned before, the production of large algae biomass is cost- and time-consuming and needs to be substituted by other storable products. The tested freeze-dried *P. viridis* meal resulted in the lowest growth rates, although the quality of the product and the composition remains unaltered, even after the process of concentration and lyophilization (Lippemeier, unpublished results). The adverse culture development is probably due to the unfavorable applicability, as it is difficult to mix the powder homogeneously in water. Furthermore, the particles and cells sink fast and the availability for the rotifers is therefore shortened compared to the other products. Similar effects were also found for other dried algae products by Lubzens et al. (1995). The *P. viridis* concentrate is another product, which can be provided as a commercial product. The quality of the single cells was unaltered and the cells stayed in the water column and were available for the rotifers (Lippemeier, unpublished results). However, this product displayed a low durability and was storable only for a few days up to two weeks. This problem can be avoided by freezing the algal concentrate and thawing the needed amount right before use. The frozen product was stored at −20 °C. There was no loss in quality compared to the *P. viridis* concentrate which was stored at 4 °C and it also led to the same or even better growth of the rotifer cultures (Table 2). Also Lubzens et al. (1995) stated that frozen *Nannochloropsis* sp. concentrate could be stored at −20 °C or −80 °C for 4 weeks and the thawed material maintained its quality and could be used for enrichment for 7 days and for cultivation for 14 days.

In conclusion *Pavlova* sp. products, especially the frozen concentrate of *P. viridis*, were found to be reasonable cultivation products for rotifers as an alternative to live algae. The frozen product features the advantages of a long storability and the preservation of product quality. Furthermore, higher algae concentrations can be applied to the rotifer cultures than with live algae cultures, which are required for the resting egg production of rotifers. However, there are still some advantages of *Nannochloropsis* sp. over the *Pavlova* products as long-term rotifer cultivation product, like more suitable cell size for ingestion and digestion in the mastax, as well as the better effects on water quality. On that account the short-term enrichment of live feed with *P. viridis* might be the more suitable application form for this alga.

Acknowledgments

This work was funded by the DBU — Deutsche Bundesstiftung Umwelt under grant agreement no. AZ 28183-34.

References

Baer, A., Langdon, C., Mills, S., Schulz, C., Hamre, K., 2008. Particle size preference, gut filling and evacuation rates of the rotifer *Brachionus* "Cayman" using polystyrene latex beads. Aquaculture 282, 75–82.

Ben-Amotz, A., Fishler, R., Schneller, A., 1987. Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. Mar. Biol. 95, 31–36.
Bengston, D.A., 2003. Status of Marine Aquaculture in Relation of Live Prey: Past, Present and Future. Blackwell Science Ltd.
Brown, M.R., 2002. Nutritional value of microalgae for aquaculture. In: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortés, M.G., Simoes, N. (Eds.), Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional deNutrición. Acúcola. 3 al 6 de Septiembre del. ancún. Quintana Roo, México.
Conceição, L.E.C., Aragão, C., Richard, N., Engrola, S., Gavaia, P., Mira, S., Dias, J., 2010. Novel methodologies in marine fish larval nutrition. Fish Physiol. Biochem. 36, 1–16.
Dhert, P., Rombaut, G., Suantika, G., Sorgeloos, P., 2001. Advancement of rotifer culture and manipulation techniques in Europe. Aquaculture 200, 129–146.
Douillet, P.A., 2000. Bacterial additives that consistently enhance rotifer growth under synxenic culture conditions: 2. Use of single and multiple bacterial probiotics. Aquaculture 182, 241–248.
Hirayama, K., 1987. A consideration of why mass culture of the rotifer *Brachionus plicatilis* with baker's yeast is unstable. In: May, L., Wallace, R., Herzig, A. (Eds.), Rotifer Symposium IV. Springer, Netherlands, pp. 269–270.
Hirayama, K., Takagi, K., Kimura, H., 1979. Nutritional effect of eight species of marine phytoplankton on population growth of the rotifer, *Brachionus plicatilis*. Bull. Jpn. Soc. Sci. Fish. 45, 11–16.
Hu, H., Gao, K., 2003. Optimization of growth and fatty acid composition of a unicellular marine picoplankton, *Nannochloropsis* sp., with enriched carbon sources. Biotechnol. Lett. 25, 421–425.
Kostopoulou, V., Vadstein, O., 2007. Growth performance of the rotifers *Brachionus plicatilis*, *B.* 'Nevada' and *B.* 'Cayman' under different food concentrations. Aquaculture 273, 449–458.
Koven, W.M., Tandler, A., Kissil, G.W., Sklan, D., Friezlander, O., Harel, M., 1990. The effect of dietary (n − 3) polyunsaturated fatty acids on growth, survival and swim bladder development in *Sparus aurata* larvae. Aquaculture 91, 131–141.
Lubzens, E., Zmora, O., 2003. Production and nutritional value of rotifers. In: Støttrup, J.G., McEvoy, L.A. (Eds.), Live Feeds in Marine Aquaculture. Blackwell Science Ltd., pp. 300–303.
Lubzens, E., Marko, A., Tietz, A., 1985. De novo synthesis of fatty acids in the rotifer, *Brachionus plicatilis*. Aquaculture 47, 27–37.
Lubzens, E., Gibson, O., Zmora, O., Sukenik, A., 1995. Potential advantages of frozen algae (*Nannochloropsis* sp.) for rotifer (*Brachionus plicatilis*) culture. Aquaculture 133, 295–309.
Nicolas, J.L., Robic, E., Ansquer, D., 1989. Bacterial flora associated with a trophic chain consisting of microalgae, rotifers and turbot larvae: influence of bacteria on larval survival. Aquaculture 83, 237–248.
Okauchi, M., Fukusho, K., 1984. Food value of a minute alga, *Tetraselmis tetrahele*, for the rotifer *Brachionus plicatilis* culture-1. Population growth with batch culture. Bull. Natl. Res. Inst. Aquacult. 5, 13–18.
Patil, V., Källqvist, T., Olsen, E., Vogt, G., Gislerød, H., 2007. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquacult. Int. 15, 1–9.
Renaud, S.M., Thinh, L., Lambrinidis, G., Parry, D.L., 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 211, 195–214.
Rothhaupt, K.O., 1990. Population growth rates of two closely related rotifer species: effects of food quantity, particle size, and nutritional quality. Freshw. Biol. 23, 561–570.
Savas, S., Guclu, Z., 2006. Filtration and ingestion rates of the rotifer *Brachionus plicatilis* fed five species of microalgae at different cell densities. Isr. J. Aquacult.-Bamidgeh 58, 39–45.
Stemberger, R.S., Gilbert, J.J., 1985. Body size, food concentration, and population growth in planktonic rotifers. Ecology 66, 1151–1159.
Støttrup, J.G., McEvoy, L.A., 2003. Live Feeds in Marine Aquaculture. Blackwell Science Ltd.
Theilacker, G.H., McMaster, M.F., 1971. Mass culture of the rotifer *Brachionus plicatilis* and its evaluation as a food for larval anchovies. Mar. Biol. 10, 183–188.
Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 34, 115–143.
Yúfera, M., 1987. Effect of algal diet and temperature on the embryonic development time of the rotifer *Brachionus plicatilis* in culture. In: May, L., Wallace, R., Herzig, A. (Eds.), Rotifer Symposium IV. Springer, Netherlands, pp. 319–322.
Yúfera, M., Pascual, E., 1980. Estudio del rendimiento de cultivos del rotifero *Brachionus plicatilis* O.F. Muller alimentados con levadura de panificacion. Investig. Pesq. 44, 361–368.
Yúfera, M., Pascual, E., 1985. Effects of algal food concentration on feeding and ingestion rates of *Brachionus plicatilis* in mass culture. Hydrobiologia 122, 181–187.