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A comparative study of byssogenesis on zebra and quagga mussels: the effects of water temperature, salinity and light–dark cycle

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The quagga mussel (*Dreissena rostriformis bugensis*) and zebra mussel (*Dreissena polymorpha*) are invasive freshwater bivalves in Europe and North America. The distribution range of both *Dreissena* species is still expanding and both species cause major biofouling and ecological effects, in particular when they invade new areas. In order to assess the effect of temperature, salinity and light on the initial byssogenesis of both species, 24 h re-attachment experiments in standing water were conducted. At a water temperature of 25°C and a salinity of 0.2 psu, the rate of byssogenesis of *D. polymorpha* was significantly higher than that of *D. rostriformis bugensis*. In addition, byssal thread production by the latter levelled out between 15°C and 25°C. The rate of byssogenesis at temperatures <25°C was similar for both species. Neither species produced any byssal threads at salinities of 4 psu or higher. At a salinity of 1 psu and a water temperature of 15°C, *D. polymorpha* produced significantly more byssal threads than *D. rostriformis bugensis*. There was no significant effect of the length of illumination on the byssogenesis of either species. Overall, *D. polymorpha* produced slightly more byssal threads than *D. rostriformis bugensis* at almost all experimental conditions in 24 h re-attachment experiments, but both species had essentially similar initial re-attachment abilities. The data imply that *D. rostriformis bugensis* causes biofouling problems identical to those of *D. polymorpha*.

Keywords: *Dreissena rostriformis bugensis*; *Dreissena polymorpha*; byssal thread production; re-attachment; biofouling; light period; salinity; water temperature

Introduction

The recent spread of the quagga mussel (*Dreissena rostriformis bugensis*) into Western Europe led to the co-occurrence of *D. rostriformis bugensis* and zebra mussel (*Dreissena polymorpha*) in Dutch inland waters (Van der Velde et al. 2010a). Such co-occurrence has already been studied in the Great Lakes of North America (eg Mills et al. 1996; Roe and MacIsaac 1997; Baldwin et al. 2002; Wilson et al. 2006; Nalepa et al. 2009) and Russia and the Ukraine for decades (eg Tzeyeb et al. 1966; Orlova et al. 2004, 2005).

D. rostriformis bugensis and *D. polymorpha* differ in the shape of their shells, with possible consequences for attachment and resistance to flow. The shell of *D. polymorpha* is flat to concave at the ventral side, while in *D. rostriformis bugensis*, the shell is tapering or convex at the ventral side (Claudi and Mackie 1994).

Both *Dreissena* species are invasive species, which can cause a decline in freshwater biodiversity, alter ecosystems by changing nutrient cycles and create expensive biofouling problems (Nalepa and Schloesser 1993; Claudi and Mackie 1994; Nalepa et al. 2009; Mackie and Claudi 2010; Van der Velde et al. 2010b). Spat and full-grown dreissenids can produce byssal threads secreted by the byssus gland in the foot of the mussel (Brazee and Carrington 2006). Both *Dreissena* species use byssal threads to attach themselves to a hard substratum such as rock, wood, other mussels, ships' hulls, hydraulic engineering structures and water intake facilities. Native adult freshwater unionids are incapable of byssogenesis and can be smothered to death by heavy colonization of *Dreissena* blocking their siphons (Ricciardi et al. 1995; Sousa et al. 2011).

Byssal threads can be distinguished as temporary and permanent, characterized by different types of plaque (Eckroat et al. 1993). Most threads are formed in the first week of attachment and there is no apparent preference for vertical or horizontal attachment

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surfaces (Eckroat et al. 1993). Besides initial settlement, secondary settlement can also take place in water intake pipelines, as young mussels in particular detach and reattach elsewhere by producing new byssal threads (Claudi and Mackie 1994). Byssal threads remaining after mussel detachment can still cause disturbance to laminar flow in pipelines (Mackie and Claudi 2010). The byssal thread plaques can cause adverse effects on iron and steel pipelines due to enhanced microbially induced corrosion by anaerobic processes (Mackie and Claudi 2010).

Ackerman et al. (1993) showed that after reattachment for 24 h, zebra mussels had a shear stress similar to that recorded after longer reattachment periods. In cooling water pipes, smaller mussels detach to move to more favourable sites since high flow velocities make them vulnerable to downstream transport, thus rapid byssal thread production is adaptive in this species. Byssogenesis is affected by environmental factors such as temperature and salinity. Other factors influencing byssogenesis include agitation, attachment, current velocity, oxygen tension and season (Clarke and McMahon 1996a, 1996b, 1996c; Rajagopal et al. 1996, 2005a). Byssogenesis has been previously studied in *D. polymorpha* (Clarke and McMahon 1996a, 1996b, 1996c; Rajagopal et al. 1996; Kobak 2001), but only one study involved both *D. polymorpha* and *D. rostriformis bugensis* (Peyer et al. 2009). In flowing water, *D. polymorpha* produced more byssal threads than *D. rostriformis bugensis*, which concurs with the observation that *D. polymorpha* is dominant in areas with a higher water velocity when both species co-occur (Peyer et al. 2009). Rajagopal et al. (1996) reported that *D. polymorpha* showed optimal byssogenesis at a temperature in the range of 15 to 20°C, while Clarke and McMahon (1996a) found that the rate of byssogenesis increased with increasing temperature up to the highest tested temperature of 30°C.

Byssogenesis of *D. polymorpha* ceased at salinities >4–7 psu during a 21-day exposure period (Rajagopal et al. 1996); data for *D. rostriformis bugensis* are unavailable. In addition, light conditions were also identified as a factor influencing byssogenesis for *D. polymorpha* (Kobak 2001), but no data are available for *D. rostriformis bugensis*. Mussel size, acclimation and differences in sampling season were found to account for variability in the rate of byssogenesis of *D. polymorpha* (Rajagopal et al. 2005a, 2005b).

The conditions that determine the relative ability of *D. rostriformis bugensis* and *D. polymorpha* to produce byssal threads are still unclear. The present study investigates the effect of temperature, salinity and light–dark cycle on byssogenesis of both *Dreissena* species in 24 h reattachment experiments in standing aerated water. The results provide insight into the possible biofouling threats of *D. rostriformis bugensis* and *D. polymorpha* under conditions similar to those investigated. The following hypotheses were tested: (1) *D. rostriformis bugensis* produces more byssal threads than *D. polymorpha* under similar temperatures; (2) byssogenesis of *D. rostriformis bugensis* and *D. polymorpha* is optimal at 15–30°C; (3) for both species increasing salinity leads to a decrease in byssogenesis; and (4) both species show a higher rate of byssogenesis under reduced duration of exposure to light.

Materials and methods

Sampling and storage

Individuals of both species were gathered from a depth of 1–2 metres in lake Kraayenbergse Plassen near Cuijk (coordinates 51° 44′ 50.9″N, 5° 50′ 54.8″E), the Netherlands. Simultaneously, water used in the experiments was collected from the field and stored in aerated tanks for a maximum of 3 weeks at 15°C. Only individuals with a shell length between 10.0 and 12.2 mm were used in the experiments (Table 1). The average (±SE) shell length of *D. rostriformis bugensis* was 11.3 ± 0.0 mm ($n = 200$; range 10.12–12.19 mm) while that of *D. polymorpha* was 11.2 ± 0.0 mm ($n = 200$; range 10.03–12.17 mm).

For each species, 150 to 200 individuals were acclimated in aerated tanks (90 × 39 × 8 cm; L × W × D) for at least 1 week. The mean water temperature and salinity in the acclimation tanks was 14°C (±0.04) and 0.2 psu, respectively. A YSI 30 multi-meter was used daily to measure water temperature and salinity.

During acclimation, mussels were not fed, but each week half of the water volume was refreshed with stored water from the lake Kraayenbergse Plassen. Only mussels that had attached to the acclimation tank were used in the experiments; all mussels were used within 3 weeks of collection. During storage and acclimation three lamps (Philips, HPI-T Plus 400 W), placed 1 m above the table, were used for illumination (12 h light:12 h dark).

Experimental design

In order to assess how temperature, salinity and light–dark cycle affect byssogenesis of *D. rostriformis bugensis* and *D. polymorpha*, several experiments were carried out in climate controlled rooms in the period of January to March 2010 (Tables 1, 2 and 3). Byssal thread production was tested at water temperatures within the ambient temperature range experienced in temperate areas (Therriault and Orlova 2010), viz. 5, 10, 15, 20 and 25°C (Tables 1 and 2). To test

Table 1. Shell length, experimental temperature and experimental salinity displayed as mean ± SE, measured between 9:00 am and 18:00 pm at the end of the experiments.

Experiment	Shell length (mm)	Experimental temperatures (°C)	Experimental salinities (psu)	Light condition (h/24 h)	Duration (h)	Replicates (#)
I Light						
1. *D. r. bugensis*	11.3 ± 0.1	15.1 ± 0.1	0.3 ± 0.0	0; 12; 24	24	10; 20; 10
2. *D. polymorpha*	11.1 ± 0.1	15.1 ± 0.1	0.3 ± 0.0	0; 12; 24	24	10; 20; 10
II Temperature						
1. *D. r. bugensis*	11.3 ± 0.1	4.7 ± n/a; 10.5 ± 0.1; 15.6 ± 0.1; 19.3 ± 0.1; 24.4 ± 0.1	0.3 ± 0.0	12	24	10; 10; 20; 10; 10
2. *D. polymorpha*	11.3 ± 0.1	5.1 ± 0.1; 10.5 ± 0.1; 15.7 ± 0.1; 19.3 ± 0.1; 24.4 ± 0.2	0.3 ± 0.0	12	24	10; 10; 20; 10; 10
III Salinity						
1. *D. r. bugensis*	11.2 ± 0.1	15.4 ± 0.0	0.3 ± 0.0; 1.1 ± 0.0; 2.1 ± 0.0; 4.1 ± 0.0; 6.2 ± 0.0; 9.3 ± 0.0; 12.4 ± 0.0	12	24	20; 20; 20; 10; 10; 10; 10
2. *D. polymorpha*	11.2 ± 0.1	15.5 ± 0.0	0.3 ± 0.0; 1.1 ± 0.0; 2.2 ± 0.0; 4.1 ± 0.0; 6.2 ± 0.0; 9.4 ± 0.0; 12.4 ± 0.0	12	24	20; 20; 20; 10; 10; 10; 10
IV Temperature × salinity						
1. *D. r. bugensis*	11.3 ± 0.1	4.6 ± 0.0; 15.6 ± 0.1; 24.3 ± 0.1	0.3 ± 0.0; 2.2 ± 0.0; 9.8 ± 0.2	12	24	10–20
2. *D. polymorpha*	11.2 ± 0.1	4.9 ± 0.1; 15.7 ± 0.1; 24.4 ± 0.1	0.3 ± 0.0; 2.2 ± 0.0; 9.8 ± 0.1	12	24	10–20

Note: The mean ± SE acclimation temperature was 14.0 ± 0.1°C and mean acclimation salinity was 0.2 psu.

Table 2. Overview of mean, SE and statistics of byssal thread production by *D. r. bugensis* and *D. polymorpha* in the light, temperature and salinity experiments.

	D. r. bugensis			*D. polymorpha*				
	Mean ± SE	Max	*n*	Mean ± SE	Max	*n*	F	*p*
Light (h)								
0	36.1 ± 4.8 (a)	59	9	34.4 ± 4.1 (a)	51	10	0.074_{ANOVA}	0.788
12	30.0 ± 4.2 (a)	63	20	32.7 ± 2.5 (a)	53	20	0.309_{WELCH}	0.582
24	28.1 ± 5.2 (a)	53	10	27.4 ± 3.6 (a)	44	10	0.012_{ANOVA}	0.913
F	0.574_{ANOVA}			1.017_{ANOVA}				
P	0.568			0.372				
Temperature (°C)								
5	10.9 ± 2.0 (a)	21	9	19.9 ± 4.9 (a)	44	9	2.925_{WELCH}	0.116
10	21.8 ± 4.3 (ab)	43	10	20.6 ± 4.8 (a)	48	10	0.034_{ANOVA}	0.855
15	30.0 ± 4.2 (b)	63	20	32.7 ± 2.5 (ab)	53	20	0.309_{WELCH}	0.582
20	16.8 ± 3.2 (ab)	29	8	23.4 ± 5.1 (a)	44	10	1.090_{ANOVA}	0.312
25	29.2 ± 4.3 (b)	54	10	44.6 ± 3.8 (b)	65	10	7.196_{ANOVA}	0.015*
F	6.402_{WELCH}			6.183_{ANOVA}				
P	0.001*			< 0.001*				
Salinity (psu)								
0.2	30.0 ± 4.2 (a)	63	20	32.7 ± 2.5 (ab)	53	20	0.309_{WELCH}	0.582
1	14.0 ± 2.6 (ab)	45	19	32.7 ± 2.3 (a)	52	19	28.799_{ANOVA}	< 0.001*
2	14.5 ± 2.4 (b)	35	18	22.6 ± 3.3 (b)	49	20	3.713_{ANOVA}	0.062
4	0	0	10	0	0	10		
6	0	0	10	0	0	10		
9	0	0	10	0	0	10		
12	0	0	10	0	0	10		
F	8.019_{WELCH}			4.498_{ANOVA}				
P	0.001*			0.015*				

Note: ANOVA or Welch tests were used to test for differences between (to right of data) or within species. *statistically significant. Letters between brackets are used to indicate significant results between treatments within a species.

Table 3. Overview of mean, SE and statistics of byssal thread production by *D. r. bugensis* and *D. polymorpha* in the combined temperature–salinity experiments.

D. r. bugensis

Salinity (psu) →	0.2			2			9			Within temperature 0.2–2 psu	
Temperature (°C) ↓	Mean ± SE	Max	*n*	Mean ± SE	Max	*n*	Mean ± SE	Max	*n*	F	*p*
5	12.8 ± 2.6	30	10	2.8 ± 1.3	13	10	0	0	10	17.963_{ANOVA}	0.001*
15	30.0 ± 4.2	63	20	19.1 ± 3.8	60	20	0	0	10	20.056_{WELCH}	< 0.001*
25	29.2 ± 4.3	54	10	26.0 ± 3.6	42	10	0	0	10	0.321_{ANOVA}	0.578

Within salinity	F_{WELCH}	*p*	F_{WELCH}	*p*
	19.631	< 0.001*	30.904	< 0.001*

D. polymorpha

Salinity (psu) →	0.2			2			9			Within temperature 0.2–2 psu	
Temperature (°C) ↓	Mean ± SE	Max.	*n*	Mean ± SE	Max	*n*	Mean ± SE	Max.	*n*	F	*p*
5	24.4 ± 6.3	65	10	5.0 ± 2.5	20	10	0	0	10	13.764_{WELCH}	0.005*
15	32.7 ± 2.5	53	20	22.6 ± 3.3	49	20	0	0	10	6.857_{ANOVA}	0.011*
25	44.6 ± 3.8	65	10	40.3 ± 6.3	75	10	0	0	10	0.001_{ANOVA}	0.977

Within salinity	F_{ANOVA}	*p*	F_{WELCH}	*p*
	9.850	< 0.001*	50.132	< 0.001*

Note: *Statistically significant.

byssogenesis at various salinities, water of the desired salinity was prepared by adding seasalt (Tropic Marin, Wartenberg, Germany) to water collected from the lake Kraayenbergse Plassen. Water with salinities of 0.2, 1, 2, 4, 6, 9 and 12 psu was used to examine byssogenesis of the dreissenids over a period of 24 h. These salinities cover the gradient from freshwater to brackish water (Tables 1 and 2). For the light experiments, three lamps (Philips, HPI-T Plus 400 W, one per 30 beakers placed 1 m above the table) were used. The effect of 24 h darkness, 12 h light:12 h dark and 24 h light on byssogenesis was tested.

To assess the combined effect of salinity and temperature on byssogenesis all possible combinations of water temperature (5, 15 and 25°C) and salinity (0.2, 2 and 9 psu) were used (Tables 1 and 3). A YSI 30 multi-meter was used to set the correct salinity at the start of each experiment. The salinity and water temperature were also measured at the end of each experiment (Table 1).

Prior to the experiments, mussels were detached from the acclimation tanks by gently cutting their byssal threads with a sharp scalpel. Any remaining byssal threads were removed from a mussel using scissors to prevent damaging the byssal gland. Each mussel was subsequently placed in a separate beaker (600 or 1000 ml SCOTT DURAN) containing 500 ml of water at the experimental temperature and salinity. A total of 90 beakers was used for the experiment, with ten replicates per tested condition. Twenty replicates were used for the experiments with a temperature of 15°C and a salinity of 0.2, 1 or 2 psu (Table 1). The beakers were aerated with an air stone placed above the mussel to maintain full oxygen saturation. The pH range observed during the experiments was 7.7–8.4. The number of byssal attachment threads produced in 24 h by each individual was observed with a magnifying glass (10×).

Statistics

Before any statistical analysis, any outliers (>1.5 times interquartile range) were identified and removed using SPSS 17. Data were tested per group for normality with a Shapiro-Wilk test and Q-Q plot. The data were then tested for homogeneity of variances with the Levene's test. If the Levene's test failed ($p < 0.05$), a Welch test with *post hoc* Games-Howell was used. For analysis, depending on the data, one-way ANOVA or Welch analysis was performed (statistical significant difference assumed at $p < 0.05$). For the salinity × temperature experiment, a Scheirer-Ray-Hare (SRH) test was performed, because data failed the assumptions for normal distribution (Q-Q-plot) or homogeneity of variances (Levene's test, $p < 0.05$) of a two-way ANOVA. The SRH test is more conservative than a two-way ANOVA (Dytham 2003).

Results

Light duration

In all three experimental conditions (24 h darkness, 12 h light:12 h dark and 24 h light) every mussel of both species produced byssal threads. No significant differences were found for the rate of byssogenesis of *D. polymorpha* and *D. rostriformis bugensis* under various light regimes (Table 2).

Temperature

Overall 80–100% of the individuals of both species produced byssal threads. *D. polymorpha* produced significantly more threads at 25°C than at lower temperatures although at temperatures below 25°C, the byssal thread production was not significantly different. *D. rostriformis bugensis* produced significantly more threads at 15°C and 25°C than at 5°C, but its rate of byssogenesis remained relatively constant with no significant differences from 10 to 25°C (Table 2). Only at 25°C did specimens of *D. polymorpha* produce significantly more byssal threads than those of *D. rostriformis bugensis* (Table 2).

Salinity

The percentage of individuals of *D. rostriformis bugensis* and *D. polymorpha* that produced at least one byssal thread was 90% and 80%, respectively, at a salinity of 2 psu, while neither species produced any byssal thread at a salinity of 4 psu. There was a significant decline in the rate of byssogenesis between the salinities 0.2 and 2 psu for both species. Only at a salinity of 1 psu was a significant difference in the rate of byssogenesis recorded between the two species; *D. polymorpha* produced more than twice as many threads as *D. rostriformis bugensis* (Table 2).

Combined effect of salinity and temperature

Byssal thread production of both species was tested at nine combinations of salinity and temperature (Figure 1; Table 3). At temperatures of 5 and 15°C, both *D. rostriformis bugensis* and *D. polymorpha* produced significantly more byssal threads at a salinity of 0.2 psu than at 2 psu, but at 25°C this difference was no longer significant for either species (Figure 1). The difference in mean thread production between 0.2 psu and 2 psu decreased for both species with rising temperature to low levels at 25°C. At a salinity of 9

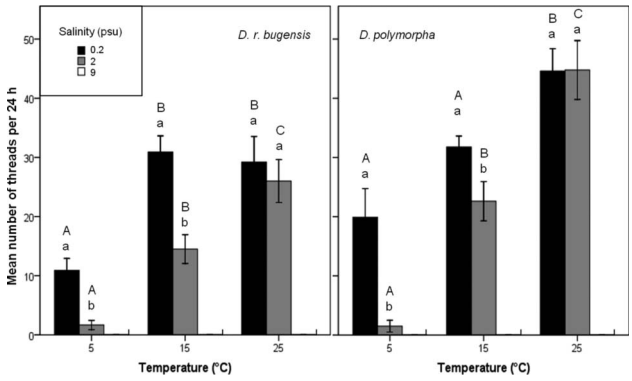

Figure 1. Mean number of byssal threads (±SE) produced by *D. r. bugensis* and *D. polymorpha* under various salinities and temperatures within 24 h. Lowercase letters (a) represent significant differences in byssal thread production between salinity levels within each temperature treatment. Uppercase letters (A) represent significant differences in byssal thread production between temperatures within each salinity treatment. For statistics see Table 3. No significant difference was found between the two species except at a salinity of 0.2 psu and 25°C (ANOVA, $p = 0.015$). No byssus thread production was observed at a salinity of 9 psu.

psu, neither species produced any threads, irrespective of the temperature.

At a salinity of 0.2 psu, *D. rostriformis bugensis* produced significantly more threads at 15°C than at 5°C, but no significant difference was found in the byssal thread production between 15°C and 25°C.

D. polymorpha produced significantly more threads at 25°C than at 15°C. At a salinity of 2 psu, *D. polymorpha* produced significantly more threads with increasing temperature (Figure 1; Table 3). A similar trend was also seen for *D. rostriformis bugensis* at this temperature. More threads were produced with increasing temperature (Table 3).

According to the SRH test, only salinity caused a significant difference in mean thread production within 24 h. There was no significant combined effect of temperature and salinity (Table 4).

Table 4. Output of the non-parametric SRH test for the combined temperature–salinity experiments.

	SS	SS/MS_{total}	d.f.	*P*-value
D. r. bugensis				
Temperature (factor)	$1.09 \cdot 10^4$	$2.69 \cdot 10^6$	2	0.260
Salinity (factor)	$5.38 \cdot 10^7$	$1.33 \cdot 10^6$	2	0.001*
Temperature × salinity (interaction)	$6.68 \cdot 10^6$	$1.65 \cdot 10^6$	4	0.800
D. polymorpha				
Temperature (factor)	$1.13 \cdot 10^4$	$2.80 \cdot 10^6$	2	0.246
Salinity (factor)	$5.03 \cdot 10^4$	$1.25 \cdot 10^6$	2	0.002*
Temperature × salinity (interaction)	$6.69 \cdot 10^6$	$1.66 \cdot 10^6$	4	0.798

Note: *Statistically significant.

Discussion and conclusions

Differences in byssal thread production in various studies can be caused by the size of the mussel, its physiological condition and by different experimental environments. Peyer et al. (2009) found similar mean numbers of byssal threads produced by zebra as well as quagga mussels in 24 h attachment assays using mussels with a shell length of 5–10 mm. In the present experiments, mussels of both species were selected with a shell length of 10–12 mm. Clarke and McMahon (1996a, 1996b, 1996c) used larger zebra mussels (mean 16–25 mm) and found lower byssal thread formation in 24 h attachment assays compared to the data contained in this study. Rajagopal et al. (1996) used zebra mussels with a shell length of 20–22 mm and also found a lower byssal thread production during a 24 h re-attachment period. Similar data were found by Eckroat et al. (1993) for zebra mussels with a shell length of mostly 21–26 mm. Although a size dependent relationship seems likely, experiments need to be conducted in a more comparable way.

In the natural habitat of *Dreissena* mussels, darkness may correlate with safer conditions. In dark crevices and on the underside of rough substrata such as stones, these species are better protected against predators, desiccation and dislodgement following wave action or currents (Kobak 2001). It was found that individuals of *D. polymorpha* move away from light and settle preferentially in dark places (Kobak 2001). The present experimental results showed 100% reattachment of both *D. polymorpha* and *D. rostriformis* within 24 h, independent of light duration. The rate of byssogenesis tended to increase slightly with decreasing light duration, but this trend was not significant. This means that the rate of byssogenesis in dark pipelines is likely to be similar to that in light conditions.

Temperature influences performance and dispersal of *Dreissena* species. Observations in the Great Lakes showed that *D. rostriformis bugensis* reached high densities and displaced *D. polymorpha* in the deeper and colder parts of the lakes (Mitchell et al. 1996; Nalepa et al. 2009). Several experiments showed that the rate of byssogenesis of *D. polymorpha* increased with temperature (5–30°C) (Clarke and McMahon 1996a) or was optimal between 10–20°C (Rajagopal et al. 1996). The temperature tolerance of *D. rostriformis bugensis* may be lower (25°C) than that of *D. polymorpha* (30°C) (Spidle et al. 1995), while other studies have reported an equal tolerance of 34°C (Therriault and Orlova 2010; Verbrugge et al. 2012).

In the experiments reported here, both *D. polymorpha* and *D. rostriformis bugensis* produced the least number of byssal threads at low temperatures, namely at 5°C. *D. polymorpha* had the highest rate of byssogenesis at the highest temperature tested ie 25°C. Clarke and McMahon (1996a) found a steady increase in the rate of byssogenesis in *D. polymorpha* up to the highest temperature tested (30°C), while Rajagopal et al. (1996) reported a decline in byssogenesis rate in this species above 20°C. In the present experiments, *D. rostriformis bugensis* produced a similar number of threads at 15 to 25°C. However, at 25°C the byssogenesis rate of *D. polymorpha* was significantly higher than that of *D. rostriformis bugensis*.

Salinity is another major factor, which determines where a species can establish and maintain populations. Both *Dreissena* species used in this experiment are freshwater species, although *D. polymorpha* has also been encountered in oligohaline brackish water (Strayer and Smith 1993). These species seem to have approximately the same tolerance for salinity. Verbrugge et al. (2012) summarized data from field studies and reported a maximum of 6 psu for *D. polymorpha* and 5 psu for *D. rostriformis bugensis*. Both species showed 100% mortality after exposure for 18 days to a salinity of 5 psu or higher (Spidle et al. 1995).

In the experiments reported here, both *D. polymorpha* and *D. rostriformis bugensis* had significantly lower rates of byssogenesis at higher salinities, with byssogenesis completely inhibited at salinities ≥ 4 psu. *D. polymorpha* produced significantly more threads within 24 h than *D. rostriformis bugensis* at a salinity of 1 psu. At a salinity of 4 psu neither species produced threads. Rajagopal et al. (1996) found that over a period of 24 h, byssogenesis ceased in specimens of *D. polymorpha* exposed to salinities between 4–7 psu. Using salinity tolerance to predict the effects of salinity on establishment/biofouling of dreissenid mussels is difficult due to large fluctuations in the salinity of river estuaries caused by tidal influences. Studies on the combined effect of salinity and temperature on byssogenesis are rarely performed (eg Rajagopal et al. 1996), let alone performed with multiple mussel species. In estuarine areas, gradients of temperature and salinity change simultaneously (Paalvast and Van der Velde 2011a, 2011b).

A Scheirer-Ray-Hare test did not show significant combination effects of salinity and temperature on the byssogenesis of either *D. polymorpha* or *D. rostriformis bugensis*, but some trends were recorded. Firstly, high temperatures seemed to mitigate the effects of an increase in salinity from 0.2 to 2 psu, resulting in no substantial decrease in the rate of byssogenesis. Secondly, the combined effect of low temperatures and increased salinity lead to a decrease in the rate of byssogenesis of both species. These results provide valuable information with respect to controlling biofouling, since the data suggest that controlling a combination of external factors is more effective compared to one factor. Further research on stacked treatments could produce valuable insights and methods to remedy biofouling.

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