

Life history traits and demographic parameters in the *Keratella cochlearis* (Rotifera, Monogononta) species complex

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Abstract A recent study based on DNA taxonomy indicated that the widespread rotifer *Keratella cochlearis* comprises several evolutionarily significant units (ESUs). Identification of ESUs based on DNA taxonomy alone is problematic and usually requires morphological, demographic, and/or ecological evidence. We isolated three haplotypes belonging to two ESUs of *K. cochlearis* and conducted life table experiments to investigate if this genetic diversity is reflected in demography. We found significant differences between haplotypes in life history traits (average lifespan, number of offspring, and percent of rejected eggs) and in demographic parameters (instantaneous growth rate, generation time, and net

reproductive rate of the populations). During the experiments, all the haplotypes produced abnormal females with a deformed lorica, which was never reported before in *K. cochlearis*. We also report the first case of an amphoteric female (producing both females and males) in *K. cochlearis*. We hypothesize that *K. cochlearis* haplotypes and thus ESUs may exhibit niche differentiation through their different life histories. The link between demographic parameters of *K. cochlearis* and niche utilization requires further research.

Keywords Life table · Cryptic species · Abnormal females · Rotifers · Lake Tovel

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Introduction

Rotifers are among the most abundant planktonic metazoans and constitute a crucial link between lower and higher trophic levels in most freshwater ecosystems around the world (Wallace et al., 2006). Rotifer biodiversity has been studied for over two hundred years, and so far about 2000 species have been described (Koste & Hollowday, 1993; Segers & De Smet, 2008). With the advent of molecular techniques and the introduction of DNA taxonomy, many rotifer species, traditionally considered as one species, proved to be complexes of cryptic species. Cryptic

species, defined as genetically distinct but morphologically difficult-to-distinguish species (Gomez et al., 2002; Fontaneto et al., 2009; Birky et al., 2011; Obertegger et al., 2012, 2014; Cieplinski et al., 2017), appear to be widespread among both microorganisms and macroorganisms and have been reported in many groups such as protists (Foissner, 2006), ants (Fournier et al., 2012), harvestmen (Arthofer et al., 2013), and rotifers (Gomez & Snell, 1996; Gomez et al., 2002; Fontaneto et al., 2009; Birky et al., 2011; Obertegger et al., 2012, 2014; Cieplinski et al., 2017). According to the niche conservatism theory, the closer the related species are, the more profound is their niche conservatism (i.e., a higher tendency to retain their ancestral traits) and the stronger is their competition (e.g., Darwin, 1859; Violle et al., 2011). Therefore, cryptic species should show strong interspecific competition and little co-existence (Wiens & Graham, 2005; Losos, 2008; Violle et al., 2011). However, co-existence of closely related species is a difficult-to-explain phenomenon (Leibold & McPeck, 2006), but has been observed in nearly 60% of rotifer complexes (Gabaldón et al., 2017). Yet, especially with small aquatic organisms we can never fully account for the n-dimensionality of the species niche, and, therefore, inferences about real co-existence are difficult.

Evidence is growing that cryptic species in rotifers often have different life history traits despite their close phylogenetic relationship and that these differences may play a role in their co-existence in the same environment (Gabaldón et al., 2015). Consequently, our knowledge on biodiversity, biogeography, and ecology of certain species might be biased because several cryptic species with different ecological requirements and characteristics are lumped into one species. Differences in life histories of closely related species that are linked to niche differentiation may thus add to the co-existence and evolution of cryptic species (Angert et al., 2009; Montero-Pau et al., 2011). Therefore, analyses of life histories in cryptic species complexes may help understand competitive abilities between those species.

Life table experiments represent one of the most widespread methods to study life history traits and population dynamics (King, 1970; Allan, 1976; Walz, 1983, 1987; Gribble & Welch, 2013; Xi et al., 2013; Xiang et al., 2016a, b). Life history traits are those parameters that are directly derived from the life table of an organism (Stearns, 1992). Demographic

parameters (also known as “population parameters”, “population traits”, etc.) are the key parameters of population dynamics (e.g., instantaneous growth rate, net reproductive rate, and generation time) (Begon et al., 1996). Various studies reported interspecific differences in life history traits for several rotifer species and cryptic species in response to abiotic factors such as salinity or temperature. Temperature is one of the most important abiotic factors influencing life histories of rotifers (Bottrell et al., 1976). Gabaldón et al. (2015) showed that the brackish water cryptic species *Brachionus manjavacas* exhibits—irrespective of salinity—higher growth rates than its sibling cryptic species *B. plicatilis*. Gabaldón and Carmona (2015) demonstrated that asexual females of *B. manjavacas* have higher survival rates in both middle and old age classes and, consequently, a longer mean lifespan than asexual females of *B. plicatilis* from the same lake. In spite of this demographic advantage of *B. manjavacas* with respect to *B. plicatilis*, the two cryptic species can co-exist stably (Gomez et al., 2002, 2007). Ciroso-Pérez et al. (2001) reported different intrinsic growth rates in three sympatric cryptic species of the *B. plicatilis* species complex that were cultured at the same temperatures. Similarly, demographic parameters were different for eight closely related Chinese populations of *B. calyciflorus* exposed to different temperatures, and these differences were linked to adaptations of populations to different environmental conditions (Ma et al., 2010).

Life history traits and demographic parameters are also influenced by biotic factors such as food quality (Korstad et al., 1989) and quantity (Robertson & Salt, 1981; Xi & Huang, 1999; Sarma et al., 2001). Hu and Xi (2008) showed that the intrinsic growth rate, generation time, and average lifespan of two strains of *B. plicatilis* and one strain of *B. calyciflorus* were all significantly different under different food regimes. Differences in life history traits and demographic parameters were observed not only between cryptic species inhabiting the same environment (e.g., Gabaldón et al., 2015) but also between geographically isolated populations of the same species of *B. calyciflorus* (Wang et al., 2014).

Compared to the well-studied *Brachionus* spp., little is known about the life history of the freshwater rotifer *Keratella cochlearis* (Gosse, 1851). This is astounding considering that *K. cochlearis* is globally

distributed in lakes and ponds and is one of the most common pelagic species worldwide (Pourriot, 1965). One of the reasons why *K. cochlearis* is understudied in contrast to *Brachionus* spp. is because it is much more difficult to culture (Lindström & Pejler, 1975; Pourriot, 1980; Stemberger, 1981). Among the few existing studies on *K. cochlearis* (Edmondson, 1965; Zimmermann, 1974; Walz, 1983; Gilbert & Stemberger, 1985; Walz, 1983, 1986, 1987), those performed by Walz (1983, 1987) are the most extensive ones. This author reported changes in the life history of *K. cochlearis* dependent on temperature and food regimes. Recently, the existence of a cryptic *K. cochlearis* species complex has been hypothesized by Cieplinski et al. (2017) based on DNA taxonomy; these authors also demonstrated that it is possible to delimit several distinct evolutionarily significant units (ESUs) based solely on morphological differences between ESUs.

Here, we investigated differences in life histories between three haplotypes of *K. cochlearis*. These haplotypes belong to two putative evolutionarily significant units (ESUs)—ESU 3 and ESU 6 of *K. cochlearis*, described in Cieplinski et al. (2017) and in Obertegger et al. (2017). We hypothesized different life history traits and demographic parameters in at least two haplotypes of *K. cochlearis* belonging to different ESUs, assuming that the existence of cryptic species was correctly inferred by DNA taxonomy.

Materials and methods

Rotifer isolation, haplotypes, and ESU

We focused on three haplotypes belonging to two ESUs that were discriminated based on their mitochondrial cytochrome oxidase subunit 1 gene (COI) by Cieplinski et al. (2017). Discrimination for these ESUs and haplotypes was later confirmed by phylogenetic analyses with a nuclear marker (internal transcribed spacer 1, ITS1) by Obertegger et al. (2017). These haplotypes were isolated from lakes Tovel, Kaltern, and Terlago (N. Italy) during a detailed sampling conducted between 2013 and 2015 (for details, see Cieplinski et al., 2017).

For simplicity, we refer to two ESUs as “ESU 3” and “ESU 6” as previously described by Cieplinski et al. (2017) and to the haplotypes as “Hap A”

(belonging to ESU 6), “Hap B,” and “Hap C” (both belonging to “ESU 3,” Table 1). The sampled lakes, although geographically relatively close, represent different environmental conditions (Table 2). Lake Tovel, despite its mid-altitude location, has the characteristics of an alpine lake (Obertegger & Flaim, 2015), while Lakes Kaltern and Terlago are lowland lakes embedded in an agricultural landscape.

The three haplotypes were regularly observed during the monthly sampling period in 2014 (supplementary material Table s1). Hap B was isolated from Lake Kaltern but was also found once in Lake Vahrn (supplementary material Table s1). Moreover, Hap A was present in Lake Tovel, Hap B in Kaltern, and Hap C in Lake Terlago in all samples indicating that these particular haplotypes are not only temporarily occurring but are in fact parts of permanent *K. cochlearis* communities in the studied lakes (Cieplinski et al., 2017).

Samples were taken at the deepest site of each lake with a 50- μ m Wisconsin-type plankton net. Rotifers were collected from Lake Terlago on September 23, 2014 and from both Tovel and Kaltern on March 2, 2015. One clonal culture per lake was established from a single female collected from that lake and continuously maintained in the laboratory. Clonal rotifer cultures were kept inside an incubator in 6-well plates (Biomedica, Vienna) in modified WC medium (Guillard & Lorenzan, 1972) at an average temperature of 14.5 °C and a 16:8 h light–dark photoperiod. The same medium was also used to cultivate *Cryptomonas* sp. strain no. 26.80 obtained from the culture collection of algae in Göttingen, Germany. This *Cryptomonas* sp. served as the only food source for all rotifer clones before and during the life table experiments. Algal concentration was measured with an electronic particle counter (CASY 1-Model TTC, Schärfe System) according to Weisse and Kirchoff

Table 1 Haplotypes used for life table experiments: Haplotype, COI-ESU (terminology for haplotypes and COI-ESUs according to Cieplinski et al., 2017), ITS1-ESU (terminology for ESUs according to Obertegger et al., 2017), and Hap (terminology for haplotypes in this study)

Lake	Haplotype	COI-ESU	ITS1-ESU	Hap
Tovel	h30	ESU 6	ESU α	A
Kaltern	h5	ESU 3	ESU β	B
Terlago	h14	ESU 3	ESU β	C

Table 2 Environmental data of sampled lakes: altitude (alti; m above sea level), area (ha), depth (m), mean summer surface temperature (temp; °C), conductivity (cond; $\mu\text{S cm}^{-1}$), and trophic state (eutrophic—eu; mesotrophic—meso; oligotrophic—oligo)

Lakes	Geographical coordinates	Alti	Area	Depth	Temp	Cond	pH	Trophic state
Tovel	46° 15' 43.2432" N 10° 56' 41.6472" E	1178	38.2	39	15	192	7.9	oligo
Kalterm	46° 22' 43.1724" N 11° 15' 50.2092" E	215	147	5	18	507	8.3	meso
Terlago	46° 5' 56.3568" N 11° 3' 21.258" E	414	11.9	10	23	289	8	eu

(1997). Most complete life table experiments with *K. cochlearis* were performed by Walz (1983, 1987) on specimen coming from the small pond Fasaneriesee in southern Germany. Walz (1983, 1987) reported 15 °C as the optimum temperature for his cultures. Therefore, we conducted all experiments at 15 °C.

Initially, many more clones and haplotypes were selected for culturing for each of the ESUs, also including various haplotypes from the same lakes. However, owing to general difficulties in culturing *K. cochlearis*, we were not able to maintain them in cultures and most of the clones died regardless of culturing efforts.

Life table experiments

Life table experiments for all three haplotypes of *K. cochlearis* were performed using exactly the same experimental setup, including food concentration, temperature, and light conditions. Depending on the size of the wells, we placed two to four flakes of cetyl alcohol on the surface of each well to reduce surface tension (see Desmarais, 1997; Stelzer, 1998), and thus to lower the probability that rotifers were caught in the surface film.

Each experiment comprised three phases:

- (1) standardization—performed in 30 Petri dishes to standardize conditions and to minimize maternal effects;
- (2) synchronization—performed in 24-well plates for female synchronization;
- (3) life table experiment—performed in 96-well plates, similarly to the experiment conducted by Walz (1983).

In monogonont rotifers, a switch from asexual to sexual reproduction is generally attributed to the accumulation of mixis-inducing proteins released into the environment by the rotifers themselves (Stelzer & Snell, 2003; Snell et al., 2006). Sun and Niu (2012)

observed for *B. calyciflorus* that maternal crowding of amictic (asexually reproducing) females can enhance the propensity of offspring to produce mictic (sexually reproducing) females. Therefore, the main purposes of the acclimation period (phase 1) were to minimize the probability of mictic female appearance in phases (2) and (3) and to standardize the starting conditions for all three haplotypes. Because the exact sex-inducing female density has not yet been described for *K. cochlearis*, the number of females used for phase (1) was based on earlier observations in our laboratory. The duration of phase (1) was long enough to rear several generations of rotifers. To initiate phase (1), five individuals were placed into 30 Petri dishes containing 30 mL of medium with abundant food (*Cryptomonas* sp. > 30,000 cells mL^{-1}) and cultured for approximately 14 days. Rotifer abundance was monitored until the total number of rotifers in all dishes reached more than ~ 300 individuals, which was a prerequisite to start phase (2).

For phase (2), single young females from phase (1) were pipetted into eight 24-well plates containing 2 mL of medium with *Cryptomonas* sp. (> 30,000 cells mL^{-1}). The criteria for selecting young females were transparency, smaller body size than adult females, and lack of eggs. These features allowed us to discriminate young females from adult ones that recently gave birth and carried no eggs. Each rotifer during phase (2) was observed two times per day, in the morning and late afternoon, to record the most proximate time of offspring production. The general purpose of phase (2) was to produce many females of similar age whose offspring born at approximately the same time were then used for phase (3); accordingly, we used the term “female synchronization” for phase (2). Newly hatched offspring from the 1st clutch (i.e., a cohort) of phase (2) females were immediately removed and used for phase (3). Phase (2) lasted for a period of approximately 14 days, which was sufficiently long enough to ensure that females produced

several generations of offspring. Only offspring from the same 1st clutch were selected (possible due to the synchronization of their mothers) for the life table experiment (phase 3) to further standardize initial conditions. For phase (3), single females born at approximately the same time were placed into wells of a 96-well plate containing 230 μL of medium and food solution with *Cryptomonas* sp. > 30,000 cells mL^{-1} . The initial number of females was always 96. Rotifers were observed twice per day to record the number of eggs, dead individuals, and the number and sex of offspring. All specimens were transported to fresh medium with food every fourth day, which represented a compromise between culture maintenance and preventing specimen loss due to death by mechanical interference. Newly hatched juveniles were removed immediately and discarded. Apart from females and males, abnormally swimming and non-loricated individuals were also counted; these specimens were called abnormal females. Amictic (parthenogenetic) eggs that did not hatch and instead decomposed in the course of several days at the bottom of the Petri dish were also counted and called rejected eggs.

Analyses of life table data

The life history traits and demographic parameters were calculated based on a sample size of 96 individuals for each haplotype. All females, irrespective of offspring production and all offspring, females, males, and abnormal females were included in calculations. Demographic parameters were calculated according to Birch (1948) and Walz (1983): average lifespan (L) was reported in days, survivorship (l_x) was the percentage of surviving females on day x , age-specific birth rate (m_x) was the fraction of all the surviving offspring on day x , and age-specific fecundity rate ($l_x m_x$) was the product of l_x and m_x .

The net reproduction rate (R_0) was the sum of $l_x m_x$ over the entire experiment:

$$R_0 = \sum l_x m_x$$

Generation time (T) is the time from hatching from an egg to producing an offspring and is calculated according to

$$T = \ln(R_0)/r$$

The instantaneous growth rate per day (r) was estimated by solving Lotka's equation (Lotka, 1907) iteratively, assuming exponential growth (see Birch, 1948):

$$\sum_{x \geq 1} e^{r(x+0.5)} l_x m_x$$

where e is the Euler constant (2.71828), x the age in days, l_x the age-specific survival rate, (i.e., the proportion of surviving females at day x), relative to the initial number of females, and m_x the age-specific fecundity rate, i.e., the mean number of offspring produced on day x by a female of age x .

In the case of R_0 , T , and r , bootstrapping was used to obtain estimates of means and standard deviation. Bootstrapping was done by randomly resampling the same sampling size ($n = 96$) with replacement from the original sample (Quinn & Keough, 2002). Bootstrapping with replacement generates robust representative statistics (Dixon, 2002) as shown for growth rates of cladocerans (Meyer et al., 1986). Here, we used 1000 bootstrapped samples.

Mean values of all life table parameters were tested for significant differences between haplotypes by non-parametric Kruskal–Wallis one-way analysis of variance. For pairwise comparisons of values which did not show normal distribution, the Dunn's post hoc test (95% family-wise confidence levels) was used. Normal distribution was found only for T , and therefore in this case ANOVA was used for analysis of variance and Tukey's post hoc test (95% family-wise confidence levels) was used for pairwise comparisons. All statistical analyses were performed using R 3.4.1 (R Core Team, 2017).

Results

Life history traits and demographic parameters of different haplotypes and ESUs

All females (i.e., amictic, mictic, females producing abnormal females) and all the offspring (females, males, and abnormal females) were included in the analyses of life table data. Excluding mictic females or females producing abnormal females did not change the results in a meaningful way (supplementary

material Table s2). All three haplotypes showed statistically significant differences in all demographic parameters except for L (Table 3). Specifically, Hap A produced more offspring (average number of offspring: 8.1 for Hap A, 2.0 for Hap B, 3.3 for Hap C) and showed a lower percentage of rejected eggs (2.15% with respect to 12.61% for hap B and 9.67% for Hap C) and a higher L compared to Hap B and Hap C (17.0 days with respect to 11.3 and 13.4 days; Table 3). Males were only observed in Hap A (Table 3). All haplotypes showed positive r with Hap A showing the highest r and Hap B the lowest r (0.23 and 0.08 days, respectively) (Table 3). T was the shortest in Hap C (7.99 days) and the longest in Hap A (9.22 days); there were significant differences between all haplotypes. Similarly, R_0 was significantly different between all haplotypes.

Age-specific survival rate (l_x) of Hap C decreased less, relative to Hap B or Hap A during the first nine days (Fig. 1). After this initial phase, Hap C showed a distinctly faster decline than Hap A and Hap B. Furthermore, after day 23 no specimens of Hap C were alive, in contrast to Hap A and Hap B. The shape of the l_x curve for Hap A and Hap B was similar, but Hap A specimens lived longer than Hap B specimens.

Hap A showed higher $l_x m_x$ and m_x values and more frequent and regular cyclical patterns (Fig. 2A1, A2) than Hap B and Hap C (Fig. 2B1, B2; Fig. 2C1, C2). In all three haplotypes, a sharp initial peak appeared after 3 to 4 days, corresponding to the time needed for specimens to reach maturity. Due to increasing mortality of the mothers, the peak height declined in all experiments until the whole population had died.

Occurrence of abnormal and amphoteric females in experiments and in routine cultures

Amictic females showing an undeveloped, non-rigid lorica, impaired swimming abilities, and short (maximum 1 day) lifespan were classified as abnormal females (Fig. 3). We exclude the possibility that these females were males because they were larger than males and had visible and moving trophi (Fig. 3c). Furthermore, both penis and setae were absent in the photographed females. The lorica of these deformed females lacked structures such as plates, ridges, and ornamentation. However, a small, deformed posterior spine was present (Fig. 3e). In routine cultures, such deformed females were very rare. During the experiments, the highest number of abnormal females was

Table 3 Demographic parameters reported for COI haplotypes: lifespan (L; days), instantaneous growth rate of the population (r ; d^{-1}), generation time (T; days), and net reproductive rate (R_0)

	Hap A	Hap B	Hap C
COI-ESU	6	3	3
L (days)	17.0 ^{aa} ± 8.49 17.5	11.3 ^{bb} ± 6.94 11.75	13.4 ^{bb} ± 3.35 13.5
Average number of offspring	8.1 ^{aa} ± 4.52 9.5	2.0 ^{bb} ± 2.02 2	3.3 ^{cc} ± 1.38 3
r (d^{-1})	0.23 ^a ± 0.001 0.228	0.08 ^b ± 0.001 0.077	0.15 ^c ± 0.001 0.149
T (days)	9.22 ^{aa} ± 0.02 9.22	9.24 ^{bb} ± 0.05 9.24	7.99 ^{cc} ± 0.03 7.99
R_0	8.2 ^a ± 0.05 8.16	2.0 ^b ± 0.03 2.03	3.3 ^c ± 0.02 3.29
Number of female offspring (total)	769	194	302
Number of male offspring (total)	7	0	0
Number of abnormal females (total)	1	1	14
% of abnormal females in all offspring	0.64 ^{ac}	0.51 ^a	4.43 ^c
% of rejected eggs	2.15 ^{aa}	12.61 ^{bb}	9.67 ^b

Reported are mean ± standard deviation (upper row values) and median (lower row value) for each parameter; all values followed by different superscripts are statistically different; values followed by a single superscript are statistically different at $P < 0.05$, and values followed by double superscript are statistically different at $P < 0.001$. COI-ESU coding is according to Cieplinski et al. (2017)

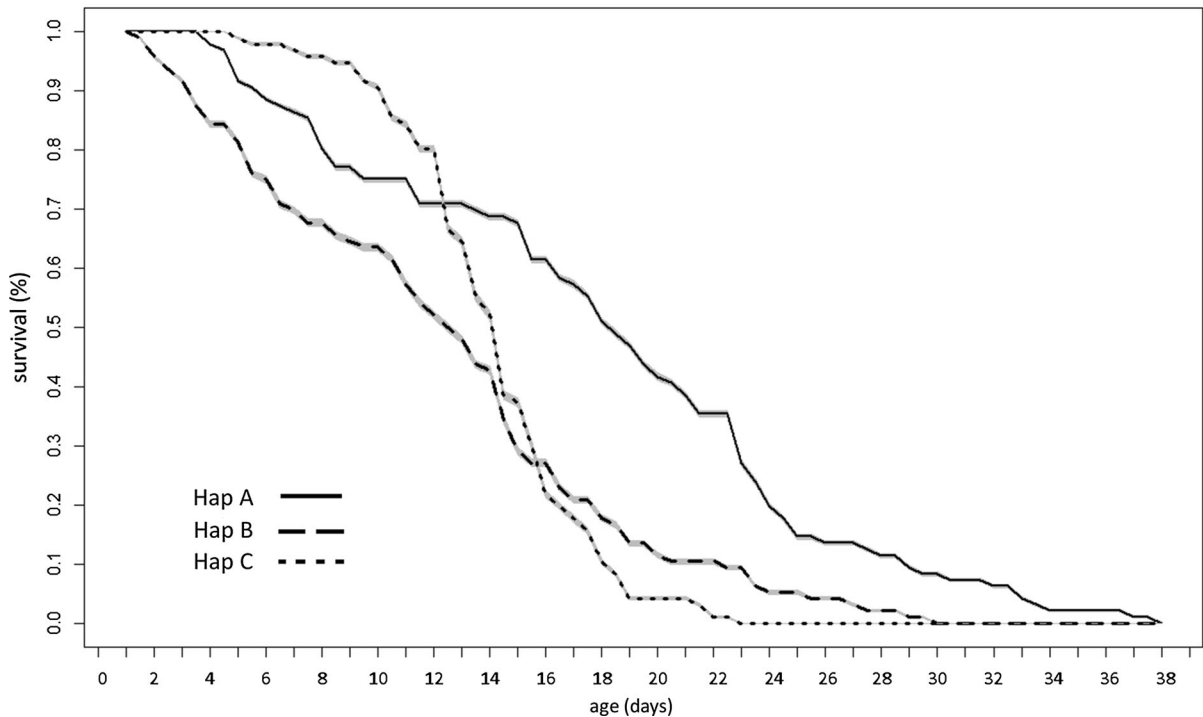


Fig. 1 Age-specific survivorship (l_x) for Hap A, Hap B, and Hap C. Hap A belongs to ESU 6 and Hap B and C to ESU, and 3. 95% confidence intervals are shown as shaded area, but are quite small

recorded for Hap C with a total of 14 specimens (Table 3). For Hap A, one amphoteric female was observed. This female produced two female offspring on days 5 and 10, and 6 male offspring on several days (i.e., 6, 8, 11, 12, 14, 19). No other amphoteric females have been observed in our cultures. The percent of amphoteric females was therefore 0.35% for all the rotifers (288 neonates for three haplotypes together). We did not observe any morphological differences between the single amphoteric female and amictic females.

Discussion

Differences in life history traits and demographic parameters in cryptic species of *K. cochlearis*

The total diversity of haplotypes of *K. cochlearis* within one ESU and the diversity of ESUs is unknown and requires further research. Even though our study did not evaluate intra-haplotype variability of demography, it is the first study that investigates

demographic differences in haplotypes in rotifer species other than *Brachionus*. We demonstrated that genetically different (see Cieplinski et al., 2017) haplotypes of *K. cochlearis* differ also demographically. Combining molecular and demographic data for cryptic species is essential to correctly delimit species using an integrative taxonomy approach recommended by Schlick-Steiner et al. (2010), Fontaneto et al. (2015), and Papakostas et al. (2016). Very few experimental studies on *K. cochlearis* exist; moreover, they did not consider cryptic diversity. These earlier studies focused on the instantaneous rate of population growth (Table 4). Only Walz (1983, 1987) provided complete life table data for *K. cochlearis*, and thus most of our comparisons relate to his studies. The instantaneous rate of population growth (r) is a comprehensive parameter and is often considered a proxy for fitness, representing the ability of a rotifer population to grow and prosper in an environment (Campillo et al., 2011). In our study, r values of the three haplotypes mostly fell within the range known from previous studies of *K. cochlearis* (Table 4) and were all positive, indicating population growth;

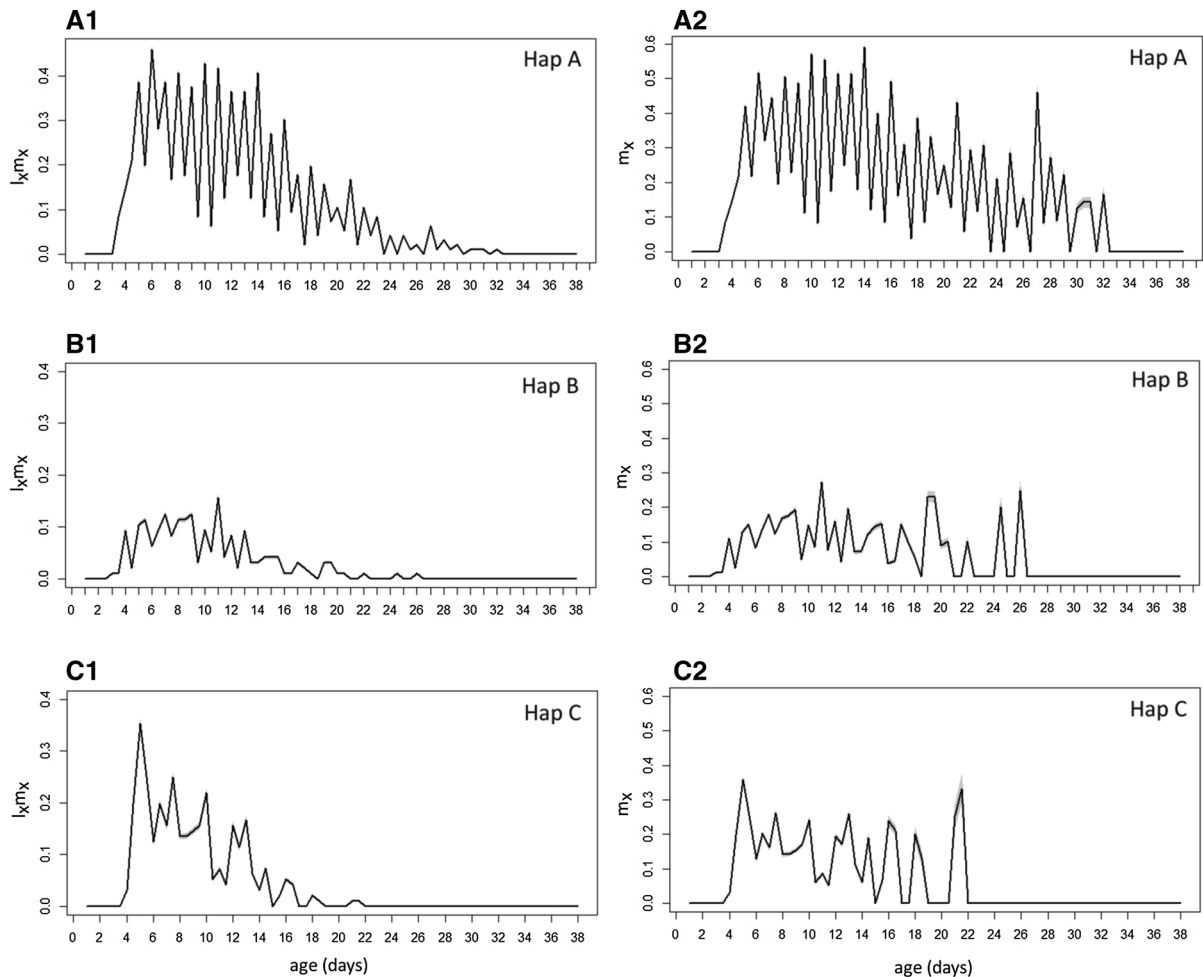


Fig. 2 Age-specific fecundity ($l_x m_x$) and age-specific birth rate (m_x) for Hap A (A1 and A2), Hap B (B1 and B2), and Hap C (C1 and C2). Hap A belongs to ESU 6 and Hap B and C to ESU, and 3. 95% confidence intervals are shown as shaded area, but are quite small

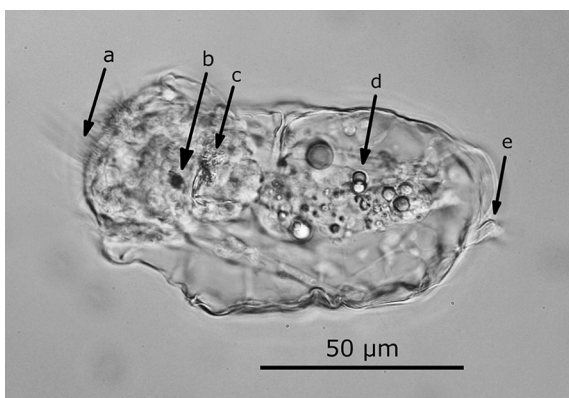


Fig. 3 Light microscopic picture in dorsal view of a deformed *K. cochlearis* female: **a** cilia, **b** eyespot, **c** trophy, **d** lipid globules, and **e** deformed posterior spine. Dorsal view. Scale bar: 50 μm

however, we also observed the lowest r value (for Hap B) ever reported for *K. cochlearis*. The r of Hap A (0.228) was also comparable with r reported by Weisse and Frahm (2001) for *K. quadrata* (0.223 ± 0.21 fed with *Cryptomonas* sp. $> 30,000$ cells mL^{-1}). Walz (1995) reported slightly higher r values for other *Keratella* species, i.e., 0.32 for *K. quadrata*, 0.3 for *K. earlinae*, and 0.28 for *K. crassa*.

The time span to reach reproductive maturity is indicated by the T value. Walz (1983) reported T for *K. cochlearis* to be 8.1 days at 15°C ; this value is slightly lower than those for Hap A and Hap B but comparable with that for Hap C. Differences in T and in r between the haplotypes were also reflected by their offspring number. Correspondence between T and offspring number was found by Ma et al. (2010) for

Table 4 Population parameters for *K. cochlearis* reported in various studies where food and temperature varied depending on the experimental setting: instantaneous growth rate of populations (r ; days), net reproductive rate (R_0), generationtime (T ; days), lifespan (L ; days), lake (refers to the lake of population's origin), lake altitude (Alti; m above sea level), lake depth (Depth; m), and trophic state (eutrophic—eu; mesotrophic—meso; oligotrophic—oligo)

r	R_0	T	L	Lake	Alti	Depth	Trophic state	Authors
$\sim 0.17_a$				Windermere N. Basin;	39;	25.1;	all lakes eu	Edmondson (1965)
				Windermere S. Basin;	39;	16.8;		
				Esthwaite;	65.2;	6.4;		
				Blelham	42	6.8		
0.35				Sempach	504	87	eu	Zimmermann (1974)
0.095	2.5	8.1	15.4	Fasaneriesee	494	5.7	eu	Walz (1983)
0.28 (food: <i>Rhodomonas</i>)				Post Pond	134	38	meso	Stemberger & Gilbert (1985)
0.35 (food: <i>Cryptomonas</i>)				Post Pond	134	38	meso	Stemberger & Gilbert (1985)
0.3				Fasaneriesee	494	5.7	eu	Walz (1986)
0.095				Fasaneriesee	494	5.7	eu	Walz (1987)
0.214				Schöhsee	22	29	meso	Weisse & Frahm (2001)
0.23;	8.2;	9.22;	17.0;	Tovel;	1178;	39;	oligo;	This study
0.08;	2.0;	9.24;	11.3;	Kaltern;	215;	5;	meso;	
0.15	3.3	7.99	13.4	Terlago	414	10	eu	

In the present study, means were reported for demographic parameters. Please note that values are as reported in original papers "Edmondson (1965) reports "population reproductive rate" which has a similar meaning as r ; its value was derived from the graph

different cryptic species of *B. calyciflorus*. Walz (1995) reported T for *K. quadrata* to be 4.8 days at 15 °C, which is comparable with T in our study.

Walz (1983) calculated $R_0 = 2.15$. In our study, R_0 for Hap B was 2.0 and that for Hap C was 3.29, while R_0 for Hap A was much higher. Such differences in amictic offspring production under identical culture conditions were also reported for the *B. plicatilis* cryptic complex (Kostopoulou & Vadstein, 2007). Lifespan (L) reported by Walz (1983) was 15.4 ± 1 days at 15°C, which is comparable to our fastest developing Hap A. Regarding reproductive curves, both $l_x m_x$ and m_x did not overlap for Hap A and for the two other haplotypes but were almost identical between Hap B and Hap C. However, l_x did not overlap between all haplotypes indicating differences in survivorship of all three haplotypes. This suggests that for *K. cochlearis* some demographical parameters may differ more between ESUs than between closely related haplotypes from the same ESU.

None of the haplotypes tested showed all life table parameters similar to the ones reported by Walz (1983, 1987). Large demographic differences in the cryptic species complex of *K. cochlearis* may indicate that the *K. cochlearis* populations previously described were composed of various ESUs and/or haplotypes that differed from those used in the present study.

In rotifers, the appearance of males has been associated with mixis-inducing proteins that are released by females when the population density reaches a species-specific threshold (Carmona et al., 1993; Stelzer & Snell, 2003, 2006; Snell et al., 2006). We observed males only in Hap A, regardless of identical culture conditions for all haplotypes. We hypothesize that this could be related to different density thresholds that trigger sexual reproduction in haplotypes (or in ESUs); this issue requires further study.

We observed some rejected, detached eggs for all the haplotypes. Keen and Miller (1977) indicated that in *K. cochlearis* amictic eggs that are always attached to their mother hatch at different intervals. This indicates that detached amictic eggs in our study were no more viable. We also excluded the possibility of those eggs being pseudo-sexual eggs (resting eggs produced in parthenogenesis) similar to those observed for *K. hiemalis* (Ruttner-Kolisko, 1946) and for *Synchaeta pectinata* (Gilbert, 1995) because these rejected, detached eggs were morphologically identical to amictic eggs and clearly decomposed after some time on the bottom of the container. Moreover, pseudo-sexual eggs have never been reported in *K. cochlearis* and neither did we observe them in our laboratory. This result is unexpected as Keen and Miller (1977) reported a hatching rate for amictic eggs of *K. cochlearis* of 100%. The percent of rejected amictic (parthenogenetic) eggs did vary significantly between Hap A and Hap B and between Hap A and Hap B but not between Hap B and Hap C. Such differences in hatching rates for different cryptic species have been found for diapausing eggs. Gabaldón et al. (2015) reported that the hatching rate of diapausing eggs differs depending on salinity between different cryptic species of *B. plicatilis* and *B. manjavacas*. In our case, culture conditions were constant; therefore, we associated varying hatching rates with population differences between the haplotypes.

The pre-experiment phases (1) and (2) lowered the maternal effect related to crowding (see Lynch & Ennis, 1983) and standardized initial conditions allowing us to observe what we interpret as phenotypic differences unrelated to culture conditions. The observed vast phenotypic and genetic diversity may result from genetically fixed, adaptive evolution (Olson-Manning et al., 2012) related to life in a fast-changing and harsh environment of alpine lakes (represented in our study by lake Tovel). Alpine lakes often experience large environmental changes within short time scales (Sommaruga, 2001), which may trigger intraspecific variation and promote changes in species composition over relatively short evolutionary time scales (Weckström et al., 2016).

Relevance of abnormal females

Abnormal and deformed females, usually appearing as a response to toxins, were reported for *K. cochlearis* by Žurek (2006), for *Platyonus patulus* by Rios-Arana et al. (2007), and for *B. calyciflorus* by Alvarado-Flores et al. (2015). While the latter two studies were performed in the laboratory experimentally exposing rotifers to toxins, the former study found deformed spines in *K. cochlearis* due to exposure to sulfides or its derivatives present in water of a mine impoundment. To the best of our knowledge, deformed females without induction of any toxins are not known for *K. cochlearis*. These deformed females did not show any similarity with males. Wesenberg-Lund (1923) described that any trace of an alimentary canal and trophi have never been observed in males of *K. cochlearis* (previously described as *Anurae cochlearis*). Moreover, as described by Wesenberg-Lund (1923), males of *K. cochlearis* have a long flexible penis with two setae at the end, and the penis cannot be withdrawn. In the present study, the larger size, the presence of trophi, and the absence of a penis and setae let us conclude that these specimens were indeed females, not males. In our study, all three haplotypes of *K. cochlearis* produced abnormal females under standard experimental conditions. Moreover, the percent of abnormal females in Hap C was much higher than that for the other two haplotypes. We cannot exclude the possibility that the genotype of Hap C had some mutations leading to a higher number of abnormal females than in the case of Hap A and Hap B. Most probably, abnormal females did not reproduce because they were never observed carrying eggs and their lifespans were shorter than that of normal females. Therefore, in the long run, the occurrence of abnormal females would result in fitness reduction of the population, relative to a population that produces only fertile females per generation.

We used identical conditions for all cultures and could not identify any proximate factor triggering the occurrence of abnormal females. Therefore, we conclude that the production of deformed females was due to intrinsic factors. One possible intrinsic factor is the accumulation of deleterious mutations (Lynch et al., 1999). Henry et al. (2011) and Barraclough et al. (2007) showed that deleterious mutations are more prevalent in asexually reproducing populations.

Furthermore, deleterious mutations can accumulate due to low genetic variance also in small, at times sexually reproducing populations (Ridley, 2008) such as in *Daphnia* (Berg, 2005). Therefore, differences in the occurrence of abnormal females between the haplotypes of *K. cochlearis* may reflect differential genetic variability and accumulation of mutations because our cultures have been kept for approximately 2 years in the laboratory. Furthermore, we cannot exclude the possibility that we accidentally selected haplotypes prone to genetic mutations.

Occurrence of amphoteric females

Amphoteric females can produce eggs both by mitosis and meiosis, and are thus able to produce both female and male offspring (King & Snell, 1977). Amphoteric females have only been described for six rotifer species (Rico-Martínez & Walsh, 2013): *Asplanchna herricki* (Mrázek, 1897), *A. priodonta* (Sudzuki, 1955), *Sinantherina socialis* (Bogoslavsky, 1958), *Conochiloides coenobasis* (Bogoslavsky, 1960), *A. girodi* (King & Snell, 1977), and *Trochospaera solstitialis* (McCullough & Lee, 1980). Therefore, to the best of our knowledge, this is the first record on the appearance of amphoteric females in the genus *Keratella*. Only by careful observation of single females for longer time periods, the existence of amphoteric females can be confirmed; therefore, it is possible that also in other genera and species amphoteric females occur. In our study, we observed only one amphoteric female corresponding to 0.35% females in our population; this is similar ratio to the ratio reported by King and Snell (1977), who observed seven amphoteric females of *A. girodi* among 1386 neonates. However, Rico-Martínez and Walsh (2013) observed three amphoteric females of *S. socialis* among only 12 neonates; the exact mechanisms behind the production of amphoteric females remain unknown (Rico-Martínez & Welsh, 2013). Therefore, more observations with different *Keratella* populations are required to investigate this phenomenon in more detail.

In conclusion, this is the first study on *K. cochlearis* that combines demography with genetics-based taxonomy and investigates demographic differences between *K. cochlearis* haplotypes and ESUs. The three investigated haplotypes showed large differences in almost all life history traits and demographic

parameters. Furthermore, smaller (and possibly biologically less relevant) differences were recorded between the two haplotypes from ESU 3, which may point to their closer relatedness. Thus, our hypothesis of significant differences in life history parameters between different haplotypes of *K. cochlearis* was confirmed. Although widespread around the world, *K. cochlearis* is an understudied species of monogonont rotifers, probably because of difficulties in culturing. Our study includes a detailed description of *K. cochlearis* culturing methods, which may be useful for future research on this species. The occurrence of abnormal and amphoteric females in *K. cochlearis* deserves further investigation. Because haplotypes used in this study were collected from various lakes, it is difficult to derive any conclusions regarding possible co-existence of these haplotypes in their natural environment. Therefore, more research is needed with more *K. cochlearis* haplotypes per ESU and ESUs derived from the same lake and season.

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