

Research Article

Effects of water depth on survival, condition and stable isotope values of three invasive dreissenid species in a deep freshwater lake

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Abstract

To examine the effects of depth on the performance of the freshwater dreissenid species *Dreissena polymorpha* and *D. rostriformis bugensis*, and the brackish water dreissenid, *Mytilopsis leucophaeata*, a controlled experiment lasting four months was conducted in a manmade freshwater lake in the Netherlands. The three species used in the experiment were collected from other water bodies in the Netherlands. Cages containing each species in separate compartments were placed at water depths of 2, 5, 10 and 17 m from November 2009 until February 2010. Samples of individuals were retrieved at the start and at approximately four-week incubation intervals, for determination of overall survival, condition and soft tissue carbon and nitrogen stable isotope ratios. In contrast to the *Dreissena* species, survival of *M. leucophaeata* decreased strongly over time at each depth. Mortality increased with increasing depth and was greatest at 17 m. Survival of *D. polymorpha* at 17 m was much greater than that of *D. rostriformis bugensis*. The condition of all species after 3.5 weeks did not differ significantly from that at the start. Surviving *M. leucophaeata* showed a significantly reduced condition at all depths after 16 weeks. Both *Dreissena* species showed an improved condition at depths of 2, 5 and 10 m after 16 weeks, the greatest improvement being found at 2 m. After 16 weeks at 17 m, both *Dreissena* species had the lowest condition index. Stable isotope ratios of soft tissue for both *Dreissena* species showed a shift in $\delta^{15}\text{N}$ within 3.5 weeks. The $\delta^{15}\text{N}$ values of mussels held at 2–10 m fell. Those held at 17 m had values similar to that initially recorded, possibly indicative of very limited food intake at that depth or a diet similar to that at their location of origin. After 16 weeks, $\delta^{13}\text{C}$ values became more negative than those at the start, in particular at 2 m. The stable isotope ratios of soft tissue of *M. leucophaeata* did not change at any depth throughout the experiment, possibly indicating lack of feeding in the freshwater lake. Changes in stable isotope values for both *Dreissena* species were reduced at 17 m. Mortality of *D. polymorpha* was lower at 17 m compared to the other two dreissenid species which indicated greater tolerance of habitat conditions at this deeper depth.

Key words: *Dreissena*; *Mytilopsis*; condition index; survival rate; stable isotopes; depth impact

Introduction

Dreissenid species are persistent invaders of estuaries, rivers and lakes throughout Europe and the United States of America, where they attach to various types of hard substratum by byssal threads (Van der Velde et al. 2010 and literature therein; Grutters et al. 2012). These species can greatly impact newly colonised waters due to their extensive filtering capacity (more than 100 mL per hour) (Roditi et al. 1996) and by attaining

densities of more than 100 000 individuals per m² (Nalepa and Schloesser 1993; Mackie et al. 1989; Van der Velde et al. 2010). They often form carpets of mussels by byssal attachment to each other's shells that overgrow other sessile and benthic species and change soft substratum into hard substratum. They can settle on the shells of unionid freshwater mussels and obstruct food intake of the unionid eventually leading to starvation and even death (Ricciardi et al. 1995; Sousa et al. 2011). Dreissenids also influence

water clarity, temperature, dissolved oxygen, nutrient concentrations, benthic carbon supply, contaminants, phyto- and zooplankton, cyanobacteria, benthic invertebrates, fish and other invasive species (Kelly et al. 2010).

When *Dreissena rostriformis bugensis* Andrusov, 1897 and *Dreissena polymorpha* (Pallas, 1771) become established in a freshwater body, both use seston as food and hard substratum for attachment. A depth zonation often develops when both species co-occur, with *D. rostriformis bugensis* being more abundant in the deeper, colder parts of water bodies than *D. polymorpha* (Mills et al. 1996; Jones and Ricciardi 2005). Mitchell et al. (1996) also observed spatial differences in abundances and maximum depth of these two species relative to depth gradients, but added that temperature tends to be correlated with depth in a lake and that this pattern may be due to higher temperatures in winter rather than lower water temperatures in summer. Although observations have shown that *D. rostriformis bugensis* can occupy deeper zones in lakes than *D. polymorpha*, comparative controlled experiments that have, to our knowledge, been conducted at depths of over 10 m are scarce (MacIsaac 1994). Many factors in a lake change with increasing depth, such as water pressure, temperature, flow velocity, oxygen supply and the quantity and quality of food. All of these factors can influence the fitness and distribution of species.

D. polymorpha and *D. rostriformis bugensis* are closely related freshwater bivalves originating from the Ponto-Caspian area. A third dreissenid species, the dark false mussel (*Mytilopsis leucophaeata* (Conrad, 1831)), originating from the Atlantic coast of North America and the Gulf of Mexico, is also dispersing across Europe. *M. leucophaeata* is characteristically a brackish water species, but has been reported to tolerate freshwater conditions for relatively long periods. *D. polymorpha*, *D. rostriformis bugensis* and *M. leucophaeata* were recorded for the first time in the Netherlands in 1826, 2006 and 1895, respectively (Van der Velde et al. 2010 and literature therein). *D. rostriformis bugensis* is thus a very recent invader of western Europe. First discovered in the Netherlands (Hollandsch Diep), it has already extended its distribution into Germany, Belgium and France, and is expanding also in eastern and central Europe. In newly invaded areas, *D. rostriformis bugensis* disperses rapidly and develops dense populations that can quickly outnumber co-occurring

D. polymorpha populations (Bij de Vaate et al. 2013; Matthews et al. 2012, 2013).

The main objective of the present study was to assess the responses of *D. rostriformis bugensis*, *D. polymorpha* and *M. leucophaeata* to increasing depth in a relatively deep freshwater lake. We hypothesised that the response to depth would differ for all three dreissenid species but would be most evident and occur faster in *M. leucophaeata*, as it would have to deal with multiple stressors, viz. depth and freshwater conditions.

An experimental set-up in a manmade freshwater lake was used to assess whether the three species would respond differently to depth-related environmental conditions. Response variables included survival, condition and feeding patterns. The latter was measured using stable isotope analyses from which diet shifts and assimilation were evaluated.

Materials and methods

The effects of water depth on the survival, condition and stable isotope values of the three dreissenid species were assessed by means of a transplantation experiment in a manmade freshwater lake called Groene Heuvels, the Netherlands (51°50'43"N, 5°41'33"E). The experiment lasted four winter months, from 4 November 2009 until 25 February 2010. This period was chosen for two reasons a) during winter stratification does not occur in the lake, which results in more homogeneous temperature and oxygen conditions at all depths, in contrast to summer when a thermocline develops and b) in winter reproduction of the introduced species is unlikely. Table 1 summarises the physico-chemical characteristics of the lake in 2009 as provided by the regional water board. The lake is a groundwater-filled, abandoned sand excavation pit with little organic matter in the sediment and with an underwater visibility of about 5 m. The lake has a surface area of 350,000 m², and has a maximum depth of 24 m. Of the three species, only *D. polymorpha* is found in the lake and it mainly occurs no deeper than 10 m.

Experimental setup

Eight rectangular cages were used in the experiments. Each cage was constructed of stainless steel strips and mesh (2 – 4 mm), and each contained eight chambers of 10 × 10 × 20 cm (L × W × H) in a 2 × 4 pattern. These cages

Table 1. Water quality data of the Groene Heuvels Lake, provided by the Rivierenland Water Board. Measurements in November were done on 17 and 26 November 2009 between 7:30 and 7:45 AM, while the whole year measurements were done in January, February, March, April, June, September, October and November 2009.

Parameter	November 2009	Whole of 2009			
	Range (n = 2)	Mean	Minimum	Maximum	n
Temperature (°C)	8.5 - 9.5	15.6	3	24	22
Conductivity (mS/m)	32 - 32	31.7	28	53	22
Dissolved oxygen (mg/l)	10.2 - 11.2	10.4	6.3	14.4	22
Dissolved oxygen (%)	90 - 97	105	64	150	22
pH	7.9 - 8.0	8.3	7.6	8.9	22
Bicarbonate (mmol/l)	2.1 - 2.2	2.2	1.8	2.4	11
Total organic carbon (mg/l)	3.8 - 3.9	4.8	3.5	8.9	11
Sulfate (mg/l)	28 - 28	28.9	27	31	11
Chl-a (µg/l)	< 4 - < 4	4.3	<4	6	11
Phosphate (mg/l)	< 0.02 - < 0.02	0.035	0.02	0.06	11
Ortho-phosphate (mg/l)	< 0.05 - < 0.05	< 0.05	<0.05	<0.05	11
Ammonium (mg N/l)	< 0.05 - 0.05	0.05	<0.05	0.06	11
Nitrate (mg/l)	< 0.05 - 0.09	0.1	<0.05	0.21	11
Nitrite (mg/l)	< 0.01 - < 0.01	< 0.01	<0.01	<0.01	11
Kjeldahl-N (mg/l)	0.4 - 0.5	0.5	0.4	0.6	11
Total N (mg/l)	0.50 - 0.53	0.53	0.41	0.66	11
Iron (µg/l)	18 - 27	31	18	65	10
Calcium (mg/l)	36 - 41	38	31	46	11
Potassium (mg/l)	2 - 2	2.3	2	3	11
Sodium (mg/l)	15 - 15	14	12	16	11
Chloride (mg/l)	14 - 14	14	14	15	11

were fitted with a lid of stainless steel mesh (4 mm) and were weighted down with a heavy concrete tile (30 × 30 × 4.5 cm; 9.5 kg) attached to the bottom. Four steel spikes (30 cm in length) ensured that the cages were firmly fixed in the sediment (Figure 1 and 2).

About 2000 individuals of each species were collected from sites in the Netherlands between late October and early November 2009. Specimens of *D. rostriformis bugensis* were collected from the Pannerdensche Kanaal (referred to in this text as *Pannerden*; 51°53'35"N, 6°01'23"E), and of *M. leucophaeata* from the Zijkanaal C canal, which connects to the Noordzeekanaal canal (referred to in this text as *ZKC*; 52°25'30"N, 4°42'07"E). Specimens of *D. polymorpha* and a further 1000 individuals of *D. rostriformis bugensis* were collected from Lake Kraayenbergse Plassen near the town of Cuijk (referred to in this text as *Cuijk*; 51°44'51"N, 5°50'55"E). *D. rostriformis bugensis* was collected from two locations in order to analyse possible effects of differences in test species' origin. The mussels were gently removed from the substrate with a sharp knife to prevent damage to the byssal gland. Individuals with a shell length over 10 mm and without malformations were selected for

the experiment and stored in aerated tanks filled with water from the water body where they were sampled, for one week at ambient temperature (14°C). Additionally, 100 specimens of each species were frozen (-20°C) until analysis and served as reference material. Shell length ranges of the mussels used in the experiment were 10.65–21.35 mm for *M. leucophaeata*, 12.50–30.61 mm for *D. rostriformis bugensis* (Cuijk), 13.50–30.24 mm for *D. rostriformis bugensis* (Pannerden) and 10.95–34.18 mm for *D. polymorpha*. Mean shell lengths ± SE were 14.96±0.14 mm for *M. leucophaeata*, 19.28±0.21 mm for *D. rostriformis bugensis* (Cuijk), 20.41±0.18 mm for *D. rostriformis bugensis* (Pannerden), and 18.67±0.18 mm for *D. polymorpha*.

The experiment in the Groene Heuvels Lake started on 4 November 2009. Two cages were placed at each of four depths (2, 5, 10 and 17 m; Figure 1). Four compartments per depth were filled with 100 individuals of one species per compartment, and a further two compartments were filled with *D. rostriformis bugensis* collected from Cuijk. One hundred individuals per species were placed both in a corner and a middle compartment to reduce positional bias as much as possible (Figure 3).

Figure 1. Experimental setup showing two cages placed at each experimental depth (2, 5, 10 and 17 m) and anchored to buoys marking their locations on the surface of the lake.

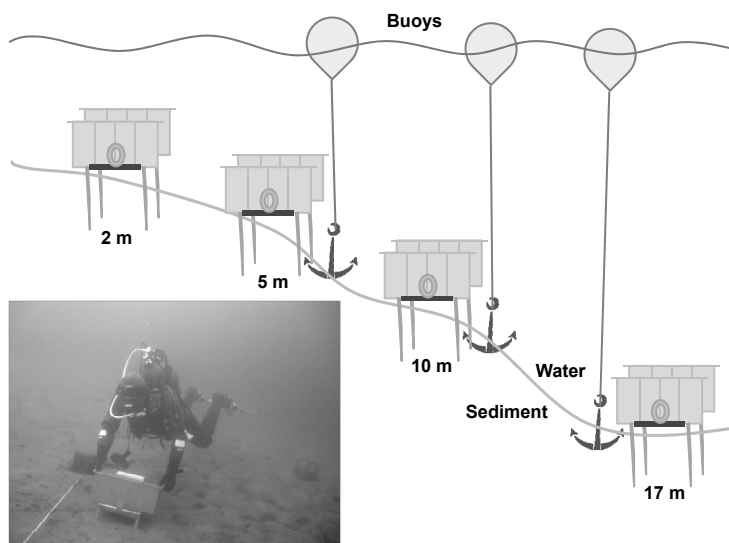
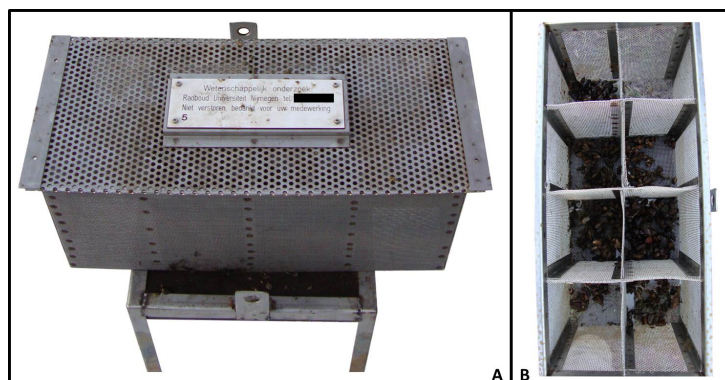


Figure 2. Cage used in the transplantation experiment: closed (A) and opened (B).



Survival

On 26 November 2009 (after 3.5 weeks of exposure), as well as on 5 January 2010 (9 weeks), 26 January 2010 (12 weeks) and 25 February 2010 (16 weeks), one compartment of each species (100 individuals) was emptied at each depth. Samples of *D. rostriformis bugensis* from Cuijk were only taken after 9 weeks and 16 weeks because of lack of sufficient specimens. For that purpose each cage was hauled to the surface by divers, emptied by two persons in a boat and then lowered and placed at the same place by divers again. The temperature at each depth was measured during mussel sampling. After sampling, mussel survival rate was determined by classifying individuals with

gaping or empty valves as dead, and those with closed or closing valves as alive. Living mussels were cleaned and stored in a freezer (-20°C). Only those collected after 3.5 and 16 weeks were used for condition and stable isotope analyses.

Condition

A condition index (CI) was calculated for each living individual mussel using formula (1), similar to that previously used by Mersch et al. (1996), Mersch and Beauvais (1997) and Smolders et al. (2004):

$$(1) \text{ Condition index} = \text{tissue dry weight (g)} / \text{shell dry weight (g)} \times 1000$$

Depth (m)

2

Cage nr. 1

MI	Drb (C)	Dp	Drb (P)
Dp	Drb (P)	MI	

5

Cage nr. 3

MI	Drb (C)	Dp	Drb (P)
Dp	Drb (P)	MI	

10

Cage nr. 5

MI	Drb (C)	Dp	Drb (P)
Dp	Drb (P)	MI	

17

Cage nr. 8

MI	Drb (C)	Dp	Drb (P)
Dp	Drb (P)	MI	

Cage nr. 2

MI	Drb (C)	Dp	Drb (P)
Dp	Drb (P)	MI	

Cage nr. 4

MI	Drb (C)	Dp	Drb (P)
Dp	Drb (P)	MI	

Cage nr. 6

MI	Drb (C)	Dp	Drb (P)
Dp	Drb (P)	MI	

Cage nr. 7

MI	Drb (C)	Dp	Drb (P)
Dp	Drb (P)	MI	

Colour legend - Compartments emptied after:

3.5 wk

9 wk

12 wk

16 wk

Figure 3. Distribution of the different species over the eight compartments of the cages and retrieval times. MI = *Mytilopsis leucophaeata*; Dp = *Dreissena polymorpha*; Drb (C) = *Dreissena rostriformis bugensis* (Cuijk); Drb (P) = *Dreissena rostriformis bugensis* (Pannerden).

An increase in CI can indicate either a relative increase of the tissue compared to the shell weight, or a decrease of the shell compared to the tissue weight. Because the ‘condition’ of the individual is only higher in the first case, we checked if the relationship between shell length (SL) and shell dry weight of a selected species was similar throughout the experiment (see *Statistics*).

In order to calculate the mean CI values per species at each depth, 50 mussels were randomly selected from the samples of 100 frozen mussels collected alive at the start of and during the experiment. They were wiped clean on a towel, weighed to determine whole fresh weight (Sartorius CP224S; $d = 0.0001$ g; Sartorius Mechatronics Netherlands BV, Utrecht, The Netherlands) and their shell lengths measured using a digital callipers (Mahr, $d = 0.01$ mm; Mahr Federal Inc., Providence, Rhode Island, USA). All remaining byssal threads were removed before the soft tissue was removed. The soft tissues and the shells were then dried separately for 48 h in aluminium cups, at a temperature of 60°C . Dried shells and tissues of each mussel were weighed and the latter stored in Eppendorf tubes (2 ml).

To compare the effects of depth on condition in each mussel species, a relative condition index

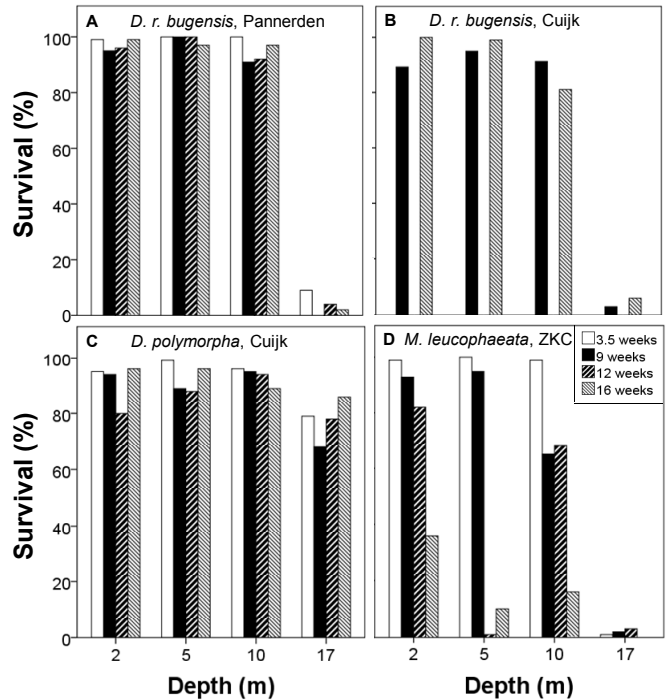
(RCI) was calculated to compensate for any species-intrinsic differences, such as possible differences in shell volume-to-weight ratios. The mean initial CI values (initial CI) were used as the basis for each RCI (Formula 2).

(2) Relative condition index = (measured species CI after exposure / species initial CI) \times 100

Diet

In order to identify differences in the diet of each species along the depth gradient, carbon and nitrogen stable isotope ratios of mussels were determined. In each batch of the 50 dried mussels used for calculating the CI, the soft tissues of 10 mussels with a shell length of about 17 mm were ground to a powder with a ball grinder (Retsch, MM301; Retsch, Haan, Germany) for stable isotope analysis. For the purpose of isotope analysis, a 0.350 mg (range of ± 0.020 mg) sample was weighed (Sartorius Micro Pro 11, $d = 0.001$ mg; Sartorius Mechatronics Netherlands BV) in a tin cup. This cup with content was closed and rolled into a ball by using a pair of pincers. These balls were analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with an elemental analyser (Thermo Fisher Scientific, EA 1110; Thermo Fisher Scientific, Waltham, Massachusetts, USA), coupled via an interface (Thermo Finnigan Conflo III) to a mass spectrometer

Figure 4. Survival of dreissenids, expressed as survival (%) per cage compartment per date per species (n = 1, comprising 100 individuals).



(Thermo Finnigan DeltaPlus). Ammonium sulphate (IEAE-N2) and sucrose (IAEA-CH6) were used as standards, while caffeine was used as a laboratory reference ($d = 0.001$).

Statistics

Before statistical analyses, outliers (> 1.5 times the interquartile range) were identified and data was tested per group for normality using a Kolmogorov-Smirnov test and Q-Q plot. The data was then tested for homogeneity of variances with Levene's test. If Levene's test failed ($\alpha = 0.05$), a Welch test with Games-Howell post-hoc was used, otherwise a 1-way ANOVA with Tukey's HSD post-hoc was used ($\alpha = 0.05$). All statistics were performed using SPSS 17 (IBM Corporation, Armonk, New York, USA).

In order to test if the assumption of the CI that the relationship between shell length and shell dry weight did not change over time and/or depth, data were first $\ln(x)$ transformed as this relationship was generally best described by an exponential function. Subsequently, a Generalized Linear Model (GLM) was used to assess if this relationship was indeed similar over time and depth for each species / population, using R 3.0.0 (R Foundation for Statistical Computing, Vienna, Austria).

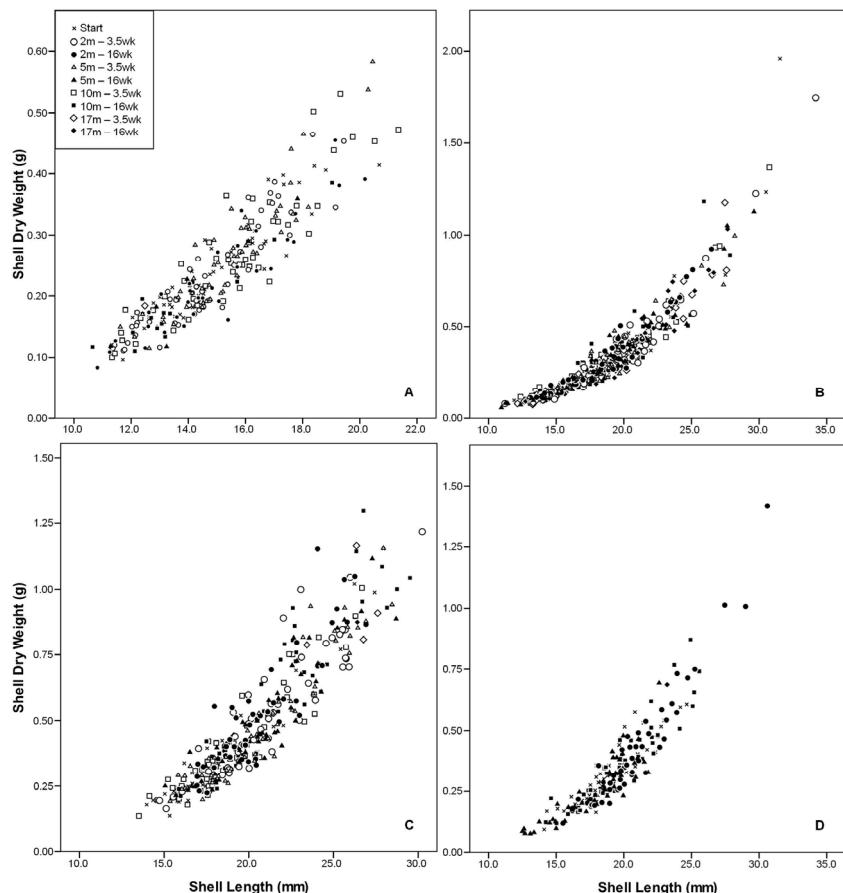
Results

The water temperature at the start of the transplantation experiment (4 November 2009) was 12 °C at the water surface, 9 °C at depths of 5 and 10 m, and 8 °C at a depth of 17 m. On 6 January 2010, the whole water column had a temperature of 4 °C, while on 26 January and 25 February 2010 it had decreased to 2–3 °C. Over the 16-week period the two *Dreissena* species in particular had reattached themselves to the available substratum (i.e., each other or the cage walls).

Survival rates differed between the three species (Figure 4). *D. polymorpha* (Cuijk) showed some gradual decline in survival with increasing depth, but survivorship at 17 m was still 79% after 3.5 weeks, which was high compared to the other two species. The percent survival of *D. rostriformis bugensis* (Pannerden) showed a strong decline to 9% at 17 m after 3.5 weeks. *M. leucophaeata* suffered even more, as its percent survival declined sharply over time at each depth. It had a high mortality rate at 17 m leading to survival of only 1% of individuals after 3.5 weeks. At shallower depths, its survival was higher than at 17 m, but still relatively low compared to the other species. A few individuals of *Dreissena*

Figure 5. Relationship between shell length and shell dry weight over time and depth for the various species:

A. *Mytilopsis leucophaeata* (ZKC);
B. *Dreissena polymorpha* (Cuijk);
C. *D. rostriformis bugensis* (Pannerden);
D. *D. rostriformis bugensis* (Cuijk).



rostriformis bugensis (Pannerden and Cuijk) and *M. leucophaeata* (ZKC) had survived the exposure to 17 m after 16 weeks. A 100% mortality rate was never reached by any species except for *D. rostriformis bugensis* (Pannerden) exposed for 9 weeks and *M. leucophaeata* (ZKC) exposed for 16 weeks to 17 m. After 16 weeks, 36 % of *M. leucophaeata* were still alive at 2 m, versus 10 % at 5 m, 16 % at 10 m but none at 17 m. The corresponding survival percentages at the various depths for *D. rostriformis bugensis* (Pannerden) were 99, 97, 97 and 2 %, respectively, for *D. rostriformis bugensis* (Cuijk) 100, 99, 81, and 6 %, respectively, and for *D. polymorpha* (Cuijk) 96, 96, 89 and 86 %, respectively.

Before analysing the CI, the assumption that the shell weight of the mussels did not decrease at any depth or over time was assessed (Figure 5). The relationship between shell length and shell weight seemed to be relatively constant in all treatments, as assumed. Only for *D. polymorpha*

(Cuijk) held at 17 m after 3.5 weeks and for *D. rostriformis bugensis* (Cuijk) held at 5 m after 16 weeks was this relationship significantly different to the mean. The intercept was only slightly lower and the slope was only slightly higher for both (Table 2). This slightly higher slope did not threaten the assumptions for the CI.

The CI of *D. rostriformis bugensis* and *D. polymorpha* collected from the same lake (Cuijk) differed at the start of the experiment. Furthermore, the CI of *D. rostriformis bugensis* collected from a canal (Pannerden) differed significantly from those collected in a lake (Cuijk). The CI of surviving individuals of *D. rostriformis bugensis* (Cuijk and Pannerden) and *D. polymorpha* (Cuijk) increased significantly after 16 weeks relative to that at the start of the experiment, with the exception of those held at a depth of 10 m or 17 m (Figure 6). In contrast, the condition *M. leucophaeata* (ZKC) significantly decreased over time.

Table 2. GLM output of the relationship between shell length and shell dry weight showing only significant different groups (species at a depth after a time). Sign. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘.’ 1.

Species; depth; time	Coefficients:	Estimate	Std. Error	t value	Pr(> t)	sign.
<i>D. rostriformis</i>	(Intercept)	-4.16774	0.198002	-21.049	<2e-16	***
<i>bugensis</i> Cuijk (Qc);	Shell Length	0.15586	0.010503	14.84	<2e-16	***
5 m;	Qc-5m-16wk	-0.66642	0.261456	-2.549	0.0116	*
16 weeks	Shell Length*Qh-5m-16wk	0.032932	0.014158	2.326	0.0211	*
<i>D. polymorpha</i>	(Intercept)	-4.04529	0.110906	-36.475	<2e-16	***
Cuijk (Z);	Shell Length	0.147137	0.00589	24.982	<2e-16	***
17 m;	Z-17m-3.5wk	-0.38005	0.157194	-2.418	0.016	*
3.5 weeks	Shell Length*Z-17m-3.5wk	0.017882	0.008303	2.154	0.0318	*

Table 3. Statistical analyses of differences in stable isotope ratios between ‘treatments’ of each species.

Species	$\delta^{15}\text{N}$				$\delta^{13}\text{C}$			
	Test	F	df	P	Test	F	df	p
<i>D. r. bugensis</i> (Cuijk)	Welch	15.468	3, 16.308	<0.001	Welch	367.409	3, 19.113	<0.001
<i>D. r. bugensis</i> (Pannerden)	ANOVA	33.922	7, 67	<0.001	Welch	253.881	7, 26.081	<0.001
<i>D. polymorpha</i> (Cuijk)	ANOVA	21.153	8, 81	<0.001	Welch	158.582	8, 33.115	<0.001
<i>M. leucophaeata</i> (ZKC)	ANOVA	1.602	6, 59	0.163	ANOVA	1.238	6, 59	0.300

The increase in CI declined with increasing depth in both *Dreissena* species. The CI values of *D. rostriformis bugensis* (Pannerden) at 2, 5 and 10 m increased significantly over time (Figure 6). At a depth of 17 m, the surviving *Dreissena* mussels had CI values similar to ‘start’ samples (*D. polymorpha* from Cuijk and *D. rostriformis bugensis* from Pannerden) or an apparently reduced condition in the case of *D. rostriformis bugensis* collected near Cuijk. However, too few individuals of *D. rostriformis bugensis* (Cuijk and Pannerden) survived at this depth to allow statistical analysis. Using the RCI allowed a comparison between species in response to depth (Figure 7). After 16 weeks of exposure, *D. rostriformis bugensis* collected from Pannerden showed significantly higher RCI values at depths of 2, 5 and 10 m compared to the other species. In contrast, *M. leucophaeata* (ZKC) showed a low RCI at all depths. A statistical comparison between mussels at 17 m was impossible due to the very low survival of all species except *D. polymorpha* (Cuijk). *D. rostriformis bugensis* (Cuijk) and *D. polymorpha* (Cuijk) had intermediate and mostly similar increases in RCI. Apparently, the condition of both *Dreissena* species collected from the same lake (i.e. Cuijk) changed in a similar way with depth.

The stable isotope values of *D. rostriformis bugensis* and *D. polymorpha* showed similar trends for both species and for the two sampled populations of *D. rostriformis bugensis* (Figure 8). Compared to their initial values (‘Start’ in Figure 8), excluding 17 m, there was a statistically significant decrease in $\delta^{15}\text{N}$ after 3.5 weeks for *D. rostriformis bugensis* (Pannerden). For *D. polymorpha* (Cuijk) this decrease was only statistically significant at 2 m (Tables 3 and 5). After 16 weeks, the $\delta^{15}\text{N}$ values of both *Dreissena* species showed a further small decline, which led to significantly lower values in both species at depths of 2 m to 10 m. Shifts in $\delta^{13}\text{C}$ values were greatest compared to initial levels after 16 weeks (Figure 8). Values of $\delta^{13}\text{C}$ became significantly more negative at all depths, but especially at 2 m, and were least at 17 m (Tables 3 and 6).

After 16 weeks, the $\delta^{15}\text{N}$ values of *D. polymorpha* (Cuijk) at 17 m were similar to those recorded at the start of the experiment (Figure 8 and Table 5). The stable isotope ratios of *M. leucophaeata* (ZKC) did not change significantly during the course of the experiment (Figure 8 and Table 5). Its $\delta^{15}\text{N}$ values remained in a range that was clearly different from those of the two *Dreissena* species (Figure 8 and Table 5).

Figure 6. Mean condition index (CI) ± SE (n ≤ 50) after 3.5 and 16 weeks at various depths, for each mussel species (A – D). Statistical analysis excluded treatments with a sample size of 1. Different lower case letters above the bars indicate significant statistical differences.

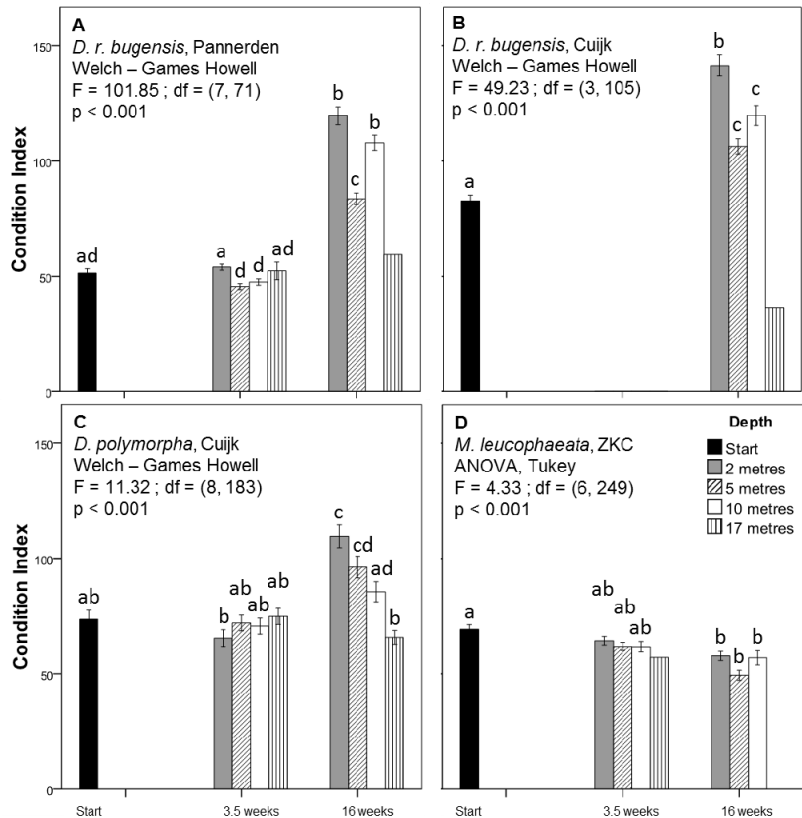


Figure 7. Mean relative condition index (RCI) ± SE (default of n = 50) of the tested mussels after 3.5 (A) and 16 weeks (B). RCI is related to the start value of a mussel species. If no error bars are displayed this indicates that only one mussel survived. Statistical comparisons were made between the three species within one treatment (retrieval * depth) and are displayed below the treatments. Different lower case letters above the bars indicate significant statistical differences. *D. rostriformis bugensis* collection sites are indicated as P (Pannderden) and C (Cuijk).

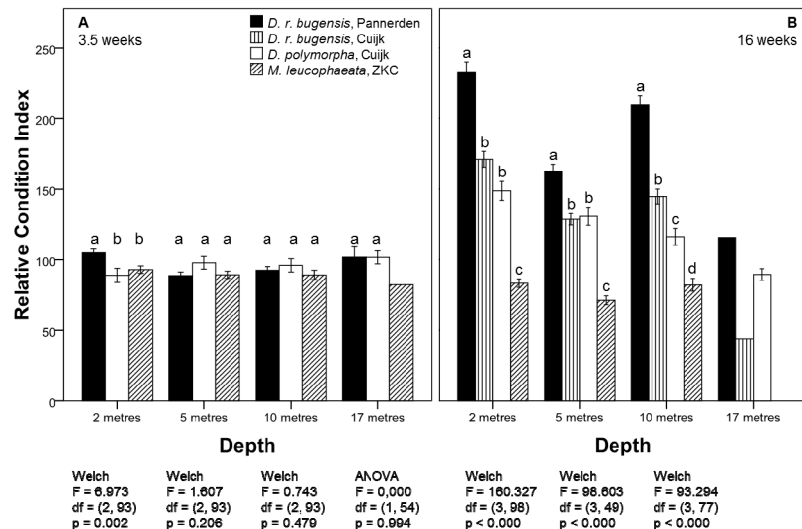


Table 4. Statistical analyses of differences in stable isotope ratios between species, within one ‘treatment’.

Time	Depth	$\delta^{15}\text{N}$				$\delta^{13}\text{C}$			
		Test	F	df	p	Test	F	df	P
Start	n/a	ANOVA	1263.970	3, 36	<0.001	ANOVA	11.605	3, 36	<0.001
	2 m	ANOVA	750.408	2, 27	<0.001	Welch	14.948	2, 13.684	<0.001
3.5 wk	5 m	ANOVA	863.069	2, 28	<0.001	Welch	24.965	2, 17.555	<0.001
	10 m	ANOVA	746.192	2, 26	<0.001	ANOVA	6.917	2, 26	0.004
	17 m	ANOVA	50.024	1, 14	<0.001	ANOVA	5.708	1, 14	0.032
	2 m	ANOVA	556.184	3, 35	<0.001	Welch	915.932	3, 16.798	<0.001
16 wk	5 m	ANOVA	202.955	3, 31	<0.001	ANOVA	37.734	3, 31	<0.001
	10 m	Welch	616.245	3, 19.496	<0.001	Welch	153.906	3, 20.230	<0.001
	17 m	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Table 5. Statistical differences in $\delta^{15}\text{N}$ isotope ratios between species (indicated by different capital letters), and between ‘treatments’ within a species (indicated by different lowercase letters in bold) tested with Tukey or Games-Howell post hoc.

Species	Start	3.5 weeks				16 weeks			
	(a)	2 m (a)	5 m (a)	10 m (a)	17 m (a)	2 m (a)	5 m (a)	10 m (a)	17 m (a)
<i>D. rostriformis bugensis</i> (Cuijk) (A)	B-a	n/a	n/a	n/a	n/a	B-b	B-b	B-b	n/a
<i>D. rostriformis bugensis</i> (Pannerden) (A)	C-a	B-b	B-c	B-bc	A-a	B-b	B-bc	B-b	n/a
<i>D. polymorpha</i> (Cuijk) (A)	B-ab	B-cd	B-bc	B-ab	B-a	B-d	B-d	B-d	ab
<i>M. leucophaeata</i> (ZKC) (A)	A-a	A-a	A-a	A-a	n/a	A-a	A-a	A-a	n/a

Table 6. Statistical differences in $\delta^{13}\text{C}$ stable isotope ratios between species (indicated by different capital letters), and between ‘treatments’ within a species (indicated by different lowercase letters in bold) tested with Tukey or Games-Howell post hoc.

Species	Start	3.5 weeks				16 weeks			
	(a)	2 m (a)	5 m (a)	10 m (a)	17 m (a)	2 m (a)	5 m (a)	10 m (a)	17 m (a)
<i>D. rostriformis bugensis</i> (Cuijk) (A)	B-a	n/a	n/a	n/a	n/a	B-b	BC-c	B-c	n/a
<i>D. rostriformis bugensis</i> (Pannerden) (A)	A-ab	A-a	A-b	A-ab	A-ab	B-c	B-d	C-cd	n/a
<i>D. polymorpha</i> (Cuijk) (A)	B-a	B-a	B-a	B-a	B-a	C-b	C-bc	B-c	d
<i>M. leucophaeata</i> (ZKC) (A)	A-a	A-a	A-a	AB-a	n/a	A-a	A-a	A-a	n/a

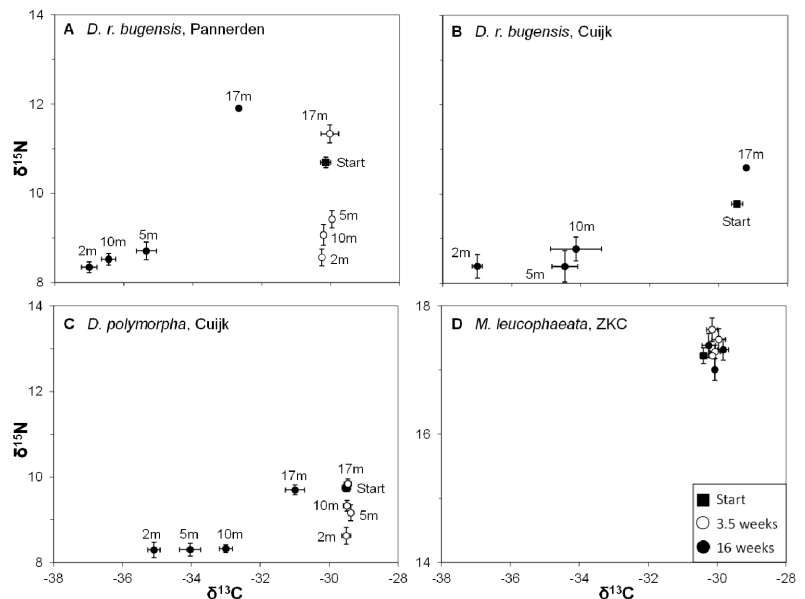
Table 7. Summary of the effects of depth on survival, condition and diet of three dreissenids after 16 weeks of exposure to different depths in a lake.

Performance indicator ↓	<i>D. polymorpha</i>	<i>D. rostriformis bugensis</i>	<i>M. leucophaeata</i>
Survival	High at 2-10 m, some decrease at 17 m	High at 2-10 m, almost none at 17 m	Decreased with time and depth
Condition	Increased with decreasing depth	Increased (more) with decreasing depth	Slight decrease over time
Diet	Decreased $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Largest shifts at shallow depth	Decreased $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Largest shifts at shallow depth	No shifts in $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$

At the start of the experiment, the two *Dreissena* species collected from Cuijk had similar stable isotope ratios. *D. rostriformis bugensis* (Pannerden) had significantly higher $\delta^{15}\text{N}$ values and more negative $\delta^{13}\text{C}$ values than specimens collected from Cuijk at the start of the experiment (Figure 8 and Tables 4, 5 and 6), but

this difference was not present after 16 weeks. The difference in $\delta^{13}\text{C}$ values between the two populations of *D. rostriformis bugensis* disappeared over a period of 16 weeks, particularly at 2 m. In addition, at 2 m depth, *D. rostriformis bugensis* (Cuijk) had significantly lower $\delta^{13}\text{C}$ values than *D. polymorpha* (Cuijk) (Figure 8 and Table 6).

Figure 8. Mean stable isotope ratios ($\text{‰} \pm \text{SE}$) for each mussel species (A. *Dreissena rostriformis bugensis* (Pannerden); B. *D. rostriformis bugensis* (Cuijk); C. *D. polymorpha* (Cuijk); D. *Mytilopsis leucophaeata* (ZKC)) prior to the experiment and following transplantation to various depths, measured after 3.5 and 16 weeks.



Discussion

Mytilopsis leucophaeata had the worst performance of the three tested species and was apparently not adapted to a prolonged exposure to this freshwater habitat. It had decreased survival both over time and with increasing depth, as well as a decrease in CI after 16 weeks, especially at increasing depth, with no shifts in stable isotope values (Table 7). Nevertheless, some specimens of this species were still alive after 16 weeks.

The survival of the two *Dreissena* species, in particular that of *D. rostriformis bugensis*, had already diminished 3.5 weeks after being transplanted to a depth of 17 m, compared to that of specimens held at 2 m. At depths ranging from 2 to 10 m, their survival did not decrease as drastically throughout the 16 week experiment. However, the increase in the CI values of both dreissenid species was negatively affected by depth, indicating that depth in general might negatively affect the fitness of these species in the long run, although not as drastically as occurred at 17 m. Thus, the shallow habitat of the lake appeared more suitable for these dreissenids in winter.

In comparison with the other tested species, *D. rostriformis bugensis* (Pannerden) showed the largest increase in RCI. This result could indicate that *D. rostriformis bugensis* (Pannerden) fed more effectively in the Groene Heuvels Lake or

that mussels from Pannerden were food-limited in their original habitat and thus performed better in the Groene Heuvels Lake, relative to their initial CI. Comparison of *D. rostriformis bugensis* and *D. polymorpha* from Cuijk revealed equal performances at 2 and 5 m. Apparently, the shallow waters of the Groene Heuvels Lake provided equally well for both species in terms of food.

On the whole, the two *Dreissena* species performed better at shallower depths in the lake, except for individuals of *D. rostriformis bugensis* which maintained better condition at 10 m than at 5 m. Since survival was apparently not lower at 5 m than at 10 m, and stable isotope ratios showed the same trends as the CI, the food source at these depths might have had an influence. The stable isotope data did not allow a conclusive answer to this supposition, however. This discrepancy in mussel condition along the depth gradient was not apparent for specimens of *D. polymorpha*, suggesting that under specific conditions, the two species do differ in their food assimilation ability. If this is the case, *D. polymorpha* could have a competitive advantage over *D. rostriformis bugensis*. Our research found differences in $\delta^{13}\text{C}$ values between the species, in contrast with the findings of Garton et al. (2005) for mussels collected from Lake Erie, USA. The more negative $\delta^{13}\text{C}$ values of *D. rostriformis bugensis* in our study could indicate a greater reliance on organisms like chemoautotrophs, as

they can display highly reduced $\delta^{13}\text{C}$ values (Doi et al. 2006). Carbon and nitrogen stable isotope signatures can also vary in time (Guzzo et al. 2011). Hence, the difference in $\delta^{13}\text{C}$ values between *D. rostriformis bugensis* and *D. polymorpha* in our study could also indicate a difference in adaptation time to new environments. This was corroborated by the finding that they did not initially differ in either nitrogen or carbon stable isotopes when collected from the same location, and thus seemed to have occupied the same food niche. However, Baldwin et al. (2002) did not find a difference in clearance rate between adults of *D. rostriformis bugensis* and *D. polymorpha* at 20°C. Their results suggest that *D. rostriformis bugensis* may grow better at naturally or filter feeder induced low food levels (Baldwin et al. 2002).

The condition indices of *D. rostriformis bugensis* and *D. polymorpha* collected from Cuijk differed at the start of the experiment (85 and 75, respectively). This could indicate either a species-specific difference in feeding efficiency in this lake or a minor difference in allometric proportions between the two species (for example a larger shell volume at equal shell dry weight). Stoeckmann (2003) found that *D. rostriformis bugensis* has a lower respiration rate and larger size than *D. polymorpha*. It also seemed to invest less in reproduction than *D. polymorpha* of similar size, so *D. polymorpha* could be a better 'r'-strategist and thus colonise new habitats first, followed by *D. rostriformis bugensis* as a slightly better competitor after the habitat has been changed slightly by *D. polymorpha* (Stoeckmann 2003).

D. rostriformis bugensis collected from a canal (Pannerden) differed significantly from those collected in a lake (Cuijk), in terms of both condition and N and C stable isotope ratios at the start of the experiments. This difference between the two populations disappeared during the experiment at 2 m depth after 16 weeks, indicating that these mussels were able to adapt to changes in food availability and habitat within a few months.

We found a large difference in survival at a depth of 17 m, at which adults of *D. polymorpha* performed much better than *D. rostriformis bugensis*. The exact mechanism underlying this outcome remains a puzzle and is worth further investigation, as several authors have reported that *D. rostriformis bugensis* displaces *D. polymorpha* in the field and especially with increasing depth (e.g. Martel et al. 2001; Mills et al. 1996;

Stoeckmann 2003). Factors contributing to the success of *D. rostriformis bugensis* could include differences in long-term growth and survival, throughout different seasons, the reproductive capacity of the two *Dreissena* species, or characteristics of the veligers (Ram et al. 2012; Karatayev et al. 2011; Martel et al. 2001). A deep water (*profunda*) form of *D. rostriformis bugensis* occurs in Russia (Orlova et al. 2005; Pavlova 2012) and in the Great Lakes of North America (Dermott and Munawar 1993). In our experiment we used *D. rostriformis bugensis* mussels collected from the littoral zone of a Dutch lake and a river canal so mussels were not likely the deep water morph.

MacIsaac (2004) found a high survival rate for both species in deep portions of Lake Erie. Our study reports differences in survival with increasing depth, which may be explained by a possibly better adaptation of *D. polymorpha* to hypoxia than *D. rostriformis bugensis*, conditions which could have been generated by the formation of ice on the lake (Alexander and McMahon 2004, McMahon and Johnson 1997). However, ice formation was never complete in our lake and ice was locally present only for a few weeks.

In summary, this study clearly showed different responses between dreissenid species as measured by survival, condition index values and stable isotope values at depths between 2 and 17 m. A different tolerance for hypoxic conditions and less suitable food at the greater depth may have caused these differences in species' response.

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