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# Increasing the levels of the essential trace elements Se, Zn, Cu and Mn in rotifers (*Brachionus plicatilis*) used as live feed

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ABSTRACT

Rotifers are a common first feeding diet for rearing marine fish larvae. However, the levels of Mn, Cu, Zn, Se and iodine found in rotifers are low and may be insufficient to meet larval fish requirements. This study investigates increasing the concentration of Mn, Cu, Zn, Se and iodine simultaneously in rotifers (*Brachionus plicatilis*) in both short term enrichments (3 h) or during batch cultures (6 days), using either organically bound or inorganic mineral sources. This study demonstrates that rotifers can simultaneously be produced with Mn, Cu, Zn and Se concentrations up to and higher than the known requirements of fish, while increasing the level of iodine in rotifers was ineffective at the concentrations tested. To produce rotifers with copepod levels of Mn, Cu, Zn and Se, only 6% of a commercial rotifer enrichment diet had to be replaced with organically bound minerals, leaving a large percentage of the rotifer diet free to deliver other important nutrients such as lipid and proteins. Rotifers enriched to copepod mineral levels and stored for 18 h retained 75–110% of their Se, Zn and Mn and 50% of their Cu. Overall, increasing rotifer mineral levels appears to be most effective when the mineral is available in an insoluble and hence ingestible form.



## 1. Introduction

Marine fish larvae fed natural zooplankton are generally superior in quality to those fed rotifers. For example, improved growth rates and survival (Busch et al., 2010, 2011; Koedijk et al., 2010; Payne et al., 2001; Rajkumar and Kumaraguru vasagam, 2006; Schipp, 2006) and large reductions in deformities (Fjelldal et al., 2009; Imsland et al., 2006) have been reported in marine fish larvae fed copepods rather than rotifers. The large differences in nutritional content between rotifers and copepods, and hence the inability of rotifers to fulfil the nutritional requirements of marine fish larvae, are suggested to be the primary reason for this (Bell et al., 2003; Evjemo and Olsen, 1997; Hamre et al., 2008a; van der Meeren et al., 2008). Thus, to raise the quality of rotifer fed fish larvae the nutritional quality of rotifers needs to be improved.

Modification of the fatty acid profile of rotifers has been carefully studied (Naz, 2008; Olsen et al., 1993; Rodriguez et al., 1997; Yamasaki et al., 2007) and has led to large improvements in larval quality (reviewed by Rainuzzo et al., 1997). However, minerals have received less attention, both in research and commercial culture. Hamre et al. (2008a) demonstrated that the level of many essential minerals in rotifers was lower than in copepods, with manganese (Mn) 2 fold, copper (Cu) 3 fold, zinc (Zn) 5 fold, selenium (Se) 33 fold and iodine (I) 10 fold lower in rotifers on average than the lowest levels found in copepods. It was then found that cod larvae fed rotifers have considerably lower whole body concentrations of I, Se and Mn than cod larvae fed copepods (Busch et al., 2010). Body mineral stores are a good indicator of mineral status (Baker, 1986), and the decreased levels in rotifer versus copepod fed cod larvae are an indication that mineral requirements of cod larvae are not fulfilled by rotifers. Further evidence that rotifers are mineral deficient, such as increasing the levels of Se and iodine in rotifers to copepod levels improved survival (Hamre et al., 2008b) and activity of Se dependent enzymes (Penglase et al., 2010) in Atlantic cod (*Gadus morhua*) larvae, was then demonstrated after the initial study of Hamre et al. (2008a). Furthermore, positive effects of copepod levels of Zn and Mn were demonstrated by Nguyen et al. (2008) for red sea bream (*Pagrus major*) larvae, and for copepod levels of Se and I by Ribeiro et al. (2011, 2012) for Senegalese sole (*Solea senegalensis*) larvae.

Thus, it is critical to develop reliable methods for manipulating essential trace minerals concentrations in rotifers for both scientific evaluation of the effects on larval fish, and also for commercial use to ensure nutritionally complete diets. To accomplish true dose response studies with minerals for fish larvae, it is important to be able to both increase and then control the level of the investigated mineral in live feed (Penglase et al., 2011). Rotifers lose many nutrients after the enrichment process, and thus the level of nutrient obtained by fish larvae ingesting the live feed at different time periods after enrichment can also change. For example rotifers have been shown to lose essential fatty acids (Naz, 2008; Rodriguez et al., 1997), and minerals

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(Matsumoto et al., 2009; Penglase et al., 2011; Srivastava et al., 2012) after enrichment. Without control of the level of nutrient in live feed, true dose response studies cannot be achieved.

This study investigated if the concentration of Cu, Mn, Zn, Se and I in rotifers could be increased simultaneously, and to what degree it is possible to control the concentration of the different minerals over time in both short term enriched (3 h) and batch cultured (6 days) rotifers. The use of organic and inorganic minerals forms was investigated and compared.

## 2. Materials and methods

### 2.1. Mineral specification

One organic and one inorganic mineral mix were made for this study. The organic mineral mix was used in the rotifer batch culturing experiments (Section 2.2.2) and short term enrichment experiments (Sections 2.2.3 and 2.2.4). The inorganic mineral mix was used as a comparison to the organic mineral mix in short term enrichment experiments (Section 2.2.3). The organic mineral mix contained three proteinated mineral products (Mn, Cu or Zn-Bioplex) and Se-yeast (Selplex) from one supplier (Alltech, Lexington, KY, USA), and one amino acid chelated mineral product from a second supplier (Frutarom, Belgium) (Table 1). The Bioplex products are based on hydrolysed soy protein and the majority of the minerals are attached to peptides with a maximum of 6–10 amino acids, but some single amino acid chelates are present due to the hydrolysation process (pers. comm. Alltech). The inorganic mineral mix was made using KI, $MnSO_4.H_2O$, $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$ and $Na_2SeO_3.5H_2O$ (Sigma-Aldrich, Germany). The total mineral level of the mineral mixtures (Table 1) was analysed by ICP-MS (Section 2.3).

#### 2.1.1. Particle size

Individual organic mineral products (except for chelated iodine) were dissolved in filtered seawater (0.45 μm, seawater as used in the rotifer stock culture, Section 2.2.1). Drops of the dispersed mineral products were placed on a glass slide and viewed using an Olympus BX51 binocular microscope fitted with an Olympus DP50 3.0 Camera and the program cell^BV.2.6 (Olympus, Germany) at 400× magnification. Particle diameter was measured for 450–550 particles from each product.

#### 2.1.2. Mineral solubility

The organic mineral mixture (Table 1) was weighed (100 mg) into 15 mL plastic tubes. Ten millilitres of filtered (0.45 μm) seawater (as per Section 2.2.1) was added to each tube, and the tubes were vortexed and placed on a shaking table at room temperature for 20 min. Tubes were then centrifuged (Eppendorf centrifuge 5810R; 4000 ×*g* for 8 min at 24 °C), the supernatant was removed and the pellet was re-suspended in 10 mL of new seawater before centrifuging and removal of the supernatant (×3). The insoluble fraction was retained and stored at −20 °C until analysis. Samples were prepared for ICP-MS analyses of minerals, by washing the retained insoluble fraction from the tubes and then acid digesting (Section 2.3). The concentration of minerals retained in the insoluble fraction was calculated from the initial weight of organic mineral mixture added to the tubes.

### 2.2. Rotifer trials

#### 2.2.1. Rotifer stock culture

A strain of *Brachionus plicatilis* (adult lorica length 202 ± 10 μm, width 173 ± 11 μm) was obtained from the Sagafjord Cod Hatchery, Stord, Norway, and was identical to that used by Penglase et al. (2011). Rotifers were cultured in 200 L tanks at 25 °C, 18–22 g $L^{-1}$, 1 μm filtered sea water with gentle aeration and at concentrations ranging from 200 to 2000 rotifers $mL^{-1}$ (average ≈ 1000 rotifers $mL^{-1}$). Rotifers were fed 3–4 times daily with 0.4 g Oriculture (Skretting, Norway) million$^{-1}$ rotifers day$^{-1}$ according to the manufacturer's directions. The rotifers were batch cultured, with water changes and tank cleaning occurring every 3–4 days. During the water change the rotifers were rinsed in a rotifer washer fitted with a 62 μm screen. For quality control, the rotifer concentration, egg ratio and viability were monitored daily. Rotifers were cultured with the above method for at least 10 days prior to use in trials.

#### 2.2.2. Rotifer batch culture trials with organic minerals

Rotifers were cultured in batches for 6 days at 25 °C with treatments consisting of Oriculture and increasing concentrations of the organic mineral mixture (Table 2). The experiment was performed in 15 L *Artemia* hatchers with initial rotifer densities of 300 individuals $mL^{-1}$ as described in Penglase et al. (2011), with the following modifications. Oriculture was mixed with sea water according to manufacture description and pipetted directly into the tanks. An organic mineral mixture solution was made by mixing 1.00 g mineral mixture with 100 mL tap water in a volumetric flask. This solution was mixed with a magnetic stirrer during its addition to tanks with a pipette. Additions rates of mineral mix to tanks were based on potential uptake and retention efficiency of minerals by rotifers ranging from 100% (lowest mineral mix addition level) to 2% (highest mineral mix addition rate) of mineral added (Table 2). Fresh solutions of mineral mixture and Oriculture were made at each feeding time. Rotifers were sampled, stored and lyophilized as per Penglase et al. (2011) before analysing for minerals (Section 2.3). The experiment was repeated in duplicate.

#### 2.2.3. Short term enrichment with organic or inorganic minerals

The enrichment trials were performed as described by Penglase et al. (2011), but with some modifications. In brief, rotifers were enriched for 3 h at 25 °C with increasing concentrations of mineral mix (Table 1) and decreasing levels of Origreen (Skretting, Norway) (Table 3) to maintain the total quantity of enrichment diet delivered to rotifers at 250 mg DW million$^{-1}$ rotifers. A mineral mixture solution was made

**Table 1**
Composition and mineral concentration of the organic mineral mix used in rotifer feeding trials.

| Product | % Included in organic mineral mix | Mineral concentration in organic mineral mix (g kg$^{-1}$ DW)[d] |
|---|---|---|
| Iodine chelate[a] | 21.3 | 11 |
| Mn proteinate[b] | 1.4 | 1.5 |
| Cu proteinate[b] | 1.7 | 1.8 |
| Zn proteinate[b] | 44.3 | 47 |
| Se-yeast[c] | 31.3 | 0.64 |

[a] 5% w/w iodine.
[b] 10% w/w Cu, Zn or Mn.
[c] 0.2% w/w Se.
[d] The inorganic mineral mix used to provide equivalent levels of minerals to the organic mineral mix in short term enrichment trials (Section 2.2.3) contained mineral concentrations of 44 g I, 5.1 g Mn, 7.4 g Cu, 231 g Zn and 2.8 g Se kg$^{-1}$ DW.

**Table 2**
Rotifer batch culture feeding rates, showing increasing levels of organic mineral mix in diets were offset by equivalent reductions in Oriculture. The resulting total feeding rates were 400 mg DW feed million$^{-1}$ rotifers day$^{-1}$ for all treatments.

| Organic mineral mix % of total diet (DW) | Feed (mg million rotifers$^{-1}$ day$^{-1}$) | | Mineral concentration in diet (mg kg$^{-1}$ DW) | | | |
|---|---|---|---|---|---|---|
| | Organic mineral mix | Oriculture | Mn | Cu | Zn | Se |
| 0 | 0 | 400 | 51 | 4.3 | 41 | 0.4 |
| 0.16 | 0.6 | 399.4 | 53 | 7.1 | 115 | 1.4 |
| 0.31 | 1.3 | 398.7 | 55 | 10 | 188 | 2.4 |
| 0.63 | 2.5 | 397.5 | 60 | 16 | 335 | 4.4 |
| 1.57 | 6.3 | 393.7 | 74 | 32 | 776 | 10 |
| 3.13 | 12.5 | 387.5 | 96 | 61 | 1511 | 20 |
| 7.83 | 31.3 | 368.7 | 164 | 145 | 3716 | 50 |

**Table 3**
Rotifer ST enrichment (3 h) feeding rates, showing increasing levels of organic mineral mix in diets were offset by equivalent reductions in Origreen. The resulting total feeding rates were 250 mg DW feed million$^{-1}$ rotifers day$^{-1}$ for all treatments.

| Organic mineral mix % of total diet (DW) | Feed (mg million rotifers$^{-1}$) | | Mineral concentration in diet (mg kg$^{-1}$ DW) | | | |
|---|---|---|---|---|---|---|
| | Organic mineral mix[a] | Origreen | Mn | Cu | Zn | Se |
| 0 | 0 | 250 | 14 | 3.3 | 37 | 0.5 |
| 0.8 | 2 | 248 | 26 | 18 | 413 | 5.6 |
| 1.5 | 3.8 | 246.2 | 37 | 30 | 741 | 10 |
| 3 | 7.5 | 242.5 | 59 | 57 | 1446 | 20 |
| 7.5 | 18.8 | 231.2 | 126 | 138 | 3559 | 48 |
| 15 | 37.5 | 212.5 | 237 | 273 | 7081 | 96 |
| 37.6 | 94 | 156 | 573 | 679 | 17,695 | 241 |

[a] An inorganic mineral mix was also fed to rotifers at the same treatment levels as the organic mineral mix, where equivalent levels of minerals in inorganic form were dissolved directly into the aqueous phase and Origreen fed at 250 mg DW per million rotifers.

by mixing 1.00 g organic mineral mixture with 100 mL tap water in a volumetric flask. This solution was mixed with a magnetic stirrer during its addition to tanks with a pipette. As a comparison, identical rotifer enrichments using an inorganic minerals mix (Table 1) to make available the same concentration of minerals rotifer$^{-1}$ as the organic mineral treatments, were performed. As with batch culture experiments (Section 2.2.2), addition rates of mineral mix to tanks were based on potential uptake and retention efficiency of minerals by rotifers ranging from 100% (lowest mineral mix addition level) to 2% (highest mineral mix addition rate) of mineral added (Table 3). Additions to the tanks were made at the rate of one tank every 10 min to allow for sampling time, and thus enable the same enrichment period for all tanks. At approximately 2.5 h a sample of rotifers from each tank was observed for differences in locomotion and mortality. Rotifers were sampled after 3 h, stored and lyophilized as per Penglase et al. (2011) before analysing for minerals (Section 2.3). The experiments were repeated; $n = 2/3$ for controls/organic minerals, $n = 2/4$ for controls/inorganic minerals ($n = 4$ for total number of controls).

*2.2.4. Short term enrichment with organic or inorganic manganese*

Analysis of results from short term (ST) enrichments showed that the Mn levels used in Section 2.2.3 resulted in insignificant responses in rotifer Mn levels. A second trial was performed using higher levels of proteinated Mn (Section 2.1) along with Origreen, or equivalent levels of inorganic Mn. The trial was performed identically to those in Section 2.2.3, but for simplicity, other minerals were not included. Proteinated Mn was included at 0, 1.2, 3, 6 or 12% replacement of Origreen in the enrichments, making treatment diets ranging from 1.2 to 12 g Mn kg$^{-1}$ (2 to 20 fold higher levels than the maximum tested in Section 2.2.3). The experiment was repeated, $n = 4/2$ for controls/treatment.

*2.2.5. Uptake efficiency of soluble versus the insoluble fraction from organic minerals in 3 h enrichments*

To investigate the difference in uptake efficiency between the soluble versus the insoluble fraction of the organic minerals, the mineral products were first separated into soluble and insoluble fractions using an upscaled version of the method described in Section 2.1.2. In brief, organic mineral mix ($\approx$1 g) was weighed into a bigger tube (50 mL plastic tube), and a larger volume of seawater (40 mL) was used in each pellet washing. The supernatant was kept. Enrichment of rotifers was then performed as in Section 2.2.4, except either the insoluble or soluble mineral fraction was used. The insoluble fraction was re-suspended in water before adding directly to the rotifer tanks. The experiments were repeated, $n = 2$–3 for controls and $n = 3$–4 for treatments.

*2.2.6. Effect of mineral enrichment on oxidation in rotifers*

To investigate if the addition of organic or inorganic mineral mixtures could lead to oxidation, rotifers ST enriched (as per method in Section 2.2.3) with either 1.5% (low dose) or 15% (high dose) of the enrichment diet as organic or the equivalent inorganic mineral mix (Table 1) were sampled and analysed for thiobarbituric acid-reactive substances (TBARS). To preserve the volatile compounds analysed with TBARS, these rotifers samples were flash frozen and stored at −80 °C until analysis. The experiments were repeated in triplicate.

*2.2.7. Mineral retention with cold storage of short term enriched rotifers*

The storage trial was performed in a similar way as described for the cold storage rotifer trial in Penglase et al. (2011), with some modifications. Briefly, rotifers were enriched for 3 h at 23 °C at a concentration of 2000 rotifers mL$^{-1}$ in a 100 L enrichment tank. Rotifers were enriched with 0.2 g Origreen million$^{-1}$ individuals and an organic mineral mixture (Table 4) predetermined to give rotifer concentrations of 3.3 mg Se, 12 mg Mn, 165 mg Zn and 33 mg Cu kg$^{-1}$ DW, which is within the range of copepod mineral levels. Rotifers were then cleaned for 30 min in warm seawater (23 °C) and then cold seawater (10 min) decreasing the temperature of the rotifers to 12.5 °C. Rotifers were then transferred into 12×15 L aerated *Artemia* hatchers (10 L volume) at a density of approximately 1400 rotifers mL$^{-1}$ in a cold room (6–7 °C). Rotifers were sampled at times 0, 1, 2, 5 and 10 h. The time 0 h samples were collected 35 min after the start of washing. The storage temperature decreased from 11 °C at the 1 h sampling to 6 °C after 10 h storage. Rotifers were sampled, stored and lyophilized as per Penglase et al. (2011) before analysing for minerals (Section 2.3). The experiment was repeated in triplicate.

*2.3. Analytical methods*

The minerals were analysed according to method described by Julshamn et al. (2004). Freeze dried samples were weighed (200 mg) into Teflon digestion vessels. Two millilitres of nitric acid (65% HNO3 Suprapur®, Merck, Germany) and 0.5 mL of hydrogen peroxide (30% $H_2O_2$, Merck, Germany) were then pipetted into each vessel and the samples were wet digested in a microwave. After microwaving, digested samples were rinsed from bombs and diluted with dd$H_2O$ to 25 mL in a volumetric flask. The samples were then analysed for minerals using inductively coupled plasma with mass spectrometry (ICP-MS; Agilent 7500 series, USA) along with blanks and standard reference material as described by Julshamn et al. (2004), with the exception that the mass of minerals measured varied with Mn (Mass 55) used in the current study. The standard reference materials used (NIST-SRM 1566, Oyster tissue, USA; TORT-2, NRC, lobster hepatopancreas, Canada) had similar concentrations of minerals as the samples analysed. Thiobarbituric acid-reactive substances (TBARS) were determined as described by Hamre et al. (2001).

*2.4. Statistics*

Statistica software (Statsoft Inc., 2008, Tulsa, USA, Ver. 8) was used for statistical analysis with ANOVA. Data were checked for homogeneity of variances using Levene's test ($p<0.05$). Significance between treatments was tested with one way ANOVA followed with Tukey's honestly significant difference (HSD) post-hoc test. Differences among means were considered significant at $p<0.05$. Models were fitted to minimise the sum of squares using GraphPad Prism (GraphPad Software, San

**Table 4**
The quantity of mineral product used in addition to 250 mg Origreen for enrichment of rotifers to or within range of copepod mineral levels before analysis of mineral retention with storage.

| Organic mineral product (mg) fed million$^{-1}$ rotifers | | | |
|---|---|---|---|
| Selplex | Mn bioplex | Cu bioplex | Zn bioplex |
| 1.5 | 5 | 0.32 | 10 |

**Table 5**
The speciation of minerals in the organic mineral mix, including the total concentration of minerals (mg $kg^{-1}$ DW), the percentage of minerals in the insoluble fraction before and after normalisation for loss of mass upon aqueous suspension.

| | Mn | Cu | Zn | Se |
|---|---|---|---|---|
| Total mineral concentration (mg $kg^{-1}$ DW) | 1502 | 1760 | 47 270 | 645 |
| % Insoluble mineral (% ISM) in organic mineral mix compared to total | 5.4 ± 0.7 | 37 ± 4 | 11.5 ± 0.3 | 86 ± 6 |
| % ISM normalised compared to total[a] | 8.7 ± 0.4 | 72 ± 3 | 29 ± 1 | 141 ± 1 |

Total mineral concentration data are $n = 1$; all other data are mean ± SD, $n = 3$.
[a] After separating from the soluble fraction, the insoluble fraction was dried, reweighed and then measured for mineral concentration, to account for total loss of mass, all minerals had statistically higher mineral retention when accounting for mass in the insoluble fraction ($p < 0.01$, one-way ANOVA).

Diego, CA, USA, Ver. 5). Modelled data were tested against the null hypothesis; Mineral X concentration in rotifer diet had no effect on rotifer mineral X content, for normality of residuals (D'Agnostino and Pearson) and for curve deviation from data points (replicates test). Data are presented as mean ± SD.

## 3. Results

### 3.1. Mineral speciation

The percentage of mineral that was insoluble upon suspension of the organic mineral products in seawater varied between minerals. Most of the Mn and Zn was soluble and low levels (<12%) of the minerals were retained in the insoluble fraction (Table 5). Copper was intermediate with approximately one third remaining insoluble, while Se, added as Se-yeast to the organic mineral mix, was largely insoluble (>85%, Table 5). After accounting for total loss of mass when the organic mineral mix was dissolved, the concentration of mineral associated with the insoluble material was a greater percentage than the insoluble mineral (Table 5). Although the difference between minerals varied for this measure, the general trend showed that for a given mass of insoluble or soluble material, more mineral was associated with the insoluble fraction, and hence the overall loss of solutes that was not associated with mineral was greater than for mineral containing solutes (Table 5).

### 3.2. Particle size

When dispersed in seawater the particle size of the insoluble fraction of the organic mineral products used in this study ranged from 2.7 to 5 μm in diameter (Table 6). The standard deviation, and hence the variability in particle size, was high compared to the mean, particularly for organic Mn (Table 6). Of all the particles measured, 100% of the organic Zn and Se particles and 99% of the organic Mn and Cu particles were less than 10 μm in diameter.

### 3.3. Rotifer enrichment with minerals

#### 3.3.1. Rotifer control diets and mineral concentrations

Only the Mn concentrations were substantially different between the control rotifer diets, with the batch culture (Oriculture) diet having 3.5 fold higher Mn levels than the enrichment (Origreen) diet (Table 7). Furthermore, only Mn levels in rotifers were significantly ($p < 0.05$) affected by diet, being approximately 20% higher in rotifers fed the culture diet (Table 7).

**Table 6**
Particle size of the insoluble fraction of the organic minerals when dispersed in seawater. Data are mean ± SD, $n = 450–550$.

| | Cu bioplex | Mn bioplex | Zn bioplex | Selplex |
|---|---|---|---|---|
| Diameter (μm) | 2.7 ± 1.6 | 3.2 ± 2.6 | 3.2 ± 1.3 | 5.0 ± 1.2 |

**Table 7**
Concentration of minerals (mg $kg^{-1}$ DW) in rotifer control diets (Oriculture and Origreen) and the rotifers fed these diets.

| | Mn | Cu | Zn | Se |
|---|---|---|---|---|
| Oriculture | 51 ± 1 | 4.3 ± 0.1 | 41 ± 1 | 0.44 ± 0.09 |
| Origreen | 14.3 ± 0.4 | 3.3 ± 0.1 | 37 ± 1 | 0.50 ± 0.01 |
| Cultured rotifers[a] | 9.9 ± 0.8b | 5.2 ± 0.3 | 71 ± 1 | 0.49 ± 0.01 |
| Enriched rotifers[b] | 8.1 ± 0.4a | 5.7 ± 0.4 | 71 ± 4 | 0.54 ± 0.05 |

Data are mean ± SD, $n = 2$ for diets, $n = 4$ for enriched rotifers and $n = 8$ for cultured rotifers.
Letters denote statistical differences in specific mineral concentration between enriched and cultured rotifers ($p < 0.05$, one-way ANOVA).
[a] Fed Oriculture for ≥10 days.
[b] Fed Origreen for 3 h.

#### 3.3.2. Rotifer batch cultures with organic minerals

No effect of diet was found on rotifer growth or egg ratio during the batch culture period (data not shown). Rotifer Mn concentrations did not increase with increasing levels of organic Mn in the batch culture diets (Fig. 1A), but rotifers did have on average 14% higher Mn levels after 3 (average 11.1 mg Mn $kg^{-1}$ DW) versus 6 (average 9.7 mg Mn $kg^{-1}$ DW) days of batch culturing irrespective of diet. Both Cu and Se levels in rotifers increased linearly with increasing dietary inclusion of organic Cu and Se, respectively (Fig. 1B and D). Meanwhile rotifer Zn levels increased non linearly, with a decreasing percentage increase in Zn in relation to increasing organic Zn inclusion in rotifer diets (Fig. 1C). No further increase in rotifer levels of Cu, Zn or Se was found after culturing the rotifers with organic mineral mix for 6 versus 3 days ($p > 0.48$), and data for 3 and 6 days could thus be combined and presented as a single curve fitting (Fig. 1B-D).

#### 3.3.3. Short term enrichment with organic or inorganic minerals

To test whether mineral speciation effected rotifer mineral concentration, rotifers were ST enriched (3 h) with diets including increasing concentration of organic mineral mix, or the equivalent mineral levels in inorganic form. A small but statistically significant ($p < 0.05$) linear increase in rotifer Mn concentrations occurred with increasing Mn additions, irrespective of whether it was organic or inorganic Mn (Fig. 2A). Rotifer Cu concentrations increased non linearly when enriched with increasing concentrations of organic Cu, but linearly with inorganic Cu (Fig. 2B). However, on visual inspection of the graph, inorganic Cu may result in a similar response in rotifer Cu concentration, but lack of data points prevented this conclusion (Fig. 2B). Rotifer Zn concentrations responded differently to Zn speciation ($p < 0.01$). Both inorganic and organic Zn resulted in a non linear response in rotifer Zn levels, similar to what was observed in batch cultured rotifers (Figs. 1C, 2C). Higher rotifer Zn concentrations were obtained with organic versus inorganic Zn at the highest enrichment levels (Fig. 2C), although as with Cu there are a lack of data points at medium enrichment levels with inorganic Zn, so it is unknown if this effect occurs before the highest enrichment level. Rotifer Se concentration increased linearly with increasing ST enrichment levels of Se-yeast or Na-Se ($p < 0.05$, Fig. 2D), but the response was increased with Se-yeast ($p < 0.01$), with rotifers containing up to 14 fold more Se at the highest enrichment levels when Se-yeast versus Na-Se was used (Fig. 2D). Due to the low response in rotifer Mn levels to the dietary Mn levels in the first enrichment trial (Fig. 2A), a second ST enrichment was performed with organic or inorganic Mn replacing Origreen in the enrichment diet (Fig. 3), without supplementation of the other minerals, to test whether it was possible to enrich rotifers with Mn. In this trial, rotifer diets ranged from 1.2 to 12 g Mn $kg^{-1}$ DW, and a positive linear response in rotifer Mn levels was observed (Fig. 3). Rotifer Mn levels increased more with organic versus

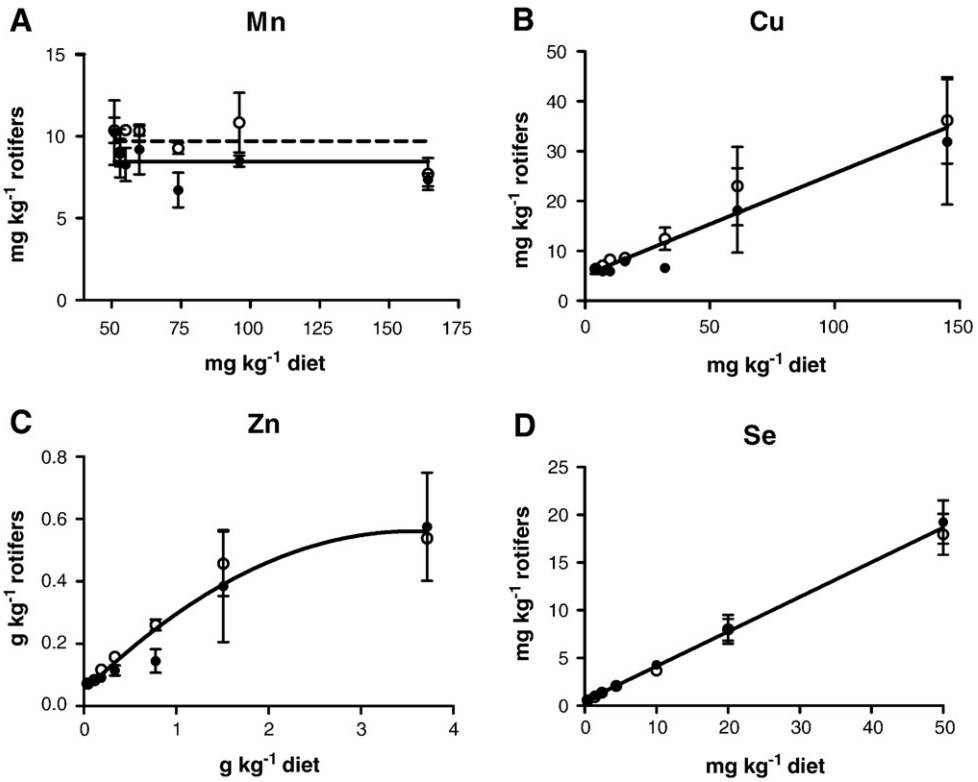


**Fig. 1.** The concentration of mineral (mg or g $kg^{-1}$ DW) in rotifers fed culture diets with increasing % of organic mineral mix for 3 (○) or 6 (●) days. Control culture diets were 400 mg Oriculture $million^{-1}$ rotifers $day^{-1}$, and treatments consisted of increasing levels of organic mineral mix up to a 7.8% replacement of the control diet (Table 2). Data are mean ± SD, $n=2$. Lines represent best fit models (first order polynomial; Se, Cu, second order; Zn, no effect (horizontal line); Mn). Data from 3 and 6 days were combined when curve fittings for the two time points were not statistically different (extra sum of squares $F$ test, $p>0.05$). $R^2$ values for the curves are, Cu = 0.74, Zn = 0.82, Se = 0.97.

inorganic Mn, with a 2.2 fold difference in rotifer Mn levels occurring at the highest levels tested (Fig. 3). The percentage of each organic mineral source required in rotifer diets to obtain minimum copepod levels is given in Table 8.

*3.3.4. Uptake efficiency of the soluble versus insoluble fraction from organic minerals in 3 h enrichment*

The minerals in several of the organic mineral products used in this study were found to be highly soluble (Table 5). To test whether increased rotifer mineral concentrations found in response to the organic minerals were a response to the soluble or insoluble fraction, or both, these two fractions were separated and used to enrich rotifers separately. The separation of the fractions resulted in the addition of different concentrations of insoluble versus soluble minerals, and hence a large difference in the *x* axes range between the two fractions for a given mineral (Fig. 4A–D). The increase in rotifer mineral concentration was higher in response to the insoluble than the soluble mineral fraction for all minerals tested (Fig. 4A–D). For example at the highest level included in rotifer enrichments, the insoluble fraction resulted in rotifers with 1.2 fold higher Mn, 2.1 fold higher Cu and 1.5 fold higher Zn levels than the soluble fraction. In contrast to the other products, the Se in the Se-yeast was largely insoluble (Table 5), and hence the levels of soluble Se added to enrichments were low (Fig. 4D). However, even at these low levels, large differences in rotifer Se content were observed, with those included in enrichments with the insoluble fraction having 7.6 fold higher Se levels than the soluble Se group (Fig. 4D).

*3.3.5. Effect of mineral enrichment on oxidative status of rotifers*

The culture feed (Oriculture) had a thiobarbituric acid relative substances level (TBARS) of 53 nmol $g^{-1}$ DW and the enrichment product (Origreen) had a TBARS of 36.1 nmol $g^{-1}$ DW (Table 9). All batches of rotifers had a low concentration of TBARS ranging from 4.7 ± 0.2 to 8.6 ± 1.3 nmol $g^{-1}$ WW, and there was no significant difference in concentration of TBARS between the enriched non mineral supplemented control group and the different groups supplemented with minerals (Table 9). However, rotifers enriched with a low dose of organic minerals had significantly higher TBARS (1.6–1.8 fold, $p<0.05$) than cultured rotifers or those enriched with a high dose of inorganic minerals (Table 9).

*3.3.6. Retention of minerals during cold storage of rotifers*

Rotifers were enriched with one level of organic mineral mix (Table 4) and then stored for up to 18 h in clean water to assess the retention of minerals over time (Fig. 5). Rotifer Mn levels increased by 10% after 18 h storage (Fig. 5). Both rotifer Se and Zn levels behaved similarly in storage, by first decreasing quickly to approximately 85% of the starting values in the first h, 80% in 5 h and 75% of the starting level after 18 h of storage (Fig. 5). Rotifer Cu levels behaved in a similar pattern but to a greater extent than the Se and Zn levels. After 1 h rotifers contained only 50% of their starting Cu level, but by 18 h they had decreased only slightly in Cu and contained 45% of their starting level (Fig. 5).

## 4. Discussion

This study differed from other studies on rotifer mineral enrichments as it investigated increasing rotifer levels of five minerals simultaneously, versus a maximum of two minerals in previous studies (Hamre et al., 2008b; Matsumoto et al., 2009; Penglase et al., 2010; Penglase et al., 2011; Srivastava et al., 2012). Furthermore, the effectiveness of organically bound versus inorganic sources of mineral for increasing rotifer mineral levels was also investigated. This study found that rotifer Cu, Zn and Se levels can be easily manipulated, while uptake and retention of Mn and iodine by rotifers were low, requiring higher addition rates

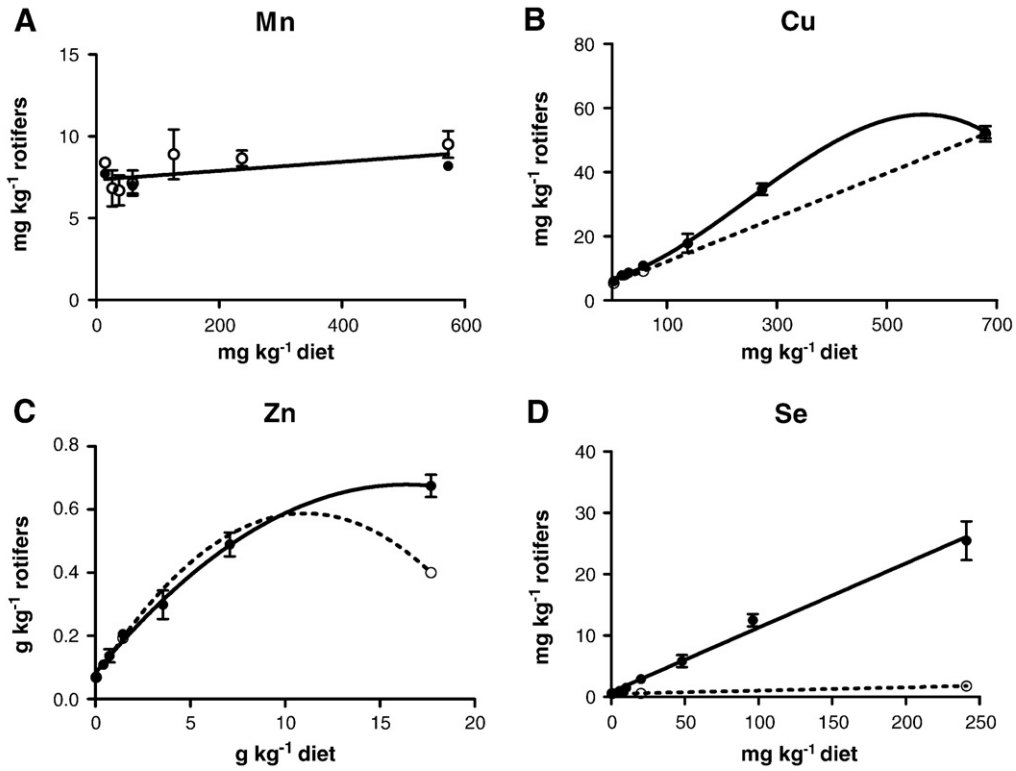


**Fig. 2.** The concentration of mineral (mg or g $kg^{-1}$ DW) in rotifers enriched with increasing % of organic (●, solid line) or inorganic (○, broken line) mineral mix for 3 h. Control enrichment diets were 250 mg Origreen $million^{-1}$ rotifers 3 $h^{-1}$ and treatments consisted of increasing levels of organic mineral mix up to a 37.6% replacement of the control enrichment diet (Table 3). An inorganic mineral mix was used to provide the same levels of mineral as the equivalent organic mineral mix treatment. Data are mean ± SD, $n=2$ for controls, $n=3$ for organic mineral treatments and $n=4$ for inorganic mineral treatments. Lines represent best fit models (first order polynomial; Mn inorganic + organic $R^2=0.17$, Cu inorganic $R^2=0.99$, Se inorganic $R^2=0.47$, organic $R^2=0.95$, second order; Zn inorganic $R^2=0.99$, organic $R^2=0.96$, third order; Cu organic $R^2=0.94$). Data from organic and inorganic mineral enrichments were combined when curve fittings were not statistically different (extra sum of squares $F$ test, $p>0.05$).

of Mn (this study) or as investigated in a related study, iodine (Srivastava et al., 2012). Overall an addition rate of around 6% of the total ST enrichment diet was required to elevate rotifers to copepod levels of Mn, Cu, Zn and Se (Table 8). A total of 1.5% replacement of the batch culture diet was required to elevate rotifers to copepod levels of Cu, Zn and Se, while Mn enrichment at this stage was unsuccessful even at the highest levels tested (164 mg Mn $kg^{-1}$ DW; Table 2). Iodine will not be discussed at length as this topic has been covered in the related study by Srivastava et al. (2012).

It has been suggested that enrichment of rotifers with minerals will be more efficient if the mineral is bound to an ingestible particle, as opposed to soluble forms (Penglase et al., 2011), and the current study supports this argument. The particle size of the dispersed non soluble fraction of the organic minerals (2.7–5 μm) was found to be within or close to the optimal ingestion size for rotifers of 4 to 10 μm (Baer et al., 2008; Hansen et al., 1997; Rothhaupt, 1990). Rotifers fed this insoluble fraction had 1.2 to 7.6 fold higher levels of mineral than rotifers fed

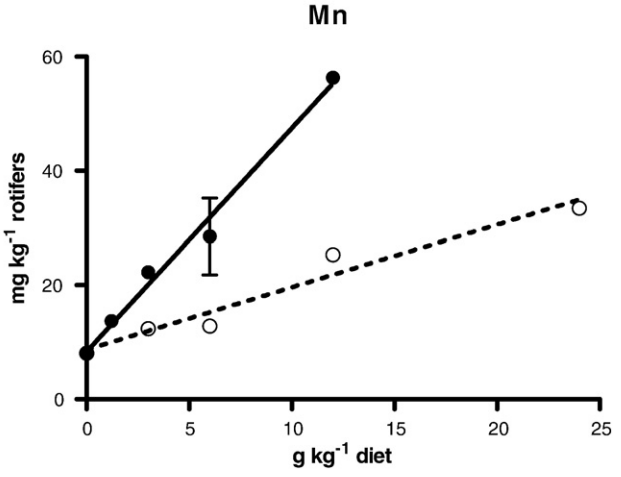


**Fig. 3.** The concentration of Mn (mg $kg^{-1}$ DW) in rotifers enriched with increasing % of organic (●, solid line) or inorganic (○, broken line) Mn for 3 h. Control enrichment diets were 250 mg Origreen $million^{-1}$ rotifers 3 $h^{-1}$, and treatments consisted of increasing levels of proteinated Mn up to a 12% replacement of the control enrichment diet. An inorganic mineral mix was used to provide levels of mineral equivalent to the organic mineral mix treatment. Data are mean ± SD, $n=4$ for controls and $n=2$ for treatments. Lines represent best fit models (first order polynomial; Mn inorganic or organic $R^2=0.96$).

**Table 8**
The percentage of rotifer batch culture or short term enrichment diet that is required to be replaced by the organic mineral mixtures to obtain rotifers with copepod levels of minerals.

| | Copepod level[a] | % of diet required | |
|---|---|---|---|
| Product | mg/kg DW | Batch culture[b] | ST enrichment[c] |
| Mn proteinate | 12[d] | ND | 0.94 |
| Cu proteinate | 12 | 0.03 | 0.07 |
| Zn proteinate | 340 | 1.12 | 3.8 |
| Se-yeast | 3 | 0.33 | 1.0 |
| Total replacement % | Not applicable | 1.5 | 5.8 |

[a] Data obtained from Hamre et al. (2008a).
[b] Total diet used was 400 mg Oriculture $million^{-1}$ rotifers $day^{-1}$.
[c] Total diet used was 250 mg Origreen $million^{-1}$ rotifers 3 $h^{-1}$.
[d] Control rotifers had above minimum copepod levels of Mn (8 mg Mn $kg^{-1}$ DW) in the current study, so higher level chosen to simulate increasing rotifer Mn content by 3–4 mg Mn $kg^{-1}$ DW.

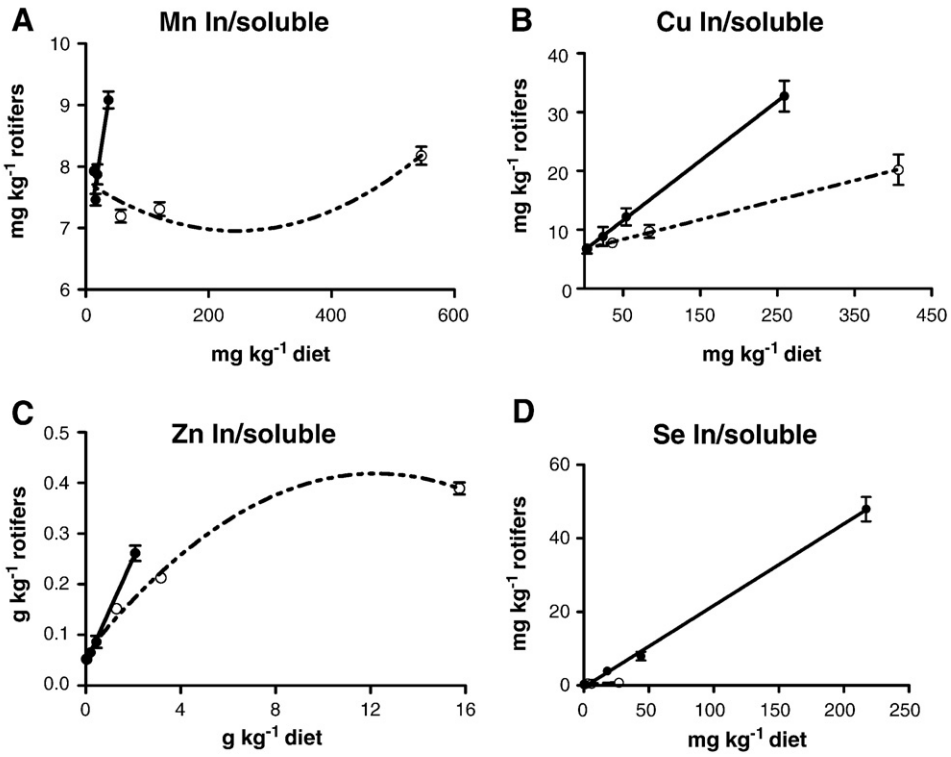


**Fig. 4.** The concentration of mineral (mg or g $kg^{-1}$ DW) in rotifers enriched (3 h) with increasing % of the soluble fraction (○, broken line) or insoluble fraction (●, solid line) of the inorganic mineral mix. Control enrichment diets were 250 mg Origreen $million^{-1}$ rotifers 3 $h^{-1}$, and treatments consisted of increasing levels of either the soluble or insoluble fraction of an organic mineral mix up to a 37.6% replacement of the control enrichment diet. Data are mean ± SD, $n = 2$–3 for controls, $n = 3$–4 for treatments. Lines represent best fit models (first order polynomial; insoluble Mn ($R^2 = 0.84$), Cu ($R^2 = 0.95$), Zn ($R^2 = 0.97$), Se ($R^2 = 0.98$), soluble Cu ($R^2 = 0.87$), Se ($R^2 = 0.55$), second order; soluble Mn ($R^2 = 0.69$), Zn ($R^2 = 0.98$)).

equivalent levels of mineral from the soluble fraction. These differences occurred even at the low insoluble Mn, Cu and Zn levels tested (Fig. 4A–C), as the majority of the mineral in these organic mineral products was soluble (95–63%). Overall, the data demonstrate that if a high percentage of the mineral does not remain associated with a particle within the size range rotifers can eat, such as the Se in Se-yeast, then the addition of inorganic minerals to ST enrichments would be as effective, cheaper and a more practical method of increasing rotifer mineral levels. However, there may be differences in the bioavailability and/or toxicity of minerals to rotifers and to the consuming fish larvae by enriching with either organic or inorganic mineral mixtures. This needs further investigations.

Uptake of the added Cu or Zn was ≈10% during ST enrichments for both inorganic and organic forms. Meanwhile the uptake of Se from the Se-yeast was 24%, which is higher but within range of the 16% uptake reported by Penglase et al. (2011). Similarly the uptake of inorganic Se was estimated to be 1% in the current study, which is higher than the 0.3% estimated in the study by Penglase et al. (2011). Overall the uptake of Se from Se-yeast is 23 (this study) to 40 (Penglase et al., 2011) fold higher than that from inorganic Se (sodium selenite). As rotifers preferentially ingest feed within a given size range (Baer et al., 2008), the higher uptake of Se from Se-yeast found in the current study may be due to increased feeding efficiency on the Se-yeast because feed fed simultaneously (Origreen and Oriculture) was of less optimal size than that used by Penglase et al. (2011) (*Chlorella* algae and fish oil).

Uptake of added Mn was around 1% for organic and 0.4% for inorganic ST enriched rotifers. As discussed, the low Mn uptake from the organic mineral form appears to be due to its high solubility, as Mn uptake by rotifers was found to occur predominately from insoluble Mn (Fig. 4A). But this does not explain why the uptake of inorganic and/or soluble Mn and Se is lower than for Cu and Zn. These four investigated minerals are ubiquitous in the environment and probably essential for rotifers, as activities of the mineral dependent enzymes glutathione peroxidise (Se), and superoxide dismutases (both Mn and Zn + Cu dependent forms) were detected in rotifer extracts (unpublished data), while Mn dependent superoxide dismutase mRNA is one of the most abundant transcripts in rotifers (Hagiwara et al., 2007). Thus mechanisms to take up, store and excrete these minerals must be present (Rainbow, 2002), and the differences observed in rotifer uptake may reflect a mineral to mineral differences in the rates of these mechanisms. Whatever the exact reason, the relatively low uptake of Mn appears to also occur in *Artemia*. For example to increase the Mn content in *Artemia* by 25 mg $kg^{-1}$ DW required

**Table 9**
Concentration of thiobarbituric acid-reactive substances (TBARS) (nmol $g^{-1}$) in rotifer feed, un-enriched rotifers (cultured), rotifers enriched with only Origreen and rotifers enriched with minerals in addition to Origreen.

| | | Control | | Organic minerals | | Inorganic minerals | |
|---|---|---|---|---|---|---|---|
| Oriculture | Origreen | Cultured | Enriched | Low dose | High dose[a] | Low dose | High dose |
| 53 | 36.1 | 4.7 ± 0.2[a] | 6.4 ± 1.2[ab] | 8.63 ± 1.3[b] | 7.5 ± 1.7[ab] | 5.9 ± 0.6[ab] | 5.5 ± 0.6[a] |

Data are mean ± SD, $n = 1$ for diets and $n = 3$ for rotifers. Values are in dry weight for rotifer feed, and wet weight for rotifers. Letters denote differences between rotifer TBARS levels (one-way ANOVA, $p < 0.05$).

[a] High dose is 15% replacement; low dose is 1.5% replacement of diet with organic or equivalent inorganic mineral mix.

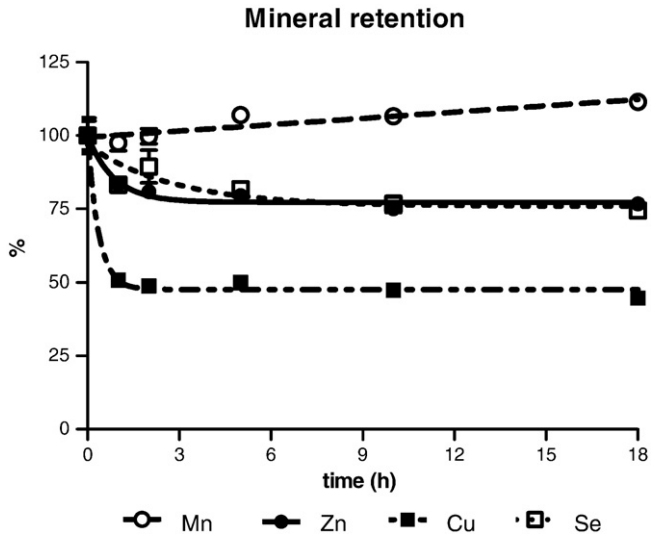


**Fig. 5.** The concentration (%) of Mn (○), Cu (■), Zn (●) and Se (□) in rotifers enriched with organic mineral mix (Table 4) and then stored in clean water for up to 18 h. Data are mean ± SD, $n = 3$. Lines represent best fit models (first order polynomial; Mn (○, segmented line, $R^2 = 0.45$), one phase decay; Cu (■, segmented line, $R^2 = 0.96$), Zn (●, solid line, $R^2 = 0.80$), Se (□, dotted line, $R^2 = 0.61$)).

additions of 240 mg of soluble Mn $L^{-1}$, while to increase Zn content by 200 mg $kg^{-1}$ DW required additions of only 100 mg soluble Zn $L^{-1}$ in a 24 h enrichment (Nguyen et al., 2008).

The Mn concentration was high in the algae based rotifer culture diet (Oriculture; 51 mg Mn $kg^{-1}$ DW, Table 7) used in the current study. As observed previously (Hamre et al., 2008a; Lie et al., 1997; Penglase et al., unpublished manuscript), algae are generally high in Mn (17–120 mg Mn $kg^{-1}$ DW) and rotifers fed primarily algae generally have high Mn levels (12–18 mg Mn $kg^{-1}$ DW). Furthermore, the high Mn levels in algae fed rotifers are within the range found in copepods (8–25 Mn $kg^{-1}$ DW), and suggest that algae fed rotifers do not need further supplementation of Mn to meet larval fish requirements. In line with this, rotifers in the current study fed oriculture had 9.9 mg Mn $kg^{-1}$ DW, which is high compared to the average 4.4 mg Mn $kg^{-1}$ DW in rotifers found by Hamre et al. (2008a). Subsequently, increasing the level of Mn was attempted on rotifers which already had relatively high levels of Mn. Furthermore, additions of Mn to batch culture diets resulted in relatively small increases in the total dietary Mn fed in relation to the change in dietary levels for other minerals tested (Table 2 and 3). Unlike Mn, iodine uptake was not investigated beyond the point of determining uptake, which was less than 2% of what was made available to rotifers. However, in a later study (Srivastava et al., 2012), uptake of iodine from the chelated iodine used in this study was found to be extremely low, at around 0.1%. In the current study, batch cultured rotifers had a decrease in mineral uptake efficiency with time, as the level of minerals did not change between 3 and 6 days, while mineral additions continued at the same rate between the 3 and 6 days period. This effect was also observed by Penglase et al. (2011) in rotifers which did not change Se concentration after 6 versus 3 days of culturing with Se-yeast.

In general, the retentions of the minerals in rotifers after ST enrichment were high, with 110 (Mn), 75 (Se or Zn) and 45 (Cu) % of the original mineral content retained after 18 h storage in clear water. This is important from a practical point of view as high rotifer mineral retention ensures fish larvae ingest the intended mineral quantity. The trend of Cu, Zn and Se retention in rotifers were similar to each other, starting with an initial quick decrease in rotifer mineral content within the first hour and followed by a relatively slow rate of mineral decrease for the remaining 17 h. This 2 phase pattern was also found in rotifers fed indigestible beads (Baer et al., 2008). In that study 40% of the beads were excreted from stored rotifers after 1 h, while 60% remained in the gut for the remaining 17.5 h. However, while the rapid initial excretion of gut contents found for beads helps explain the initial rapid loss of mineral in the current study, the behaviour of the digestible particles fed in this study is probably different from the indigestible beads during the last 17 h. As discussed, the investigated minerals are probably essential for rotifers, and hence are probably actively taken up and readily proceed through existing metabolic pathways. Manganese behaved differently to the other minerals, as rotifer Mn levels increased slightly over the 18 h storage period. The slight increase could be explained by maintenance of absolute Mn quantity per rotifer together with rotifer mass loss from excretion. Overall the Mn result is interesting, because on one hand it was found that enriching rotifers with Mn was difficult, but on the other it was found that once elevated with Mn, it appears to remain tightly bound. The retention results of minerals differ somewhat from previous reports. For example Matsumoto et al. (2009) found rotifers fed Zn enriched *Chlorella* (green microalgae) had 50% Zn retention after 24 h storage, versus the 75% Zn retention after 18 h storage in the current study. No loss in Se was found after 18.5 h storage of Se-yeast fed rotifers by Penglase et al. (2011), versus the 25% loss observed in the current study, despite the use of an identical rotifer strain, Se-yeast and similar methods in the two studies.

One factor that did differ between this and the study by Penglase et al. (2011) is the presence of the increased levels of Zn, Mn and Cu in the current study. As rotifer mineral levels were increased simultaneously in this study, it is not possible to determine if the minerals affected one another's uptake or excretion in rotifers. However, such effects have been demonstrated previously. Matsumoto et al. (2009) found that Zn enrichment of rotifers reduced rotifer Mn levels, and a trend of reduced rotifer Cu levels was also observed. An antagonistic effect between Zn and Mn was also found in *Artemia*, as when enriched with Zn and Mn, *Artemia* had lower levels of Mn than when enriched with Mn alone (Nguyen et al., 2008). Furthermore the same study found that Zn enriched *Artemia* had lower iron contents. Feeding rotifers Se-yeast during 3 h enrichments decreased mercury concentration in rotifers, while culturing rotifers for 6 days with Se-yeast resulted in lower rotifer levels of both arsenic and cadmium (Penglase et al., unpublished manuscript). Therefore, mineral interactions do appear to occur, and affect specific mineral concentrations, in rotifers. Thus efforts to increase one mineral in rotifers may require the co-enrichment with other minerals to maintain their concentrations. This may be particularly important in feeding studies focusing on one mineral, where a minimal change in other parameters is required.

The current study did not investigate mineral speciation in rotifers. The distribution and speciation of minerals is particularly important if extrapolating the mineral concentration found in copepods directly for use in rotifers as fish larvae feed. It has been shown that Se in rotifers fed Se-yeast is bioavailable to fish larvae (Penglase et al., 2010), and is most likely present in rotifers as protein incorporated selenomethionine (Penglase et al., 2011). Overall, minerals in enriched rotifers appear to be more bioavailable than those in copepods. For example Atlantic cod (*Gadus morhua*) larvae fed rotifers with copepod levels of Se were shorter than controls in the early larval period, while those fed copepod levels of iodine (Penglase et al., in press) or Cu (Penglase et al., unpublished manuscript) displayed signs of iodine or Cu toxicity, respectively. Meanwhile the superior performance of cod larvae fed copepods (Busch et al., 2010, 2011; Finn et al., 2002; Koedijk et al., 2010) suggests that copepods *per se* do not induce mineral toxicity. Mineral uptake by fish from copepods has been demonstrated to occur largely from the soft body parts, but the majority of copepod minerals are bound in forms with low bioavailability in the chitin exoskeleton (Reinfelder and Fisher, 1994). Furthermore, it is known that time is an important variable for uptake and retention of Se in rotifers (Penglase et al., 2011), and rotifers with similar levels of minerals from batch culture or short term enrichment trials in the current study may have different levels of bioavailable minerals. The industry protocol for rotifer enrichment is usually between 2 and 4 h. In this study only 3 h enrichment was

investigated. A change in protocol to 2 h enrichment may therefore change both the uptake, retention and the bioavailability of the minerals.

Factors that favour high rates of lipid oxidation such as increased temperature, light, aeration and lipid emulsions with high water to oil surface interfaces (Laguerre et al., 2007) are available under conditions standard to rotifer production, particularly in ST enrichments. Furthermore, ionic forms of minerals, particularly the transition metals which includes Mn, Cu and Zn, can increase the rate of lipid oxidation (Laguerre et al., 2007). Surprisingly, we found few effects of mineral additions to the level of TBARS, a measure of one of the end products from lipid oxidation (Hamre et al., 2001), as rotifers had similar TBARS to controls when enriched with minerals. The reason why rotifers enriched with a low dose of organic minerals had higher TBARS than cultured control or rotifers enriched with a high dose of inorganic minerals is unclear, as at the least, the higher levels of transition metals added to the inorganic mineral treatment would be expected to have increased levels of oxidative agents. The levels of TBARS were similar between rotifers and their feed when taking into account moisture content differences, and overall TBARS were in the low to medium range found previously in fish feed (Hamre et al., 2001).

This study was aimed at a practical application of addressing possible trace element deficiencies of rotifers in comparison to copepods (Hamre et al., 2008a), which would require a protocol to enrich rotifers with several minerals simultaneously. To be implemented into existing industry protocols, the feeding of minerals to rotifers has to occur either during the continuous culturing phase and/or in the 2–4 hour enrichment period. The current study demonstrates that the levels of Zn, Cu and Se could be effectively increased at both stages, but Mn could only be increased in the ST enrichment stage. The batch culture period investigated in this study was a maximum of 6 days, while rotifers are typically cultured continuously for months to years. On a longer term, the high mineral concentration needed to enrich to copepod levels may be toxic to the rotifers. The growth rate and concentration of rotifers in commercial production are also much higher than in this study. Therefore, until longer term consequences of the change in diet on rotifers are investigated, the implementation of any protocol in the ST enrichment period is suggested. The linear increase in rotifer mineral content at levels that achieved copepod levels means that implementing a protocol for increasing rotifer contents of Mn, Cu, Zn, Se (this study) and iodine (Srivastava et al., 2012) would require a simple dose response trial specific for the hatchery, in order to determine how much of each mineral is required in the enrichment.

## 5. Conclusion

Increasing the levels of Cu, Zn, and Se in rotifers to copepod levels was possible during both the culture and enrichment stages of rotifer production using organic mineral supplements at less than 5% replacement of total feed. Manganese could only successfully be increased in the ST enrichment phase, increasing the total replacement of feed with organic minerals to 6%. Furthermore, it was demonstrated that uptake of minerals by rotifers was greatest when associated with the insoluble fraction within the size range that can be ingested by rotifers. Overall retention rates of minerals in rotifers post enrichment were between 50 and 110%. Furthermore rotifer mineral levels were relatively stable from 1 to 18 h post enrichment. The high and stable retention of minerals in rotifers will allow future use in dose response feeding trials with fish larvae.

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