

Original Research Article

Comment [H1]:

Zooplankton: A Valuable Environmental Indicator Tool in Reservoir Ecological Management?

ABSTRACT

The trends in zooplankton community were assessed in response to the variations in the trophic state and water quality, in Serra Serrada, a shallow reservoir, with seasonal water level fluctuations, located at the Portuguese part of River Douro Basin. Various ratios were also calculated: (1) the ratio of crustacean abundance to rotifer abundance (NCrust/NRot); (2) the ratio of large (>10 µg) cladoceran abundance to total cladoceran group abundance (NLarge-Clad/NClad); (3) The trophic state indices of rotifer abundance. The reservoir's hydrological cycle was characterized by three phases. The maximum level phase lasted from January to the beginning of June, the emptying phase from mid-June to the beginning of September and the minimum level phase between mid-September to the beginning of the first autumn/winter rain events. The highest values for total phosphorus, soluble reactive phosphorus, nitrate, and 'chlorophyll a' were found during the minimum level phase. Rotifera was the most abundant taxa, except in summer and in autumn where Cladocera and Copepoda were dominant. The ordination space defined by the first two RDA axes accounted for 84.2% of species– environment relations and represented 25.4% of the variation in species data. The ratio NCrust/NRot showed the highest role of rotifers in the zooplankton community in the low level phase. The ratio NLargeClad/NClad showed a decreasing trend in the abundance of large-sized cladocerans for the same mentioned period. Redundancy analysis (RDA) revealed a strong influence of temperature, chlorophyll a, soluble reactive phosphorus and total phosphorus to the observed significant associations between the zooplankton assemblage and environmental variables. Therefore, these indices are suitable to be used in reservoir management as rapid tools to evaluate the effects of environmental disturbances with a concern to the reservoir ecological integrity.

Keywords: shallow reservoir; seasonal water level fluctuations; nutrients; zooplankton community; environmental indicator.

1. INTRODUCTION

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The physical, chemical and biological features in the reservoirs are influenced by seasonal surface level fluctuations, which are associated with anthropogenic utilization [1,2,3,4,5,6]. Such fluctuations are frequent in the reservoirs located in Portugal, where the seasonal rain events occur in a very irregular pattern. Local human populations of these areas intensively use the stored reservoir water for irrigation, recreation, urban necessities and in hydroelectric power supply. According to European Union (EU) Water Framework Directive, the ecological status is an expression of the quality of the structure and functioning of aquatic ecosystems. This is assessed by a series of biological quality elements complemented with a set of chemical and hydro-morphological quality data. Zooplankton, despite of not being recognised as a biological quality element, it shows quick responses to environmental changes and so, it can be a valuable indicator of ecological conditions. This value stems from their position in the food web, sandwiched between the top-down regulators (fish) and bottom-up factors (phytoplankton), thus providing information about the relative importance of top-down and bottom-up control and their impact on water clarity [7]. Consequently, few studies have been undertaken to assess and understand the responses of zooplankton communities to reservoir water level fluctuations [8,9,10,11,12,13]. Serra Serrada reservoir is located in the Iberian Peninsula, within the Portuguese part of the River Douro catchment. In this region, precipitation occurs mainly in autumn and winter in an irregular way, with one wet winter usually followed by a few dry ones. This reservoir is subjected to intense water use for hydroelectricity production and for urban supply. Thus, the

hydrological cycle of this reservoir was characterised by the following phases: (1) Maximum level 32
phase, from January to the beginning of June; (2) Emptying phase, from mid June to the beginning of
33 September; (3) Minimum level phase, from mid September to the beginning of the first
autumn/winter
34 rain events. Therefore, the objectives of this study were to: (a) examine responses of zooplankton to

changes in environmental variables as a consequence of water level fluctuations and (b) compare the values obtained for Carlson's Trophic State Index (based upon physical and chemical variables) to values obtained by several zooplankton metrics, in order to assess the role of zooplankton as an environmental indicator.

2. MATERIAL AND METHODS

2.1 Study site

Serra Serrada (Latitude: 41°57'12"N; Longitude: 6° 46' 44") is located on the granitic bedrock at an altitude of 1300 m at NE Portugal (Figure 1). The total capacity of the reservoir, spreading over 25 ha is $1680 \times 10^3 \text{ m}^3$ and it was filled for the first time in 1995 for urban supply and to generate hydroelectric power. As a result of this, accentuated water level fluctuations occur. The annual range of water level variation is between 8 and 10 m (Figure. 2). The maximum depth is 17 m and the mean depth is around 6.7 m.

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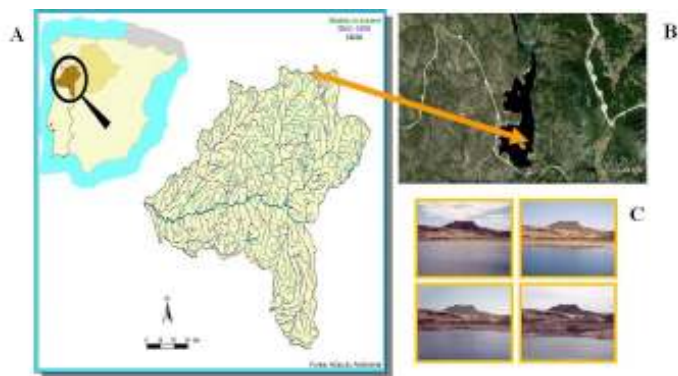


Fig.1. Location of Serra Serrada Reservoir in the Iberia. Peninsula context (A, B). Hydrological cycle (C).

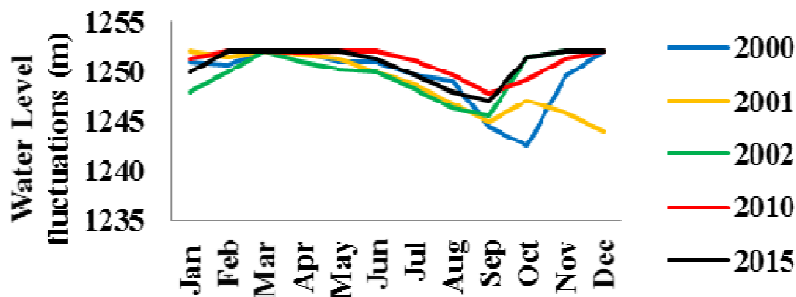


Fig. 2. Pattern of water level fluctuations during the study period

source: [14]

Thermal stratification was observed from June to August/ beginning of September. Disruption of stratification was coincident with the lowest water level. During the period of study this reservoir was considered meso-eutrophic. Direct human influence on S. Serrada impoundment was considered negligible. There are no villages, there has been no agricultural activity in the area for approximately 20 years and recreational activities are reduced to a minimum. However, in the catchment basin grazing can be very intense during the summer months. Consequently, this area is very often subjected to wild fires mainly induced by shepherds to obtain better grazing. Further information concerning morphological and hydrological characteristics of this reservoir can be found in [15].

2.2 Field sampling and laboratory analysis

Water samples were collected monthly in winter and biweekly in summer, from January 2000 to December 2002 at one single sampling station, located at the deepest point of the reservoir. Both, in 2010 and in 2015 samples were obtained seasonally. Main trends in zooplankton and environmental variables were analysed on the basis of three hydrological phases: the maximum level phase, the emptying phase and the minimum level phase. The total phosphorus (TP), chlorophyll a (CHL a) and water transparency were determined to evaluate the reservoir trophic state according to [16], and also the changes in it across the reservoir hydrological cycle. Other environmental variables such as water temperature, dissolved oxygen, conductivity, pH, as well as nitrate (N-NO₃), ammonium ion (N-NH₄) and ammonia gas (N-NH₃) concentrations, were measured "in situ". The soluble reactive phosphorus (SRP) was also determined. Zooplankton samples were obtained on each sampling date with the help of two vertical hauls using a Wisconsin type net of 64 µm mesh size. Animals were anaesthetised with carbonated water and preserved in sugar-saturated formaldehyde (4% final concentration). Depending on the density, zooplankton samples were counted in 5, 10, 20 ml subsamples or in total. Animals were identified to the genus/species level.

2.2 Data analysis

To evaluate the association between taxa composition and environmental variables, a Redundancy Analysis (RDA) was performed. A linear model of ordination was used, because a preliminary Detrended Correspondence Analysis (DCA) resulted in turnovers < 2 SD. According to [17], this is the recommended criterion for choosing linear models of ordination instead of uni-modal models. Relative abundance of the most representative zooplankton taxa were transformed to log (x+1). For the purpose of the statistical analyses, all *Keratella* species were retained in a single category. Environmental variables showing skewed distributions were also log (x+1) transformed. In RDA, a forward selection procedure was used to add significant explanatory variables to the model. Those were added in the order of the greatest additional contribution to the total variance explained. Statistical significance in RDA was assessed by Monte-Carlo permutation tests (9999 permutations). The computer program CANOCO version 4.5 was used to perform both the preliminary DCA and the RDA. A Kruskal-Wallis test was performed for each environmental variable to determine whether the mean values obtained at the maximum level phase, at the emptying phase and at the minimum level phase were significantly different. This statistical analysis was performed using SPSS 20. To illustrate the role of zooplankton as a valuable environmental indicator, several zooplankton metrics were studied: (1) The trophic state index of rotifer abundance [18]; (2) the ratio of crustacean abundance to rotifer abundance (N_{Crust}/N_{Rot}) and (3) the ratio of large (>10 µg) cladoceran abundance to total cladoceran group abundance (N_{Large-Clad}/N_{Clad}) [19].

3. RESULTS

3.1 Zooplankton community

The present research identified: 13 taxa of Rotifera, 12 Cladocera and 3 Copepoda (Table 1). However, zooplankton community was dominated only a few species (10) (Table 2). Of all the zooplankton species found in S. Serrada only the most representative were considered in the RDA.

Table 1. Composition of the zooplankton community in S. Serrada

Rotifera	Cladocera	Copepoda
<i>Asplanchna priodonta</i>	<i>Alona affinis</i>	<i>Eucyclops serrulatus</i>
<i>Collotheca</i> sp.	<i>Alonacostata</i>	<i>Macrocyclops albidus</i>
<i>Conochilus</i> sp.	<i>Alona quadrangularis</i>	<i>Tropocyclops prasinus</i>
<i>Euchlanis</i> sp.	<i>Alona rectangula</i>	Nauplii
<i>Gastropus</i> sp.	<i>Alonella nana</i>	
<i>Hexarthra</i> sp.	<i>Bosmina longirostris</i>	
<i>Keratella cochlearis</i>	<i>Ceriodaphnia quadrangula</i>	
<i>Keratella</i> gr. <i>quadrata</i>	<i>Chydorus sphaericus</i>	
<i>Keratella cochlearis</i> var. <i>tecta</i>	<i>Daphnia longispina</i>	
<i>Ploesoma hudsoni</i>	<i>Drepanothrix dentata</i>	
<i>Polyarthra</i> sp.	<i>Holopedium gibberum</i> *	
<i>Synchaeta</i> gr. <i>Simocephalus</i> sp.		
<i>Trichocerca</i> sp.		

Comment [H4]: Author is not given. Example: Gosse, 1850

Comment [H5]: *Alona rectangula* changed= *Coronatella rectangula* (Sars, 1862)

Comment [H6]: *Keratella quadrata*

Comment [H7]: *Keratella cochlearis tecta*

Comment [H8]: *Synchaeta pectinata*

*only detected in January 2010.

Comment [H9]: Author names of all species must be given on the table 1.

Table 2. The most common taxa of the zooplankton community quantified using the mean ($\pm$ SD) of the relative abundances during: (1) the maximum level phase; (2) the emptying phase; and (3) the minimum level phase. Taxa abbreviations used in the RDA are indicated in brackets.

	1	2	3
Rotifera	68.43 $\pm$ 33.23	30.37 $\pm$ 28.65	47.57 $\pm$ 23.72

Cladocera	19.46±23.22	19.46 ± 22.58	11.28±7.81
Copepoda	13.71±18.05	50.17 ± 28.46	40.00±23.36
Rotifera			
<i>Asplanchna</i> (Aspl.)	20.89 ± 29.48	4.37 ± 8.92	6.03 ± 12.33
<i>Conochilus</i> (Cono.)	26.46 ± 33.55	2.59 ± 7.46	3.67 ± 6.40
<i>Gastropus</i> (Gast.)	0.21 ± 0.83	0.47 ± 1.72	0.35 ± 0.63
<i>Hexarthra</i> (Hexa.)	0.05 ± 0.15	1.08 ± 1.72	0.19 ± 0.60
<i>Keratella</i> (Kera.)	7.38 ± 14.04	16.82 ± 28.21	16.06 ± 24.05
	12.15 ± 25.64	1.15 ± 2.08	6.42 ± 10.30
<i>Polyarthra</i> (Poly.)			
<i>Synchaeta</i> (Sync.)	2.20 ± 4.70	2.63 ± 3.84	17.83 ± 19.74
Cladocera			
<i>Ceriodaphnia</i> (Cqua.)	6.97 ± 18.41	22.17 ± 24.89	7.71 ± 8.19
<i>Daphnia</i> (Dlon.)	4.94 ± 13.37	0.42 ± 0.76	1.90 ± 2.31
Copepoda			
<i>Tropocyclops</i> (Tptra.)	6.82 ± 15.33	17.43 ± 22.30	26.76 ± 30.55
Nauplii (Naup)	11.93 ± 19.76	30.87 ± 28.53	13.00 ± 13.42

The forward variable selection approach to the RDA revealed a strong influence of temperature ($P = 0.005$), and CHL a ($P = 0.007$), SRP ($P = 0.01$) and TP ($P = 0.03$) concentrations, to the observed significant associations between the zooplankton assemblage and environmental variables (MonteCarlo test; $P = 0.005$). The ordination space defined by the first two RDA axes (Figure 3) accounted for 84.2 % of species-environment relation and represented 25.4 % of the variation in species data. The distribution of sample scores in this ordination space reflected a clear distinction between the maximum level phase and the emptying/minimum level phases. However, the distinction between the latter two phases was not so clear. *Daphnia* were associated with lower temperatures and the maximum level phase. *Ceriodaphnia* were clearly related to the emptying phase, when temperature was higher. *Conochilus* and *Asplanchna* were associated with low values of TP and CHL a (maximum level phase). *Polyarthra* abundance was also high during this period. However, in the ordination space, this species was associated with high concentrations of TP. In fact, the abundance of this rotifer was higher after strong rain events when TP concentrations concomitantly increased. *Gastropus*, *Keratella*, *Hexarthra*, *T. prasinus* and nauplii abundances were related to the increase in system instability and to the higher TP and CHL a concentrations during the emptying and the minimum level phases.

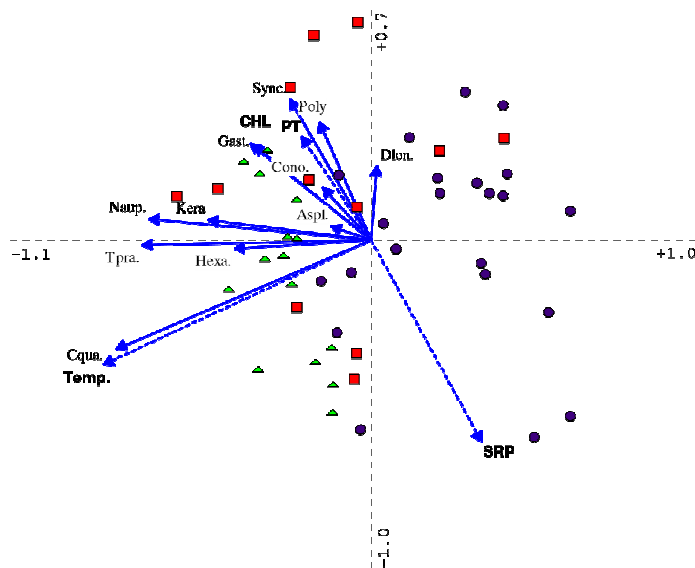


Fig. 3. RDA ordination diagram depicting the effects of environmental variables on Zooplankton community. Circles are the scatters for samples taken at the maximum level phase; triangles are the scatters for samples taken at the emptying phase and squares are the scatters for samples taken at the minimum level phase. Dashed arrows are the species and solid arrows are the environmental variables evidencing significant correlations with the canonical axes. Codes for environmental variables are given in Table 3, codes of zooplankton are given in Table 2.

3.2 Environmental variables and Carlson's Trophic State Index

Mean TP, N-NO₃ and CHL a concentrations, and conductivity were the highest during the minimum level phase. On the other hand, water transparency was the lowest during the same period. SRP mean concentrations were also slightly higher during this period than during the other months of the year. However, those differences were not statistically significant. TP, conductivity and CHL a decreased during the maximum level phase. Mean N-NO₃ decreased during the emptying phase. NNH₃ concentrations were always below detection limits (Table 3). During the period of study and depending on precipitation intensity, S. Serrada reached the maximum level phase in one or two weeks. Thus, at the beginning of the maximum level phase, TP, N-NO₃ and CHL a concentrations were high, decreasing afterwards.

Table 3. Mean $\pm$ SD values of the environmental variables and minimum-maximum range for pH obtained during the maximum level phase (1), the emptying phase (2) and the minimum level phase (3), and the results of the Kruskal-Wallis test. Abbreviations used in the RDA are indicated in brackets.

Variables	1	2	3	P
Water transparency (m) (Secc.)	2.9 $\pm$ 0.9	2.9 $\pm$ 1.0	1.7 $\pm$ 0.4	**
Water temperature (°C) (Temp.)	9.3 $\pm$ 5.5	19.4 $\pm$ 1.3	10.2 $\pm$ 4.1	**

Dissolved oxygen (mg l ⁻¹) (DO)	8.6 ± 1.6	8.4 ± 0.9	8.6 ± 1.5	NS
Conductivity (µS cm ⁻¹) (Cond.)	6.0 ± 1.5	8.3 ± 0.8	8.1 ± 1.5	**
pH (PH)	6.9-7.4	5.4-8.1	7.0-8.5	NS
N-NO ₃ (mg l ⁻¹) (NO ₃)	5.8 ± 8.7	0.9 ± 0.8	13.3 ± 15.2	**
N-NH ₄ (mg l ⁻¹) (NH ₄)	0.2 ± 0.5	0.1 ± 0.2	0.4 ± 0.8	NS
TP (µg l ⁻¹) (TP)	59.9 ± 25.3	70.1 ± 19.2	82.0 ± 11.4	**
SRP (µg l ⁻¹) (SRP)	8.7 ± 7.5	6.6 ± 4.7	9.5 ± 7.2	NS
Chlorophyll a (µg l ⁻¹) (CHL a)	1.7 ± 1.3	1.9 ± 1.6	8.0 ± 4.7	**

* $p < 0.05$; ** $p < 0.01$; NS: not significant

According to Carlson's Trophic State Index, this reservoir is meso-eutrophic (with a tendency to increase in all components of the index towards the emptying and minimum level phases) in all phases of its hydrological cycle (Table 4).

3.2 Zooplankton metrics

The trophic state index for rotifer abundance also characterized the reservoir as meso-eutrophic. The other metrics (crustacean abundance to rotifer abundance and large cladoceran abundance to total cladoceran abundance) further supported the dominance of rotifers, which is typical of aquatic systems exposed to high levels of disturbance (Table 4). Despite of the rotifer dominance in all phases (except in the emptying) TSI_{N rot} increased in the low level phase, when TP, SRP and CHL a concentrations increased and water transparency decreased. Crustacean/Rotifer ratio also followed the same variation pattern. Results for Large Cladocera/total Cladocera ratio were not so elucidative. In fact, these results could also have been influenced by variations in water temperature. Daphnia prefer cold waters, whereas *Ceriodaphnia* prefer warm waters. In general, results obtained for Carlson's Trophic Index and for zooplankton metrics were all in the same line.

Table 4. Mean ± SD values of Carlson's Trophic State Index and of zooplankton metrics range for pH obtained during the maximum level phase (1), the emptying phase (2) and the minimum level phase (3).

	1	2	3
CARLSON'S TROPHIC INDEX			
TSI (SD)	45.5±5.0	45.5±6.0	53.1±3.8
TSI (TP)	62.0±6.0	64.9±4.1	67.6±2.2
TSI (CHL)	31.4±10.4	33.9±9.9	49.7±5.2

Comment [H10]: cladoceran must change as cladocera

Comment [H11]: cladoceran must change as cladocera

ZOOPLANKTON METRICS

TSI_{N rot}	63.0 ± 12.1	65.5±8.3	70.3±7.2
Ncrus/Nrot	5.5±19.4	15.0±33.5	2.8±6.1
NLargeClad/NClad	0.3 [±] 0.3	0.1 [±] 0.2	0.4 [±] 0.4

4. DISCUSSION

Actually, S. Serrada reservoir is a highly disturbed system. The increase in TP, SRP and CHL concentrations and the decrease in water transparency during the emptying and minimum level phases could have been a consequence of the increment of suspended particulate material in the water column. This must be due to the water turbulence generated during the emptying phase, plus the disruption of stratification at the end of this phase. Moreover, the exposure of littoral sediments to cycles of drying and wetting, as a consequence of water level fluctuations, could have had implications on nutrient cycling, namely on phosphorus availability. In fact, in reservoirs where water level fluctuations were accentuated and where refilling has taken place over a short period of time, littoral sediments that were periodically exposed during cycles of drying and wetting have less capacity to adsorb nutrients than those that remained inundated [20, 21, 3, 4, 5].

Despite of the high internal disturbance, zooplankton community is composed of several rare species (in the context of Iberian Peninsula) such as *Drepanothrixdentata*, *Ceriodaphnia quadrangular* and *Holopediumgibberum*. *Holopedium* (typical of oligotrophic waters) was only detected in and after 2010 [22]. However, the community is always dominated by rotifers and other specialists in small particle feeding, whose food preferences are mostly comprised of detritus-bacteria and phytoplankton. Most zooplankters including *Ceriodaphnia* and *T. prasinus*, can only take particles smaller than 20 µm [19]. Those assemblage patterns are typical in reservoirs subjected to periodic water level variations [24,25, 12, 13]. According to those authors, the above mentioned species evidence adaptive advantages over larger Cladocera and Copepoda, not only on account of their feeding behaviour but also because they have shorter generation times and greater tolerance to large amounts of organic matter in the water. An exception was *Conochilus*, which according to [26], is typically associated with environments poor in organic matter.

5. CONCLUSION

The present research has shown that zooplankton community has indicative ability. When the system was more oligotrophic (maximum level phase), indicatory species of oligotrophic waters, such as *Daphnia longispina*, *Holopedium* and *Conochilus* increased their densities; when the system became more eutrophic (emptying phase and minimum level phase) the dominant taxa were typical of eutrophic waters, such as *Keratella*, *Gastropus* and *T. prasinus*. These trends were also reflected by zooplankton metrics, which were in line with Carlson's Trophic State Index.

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Comment [H12]: Discussion section should be expandend and rewrite

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