



Abundance and biomass of rotifers in relation to the environmental factors in geothermal waters in Southern Tunisia

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ABSTRACT

The spatial and temporal dynamics of rotifers in relation to the physico-chemical parameters in Fish-Culture Research Station (Southern Tunisia) were studied monthly from February 2005 to January 2006. Thirteen rotifer species were found: *Brachionus urceolaris*, *Brachionus calyciflorus*, *Brachionus* sp., *Lecane stichaea*, *Lecane rhytida*, *Lecane* sp., *Hexarthra mira*, *Rotaria tardigrada*, *Conochiloides natans*, *Trichocerca marina*, *Keratella quadrata*, *Keratella cochlearis* and *Notommata codonella*. The most dominant rotifer was *B. urceolaris* (76% of total abundance). Rotifer density and water temperature were negatively correlated ($r = -0.94$, $n = 12$, $p = 0.001$). The highest abundance of rotifers was found in basin 4 (1.5×10^5 ind m^{-3} , in June 2005).

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1. Introduction

Tunisia has an important geothermal water situated in the N-E, N-W and in the South. These geothermal waters have a potential use for heating, sericulture and pisciculture (Kairouani and Hassairi, 2002). Fish-Culture Research Station, Bechima-Gabes (Southern Tunisia), was created in 1990, to optimize the rearing Nile tilapia, *Oreochromis niloticus* (Linné, 1758), in Tunisian fish farms (Azaza et al., in press). This species belonging to the Egyptian stock was imported from Libya (Turki and Kraïem, 2002; Azaza et al., 2008) and was introduced in Tunisia in 1999 to take advantage of the geothermal water resources in the south of the country, which had hitherto been underutilized for aquaculture purposes (Azaza et al., 2006; 2008). The thermophilic and eurytherm *O. niloticus* (Azaza et al., 2008) has been often regarded as one of the excellent candidates for intensive aquaculture production (Hussain et al., 2000; Li et al., 2001; Rutten, 2005; Azaza et al., in press). Further development of tilapia farming will depend on the availability of food resources (Azaza and Kraïem, 2005). In the wild, tilapia are generally omnivores (Šetlikova and Adámek, 2004; Oso et al., 2006), feeding on phytoplankton (Turker et al., 2003), zooplankton, especially rotifers (Preston and Snell, 2001; Wallace and Snell, 2001) and zoobenthos

(Adámek and Sukop, 1995). Tilapia are also capable of digesting cyanobacteria such as *Microcystis aeruginosa* (Mohamed et al., 2003; Lu et al., 2006). Rotifers are the first colonizers of newly constructed basins (Khattabi et al., 2006) and reservoirs (Thouvenot et al., 2000). They constitute a privileged food in shrimp and larvae rearing (Shields, 2001; Omholt Alver et al., 2008) because of their small size (Yoshinaga et al., 2001; Haoyuan and Yilong, 2008), nutritional quality (Abatzopoulos et al., 2002), peak motility (Snell and Carrillo, 1984) and high reproduction rate (Sarma and Nandini, 2001; Hurtado-Bocanegra et al., 2002). One species of the *Brachionus* species complex (Papakostas et al., 2006) is usually the first kind of food in intensive culture. Therefore rotifers constitute an important part in the food ration of the tilapia (Veverica et al., 1991; Dabbadie, 1996).

To our knowledge, this is the first contribution on the spatial and temporal distribution of rotifer assemblages in geothermal waters. In the present study, abundance and biomass of rotifers together with abiotic parameters (water temperature, pH, dissolved oxygen, salinity and nutrients) and biotic parameters (phytoplankton abundance and chlorophyll *a*) were estimated in eight basins with geothermal waters of “Bechima station” in order to address the following questions: (i) Do the species richness and abundance of rotifers decrease with temperature increase? (ii) Are there eurythermic forms? (iii) How the rotifers can be used in Tunisian aquaculture industry as live food for Nile tilapia, *Oreochromis niloticus* L.

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2. Materials and methods

2.1. Study site

This study was carried out at the National Institute of Marine Sciences and Technologies (INSTM), Fish-Culture Research Station, Bechima-Gabes (Southern Tunisia, about 33°91'N and 9°73'E). Each tank was part of an open circulation system with a common water reservoir. The tanks were supplied with water coming from a geothermal source after undergoing a cooling process in a large storage tank located upstream of the experiment system. In all 12 tanks used, water was constantly replaced by continuous flow at a rate of 4–6 l min⁻¹ per tank to provide oxygen and remove excess of nitrogenous waste (Azaza et al., in press). In addition, the tanks were siphoned daily, before the first feeding, to remove faecal material and they were thoroughly scrubbed and completely flushed fortnightly. Water quality remained within limits recommended for Nile tilapia culture (Azaza et al., in press).

2.2. Sampling

Eight basins of very different temperature were chosen for sampling. These basins were interconnected: B1 (exit of the cooling), B2 and B3 (cooling basins to provide water at a temperature suitable for optimal breeding of fish: 28–32 °C), B4 (a larger basin 800 m³), B5, B6, B7 (300 m² basins under greenhouse conditions) and B8 (settlement basin of 32 m³). These basins are shallow (100–150 cm deep). Samples were collected with a Van Dorn-type closing bottle approximately 50 cm below the surface of each of the eight basins, every month, between the months of February 2005 and January 2006 (96 samples). Water samples were collected and immediately filtered through a 50 µm mesh net. The rotifers retained by filtering 50 l of water were transferred to a sterile 250 ml flask and fixed with formal (5% final concentration). Samples were collected and stored separately in the dark and cold, for the analysis of biotic and abiotic variables, except for temperature, pH, dissolved oxygen and salinity, which was measured *in situ*. For the biological parameters, two series of samples were collected: the first series was fixed in Lugol's iodine (final concentration 1% v/v) for identification and counts of phytoplankton cells and the second series was used for assaying pigments (chlorophyll *a*).

2.3. Analysis of physico-chemical variables

Temperature, pH, dissolved oxygen and salinity were measured in the field using portable meters (Multi 340i/SET). Samples for dissolved inorganic nitrogen (nitrite: NO₂⁻, nitrate: NO₃⁻, ammonium: NH₄⁺) and orthophosphates (PO₄³⁻) were stored at -20 °C before analysis with an automatic BRAN and LUE BBE-type 3 analyzer. Concentrations were determined colorimetrically according to Grasshof (1983).

2.4. Analysis of biotic variables

The phytoplankton counts were conducted using an inverted microscope, according to Utermöhl's method (Utermöhl, 1958). The different species encountered were identified from morphological criteria. Samples for chlorophyll *a* analysis were filtered by vacuum filtration onto a 0.45 µm pore size filter and 47-mm-diameter glass fibre filter Whatman, GF/F. Pigments analysis was performed by spectrophotometry, after extraction of the pigments in acetone. The concentrations were then estimated using the equations of Lorenzen (1967). Rotifers were counted under a Leica

inverted microscope after sedimentation of a 20–50 ml sample. In most cases, rotifers were identified to species level using the keys of Koste (1978) and Pourriot and Francez (1986). Rotifer biomass calculated from mean biovolumes of each taxon was estimated from appropriate geometric shapes (Bottrell et al., 1976). The densities and biomasses were expressed in ind m⁻³ and µgC m⁻³, respectively.

2.5. Statistical analyses

The data recorded in this study were examined with a normalized principal component analysis (PCA) (Doledec and Chessel, 1992). Simple log(*x*+1) transformation was applied to data in order to stabilize the variance (Frontier, 1973). Pearson's Correlation Analysis was performed to evaluate the relationships between physico-chemical variables and rotifer densities. It was considered significant at *p* < 0.05. In addition, One-way ANOVA was applied to identify significant differences (*p* < 0.001, *p* < 0.01, *p* < 0.05) between studies for physical-chemical variables. ANOVA tests were made using XL stat software.

3. Results

3.1. Physical-chemical analyses

The mean values of the main physical and chemical parameters measured are shown in Table 1. Water temperature decreased from B1 (mean ± sd = 41.3 ± 4.1 °C) to basin B4 (mean ± sd = 27.5 ± 4.9 °C). In basins B5, B6, B7 and B8, the temperatures were constant and averaged 30 °C. Such high values are usual in arid to semi-arid zones. Dissolved oxygen concentrations were similar in B1, B2, B3, B4, B5 and B8, and averaged 4 mg l⁻¹. The lowest values were recorded in basins B6 (2.3 ± 1.2 mg l⁻¹) and B7 (2.1 ± 1.1 mg l⁻¹). pH values varied from 7.6 ± 0.2 (in B6) and 7.6 ± 0.4 (in B7) to 8.1 ± 0.2 (in B2). Salinity values were constant, averaged 1.9 ± 0.1 g l⁻¹. Nutrient concentrations did not differ significantly among the eight basins (ANOVA, *p* < 0.05). Ammonium (76%) constituted the most important fraction of the total nitrogen. Highest values were recorded in B5 (mean ± sd = 16.1 ± 27.0 µatg l⁻¹), whereas nitrite and nitrate concentrations were high in B8 and averaged 1.2 ± 1.1 and 2.0 ± 1.4 µatg l⁻¹, respectively. Orthophosphate concentrations varied from 0.4 ± 0.4 µatg l⁻¹ (in B2) to 0.7 ± 0.5 µatg l⁻¹ (in B3) and 0.7 ± 0.3 (in B5). N/P ratios varied from 7.8 ± 6.3 in B3 to 13.9 ± 22.2 in B2.

3.2. Spatial and temporal distribution of phytoplankton

Thirty-nine taxa of phytoplankton were identified in this study, amongst which cyanobacteria were numerically dominant (15). The remaining taxa were diatoms (12), dinoflagellates (8) and chlorophytes (4).

In basin B1, the total phytoplankton abundance varied between 4 (September) and 1.1 × 10⁴ cells l⁻¹ (August), the mean value being 0.2 ± 0.2 × 10⁴ cells l⁻¹ (Fig. 1). The dinoflagellate (*Polykrikos* sp.) dominated the phytoplankton community from February to June (80–100% of the total phytoplankton cells) but being replaced by the cyanobacteria (*Anabaena* sp., *Chroococcus* sp., *Merismopedia* sp., *M. aeruginosa*, *Oscillatoria* sp., *Planktothrix* sp., *Pseudoanabaena* sp. and *Spirulina subsalsa*) from July to January (50–100% of the total phytoplankton cells) (Fig. 2a).

In basin B2, the total phytoplankton abundance ranged from 5 (September) to 3.6 × 10³ cells l⁻¹ (May) (mean ± sd = 1.0 ± 1.0 × 10³ cells l⁻¹) (Fig. 1). Cyanobacteria (*Microcystis* sp., *Chroococcus*

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