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

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Abstract

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

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

Introduction

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

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

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

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

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

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

Methods

Origin and acclimation of bivalves

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

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

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

Temperature and light were measured three times a day in each aquarium using a hand-held thermometer (Oakton® RTD thermometer) and a PAR meter (Quantum Flux® Apogée), respectively. Conductivity, pH, dissolved oxygen, total dissolved solids (TDS) were monitored 5 times a week in each aquarium using a logging multiparameter meter (HANNA® Hi 9829). Additional measurements were regularly taken in each aquarium for NH_3 and NO_2^- (HACH® SL 1000). Values of water parameters are summarized in Table 1.

Algae culture

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

Experimental procedures

To assess the effects of food concentration on clearance rates of *C. fluminea* and *L. ovata*, two experiments were performed under the same temperature (10, 15, 20 and 25 °C) and light conditions (19 ± 2 µmol m^{-2} s^{-1} and 0 ± 0 µmol m^{-2} s^{-1}). In Experiment 1, bivalves were acclimated to temperature and food ration (1.2 x 10^7 cells ind^{-1} d^{-1}),

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

In each experiment, eight individuals of each bivalve species were randomly selected for each experimental 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 ± 1.2 mm shell length). On the day of the experiment, each individual was weighed and placed in a food-grade PET container filled with 200 mL of stream water. Temperature was kept constant in the containers at targeted experimental treatments by using chilled or heated water baths. Three containers per temperature treatment were used as controls with no bivalve. Slight air bubbling in each container kept water well-circulated. Bivalves were acclimated to experimental containers for 4 h. The sample sizes (i.e. n = 8 per temperature for each species) followed the recommendations from (Salerno et al., 2018).

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

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

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

Sample analysis

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

Computations and statistics

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

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

Where V is the volume of water in the PET container (mL), n is the number of individuals per container (n=1), t is the length 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 average initial and final cell concentrations respectively in the containers used as controls (with no bivalve). Based on calculations, all the individuals whose valves were closed at the end of the experiment and with CR < 1 mL g^{-1} h^{-1} were considered as non-filtering.

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

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{t2-t1}\right)}$$

Where R_1 is the clearance rate at t_1 temperature, and R_2 is the clearance rate at t_2 temperature.

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

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

Results

General

Overall, across all temperature, light and food conditions, clearance rates for *C. fluminea* (whole-body wet weight: 2.10 ± 0.34 g, mean ± SD; min-max: 1.37-2.80 g) ranged from 0 to 591 mL g^{-1} h^{-1} (median value: 181 mL g^{-1} h^{-1}) equiv. to 0 to 1243 mL ind^{-1} h^{-1} (median value: 385 mL ind^{-1} h^{-1}). Clearance rates for *L. ovata* (whole-body wet weight: 0.56 ± 0.13 g, mean ± SD; min-max: 0.33-0.89 g) ranged from 0 to 905 mL g^{-1} h^{-1} (median value: 233 mL g^{-1} h^{-1}) equiv. to 0 to 507 mL ind^{-1} h^{-1} (median value: 133 mL ind^{-1} h^{-1}).

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

higher than those calculated for *L. ovata* with values 0 to 6517 mL g soft parts^{-1} h^{-1} (median value: 1991 mL g soft parts^{-1} h^{-1}) against 0 to 3234 mL g soft parts^{-1} h^{-1} (median value: 834 mL g soft parts^{-1} h^{-1}) in *L. ovata*.

In the following sections, results are presented by experiment (i.e. Experiment 1 performed at 10,000 cells mL^{-1} and Experiment 2 performed at 100,000 cells mL^{-1}). As indicated in "Computations and statistics" section, combined results from the two experiments were used to estimate the relative contribution of food, temperature and light on clearance rate.

Experiment 1

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

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

Experiment 2

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

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

Relative contribution of food, temperature and light on clearance rate

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Conclusion

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

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

Authors Contributions

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

Data Availability Statement

Data are available upon personal request.

Conflict of Interest Statement

Authors declare no conflict of interest.

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Table 1. Summary of water quality parameters measured for the four temperature conditions during the experiment. Values are means ± SD. DO: dissolved oxygen, TDS: total dissolved solids.

Parameters	Experimental treatment			
	10°C	15°C	20°C	25°C
Temperature (°C)	10.3 ± 0.1	14.9 ± 0.2	20.3 ± 0.2	24.9 ± 0.2
pH	8.02 ± 0.10	8.05 ± 0.11	8.08 ± 0.12	8.26 ± 0.13
DO saturation (%)	92.2 ± 2.3	91.9 ± 2.4	91.8 ± 2.5	92.4 ± 2.0
DO (mg L^{-1})	9.82 ± 0.42	8.96 ± 0.28	8.03 ± 0.25	7.49 ± 0.27
Conductivity A (µS cm^{-1})	162 ± 16	164 ± 18	173 ± 19	178 ± 24
TDS (mg L^{-1})	85 ± 11	85 ± 11	88 ± 12	89 ± 12
NH_3-N (mg L^{-1})	0.05 ± 0.04	0.03 ± 0.02	0.02 ± 0.03	0.02 ± 0.02
NO_2^- (mg L^{-1})	0.014 ± 0.019	0.019 ± 0.015	0.005 ± 0.004	0.004 ± 0.008

Table 2. Three-way mixed analysis of variance (ANOVA) results. Effect of light (i.e. 'dark' and 'light' within-subjects factor) and temperature (10, 15, 20 and 25 °C) on the clearance rates of bivalves of two different species (*C. fluminea* and *L. ovata*) at two different concentrations of food: 'low' food (i.e. 10,000 cells mL^{-1}) and 'high' food (i.e. 100,000 cells mL^{-1}) experiments.

Effect	Degrees of freedom		Low food		High food	
	numDF	denDF	F-value	p-value	F-value	p-value
Temperature	3	56	6.36	**< 0.001**	25.31	**< 0.001**
Species	1	56	77.77	**< 0.001**	20.81	**< 0.001**
Light	1	56	0.01	**0.913**	37.54	**< 0.001**
Temperature x Species	3	56	5.29	**0.003**	3.29	**0.027**
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

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

Predictor	*C. fluminea*		*L. ovata*	
	Estimate (± SD)	p-value	Estimate (± SD)	p-value
Intercept	41.22 (46.06)	0.374	-118.10 (35.01)	**0.001**
Temperature	10.53 (2.62)	**< 0.001**	14.01 (2.07)	**< 0.001**
Food (Low)	-4.41 (28.48)	0.877	334.25 (25.14)	**< 0.001**
Light (Light)	-58.03 (24.59)	**0.022**	-46.57 (20.81)	**0.029**

Figure captions

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

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

Fig. 3. Percent of individuals filtering during the 'low food' (i.e. 10,000 cells mL^{-1}) experiment for *Corbicula fluminea* and *Lampsilis ovata* exposed to two light intensities (i.e. 'dark': 0 ± 0 µmol m^{-2} s^{-1} and 'light': 19 ± 2 µmol m^{-2} s^{-1}) and four temperatures (10, 15, 20 and 25 °C).

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

Fig. 5. Percent of individuals filtering during the 'high' food (i.e. 100,000 cells mL^{-1}) experiment for *Corbicula fluminea* and *Lampsilis ovata* exposed to two light intensities (i.e. 'dark': 0 ± 0 µmol m^{-2} s^{-1} and 'light': 19 ± 2 µmol m^{-2} s^{-1}) and four temperatures (10, 15, 20 and 25 °C).

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

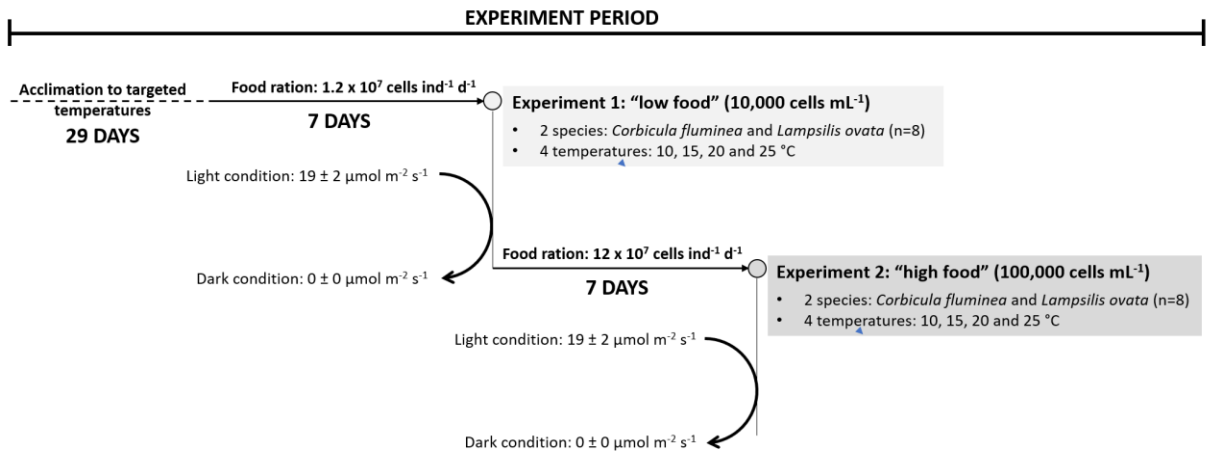


Figure 1

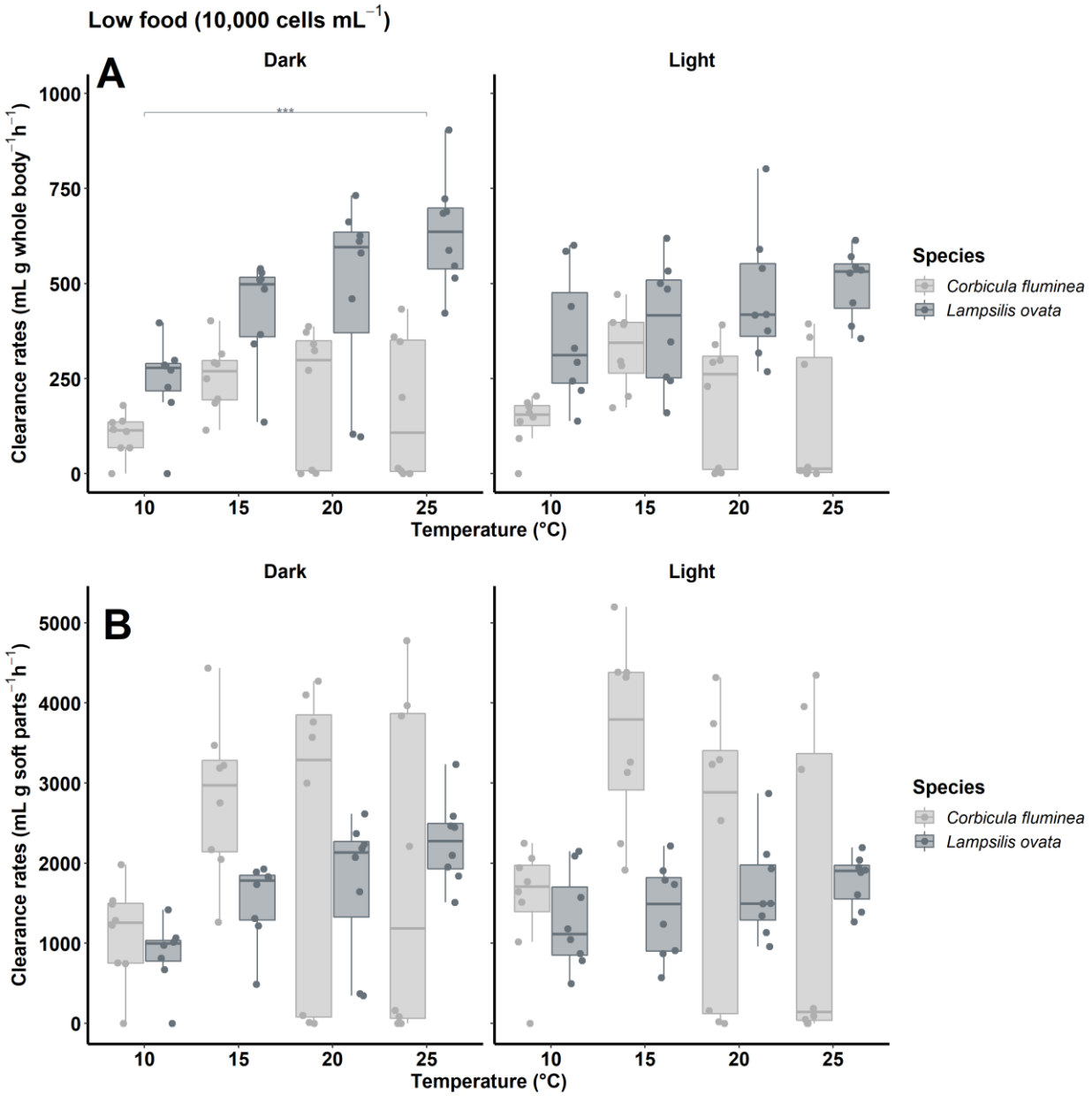

Low food (10,000 cells mL^{-1})
A
Dark
Light
***
Clearance rates (mL g whole body^{-1}h^{-1})
1000
750
500
250
0
10
15
20
25
Temperature (°C)
Species
Corbicula fluminea
Lampsilis ovata
B
Clearance rates (mL g soft parts^{-1}h^{-1})
5000
4000
3000
2000
1000
0

Figure 2

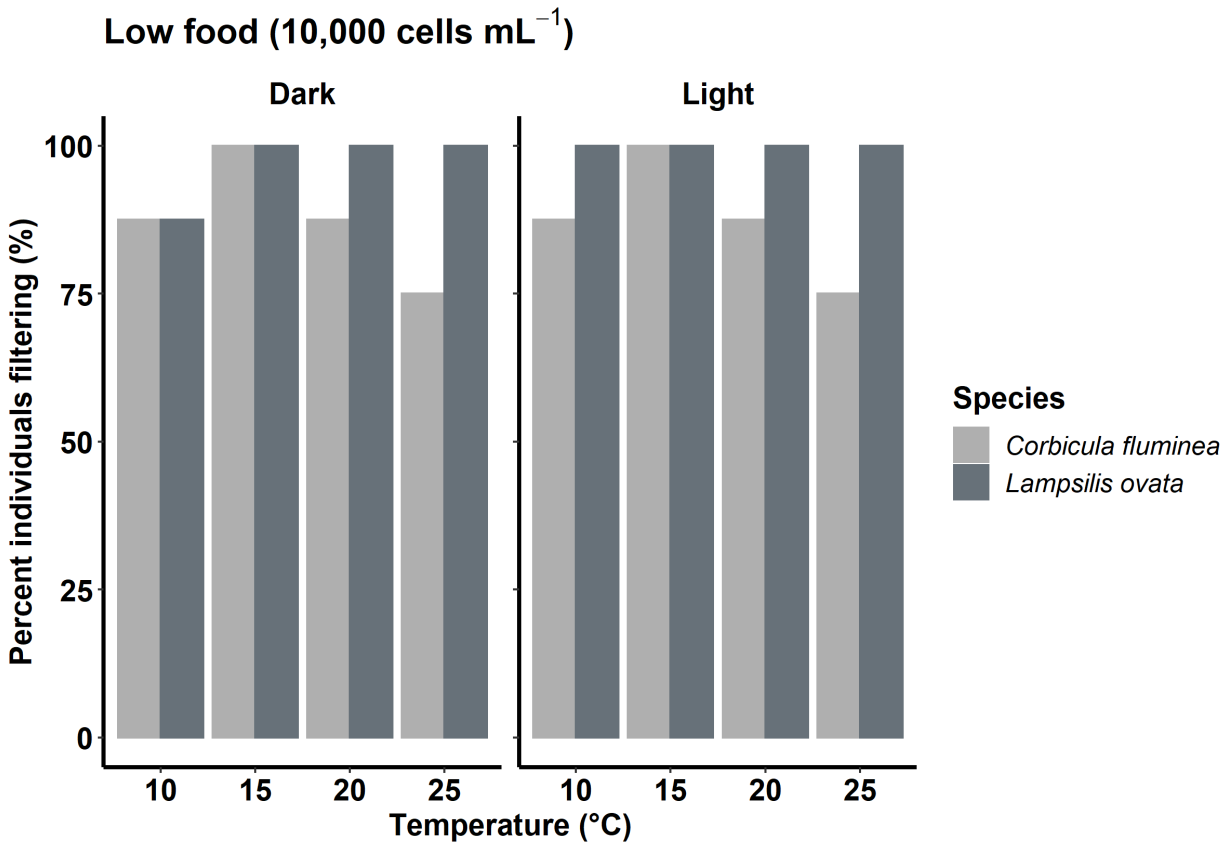
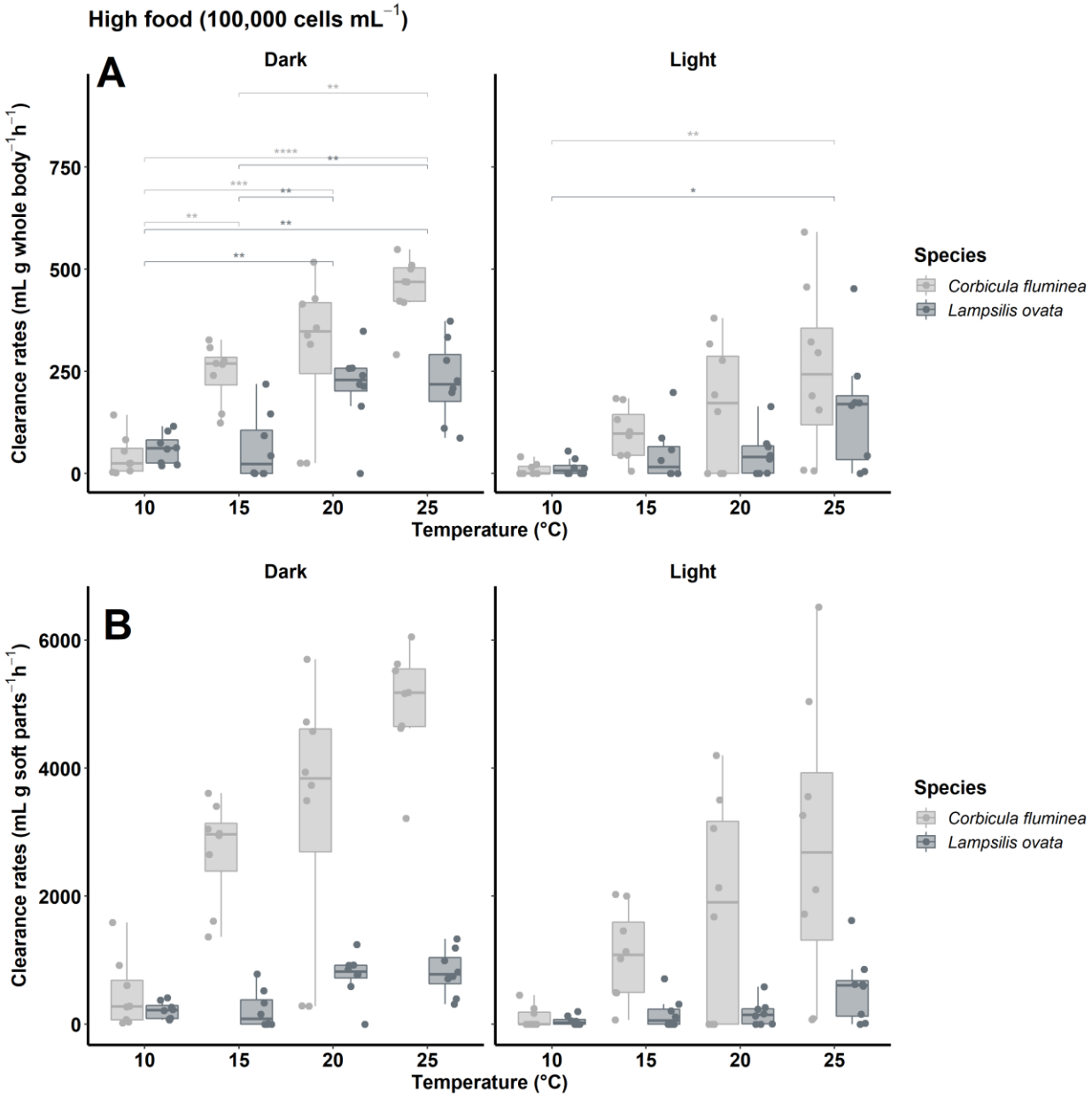

Low food (10,000 cells mL^{-1})
Dark
Light
Percent individuals filtering (%)
100
75
50
25
0
10
15
20
25
10
15
20
25
Temperature (°C)
Species
Corbicula fluminea
Lampsilis ovata

Figure 3


High food (100,000 cells mL^{-1})
A
Dark
Light
Clearance rates (mL g whole body^{-1}h^{-1})
750
500
250
0
10
15
20
25
Temperature (°C)
Species
Corbicula fluminea
Lampsilis ovata
B
Clearance rates (mL g soft parts^{-1}h^{-1})
6000
4000
2000

596 Figure 4

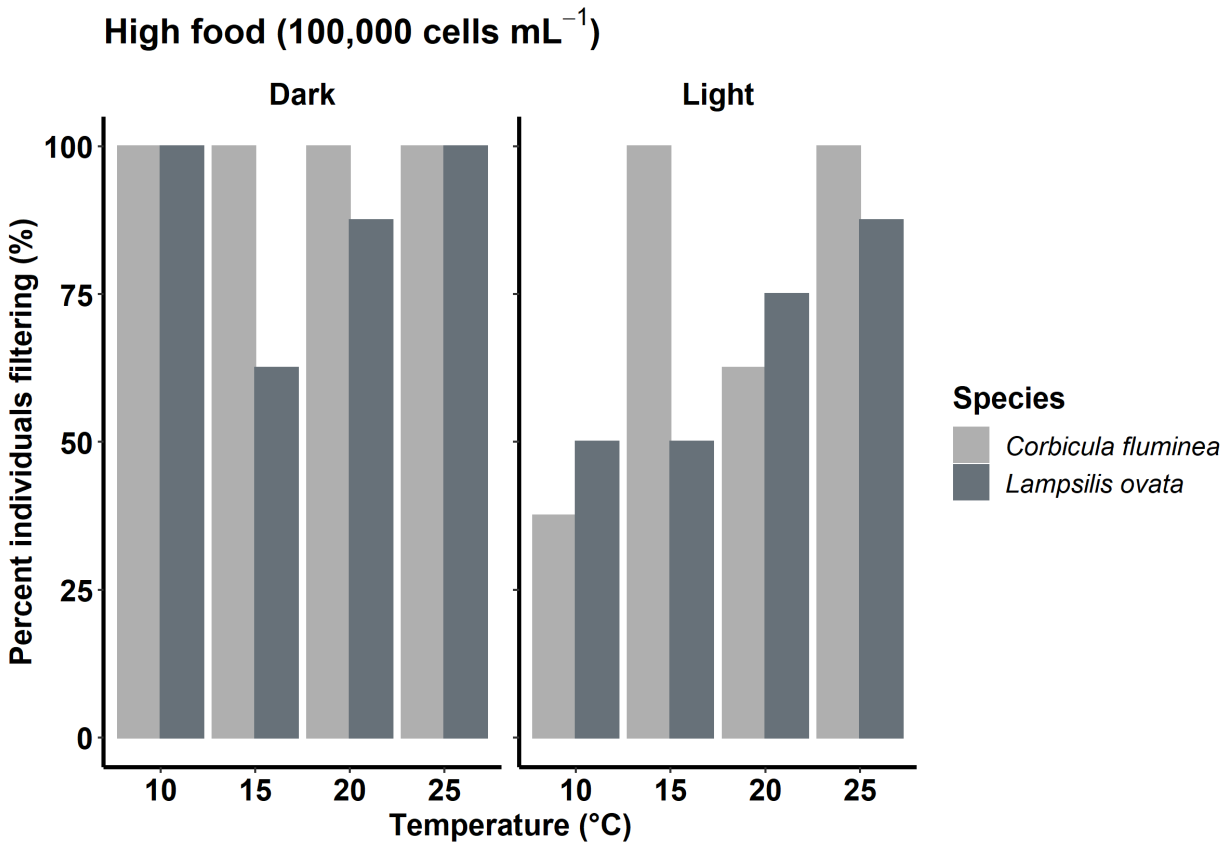

High food (100,000 cells mL^{-1})
Dark
Light
Percent individuals filtering (%)
100
75
50
25
0
10
15
20
25
Temperature (°C)
Species
Corbicula fluminea
Lampsilis ovata

Figure 5

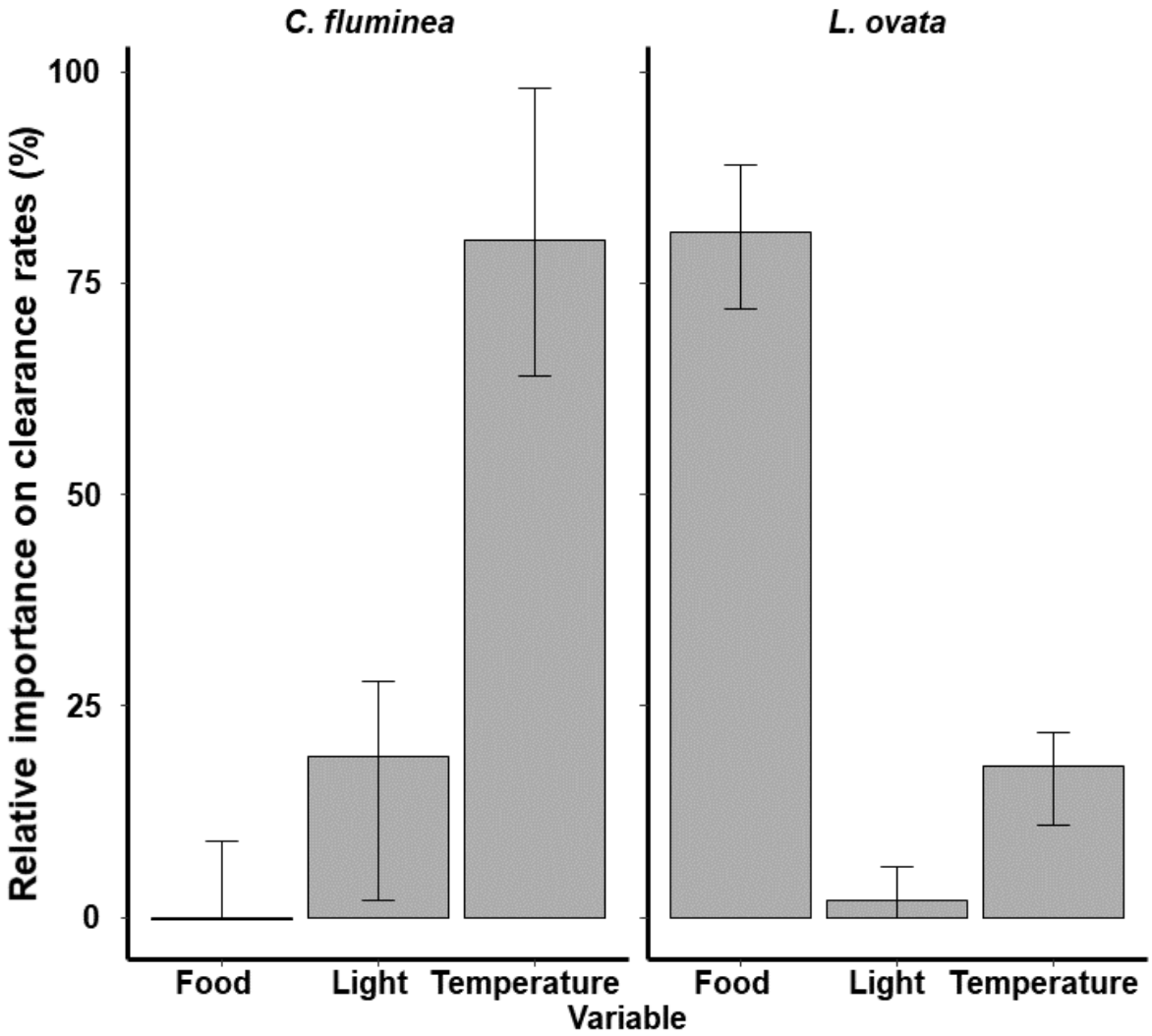

C. fluminea
L. ovata
Relative importance on clearance rates (%)
100
75
50
25
0
Food
Light
Temperature
Food
Light
Temperature
Variable

Figure 6