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1                   **The effects of food quantity, light, and temperature on clearance rates**  
2                   **in freshwater bivalves (Cyrenidae and Unionidae)**

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29 **Abstract**

30 Assessing the environmental variables that influence freshwater bivalve filtration activity is key to better understand  
31 the functioning of stream ecosystems. In the present study, the effects of light ( $19 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $0 \pm 0 \mu\text{mol}$   
32  $\text{m}^{-2} \text{s}^{-1}$ ) and temperature (10, 15, 20 and 25 °C) on the clearance rates of two bivalve species: the Asian clam  
33 *Corbicula fluminea* (Cyrenidae) and pocketbook *Lampsilis ovata* (Unionidae) were assessed under controlled  
34 laboratory conditions at two different concentrations of algal food (i.e. 10,000 cells  $\text{mL}^{-1}$  and 100,000 cells  $\text{mL}^{-1}$ ).  
35 Clearance rates for *C. fluminea* varied from 0 to 491  $\text{mL g}^{-1} \text{h}^{-1}$  while the observed range for *L. ovata* was 0 to 905  
36  $\text{mL g}^{-1} \text{h}^{-1}$ . We found that the relative contribution of the tested variables was species-dependent. While temperature  
37 plays a major role in the clearance rates of *C. fluminea*, food concentration was the most significant variable in the  
38 clearance rate of *L. ovata*. Our results confirm the complex interactions between abiotic and biotic factors in  
39 freshwater bivalve filtration activity. Overall, clearance rates are highly variable especially in *C. fluminea* and  
40 presumably regulated by other untested factors suggesting high plasticity in filtration.

41

42 **Keywords:** Asian clam, Environmental variables, Filtration, Freshwater mussel, Pocketbook

## 43 **Introduction**

44 Freshwater bivalves are ecosystem engineers that feed by filtering suspended particles, including phytoplankton,  
45 zooplankton, bacteria and fine organic detritus (Nichols et al., 2005; Haag, 2012; Marescaux et al., 2016) from the  
46 water column. Through their filtration activity, freshwater bivalves can significantly modulate the availability of  
47 resources to other organisms. For example, by filtering suspended particles out of the water column and transferring  
48 these resources to substrates (i.e. as feces or pseudofeces), they play an important role in benthic-pelagic coupling in  
49 stream systems (Howard & Cuffey, 2006) and can significantly affect water quality (Pigneur et al., 2014).

50 In addition to affecting energy and nutrient recycling and storage, freshwater mussels can create structural habitat  
51 and can affect food web structures. Thus, losses or overabundance of bivalves may lead to, among other things, a  
52 disruption in trophic networks or a deeply modified habitat for other organisms (Marescaux et al., 2016; Vaughn,  
53 2018). In addition, because bivalve filtration activity affects the dynamics of contaminants in streams, there are  
54 growing considerations regarding the use of freshwater bivalves for contaminant mitigation or bioremediation in  
55 lakes and industrial effluents (Bianchi et al., 2014; Rosa et al., 2014; Domingues et al., 2020). For all of the  
56 aforementioned reasons, a whole field of research is dedicated to the assessment of filtration rates in bivalves.

57 The filtration rates of freshwater bivalves are affected by environmental parameters including both abiotic and biotic  
58 factors. Among the abiotic factors, several studies have already shown that temperature plays an important role for  
59 many species (Aldridge et al., 1995; Lim et al., 2005; Loayza-Muro & Elias-Letts, 2007) while other work  
60 demonstrated effects of pH, water velocity and turbidity (Ackerman, 1999; Loayza-Muro & Elias-Letts, 2007;  
61 Tuttle- Raycraft & Ackerman, 2019). In addition, recent evidence shows that light may affect filtration rates in the  
62 Asian clam *Corbicula fluminea* (Müller, 1774) (Hills et al., 2020). Numerous studies have also shown that biotic  
63 factors such as food availability and particle type (Sprung & Rose, 1988), as well as reproduction and growth cycle  
64 (Hornbach et al., 1984; Viergutz et al., 2012) can influence filtration rates.

65 Although a considerable amount of data is available regarding filtration rates on widespread invasive species such as  
66 *Corbicula* and *Dreissena* species (see Marescaux et al., 2016), limited attention is paid to Unionid mussels. Overall,  
67 the filtering capacity of Unionidae is often reported to be lower than the filtration capacity of *Corbicula*. Indeed, in  
68 Unionidae, individual filtration rates for various species usually range between 18 and 2,000 mL ind<sup>-1</sup> h<sup>-1</sup>  
69 (Tankersley & Dimock, 1993; Gatenby et al., 1996; Tankersley, 1996; Loayza-Muro & Elias-Letts, 2007; Gatenby  
70 et al., 2013; Mistry & Ackerman, 2017, 2018; Tuttle-Raycraft & Ackerman, 2018, 2019) though filtration rates of up

71 to 10,000 mL ind<sup>-1</sup> h<sup>-1</sup> were reported for the mucket *Actinonaias ligamentina* (Lamarck, 1819) (>100 g, Baker &  
72 Hornback, 2001) compared to filtration rates between 29 and 3,000 mL ind<sup>-1</sup> h<sup>-1</sup> for *Corbicula* species (see  
73 Marescaux et al., 2016). Nevertheless, there are some discrepancies in the data available as shown by the wide  
74 variation in measured filtration rates. Indeed, very few studies have directly compared the filtration rates of different  
75 bivalves, especially for freshwater species, in the same study using the same methodology (Kryger & Riisgård,  
76 1988; Marescaux et al., 2016), making it difficult to compare filtration rate values among species and assess how  
77 environmental variables affect them.

78 In this study, we aimed to test the effects of light and temperature on the clearance rates (i.e. volume of water  
79 cleared of suspended particles per unit of time (Riisgård, 2001) of two bivalve species: the Asian clam *C. fluminea*  
80 (Cyrenidae) and the pocketbook mussel *Lampsilis ovata* (Say, 1817) (Unionidae) under controlled laboratory  
81 conditions at two different concentrations of algal food: ‘low’ food (i.e. 10,000 cells mL<sup>-1</sup>) and ‘high’ food (i.e.  
82 100,000 cells mL<sup>-1</sup>). Using collected data from two experiments, the relative contribution of each tested variable (i.e.  
83 food, temperature and light) on the clearance rate of each species was assessed.

84

## 85 **Methods**

### 86 Origin and acclimation of bivalves

87 Two-year-old juvenile *L. ovata* (pocketbook) and adult *C. fluminea* (Asian clam) were chosen in order to compare  
88 native to exotic species in the USA. *C. fluminea* were collected from Sewee Creek in Meigs county, TN, USA, while  
89 *L. ovata* came from Tennessee Wildlife Resources Agency’s hatchery, Cumberland River Aquatic Center (TWRA’s  
90 C-RAC). Bivalves were brought to the laboratory in a cooler with an air bubbler. No mortality was recorded during  
91 transportation. The bivalves were kept in a 700-L tank supplied with flow-through water from First Creek on the  
92 Oak Ridge Reservation in Oak Ridge, TN (water renewal: 50-150 L h<sup>-1</sup>; ambient temperature: 15-18 °C; light/dark:  
93 12h/12h), and acclimated to the laboratory for at least three months prior to the experiment. In addition to the supply  
94 of food particles coming from the water inlet (i.e. ~1,000 particles mL<sup>-1</sup>), bivalves were fed a daily diet of fresh  
95 algae: *Chlamydomonas reinhardtii* (Dangeard, 1888) and *Navicula* sp. (approx. 7 x 10<sup>7</sup> cells ind<sup>-1</sup> d<sup>-1</sup>) using a  
96 peristaltic pump.

97 Four weeks before the experiment, individuals of each species were randomly placed in four 40-L closed-system  
98 aquaria (n = 8 per species) and acclimated to the targeted temperatures (10, 15, 20 and 25 °C). Each aquarium was

99 equipped with an airlift foam filter and a plastic tray containing a 1-cm layer of fine gravel (1-4 mm) to encourage  
100 natural burrowing activity of the bivalves. During the beginning of the acclimation period, temperatures were  
101 gradually adjusted ( $\sim 1\text{ }^{\circ}\text{C d}^{-1}$ ) and then stabilized at the targeted temperatures for at least 20 days before starting the  
102 experiments. Each aquarium was placed in a large water bath to aid temperature control of the small volume. During  
103 the aquaria-acclimation period, bivalves were fed exclusively on *C. reinhardtii* ( $1.2 \times 10^7$  cells  $\text{ind}^{-1} \text{d}^{-1}$ ) and daily  
104 water renewals (i.e. 10-20%) were performed.

105 Temperature and light were measured three times a day in each aquarium using a hand-held thermometer (Oakton®  
106 RTD thermometer) and a PAR meter (Quantum Flux® Apogée), respectively. Conductivity, pH, dissolved oxygen,  
107 total dissolved solids (TDS) were monitored 5 times a week in each aquarium using a logging multiparameter meter  
108 (HANNA® Hi 9829). Additional measurements were regularly taken in each aquarium for  $\text{NH}_3$  and  $\text{NO}_2^-$  (HACH®  
109 SL 1000). Values of water parameters are summarized in Table 1.

110

#### 111 Algae culture

112 The unicellular green algae *C. reinhardtii* (5-6  $\mu\text{m}$  in diameter) was selected for its availability and ease of  
113 maintenance in laboratory conditions. This chlorophyte remains in suspension because it is flagellated, facilitating  
114 the homogeneity of the particles requires to properly estimate clearance rates. Although this species has been shown  
115 to be filtered by both clams and mussels (Boltovskoy et al., 1995; Gatenby et al., 1996), is not among the dominant  
116 species in the diet of such bivalves and no nutritional analysis has been performed in the present study. *C.*  
117 *reinhardtii* culture was grown in 10-L clear Nalgene carboys containing WC medium (Guillard, 1975) (temperature:  
118  $23\text{-}24\text{ }^{\circ}\text{C}$ ; light intensity:  $150\text{-}200\text{ }\mu\text{mol m}^{-2} \text{s}^{-1}$ ; light/dark: 12h/12h). Algal concentrations were evaluated by  
119 imaging flow-cytometry (see “Sample analysis” section for details). For the entire experiment (i.e. acclimation and  
120 clearance rates assessment), the algae used were harvested during their exponential growth phase (i.e. after  $7 \pm 2$  d at  
121  $1.4 \pm 0.5 \times 10^6$  cells  $\text{mL}^{-1}$ ; mean  $\pm$  SD).

122

#### 123 Experimental procedures

124 To assess the effects of food concentration on clearance rates of *C. fluminea* and *L. ovata*, two experiments were  
125 performed under the same temperature (10, 15, 20 and  $25\text{ }^{\circ}\text{C}$ ) and light conditions ( $19 \pm 2\text{ }\mu\text{mol m}^{-2} \text{s}^{-1}$  and  $0 \pm 0$   
126  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). In Experiment 1, bivalves were acclimated to temperature and food ration ( $1.2 \times 10^7$  cells  $\text{ind}^{-1} \text{d}^{-1}$ ),

127 then clearance rates were assessed using *C. reinhardtii* (dry weight of 83 pg cell<sup>-1</sup>, Pickhardt & Fisher, 2007) at a  
128 concentration of 10,000 cells mL<sup>-1</sup> (i.e. 0.8 mg (dry weight) L<sup>-1</sup>; ‘low food’ condition). In Experiment 2, after an  
129 additional week of acclimation to a 10-fold increase of the food ration (12 x 10<sup>7</sup> cells ind<sup>-1</sup> d<sup>-1</sup>), clearance rates were  
130 assessed at the second *C. reinhardtii* concentration (i.e. 100,000 cells mL<sup>-1</sup> or 8 mg (dry weight) L<sup>-1</sup>; ‘high food’  
131 condition). The detailed experimental schedule is described in Fig. 1.

132 In each experiment, eight individuals of each bivalve species were randomly selected for each experimental  
133 temperature (*C. fluminea*: 2.06 ± 0.35 g wet wt, 16.9 ± 0.8 mm shell length; *L. ovata*: 0.54 ± 0.13 g wet wt, 16.4 ±  
134 1.2 mm shell length). On the day of the experiment, each individual was weighed and placed in a food-grade PET  
135 container filled with 200 mL of stream water. Temperature was kept constant in the containers at targeted  
136 experimental treatments by using chilled or heated water baths. Three containers per temperature treatment were  
137 used as controls with no bivalve. Slight air bubbling in each container kept water well-circulated. Bivalves were  
138 acclimated to experimental containers for 4 h. The sample sizes (i.e. n = 8 per temperature for each species)  
139 followed the recommendations from (Salerno et al., 2018).

140 At the start of the experiment, concentrated live *C. reinhardtii* cells were spiked into each plastic container to add  
141 the targeted initial concentration (see above). Algae cells were concentrated by centrifugation (5000 RPM for 5 min)  
142 and resuspension in Milli-Q water. The use of concentrated algae allowed a reduction of spike volume (i.e. ~500-  
143 600 µL) and avoided significant changes in water volume to preserve physical and chemical parameters.

144 The cell concentration of the initial spike was checked immediately by analyzing a 3-mL sample from three  
145 randomly selected containers in each temperature treatment using flow imaging cytometry (see “Sample analysis”  
146 section). A 4-mL sample was taken from each plastic container five minutes after the algae spike and then a second  
147 4-mL sample at the end of the 35-minute filtration period. Samples were placed in 5-mL snap-cap centrifuge tubes  
148 containing 800 µL of 10% formalin solution, a common preservative for phytoplankton samples (Mukherjee et al.,  
149 2014) and mixed immediately.

150 In a single day, each experiment was first performed in light conditions (i.e. at 2PM: 19 ± 2 µmol m<sup>-2</sup> s<sup>-1</sup>). Each PET  
151 container was cleaned and rinsed with dechlorinated tap water, refilled with stream water at the appropriate  
152 temperature, and the same organisms were acclimated for another 4-h period. The same experiment was then  
153 repeated in dark conditions (i.e. at 8PM: 0 ± 0 µmol m<sup>-2</sup> s<sup>-1</sup>). This protocol, without photoperiod modification,  
154 maintains the circadian rhythm in the studied bivalves throughout the experiments.



155

156 Sample analysis

157 Algal cell counts were performed by flow imaging cytometry (FlowCam® Benchtop B3 Model) following a  
158 standard procedure within 30 days of sampling to limit the effect of preservative. The preserved samples were  
159 analyzed in AutoImage mode in which particles of the flow sample are imaged and captured at a regular interval,  
160 with no fluorescence measurements being taken. Therefore, every particle (phytoplankton, aggregates, inorganic,  
161 and so on) ranging from 2 to 50 µm equivalent spherical diameter (ESD) was counted and imaged. For each sample,  
162 a maximum of either 10,000 particles or 1 mL were analyzed. A 10X objective was used in the sample analysis, and  
163 the instrument count-calibration was checked using beads of a known size (Zarauz & Irigoien, 2008). Invalid  
164 pictures (i.e. bubbles, repeated images) were removed from the image database, through visual recognition. Each  
165 sample was run in at least duplicate, but if the coefficient of variation was  $\geq 20\%$  between measurements, a third  
166 measurement was taken. In that case, the two results within the variation limits were used in the mean calculation; if  
167 all three results were outside variation limits, then the mean was calculated from all three. Mean values were then  
168 used for clearance rate calculations.

169

170 Computations and statistics

171 Clearance rates (expressed as mL g<sup>-1</sup> h<sup>-1</sup>) were calculated two ways, using whole-body wet weight and soft parts wet  
172 weight, according to the following equation modified from Coughlan (1969) (see Mistry & Ackerman, 2018):

173 
$$CR = \frac{V}{nt} \left( \ln \frac{C_i}{C_f} - \ln \frac{C'_i}{C'_f} \right)$$

174 Where  $V$  is the volume of water in the PET container (mL),  $n$  is the number of individuals per container ( $n=1$ ),  $t$  is  
175 the length of the experiment (h),  $C_i$  and  $C_f$  are initial and final cell concentrations respectively in the container with  
176 bivalves while, and  $C'_i$  and  $C'_f$  are average initial and final cell concentrations respectively in the containers used as  
177 controls (with no bivalve). Based on calculations, all the individuals whose valves were closed at the end of the  
178 experiment and with  $CR < 1$  mL g<sup>-1</sup> h<sup>-1</sup> were considered as non-filtering.

179 Temperature coefficient ( $Q_{10}$ ) values were calculated using the following equation:

$$Q_{10} = \left( \frac{R_2}{R_1} \right)^{\frac{10}{t_2 - t_1}}$$

180 Where  $R_1$  is the clearance rate at  $t_1$  temperature, and  $R_2$  is the clearance rate at  $t_2$  temperature.  
181 For each food concentration test (i.e. ‘low’ and ‘high’ food), the data were analyzed using a three-way mixed  
182 Analysis of Variance (ANOVA) to examine the effect of light (i.e. ‘dark’ and ‘light’ within-subjects factor) and  
183 temperature (10, 15, 20 and 25 °C) on the clearance rates of the two bivalve species (*C. fluminea* and *L. ovata*). All  
184 two-way and three-way interactions were examined ( $\alpha = 0.05$ ). The data were first checked for normality (Shapiro’s  
185 test) and homoscedasticity (Levene’s test). T-tests were then used to identify pairwise differences for significant  
186 factors in the model. P-values were then adjusted using the Bonferroni multiple testing correction method. All  
187 statistical analyses were performed in R v. 3.6.3 (R Development Core Team, 2020), using the packages *lmerTest*,  
188 *rstatix* and *tidyverse*.

189 The relative contribution of each variable (food, temperature, and light) on the clearance rates of each species was  
190 assessed from the data of the two experiments through the *lmg* approach (Groemping, 2007) using the package  
191 *relaimpo*. This package does not cover linear mixed models, so we accommodated clustered data to a design that  
192 contains clusters by applying a generalized linear model (GLM) with the *svyglm* function from the package *survey*  
193 with a linear link function and gaussian distribution. The bootstrapping approach was subsequently used to test  
194 significant differences in the contribution of each variable in the model; this takes care of the dependence between  
195 the data from the same individuals.

196

## 197 **Results**

### 198 **General**

199 Overall, across all temperature, light and food conditions, clearance rates for *C. fluminea* (whole-body wet weight:  
200  $2.10 \pm 0.34$  g, mean  $\pm$  SD; min-max: 1.37-2.80 g) ranged from 0 to 591 mL g<sup>-1</sup> h<sup>-1</sup> (median value: 181 mL g<sup>-1</sup> h<sup>-1</sup>)  
201 equiv. to 0 to 1243 mL ind<sup>-1</sup> h<sup>-1</sup> (median value: 385 mL ind<sup>-1</sup> h<sup>-1</sup>). Clearance rates for *L. ovata* (whole-body wet  
202 weight:  $0.56 \pm 0.13$  g, mean  $\pm$  SD; min-max: 0.33-0.89 g) ranged from 0 to 905 mL g<sup>-1</sup> h<sup>-1</sup> (median value: 233 mL g<sup>-1</sup>  
203 h<sup>-1</sup>) equiv. to 0 to 507 mL ind<sup>-1</sup> h<sup>-1</sup> (median value: 133 mL ind<sup>-1</sup> h<sup>-1</sup>).

204 Results are noticeably different when the calculation of the clearance rates are based only on the soft-parts wet  
205 weight, the organic fraction involved in filtration activity, which represents  $9 \pm 2\%$  ( $n = 10$ ) of the total body weight  
206 in *C. fluminea* and  $28 \pm 4\%$  in *L. ovata* ( $n = 5$ ). In this case, the clearance rates of *C. fluminea* are systematically

207 higher than those calculated for *L. ovata* with values 0 to 6517 mL g soft parts<sup>-1</sup> h<sup>-1</sup> (median value: 1991 mL g soft  
208 parts<sup>-1</sup> h<sup>-1</sup>) against 0 to 3234 mL g soft parts<sup>-1</sup> h<sup>-1</sup> (median value: 834 mL g soft parts<sup>-1</sup> h<sup>-1</sup>) in *L. ovata*.

209 In the following sections, results are presented by experiment (i.e. Experiment 1 performed at 10,000 cells mL<sup>-1</sup> and  
210 Experiment 2 performed at 100,000 cells mL<sup>-1</sup>). As indicated in “Computations and statistics” section, combined  
211 results from the two experiments were used to estimate the relative contribution of food, temperature and light on  
212 clearance rate.

213

#### 214 Experiment 1

215 The first experiment was performed using a low concentration of food (i.e. 10,000 cells mL<sup>-1</sup>). In this treatment,  
216 combining all the temperature and light conditions, clearance rates ranged from 0 to 472 mL g<sup>-1</sup> h<sup>-1</sup> (median value:  
217 186 mL g<sup>-1</sup> h<sup>-1</sup>) for *C. fluminea* (n = 64) while median clearance rate of *L. ovata* (n = 64) was 2.5-fold higher (i.e.  
218 455 mL g<sup>-1</sup> h<sup>-1</sup>) and values were ranging from 0 to 905 mL g<sup>-1</sup> h<sup>-1</sup> (Fig. 2). Interestingly, the proportion of individuals  
219 filtering during this experiment also differed according to species. While most of the *L. ovata* filtered regardless of  
220 the temperature and light condition (88-100% of individuals filtering), the proportion of individuals filtering was  
221 more variable for *C. fluminea*: in both light and dark conditions, 100% of the individuals filtered at 15 °C , but only  
222 88% at 10 and 20 °C and 75% at 25 °C (Fig. 3).

223 A three-way mixed ANOVA was performed to evaluate the effects of temperature and light on the clearance rates of  
224 the two bivalve species. The three-way interaction between temperature, light and species was not significant (F(3,  
225 56) = 0.74, *P* = 0.535), but a statistically significant simple two-way interaction was found between temperature and  
226 species, (F(3, 56) = 5.29, *P* = 0.003) indicating that effect of temperature is species dependent. Thus, while there  
227 were significant effects of temperature on clearance rates of *L. ovata* in the dark (F(3, 28) = 7.10, *P* = 0.001), no  
228 statistical difference was observed for *C. fluminea* maintained in the dark (F(3, 28) = 1.77, *P* = 0.176). For both  
229 species, no significant effect of temperature was found in light condition. Pairwise comparisons tests revealed that  
230 for *L. ovata* in the dark, clearance rates were significantly different between 10 °C and 25 °C (*P* < 0.001). There was  
231 no significant difference for other temperatures (*P* > 0.05, Fig. 3). In low food conditions, Q<sub>10</sub> values were 1.56  
232 (dark) and 1.08 (light) in *C. fluminea* compared to 1.25 to 1.73 in dark and light conditions respectively in *L. ovata*.

233

#### 234 Experiment 2

235 The second experiment was performed after one week of acclimation to a ten-fold higher daily food ration to assess  
236 the clearance rate at a high concentration of food (i.e. 100,000 cells mL<sup>-1</sup>). When all temperature and light  
237 conditions were combined, the clearance rates ranged from 0 to 591 mL g<sup>-1</sup> h<sup>-1</sup> (median value: 154 mL g<sup>-1</sup> h<sup>-1</sup>) for *C.*  
238 *fluminea* (n = 64) and 0 to 453 mL g<sup>-1</sup> h<sup>-1</sup> (median value: 62 mL g<sup>-1</sup> h<sup>-1</sup>) for *L. ovata* (n = 64, Fig. 4). While median  
239 values were similar at the two food concentrations in *C. fluminea*, median values for *L. ovata* clearance rate was  
240 more than 7-fold higher at the 'high' food concentration than the one observed at the 'low' food concentration. The  
241 proportion of individuals filtering also differed between the two experiments. In the 'high' food condition, in dark,  
242 all the *C. fluminea* filtered at all temperatures. For the same species, filtration activity was highly dependent on  
243 temperature and light condition (38% of individuals filtered at 10 °C vs. 100% at 15 and 25 °C). For *L. ovata*, in the  
244 dark, all the individuals filtered at the extreme temperatures (i.e. 10 °C and 25 °C) while this proportion decreased to  
245 88% and 63% at 20 °C and 15 °C, respectively. The proportion of *L. ovata* individuals filtering in light increased  
246 with increasing temperature from 50% at 10 °C and 15 °C to 88% at 25 °C (Fig. 5).

247 As for the 'low' food concentration tested, a three-way mixed ANOVA was used to evaluate the effects of  
248 temperature and light conditions on the clearance rates *C. fluminea* and *L. ovata* revealed that the interaction  
249 between temperature, light and species was not significant (F(3, 56) = 1.71, *P* = 0.176, Table 2). In contrast, the two-  
250 way interaction between temperature and species was significant (F(3, 56) = 3.29, *P* = 0.027). There were significant  
251 effects of temperature on clearance rates of *C. fluminea* in the dark (F(3, 28) = 19.6, *P* < 0.001) and in the light (F(3,  
252 28) = 4.83 *P* = 0.008). Significant effects were also found for *L. ovata* in the dark (F(3, 28) = 9.46, *P* < 0.001) and in  
253 the light (F(3, 28) = 4.83, *P* = 0.017). In the dark condition, clearance rates gradually increased with increasing  
254 temperatures for the two species (*P* < 0.01). In the light condition, clearance rates of the two species were only  
255 significantly different between the extreme temperatures tested (i.e. 10 °C and 25 °C, *P* < 0.05, Fig. 4). In high food  
256 conditions, Q<sub>10</sub> values differed only slightly with light in *C. fluminea* with values of 4.81 in the dark and 4.53 in the  
257 light while a broader range was observed for *L. ovata* (i.e. Q<sub>10</sub> of 2.41 in dark and 3.32 in light condition).

258

259 Relative contribution of food, temperature and light on clearance rate

260 GLM models were implemented using the data from each experiment to assess the effect of explanatory variables  
261 (i.e. food, temperature and light) on clearance rates (Table 3). The lmg approach was then used to estimate the  
262 relative contribution of each variable. For *C. fluminea*, the GLM revealed that temperature (*P* < 0.001) and to a

263 lesser extent light ( $P = 0.022$ ) played a significant role in clearance rates, but clearance rates were not affected by  
264 the 10-fold difference in food concentration ( $P = 0.87$ ). Interestingly, for *C. fluminea* only 16% of the variance  
265 observed in clearance rates was explained by the model. Within the explained variance (16%), temperature had the  
266 highest contribution (80%, CI: 64-98%) while light had moderate and food had virtually no effect on the clearance  
267 rate (19%, CI: 2-28% and 0%, CI: 0-9%, respectively). For *L. ovata*, clearance rate was significantly affected by  
268 food, temperature ( $P < 0.001$ ) and to a lesser extent light ( $P = 0.048$ ) with a high proportion of the observed variance  
269 explained by these three variables (67%). Within the explained variance, food clearly has the highest contribution  
270 (81%, CI: 72-89%) while temperature and light have moderate effects on the clearance rate (18%, CI: 11-22% and  
271 2%, CI: 0-6%, respectively, Fig. 6).

272

## 273 **Discussion**

274 Our experimental approach addresses the critical need to examine the relative contribution of biotic and abiotic  
275 factors in the clearance rates of freshwater bivalves. To the best of our knowledge, this is the first study to examine  
276 the effects and relative contribution of light, temperature and food in the clearance rates of two freshwater bivalve  
277 species: the invasive Asian clam *C. fluminea* (Cyrenidae) and the native pocketbook *L. ovata* (Unionidae).

278 Overall, the filtration rates observed in our study were mostly in the range of those previously reported. In their  
279 review, Marescaux et al. (2016) reported clearance rate values ranging from 29 mL ind<sup>-1</sup> h<sup>-1</sup> (Boltovskoy et al.,  
280 1995) to 3,252 mL ind<sup>-1</sup> h<sup>-1</sup> (Viergutz et al., 2012) in *C. fluminea*. This wide range suggests plasticity in the filtration  
281 activity of *C. fluminea* in response to environmental changes. Less information is available in the literature for *L.*  
282 *ovata*, but recent studies highlighted the high filtration rate capacity of other *Lampsilis* species (*L. fasciola*  
283 (Rafinesque, 1820) and *L. siliquoidea* (Barnes, 1823), 250-2,000 mL ind<sup>-1</sup> h<sup>-1</sup>) compared to other freshwater mussels  
284 species such as *Villosa iris* (Lea, 1829) and *Ligumia nasuta* (Say, 1817) of similar size (18-400 mL ind<sup>-1</sup> h<sup>-1</sup> and  
285 150-500 mL ind<sup>-1</sup> h<sup>-1</sup>, respectively; Mistry & Ackerman, 2018; Tuttle-Raycraft & Ackerman, 2018, 2019). Although  
286 we used smaller individuals of *L. ovata* than the studies performed on other *Lampsilis* species (14-19 mm shell  
287 length in the present study vs. ~50-120 mm shell length for previous studies), we confirmed the high filtration  
288 capacity of this species seen in previous studies.

289 While we found filtration rates of the two studied species are highly dependent on environmental conditions,  
290 interestingly, when only the organic biomass of bivalves is considered in the calculation of the rate clearances, *C.*

291 *fluminea* systematically showed a higher filtration capacity than that of *L. ovata*. This observation is true regardless  
292 of the temperature, light and food conditions that we tested. This result demonstrates the importance of taking into  
293 account the organic mass (sometimes expressed in g of C, e.g. Marescaux et al., 2016) in the calculation of clearance  
294 rates in bivalves. Bivalve clearance rates can vary in proportion to gill surface area (Galbraith et al., 2009). Payne &  
295 Miller (1995) found that the allometric relationship between gill surface area (GA, mm<sup>2</sup>) and shell length (SL, mm)  
296 for *C. fluminea* can be approximately described by a linear relationship based on a regression model:  $GA = 63.2 +$   
297  $0.78 \times SL^2$ . Similarly, Galbraith et al. (2009) found in four species of Unionids that, on average,  $GA = 2.21 \times SL$ .  
298 Using these equations, we estimated that gill surface areas were  $285 \pm 20 \text{ mm}^2$  in *C. fluminea* used in the present  
299 study and only  $36 \pm 3 \text{ mm}^2$  in *L. ovata*. Although these estimates need to be confirmed by measurements, such  
300 differences suggest that clearance rates performances of *C. fluminea* may be explained, at least partially, by a large  
301 gill surface area compared to Unionids.

302 Effects of numerous factors on the clearances rates of *Corbicula* and Unionidae species have been studied including  
303 abiotic factors such as temperature (Aldridge et al., 1995; Lim et al., 2005; Loayza-Muro & Elias-Letts, 2007), pH,  
304 water velocity, and turbidity (Ackerman, 1999; Loayza-Muro & Elias-Letts, 2007; Tuttle- Raycraft & Ackerman,  
305 2019), and biotic factors including food quality and quantity (Sprung & Rose, 1988), and life-history traits such as  
306 reproduction and growth cycle (Hornbach et al., 1984; Viergutz et al., 2012). However, the interactions between the  
307 different factors and their relative contribution in clearance rates remain largely unknown. In the present study, we  
308 found that contribution of each tested variable is species dependent. While temperature was the main variable  
309 affecting the clearance rates with virtually no change related to food concentration in *C. fluminea*, food  
310 concentration plays a major role in clearance with a limited role of temperature in *L. ovata*. For both species, the  
311 effects of light remain limited although changes in light conditions did affect the clearance rate in *C. fluminea*. Such  
312 findings highlight the complex interactions between abiotic and biotic factors that potentially affect bivalve  
313 clearance rates.

314 Among abiotic factors, temperature is key because bivalves are poikilothermic organisms, responding to temperature  
315 changes through behavioral and physiological adaptations. For example, Rodland et al. (2008) found that  
316 temperature (from 10 to 34 °C) affects frequency and duration of shell closure in two freshwater mussels: *Anodonta*  
317 *cygnea* (Linnaeus, 1758) and *Margaritifera falcata* (Gould, 1850), with a duration of intervals of valve closure  
318 decreasing in both species as temperature rises. These results are in accordance with Block et al. (2013) who found

319 that latency in valve opening and foot extension was highest at the lowest experimental temperature (10 °C)  
320 compared to the other experimental treatments (i.e. 20 and 30 °C) in their study. Regarding the effects of  
321 temperature on bivalve metabolism, Vidal et al. (2002) highlighted that detoxification mechanisms in *C. fluminea*  
322 are affected by change in temperatures (10 and 20 °C). In this study, we found that temperature effects on clearance  
323 rates were species-dependent and dependent on light and food conditions. This is especially true for *C. fluminea*:  
324 while at low food concentration (i.e. 10,000 cells mL<sup>-1</sup>), no effect of temperature was found, we found an increase in  
325 clearance rates at increasing temperatures (from 10 to 25 °C) at the high food concentration (i.e. 100,000 cells mL<sup>-1</sup>).  
326 These results are in accordance with Lim et al. (2005) who found that clearance rates of *C. fluminea* remained close  
327 to 0 at 5 °C, increased linearly with water temperature up to approx. 25 °C, and drastically fell at temperatures above  
328 25 °C. This suggests that the range of temperatures we studied covers the thermal preference of this species. For *L.*  
329 *ovata*, we found a similar pattern in response to temperature changes. Such results are consistent with the field study  
330 of Vanderploeg et al. (1995) who found a positive non-linear relationship between filtration rates in *L. radiata*  
331 (Gmelin, 1791) and temperature measured in their habitat (from 8 to 25 °C). In a recent laboratory study, Malish &  
332 Woolnough (2019) found that clearance rates of *L. cardium* were higher in a trial performed in June (25.0-26.5 °C)  
333 than during the same experiment performed in May (17.0-18.5 °C). In the present study, the effects of temperature  
334 on the filtration activity of the studied species were highlighted through the calculations of temperature coefficient  
335 (Q<sub>10</sub>). Overall, the average Q<sub>10</sub> was 2.12 for *C. fluminea* compared to 1.56 for *L. ovata*. Nevertheless, Q<sub>10</sub> was  
336 strongly variable depending on the experimental conditions (i.e. food availability and light intensity) with values  
337 ranging from 1.08 to 4.81 for *C. fluminea* and from 1.25 to 3.31 for *L. ovata*. These results suggest that the intensity  
338 of the effects of temperature is modulated by other environmental conditions.

339 For both species, the effects of temperature in the two food conditions were light-dependent. Effects of temperature  
340 were stronger in the dark (i.e.  $0 \pm 0 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) than in the light (i.e.  $19 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). These results are in line  
341 with field observations showing that seasonal differences in clearance rates of freshwater bivalves were not  
342 necessarily only related to temperature changes but also to the combined effects with other factors (Viergutz et al.,  
343 2012). Here we show that the effects of temperature on clearance rate differ between the two studied species and are  
344 also closely related to light and to food availability.

345 There is limited information available regarding the effects of light on clearance rates in freshwater bivalves;  
346 however, there are a few relevant studies. For example, Ortmann & Grieshaber (2003) used gaping activity in *C.*

347 *fluminea* to estimate filtration activity in the field and reported a circadian rhythm with valves being closed in the  
348 morning but open in the afternoon. In addition, Duchini et al. (2015) found that *Limnoperna fortunei* (Dunker, 1857)  
349 is more mobile in darkness than in light. Kobak & Nowacki (2007) found that *D. polymorpha* shows light avoidance  
350 behavior. Hills et al. (2020) found higher clearance rates in the dark for *C. fluminea* than *Utterbackia imbecillis*  
351 (Say, 1829). The present study confirmed differences observed in the effects of light in clearance rates between *C.*  
352 *fluminea* and Unionidae species.

353 Food quantity is one of the most studied biotic factors in the assessment of filtration activity in freshwater bivalves  
354 (e.g. Roper & Hickey, 1995; Lim et al., 2005; Gatenby et al., 2013). In the present study, we found that clearance  
355 rates for *C. fluminea* are overall higher at the highest concentration of food tested. Interestingly, we also found that  
356 the effects of temperature on clearance rates are most pronounced at the highest concentration of food tested. This  
357 can be related to the reduced or even zero filtration observed whatever the temperature in a large proportion of  
358 individuals exposed to a low concentration of food. Altogether these results suggest that *C. fluminea* is efficient in  
359 regulating its filtering activity according to the available food resource and that the filtration activity is a trade-off  
360 between the energy expended and that acquired by the ingestion of filtered particles (Ortmann & Grieshaber, 2003).  
361 Interestingly, an opposite trend was observed in *L. ovata* with lower clearance rates measured at the highest food  
362 concentration. To our knowledge, there is no similar study in the literature for this species. We assume that such  
363 difference may be related to lower energy requirements of individuals of *L. ovata* (~ 0.5 g) compared to *C. fluminea*  
364 whose total weight is 4-fold higher (~ 2 g). Thus, the energy requirements are likely to be covered more quickly for  
365 *L. ovata* when the availability of food is high. Nevertheless, further investigations assessing the metabolism of these  
366 species through a wide range of temperatures and food concentrations are needed to support this assumption.

367 In North American freshwater ecosystems, native Unionids coexist with exotic bivalve species such as *C. fluminea*  
368 and *Dreissena polymorpha* (Pallas, 1771). The threatened status of Unionid mussels has been attributed, along with  
369 other factors such as space and habitat availability, to food competition with exotic and invasive bivalve species  
370 (e.g., Ferreira-Rodríguez et al., 2018; Strayer & Malcom, 2018). Although experimental evidence of this  
371 competition between species is rare in the literature thus far, field investigations have highlighted the declines of  
372 Unionid populations due to exploitative competition for food after invasion by *Corbicula* and *Dreissena* species  
373 (Burlakova et al., 2014; Strayer & Malcom, 2007). Comparative studies on food preferences in Unionids and  
374 *Corbicula* and *Dreissena* have demonstrated that these bivalves compete for the same food resources, and this may



375 result in the reduced availability of preferred and nutritious food items for Unionid mussels. In the present study, we  
376 highlighted that *C. fluminea* has a great ability to modulate filtration rate depending on the environmental conditions  
377 presumably to optimize food intake. These results are not surprising considering that *Corbicula* has a much faster  
378 growth rate than Unionids, which have longer lifespans and later sexual maturation (Anthony et al., 2001), requiring  
379 high energy.

380 In combination with food competition, our findings suggest that differential responses to changing environmental  
381 conditions in filtration rate between *Corbicula fluminea* and native mussels may be key to understanding the decline  
382 of Unionids populations after invasions by Asian clams. Nevertheless, climate change may strongly affect current  
383 coexistence and competitive relationships between Unionids and *Corbicula* species. Indeed, we found that *C.*  
384 *fluminea* filtration is strongly driven by temperature. Thus, it is crucial to take temperature into account when  
385 discussing food competition, because the metabolic rate, and thus the requirement for food, generally increases with  
386 rising temperature in bivalves (Vohmann et al., 2010). An increase of 5°C results in an approximately two-fold  
387 increase in energy demand in *C. fluminea* (Ortmann & Grieshaber 2003). Increasing temperature under limited food  
388 conditions can lead to a negative energy balance and thus to an enhanced starvation (Vohmann et al., 2010).  
389 Compared to many other bivalves, *C. fluminea* is less able to compensate in energy losses at high temperatures by  
390 decreasing its metabolic activity (McMahon 2002). Although less information is available regarding metabolism in  
391 Unionids, such findings illustrate that climate change may also change filtration in bivalves and ultimately affect  
392 competition between species.

393

#### 394 **Conclusion**

395 In the present study, we confirmed through multi-parameter laboratory experiments performed in controlled  
396 conditions that complex interactions between abiotic and biotic factors affect freshwater bivalve filtration activity.  
397 Overall, clearance rates are highly variable in *C. fluminea* and presumably regulated by additional untested factors,  
398 suggesting high plasticity in filtration of this species. Such findings are useful to understand field observations  
399 where temporal variations of clearance rates in bivalves are often difficult to link to a unique environmental  
400 variable. Furthermore, our study provides data for modeling bivalve filtration in stream systems highlighting that  
401 filtration activity is not constant through time and potentially highly variable between individuals especially in *C.*  
402 *fluminea*. Refining models by using such data may avoid filtration rate overestimation, particularly when clams

403 switch to pedal feeding at low phytoplankton concentrations (Marescaux et al., 2016). Such findings help to properly  
404 evaluate the potential of such organisms to mitigate/bioremediate contamination of anthropogenic origins in such  
405 fragile ecosystems.

406

#### 407 **Authors Contributions**

408 S.P. and A.H. designed and performed the experiments and analyzed samples. S.P. performed statistical analyses,  
409 interpreted the results and wrote the first draft of the paper. T.J.M. supervised the experiments. T.J.M. and A.H.  
410 corrected and improved the manuscript.

411

#### 412 **Data Availability Statement**

413 Data are available upon personal request.

414

#### 415 **Conflict of Interest Statement**

416 Authors declare no conflict of interest.

417

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549 samples, analyzed by means of an automatic counting method. Journal of Plankton Research 30: 1297-  
550 1303.

551 Table 1. Summary of water quality parameters measured for the four temperature conditions during the experiment.

552 Values are means  $\pm$  SD. DO: dissolved oxygen, TDS: total dissolved solids.

Parameters	Experimental treatment			
	10°C	15°C	20°C	25°C
Temperature (°C)	10.3 $\pm$ 0.1	14.9 $\pm$ 0.2	20.3 $\pm$ 0.2	24.9 $\pm$ 0.2
pH	8.02 $\pm$ 0.10	8.05 $\pm$ 0.11	8.08 $\pm$ 0.12	8.26 $\pm$ 0.13
DO saturation (%)	92.2 $\pm$ 2.3	91.9 $\pm$ 2.4	91.8 $\pm$ 2.5	92.4 $\pm$ 2.0
DO (mg L <sup>-1</sup> )	9.82 $\pm$ 0.42	8.96 $\pm$ 0.28	8.03 $\pm$ 0.25	7.49 $\pm$ 0.27
Conductivity A ( $\mu$ S cm <sup>-1</sup> )	162 $\pm$ 16	164 $\pm$ 18	173 $\pm$ 19	178 $\pm$ 24
TDS (mg L <sup>-1</sup> )	85 $\pm$ 11	85 $\pm$ 11	88 $\pm$ 12	89 $\pm$ 12
NH <sub>3</sub> -N (mg L <sup>-1</sup> )	0.05 $\pm$ 0.04	0.03 $\pm$ 0.02	0.02 $\pm$ 0.03	0.02 $\pm$ 0.02
NO <sub>2</sub> <sup>-</sup> (mg L <sup>-1</sup> )	0.014 $\pm$ 0.019	0.019 $\pm$ 0.015	0.005 $\pm$ 0.004	0.004 $\pm$ 0.008

553



554 Table 2. Three-way mixed analysis of variance (ANOVA) results. Effect of light (i.e. ‘dark’ and ‘light’ within-  
 555 subjects factor) and temperature (10, 15, 20 and 25 °C) on the clearance rates of bivalves of two different species (*C.*  
 556 *fluminea* and *L. ovata*) at two different concentrations of food: ‘low’ food (i.e. 10,000 cells mL<sup>-1</sup>) and ‘high’ food  
 557 (i.e. 100,000 cells mL<sup>-1</sup>) experiments.

Effect	Degrees of freedom		Low food		High food	
	numDF	denDF	F-value	p-value	F-value	p-value
Temperature	3	56	6.36	< <b>0.001</b>	25.31	< <b>0.001</b>
Species	1	56	77.77	< <b>0.001</b>	20.81	< <b>0.001</b>
Light	1	56	0.01	<b>0.913</b>	37.54	< <b>0.001</b>
Temperature x Species	3	56	5.29	<b>0.003</b>	3.29	<b>0.027</b>
Temperature x Light	3	56	1.77	0.164	2.38	0.079
Species x Light	1	56	0.41	0.525	2.77	0.102
Temperature x Species x Light	3	56	0.74	0.535	1.71	0.176

558

559 Table 3. Generalized linear model (GLM) for *C. fluminea* and *L. ovata*. Effects of each explanatory variable (i.e.  
 560 food, temperature and light) on bivalve clearance rates. Coefficient of determination  $R^2$  was 0.16 for *C. fluminea* and  
 561 0.67 for *L. ovata*.

Predictor	<i>C. fluminea</i>		<i>L. ovata</i>	
	Estimate ( $\pm$ SD)	p-value	Estimate ( $\pm$ SD)	p-value
Intercept	41.22 (46.06)	0.374	-118.10 (35.01)	<b>0.001</b>
Temperature	10.53 (2.62)	<b>&lt; 0.001</b>	14.01 (2.07)	<b>&lt; 0.001</b>
Food (Low)	-4.41 (28.48)	0.877	334.25 (25.14)	<b>&lt; 0.001</b>
Light (Light)	-58.03 (24.59)	<b>0.022</b>	-46.57 (20.81)	<b>0.029</b>

562

563 Figure captions

564

565 **Fig. 1.** Diagram of the protocol used to test effects of temperatures (10, 15, 20 and 25°C) on the clearance rates of  
566 *Corbicula fluminea* and *Lampsilis ovata*. Experiments were repeated the same day using the same individuals at two  
567 light intensities (i.e. ‘dark’:  $0 \pm 0 \mu\text{mol m}^{-2} \text{s}^{-1}$  and ‘light’:  $19 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) first at ‘low’ concentration of food  
568 (i.e. 10,000 cells  $\text{mL}^{-1}$ ) and then, one week later at ‘high’ concentration of food (i.e. 100,000 cells  $\text{mL}^{-1}$ ) after  
569 acclimation to a 10-fold higher food daily ration.

570

571 **Fig. 2.** Effects of temperatures (10, 15, 20 and 25°C) on the clearance rates, expressed in g of whole body wet  
572 weight (A) and g of soft parts wet weight (B), of *Corbicula fluminea* and *Lampsilis ovata* exposed to two light  
573 intensities (i.e. ‘dark’:  $0 \pm 0 \mu\text{mol m}^{-2} \text{s}^{-1}$  and ‘light’:  $19 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and low concentration of food (i.e. 10,000  
574 cells  $\text{mL}^{-1}$ ). Symbols denote significant differences. Statistical differences printed in panel A are identical for panel  
575 B.

576

577 **Fig. 3.** Percent of individuals filtering during the ‘low food’ (i.e. 10,000 cells  $\text{mL}^{-1}$ ) experiment for *Corbicula*  
578 *fluminea* and *Lampsilis ovata* exposed to two light intensities (i.e. ‘dark’:  $0 \pm 0 \mu\text{mol m}^{-2} \text{s}^{-1}$  and ‘light’:  $19 \pm 2 \mu\text{mol}$   
579  $\text{m}^{-2} \text{s}^{-1}$ ) and four temperatures (10, 15, 20 and 25 °C).

580

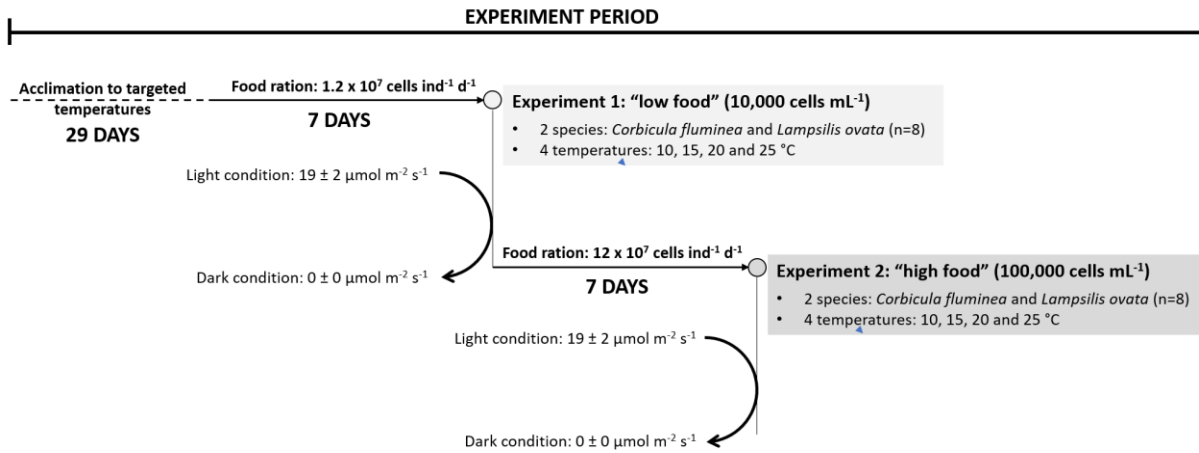
581 **Fig. 4.** Effects of temperature (10, 15, 20 and 25 °C) on the clearance rates, expressed in g of whole body wet  
582 weight (A) and g of soft parts wet weight (B), of *Corbicula fluminea* and *Lampsilis ovata* exposed to two light  
583 intensities (i.e. ‘dark’:  $0 \pm 0 \mu\text{mol m}^{-2} \text{s}^{-1}$  and ‘light’:  $19 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and high concentration of food (i.e.  
584 100,000 cells  $\text{mL}^{-1}$ ). Symbols denote significant differences. Statistical differences printed in panel A are identical  
585 for panel B.

586

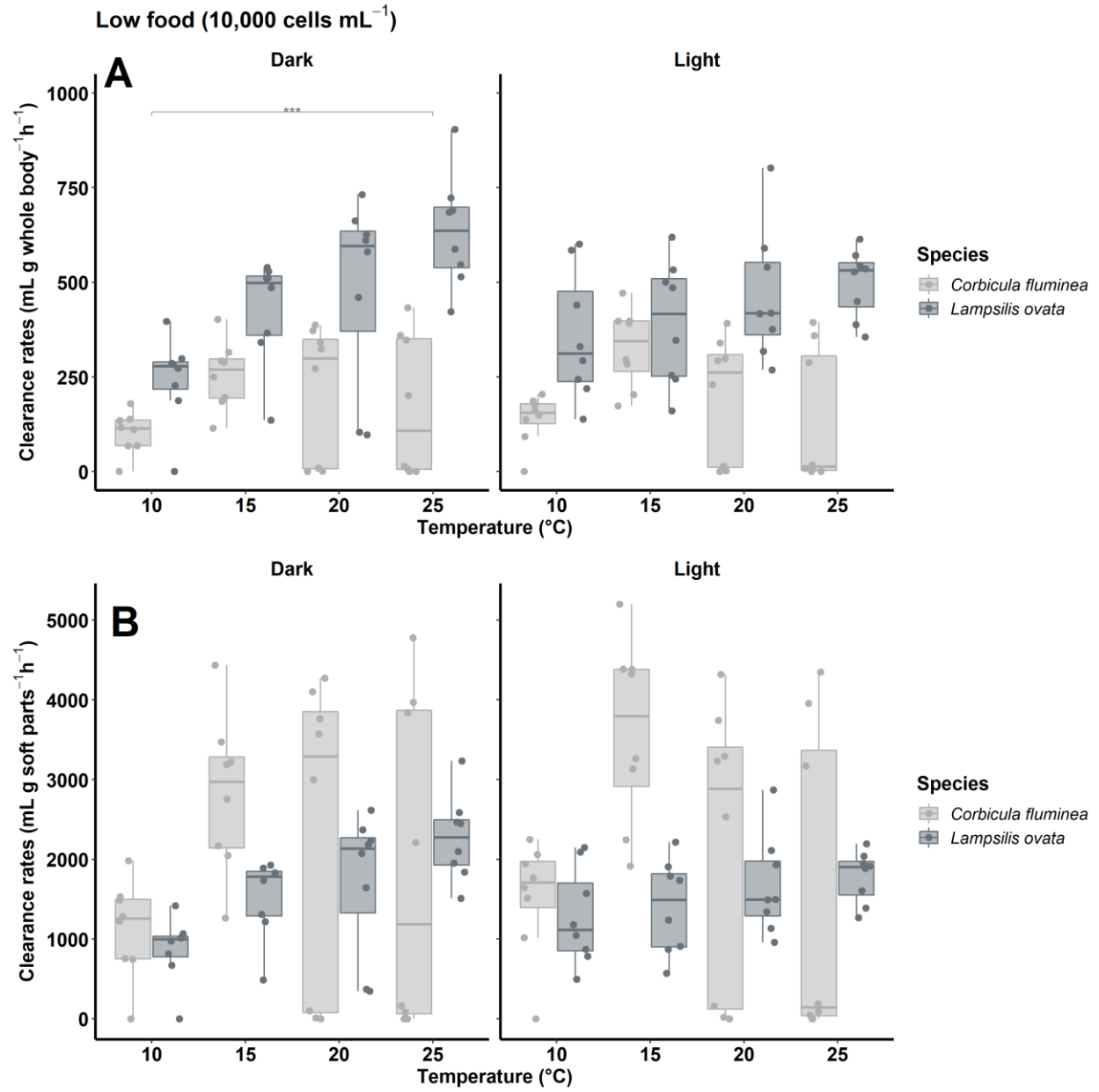
587 **Fig. 5.** Percent of individuals filtering during the ‘high’ food (i.e. 100,000 cells  $\text{mL}^{-1}$ ) experiment for *Corbicula*  
588 *fluminea* and *Lampsilis ovata* exposed to two light intensities (i.e. ‘dark’:  $0 \pm 0 \mu\text{mol m}^{-2} \text{s}^{-1}$  and ‘light’:  $19 \pm 2 \mu\text{mol}$   
589  $\text{m}^{-2} \text{s}^{-1}$ ) and four temperatures (10, 15, 20 and 25 °C).

590

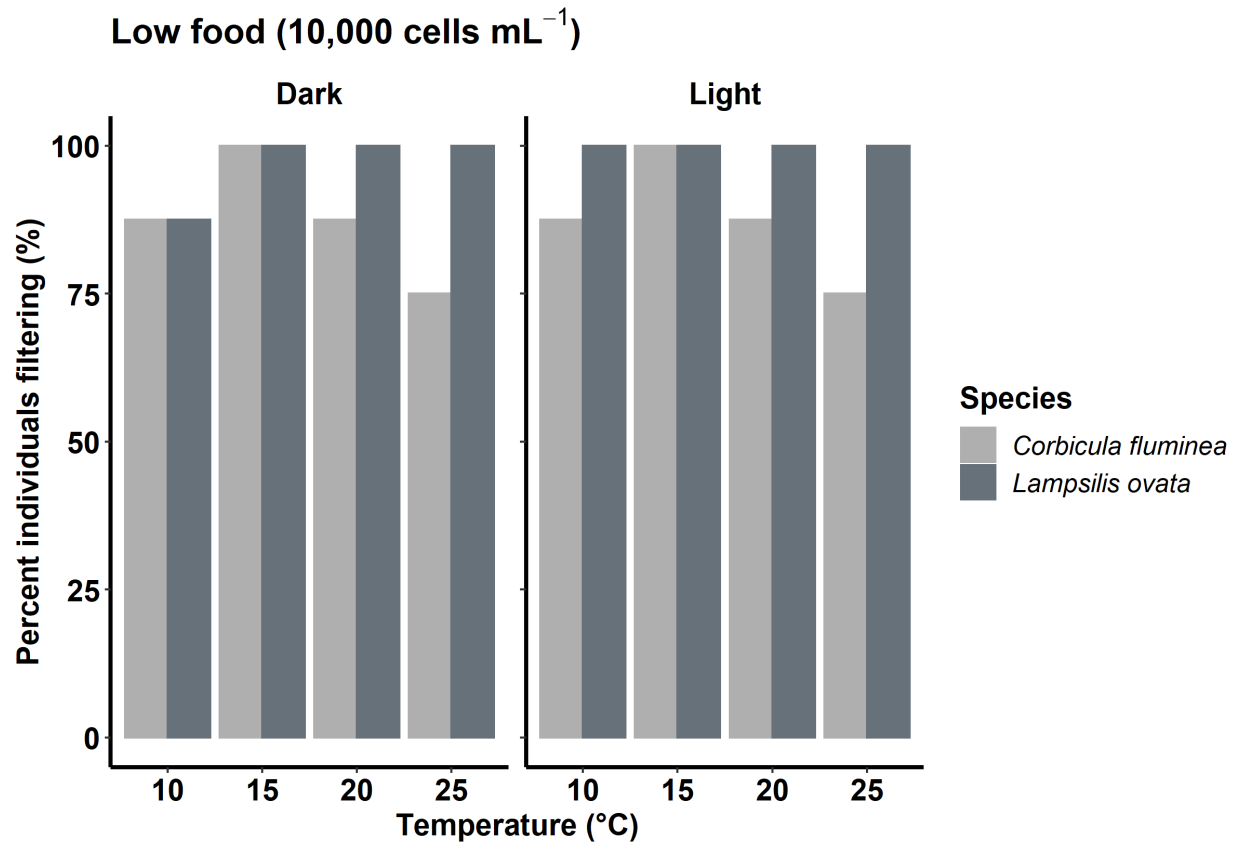
591 **Fig. 6.** Relative contribution of each explanatory variable (i.e. food, temperature and light) on the clearance rates of  
592 each species: *Corbicula fluminea* and *Lampsilis ovata*. Values are means with 95% bootstrap confidence intervals.



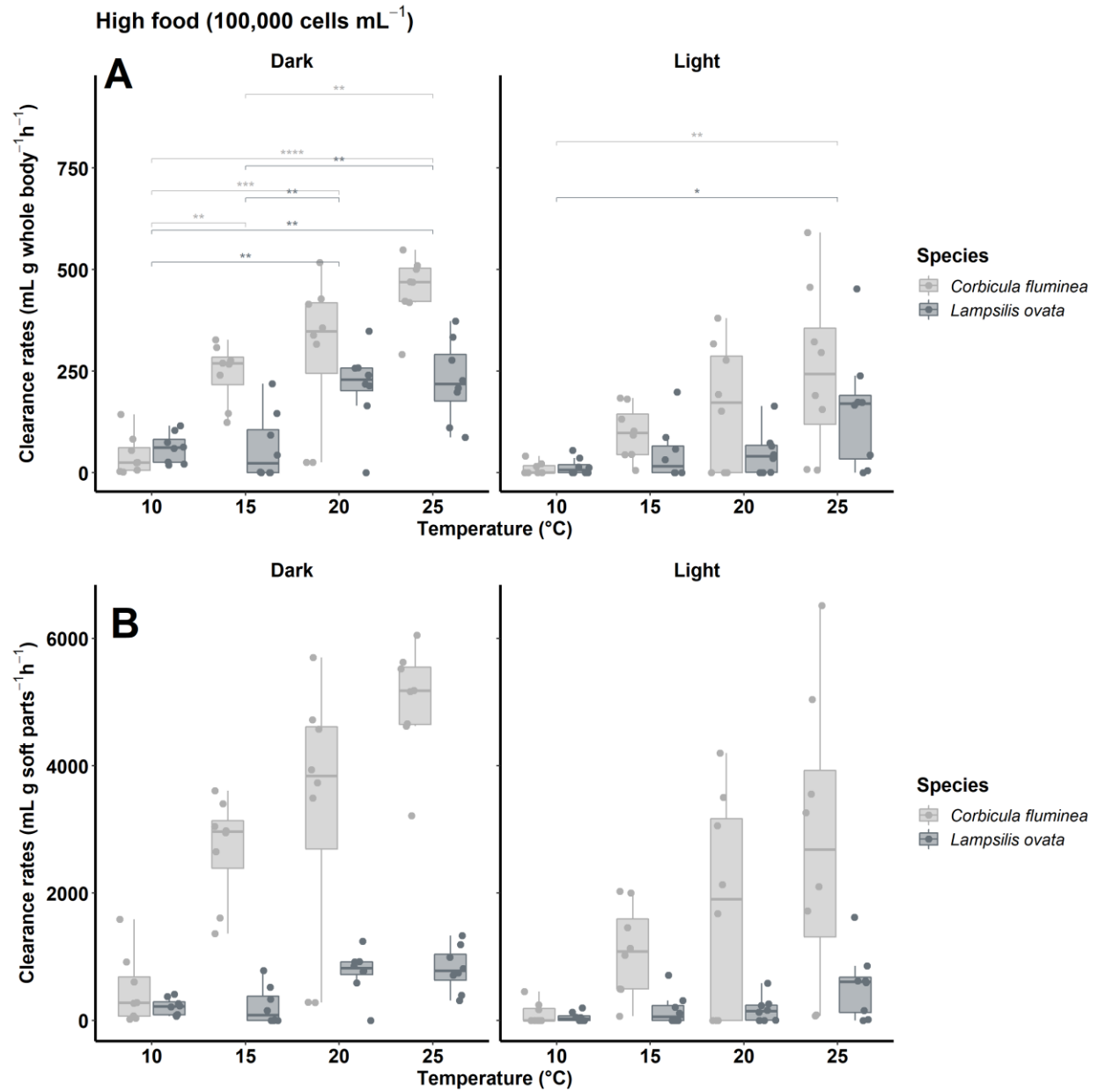
593 Figure 1



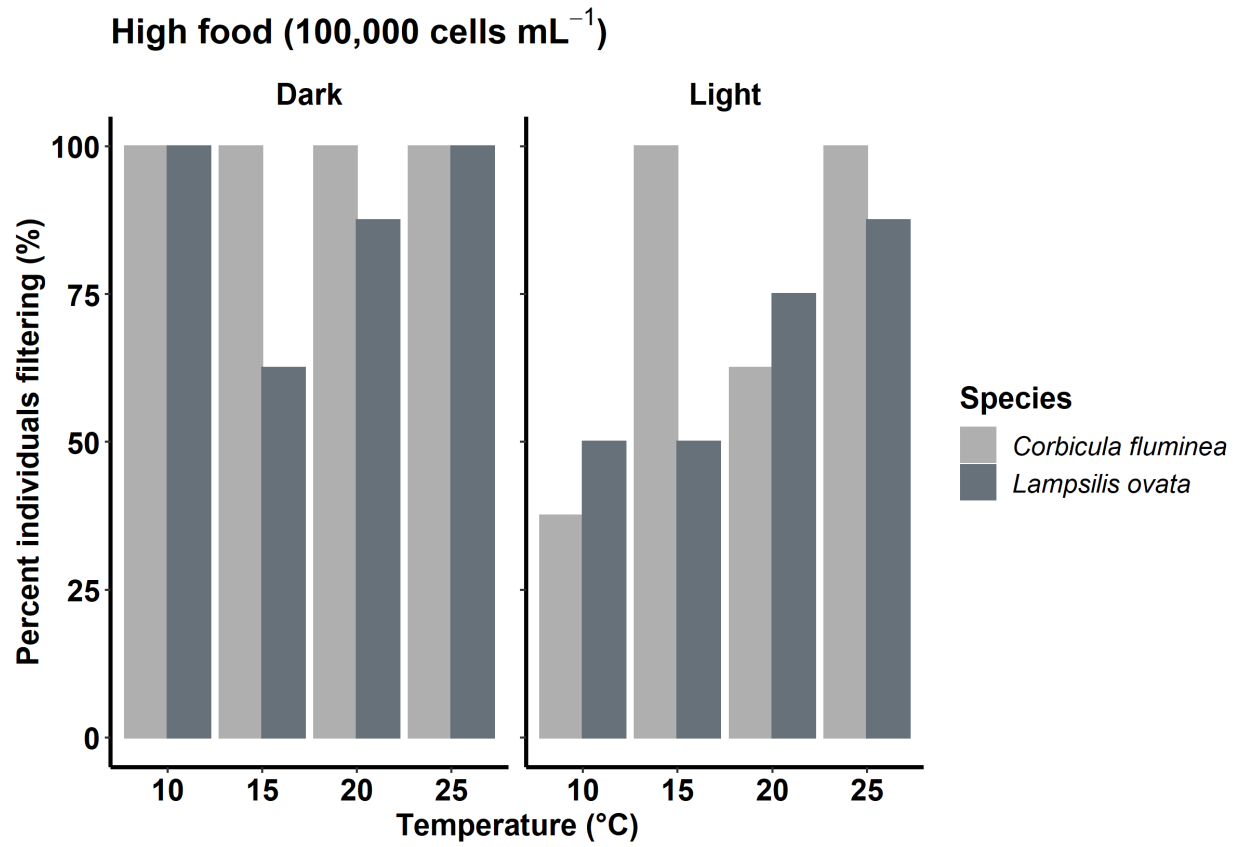
594 Figure 2



595 Figure 3

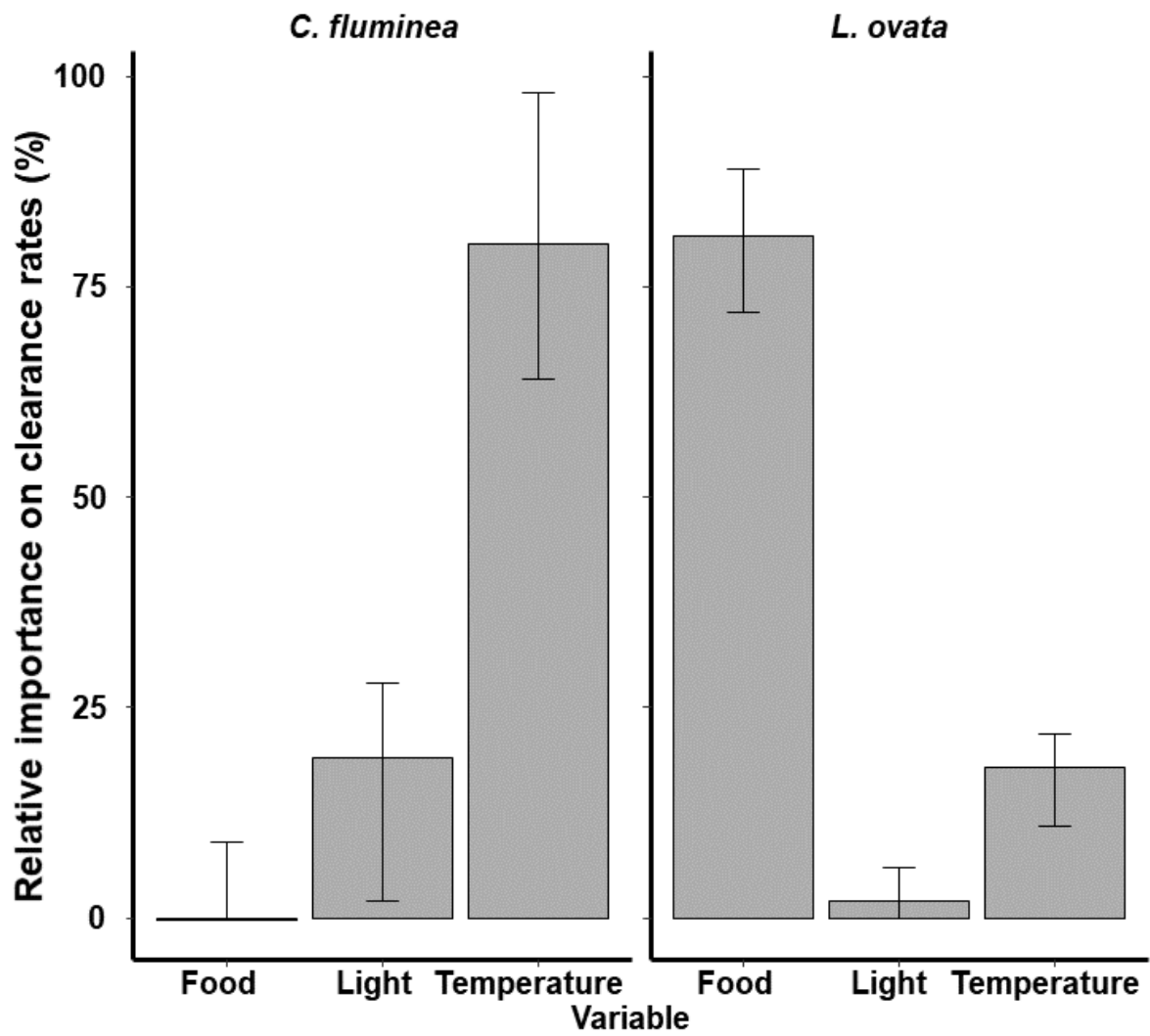


596 Figure 4



597 Figure 5





598 Figure 6