

# Clearance rates of freshwater bivalves Corbicula fluminea and Utterbackia imbecillis in the presence and absence of light

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24					

### 25 Abstract

Light affects bivalve behavior and gaping activity, but its potential effects on bivalve filtration remain 26 poorly investigated. In this study, clearance rates of two bivalve species: the Asian clam, Corbicula 27 *fluminea* and paper pondshell, *Utterbackia imbecillis* were assessed in light ( $6.5 \pm 0.5 \mu$ mol m<sup>-2</sup> s<sup>-1</sup>) and 28 dark (0.0 µmol m<sup>-2</sup> s<sup>-1</sup>) conditions. We found that clearance rates remained similar between C. fluminea (50 29  $\pm$  18 mL g<sup>-1</sup> h<sup>-1</sup>) and U. *imbecillis* (41  $\pm$  10 mL g<sup>-1</sup> h<sup>-1</sup>) during light conditions. However, C. fluminea (110 30  $\pm$  15 mL g<sup>-1</sup> h<sup>-1</sup>) filtered significantly faster than U. *imbecillis* (24  $\pm$  6 mL g<sup>-1</sup> h<sup>-1</sup>) in the dark condition (p < 31 32 0.05). The results indicated that light might affect the filtration activity in freshwater bivalves differently, 33 depending on species.

- 34
- 35 Keywords: Dark, filtration rate, light, mussel, Unionidae

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### 46 **Conflict of Interest**

- 47 Authors declare no conflict of interest.
- 48

### 49 Availability of data and materials

50 Data will be available upon request.

51

#### 52 Authors' contributions

53 ADH planned and conducted experiments, supervised by TJM. DH helped provide U. imbecillis. ADH

and SP analysed data, conducted statistical analysis, and wrote the paper. DH and TJM helped revise

55 writing.

#### 56 Introduction

North America has the highest diversity of freshwater mussel species in the world, but over the past several 57 58 decades these animals have become among the most threatened group of organisms globally with over 70% 59 of species vulnerable to extinction or already extinct (Vaughn & Taylor 1999; Haag & Williams 2014). 60 Bivalves are filter feeders and play important roles in freshwater ecosystems through biogeochemical 61 cycling of nutrients and contaminants (Geist 2010; Haag 2012). Therefore, they are increasingly used as 62 sentinels of water quality in ecological remediation of contaminated aquatic systems (Rosa et al. 2014; Otter et al. 2015). Studies on filtration rates (FRs) in freshwater bivalves under different environmental 63 64 conditions are needed to assess their ecological impact and potential use in bioremediation.

Current knowledge of the FRs of freshwater bivalve species in North America is based on a limited number of species. While a considerable amount of data has been published on FRs of exotic *C*. *fluminea* and *Dreissena* species (Marescaux et al. 2016; Pigneur et al. 2014; Rosa et al. 2014; Viergutz et al. 2012), much less attention has been paid to native freshwater mussels, particularly Unionid species. Approximately only 8 species of freshwater mussels have been studied for FR assessment in laboratory experiments in North and South America (Table 1).

71 negatively impacted native bivalve abundance *C. fluminea* has and diversity in North 72 American freshwater ecosystems (Ferreira-Rodriguez et al. 2018). The FR of C. fluminea is often reported to explain their ability to compete with other bivalve species (Pigneur et al. 2014). Several studies, 73 74 conducted mostly in the laboratory, have reported a wide range of individual FRs for C. fluminea, 75 between 29 and  $< 3000 \text{ mL ind}^{-1} \text{ h}^{-1}$  (Viergutz et al. 2012) while FRs in Unionidae usually ranges between 18 and 2000 mL ind<sup>-1</sup> h<sup>-1</sup> although FRs up to <10,000 mL mL ind<sup>-1</sup> h<sup>-1</sup> were measured in 76 77 large individuals (up to 259 g, Table 1). Nevertheless, very few studies assessed the FRs of the different 78 species at the same time while using the same protocol, making it difficult to compare experimental data 79 (Marescaux et al. 2016).

Laboratory studies have highlighted that FRs in freshwater mussels vary depending on biotic and abiotic
factors. These include food availability, sex and reproductive cycle (Tankersley 1996; Gatenby et al.

2013), pH, temperature, turbidity and water velocity (Loayza-Muro and Elias-Letts 2007; Mistry and
Ackerman 2018; Tuttle- Raycraft and Ackerman 2019). Gatenby et al. (2013) found that *Villosa iris*adjusted FRs depending on the availability of food. More recently, Mistry and Ackerman (2018)
experimentally assessed FRs in four Unionidae species and found that FR increased with water velocity and
algae flux up to a saturation level.

Field studies have revealed seasonal changes in FRs in several bivalve species. Viergutz et al. (2012) found that seasonal variability of FR in *C. fluminea* surpasses direct temperature effects. These findings demonstrate that other factors besides temperature and body mass can strongly affect FRs. Additionally, light is also known to affect the behavior of bivalves involving closure or retraction of siphon and valve adduction (Wilkens 2008). Morton (2008) reported that photoreceptors in bivalves are not uncommon. However, no experimental study has been conducted to assess the effects of light intensity on the FRs of freshwater bivalves.

94 In this study, we aimed to determine the FRs of an invasive species *C. fluminea* and a poorly studied native 95 species *U. imbecillis* under controlled light conditions. Filtration activity was assessed through the 96 determination of clearance rates of the studied species (i.e. volume of water cleared of suspended particles 97 per unit of time (Riisgård 2001).

98

### 100 Bivalves and algae culture

Adult *C. fluminea* and *U. imbecillis* were used to compare FRs in native and invasive species. All the bivalves were collected in the field in July 2018. *C. fluminea* (weight:  $3.50 \pm 0.35$  g wet wt, shell length:  $20.2 \pm 0.9$  mm) were collected from Sewee Creek in Meigs county, TN, USA while *U. imbecillis* (weight:  $21.2 \pm 8.2$  g wet wt, shell length:  $62.8 \pm 7.6$  mm) were collected by Tennessee Wildlife Resources Agency (TWRA) from Sumner Sportsman Club Lake in Portland, TN, USA and brought to the laboratory in a cooler with a bubbler. No mortality was recorded during transportation. 107 The bivalves were kept in a 450-L tank (flow-through circuit of dechlorinated tap water, water renewal: 50-108 170 L h<sup>-1</sup>; ambient temperature: 14-28 °C; light intensity: 13-24  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>; light/dark: 14h/10h) and 109 acclimated to laboratory conditions for four weeks prior to the experiment. Two weeks prior to each test, 110 bivalves were fed a daily ration of a mix of the chlorophyte *Chlamydomonas reinhardtii* and the diatom 111 *Navicula sp.* (3 - 5 x 10<sup>6</sup> cells g<sup>-1</sup> d<sup>-1</sup>) using medical IV drip bags for continuous feeding.

112 The unicellular green algae, *C. reinhardtii* (5-6  $\mu$ m of diameter) was reported to be efficiently filtered by 113 both clams and mussels (Boltovskoy et al. 1995; Gatenby et al. 1996) and was used in this clearance rate 114 experiments. *C. reinhardtii* was cultured in 10-L clear Nalgene carboys containing WC medium 115 (temperature: 23-24°C; light intensity: 150-200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>; light/dark: 12h/12h). Algal concentrations 116 were evaluated by counting cells using a flow imaging cytometer (FlowCam® Benchtop B3 Model).

117

#### 118 Experimental procedures

119 Clearance rates of each species were determined following the methods of Riisgård (2001). Individuals of 120 both species were placed in separate clear plastic containers with dechlorinated tap water (100 mL in round 121 punch cups for *C. fluminea* and 500 mL in Lee's specimen containers for *U. imbecillis*) with aeration. One 122 container was kept with no bivalve and used as a control. Preliminary tests were carried out and confirmed 123 that there was no significant difference in cell concentrations between control containers (without bivalves, 124 n = 3), so we used only one control container for each of the experiments. Bivalves were allowed to 125 acclimate until active filtering was observed (approx. 30 min).

After the acclimation period, live *C. reinhardtii* was centrifuged (5000 RPM for 5 min) and spiked to each plastic container to reach the targeted initial concentrations. The use of concentrated algae allowed for the reduction of spike volume (approximately 3 mL) and to avoid significant changes of water volume and physio-chemical parameters. Subsamples of 1 mL water were taken from each plastic container five minutes after the algae spike and then at 5 to 15 min intervals (based on visual color changes in the plastic containers). The filtration was assessed for up to 1 h. The same sampling procedures were conducted for the experiments in the dark condition where no visual observation was possible. Samples were placed in
1.5-mL tubes with 200 µL of 10% formalin solution and mixed immediately. Analyses of the samples were
performed using by flow imaging cytometry (FlowCam® Benchtop B3 Model).

135 The experiments were performed for both species in the dark (0.0  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) with the initial cell concentrations of C. reinhardtii  $(2.5 \pm 0.2 \text{ x } 10^5 \text{ cells mL}^{-1})$  and in the light  $(6.5 \pm 0.5 \text{ } \mu\text{mol } \text{m}^{-2} \text{ s}^{-1})$  with the 136 initial cell concentrations of C. reinhardtii ( $4.0 \pm 0.2 \times 10^5$  cells mL<sup>-1</sup>). The sample sizes, the number of 137 138 individuals tested per treatment, (n = 7 in dark, 8 in light for C. fluminea and n = 6 in dark, 8 in light for U.139 *imbecillis*) followed the recommendations from (Salerno et al. 2018). Light intensity was measured using 140 a PAR meter (Quantum Flux<sup>®</sup> Apogée). Temperature was maintained at  $22 \pm 0.5$  °C during the experiment. 141 Dark experiments were done in the evening, after the daily acclimated dark time began. Likewise, light 142 experiments were done during the day according to the acclamation period light pattern.

Algae concentrations in a similar range showed no influence on growth and survival of juvenile mussels of *V. iris*  $(3.5 \times 10^4 - 1.8 \times 10^5 \text{ cell mL}^{-1})$  (Hua et al. 2013). However, Gatenby et al. (2013) found that high and low food concentration can affect FR of adult *V. iris*. So in the present study, since cell concentrations of *C. reinhardtii* differed for the experiments performed in light and dark conditions, the effects of light and dark condition on FRs within the same species were not evaluated statistically. Only the FRs between species within the same light condition (light and dark) were statistically tested.

149

#### 150 Data analysis

151 Cell concentrations (cells mL<sup>-1</sup>) were analyzed in time (minutes) series. Kinetics were fitted with an 152 exponential model and linearized by log transformation. Only regressions with  $R^2 \ge 0.85$  were considered 153 for clearance rate calculations (Hansen et al. 2011).

154 Clearance rates (expressed as mL  $g^{-1} h^{-1}$ ) were calculated according to the following equation (Mistry and 155 Ackerman 2018):

156 
$$CR = \frac{V}{nt} \left( ln \frac{C_i}{C_f} - ln \frac{C'_i}{C'_f} \right)$$

Where *V* is the volume of water in the plastic container (mL), n is the number of individual per container, *t* is the time of the experiment (h),  $C_i$  and  $C_f$  are initial and final cell concentrations respectively in the container with bivalves while, and  $C'_I$  and  $C'_f$  are initial and final cell concentrations respectively in the containers used as controls (with no bivalve). The result from this equation was then divided by the total wet weight (g) of the corresponding individual to normalize results from these two differently sized species. Kinetics of FRs were fitted using nonlinear regression routines and iterative adjustment. Nonlinear regressions and statistics were performed in R freeware 3.5.2 (R Development Core Team 2020).

164 Data for clearance rates were first tested for normality (Shapiro's test). A Student's t-test was then used to 165 assess significance of differences between the two species ( $\alpha = 0.05$ ).

166

#### 167 Results and Discussion

We found that *C. fluminea* filtered significantly faster ( $110 \pm 15 \text{ mL g}^{-1} \text{ h}^{-1}$ , n = 7) than *U. imbecillis* ( $24 \pm 6 \text{ mL g}^{-1} \text{ h}^{-1}$ , n = 6) in the dark condition (t = 12.64, p < 0.001). During light conditions, no significant difference was found in clearance rates in *C. fluminea and U. imbecillis* (t = 1.27, p = 0.23) although the average clearance rates were slightly higher in the *C. fluminea* ( $50 \pm 18 \text{ mL g}^{-1} \text{ h}^{-1}$ , n = 8) than those in the *U. imbecillis* ( $41 \pm 10 \text{ mL g}^{-1} \text{ h}^{-1}$ , n = 8) (Fig. 1).

173 This noticeable difference of FRs in the dark was also revealed in the filtration kinetics of the two species (Fig. 2). The cell concentration of C. reinhardtii decreased rapidly in the cups with C. fluminea, while it 174 175 did not show the same pattern in containers with U. imbecillis in the dark condition. During the first 20 176 minutes, C. fluminea filtered more than 50% of algal cells while U. imbecillis only filtered less than 25%. At the end of the filtration period in the dark, only  $4 \pm 2\%$  of C. reinhardtii cells remained in the cups with 177 178 C. fluminea while  $36 \pm 8\%$  of C. reinhardtii cells remained in the cups with U. imbecillis. Filtration patterns 179 in the light remained very similar between the two species. The results indicated that C. fluminea had much 180 higher FRs (over 4.5 times) than U. imbecillis during the dark condition in this study. This finding suggests that the FRs of the invasive species C. fluminea may be advantageous at night compared to native species. 181

182 FRs of C. fluminea in this study agree with previous measurements conducted in laboratory settings (78-183 486 mL ind<sup>-1</sup> h<sup>-1</sup>) (see Marescaux et al. 2016 for review). The FRs in U. imbecillis (360-1169 mL ind<sup>-1</sup> h<sup>-1</sup>) 184 are close to those in other Unionidae species (Table 1), (Mistry and Ackerman 2018; Tuttle-Raycraft and 185 Ackerman 2018, 2019). There are only a few studies on freshwater bivalve filtration regarding the influence 186 of light, most of which focus on invasive species. Ortmann and Grieshaber (2003) used gaping activity in 187 C. fluminea to estimate filtration activity in the field and reported a circadian rhythm with valves being 188 closed in the morning and opened in the afternoon. They assumed this phenomenon was due to the food 189 availability. The invasive golden mussel Limnoperna fortune, voluntarily detached its byssal threads, 190 crawled, and reattached more often in darkness than in light (Duchini et al. 2015) while invasive zebra 191 mussel Dreissena polymorpha and quagga mussel D. rostriformis bugensis showed no preference between 192 light regimes in byssal thread production (Grutters et al. 2012). Kobak and Nowacki (2007) found that D. 193 polymorpha tried to avoid any light. Altogether, these contrasting results indicate that response to light in 194 FRs in bivalves is species-dependent.

195 Limited investigations have been performed to study light effects in Unionids. Nevertheless, Haag and 196 Warren (2000) studied mantle lure displays as an endpoint for the combined effects of light and fish 197 presence in Villosa nebulosa and V. vibex. They found that presence or absence of fish had no effect on 198 display, but V. nebulosi displayed mainly at night while V. vibex displayed mainly during daytime 199 suggesting that behavior of Unionids species may be affected by light. In our study, C. fluminea exhibited 200 higher FRs than the Unionid U. imbecillis in the dark. This exotic species was already known for its high 201 FRs as well as for its ability to feed on smaller particles than most native freshwater bivalves such as 202 Unionidae (Marescaux et al. 2016 and citations within). The two species in this study can occupy the same 203 habitat and burrowing depth. While our organisms did not originate from the same location and competition 204 was not analyzed in our study, food competition would be possible in their natural habitat. Our study could 205 provide preliminary data for light conditions under which competition might occur.

This study provides new insights regarding the influence of light on the filtration activity of two bivalve species, *C. fluminea* and *U. imbecillis*, highlighting the complexity of bivalve behavioral response to light and suggests differing niche capabilities between them.

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307

	Shall langth	Whole body	Filtration rates	
Species	(mm)	wet weight (g)	$(mL ind^{-1} h^{-1})$	Reference
Actinonaias ligamentina		54 159	2700 11000*	(Baker & Hornbach 2001)
Actinonalas ligamentina	-	54-159	2700-11000*+	(Baker & Hornbach, 2001)
Amblema plicata	-	78-259	800-5000*+	(Baker and Hornbach 2001)
Anodontites trapesialis	90-100	-	700-1000	(Loayza-Muro and Elias-Letts 2007)
	$67\pm101$	-	500-2000*	(Mistry and Ackerman 2018)
Lampsilis fasciola	51-79	-	250-650*	(Tuttle-Raycraft and Ackerman 2018)
	0.3-0.4	-	< 1**	(Mistry & Ackerman 2017)
	$112 \pm 83$	-	600-2000*	(Mistry and Ackerman 2018)
Lampsilis siliauoidea	95-123	-	400-900*	(Tuttle- Raycraft and Ackerman 2018)
	80-110	-	300-1200*	(Tuttle-Raycraft and Ackerman 2019)
	0.3-0.4	-	< 3**	(Mistry and Ackerman 2017)
	$58\pm 62$	-	200-500*	(Mistry and Ackerman 2018)
Ligumia -suta	50-70	-	150-300*	(Tuttle-Raycraft and Ackerman 2018)
	0.2	-	< 1**	(Mistry and Ackerman 2017)
Due an e den e stansets	-11.5-15.5 -		500-2000*	(Tankersley 1996)
Pyganoaon cataracia		-		(Tankersley & Dimock 1993)
	37-51	-	18-55*	(Gatenby et al. 2013)
Villogg inis	$60 \pm 45$	-	250-400*	(Mistry and Ackerman 2018)
viiiosa iris	53-68	-	150-200*	(Tuttle-Raycraft and Ackerman 2018)
	0.4	-	< 2**	(Mistry and Ackerman 2017)

#### Table 1 Filtration rates of freshwater mussels from North and South America measured in various 308

#### experimental studies. 309

310 \* Graphical estimation

311

\*\* Measured on new metamorphosed mussels + Estimated from mL g<sup>-1</sup> h<sup>-1</sup> with average weight 312

313 Figure captions

314

- **Fig. 1** Clearance rates assessed for the two tested species (the Asian clam *Corbicula fluminea* in dark gray
- and the paper pondshell *Utterbackia imbecillis* in light gray) between August 2018 and March 2019 under
- 317 two light intensity conditions: (A) "Light" (6.5  $\pm$  0.5  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) and (B) "Dark" (0.0  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>).
- 318 Whiskers indicate the minimum and maximum values, black lines indicate the median, and boxes represent
- 319  $25^{\text{th}}$  and  $75^{\text{th}}$  percentile.

320

**Fig. 2** Combined kinetics of all FRs for the two tested species *Corbicula fluminea* and *Utterbackia imbecillis* under two light conditions: (A) "Light" ( $6.5 \pm 0.5 \mu$ mol m<sup>-2</sup> s<sup>-1</sup>) and (B) "Dark" ( $0.0 \mu$ mol m<sup>-2</sup> s<sup>-1</sup>)

323 <sup>1</sup>).







Fig. 2