Functional response and size-selective clearance of suspended matter by an invasive mussel

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HIGHLIGHTS

- Golden mussels have a type I functional response.
- Suppressive effect on suspended matter is mussel abundance-dependent.
- Size of suspended particles affects their capture efficiency by golden mussels.

GRAPHICAL ABSTRACT
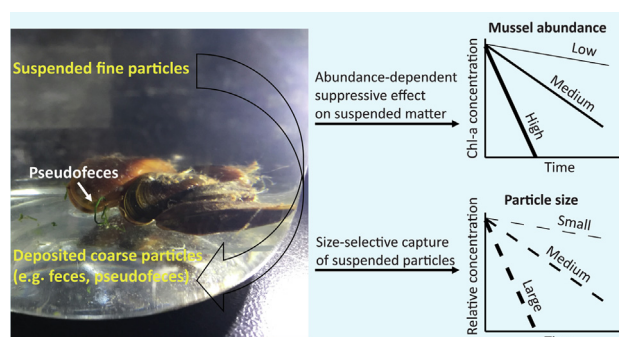
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ABSTRACT

Filter feeding activities link suspension feeders with their environment and underpin their impact on aquatic ecosystems. Despite their ecological and economic impacts, the functional response and size-selective capture of suspended particulates have not been well documented for the golden mussel *Limnoperna fortunei*. Here we demonstrated that golden mussels had a type I functional response, with an attack rate $a = 0.085$ and negligible handling time (h). Clearance rate ranged between 72.6 ± 27.0 and 305.5 ± 105.9 mL ind.$^{-1}$h^{-1} (Mean $\pm$ S.E.), depending on food concentrations, which exhibited an inverse relationship with clearance rate. Presence of golden mussels suppressed chlorophyll *a* concentration in experimental mesocosms, the extent of which was dependent on mussel abundance. Concentration of suspended particles in experimental mesocosms experienced a sharp initial decline across all size categories (≤ 1–>50 μm), though with increased final concentration of large particles (>25 μm), indicating packaging and egestion by golden mussels of fine particles (down to ≤ 1 μm). Capture efficiency of quantitatively-dominant suspended matter (≤ 1–50 μm) by golden mussels was inversely related to particle size. Animal abundance, particle size, and their interaction (abundance $\times$ particle size) determined the extent to which matter was removed from the water column. Presently *L. fortunei* occurs primarily in the southern end of the central route of South to North Water

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Diversion Project (China), but the species is spreading north; we anticipate that impacts associated with filtering of *L. fortunei* will correspond with local population abundance along this gradient.

1. Introduction

Aquatic ecosystems worldwide have endured a series of high-profile species invasions (Bobeldyk et al. 2015; Carlton et al. 2017). Invasive suspension feeders including many bivalves represent can profoundly influence invaded environments (Gili & Coma 1998; Boltovskoy & Correa 2015). Through a variety of interactions with local abiotic conditions and biological communities, suspension feeding invaders serve as potential ecosystem engineers (e.g., MacIsaac 1996; Gili & Coma 1998; Boltovskoy & Correa 2015; Linares et al. 2017). For example, their filter-feeding activities deposit suspended matter from the water column to the benthos (Nishizaki & Ackerman 2017), leading to food depletion of pelagic habitats but supplementation of benthic ones, alteration of geochemical cycling, enhancement of water clarity and of aquatic macrophytes, and shifts of resource utilization in food webs (Hecky et al. 2004; Boltovskoy & Correa 2015; Karatayev et al. 2015; Fera et al. 2017; Knight et al. 2018). Furthermore, many invasive bivalves change physical habitats by forming massively dense aggregates (Gili & Coma 1998; Boltovskoy & Correa 2015). Generally, these impacts will be highly distinguished in systems where no native counterparts of the invaders exist (Ricciardi & Atkinson 2004).

Furthermore, the filter-feeding invaders link their overall impacts to their abundance and per capita clearance rate (CR), the latter of which is defined as the amount of water cleared of food per individual per hour (Darrigran & Damborenea 2010; Karatayev et al. 2015). CR is impacted by many factors, notably food concentration (e.g., Riisgård et al. 2011; Sarnelle et al. 2015; Tokumon et al. 2015). The response of CR to varying food levels, particularly at low concentration, reflects resource uptake and has enormous implications of their interactions with environment (Lehman 1976; Gili & Coma 1998). Given that food availability may differ in space and time, it could contribute to invasion success by influencing nutritional status of animals and, if sufficient, allow for a wide distribution of invaders (e.g., Brown et al. 2013; Laverty et al. 2015; Bracken, 2017; Dick et al. 2017). Furthermore, aquatic systems with sufficient and/or quality food resources may sustain larger populations than those with limited resources (see review by Vaughn & Hakenkamp 2001). Apart from substantial deposition of suspended matter, many suspension feeders (e.g., bivalves) demonstrate size-selective clearance of suspended particulates, which, in turn, may alter the size distribution of suspended matter in the water column (Ward & Shumway 2004) and alter composition of phytoplankton communities (Vanderploeg et al. 2001, Cataldo et al. 2012). Thus, a comprehensive understanding of the filter-feeding behaviors, including the response of CR, to varying food levels and size-selective removal of suspended matter, can inform predictions of the invasiveness and potential impacts of filter-feeding invaders.

The golden mussel *Limnoperna fortunei* is a suspension-feeding, invasive bivalve in freshwater ecosystems in Asia and South America (Ricciardi 1998; Xia et al. 2018). It serves as an ecosystem engineer and exerts profound ecological impacts on many invaded habitats (Darrigran & Damborenea 2010; Boltovskoy & Correa 2015). Furthermore, its fouling impairs many submerged systems that use raw water, including drinking water supply systems, hydropower plants, irrigation stations, and shipping industries (Xu et al. 2014; Nakano et al. 2015; Chakraborti et al. 2016; Li

et al. 2019). Trans-oceanic spread of the species has been facilitated by discharge of contaminated ballast water, and possibly by fouling of vessels or by the aquaculture trade (Boltovskoy 2015; Nakano et al. 2015). Inland spreading has been facilitated by anthropogenic hydraulic programs such as dam construction (Nakano et al. 2015) and water transfer projects (Xu et al. 2014; Zhan et al. 2015). For example, the > 1200 km long central route of the South to North Water Diversion Project (SNWDP) in China connects areas inhabited by the golden mussel in the south (donor) to uninhabited water bodies in the north (recipient), facilitating dispersal of this species (Zhan et al. 2015). Relative to natural ecosystems, such anthropogenic hydraulic facilities may support much denser populations of golden mussels owing to vast amounts of concrete which could serve as settling substrate and result in great potential impacts (Xu et al. 2014; Nakano et al. 2015). To our knowledge, the golden mussel is the only biofouling, filter-feeding bivalve in this region and the potential recipient water environments of the SNWDP.

The filter-feeding behavior of golden mussels has been measured in several studies, particularly in invasive populations in South America (see Boltovskoy 2015). Factors such as animal size, temperature, food types, and inorganic matter influence their CR (Sylvester et al. 2005; Pestana et al. 2009). However, the functional response has not been explored. Also, there is a paucity of knowledge regarding the species' impact on different size classes of suspended matter. In this study, we tested the CR of golden mussels exposed to varying food concentrations and investigated their functional response. We also tested their suppressive effects on phytoplankton in mesocosms and their potential to alter the size distribution of suspended matter.

2. Materials and methods

2.1. Study design

We first tested the CR of the golden mussels under varying levels of algae concentration using nontoxic monospecific algae *Chlorella vulgaris* cultured in laboratory, which was followed by modeling the functional response of the species. Next, we conducted two independent 7-day experiments in mesocosms to investigate the suppressive effects of golden mussels on growing algae (measured as chlorophyll *a* concentration) (experiment 1), and size-selective clearance of suspended particles of various sizes (experiment 2), respectively. Specifically, algae food was mimicked by adding commercial green algae to experimental tanks daily in experiment 1, and suspended matter with varying sizes was prepared by collecting lake seston in experiment 2. Given that abundance of golden mussels in natural environment varies widely (e.g., 85–1.7×10^5 ind. m^{-2}, Boltovskoy 2015), we set different animal densities in the two experiments.

2.2. Animal collections

Golden mussels were collected from the underside of a floating dock in Danjiangkou Reservoir, China (32°45′N, 111°35′E) by carefully cutting the byssal threads. Mussels were transported in coolers to laboratory and raised in dechlorinated water at 24 °C in a 60-L fish tank. Typical adult-size individuals were separated from mussel aggregates by carefully cutting byssal threads, and were

subsequently placed on glass slides to allow reattachment. Commercial fish food (~50 mL concentrated algae culture (*Chlorella* sp.), concentration > 10^6 cells mL^{-1}) was fed to animals daily before experiments.

2.3. Functional response test

Nontoxic *Chlorella vulgaris* was served as food for golden mussels to test their CR. Algae was purchased from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences, and cultured following the provider's instructions. Food suspensions with varying algae concentrations were prepared by adding algae culture to dechlorinated tap water. Specifically, a total of nine algae concentrations (i.e., 276–37077 cells mL^{-1}) were prepared (and tested) by adding 20, 30, 50, 60, 120, 240, 500, 1000, and 2000 µL of cultured algae to 1.8 L dechlorinated tap water, respectively (Table S1). The food concentration range was reflective of what was observed in the potential receiving water bodies of the SNWDP (e.g., Miyun Reservoir and Tuancheng Lake, Beijing). Slides with a single average-size (~15 mm) mussel that firmly attached were exposed in food suspension for 24 h prior to a 6 h gut clearance interval in filtered (0.22 µm pore size) dechlorinated tap water. Animals were randomly assigned to different food concentrations. Only typical adult-size animals were used though each individual size was not measured. Tests were carried out with five experimental replicates for each food concentration and three no-animal controls in beakers with 200 mL food suspension. Beakers were placed on a shaker at 100 rpm (revolutions per minute) which mixed water but did not affect animals' filtration activities during the experiment. Animals were active (i.e., ventilating) and most started to filter water within one minute of being placed into a beaker. We started timing once a minimum of three animals began filtering and terminated after 10 min according to pilot tests. We collected 1 mL of food suspension for initial algae concentration from each beaker immediately after animals were introduced, and this was repeated at the end of experiment for the final concentration. The concentration of algae was measured immediately after collection of each batch (i.e., initial and final) of samples using a BD Accuri C6 flow cytometry instrument (Becton, Dickinson and Company, US). 200 µL of each mixed sample was loaded to quantify algae, and a 5 s sample shake for every two samples was programmed to reduce algae settlement during measurement. Cell diameter of *C. vulgaris* was 2.54 ± 0.56 µm (Mean ± S.D., n = 118), which was measured under a microscope (10 × 40, Olympus CX41, Japan).

Ingestion rate (IR) (i.e., the number of algae cells consumed per mussel per hour) was calculated as:

$$IR = V * \frac{[(Ci - Cf) - (Ci' - Cf')]}{N * T}$$

which was adopted from Tokumon et al. (2015), where V is the algae suspension volume (mL), N is the number of animals in each experimental beaker, T is the experimental duration (h), Ci and Cf are initial and final algae concentration (cells mL^{-1}) in each experimental beaker, while Ci' and Cf' are the average initial and final algae concentration of three control beakers, respectively. CR in each experimental beaker was calculated following Coughlan (1969):

$$CR = V * \frac{\left[Ln\left(\frac{Ci}{Cf}\right) - Ln\left(\frac{Ci'}{Cf'}\right)\right]}{N * T}$$

Functional response (FR) of the golden mussel was tested and modeled using the R package "frair", in which the type I FR was chosen to fit the FR curve following a preliminary comparison with the Rogers type II FR (Pritchard, 2017). Non-parametric bootstrap

(n = 2000) was conducted to generate 95% confidence intervals for the fitted FR curve, and statistical analysis was performed in R 3.5.1 (R core team, 2018). Two of the five replicates in each of three lowest algae concentration treatments (i.e., 276, 491, and 908 cells mL^{-1}) and one in each of two high algae concentration treatments (i.e., 3780 and 19,035 cells mL^{-1}) were negative and were discarded. The former problem was likely due to sampling and (or) enumeration bias, while the latter was unclear as we tried to reduce algae settlement during the experiment.

2.4. Suppressive effect on growing algae test

Four golden mussel aggregates with various individuals (n = 1, 4, 8, 16) and similar size [medium: ~12–18 mm shell length, no significant difference among aggregates ($F_{3, 25}$ = 1.21, P = 0.325, One-way ANOVA)] – which was measured after experiment – were used to graze on commercial fish food (*Chlorella* sp. cells). Each mussel clump was placed at the center of a round plastic bucket containing 10 L dechlorinated tap water (~30 cm deep). Each bucket was aerated with an air stone placed at the bottom by the wall ~ 10 cm away from the mussel clump. Only one air stone was used per tank to reduce potential breakdown of large particles such as feces and (or) pseudofeces produced, which may bias clearance of suspended particles with varying sizes, though settling of suspended particles may be inevitable as a result. Three control tanks without animals were arranged with the same setup to correct the effect of natural settling on suspended matter. Given that the food used in this experiment was a single algal species, we used chlorophyll *a* as a proxy of algal density (Gitelson et al. 1999). Before animals were introduced, chlorophyll *a* concentration in each bucket was adjusted to the same level (2–3 µg L^{-1}, day 0 in Fig. 3 A). Chlorophyll *a* concentration was measured *in situ* (~10 cm deep) using a handheld fluorometer (Turner Designs, U.S.). Water temperature and pH (multiparameter water quality sonde, MYRON, USA), and dissolved oxygen (DO meter, HACH, USA) were also monitored throughout the experiment. All measurements were carried carefully without re-suspending materials from the bottom. 10 mL of the concentrated *Chlorella* sp. was added to each tank following daily measurements (i.e., once per day) throughout the experiment. At the end of the experiment (day 7), a 500 mL water sample from the subsurface of each tank was collected to measure particle size of suspended matter using a particle counter (PAMAS Water Viewer, Germany), and the average of three reads for each sample was used in data analysis. The change percentage of suspension particulates for each size category relative to no-animal controls was calculated as:

$$Change\ percentage = \frac{Ct - Cc}{Cc} * 100\%$$

where Ct is the concentration for each experimental mesocosm, and Cc is the average of all controls without animals.

2.5. Size-selective clearance of suspended particulates test

Lake-water was collected from the surface layer of Kunming Lake (Beijing, China) and transported to laboratory within one hour. Prior to use, large inorganic particles were removed by a 2-hour settlement in laboratory, and the initial chlorophyll *a* concentration and water pH were ~ 66 µg L^{-1} and 8.05, respectively. Lake water was mixed and distributed into six tanks (10 L each), and each tank was aerated by the same settings in 2.4. Four mussel clumps with wet weights of 1.96 g (T1, 4 mussels), 10.1 g (T2, 17 mussels), 16.2 g (T3, 31 mussels) and 33.1 g (T4, 100 mussels) – which were weighted prior to test – were randomly assigned into 4 tanks (experimental) and the remaining (2 tanks) served as controls. Only two tanks were used for controls due to a limitation in

tank availability. Here the average wet weight of mussel in T4 (0.331 g) was lighter than in the three groups with less dense animals, while it was reflective of adult mussel size (e.g., >15 mm shell length) according to our bivalve biometric analysis using mussels collected from the same site and around the same time (Z. Xia, unpublished data). Chlorophyll *a* concentration and size of suspended particulates in each tank were measured *in situ* daily between days 0 and 7 using methods described in 2.4. No additional food was provided to animals, and no mortality was observed until day 7 (i.e., two mussels died in T1 and one in T3, respectively), which was not included in analysis to avoid bias. The size of suspended particulates was partitioned into eight default categories of the particle counter (i.e., ≤1, 1–2, 2–4, 4–8, 8–15, 15–25, 25–50, and > 50 μm), and the change percentage of each size category was calculated as described in 2.4. Capture efficiency of each size category of suspended particles was characterized as the removal rate (%) on the first day since introduction of golden mussels. To explore factors affecting removal rate on the first day, we applied a generalized linear model to project the change percentage of suspended particles as a function of animal abundance, particle size, and their interaction (i.e., abundance × particle size). To simplify modeling, we reduced the number of variables for particle size by combining the default categories into three new ones (i.e., ≤2, 2–25, and > 25 μm) within each the original categories exhibited similar effects on capture efficiency.

3. Results

3.1. Clearance rate and functional response of golden mussel

Average attenuation rate of algae density in control beakers was 2.9% (range: 1.1–4.8%) during the experiment, suggesting sufficient mixing of food suspension and full access of mussels to the food resource. CR of golden mussels tested with nontoxic strain of *Chlorella vulgaris* differed among algae concentrations, ranging from a high of 305.5 ± 105.9 mL ind.$^{-1}$h^{-1} to a low of 72.6 ± 27.0 (Mean ± S.E.) (Fig. 1). Specifically, CR was highest at low food concentrations (e.g., 491–908 cells mL^{-1}) and declined as algae concentration increased (Fig. 1), though no statistical significance among food levels ($F_{8, 28} = 1.95$, $P = 0.091$, One-way ANOVA) and greater variance at low food levels were found. As expected, golden mussels exhibited a linearly-increasing IR with increasing algae concentration, resulting in a type I functional response ($F_{1, 35} = 41.3$, $R^2 = 0.541$, $P < 0.0001$, Fig. 2). The estimated attack rate was $a = 0.502$ ($P < 0.001$) with a negligible handling time (h) (Pritchard, 2017), indicating a saturation point higher than the maximum algae density in our experimental setup (i.e., ~37000 cells mL^{-1}).

3.2. Chlorophyll a clearance

In experiment 1 for which the mussels were daily fed by adding algae, chlorophyll *a* concentration in control tanks contentiously increased. However, presence of golden mussels demonstrated suppressive effects on this pattern, and animal abundance was positively related to the extent of chlorophyll change (Fig. 3 A). Specifically, clumps of animals (i.e., n = 4, 8, and 16) constrained the increase of chlorophyll *a* concentration, resulting in a significantly lower final concentration relative to controls lacking mussels (1.07 ± 0.43 *vs.* 18.22 ± 0.71 μg L^{-1}, Mean ± S.E.) ($t_4 = 20.6$, $P < 0.0001$, Student *t*-test). In contrast, the one-animal treatment had very limited effects ($t_2 = 1.43$, $P = 0.29$, One-sample *t*-test) and chlorophyll *a* concentration consistently increased throughout the experiment (Fig. 3 A). The highest chlorophyll *a* concentration
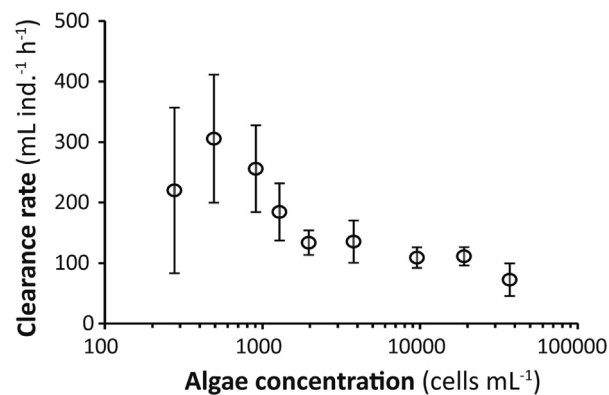

Fig. 1. Clearance rate (Mean ± S.E.) of golden mussels in aquaria (24 °C) at varying algae concentrations.
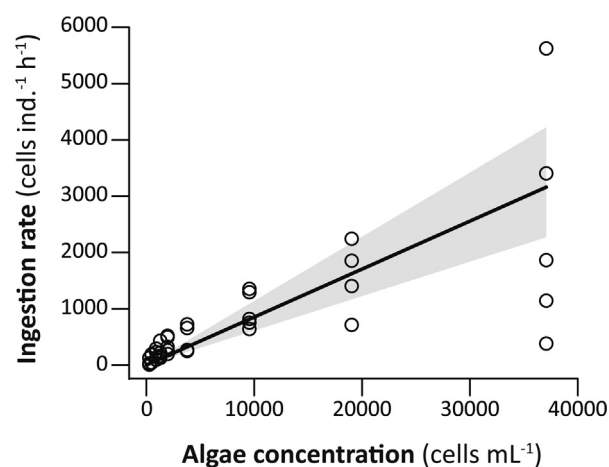

Fig. 2. Fitted functional response (with 95% confidence interval, gray area) of golden mussels. Open circles indicate the actual ingestion rate.

achieved was inversely related to animal abundance, with 17.2, 6.7, 5.5, and 3.4 μg L^{-1} in mesocosms with 1, 4, 8, and 16 individuals, respectively (Fig. 3 A). Temperature (23.9 ± 0.3 °C), pH (8.07 ± 0.12) and DO (104 ± 3.2%) (Mean ± S.E.) were relatively stable throughout the experiment, suitable for golden mussels (e.g., Boltovskoy 2015). Similar effects were found for golden mussels exposed in lake water where no additional food was added. Chlorophyll *a* concentration decreased in all tanks (including controls) while the presence of golden mussels facilitated the attenuation process. For example, the chlorophyll *a* concentration became undetectable at day 2 in tanks with n = 17, 31, and 100, day 3 with n = 4, and day 5 with n = 0 mussels, respectively (Fig. 3 B). Even in the tank with the lowest number of mussels (n = 4), a significantly reduced chlorophyll *a* was observed (i.e., days 1–4, $t_3 = 3.4$, $P = 0.042$, Paired-sample *t*-test).

3.3. Size-selective clearance of suspended particulates

Golden mussels substantially removed suspended matter from the water column and altered its size distribution relative to controls lacking animals (Figs. 4 & 5). At the end of experiment 1 (day 7) for which algae was added, the concentration of suspended particulates in tanks with mussels declined substantially relative to controls across most size categories (Fig. 4). Animal abundance ($F_{3, 64} = 562.3$, $P < 0.001$), particle size ($F_{7, 64} = 271.7$, $P < 0.001$), and their interaction (abundance × particle size) ($F_{21, 64} = 341.6$, $P < 0.001$) (Two-way ANOVA) were significantly related to the
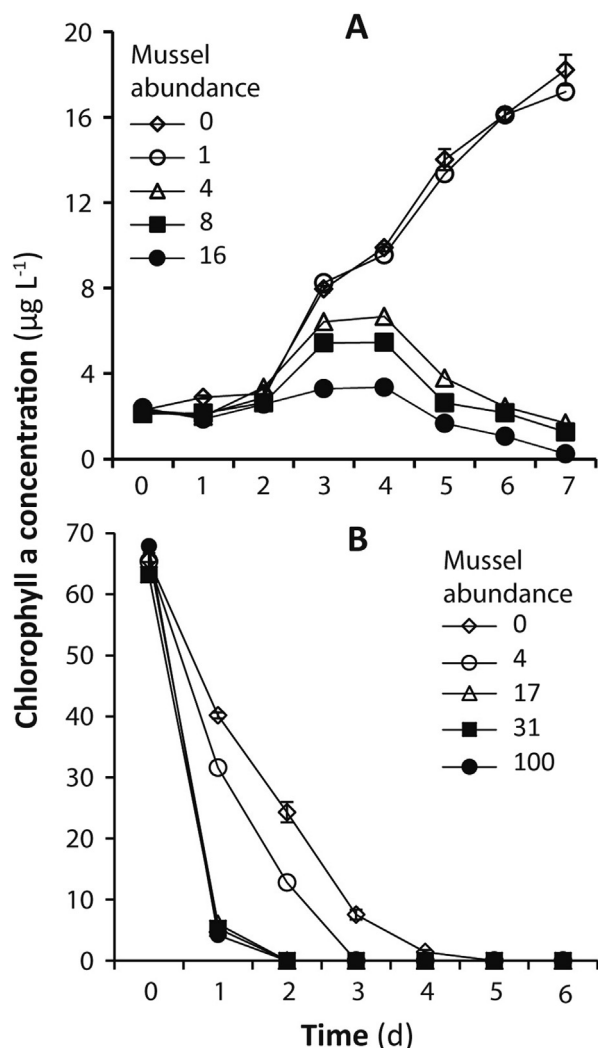
Fig. 3. Chlorophyll *a* concentration in mesocosms in which golden mussel aggregates with different number of individuals were (A) fed daily or (B) not fed during the experiment. Test water was irradiated tap water in (A) and raw lake water in (B). Error bars indicate standard errors of the average concentration in no-animal control mesocosms.

change percentage. Specifically, concentration of suspended particles declined significantly with animal abundance (P < 0.001) except between 4- and 8- individual treatments (P = 0.997, Tukey's post hoc test). Suppressive effects on suspended particles were observed across all size categories, with the exception of the > 25 μm particles in the 16-individual treatment, which yielded remarkably higher concentrations than controls (Fig. 4).

In experiment 2 for which no additional food was added, the concentration of suspended particles in control tanks attenuated with time (Table S3). However, tanks with mussels experienced much sharper initial decline of suspended particles relative to no-mussel controls after which patterns became less distinguishable (Fig. 5). Specifically, the majority of suspended particulates removed occurred within the first three days, while this process was prolonged for the treatment with the lowest animal abundance (n = 4) (Fig. 5), similar to patterns of chlorophyll *a* (Fig. 3 B). In most cases, the concentration dropped sharply on day 1 (Fig. 5), during which the animal abundance, particle size, and their interaction demonstrated significant relationships with the change percentage, explained 72.6% of deviance (Table 1). Specifically, animal abundance = 17 and greater, and particle size > 25 μm exhibited a significant negative relationship with change percentage of

suspended particles. Interactions between abundance and particle size, however, exhibited either negative (i.e., abundance = 17 or = 31 × size 2–25 μm) or positive (i.e., abundance = 31 or = 100 × size > 25 μm) relationships with change percentage (Table 1). As a result, capture efficiency of suspended particles was positively related to particle size (Fig. 6). Change percentages of suspended particles > 25 μm were significantly related to the interaction term in treatments of mussel abundance = 31 and = 100 (Table 1), which were not considered the capture efficiency to avoid bias (Fig. 6).

4. Discussion

The functional response of species plays a central role in understanding their interactions with food resources. For invasive species, functional response can be used to evaluate impact potential relative to either native or other invasive counterparts (e.g., Dick et al. 2017; Hoxha et al. 2018) and has been proposed as a universal trait to predict impacts (Dick et al. 2017). Golden mussels exhibited a type I functional response (Fig. 2), consistent with existing studies on many suspension feeders (Jeschke et al. 2004, but see Sarnelle et al. 2015). The measured FRs indicate a strong clearance potential on suspended matter (e.g., phytoplankton and organic debris) as the mussels demonstrated a linear IR across the wide range of food concentrations used (Pritchard, 2017). Our results demonstrated strong influences of food concentration on CR – highest at low food concentrations and reduced at higher food concentrations (Fig. 1) – consistent with numerous existing studies (e.g., Riisgård et al. 2011; Tokumon et al. 2015). This is mainly owing to low energy needed during filtering activities in bivalves, allowing individuals to filter at maximum rates (Willows 1992; Gili & Coma 1998). Such characteristics may maximize the energy gain of golden mussels. It should be noted that reduced CR is possible when golden mussels encounter long-term starvation (e.g., no food supplied). For example, golden mussels can tolerate up to 125 days of starvation, during which the animals reduced filtering activities by closing valves (Cordeiro et al. 2016). A similar phenomenon was observed in blue mussels, which was proposed as an energy-saving mechanism (Riisgård & Larsen 2015).

The concentration of suspended particulates across all size categories initially declined following introduction of golden mussels (Fig. 5), indicating that the mussel is capable of capturing a broad array of particles. The extent to which the suspended particulates were removed was highly dependent on animal abundance (Figs. 5 & 6). During the first day of exposure, we observed different removal rates for different size categories, indicating differing capture efficiencies (Fig. 6). Generally, fine particles (e.g., <2 μm) were captured at lower efficiency (Fig. 6, n = 4 & 17). However, a high overall clearance rate can be achieved if animals are present at very high abundance (e.g., n = 100, Fig. 6, Table 1). In experiment 2, the use of lighter wet weighted mussels in T4 (i.e., n = 100) might not underestimate the overall clearance effect (see Pestana et al. 2009). In both experiments, the concentration of fine particles declined despite initially being more abundant (Tables S2 &S3), while large particles (e.g., >25 μm) had higher final concentrations than in controls (Figs. 4-5). These findings are consistent with the view that golden mussels package fine suspended matter to coarse particles. The increased coarse particles in the tanks with high mussel abundance likely resulted from the ejection of feces through the exhalant siphon and/or pseudofeces through the inhalant siphon, which has been suggested to improve local mixing of the benthic layer near mussel aggregates (MacIsaac & Rocha 1995; Nishizaki & Ackerman 2017). In contrast, such patterns were not observed in tanks with low mussel abundance, which was likely because of less coarse particles produced and limited total ejection effect
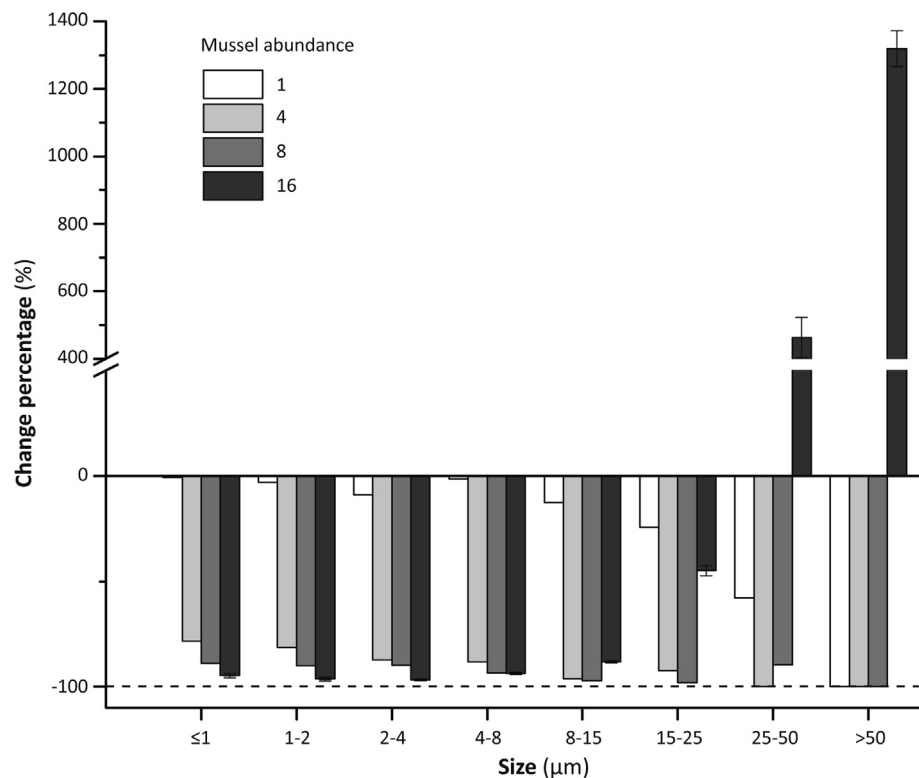
Fig. 4. Change percentage (Mean ± S.E.) of concentration of suspended particulates in mesocosms with golden mussels relative to no-animal controls, showing results of each size category at the end (d7) of the experiment. Error bars indicate standard error of three replicates for each sample.

by a lower number of mussels. Golden mussels exhibited effective clearance of particles between 2 and 25 μm (Fig. 6), which are the dominant components of suspended organic matter in many natural environments that cause light attenuation (Davies-Colley & Smith 2001). Despite being less effective, fine particles (i.e., <2 μm) were also suppressed and retained at a relatively low level (Figs. 4 & 5). Therefore, massive clearance of these particles from the water column could improve light penetration and change the underwater light climate (Boltovskoy & Correa 2015). Apart from the improvement of light penetration, size-selective capture of particles by golden mussels may cause unbalanced consumption of phytoplankton in natural environments, altering size composition of algae (e.g., Cataldo et al. 2012; Frau et al. 2016). Removal of most edible algae could adversely affect zooplankton and fishes dependent on it.

Similar to suspended particles, we observed strong abundance-dependent clearance of chlorophyll *a* concentration by golden mussels. Specifically, mussels at low abundance exhibited a limited suppressive effect on growing algae (Fig. 3). However, suppression (Fig. 3 A) and removal rate (Fig. 3 B) were increasingly pronounced as mussel abundance increased. In experiment 2, chlorophyll *a* concentration of no-animal control declined, which was likely due to settling of suspended particles and difference of environmental condition between field and experimental tanks. However, the attenuation rate was much faster in tanks with mussels (Fig. 3 B). This highlights the important role of mussel abundance in their overall impact (MacIsaac et al. 1992; Gili & Coma 1998; Linares et al. 2017). The massive clearance of chlorophyll *a*, which is a surrogate for organic matter, may lead to its large-scale depletion in pelagic environments (Officer et al. 1982; Boltovskoy & Correa 2015). Consequently, this may restrain the ingestion rate of total suspended matter due to food concentration declines (Fig. 2) and reduce the growth rate of golden mussels due to food resource deficits (Riisgård & Larsen 2015; Sarnelle et al. 2015). This process

may transform stoichiometry in the surrounding water and enhance the mismatch between golden mussel requirements and available resources (Bracken, 2017). It should be noted that the depletion of suspended matter in pelagic systems can be diminished if hydrodynamic conditions do not permit (e.g., strong re-suspension of sediment in lotic environment or when water column is stratified) or if nutrient cycling is enhanced, stimulating phytoplankton growth (Cataldo et al. 2012; Rowe et al. 2017).

Estimation of total impacts of filter-feeding mussels on surrounding suspended matter relies on proper hydrodynamic conditions that allow for sufficient access of mussels to food resources (MacIsaac et al. 1999; Ackerman et al. 2001). We used one air stone to aerate aquaria in experiments 1 and 2 because we expected to reduce potential breakdown of coarse particles produced. Such settings resulted in gradual loss of suspended particles in no-animal tanks, suggesting insufficient mixing to remain food level in the water column. Reduced food resources may cause an increased clearance rate but reduced ingestion rate of suspended matter by golden mussels (Figs. 1 & 2), leading to underestimation of overall removal rate of suspended matter by dense mussel clumps due to refiltration. Therefore, the mussel abundance-dependent removal of suspended matter should be even strengthened given the higher removal of suspended matter by more abundant mussels that derived from the current settings. It should be noted that insufficient mixing in natural water bodies may be common, and a simplified scale-up of per capita effect derived from well-mixed mesocosms will likely overestimate the true impacts in natural environment (e.g., MacIsaac et al. 1999; Ackerman et al. 2001).

The large SNWDP water diversion project in China addresses the serious problem of regional water scarcity, though it may facilitate species dispersal into new regions (Xu et al. 2014; Zhan et al. 2015). In the case of golden mussels in the central route of SNWDP, several characteristics can amplify impacts associated with this suspension-feeding mussel. First, extensive concrete channels pro-
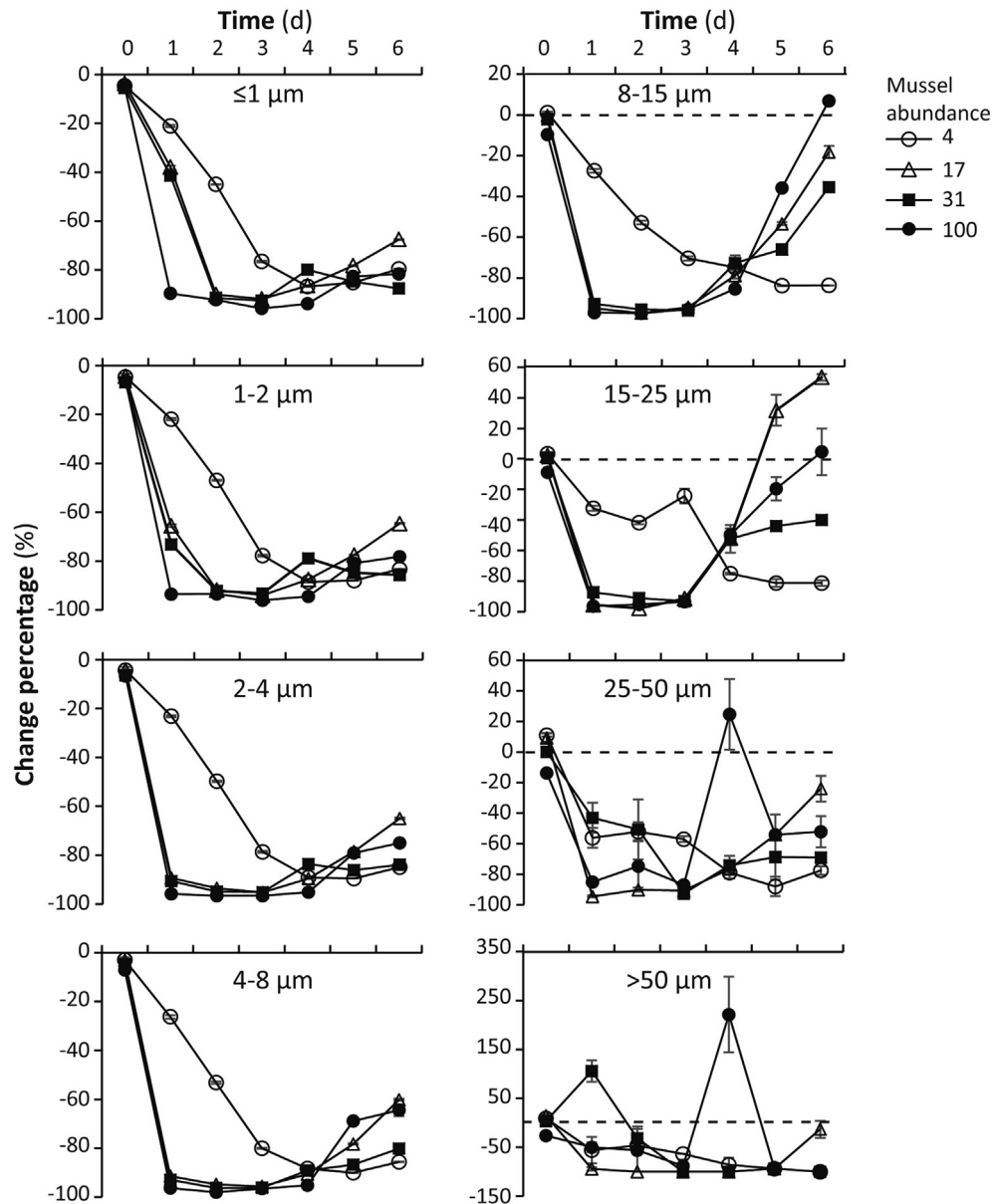
Fig. 5. Change percentage (Mean ± S.E.) of concentration of suspended particulates in mesocosms with golden mussels related to no-animal controls, showing daily results of each size category in the no-feeding experiment. Error bars indicate standard error of three replicates for each sample.

Table 1
Summary of generalized linear modelling of change percentage (%) of suspended particles on day 1 of experiment 2 as a function of animal abundance (Abundance, # of individual), size of suspended particles (Size, μm), and their interactions (Abundance × Size), showing results of only significant variables. Significance level: *** 0.001, ** 0.01, · 0.1.

Variable	Est. coefficient (S.E.)	t-value	P-value	Deviance explained
Intercept	−21.49 (9.65)	−2.23	0.029*	72.6%
Abundance = 17	−30.26 (13.65)	−2.22	0.029*	
Abundance = 31	−35.79 (13.65)	−2.62	0.01*	
Abundance = 100	−70.08 (13.65)	−5.14	1.8e-6***	
Size > 25	−34.49 (13.65)	−2.53	0.103*	
Abundance = 17 × Size 2−25	−35.24 (16.72)	−2.11	0.038*	
Abundance = 31 × Size 2−25	−27.84 (16.72)	−1.67	0.099·	
Abundance = 31 × Size > 25	123.13 (19.30)	6.38	9.2e-9***	
Abundance = 100 × Size > 25	58.55 (19.30)	3.03	0.003**	

vide an ideal substrate for attachment, allowing for widespread establishment of the species (Xu et al. 2014; Nakano et al. 2015; Zhan et al. 2015; Li et al., 2018). Second, upstream production

and export of trillions of propagules allow seeding of both newly and previously colonized areas to the north (Zhan et al. 2015). Environmental DNA analyses have detected *L. fortunei* at high
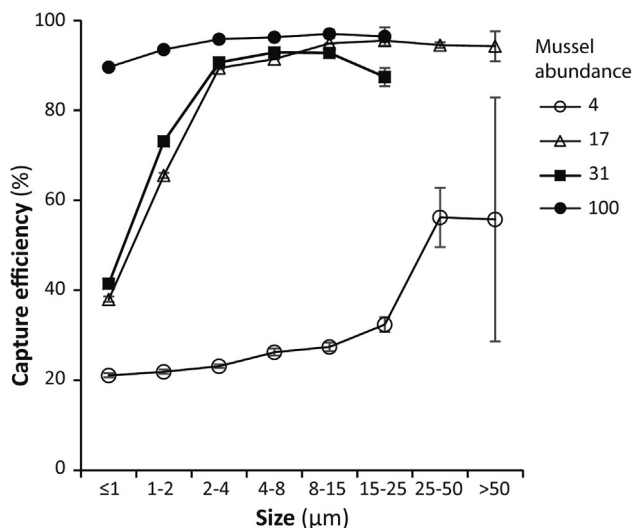
Fig. 6. Capture efficiency (Mean ± S.E.) of suspended particles of varying size, showing results of removal rate during the first day. Error bars indicate standard error of three replicates for each sample. Note that the data of size > 25 μm in treatments mussel abundance = 31 and = 100 was not shown because the removal rate (capture efficiency) was significantly related to the interaction between animal abundance and particle size (Table 1).

and low prevalence in southern and northern extents of the SNWDP project (Z. Xia, unpublished data). Third, water flow continually renews food resources and eliminates some waste products, allowing for dense local mussel populations (Arkema 2009). Collectively, these factors will result in a pronounced short-term gradient in both spatial distribution and population density from south to north. We anticipate that filtering impacts will correspond directly with mussel distribution and abundance, being highest in the south and low to non-existent in the northern parts of the project. In time, the species' distribution might infill in the north, in which case impact would rise in that region as well.

Large populations of filter-feeding, biofouling bivalves have been associated with occurrence of harmful cyanobacteria (e.g., Vanderploeg et al. 2001; Cataldo et al. 2012). In lakes Erie and Huron, for example, Vanderploeg et al. (2001) demonstrated that increased occurrence of *Microcystis aeruginosa* followed establishment of the zebra mussel (*Dreissena polymorpha*). Experiments highlighted that mussels differentially rejected toxic *M. aeruginosa* colonies in pseudofeces, which could later be re-suspended unharmed, while edible phytoplankton taxa were ingested (Vanderploeg et al. 2001). Similarly, mesocosm studies in Salto Grande reservoir, Argentina, demonstrated that high *L. fortunei* abundance significantly enhanced the cell density, proportion of colonial cells, and colony size of *Microcystis* spp. In both studies, impact of grazing mussels was highest when mussel abundance was high. As *L. fortunei* spreads through the SNWDP project, it will be important to monitor the occurrence and abundance of toxic cyanobacteria.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix A. Supplementary data

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