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Leptodora kindtii survival in the laboratory

Radka Pichlová[1,2,*], Anke Weber[3,4] and Brigitte Gosser[3]
[1]Faculty of Biological Sciences, University of South Bohemia, Branišovská 31 370 05 České Budějovice, Czech Republic; [2]Current address: CILER, University of Michigan and Great Lakes Environmental Research Laboratory/NOAA, 2205 Commonwealth Blvd. Ann Arbor, MI 48105, USA; [3]Netherlands Institute of Ecology (NIOO-KNAW), Centre for Limnology, Rijksstraatweg 6, 3631 AC Nieuwersluis, The Netherlands; [4]Current address: TNO Environment, Energy and Process Innovation, PO Box 57, 1780 AB Den Helder, The Netherlands; [*]Author for correspondence (fax: +1-734-741-2055; e-mail: pichlova@umich.edu)

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Abstract

Leptodora kindtii, a pelagic predatory cladoceran, suffers high mortality on transfer to laboratory, which makes the experimental work difficult. We investigated the causes of high mortality, using four variables: water volume, animal density, light intensity, and origin of water for culturing, i.e., water from native or a non-native lake. For the experiments we used *Leptodora* and water from Lake Loosdrecht and Lake Maarsseveen (The Netherlands). Water was found to be the most important factor; the animals did not necessarily do better in lake water from which they were collected. Water volume and animal density were of limited importance, and light intensity did not affect survival.

Introduction

The predatory cladoceran *Leptodora kindtii* is a large plankton species common in lakes of the Northern temperate zone (Rivier 1998). Its importance in food webs of lakes and reservoirs has been reported repeatedly (e.g., Lunte and Luecke 1990; Branstrator and Lehman 1991; Herzig 1994). However, detailed studies of its predatory behavior under controlled conditions are still scarce (Herzig and Auer 1990; Pichlova and Vijverberg 2001). Most of the workers have reported high mortality of animals under laboratory conditions (e.g., Mordukhai-Boltovskaya 1956; Havel 1985; Burkhardt and Lehman 1994), but cause of this is not known yet.

We used four parameters that we considered important for studying *Leptodora* survival: 1) volume of water in a beaker, 2) density of animals in a beaker, 3) light intensity, and 4) origin of culture water. *Leptodora*, being a large animal, is reported to need a large space for survival (Mordukhai-Boltovskaya 1957), though this has never been demonstrated. The highest densities in field samples reached is 2–3 ind./l (Gulati et al. 1992), however, we do not know if crowding (physical contact of the animals) can influence survival. Regarding light, *Leptodora* is known to be positively light sensitive; it tends to move more actively in high light conditions (e.g., Herzig and Auer 1990; pers. observation). Finally, testing of water origin as a factor to concern was based on our previous preliminary experience. With this study we aim at investigating factors that can result in improved maintenance of *Leptodora* in laboratory for experimental work.

Table 1. General parameters of Lakes Loosdrecht and Maarsseveen. References: [1]Gulati et al. (1992), [2]Hofstra and van Liere (1992), [3]Kersting (1981), [4]Swain et al. (1987), [5]van Donk (1983), [6]van Donk (1987), [7]personal observation.

Parameters	Lake Loosdrecht	Lake Maarsseveen
Origin of lake	peat-digging[1]	peat-digging, later excavation of sand[6]
Area (ha)	979[1]	70[5]
Mean depth (m)	1.85[1]	12.1[4]
Main source of water	Amsterdam-Rhine canal[2]	groundwater, seepage[4]
Trophy	eutrophic[2]	meso-oligotrophic[6]
Mean Secchi disc depth (m)	0.3–0.5[1]	3.5–8[4]
Color of water	yellow-brown[7]	transparent[7]
pH (summer average)	8.9[2]	8.2[4]
Total phosphorus (mg l^{-1})	0.1[2]	0.02[6]
Salinity (mg l^{-1})	305[2]	310[4]
Oxygen concentration (mg l^{-1}) (summer average)	10.4[1]	9.0 (in upper 5 m)[3]
Chlorophyll content (μg l^{-1})	93 $\pm$ 18[1]	0.5–4[5]
Average *Leptodora* density during summer season (ind. l^{-1})	0.5–1.5[1]	0.1[7]

Material and methods

Leptodora: collecting, keeping, and handling

The animals and lake water were collected one day prior to experiments from two lakes in the vicinity of Nieuwersluis (The Netherlands): the eutrophic Lake Loosdrecht (LL) and the oligotrophic Lake Maarsseveen (LM); general limnological characteristics of the lakes are presented in Table 1. The experiments were carried out in August 1998. *Leptodora* were sampled using horizontal net hauls of two hoop plankton nets (mesh size 1 mm) with 5-l plastic cod ends (a bottle attached to a narrow end of a plankton net), mounted on a pole, which was slowly towed through water (less than 1 m s^{-1}) (Vijverberg 1991), and then transferred carefully to the laboratory directly in the cod ends. The samples were diluted with water from the corresponding lake, and *Leptodora* were acclimated to lab conditions without food for 24 hrs in rectangular 25-l glass aquaria. To prevent *Leptodora* from continuously striking against the aquaria walls, especially corners, the vessels were kept under low and diffused light (0.07 μmol m^{-2} s^{-1}), at 16:8 hrs light and dark, and the corners of aquaria were covered with black foil. The acclimation temperature (17.5 $\pm$ 1 °C) was similar to that in the lakes. Oxygen concentration in the aquaria did not decrease below 90% of its initial concentration; the lowest concentration measured was 8.5 mg O_2 l^{-1}.

Experimental design

The acclimated *Leptodora* were transferred to beakers filled with filtered lake water (0.45 μm membrane filter; Schleicher and Schuell) using a wide-mouth pipette. The animals were not fed during the experiments. Although the starvation is likely to decrease the survival time, we decided not to feed the animals to ascertain that all animals were under similar conditions during the experiments. *Leptodora* feed discontinuously, and their foraging success depends on the capture rate and handling time of the prey. Consequently, the presence of prey and differential feeding ability can affect the outcome of the experiments. We used only adult females, i.e., mature animals measuring at least 5.0–6.5 mm in length (Andrews 1953). To avoid the release of newborns during the experiment, only females without brood or with early stage eggs were used. The experiments lasted for 72 or 84 hrs, during which the animals were checked every 12 hrs for survival. An overview of the performed experiments and tested treatments is shown in Table 2. The design was partially crossed.

Statistical analysis

We used a sample comparing function in a survival analysis module of the Statistica package (Statsoft Inc. 1995) for analyzing differences in surviving pattern among treatments. This method considers: (1) that some of the animals were still alive at the end of the experiment (72 or 84 hrs), and that their survival time was unknown (so called 'censored'), and (2) that others died during the experimental period and their

Table 2. Overview of all experiments done. Numbers of replicates per treatment are given.

		Leptodora origin				
		Loosdrecht		Maarsseveen		
		Water origin		Water origin		
Tested factor	Treatment	Loosdrecht	Maarsseveen	Loosdrecht	Maarsseveen	Parameters kept constant
Volume (ml)	100	15	33	–	–	*Leptodora* density (= 1 ind.)
	250	18	33	–	32	Light intensity (= 16h L:8h D
	500	10	20	–	–	high)
	800	10	20	–	12	
Leptodora	1	10	20	–	12	Volume (= 800 ml) Light
density (# of ind.	5	3	5	–	3	intensity (= 16h L:8h D high)
in beaker)	10	3	4	–	3	
	15	3	4	–	3	
	20	3	4	–	2	
Light intensity	No light	8	21	–	8	*Leptodora* density (= 1 ind.)
	16h L:8h D low	8	21	–	8	Volume (= 250 ml)
	8h L:16h D high	8	21	–	–	
	16h L:8h D high	6	21	–	8	
	Permanent high	8	14	–	8	
Water origin		20	20	20	22	*Leptodora* density (= 1 ind.) Volume (= 250 ml) Light intensity (= 16h L:8h D high)

survival time was known (uncensored). To compare more than two treatments within an experiment we performed a nonparametric multiple-sample test, while Cox's *F*-test (recommended as best for relatively small *n*, Statsoft Inc. 1995) was applied for comparing only two treatments. The Cox's *F*-test was used also for a post-hoc comparison of pairs of treatments within a tested factor when the multiple-sample test showed a significant difference. In the density experiment, the three to five replicates of 5, 10, 15 and 20 animals were pooled for analysis; in all other experiments all observations within one treatment represented one pool.

Results

Survival of *Leptodora* from Lake Loosdrecht was not significantly affected by water volume in either Lake Loosdrecht water (LLW) (Table 3, Figure 1a) or in Lake Maarsseveen water (LMW) (Table 3, Figure 1c). However, *Leptodora* from Lake Maarsseveen survived in LMW significantly better in 800 ml than in 250 ml (Table 3, Figure 1b).

The analysis of density experiments revealed significant dissimilarity among treatments in sets with animals kept in their native water (Table 3, Figure 2a,b). Though the post-hoc pair comparison within these sets resulted in several significant differences, we could not conclude if density affected survival (Table 4). The survival of *Leptodora* from LL in LMW was similar at all densities (Table 3, Figure 2c).

Light intensity had no significant effect on *Leptodora* survival (Table 3, Figure 3), irrespective of *Leptodora* and lake water in the treatment.

Water origin appeared to be an important variable for *Leptodora* survival. *Leptodora* from LL survived significantly longer in LLW than in LMW (Table 3). Only a few animals from LL died in their original water within the first 36 hrs. In contrast, in LMW there was nearly 90% mortality of LL animals under similar duration (Figure 4). The survival significantly differed also for *Leptodora* from LM exposed to LLW and LMW, respectively (Table 3, Figure 4). Surprisingly, *Leptodora* from LM survived longer in LLW than in their native LM water. There was no significant difference in survival between LL and LM *Leptodora* in Lake Loosdrecht water (Figure 4), while in

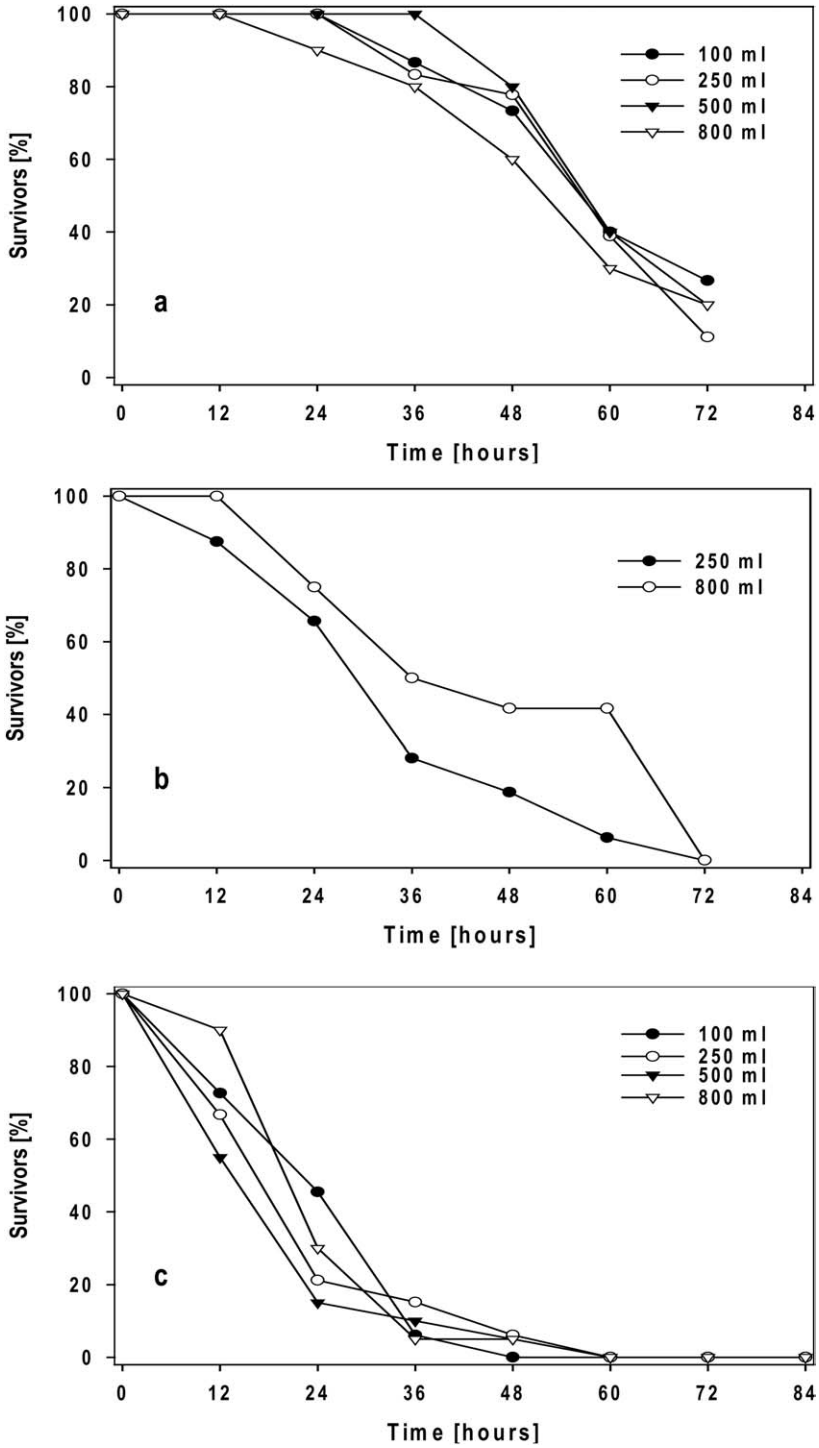

Figure 1. Volume effect: single *Leptodora* in water volumes of 100 ml, 250 ml, 500 ml and 800 ml in 1000 ml beakers. *Leptodora* from Lake Loosdrecht in Lake Loosdrecht water (a), *Leptodora* from Lake Maarsseveen in Lake Maarsseveen water (b), *Leptodora* from Lake Loosdrecht in Lake Maarsseveen water (c).

Table 3. Results of survival statistical analysis for studied effects. (LL – Lake Loosdrecht, LM – Lake Maarsseveen). 'Uncensored' number of observations means number of animals dead by the last counting, while animals 'censored' were still alive at the moment of the last counting.

Effect	*Leptodora*	Water	Number of observations			d.f.	χ^2	F	P
			Total	Uncensored	Censored				
Volume	LL	LLW	53	43	10	3	1.08		0.783
		LMW	106	105	1	3	5.27		0.153
	LM	LLW	–						
		LMW	44	43	1	0		2.11	*0.027*
Density	LL	LLW	160	157	3	4	16.38		*0.003*
		LMW	225	218	7	4	0.66		0.956
	LM	LLW	–						
		LMW	142	130	12	4	13.98		*0.007*
Light	LL	LLW	38	36	2	4	5.75		0.219
		LMW	98	89	9	4	4.84		0.304
	LM	LLW	–						
		LMW	32	32	0	3	4.16		0.245
Water	LL	LLW LMW	40	39	1	0		4.62	*< 0.001*
	LM	LLW LMW	42	40	2	0		2.61	*0.018*

Lake Maarsseveen water LM *Leptodora* survived better than animals from LL.

Discussion

The lack of a hard carapace makes *Leptodora* very vulnerable to handling and manipulation. Their high mortality in laboratory is probably largely caused by cumulative stress of collection and transfer. We hypothesize though that one or more key factors of the laboratory environment are crucial for extension of *Leptodora* survival. In general, the rather short total period of survival (max. 4.5 days incl. acclimation period) in our experiment could have been negatively affected by starving. Nevertheless, under the similar handling and starvation conditions, the survival of the animals differed for different manipulated factors.

It is surprising that light intensity did not produce any effects, since published reports (Andrews 1953; Wolken and Gallik 1965) and our unpublished data suggest that light is an important factor in *Leptodora* activity. However, we did not observe that enhanced activity at higher light intensities would decrease the survival of the animals. We did not, however, investigate the effect of qualitative characteristics of light on *Leptodora*. Contrary to a common assumption that large animals like *Leptodora* would do better in larger volume and lower density, we did not find any strong evidence for it.

The effect of water origin (Figure 4) was strong in all experiments, including those focused primarily on factors other than origin of water. Irrespective of differences within the tested treatments, the overall shape of survival curves followed similar patterns based on used *Leptodora* and lake water (Figure 1–3). Our finding on the effect of water type is significant, more so because survival of *Leptodora* in water from lake of its origin was not necessarily higher. On the contrary, *Leptodora* originating from Lake Maarsseveen survived better in water from Lake Loosdrecht than in lake water of its origin. Water from the same lake as the tested animal is generally considered to be the best medium for any experimental work (Peters and de Bernardi 1987). Here we show that for *Leptodora* it is not always true, and that one should consider use of water from non-native lake(s) for better survival. The filtered Lake Loosdrecht water obviously contains some substances that keep *Leptodora* longer alive in the laboratory, regardless of animals' origin, while Lake Maarsseveen lacks that. Such a substance from LLW needs to be identified in future studies.

We are aware that water quality data for Lakes Loosdrecht and Maarsseveen (Table 1) are rather limited and not from the period of our *Leptodora* study. Therefore, we can only speculate about

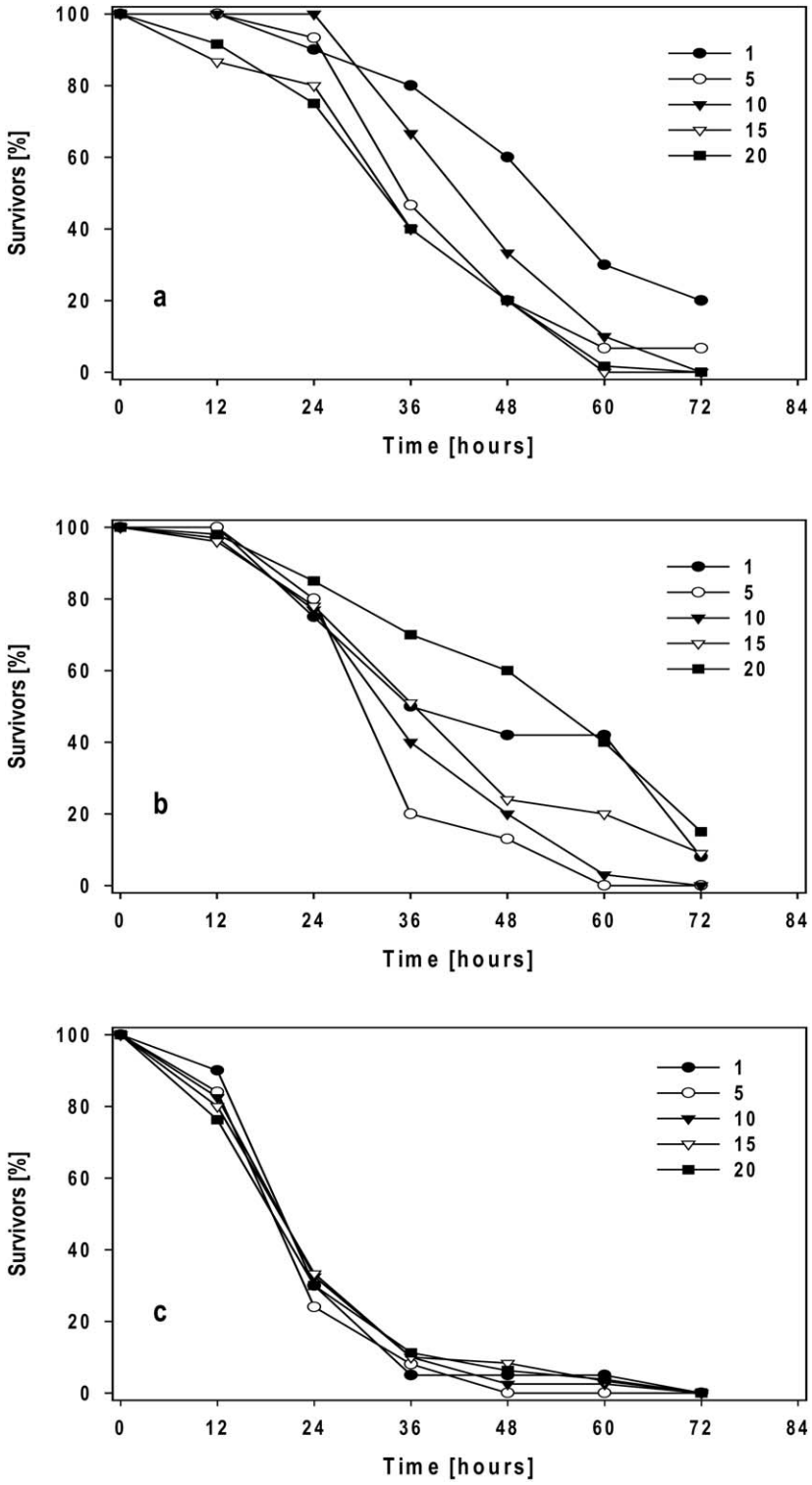

Figure 2. Density effect: densities of 1, 5, 10, 15 and 20 individuals in 800 ml of water. *Leptodora* from Lake Loosdrecht in Lake Loosdrecht water (a), *Leptodora* from Lake Maarsseveen in Lake Maarsseveen water (b), *Leptodora* from Lake Loosdrecht in Lake Maarsseveen water (c).

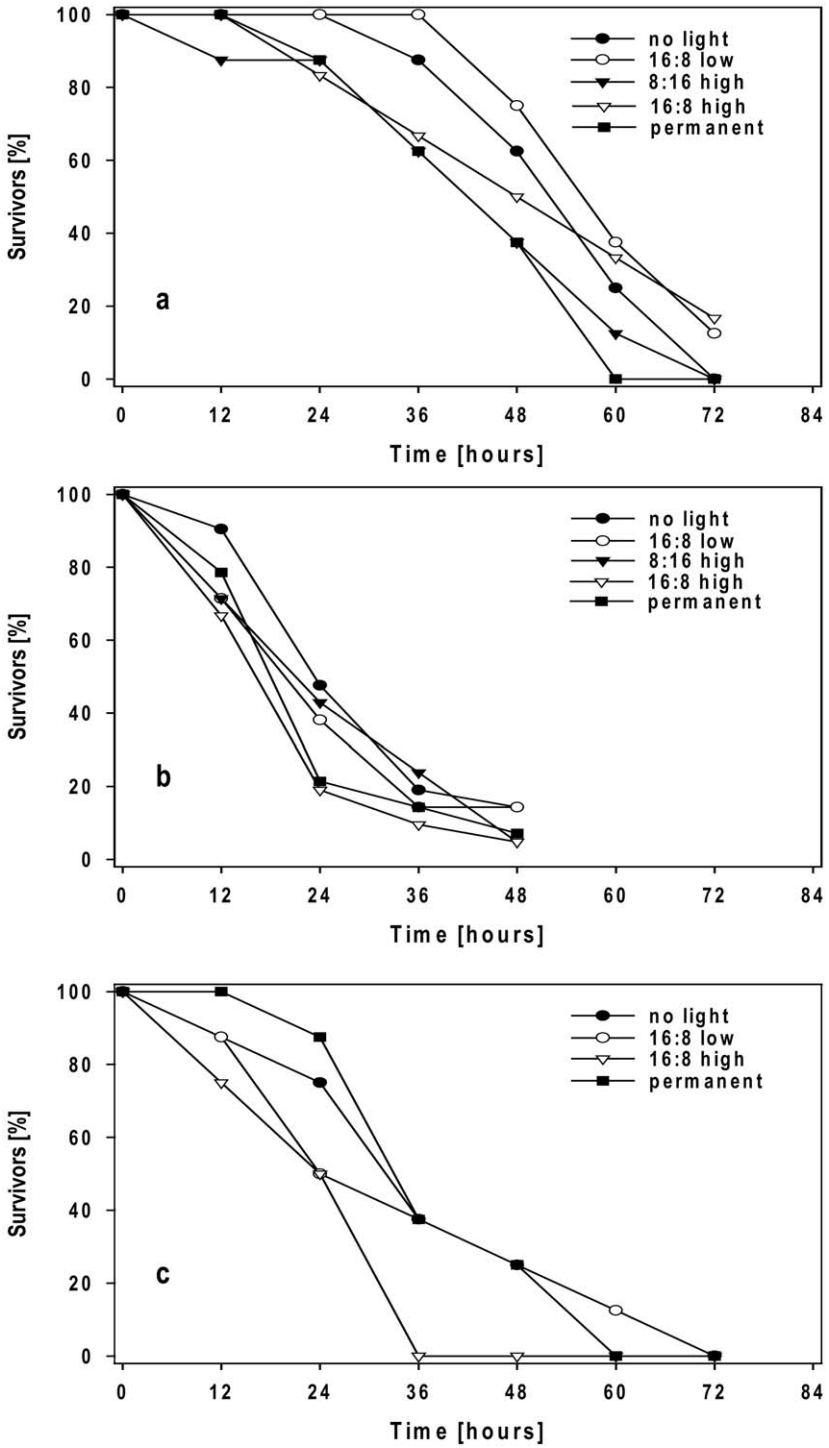

Figure 3. Light effect: single *Leptodora* in five levels of light intensity (no light, 16h L:8h D low light (0.07 μmol m^{-2} s^{-1}), 8h L:16h D high intensity light (10.3 μmol m^{-2} s^{-1}), 16h L:8h D high intensity light (10.3 μmol m^{-2} s^{-1}), permanent high intensity light (10.3 μmol m^{-2} s^{-1}). *Leptodora* from Lake Loosdrecht in Lake Loosdrecht water (a), *Leptodora* from Lake Maarsseveen in Lake Maarsseveen water (b), *Leptodora* from Lake Loosdrecht in Lake Maarsseveen water (c).

Table 4. Post-hoc pair comparison of density experiments, which showed statistically significant dissimilarity. UnC = uncensored, C = censored.

Density treatments pairs tested	LL *Leptodora* in LL water					LM *Leptodora* in LM water				
	Number of observations			F	P	Number of observations			F	P
	Total	UnC	C			Total	UnC	C		
1 × 5	25	22	3	1.80	0.072	25	23	2	1.23	0.217
1 × 10	40	38	2	1.40	0.160	42	41	1	1.09	0.277
1 × 15	55	53	2	2.69	*0.007*	57	51	6	0.36	0.719
1 × 20	70	68	2	2.74	*0.006*	52	45	7	0.77	0.439
5 × 10	45	44	1	1.33	0.185	45	45	0	0.66	0.509
5 × 15	60	59	1	0.85	0.395	60	55	5	1.36	0.175
5 × 20	75	74	1	1.00	0.316	55	49	6	2.97	*0.003*
10 × 15	75	75	0	2.68	*0.007*	75	70	5	0.89	0.372
10 × 20	90	90	0	2.90	*0.004*	70	64	6	3.21	*0.001*
15 × 20	105	105	0	0.09	0.93	85	74	11	2.26	*0.024*

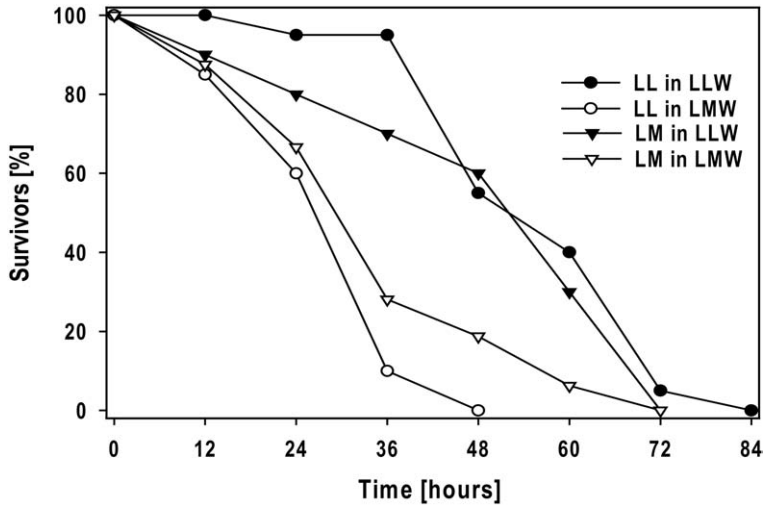

Figure 4. Water effect: single *Leptodora* originated either from Lake Loosdrecht (LL) or Lake Maarsseveen (LM) kept in water from Lake Loosdrecht (LLW) or from Lake Maarsseveen (LMW).

possible causes of the differences in *Leptodora* survival due to water quality differences in the two lakes. Lakes Loosdrecht and Maarsseveen differ greatly from one another in trophic states: in total phosphorus, chlorophyll content and Secchi disc depth (Table 1), and are therefore also likely to differ in contents of dissolved organic matter (DOM) and particulate organic matter (POM). Kulikov et al. (1992) suggested that *Leptodora* might possibly absorb some DOM through penetration across the very thin body surface. This would supplement the carbon (= energy) in the starving animals, here much more in Lake Loosdrecht than in Lake Maarsseveen water. Lake Loosdrecht has higher dissolved organic material mainly due to more dissolved humic acids. The humic acids may have some buffering activity or may sequester some substances including nutrients (Gulati, pers. comm.), and therefore influence qualities of water important for better *Leptodora* survival. These hypotheses, however, require further testing.

Because the salinity and pH differences between the lakes (Table 1) are minimal they do not help us to explain the discrepancies in survival rates. Oxygen is another potentially inconsistent parameter. Moshiri et al. (1969) had reported that below 8 mg l^{-1}, the animals are stressed or at least abnormally inactive. Though we did not continuously monitor the oxygen concentration in the acclimation aquaria for *Leptodora*, oxygen concentrations were not lower than 8.5 mg O_2 l^{-1}. Moreover, the experiments were car-

ried out at a medium temperature (17.5 ± 1 °C), which makes oxygen depletion less likely.

The response of the *Leptodora* populations from two lakes to origin of water and density differed possibly because of differences in genetic, population-related characteristics of these populations. The genotype-related differences in phenotypic response to some stress has been documented several times in filter-feeding cladocerans (e.g., Bachiorri et al. 1991; Epp 1996; Weber and Declerck 1997; Barata et al. 2000). Therefore, the success of keeping *Leptodora* in the lab might also depend on the characteristics of the source population and not only on culture conditions.

Conclusions

We found that the culture water used is an important factor for survival of starved *Leptodora* in the laboratory, though obviously no clear-cut explanations can be given for the observed differences in mortality rate. Water volume and density of animals were of a limited importance and light intensity seemed to play no significant role.

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