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Temperature-dependent demographic differences in sessile rotifers of the genus *Limnias* (Rotifera: Gnesiotrocha)

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Abstract

Aim : Rotifer research on sessile taxa has received less attention because they are not easy to identify in fixed samples. In the Lake Xochimilco, a Ramsar site in Mexico City, three morphotypes of *L. ceratophylli* and a single morphotype of *L. cf. melicerta* occur in different densities. The aim of this study was to test if temperature was responsible for the differences in the population densities of these morphotypes.

Methodology : The present study was carried out using population growth method consisting of 4 treatments (3 morphotypes of *L. ceratophylli* and one of *L. cf. melicerta*) at 20 and 25°C. Experiments were carried out in 50 ml glass jars containing 25 ml synthetic medium with *Chlorella vulgaris* as food. The population growth rates (*r*) were derived. Analysis of variance (ANOVA) and post-hoc tests were used to quantify the intra- and interspecific differences in the population growth rates.

Results : The temperature levels for optimal population growth rates differed among the morphotypes of same species. At 20°C, the morphotype 1 had the highest *r* (0.23 d⁻¹) similar to that of *L. cf. melicerta*, while morphotype 3 had the lowest (0.15 d⁻¹). For *L. cf. melicerta*, the *r* was higher at 25°C than at 20°C. Of three morphotypes of *L. ceratophylli*, morphotype 3 had the highest *r* at 25°C similar to that of *L. cf. melicerta* at same temperature.

Interpretation : There were significant differences among growth patterns within the morphotypes of *L. ceratophylli*, depending on culture temperature. These trends highlight the relative importance of environmental variables in differentiating morphotypes of a sessile species complex which could explain their possible seasonal changes in the natural waterbodies.

Key words: *Limnias*, Morphotypes, Ramsar site, Sessile rotifers, Temperature dependent

Morphotypes in sessile rotifers from
Lake Xochimilco, a Ramsar site

Limnias ceratophylli

L. melicerta

Ecological differentiation
approach

Population growth experiment:
Temperatures: 20 and 25°C

Indicators:
Population growth rate
Peak of maximal population growth
Density-dependence and growth rate

Intra-interspecific
identification

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Introduction

Rotifers, in terms of species richness and/or numbers often dominate freshwater zooplankton. The limnetic and periphytic zones of epicontinental waters usually have both high species diversity and density (Wallace *et al.*, 2016). While most rotifers lack larval stages, some species, especially the sessile taxa, exhibit larval forms and thus have peculiar characteristics in their ecological and evolutionary history (Kutikova, 1995). The Superorder Gnesiotrocha has species with different life history stages, including free-swimming larval stages. Although a few species (ca. 25) of Gnesiotrocha form true colonies, many (ca. 100) are typically sessile and attached to macrophytes (Wallace *et al.*, 2016). Rotifer research is largely biased towards planktonic rotifers. Sessile rotifers have received considerably much less attention because they lack a lorica, and therefore are not easy to identify to species or even genus level based on morphology in fixed samples (Segers *et al.*, 2010).

The trophi structure (malleoramate) is also similar for most taxa and a specialized study (e.g., scanning electron microscopy) is needed to obtain a higher resolution of the trophi and recognize the rotifers based on this characteristic (Meksuwan *et al.*, 2015). Even in such cases, the results are not entirely satisfactory for certain genera such as *Filinia* (Wulfsen and Ahlrichs, 2012). Underestimation of global species diversity in the phylum Rotifera is thus attributed to the limitations in the use of morphological taxonomy (Kaya *et al.*, 2009). In populations with morphological stasis or those that lack clearly identifiable intra-populational morphological attributes, the existence of cryptic species has been documented using molecular analysis. For example, *Brachionus plicatilis* species complex where the taxa differ slightly in body size and lorica spines is now reported to compose of 15 species using this integrated approach (Mills *et al.*, 2017). Current practice in rotifer taxonomy includes integration of morphological, ecological, demographic and molecular approaches (Fontaneto *et al.*, 2007; Jiménez-Contreras *et al.*, 2017). Cryptic species have been recognized in just about 40 rotifer species mainly belonging to the families Brachionidae and Lecanidae and class Bdelloidea (Gabaldón *et al.*, 2017). Application of demographic parameters involve establishment of culture systems. Once such techniques are available, it is possible to understand the different aspects of biology of these organisms (Sarma *et al.*, 2017).

Amongst the several biotic and abiotic factors that influence the dominance of rotifers is temperature which influences their population both spatially and temporally. Cryptic species respond differentially to the influence of these biotic and abiotic factors in a given ecosystem (Gabaldón *et al.*, 2017). Temperature, one of the important abiotic factors also affects life history traits, mostly documented from a few members of Brachionidae (Sarma *et al.*, 2005). The genus *Limnias* consists of 8 species (Meksuwan *et al.*, 2018; Wallace *et al.*, 2018) with worldwide distribution. Studies on *Limnias* have revealed unique characteristics within the phylum

Rotifera, including the origin and development of the tube in different sessile species, the presence of unusual form of exocrine glands, phylogenetic history of this group through molecular analysis and ecological aspects through demographic study (Sarma *et al.*, 2017; Yang and Hochberg, 2018).

Limnias is also commonly distributed in the Mexican freshwaters. It has been reported from the Chihuahuan Desert (Walsh *et al.*, 2008), Central Mexico and the Yucatan Peninsula (Sarma, 1999). In the Lake Xochimilco, a Ramsar site in Mexico City, three morphotypes of *Limnias ceratophylli* and one morphotype of *L. cf. melicerta* are frequently encountered in different densities. Based on the field samples, it is known that temperature strongly affects the abundance of rotifers (Muñoz-Colmenares *et al.*, 2017). However, laboratory studies are still needed to confirm this, especially for sessile taxa. In view of the above, the present study aimed to test the effect of temperature on the population growth parameters of three well-recognizable morphotypes of *Limnias ceratophylli* complex and *L. cf. melicerta* isolated from Lake Xochimilco.

Materials and Methods

Sample collection: *Limnias cf. melicerta* and three morphotypes of *Limnias ceratophylli* were originally obtained from the Lake Xochimilco. Since these sessile rotifers were attached to the roots of water hyacinth (*Eichhornia crassipes*), the plants with roots were collected and transported in 250 ml flasks to the laboratory where they were examined for *Limnias*. Using a stereoscopic microscope, the species was isolated individually and identified including the morphotype under a compound microscope. The isolated individuals were placed in a 10 ml glass containers with 5 ml of re-constituted moderately hard water (the EPA medium, Weber, 1993) and green alga, *Chlorella vulgaris* as food. The alga was batch-cultured on defined medium (Bold's basal, Borowitzka and Borowitzka, 1988). Log phase alga was harvested, centrifuged and re-suspended in a small volume (5 ml) of distilled water. The algal density was estimated using a haemocytometer and required density was obtained by diluting with EPA medium (Sarma *et al.*, 2017). As the population density of each specie increased, the individuals were transferred to progressively larger volumes.

Population growth experiments and analyses: Experiments were carried out in 50 ml capacity glass jars containing 25 ml EPA medium with 1×10^6 cells ml^{-1} of *Chlorella*. Five individuals (age: one day after completion of free larval stage) of *L. cf. melicerta* or one of the three morphotypes of *L. ceratophylli* were introduced into each jar. For each treatment, five replicates were set. The test jars were transferred to temperature-controlled chambers set at 20 and 25°C. Thus, 40 test jars were used (4 treatments (3 morphotypes of *L. ceratophylli* + *L. cf. melicerta*) X 2 temperatures X 5 replicates). Following the initiation of growth experiment, the population density was estimated every day in each replicate and the medium was 100% replaced with fresh EPA medium with the

chosen algal level. The rotifer growth experiment was terminated after 24 days by which time most populations began to decline and/or reached an asymptote in each replicate.

From the population density data, the growth rate (r) was derived during the exponential phase by the formula given below:

$$r = \frac{\ln N_t - \ln N_0}{t}$$

Where, N_t is the final density (after time t), N_0 is the initial density and t is the time in days. One-way ANOVA was applied to quantify the differences in the growth rates of different treatments. Post-hoc analysis (Tukey test) was applied where needed for multiple comparisons using SigmaPlot 11.0. In order to detect the effect of increasing population density on the population growth rate, the plotted data of population density were plotted against the daily growth rate following Kerfoot *et al.* (1985).

Results and Discussion

The three morphotypes of *Limnias ceratophylli* differed in the length of the pair of dorsal antennae and presence/absence of a gap in the ciliated corona (Fig. 1). These morphotypes, despite being cultured under similar laboratory conditions for several generations, retained these characteristics. In addition, no morphologically distinct forms of *L. cf. melicerta* were observed in the field samples or from the laboratory cultures. On the other hand, for *L. ceratophylli* three morphologically distinct and stable forms were observed: morphotype 1 had long dorsal antennae while morphotype 2 had no visible dorsal antennae and morphotype 3 had a small notch at corona. These features remained unchanged during the culture period. Also, the occurrence of three morphotypes at same time from lake Xochimilco was rare. Generally, morphotype 1 was found throughout the year, while morphotype 2 during winter and morphotype 3 during warmer months (April-March) of the year.

The population growth patterns of *Limnias cf. melicerta* and three morphotypes of *Limnias ceratophylli* varied depending on the culture temperature; at 20° C, morphotype 1 of *L. ceratophylli* reached the highest density (ca. 15 ind. ml⁻¹) towards the end of the experiment, morphotype 2 reached density of 2 ind. ml⁻¹, while morphotype 3 had the lowest density (0.4 ind. ml⁻¹); *Limnias cf. melicerta* had intermediate peak density (8 ind. ml⁻¹). With an increase in temperature (25 °C), *L. cf. melicerta* had the highest density (33 ind. ml⁻¹). Morphotype 3 had a peak density of about 16 ind. ml⁻¹ at this temperature and reached asymptote after 20 days. Morphotype 1 had the lowest density (1 ind. ml⁻¹) under this treatment (Fig. 2), this indicates that temperature levels for optimal growth conditions differed among the morphotypes of same species.

The time taken to reach peak densities in different treatments depended significantly on the temperature. At 20°C,

Fig. 1: Three morphotypes of *Limnias ceratophylli* (A-C) and one morphotype of *L. cf. melicerta* (D) collected from the Lake Xochimilco, Mexico.

morphotype 3 of *L. ceratophylli* reached maximum density by day 10 as compared to morphotype 1 (day 24) and *L. cf. melicerta* (day 24). On the other hand, at 25°C, morphotype 1 reached its highest density after about 2 weeks following the initiation of tests, while for other treatments, the maximal densities were

Fig. 2: Population growth curves of three morphotypes of *Limnias ceratophylli* and one morphotype of *L. cf. melicerta* at 20°C (open circles) and 25°C (closed circles). Data are mean of five replicates $\pm$ SE.

observed towards the end of the experiment (Fig. 3). Population growth rate (r) of all the treatments was significantly different (ANOVA, $p < 0.05$) between the two temperatures. At 20°C, morphotype 1 had the highest r (0.23 per day) with no significant differences compared to *L. cf. melicerta* ($p > 0.05$), while morphotype 3 had the lowest (0.15). For *L. cf. melicerta*, the r was

higher at 25°C than at 20°C. Among the three morphotypes of *L. ceratophylli*, morphotype 3 had the highest r at 25°C but was not significantly different from that of *L. cf. melicerta* at same temperature ($p > 0.05$), while morphotype 1 had the lowest r (Fig. 4). When the population densities of three morphotypes of *L. ceratophylli* and *L. cf. melicerta* were plotted as function of daily

Fig. 3: Maximum density (ind. ml⁻¹) of three morphotypes of *Limnias ceratophylli* and *L. cf. melicerta* at 20 °C (white bars) and 25 °C (black bars). Data are mean of 5 replicates $\pm$ SE. Data bars carrying similar alphabets are not significantly different ($p > 0.05$, Tukey test).

growth rates, significant curvilinear was observed ($p < 0.05$) for all treatments, except morphotypes 3 and 1 at 20 and 25 °C, respectively (Fig. 5). Sessile rotifers represent less than 10% of the subclass Monogononta with around 100 described species in 3 families (Wallace *et al.*, 2016). Although common and abundant, they are rarely reported in rotifer field studies. Edmondson (1940)

found 55 sessile species in 90 locations. Koste (1975) reported 19 sessile rotifers associated with *E. crassipes*. Recently, Meksuwan *et al.* (2018) also reported new species of sessile rotifers from Thailand. Only 17 species of sessile rotifers have been reported from Mexico probably due to difficulty in recognizing fixed specimens (Sarma, 1999; Muñoz-Colmenares *et al.*, 2017;

Fig. 4: Population growth rate (r , per day) of three morphotypes of *Limnias ceratophylli* and *L. cf. melicerta* at 20 °C (white bars) and 25 °C (black bars). Data are mean of 5 replicates $\pm$ SE. Data bars carrying similar alphabets are not significantly different ($p > 0.05$, Tukey test).

Fig. 5: Polynomial correlation (better adjusted to the data) between population density and daily growth rate of population of three morphotypes of *Limnias ceratophylli* and *L. cf. melicerta* at 20 °C (white dots) and 25 °C (black dots).

Nandini *et al.*, 2017). The application of molecular tools for systematics of sessile rotifers, including *Limnias*, has been gaining momentum (Kordbacheh *et al.*, 2018). Despite this, the systematics of this and other genera of sessile rotifers is still uncertain, which could underestimate a great diversity of

Gnesiotrocha (Wallace *et al.*, 2018). This work is perhaps first approach showing the ecological differences of different morphological types of *Limnias* spp. when cultured under similar laboratory conditions. Peak population density and rate of population increase are significant variables of rotifers,

sensitive to changes in temperature. Fernández-Araiza *et al.* (2005) observed that temperature (22°C vs 28°C) had a significant effect on the population abundance and rate of population increase of different brachionid rotifers. In this work, the peak population densities of three morphotypes differed significantly when cultured at 20 or 25°C. The effect of temperature on the peak densities of *L. cf. melicerta* also showed significant differences when cultured at 20 and at 25°C. Data on the abundance of field-collected sessile rotifers differ strongly not only due to the type of macrophyte with which they are associated but also due to the methods followed in deriving the rotifer densities. For example, epiphytic species abundance can be estimated using wet weight of macrophytes or their root diameter (Li *et al.*, 2009; Ferreira *et al.*, 2013). In addition, most sessile rotifers are attached at a specific site of the macrophytes (e.g., leaves, stems, roots or decaying parts). In such cases, density cannot be estimated without a certain degree of ambiguity.

On the other hand, laboratory tests revealed certainty in density estimations since the culture volume is usually fixed. Data on the rate of population increase per day are known mostly from planktonic rotifers of the Family Brachionidae. Most brachionid rotifers have *r* ranging from 2 to 0.2 per day when cultured on near optimal and toxicant-free conditions. The *r* values of sessile rotifers are rarely reported, although the population dynamics of these rotifers are well-studied since early 1940s (Edmondson, 1944). Recent studies show that the *r* values of sessile rotifers including *L. cf. melicerta*, are rather low (<0.2 per day) and can be strongly affected by abiotic factors such as temperature as shown in this study. The population growth rate of different morphotypes observed in this study are within the range reported for sessile rotifers. Sarma *et al.* (2017) documented that the *r* value of *Limnias* varies between 0.12 and 0.16 d⁻¹, based on population studies, and 0.29 and 0.38 d⁻¹ based on life table demography. *Limnias* species also have lifespan longer than a month and with a life expectancy at birth of 23 - 33 days. Daily growth rates, when plotted as function of population density, generally show an inverse linear or curvilinear relation for any genera of zooplankton such as *Daphnia*, *Brachionus*, *Lecane* and *Lepadella* (Gama-Flores *et al.*, 2004; Sarma *et al.*, 2010). Under limiting food conditions, decrease in growth rates are attributed to reduced fecundity. However, under unfavorable temperature conditions, food consumption is lowered, and therefore population densities remain low which results in lack of any significant relation between population density and daily growth rates. This was observed in this study for morphotypes 3 and 1 at 20°C and 25 °C, respectively. This suggests that factors affecting population abundance would also influence the relation between population density and daily growth rates. The trends observed in this work highlight the relative importance of environmental variables in differentiating morphotypes and population level parameters of a sessile species complex which will help understand their coexistence in nature.

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