Tesis de Doctorado del Programa de Desarrollo de las Ciencias Básicas

(PEDECIBA)

Ecología de *Diplodon parallelopipedon* en Laguna del Sauce y sus implicancias en técnicas de biomanipulación

Msc. Soledad Marroni

Tutores: Carlos Iglesias/Néstor Mazzeo
2019

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Universidad de la República

Programa de Desarrollo de las Ciencias Básicas

Área Biología

Sub-área Ecología y Evolución

La tesis de Doctorado "Ecología de *Diplodon parallelopipedon* en Laguna del Sauce y sus implicancias en técnicas de biomanipulación" de Magister María Soledad Marroni Plata ha sido aprobada con Mención por el siguiente tribunal evaluador:

Dr. Alvar Carranza (CURE, UdelaR)

Dra. Carla Kruk (CURE, UdelaR)

Dr. Diego Lercari (CURE, UdelaR)

Contenido

Agradecimientos

Lista de Publicaciones

1-2016- *Interactions between bivalves and zooplankton. Competition or intraguild predation? Implications for biomanipulation in subtropical shallow lakes.* Soledad Marroni, Carlos Iglesias, Juan Pablo Pacheco, Juan Clemente, Néstor Mazzeo. Marine and Freshwater Research, 67, 1–8. Artículo 1.

2- 2019- *Effects of temperature and food availability on the filtration and excretion rates of Diplodon parallelopipedon (Unionidae). Implicances for biomanipulation techniques in subtropical shallow lakes.* Soledad Marroni, Néstor Mazzeo and Carlos Iglesias. *Water.* Artículo 2. Submitted.

3- Marroni et al. 2019. *Can nutrient release and/or consumption by D. parallelopipedon bust phytoplankton development?* Soledad Marroni, Néstor Mazzeo & Carlos Iglesias. Manuscrito 1

4- Marroni et al. 2019. *Pedal vs. Filtration feeding contribution to bivalve´s biomass, an example using Stable Isotopes analysis and Diplodon parallelopipedon*. Manuscrito 2

Resumen

El aporte externo de nitrógeno y fósforo asociado a múltiples actividades antrópicas condiciona diversos procesos que provocan un serio deterioro de la calidad del agua, principal problemática ambiental a escala global y en el Uruguay. Este fenómeno, denominado eutrofización, produce un crecimiento excesivo de los productores primarios, muchas veces con fuertes incrementos en la frecuencia y magnitud de floraciones fitoplanctónicas potencialmente tóxicas (Kruk et al 2019). Existen múltiples técnicas de rehabilitación de los sistemas afectados que incluyen desde el control del ingreso de nutrientes así como la carga interna asociada al sedimento, y múltiples técnicas focalizadas en la reversión de las consecuencias adversas. Dentro de este último grupo, se encuentra las técnicas de biomanipulación que básicamente consiste en aumentar la presión de consumo sobre el fitoplancton dentro del sistema. Tradicionalmente, dicha alternativa se ha focalizado en maximizar la herbívora por parte del zooplancton modificando la estructura de la trama trófica, particularmente la presión de depredación por parte de los peces planctívoros. Dicho objetivo puede lograrse por la extracción directa de estos peces y/o incrementando la abundancia de peces piscívoros. La aplicación de las técnicas clásicas de biomanipulación en sistemas templados presenta múltiples dificultades y desafíos asociados a diversas características de las comunidades de peces, en este marco, resulta pues relevante explorar otras alternativas. Los bivalvos filtran el fitoplancton y el zooplancton suspendidos en la columna de agua y pueden desempeñar un rol clave en su control e incidir en como dicha producción primaria se canaliza en la trama trófica. El sistema Laguna del Sauce es el mayor lago somero del Departamento de Maldonado, fuente de abastecimiento de agua potable para la mayoría de sus habitantes, presenta 3 especies de bivalvos filtradores, uno nativo (*Diplodon parallelopipedon*) y dos exóticos (*Corbicula fluminea* y *Limnoperna fortunei*). Laguna del Sauce es un sistema eutrófico que presenta floraciones fitoplanctónicas periódicas y un crecimiento excesivo de plantas sumergidas en Laguna del Potrero.

El objetivo general de la presente tesis: evaluar la capacidad potencial del bivalvo filtrador nativo *Diplodon parallelopipedon* como controlador de la biomasa fitoplanctónica en escenarios de eutrofia con dominancia de cianobacterias. Para alcanzar el objetivo planteado en esta tesis se realizó un abordaje tanto experimental como empírico. Para evaluar la tasa de filtración, con diferentes temperatura y cantidad y calidad de alimento se realizaron actividades experimentales. Mientras que para determinar la importancia relativa de las posibles vías de alimentación se realizaron análisis de isotopos estables con un muestreo de campo Para entender si los bivalvos funcionan como consumidores o promotores de cianobacterias, se realizaron experimentos donde se contrastaron las tasas de filtración en presencia de cianobacterias *Microcystis sp.* y de algas palatables. Finalmente, se abordó experimentalmente si el tipo de interacción que se establece entre el zooplancton y *D. parallelopipedon* es de competencia o de depredación.

Los principales resultados indican que *D. parallelopipedon,* la tasa de filtración presenta una relación no-lineal con la temperatura, presentando un óptimo en el entorno de los 20^0C y es capaz de consumir una amplia gama de partículas, incluyendo diversos grupos de microalgas, cianobacterias y zooplancton, ejerciendo un fuerte control sobre el fitoplancton. Sin embargo, se observó un patrón diferente en el caso de las tasas de excreción de fosforo reactivo soluble y amonio, existiendo aquí si una relación directa con la temperatura. Por otra parte, la alimentación pedal presenta un mayor aporte a la biomasa de *D. parallelopipedon.* Experimentalmente se demostró que especies como *Microcystis aeruginosa* no son consumidas por *D. parallelopipedon* debido a que por su capacidad de flotación escapan del proceso de filtración. Este consumo diferencial de *D. parallelopipedon* favorecería el desarrollo de cianobacterias que regulan su flotabilidad, de forma directa al evitar la filtración e indirectamente al eliminar a sus competidoras. Estos últimos pueden ser de menor entidad en sistemas mezclados o polimícticos como Laguna del Sauce, donde el control de la flotación

podría ser contrarrestada por el efecto de mezcla provocado por el viento. Otra vía por la que la actividad de los bivalvos promovería un mayor desarrollo del fitoplancton es por la liberación de nutrientes y sus impactos en las tasas de su recirculación en el sistema. *D. parallelopipedon* presenta un consumo diferencia del zooplancton. En este sentido consume el zooplancton de pequeño tamaño. Este consumo diferencial de *D. parallelopipedon* sobre el zooplancton es otro factor importante para favorecer la presión de consumo sobre el fitoplancton.

Los efectos evaluados sobre el consumo de fitoplancton son positivos desde la perspectiva de las técnicas de biomanipulación, aunque, existen importantes desafíos a explorar en función de los resultados y las posibilidades reales de su implementación. Por ejemplo: conocer el ciclo de vida de esta especie evaluar la capacidad de los bivalvos de acumulación y/o del daño que podría experimentar al estar expuestos de forma puntual o crónica a cianotoxinas. Además se deberán realizar pruebas de campo sobre la implementación de bivalvos en los sistemas. En este sentido son también necesarios construcción de modelos matemáticos para la estimación de cuantos bivalvos son necesarios por metro cuadrado para lograr el efecto deseado en el ecosistema

Abstract

Contribution of nitrogen and phosphorus associated with multiple anthropic activities conditions various processes that cause a serious deterioration of water quality. It is the main environmental problem on a global scale and in Uruguay. This phenomenon, called eutrophication, produces an overgrowth of primary producers, often with strong increases in the frequency and magnitude of potentially toxic phytoplankton blooms. There are multiple rehabilitation techniques, many of which consist of the control of both external and internal nutrient loading and biomanipulation. The classic biomanipulation strategy is to increase plankivorous fish, to favor the consumption of phytoplankton by the zooplankton. This objective can be achieved by the direct extraction of these fish and / or by increasing the abundance of piscivorous fish. The application of classical biomanipulation techniques in warm systems presents multiple difficulties and challenges associated with various characteristics of fish communities, in this context, it is therefore relevant to explore other alternatives.

Bivalves filter phytoplankton and zooplankton suspended in the water column and can play a key role in their control and influence how such primary production is channeled into the trophic plot. The Laguna del Sauce system is the largest shallow lake in the Department of Maldonado, a source of drinking water for most of its inhabitants, has 3 species of filter bivalves, one native (*Diplodon parallelopipedon*) and two exotic species (*Corbicula fluminea* and *Limnoperna fortunei*). Laguna del Sauce is a eutrophic system that presents periodic phytoplankton blooms in the Laguna del Sauce subsystem and excessive growth of submerged plants in Laguna del Potrero. This system constitutes the model of analysis for the general objective of the present thesis: to evaluate the potential capacity of the native bivalve filter *Diplodon parallelopipedon* as a controller of phytoplankton biomass in eutrophic scenarios with cyanobacterial dominance.

To achieve the objectives set out in this thesis, an experimental and empirical field activities approach was carried out. To evaluate the filtration rate, with different temperatures and quantity and quality of food, perform experimental activities. While to determine the relative importance of possible feeding routes, stable isotopes will be analyzed with field sampling. To understand whether bivalves function as consumers or promoters of cyanobacteria, experiments should be conducted where filtration rates were tested in the presence of cyanobacteria *Microcystis sp.* and palatable seaweed. Finally, it was experimentally addressed if the type of interaction that was established between zooplankton and *D. parallelopipedon* was of competence or predation.

The main results found indicate that *D. parallelopipedon* is able to consume a wide range of particles, including various groups of microalgae, cyanobacteria and zooplankton, exerting strong control over phytoplankton. The filtration rate has a non-linear relationship with temperature, presenting an optimum in the environment of 20^0C. However, a different pattern was observed in the case of excretion rates of soluble reactive phosphorus and ammonium, existing here a direct relationship with temperature. On the other hand, the pedal feed has a greater contribution to the biomass of *D. parallelopipedon.* Experimentally it was shown that *D. parallelopipedon* species such as *Microcystis aeruginosa* are not consumed by *D. parallelopipedon* because of their flotation capacity they escape the filtration process. This differential consumption of *D. parallelopipedon* would favor the development of cyanobacteria that regulate its buoyancy, directly by preventing filtration and indirectly by eliminating its competitors. The latter may be of lesser importance in mixed or polymictic systems such as Laguna del Sauce, where flotation control could be counteracted by the effect of mixing caused by wind. Another way in which the activity of the bivalves would promote a greater development of phytoplankton is by the release of nutrients and their impacts on the rates of their recirculation in the system.

D. parallelopipedon presents a difference consumption of zooplankton. In this sense it consumes the small zooplankton. This differential consumption of *D. parallelopipedon* on zooplankton is another important factor in favoring the consumption pressure on phytoplankton.

Effects evaluated on phytoplankton consumption are positive from the perspective of biomanipulation techniques; however, there are important challenges to explore depending on the results found the effects evaluated on phytoplankton consumption are positive from the perspective of biomanipulation techniques, however, there are important challenges to explore based on the results found and the real possibilities of their implementation. For example: knowing the life cycle of this species assessing the ability of accumulation bivalves and / or the damage that could be experienced by being punctually or chronically affected to cyanotoxins. In addition, field tests on the implementation of bivalves in the systems are also required. In this sense, it is also necessary to build mathematical models for the precision of how many bivalves are needed per square meter to achieve the desired effect on the ecosystem

1- Introducción

1.1 Lagos someros

El agua es un recurso natural esencial para la vida en el planeta y la sostenibilidad de las sociedades humanas. Sus múltiples usos involucran diversas actividades como consumo de agua potable, recreación, alimentación, riego y procesos industriales, entre otros (Dodds et al. 2013). Los ecosistemas acuáticos, en particular los cuerpos de agua dulce, que proveen estos servicios ecosistémicos están sometidos a fuertes presiones antrópicas que alteran su funcionamiento (Carpenter 2009). El cambio en el uso de la tierra, así como la urbanización generada en el entorno de los cuerpos de agua, tienen un fuerte impacto sobre la cantidad de nutrientes que ingresan al ecosistema, el excesivo aporte externo de nutrientes (eutrofización) condiciona el deterioro de la calidad del agua e interfiere con los procesos de potabilización (Jeppesen et al. 2010). Además, puede afectar de forma directa la salud humana por la aparición de floraciones de cianobacterias tóxicas (Carmichael 2001; Azevedo et al. 2002).

La eutrofización de los cuerpos de agua es un fenómeno global y se produce por un aumento del ingreso de nitrógeno y fósforo los ecosistemas, dichos aportes pueden ser puntuales (falta de saneamiento/desechos industriales) o difusa (escorrentía superficial de las actividades agrícolas-ganaderas). El aumento del aporte de nutrientes provoca un incremento de la productividad primaria del sistema pudiendo incluso ocasionar cambios en la estructura trófica del sistema (a medida que el proceso se intensifica), alterando la biodiversidad y los ciclos bio-geoquímicos (Jeppesen et. al. 2005).

En los ecosistemas acuáticos poco profundos (someros) pueden darse en un rango intermedio de concentración de nutrientes dos conformaciones alternativas: una denominada de aguas turbias y otra de aguas claras (Hipótesis de los Estados Alternativos, HEA, Scheffer 2004, Fig. 1). El estado denominado claro se caracteriza por una mayor transparencia del agua y por la dominancia de macrófitas sumergidas dentro del grupo de productores primarios. Por su

parte, en la configuración turbia, el sistema puede presentar una elevada biomasa fitoplanctónica o una importante concentración de material en suspensión (Scheffer 2004). Los sistemas acuáticos pueden presentar otras configuraciones alternativas, por ejemplo la dominancia de plantas flotantes libres (Scheffer & Carpenter 2003, Meerhoff & Mazzeo 2004) o cianobacterias dentro del fitoplancton (Scheffer et al. 1997). La persistencia en el tiempo de las configuraciones o regímenes señalados dependen de la interacción de un conjunto de factores, entre los que se destacan la disponibilidad de recursos (luz y nutrientes) y la presión de herbivoría (Fig. 1), así como mecanismos químicos y biológicos de retroalimentación negativos y positivos.
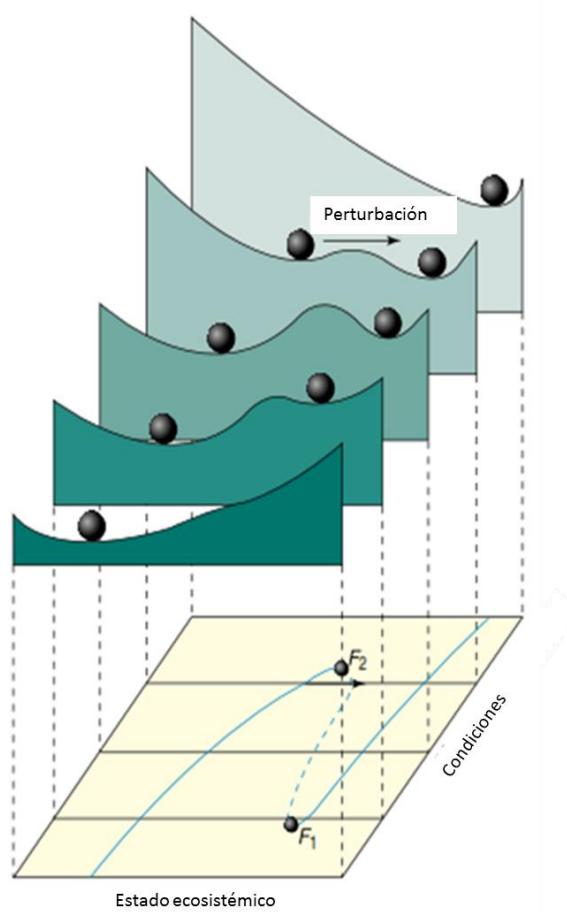
Figura 1. Teoría de los estados alternativos. El sistema exhibe un comportamiento de histéresis debido a que el sistema puede presentar diferentes biomasas fitoplanctónicas con la misma carga de nutrientes en la columna de agua. La respuesta no solo depende de la condición actual sino también de la trayectoria previa del sistema. Oligotrofización=empobrecimiento de nutrientes. Eutrofización = enriquecimiento de nutrientes. Esquema tomado de Scheffer & Carpenter (2003).

La teoría de los estados alternativos es un ejemplo de cambios de régimen donde ocurre histéresis, existen otros tipos de cambios de régimen donde esta condición no necesariamente se cumple (Dakos et al. 2015). En los lagos someros los cambios de régimen ocurren de manera brusca o catastrófica (Scheffer & Carpenter 2003), un aumento de la llegada de nutrientes al ecosistema no tiene ninguna consecuencia visible de forma inmediata (pero disminuye su resiliencia), solo suceden cuando se supera un cierto umbral de nutrientes y varios mecanismos de retroalimentación se desactivan. En un rango intermedio de nutrientes es posible que una perturbación determine el pasaje de un estado al otro, por ejemplo la escorrentía superficial de herbicidas provoca una alteración drástica de la comunidad de plantas sumergidas promoviendo el pasaje de una configuración clara a otra turbia (Scheffer & Carpenter 2003). En este mismo sentido, un aumento de la tasa de herbivoría o forrajeo puede controlar una elevada biomasa fitoplanctónica sin que ocurran modificaciones asociados al aumento de la carga externa o interna de nutrientes (Perrow et al. 1997; Scheffer et al. 2003).

En los ecosistemas templados, donde estos procesos han sido estudiados en detalle y con múltiples aproximaciones (Scheffer 1998; Smith 1998; Smith et al. 2006; Jeppesen et. al. 2005), se ha constatado que el ingreso de nutrientes puede generar un cambio en la estructura de la comunidad de peces. Sistemas que originalmente cuentan con poblaciones abundantes de peces piscívoros sufren la pérdida total o parcial de este componente a medida que el proceso se desarrolla, quedando dominado por peces planctívoros en términos de abundancia y biomasa (Jeppesen et al. 2005). La disminución de la presión de depredación sobre los peces planctívoros condiciona indirectamente una mayor presión de consumo sobre el zooplancton por un efecto en cascada trófica (Carpenter et al. 1985) determinando, indirectamente, un aumento de la biomasa fitoplanctónica. En los casos más extremos, el fitoplancton puede pasar a estar dominado por cianobacterias y la ocurrencia de floraciones de especies potencialmente tóxicas es un fenómeno frecuente en estos casos, pudiendo llegar a afectar la

salud humana por la ingesta de toxinas, ya sea por consumo directo de agua, baños, consumo peces o bivalvos contaminados, etc. (Azevedo et al. 2002).

Diversas estrategias se han implementado para mitigar los efectos de la eutrofización. Por un lado, es ampliamente reconocido que resulta imprescindible la reducción del ingreso de nitrógeno y fósforo al ecosistema para que el éxito de cualquier otra acción resulte productiva (Cooke et al. 1993; Søndergaard et al. 2002). Sin embargo, la reducción del ingreso de nutrientes a los sistemas acuáticos, no permite por si solo el retorno del sistema a la condición previa a la eutrofización. Existe así evidenciada la existencia de una serie de mecanismos de retroalimentación tanto químicos como biológicos instalados en el sistema que impiden el retorno a las condiciones previas (Moss 1992; Scheffer et al. 1993; Søndergaard et al. 2002). Se requiere de medidas adicionales vinculadas fundamentalmente al manejo y control de la carga interna de nutrientes así como a la re-estructura de la trama trófica (Jeppesen et al. 2007).

1.2 Rol funcional de los bivalvos en los ecosistemas

Los bivalvos son un componente fundamental del ecosistema debido a las múltiples interacciones que presentan con otros organismos. Son capaces de consumir, por filtración, una amplia variedad de material particulado y organismos (fitoplancton y zooplancton) suspendidos en la columna de agua (Fig. 2) (Zangh et al. 2010; Davenport et al. 2011; Peharda et al. 2012; Marroni et al. 2014; Marroni et al. 2016), así como materia orgánica del sedimento de forma directa por alimentación pedal (Vaughn & Hakenkamp 2001; Marroni et al. 2014). Esta posibilidad de utilizar dos fuentes alternativas permite a algunas especies de bivalvos, a diferencia de lo que ocurre en general con el zooplancton, mantener altas abundancias e incluso sobrevivir en el sistema aunque no haya fitoplancton en la columna de agua por la alimentación pedal (Vaughn & Hakenkamp 2001; Marroni et al. 2014).
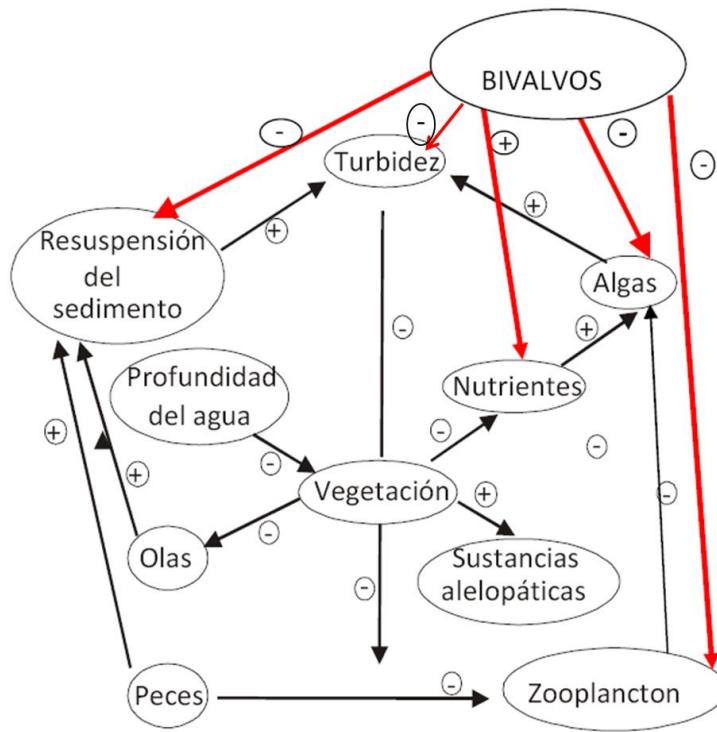
Figura 2. Interacciones directas e indirectas de los bivalvos sobre los componentes del ecosistema. Modificación del esquema original de Scheffer (1998).

En ecosistemas acuáticos donde existen grandes abundancias, los bivalvos filtradores constituyen uno de los principales consumidores de los productores primarios (Officer et al. 1982; Vaghun & Hakenkamp 2001). Las tasas de filtración de las poblaciones de bivalvos pueden ser considerables, alcanzando entre un 10-100 % de la columna de agua por día del lago (Olivie & Mitchell 1995; Strayer et al. 1999; Eliott et al. 2008). Se ha demostrado que la tasa de filtración depende, entre otros factores, de la concentración de las partículas en la columna de agua y de la temperatura del agua (Winter 1976). Algunos estudios indican que los bivalvos pueden regular su tasa de filtración para optimizar la energía obtenida (Bayne et al. 1987, 1988, 1989, 1993; Hawkins et al. 1996; Bayne 1998, 2001). La regulación de la tasa de filtración se da a través de la selección del alimento, la tasa de ingestión, la digestión y la absorción de nutrientes (Bayne et al. 1993, Bayne 1998). Por otra parte, los bivalvos son organismos ectotermos, por lo cual la tasa de filtración es dependiente de la temperatura

(Widdows 1978; Jørgensen 1990), siendo la respuesta más aceptada un incremento lineal de la tasa de filtración con un incremento de la temperatura.

Los bivalvos cumplen un rol importante inmovilizando nutrientes disponibles en la columna de agua, incorporándolos al sedimento (Vaguhn et al. 2007; Howard & Cuffey 2006). En el proceso de filtración, solo parte de las partículas filtradas son retenidas, mientras que el resto son excretadas como heces o pseudoheces. Éstas provocan una drástica transformación de la estructura del sedimento, junto con un cambio en la composición de la comunidad bentónica (Vaguhn & HakenKamp 2001). La bioturbación del sedimento, producida por el movimiento y alimentación de los bivalvos, genera un aumento del contenido de agua, conjuntamente con la homogeneización del sedimento y un incremento en la concentración de oxígeno disuelto (McCall et al. 1979). Está interacción entre los bivalvos y la interfase agua-sedimento, permite además la liberación de nitrato, junto con la retención de carbonato de calcio por el sedimento y retenido en las valvas (Matisoff et al. 1985).

D. parallelopipedon

Diplodon sp. es un género de bivalvos de agua dulce ampliamente distribuido en América del Sur. En Uruguay existen varias especies distribuidas en varios a sistemas acuáticos como por ejemplo: el Río Uruguay y sus afluentes, Laguna del Sauce, Cuenca de la Laguna Merin, cursos de agua con pendiente atlántica (Figueiras, 1965). *D. parallelopipedon* está ampliamente distribuido en la Laguna del Sauce, donde también co-exsite, con otros bivalvos, *Corbicula fluminea* (no-nativo) y *Limnoperna fortunei* (exótico-invasor).

Los bivalvos del género *Diplodon sp.* presentan una estrategia de vida de tipo K, con una baja tasa de crecimiento y largo tiempo generacional llegando hasta los 90 años de vida (Anthony et al. 2001; Soldati et. al., 2009). *D. parallelopipedon* registra tallas de hasta 10cm de longitud valvar. En el sistema Laguna del Sauce la población está dominada por individuos adultos con

escases de juveniles (Marroni 2006). El ciclo de vida es complejo con una fase larval parasita (gloquideo), que utiliza un pez como hospedero y luego de completar su desarrollo se desprende del pez y continúa su desarrollo en el sedimento (Bonetto & Tassara, 1987/8; Bauer & Wächtler, 2001). La reproducción es sexuada, la fecundación se produce en el interior de la hembra en el marsupio, donde los embriones crecen hasta ser liberados como gloquideos (Bauer & Wächtler, 2001; Strayer, 2008). Se desconocen predadores para el sistema Laguna del Sauce. Par la especie otra especie del mismo género, *D. chilensis* se encontró que el crustáceo *Aegla sp*. es su principal predador*.*

Durante la tesis de Maestría se estimó la Tasa de Filtración de *D. parallelopipedon* con dos especies de fitoplancton: *Ankistrodesmus sp.* (palatable) y *Plankthotrix agardhii* (poco palatable), y a la misma temperatura (20^0C). Adémas se detreminó el consumo de materia orgánica del sedimento a través de la alimentación pedal (Marroni et al. 2014).

1.3 Biomanipulación

La biomanipulación se define como la modificación de la trama trófica con un objetivo determinado (Shapiro et al. 1975). El control de la abundancia y biomasa de los peces planctívoros constituye una de las estrategias claves para aumentar el consumo de fitoplancton, fue aplicada en lagos con relativo éxito por Moss (1992), donde se favoreció el aumento de la abundancia y biomasa del zooplancton herbívoro, provocando un mayor control de la biomasa fitoplanctónica. A partir de los años 1990, la remoción directa de planctívoros y/o la re-introducción de peces piscívoros (introduciéndolos y/o por la restauración de la vegetación sumergida) constituyó el enfoque predominante de las estrategias de rehabilitación en los sistemas templados (Perrow et al. 1997; Meijer et al. 1999; Jeppesen et al. 2005; Gulati et al. 2008). La mayoría de los ejemplos de biomanipulación se refieren a casos en el hemisferio Norte, donde la presencia de cladóceros de mediano y gran porte, fundamentalmente del género *Daphnia* domina la estructura de los herbívoros (Meijer

et al. 1999, Jeppesen et al. 2005; Wilson & Chislock 2013). Por otra parte, la manipulación de la cadena trófica ha sido más exitosa en lagos someros pequeños, pero su efectividad a largo plazo es variable (Jeppesen et al. 2012). En lagos donde se observó un gran cambio debido a la remoción de peces, entre 5-10 años después se observa un gradual retorno al estado turbio junto con una mayor abundancia de peces zooplanctívoros, particularmente si en ese período no se produjo la reducción de los aportes externos e internos de nutrientes (Jeppesen et al. 2012).

Por otra parte, cuando se intentó aplicar estas técnicas en sistemas subtropicles quedaron evidenciadas la existencia de algunas limitaciones (Jeppesen et al. 2005; 2010; Kosten et al. 2009). Actualmente existe amplio acuerdo en que la manipulación de la comunidad de peces en nuestra región resulta más compleja debido a una mayor riqueza de especies, una mayor complejidad funcional (prevalencia del hábito omnívoro dentro del grupo), la dominancia de especies de pequeño tamaño y sus preferencias de uso del espacio (Fernando 1994; Meerhoff et al. 2007a y b, Teixeira de Mello et al. 2009; González-Bergonzoni et al. 2012). Además, la evidencia generada, indica que las características presentadas por la comunidad de peces de regiones sub tropicales determinan una mayor presión de consumo sobre el zooplancton. Por lo cual la comunidad zooplanctonica estádominada por especies de pequeño tamaño, con la ausencia casi absoluta de los grandes herbívoros (como *Daphnia*) en los períodos de mayor producción fitoplanctónica. Solamente en condiciones de remoción total de peces planctívoros o condiciones particulares con estructuras tróficas muy simplificadas, se ha podido comprobar el éxito de los enfoques clásicos de la biomanipulación (Fig. 3) (Mazzeo et al. 2010; Iglesias et al. 2011).

En el caso de los sistemas sub tropicales resulta relevante explorar otros mecanismos adicionales de consumo de fitoplancton para acelerar los procesos de rehabilitación. En este sentido, los bivalvos filtradores son eficientes removiendo el fitoplancton de la columna de

agua. En ecosistemas eutróficos constituyen uno de los principales consumidores de los productores primarios (Officer et al. 1982; Vaghun & Hakenkamp 2001).

La introducción del bivalvo *Corbicula sp.* ha provocado cambios tanto cuantitativos como cualitativos en la estructura y funcionamiento de sistemas acuáticos (Hawng et al. 2004). Existen varios ejemplos donde luego de la introducción de bivalvos se incrementa la transparencia del agua así como disminuye la biomasa fitoplanctónica (Reeders et al. 1993; Cohen et al. 1984; Phelps 1994; Holland 1993; Caraco et al. 1997; Karatayev et al. 1997; Cataldo et al. 2012). También se ha reportado que el aumento de la transparencia del agua favoreció el establecimiento y expansión de las macrófitas (Griffiths 1992; MacIsaac 1996; Phelps 1994; Zhu 2006). Otros cambios producidos por la introducción de *D. polymorpha* se refieren a la composición tanto fito como zooplactónica (Bastviken et al. 1998; Ten Winkel & Davids 1982; Pace et al. 1998; Cataldo et al. 2012). En este sentido, el consumo y/o promoción de cianobacterias tóxicas por parte de bivalvos (por ej. *D. polymorpha*) es objeto de particular interés. Alguno antecedentes demuestran la promoción (Knoll et al. 2008; Raikow et al. 2004; Sarnelle et al. 2010; Vanderploeg et al. 2001), aunque por el contrario otros estudios indican que son capaces de consumirlas (Baker et al. 1998; Dionisio Pires et al. 2004, 2005; Smith et al. 1998).

A la luz de esta evidencia, diversos estudios sugieren la posibilidad de utilizar a los bivalvos como herramienta de biomanipulación (Ogilvie & Mitchell 1995; Dionisio Pires et al. 2007) alternativa y/o complementaria al enfoque clásico. Sin embargo su utilización requiere de un mayor conocimiento de la biología y ecología, particularmente de las especies nativas candidatas a ser utilizadas con este fin (Fig. 3).
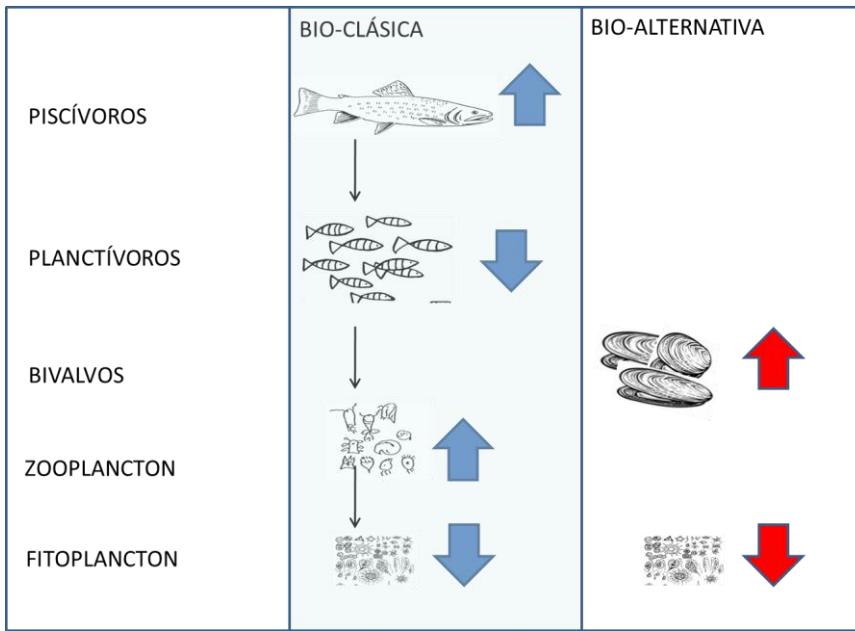
Figura 3. Esquema teórico de biomanipulación (BIO) de la cadena trófica: clásica (aumento de piscívoros/reducción de planctívoros/aumento de zooplancton/reducción de fitoplancton) y alternativa (introducción de bivalvos/reducción de fitoplancton).

2-Justificación, Hipótesis y Objetivo General

En general se conoce mucho sobre la ecología de bivalvos exóticos invasores como *Limnoperna fortunei* (mejillón dorado), *Corbicula fluminea* (almeja asiática) o *Dreissena polymorpha* (mejillón cebra) y sus efectos en el ecosistema a partir de su introducción. Por otro lado poco se conoce sobre el efecto de los bivalvos nativos en los ecosistemas. El principal problema que sufren los cuerpos de agua en nuestro país es el proceso de eutrofización debido al ingreso de nitrógeno y fósforo proveniente de las actividades antrópicas. Una de las estrategias que se ha implementado con éxito en ecosistemas templados es la biomanipulación. Sin embargo, cuando aplicamos estas técnicas en sistemas subtropicales han sido poco exitosas. En este sentido la utilización de bivalvos filtradores es un herramienta alternativa de biomanipulación capaz de logra efectos de control de biomasa fitoplanctónica. Para la implementación de esta estrategia es necesario el conocimiento sobre la ecología de los bivalvos filtradores nativos presentes en nuestros ecosistemas. *D. parallelopipedon* es una especie nativa que está presente en el sistema Laguna del Sauce, junto con otros bivalvos exóticos (*C. fluminea/ L. fortunei*). Resulta esencial conocer aspectos de su biología como por ej: tasa de filtración y

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excreción con diferentes temperaturas y/o concentración de alimento; su ciclo de vida; interacciones con otras comunidades entre otros.

Hipótesis General:

D. parallelopipedon es un organismo filtrador capaz de consumir un rango amplio de partículas suspendidas en la columna de agua, existirá un balance favorable entre su tasa de filtración de fitoplancton versus el consumo de organismos no blanco y la remineralización de nutrientes que harán posible implementar su uso como herramienta de biomanipulación.

Objetivo general:

Evaluar la capacidad de consumo del bivalvo filtrador nativo, *Diplodon parallelopipedon*, como control de la biomasa fitoplanctónica en escenarios de eutrofia con dominancia de cianobacterias, así como la interacción con otras comunidades y sus implicancias como herramienta de biomanipulación.

3-Preguntas a responder, objetivos específicos e hipótesis:

Para poder evaluar a *D. parallelopipedon* como herramienta de Biomanipulación es necesario conocer y/o profundizar algunos aspectos de su ecología para entender su rol en el ecosistema.

1-¿La tasa de filtración y excreción de *D. parallelopipedon* varían en función de la cantidad de alimento disponible y/o con la temperatura del agua? Artículo científico 2.

2-¿Cuánto aportan a la nutrición de *D. parallelopipedon* (en términos de aporte de biomasa) la materia orgánica del sedimento a través del pie filtrador y la filtración de las partículas de la columna de agua? Manuscrito 2.

3-¿*D. parallelopipedon* favorece la presencia de cianobacterias a través de la liberación de nutrientes y/o a través de un consumo diferencial? Manuscrito 1.

4- ¿Cómo es la interacción entre el zooplancton y *D. parallelopipedon*? Competencia? Depredación? Artículo científico 1.

Objetivos específicos

Hipótesis 1a. Las tasas de filtración de *D. parallelopipedon* está condicionada por la concentración de alimento disponible. Cuando se alcanza un umbral determinado las valvas se cierran para proteger al sistema digestivo de una saturación por lo cual la tasa de filtración disminuye.

Predicción: La tasa de filtración aumenta cuando la concentración de alimento es mayor, alcanzando un umbral a partir del cual disminuye..

Objetivo específico 1b. Determinar cómo varía la tasa de filtración de *D. parallelopipedon* en función de cambios en la temperatura del agua.

Hipótesis 1b. La tasa de filtración es fuertemente condicionada por el rango de temperatura observado. Cuanto mayor es la temperatura hay una mayor actividad metabólica, y por lo tanto mayor es la tasa de filtración.. **Predicción**: Existe una relación lineal entre la tasa de filtración y la temperatura en el rango entre 10-30 ⁰C. Estudios previos de filtración con bivalvos indican una relación lineal con la temperatura.

Objetivo específico 2. Evaluar la tasa de excreción de nutrientes de *D. parallelopipedon* y su dependencia con la temperatura.

Hipótesis 2. El proceso de excreción depende de múltiples factores externos, la temperatura constituye uno de los controles claves. A mayor temperatura mayor actividad metabólica por lo tanto mayor liberación de excreción. **Predicción:** La tasa de excreción de nutrientes varía linealmente con la temperatura en el rango entre 10-30⁰C.

Objetivo específico 3. Evaluar las tasas de excreción de nutrientes en función de la concentración de alimento.

Hipótesis 3. Los mecanismos se excreción son dependientes de la concentración de alimento disponible. A mayor concentración de alimento ingerido mayor liberación de nutrientes.

 Predicción: La tasa de excreción de nutrientes aumenta de forma lineal cuanto mayor sea la concentración de alimento mayor será la liberación de nutrientes.

Objetivo específico 4. Analizar la importancia relativa de las dos posibles vías de alimentación que presenta *D. parallelopipedon*, materia orgánica del sedimento a través del pie filtrador vs. filtración de partículas en suspensión en la columna de agua.

Hipótesis 4. *D. parallelopipedon* es capaz de alimentarse a través de la filtración del la columna de agua y a través dela alimentación pedal. . **Predicción:** La alimentación de materia orgánica

a través del pie filtrador representa un aporte menor de biomasa asimilada frente a la filtración de partículas suspendidas en la columna de agua.

Objetivo específico 5. Evaluar si *D. parallelopipedon* es capaz de consumir cianobacterias de gran tamaño y con capacidad de regular su flotación.

Hipótesis 5. *D. parallelopipedon* consume una gran diversidad de tamaños de partículas y organismos de la columna de agua. Predicción: *D. parallelopipedon* consume cianobacterias como *Microcystis sp.*

Objetivo específico 6. Determinar si *D. parallelopipedon* facilita la proliferación de cianobacterias a través de la liberación de nutrientes.

Hipótesis 6. *D. parallelopipedon* promueve la proliferación algal a través de la liberación de nutrientes especialmente amonio y fosforo reactivo soluble. **Predicción:** La liberación de nutrientes de *D. parallelopipedon* favorece el desarrollo de cianobacterias.

Objetivo específico 7. Determinar si las interacciones entre *D. parallelopipedon* y el zooplancton corresponden a competencia por recursos o depredación intragremio.

Hipótesis 7. Debido a que *D. parallelopipedon,* consume un amplio rango de partículas, es capaz de consumir zooplancton de pequeño tamaño debido a su limitada capacidad de nado y escape. **Predicción**: *D. parallelopipedon* disminuye la abundancia de los componentes del zooplancton de menor tamaño

4. Estrategia de investigación

A través de la combinación de abordajes experimentales y empíricos se analizan las preguntas e objetivos indicados, procurando dar respuesta a las distintas preguntas formuladas y finalmente comprobar o rechazar las hipótesis planteadas. Las tasas de filtración y de excreción *D. parallelopipedon* se analizaron mediante experimentos en función de la disponibilidad de alimento y la temperatura del agua (BOX 1 y 2). Se colectaron muestras en el sistema laguna del Sauce de: *D. parallelopipedon*, agua y sedimento para el análisis de isotopos estables y determinar los contenidos de isótopos estables de carbono y nitrógeno. En base a estos datos se aplicaron modelos de mezcla (*mixing models*), para determinar la importancia relativa de las fuentes de alimentación (alimentación pedal vs filtración de la columna de agua) a la biomasa de *D. parallelopipedon* (BOX 3). Para entender si los bivalvos funcionan como consumidores o promotores de cianobacterias, se realizaron experimentos donde se contrastaron las tasas de filtración en presencia de cianobacterias *Microcystis sp.* y de algas palatables (BOX 4). Finalmente, se abordó experimentalmente si el tipo de interacción que se establecía entre el zooplancton y *D. parallelopipedon* era de competencia o de depredación (BOX 5).

5-Efectos de la temperatura y la concentración de alimento sobre la tasa de filtración y excreción de nutrientes de *Diplodon parallelopipedon*

5.1 Tasa de Filtración

La tasa de filtración es un atributo ecológico importante que permite cuantificar el impacto de los bivalvos en el consumo de recursos y/o presas. La filtración es controlada negativa o positivamente por múltiples factores, por ejemplo la temperatura, concentración de alimento (tipo y calidad), régimen de flujo, morfología y tamaño de las presas (Bayne et al. 1977; Paterson 1984; Cranford 2001; Hawkins et al. 2001; Widdows 2001; Riisgård et al. 2003).

La temperatura es un factor importante en la regulación de la tasa de filtración de los bivalvos y está positivamente relacionada en el rango temperatura observados en los cuerpos de agua continentales. Algunos estudios confirman una relación lineal entre la temperatura y la tasa de filtración (Riisgård & Seerup 2003; Petersen et al. 2003; Kittner & Riisgård 2005), mientras que otros sugieren la existencia de relaciones no lineales. En estos casos se llega a un óptimo, luego del cual se produce un decrecimiento en la tasa de filtración (Schulte 1975, Aldbridge et al. 1995; Vanderploeg et al. 1995; Masilamoni et al. 2002; Viergutz et al. 2007; Fulford et al. 2007; Marissa 2016).

Para comprender como varía la tasa de filtración de *D. parallelopipedon* con la temperatura, se seleccionó un rango de temperaturas registradas a lo largo del año en el sistema Laguna del Sauce y se realizaron experimentos de laboratorio para analizar como varía la tasa de filtración con la temperatura y la concentración de alimento (BOX 1). Los resultados indican que la tasa de filtración presenta una relación no lineal con la temperatura (Fig. 4). La predicción 1b fue rechazada, la tasa de filtración de *D. parallelopipedon* varió no linealmente en el rango de temperatura del agua analizado. Las temperaturas más bajas 10-15°C presentan los menores valores de tasas de filtración, no encontrándose diferencias significativas entre estos (p=0.98). La máxima tas de filtración se registró a los 20°C habiéndose observado la mayor tasa de filtración a los 20 °C (Fig. 4). Las diferencias entre los resultados encontrados y estudios previos que indican una relación lineal entre la tasa de filtración y la temperatura (Riisgård & Seerup 2003; Petersen et al. 2003; Kittner & Riisgård 2005) pueden deberse a que los rangos de temperatura utilizados en estos trabajos sean diferentes y que en un mayor rango de temperatura como el utilizado aquí la relación encontrada sea no sea lineal. Los patrones de respuesta de la tasa de filtración con la temperatura encontrados son similares a los reportados en otros bivalvos: *Dreissena polymopha* y *Corbicula fluminea*, con óptimos en la

tasa de filtración a 22.9°C y 25°C respectivamente (Lei 1996; Veirguzt et al. 2007; Weitered 2008).
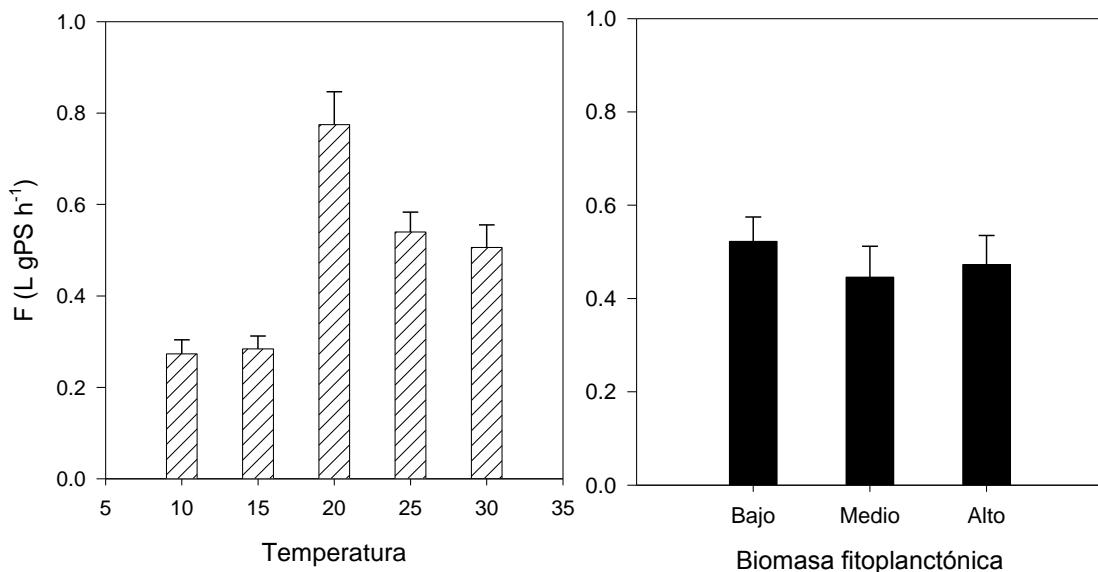
Figura 4. Tasa de filtración (TF) en función de la temperatura (izquierda) y la biomasa fitoplacntonica (derecha). La tasa de filtración corresponde a los promedios de los tratamientos en cada caso y esta expresada en unidades de biomasa PS (Peso Seco). Temperatura: El máximo de la tasa de filtración se registró a los 20°C (0.78L g PS^{-1} h^{-1}). TF vs Tempertura ($F_{(4,38)}$=16.56, p=0.002; post hoc test: 10=15<25=30<20). Biomasa fitoplanctónica: la tasa de filtración no varió significativamente con la concentración de fitoplancton TF vs Biomasa fitoplanctónica ($F_{(2,38)}$=1.29, p=0.28; post hoc test: bajo=medio=alto). Modificado del artículo 3.

Los resultados coinciden con modelos predictivos que indican un decrecimiento en la tasa de filtración particularmente en temperaturas altas en otras especies (Cerco & Noel 2005; Fulford et al. 2007). Esta disminución puede asociarse a una situación de estrés fisiológico, ya que las temperaturas extremas pueden afectar a los individuos provocando cambios en el metabolismo y hasta la propia muerte (Kennedy & Mihursky 1971; Anestis et al. 2007).

Box 1. Tasa de filtración de *D. parallelopipedon.*

Diseño Experimental

Los experimentos de filtración se realizaron con cinco temperaturas diferentes (10-15-20-25-30°C) y con tres biomasas fitoplactónicas (alta, media y baja). La tasa de filtración se midió siguiendo el procedimiento de Riisgård et al. (2001). Se utilizaron recipientes de 6 litros de capacidad, llenados con 4 litros de cultivo algal dominado por *Ankistrodesmus sp.* En cada recipiente se introdujeron 4 bivalvos en un canasto plástico con sedimento sin materia orgánica (Fig 1.1). Los tratamientos fueron asignados al azar para la biomasa fitoplactónica: Bajo (Chlo a= 2,5-5 μgl^{-1}), medio (Chlo a= 10-15 μgl^{-1}) y alto (Chlo a= 20-30 μgl^{-1}) y para temperatura. Se realizaron 5 réplicas y tres controles por cada tratamiento. Previo a cada experimento los animales fueron aclimatados durante 2 semanas para cada temperatura.

Figura 1.1. Canasto plástico donde fueron colocados los individuos de *D. parallelopipedon*

Estimación de la Tasa de Filtración

La tasa de filtración fue estimada por el método de aclaramiento, como el volumen de agua aclarado por unidad de tiempo. Siguiendo las recomendaciones de Riisgård (2001), que consiste en mantener constante la concentración de biomasa algal dentro de un cierto rango, con el agregado periódico de un volumen fijo de *Ankistrodesmus sp.* El agregado de fitoplancton se determinó en experimentos piloto cada 45 min (20, 25, 30°C) y 90 min (10-15°C).

Tasa de Filtración (TF): fue determinado como el decrecimiento exponencial de concentración de fitoplancton, medido como la concentración de clorofila *a in vivo* (Chl-*a*) usando espectrofotómetro (Aquafluor Handheld Fluorometer Turner Designs)

$$TF= a\,V/n,$$

donde V=volumen de agua, n=número de bivalvos, y a pendiente de la gráfica de la regresión lineal del semi log de la concentración vs. tiempo. TF fue expresada en términos de biomasas usando las relaciones disponibles largo-peso (Marroni et al. 2014).

La tasa máxima de filtración para *D. parallelopipedon* observada experimentalmente en los experimentos fue: 0.780 L g PS^{-1} h^{-1}, es mayor en comparación con otras especies de bivalvos exóticos invasores: *Dreissena polymorpha* 3.8-6.94 mL mg PS^{-1}h^{-1} 22 °C (Diggins 2001), *Corbicula fluminea* 2-20 mL mg PS^{-1}h^{-1} 22-24 °C (Buttner & Heidinger 1981; Silverman et al. 2007) o *Limnoperna fortunei* 1.5-3 mL mg PS^{-1}h^{-1} 24-25 °C (Cataldo et al. 2011).

La evidencia experimental permite entonces sostener que *D. parallelopipedon* puede ejercer fuerte control descendente (Top-Down) del fitoplancton en un amplio rango de temperaturas, incluso en las temperaturas medias del año. Las bajas tasas de filtración observados a las menores temperaturas pueden corresponder a períodos de inactividad de filtración, que se ajustan a estrategias de ahorro energético (Luruman 2014).

Otro de los factores que puede afectar la tasa de filtración es el tipo y cantidad de alimento disponible (Navarro et al. 1994; Navarro et al. 1996; Hawkins et al. 1996; Urrutia et al. 1996; Tokumon et al. 2015; Marescaux et al. 2016), particularmente un incremento en la tasa de filtración puede observarse en respuesta a un incremento en la disponibilidad de fitoplancton asegurándose un máximo en la tasa de ingestión (Winter 1978; Schetule 1975; Fiala-Medioni et al. 1983; Bayne et al. 1989). Los resultados obtenidos no verifican la hipótesis 1a, ya que no se encontraron diferencias en la tasa de filtración en función de la cantidad de alimento (Fig. 4). Por otra parte, la calidad del alimento es otro factor que puede influir en la capacidad de filtración de una especie. *D. parallelopipedon* es una especie que presenta un amplio rango de filtración desde algas pequeñas hasta filamentos de cianobacterias (Marroni et al 2014). En este sentido, el alimento utilizado corresponde a un alga palatable *Ankistrodesmus sp*, en un rango de biomasa de 5-30 chl *a* µg L^{-1} (representativo de sistemas meso y eutróficos). La ausencia de la respuesta esperada podría estar explicado en el hecho de que haber utilizado un rango demasiado pequeño de concentración como para observar el patrón inicialmente planteado en las hipótesis y predicciones.

5.2 Liberación de nutrientes

Tasa de excreción

Los bivalvos transfieren nutrientes y energía desde la columna de agua hacia los sedimentos a través de la actividad de filtración y estimulando la producción de otros niveles tróficos (Howard & Cuffey 2006; Spooner & Vaughn 2006; Vaughn et al. 2007). En este sentido el rol de los bivalvos en el ciclo de nutrientes ha sido poco estudiado. Los bivalvos consumen organismos y material suspendido en la columna de agua y en este proceso parte de estos nutrientes es asimilada formando parte de la biomasa y otra parte es excretada al medio. Estos últimos pueden ser depositados como heces o pseudoheces y/o liberados en forma de nutrientes disueltos (Vaughn & Hakenkamp 2001). Por otra parte, los bivalvos pueden acumular nutrientes, estos pueden ser capturados, almacenados en sus tejidos y un tiempo después liberados por muerte masiva de los individuos. La muerte masiva de bivalvos se puede producir por variaciones en las condiciones ambientales temperatura, falta de oxígeno o enfermedades entre otros dejando así disponible grandes cantidades de nutrientes (Vaughn et al. 2007; Sousa et al. 2012).

Para comprender cómo es la dinámica de la tasa de excreción de *D. parallelopipedon*, se realizaron experimentos de laboratorio para explorar las relaciones con la temperatura y la concentración de alimento (BOX 2). El rango encontrado para la tasa de excreción de PT fue 0.98-2.37 μg P g PS^{-1}h^{-1}. La tasa de excreción de PT fue influenciada por la temperatura ($F_{(4,68)}=10.84$, p=0.00). Sin embargo, no se encontraron diferencias significativas en las temperaturas más bajas (p=0.84), el mayor valor se observó a los 20°C y luego a temperaturas mayores la tasa de excreción permaneció constante (20-30°C, p=0.54) (Fig. 5A). Cuando se analizó la tasa de excreción de PT en función de las diferentes concentraciones de alimento no se encontraron diferencias significativas entre los tratamientos ($F_{(2,68)}=0.29$, p=0.6) (Fig. 5B).

La tasa de excreción de NT mostró un patrón similar al de PT, con un aumento significativo a medida que se incrementa la temperatura ($F_{(4,68)}=10.31$, p=0.000). Los valores más bajos se registraron a 10°C (5.44μg N g PS^{-1}h^{-1}), mientras que no se registraron diferencias significativas entre 10-15°C (p=0.38). Los mayores valores se encontraron a 30°C (28 μg N gPS^{-1} h^{-1}), sin embargo, no se registraron diferencias significativas entre 20, 25 and 30°C (p=0.34) (Fig. 5C). Considerando la cantidad de alimento disponible en los diferentes tratamientos, la tasa de excreción de NT no presentó diferencias significativas ($F_{(2,68)}=0.93$, p=0.39) (Fig. 5D).

Box 2. Estimación liberación de nutrientes

Estos experimentos se realizaron a continuación de los experimentos de filtración. En 500 ml de agua comercial se colocaron 3 bivalvos por recipiente. Respetando el tratamiento asignado en la tasa de filtración de concentración de fitoplancton (bajo, medio, alto) and temperatura (10-15-20-25-30°C). Se realizaron 5 réplicas de cada tratamiento y 3 controles consistieron en los recipientes con agua comercial sin bivalvos. Los experimentos fueron realizados en oscuridad y la duración de 6 horas para cada temperatura y concentración de alimento (Conroy et al. 2005). PT y NT se determinaron de acuerdo Valderrrama (1981). La tasa de excreción fue expresada en términos de microgramo de N o P por gramo de peso seco (mg g DW h^{-1}).

Para determinar que fracciones de nutrientes que se están liberando se consideraron 3 temperaturas (15-20-25°C) y se consideró la concentración media de fitoplancton.

Figura 2.1 Diseño del experimento tasa de excreción.

Se analizaron las facciones de totales de fósforo (PT) y disueltas (SRP, fósforo reactivo soluble), así como las fracciones totales de nitrógeno (NT) e inorgánicas disueltas (NH$_3^+$, NO$_3^-$ y nitrógeno total disuelto NTD). Para determinar la tasa de excreción se realizó el mismo procedimiento descripto anteriormente.
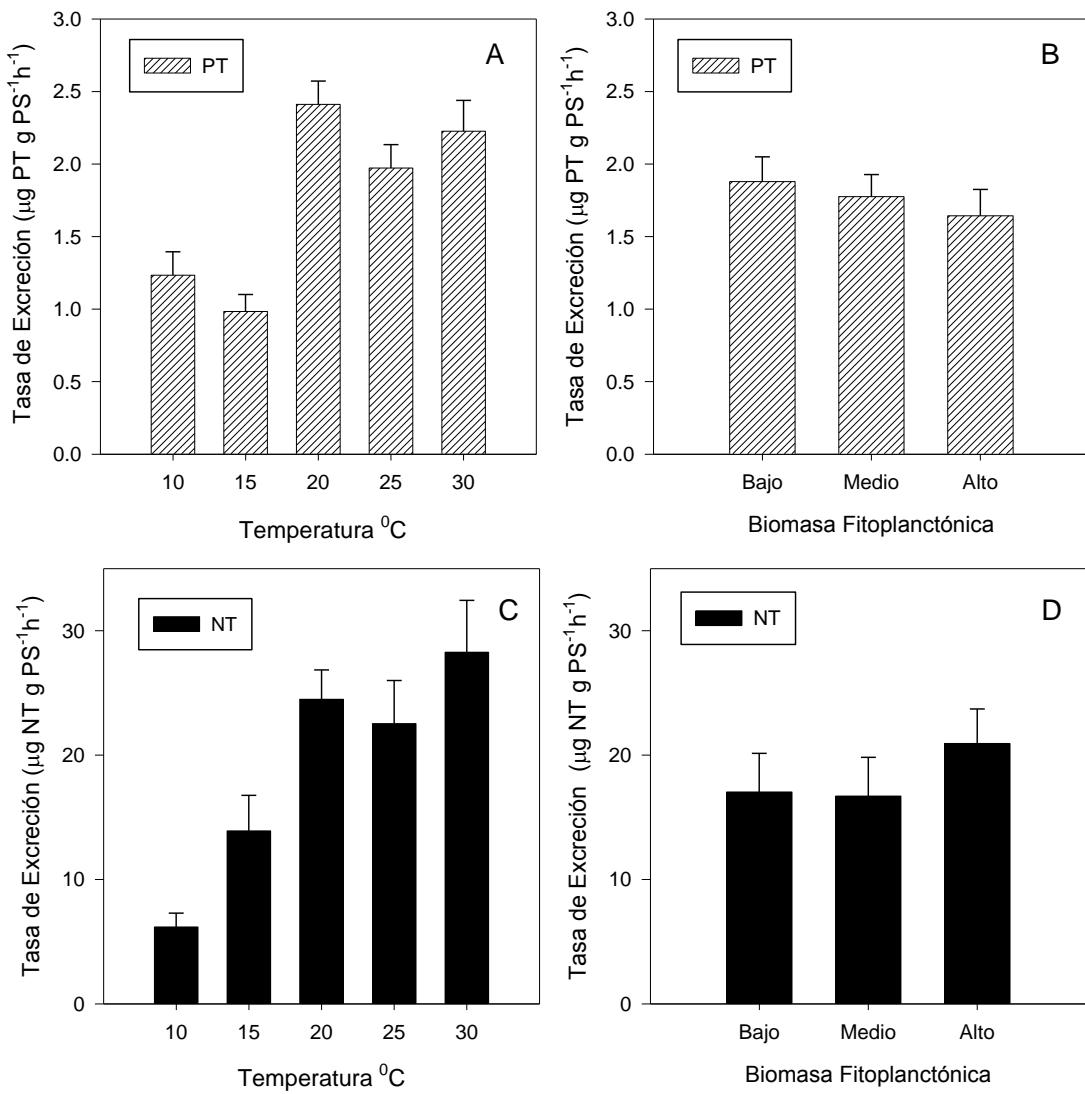
Figura 5. Tasa de excreción de fósforo total (PT) y nitrógeno total (NT) (μg g PS^{-1} h^{-1} of PT/NT) de *D. parallelopipedon* versus temperatura (derecha) y biomasa fitoplanctónica (izquierda). Los valores presentados en ambas gráficas corresponden al promedio del valor registrado en los tratamientos considerados. La tasa de excreción para ambos nutrientes aumenta con la temperatura. No se observaron diferencias significativas entre los tratamientos de biomasa fitoplanctónica (alta, media, baja). Se indica el error estándar. Tomado de artículo 3.

Si consideramos las fracciones liberadas encontramos que la mayor liberación de fósforo

ocurre a los 20 ºC en forma de SRP (fósforo reactivo soluble). La mayor liberación de nitrógeno

se constató en forma de NH_4^+ a los 25 °C (Fig. 6). El rango de la tasa de excreción de SRP fue de

0.9-1.2 μg g PS^{-1} h^{-1} y NH_4^+ de 8.4-12.1 μg g PS^{-1} h^{-1} (Tabla 1).
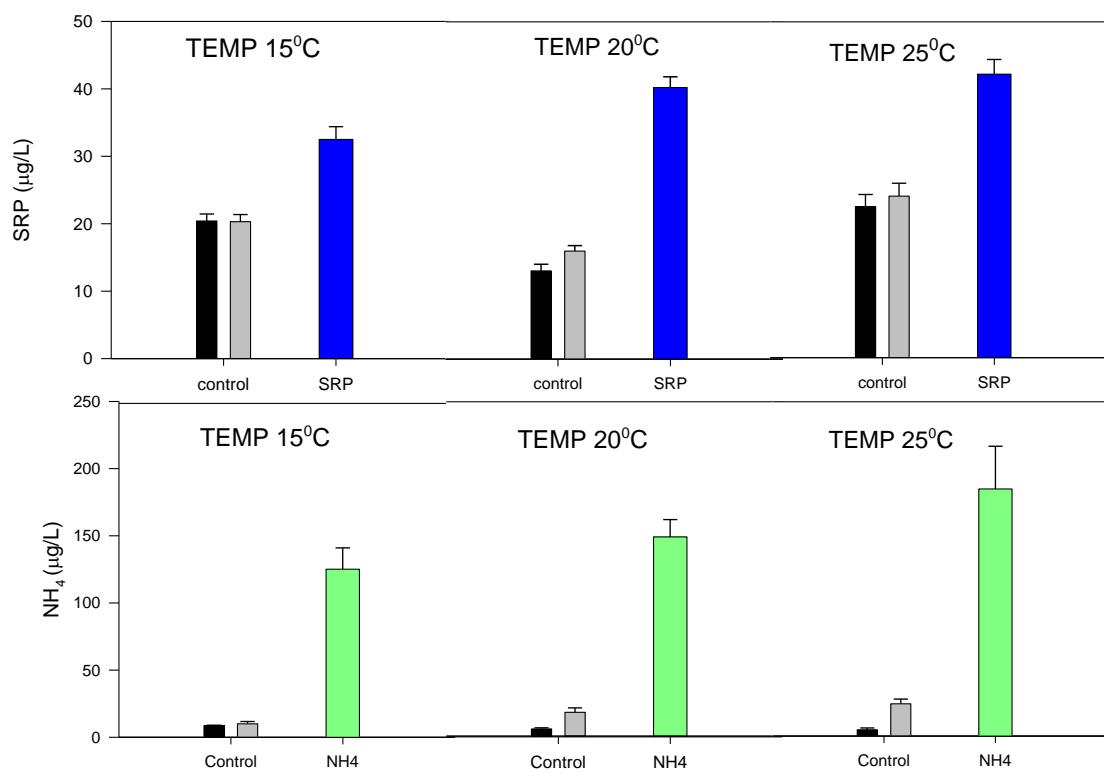
Figura 6. Excreción de fósforo reactivo soluble (SRP) (μg/L) y amonio (μg/L) por *D. parallelopipedon* a diferentes temperaturas (15, 20, 25^{0}C). Tratamientos: concentración inicial (barras negras); control final (barras grises), SRP (azul) y NH_4 (verde). El mayor aumento de SRP se registró a los 20^{0}C, mientras que para NH_4 correspondió a los 25^{0}C. Modificado de manuscrito 1.

Tabla 1. Tasa de excreción promedio (μg g PS[1] h^{-1}) error estándar está indicado entre paréntesis () de las distintas fracciones de fósforo disueltas (SRP, fósforo total disuelto (PTD)), así como las fracciones inorgánicas disueltas nitrógeno (NH_3^+, NO_3^- y nitrógeno total disuelto NTD) a diferentes temperaturas (15, 20, 25 ^{0}C). Tomada de manuscrito 2

	SRP	PTD	PT	NO3	NH4	NT
15	0.95 (0.48)	1.1 (0.6)	0.8 (0.20)	0.3 (13)	8.4 (1.6)	15.9 (6.1)
20	1.5 (0.34)	1.6 (0.5)	1.4 (0.20)	-1.9 (2.37)	5.2 (1.5)	11.4 (3.7)
25	1.4 (0.49)	1.6 (0.7)	1.0 (0.25)	-8.4 (4.63)	12.1 (3.45)	17.6 (8.3)

Los bivalvos pueden ejercer un control top-down sobre el fitoplancton, sin embargo también pueden favorecer su desarrollo mediante la re-mineralización de a través de la excreción de nutrientes. Los resultados indican que *D. parallelopipedon* libera nutrientes principalmente fósforo reactivo soluble y amonio (Tabla 1). Estos pueden ser asimilados rápidamente por el fitoplancton favoreciendo su desarrollo. En este trabajo se consideraron las formas totales y disueltas de fósforo y nitrógeno disueltos excretados por *D. parallelopipedon*, y no se contabilizaron las formas particuladas como heces y seudoheces. I Los valores de tasa de excreción de *D. parallelopipedon*, encontrados fueron bajos si los comparamos con especies exóticas como *L. fortunei* o *D. polymorpha* (Arnott & Vanni 1996; Cataldo et al. 2012). Además, encontramos un aumento de la tasa de excreción simultáneamente con una disminución de la tasa de filtración exhibida para la temperatura más alta aplicada (Fig. 4). Esta observación nuevamente sugiere que los bivalvos a altas temperaturas pueden estar estresados y podrían estar descomponiendo sus proteínas, excretando amoníaco (Spooner & Vaughn 2008) que no proviene de la remineralización de los alimentos ingeridos

6-Importancia relativa en la producción de biomasa de *D. parallelopipedon* de la alimentación por filtración y la alimentación pedal

Los bivalvos resultan también clave en el funcionamiento ecosistémico ya que transfieren materia orgánica y nutrientes desde y hacia la columna de agua, interaccionando con las comunidades planctónicas, bentónicas y nectónicas, estimulando la producción primaria y secundaria (Howard & Cuffey 2006; Spooner & Vaughn 2006; Vaughn et al. 2007). Son organismos filtradores omnívoros capaces de alimentarse de una gran variedad de material suspendido en la columna de agua, tales como: bacterias, fitoplancton e incluso zooplancton (Zangh et al. 2010; Davenport et al. 2011; Peharda et al. 2012; Artículo 2). Por otra parte, algunos bivalvos son capaces de alimentarse además de materia orgánica particulada del sedimento a través de mecanismos denominados alimentación pedal (Vaughn & Hakenkamp 2001; Nicholson et al 2005; Marroni et al. 2014). Nuestros resultados indican que *D. parallelopipedon* puede consumir entre 131-181 mg de MO g $PS^{-1}dia^{-1}$ (Marroni et al. 2014). Esta alimentación consiste en tomar partículas del sedimento y transportarlas hasta el tracto digestivo (Brendelberger & Klauke 2009), existen distintas estrategias de cómo utilizan el pie filtrador para captar partículas, descritas en detalle en Reid et al. (1992) y Nicholson et al. (2005).

Para entender la importancia relativa de estas vias de alimentación se analizaron las composiciones isotópicas de N y C en muestras de músculo de *D. parallelopipedon* provenientes de sectores con bajo (0-10% MO) y alto (20-30% MO) contenido de materia orgánica del sistema Laguna del Sauce, así como muestras de sedimentos y seston de la columna de agua (Box 3).
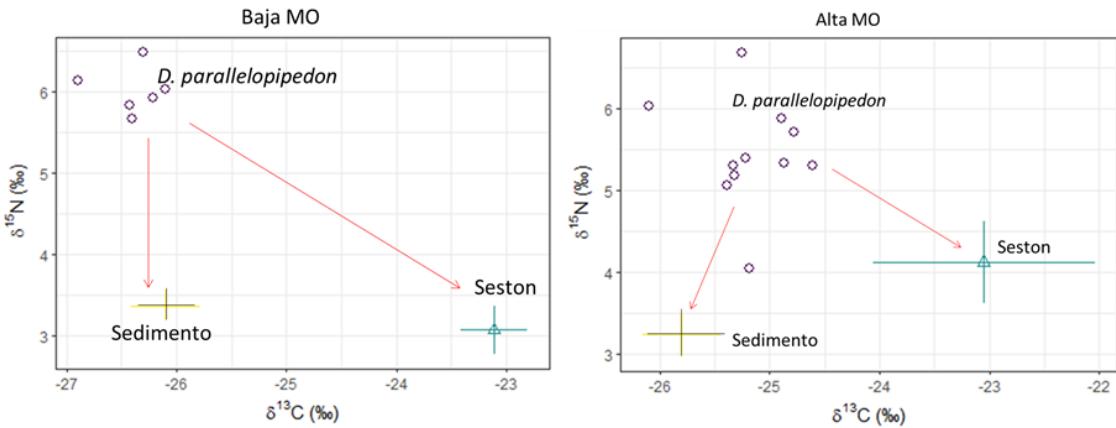
Figura 6. Análisis de isotopos estables de δ^{15}N y δ^{13}C, valores medios ‰ ± SD), el consumidor *D. parallelopipedon* (círculos) y las fuentes de alimento: seston y sedimento (Cruces). Los dos sectores de la Laguna con bajo (izquierda) y alto (derecha) contenido de materia orgánica. Los resultados en ambos casos indican un mayor aporte de materia orgánica del sedimento en la composición de la biomasa de los bivalvos. Tomado de manuscrito 2.

Cuando analizamos la importancia relativa de las variables seston y materia orgánica del sedimento a través de un análisis de **mixing models** encontramos que el sedimento r tiene una mayor importancia relativa que en el seston en la generación de biomasa de *D. parallelopipedon* en ambos sectores de la Laguna (Fig. 6)*. La importancia relativa del recurso materia orgánica del sedimento varía entre 75% a un 98%. Mientras que el aporte del seston a la biomasa de *D. parallelopipedon* se encontró en una menor proporción máxima de un 25%.

Alguno de los problemas que surgen en los análisis de isotopos estables es la estabilidad en el tiempo de las señales isotópicas del fitoplancton y su contaminación con detritus (Jardin et al 2014). Una solución propuesta por Jardin et al. (2014) al problema planteado es la utilización de señales integradas en otros organismos que consumen la fuente de alimento de nuestro interés. En este sentido y en un posterior análisis de los datos, consideramos a la Laguna del Sauce en su conjunto y señales alternativas de fuentes de alimento de *D. parallelopipedon*. Para la filtración de la columna de agua utilizamos una señal integrada, representada por la especie *L. fortunei,* bivalvo filtrador exótico invasor presente en el sistema y que se alimenta exclusivamente de seston. Para la señal de materia orgánica mantuvimos la misma alternativa

inicial ya que es estable en el tiempo. Los resultados encontrados considerando las variantes

señaladas fueron similares a los presentados en la Fig. 6. El mayor aporte a la biomasa del *D. parallelopipedon* se produce por parte de la materia orgánica del sedimento (Fig.7).
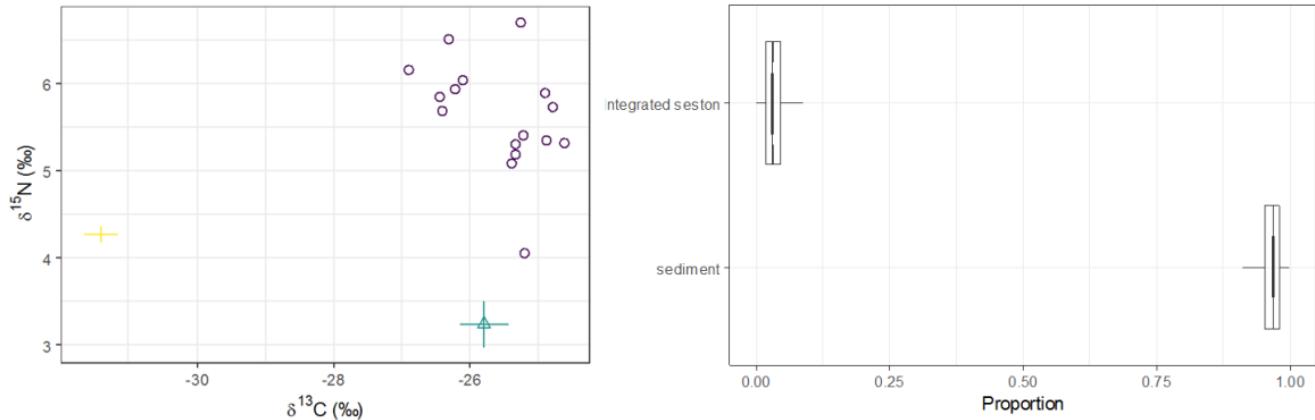
Figura 7. Izquierda: análisis de isotopos estables de δ^{15}N y δ^{13}C, valores medios ‰ ± SD., Consumidor *D. parallelopipedon* (círculos) y las fuentes de alimento cruces (señal integrada de seston corresponde a ejemplares de *L. fortunei* (azul) /sedimento (amarillo)). Derecha: **Mixing Model**: Boxplot del resultado de la importancia relativa de las dos fuentes de alimento asociado a la producción de biomasa de *D. parallelopipedon*. Nuevamente se observa que le mayor aporte a la biomasa de *D. parallelopipedon* proviene de materia orgánica del sedimento. Tomado de manuscrito 2.

Los bivalvos son generalmente considerados como organismos filtradores, sin embargo los

resultados obtenidos indican una mayor importancia de la vía de alimentación pedal en *D. parallelopipedon* en comparación a la filtración. Estos resultados son congruentes con los

resultados de Stábile (2017) en Laguna del Sauce, donde constata una menor importancia de la

producción primaria fitoplanctónica en las señales isotópicas de los herbívoros, consumidores

primarios y secundarios, tanto bentónicos como nectónicos. La limitación de luz en la columna

de agua por largos períodos, el aporte de materia orgánica de los humedales asociados a los

principales tributarios, así como la hojarasca de la vegetación arbórea litoral dominante

(Sauce, especie caducifolia) condicionan los patrones observados. Resultados similares en

bivalvos por ejemplo: *C. fluminea* han sido observado previamente y destacan la importancia

de la alimentación pedal para el metabolismo de los bivalvos (Raikow & Hamilton 2001; Klauke

2009). Los resultados del análisis de *mixing models* son similares a los resultados de Raikow & Hamilton (2001), donde indican que la contribución de la alimentación pedal es de un 80% en relación la filtración de los bivalvos.

Nuestros análisis no nos permiten discriminar si la alimentación pedal es la responsable de toda la MO consumida por *D. parallelopipedon.* Esta MO puede provenir de la re suspensión del sedimento y ser ingerida via filtración de la columna de agua. Algunas especies como *L. fortunei* presentan solo alimentación por filtración de la columna de agua.

7. Rol del bivalvo *Diplodon parallelopipedon* en la dominancia de cianobacterias potencialmente tóxicas

La dominancia de cianobacterias en los sistemas acuáticos eutróficos es un fenómeno cada vez un fenómeno más frecuente (Wilson & Chislock 2013). Las cianobacterias han desarrollado estrategias competitivas para una mayor eficiencia en la captación de recursos como la luz y los nutrientes. A modo de ejemplo, en condiciones de baja intensidad de luz pueden modificar su posición en la columna de agua a través de la flotación lo que les permite optimizar la fotosíntesis. Por otra parte, son capaces de vivir en amplios rangos de temperatura. (Wolfe 2000; UNESCO 2009).

Las toxinas liberadas por las cianobacterias son altamente tóxicas y afectan tanto a los animales como al hombre (Leflaive & Ten-Hage 2007; Azevedo et al. 2002). Estas sustancias llamadas cianotoxinas están agrupadas en hepatotoxinas, neurotoxinas y dermotoxinas de acuerdo con su mecanismo de acción (Carmichael 1992). Microcystina-LR es una hepatotoxina ampliamente distribuida que inhibe a la proteína fosfatasa generando deformación celular, necrosis, hemorragia intrahepática y muerte celular. La exposición prolongada a estas toxinas puede generar daños acumulativos (Carmichael 1992; 1994; Harada et al. 1996; Codd et al. 1999; Zaccaroni & Scaravelli 2008).

Las cianobacterias tienden a dominar el fitoplancton en condiciones particulares, como por ejemplo en condiciones variadas de relación N/P, elevadas temperaturas, períodos de estratificación de la columna de agua, o en presencia de comunidades zooplanctónicas dominadas por pequeños organismos (Leflaive & Ten-Hage 2007; UNESCO 2009). En nuestro país se han registrado floraciones de cianobacterias en diversos ecosistemas, las mismas ocurren principalmente en verano (Bonilla et al. 1995; Bonilla 1997; Kruk & De León 2002; Kruk et al. 2003; Gonzales-Madina et al 2018).

En varios casos se ha relacionado la aparición de floraciones y/o blooms (de cianobacterias con la introducción en el sistema acuáticos de especies exóticas de bivalvos (Vanderploeg et al. 2002; Raikow et al. 2004). En este sentido, estudios recientes indican que *Limnoperna fortunei* es capaz de modificar la proporción y concentración de nutrientes, promoviendo la agregación de *Microcystis spp*. y favoreciendo la aparición de blooms tóxicos de cianobacterias (Cataldo et al. 2012). *D. polymorpha*, otro bivalvo invasor ampliamente estudiado, presenta un consumo diferencial del fitoplancton por lo cual se produce un cambio en la composición y abundancia de las comunidades planctónicas (Holland 1993). Se ha sugerido que este consumo diferencial es el que provoca la aparición de floraciones algales tóxicas de *Microsystis spp*. (Vanderploeg et al. 2002; Raikow et al. 2004; Sarnelle et al. 2005). Por otro lado, ciertos estudios indican que *Dreissena sp.* es capaz de coexistir y consumir *Microsystis spp*. (Caraco et al. 1997; Baker et al. 1998; Dionisio Pires & Van Donk 2002; Dionisio Pires et al. 2004). En el caso de exposición prolongada a Microcystis-LR por parte de *Dreissena polymorpha*, no se constataron modificaciones en su tasa de filtración (Vanderploeg et al. 2001) pudiendo incluso bioacumular esta sustancia en sus tejidos, sin causar la muerte del organismo (Dionisio Pires et al. 2004).

D. parallelopipedon es capaz de consumir un amplio rango de partículas. Nuestros resultados indican que es capaz de consumir partículas de pequeño tamaño como *Ankistrodesmus sp.* hasta *Planktothrix agardhii* (Marroni et al 2014) (Fig. 8). Con el objetivo de determinar si *D.*

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parallelopipedon favorece la presencia de cianobacterias a través de un consumo diferencial se realizaron experimentos de filtración (VER BOX 4). Nuestros resultados indican que existe un consumo diferencial de fitoplancton (Fig 9. arriba)*. Diplodon parallelopiepdon* no es capaz de consumir CMA (complejo de *M. aeruginosa*) cuando se encuentra formando colonias debido a la capacidad de flotar y mantenerse en la superficie. En esta configuración *D. parallelopipedon* no puede consumirlas*.* Por otra parte, es capaz de consumir y controlar un alga palatable como *Cryptomonas sp.* (Fig. 9. abajo).
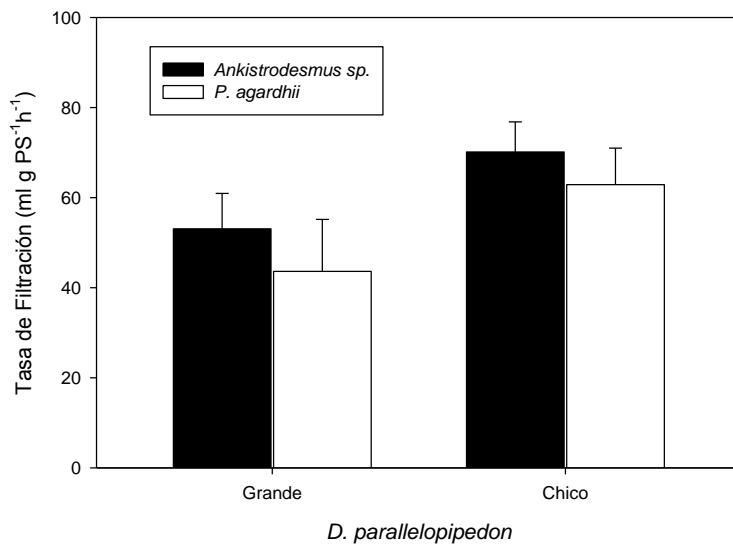

Figura 8. Comparación de la tasa de filtración (ml g PS h^{-1}) de *D. parallelopipedon* con dos tamaños grande (GR 7-9cm) y chico (CH, 5-6cm). Tratamientos: *Ankistrodesmus sp*. (negra) y *P. agardhii* (blanco)*.* No hay diferencias significativas entre la tasa de filtración de ambas especies de fitoplancton. Se muestra el promedio y el Error estándar. Tomado Marroni et al 2014

Box 4. Consumo diferencial de fitoplancton

Para determinar el diferencial de consumo de *D. parallelopipedon,* se realizó un experimento de laboratorio en condiciones controladas de temperatura (20 ° C) y oscuridad que simulan condiciones naturales, durante las pruebas de 1 h. Se utilizaron acuarios de 1 litro de capacidad y se llenaron con 500 ml de mezcla de fuentes de alimentos. En cada acuario se colocó 1 individuo de *D. parallelopipedon* (60-80 cm). Los bivalvos seleccionados fueron hambreados 24 h antes de comenzar la prueba. Se ofrecieron diferentes fuentes de alimentos: 1- conjunto de fitoplancton: dominados por *Cryptomonas sp.* (cultivados en el laboratorio) (C) y 2-población salvaje de *Microcystis aeruginosa* (MC). Se mezclaron ambas fuentes de alimentos para hacer un gradiente de concentración relativa (%) entre MC y C. Los tratamientos fueron: 1- 100% MC, 2: 75% MC-25% C, 3 50% MC-50% C, 4: 25% MC-75% C y 5: 100% C. Se realizaron 3 replicas y 3 controles por tratamiento. Los controles consistieron en el tratamiento sin bivalvos. Al comienzo y al final del experimento, se tomaron muestras de agua (Fijaron con lugol) para contar con una microscopía invertida de luz, y estas muestras se tomaron para determinar qué fracciones se consumidas en cada tratamiento. El conteo Se realizó en cámaras de sedimentación, se contaron campos aleatorios hasta contar 100 individuos o colonias de las especies más frecuentes.
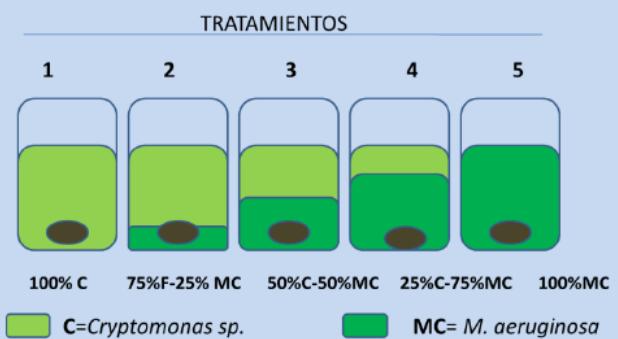
Figura 4.1 Diseño experimental de consumo diferencial de fitoplancton. Se realizó un gradiente de concentración entre *Cryptomonas sp.* (C) y *M. aeruginosa* (MC)
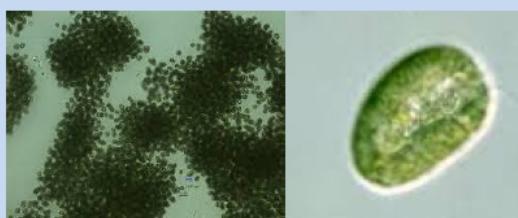
Figura 4.2. Imagen de *M. aeruginosa* colonia (derecha) y *Cryptomonas sp.* unicelular (izquierda)
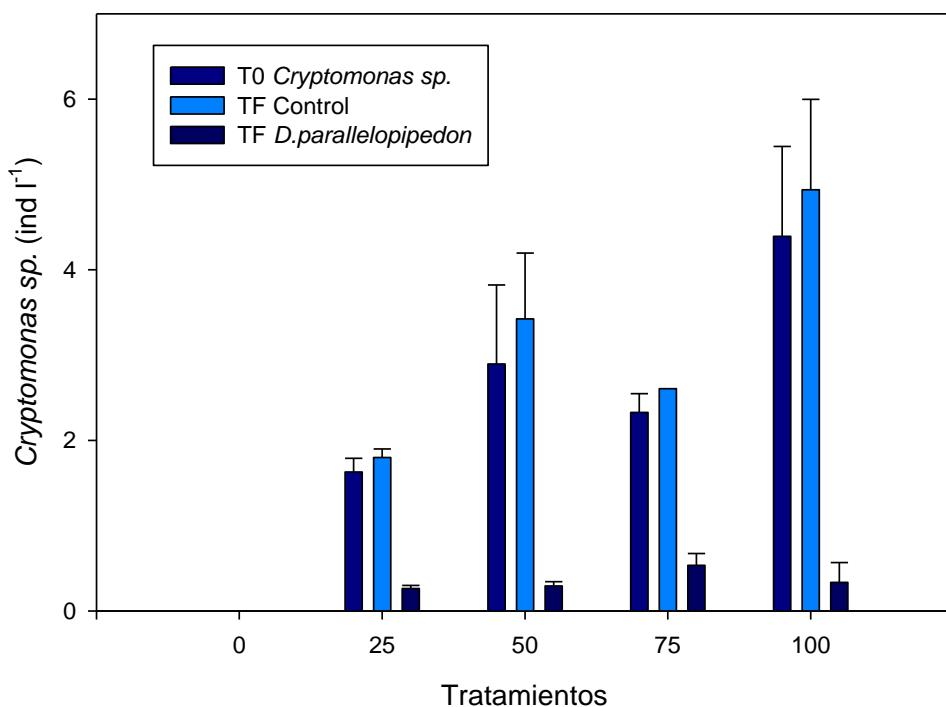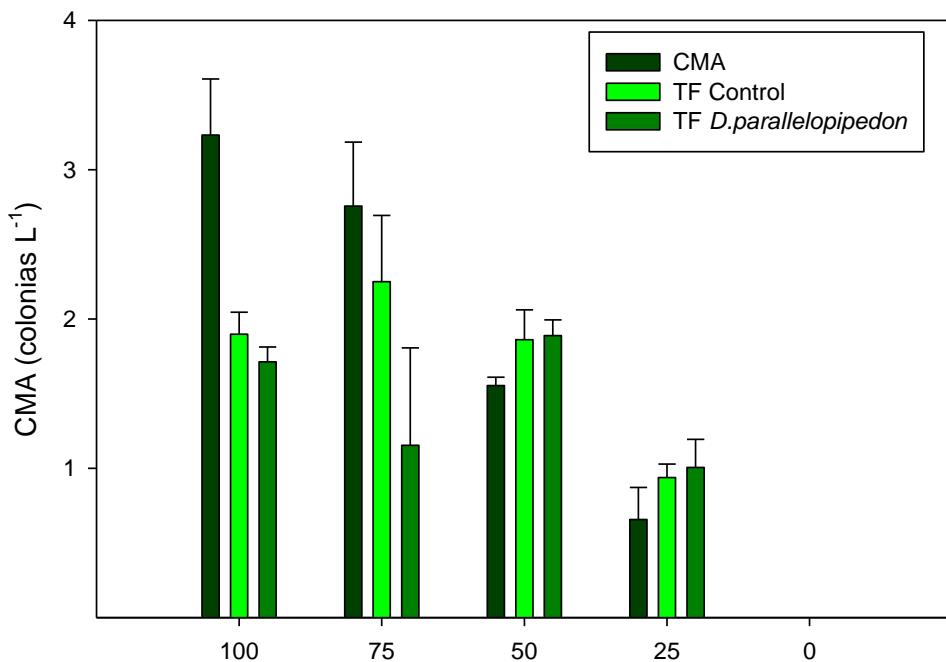
Figura 9. Experimento de consumo diferencial CMA (complejo de *M. aeruginosa* (M, arriba) y *Cryptomonas sp*. (C, abajo). Tratamientos 1: 100% M, 2: 5%M-25% C, 3: 50%M-50%C, 4: 25%M-75%C 5: 100%C. En la gráfica de arriba se puede observar como *D. parallelopipedon* es incapaz de consumir colonias CMA. Mientras que en la gráfica de abajo se pue de observar un consumo efectivo de *Cryptomonas sp.* Tomado del manuscrito 1.

Algunas investigaciones indican que los bivalvos son capaces de consumir cianobacterias como *M. aeruginosa* (Vanderploeg et al. 2002; Raikow et al. 2004; Sarnelle et al. 2005). Sin embargo, esos experimentos se realizaron con cultivos de laboratorio donde las células no presentaron mucilago y por lo tanto no son capaces de formar colonias. En el experimento presentado aquí se intentó reflejar las condiciones naturales en el cual se encuentra CMA , es decir formando colonias, en estas condiciones *D. parallelopipedon* no fue capaz de consumir colonias de CMA. Por otra parte, *D. parallelopipedon* es capaz de consumir filamentos de cianobacterias como *Planktothrix agardhii* distribuidos en toda la columna de agua (Marroni et al 2014).

7.1 Efecto de la liberación de nutrientes y CMA

Nuestros resultados indican que la liberación de nutrientes de *D. parallelopipedon* (0.95-1,5 μg de P g PS^1 h^{-1}/8.4-12.1 μg de Nh_4 g PS^1 h^{-1}, 15-25^{0}C) es alta comparada con las exhibidas por otras especies exóticas como *Dreissena polymorpha* (Conroy et al. 2005). Sin embargo, las tasas de excreción de PT y NT fueron más bajas en comparación con las informadas para *L. fortunei* (39.88 μg de P g PS^1 h^{-1}/ 51.93 μg de Nh_4 g PS^1 h^{-1}(20-25^{0}C)) en experimento de mesocosmos (Cataldo et al. 2012). Más aún, en nuestro caso se constató un aumento de la tasa de excreción simultáneamente con una disminución de la tasa de filtración exhibida para la temperatura más alta aplicada (Fig. 4/Fig. 5).

D. parallelopipedon no es capaz de ejercer un control top-down sobre CMA ya que no es capaz de consumirla. Por otro lado, toma más relevancia en este caso la liberación de nutrientes que favorecer el crecimiento de CMA. Estos nutrientes son rápidamente asimilados y fundamentales para el desarrollo del fitoplancton (Tabla 1). Además, en un escenario dominado por *P. agardhii*, el control top-down sería la principal fuerza de control mientras que el control bottom up tendría un rol secundario. Estos son resultados de experimentos en laboratorio, para complementar está investigación hay que analizar estudios de campo y

estimar mediante modelos matemáticos la cantidad de bivalvos por m^2 necesarios para lograr en el campo, los resultados obtenidos en el laboratorio.

8. Interacción entre bivalvos y el zooplancton

Los bivalvos han sido considerados organismos filtradores principalmente. En este sentido resulta importante entender la relación entre el zooplancton y *D. parallelopipedon* ya que ambos son consumidores de fitoplancton. Además, algunos bivalvos han sido reportados como consumidores de zooplancton, ej: *Corbicula leana* and *Limnoperna fortunei* (Hwang et al 2004; Rojas Molina et al 2012). Los bivalvos y zooplancton pueden ser considerados competidores pueden ser considerados competidores (Dame 1996), o presentar una dinámica de predación intra gremio (Polis et al. 1989; Polis and Holt, 1992; Wong et al. 2006; Davenport et al 2011; Peharda et al 2012). Está relación se analizó mediante experimentos en el laboratorio (VER BOX 5). Los resultados de la presente tesis indican una disminución significativa del zooplancton en los tratamientos con bivalvos (*D. parallelopipedon* y *C. fluminea*) ($F_{(1,8)}$=26.76; p<0.05). La fracción del zooplancton que presentó la mayor disminución fueron los microfiltradores (rotíferos y nauplios de copepodos) ($F_{(1,8)}$=22,42;p<0,05). Los organismos mesofiltradores (*Bosmina* sp. y otros cladoceros) y los copépodos ciclopoides no presentaron diferencias significativas entre los tratamientos y los controles finales (Fig. 10). En simples términos, *D. parallelopipedon* es capaz de consumir organismos de pequeño tamaño, microfiltradores del zooplancton, pero los mesofiltradores y los copépodos ciclopoides no fueron consumidos por *D. parallelopipedon*, probablemente debido a su capacidad de nado y escape (Wong et al. 2006).
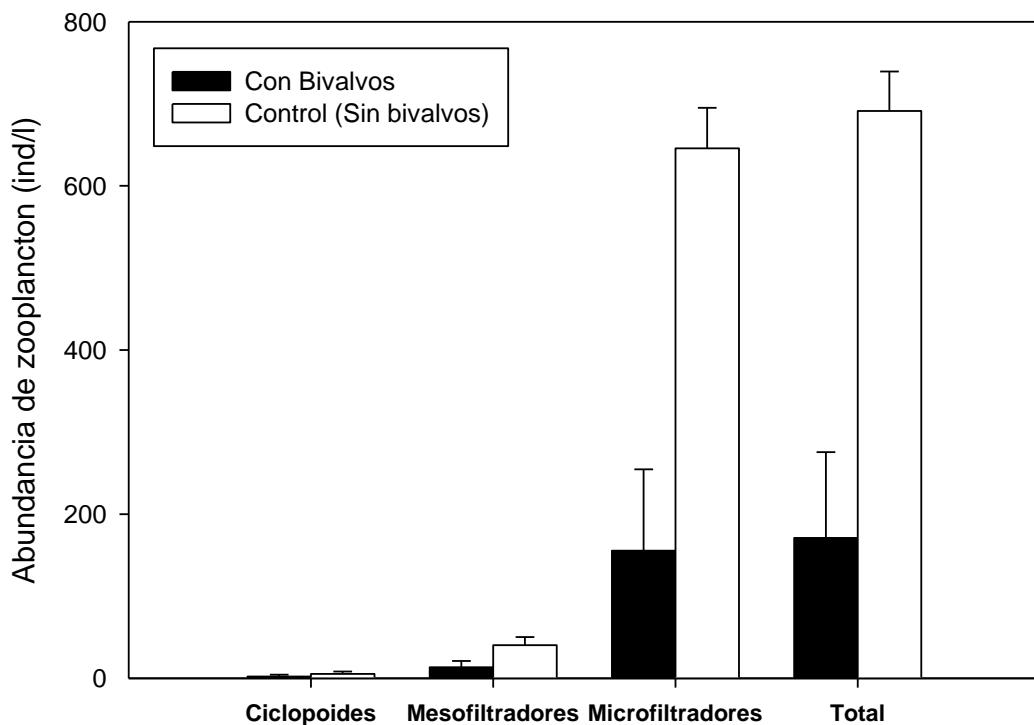
Figura 10. Resultados del experimento interacción entre bivalvos (*D. parallelopipedon,* D y *C. fluminea*, C) y zooplancton (ZOO). Se indica el valor final del total del zooplancton y por separado las fracciones de los grupos morfo-funcionales en cada uno de los tratamientos. Tratamientos: bivalvos (D+C+ZOO) y el control zooplancton (sin bivalvos). Las barras corresponden al valor medio y se indican al error estándar. Tomado de Marroni et al 2016.

El consumo diferencial de zooplancton de pequeño tamaño afecta la estructura de tallas de la comunidad y tiene como consecuencias un aumento de las tallas del zooplancton presente en el sistema. En este sentido, luego de la invasión *L. fortunei* en el río Paraná se observó una disminución de la clorofila a y un aumento de las tallas del zooplancton (Rojas & José de Paggi 2008). El efecto final de la introducción de bivalvos resultaría entonces no solo del efecto directo de remoción sobre el fitoplancton sino que además provocaría un aumento en las tallas del zooplancton, lo cual de forma indirecta afectaría también negativamente al fitoplancton al potenciar una mayor capacidad de filtración (Wong et al. 2006). La evidencia del consumo de rotíferos y nauplios ha sido reportado para otras especies de bivalvos, como *Mytilus edilus* (Wong & Levinton 2006), *Dreissena polymorpha* (Wong et al. 2003), *Corbicula.*

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leana (Hwang et al. 2004) y *Limnoperna fortunei* (Molina et al. 2012). En contraste, algunos estudios previos confirmaron el consumo de cladoceros y copépodos por bivalvos después del análisis de contenido estomacal (Zeldis et al. 2004; Wong & Levinton 2006). En el presente estudio se pudo ratificar experimentalmente la existencia de un efecto diferencial de bivalvos sobre el zooplancton dependiente del tamaño de éste último, constituyendo una depredación intragremio (Marroni et al 2016) con respecto a los organismos de menor talla al estar depredando sobre los nauplios y rotiferos con quien comparte el recurso fitoplancton (Fig. 11). En el caso de los organismos de mayor tamaño la relación ecológica establecida entre los bivalvos y los cladoceros y copepodos se trataría de una competencia por el recurso fitoplancton (Fi g. 11).
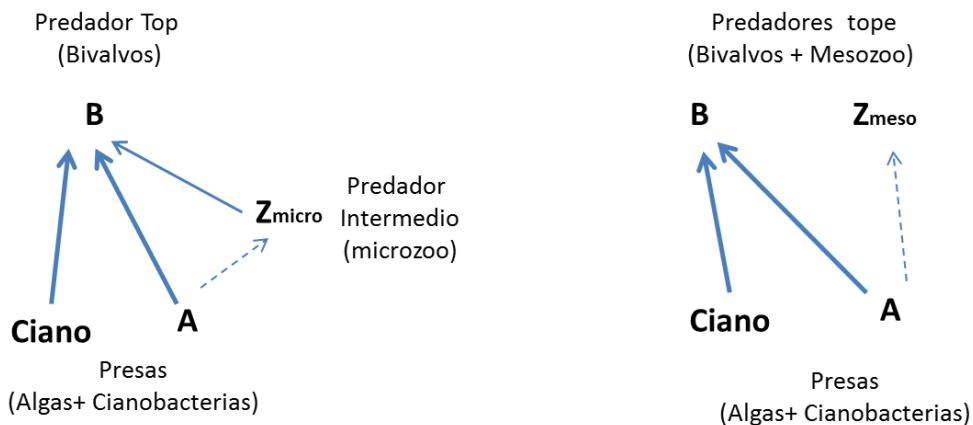
Figura 11. Esquema de las interacciones biológicas entre Bivalvos, zooplancton y presas. Las flechas indican el consumo de presas potenciales: algas comestibles (A) y cianobacterias (Ciano), por depredadores intermedios (microzooplancton) y superiores (bivalvos y mesozooplancton). La depredación intragremio se estableció entre bivalvos y microzooplancton (derecha). Las algas comestibles son presas compartidas. Entre bivalvos y mesozooplancton, no hay predación intragremio en las actuales condiciones experimentales. Las cianobacterias aparentemente solo fueron atacadas por bivalvos. Tomado de Marroni et al 2016.

9. Implicancias para la biomanipulación

La filtración de la columna de agua es uno de los principales servicios ecosistémicos que brindan los bivalvos. Cuando estos se encuentran en grandes densidades son capaces de filtrar grandes volúmenes de agua (Fulford et al. 2007; Vaughn 2017). Los bivalvos son herramientas útiles para la biomanipulación ya que pueden reducir efectivamente la biomasa de fitoplancton (Olivie & Mitchell 1995; Reeders & Bij de Vaate 1990; Reeders et al. 1993; Soto & Mena 1999; Richard & Sergej 2003; Hwang et al. 2004; Stadmark & Conley 2011; Dionisio Pires et al. 2007).

Este trabajo buscó profundizar aspectos claves de *D. parallelopipedon* procurando comprender su rol en el ecosistema y analizar su utilización como herramienta de biomanipulación. La evidencia aquí contenida tiene varias implicaciones para el diseño de posibles técnicas de biomanipulación basadas en el uso de bivalvos en general y particularmente para el caso de esta especie autóctona. Los resultados mostraron que *D. parallelopipedon* tiene potencial para ser utilizado como herramientas de biomanipulación, si se considera su capacidad de filtración pero su uso potencial dependerá también del grupo fitoplanctónico presente en el ecosistema.

Las estrategias clásicas de biomanipulación incluyen el favorecimiento de la comunidad zooplanctónica, especialmente el cladocero *Daphnia sp.* Debido a las diferentes estructuras de las comunidades en los sistemas subtropicales la aplicación de las aproximaciones clásicas de biomanipulación han sido poco exitosas (Mazzeo et al. 2010; Iglesias et al. 2011). En los ejemplos de biomanipulación clásicos, cuando el fitoplancton desaparece también lo hace *Daphnia sp.* (siguiendo el clásico ciclo depredador-presa), permitiendo así al fitoplancton recolonizar el sistema (Scheffer 2004). Por otro lado, la utilización alternativa de bivalvos tiene ventajas sobre el esquema clásico de la biomanipulación. *D. parallelopipedon* tienen dos posibles fuentes de alimentación, filtración de la columna de agua y alimentación pedal, de esta manera si el fitoplancton desaparece ellos tienen una fuente alternativa de alimento

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pudiendo sobrevivir en situaciones donde no haya fitoplancton. De acuerdo con nuestros resultados la alimentación pedal es un componente importante en la biomasa de *D. parallelopiepdon*. Esta estrategia les permite persistir en el tiempo, y no proporciona ventanas de oportunidad para fitoplancton ya que están siempre presentes y activos durante todo el año en el sistema (Marroni et al. 2014).

Además, *D. parallelopipedon* puede consumir microzooplancton. Por lo tanto, la introducción de bivalvos no solo tendría efectos directos sobre el fitoplancton, sino también podría conducir a un aumento en el tamaño de la comunidad de zooplancton, que puede afectar indirecta y negativamente al fitoplancton (Marroni et al 2016).

La temperatura es el principal factor que afecta tanto la tasa de filtración como la de excreción. La tasa de filtración no varía linealmente con la temperatura, presenta un óptimo de filtración a los 20 ^{0}C. A mayores temperaturas las tasas son menores, posiblemente los bivalvos se estresan y disminuyen su actividad metabólica. Cuando analizamos la liberación de nutrientes, encontramos que los principales nutrientes libera dos son SRP y amonio. A mayores temperaturas los bivalvos se estresan y su tasa de filtración se reduce mientras que la remineralización de nutrientes se incrementa simultáneamente. En escenarios con temperaturas más elevadas *D. parallelopipedon* promueve el desarrollo de la biomasa de fitoplancton ya que los nutrientes liberados son potencialmente consumidos por el fitoplancton. Al mismo tiempo, la liberación de nutrientes favorece el crecimiento de las cianobacterias. De la siguiente manera CMA podría encontrar una forma fácil de usar los nutrientes remineralizados por *D. parallelopipedon*, ya que evitan la herbivoría en las capas superficiales, aprovechan la remoción de los competidores por filtración y el uso de nutrientes disponible en la columna de agua. En cualquier caso, nuestro conocimiento es aún escaso y se necesitará más investigación para dilucidar si esta novedosa técnica de biomanipulación que

utiliza bivalvos nativos es capaz de generar cambios de régimen en un rango intermedio de nutrientes.

D. parallelopipedon podría ser utilizado como una posible herramienta de biomanipulación, sin embargo, en ambientes con floraciones periódicas de CMA algunas consideraciones deben ser tenidas en cuenta. Además, el control de CMA a través de técnicas de biomanipulación es complejo ya que lograr un control top-down depende de múltiples factores (Triest et al 2015). Las estrategias combinadas como la introducción de bivalvos, peces piscívoros junto con macrófitas, podrían ser la mejor opción para mitigar los efectos de eutrofización. En este sentido, la introducción de bivalvos y macrófitas podría ser una buena estrategia, ya que las macrófitas sumergidos pueden contrarrestar y reducir efectivamente el efecto de liberación de nutrientes que se ha mostrado en este trabajo y resultar así en un efecto positivo de restauración (Wang et al 2018).

10. Conclusiones y Perspectivas

D. parallelopipedon es capaz de ejercer un control descendente o top-down sobre la gran mayoría de organismos presentes en el fitoplancton continental. Asimismo, es capaz de incidir en los controles bottom-up por la liberación de nutrientes, principalmente nitrógeno y fósforo en sus formas inorgánicas solubles, directamente utilizables por el fitoplancton. Por otra parte, *D. parallelopipedon* es incapaz de consumir CMA cuando se encuentra formando colonias, ya que en su forma colonial posee la capacidad de regular su flotación en la columna de agua lo que le permite evadir el ser consumido por los bivalvos. En este caso, el efecto positivo provocado por la excreción de nutrientes favorecería de forma diferencial el crecimiento de estas cianobacterias con capacidad de evasión de la depredación. Este aspecto es muy relevante en la aplicación de una estrategia para lograr la manipulación efectiva. Una alternativa según consideraciones de Triest et al 2015, podría ser la introducción de *D.*

parallelopipedon junto con macrófitas capaces de consumir los nutrientes liberados y ayudar a mantener la estabilidad una vez conseguida una disminución de la biomasa fitoplanctónica.

Independiente de las alternativas a explorar, es necesaria información complementaria sobre la capacidad de los bivalvos de acumulación y/o del daño que podría experimentar al estar expuestos de forma puntual o crónica a cianotoxinas en caso de producirse una floración algal tóxica. En este sentido también es necesario realizar experimentos que permitan confirmar que *D. parallelopipedon* es capaz de consumir células individuales en las etapas de formación inicial. Este punto podría ser clave en cuanto a la filtración preventiva de estas células. La determinación del ciclo de vida es también un aspecto importante en el establecimiento de cultivos de bivalvos que le permitan su cría para la posterior introducción en los ecosistemas. También se deberán realizar pruebas de campo sobre la implementación de bivalvos en los sistemas. En este sentido son también necesarios la elaboración de modelos matemáticos que permitan estimar cuantos bivalvos son necesarios por metro cuadrado para lograr el efecto deseado en el ecosistema.

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Interactions between bivalves and zooplankton. Competition or intraguild predation? Implications for biomanipulation in subtropical shallow lakes..

Artículo 1.

Interactions between bivalves and zooplankton: competition or intraguild predation? Implications for biomanipulation in subtropical shallow lakes

Soledad Marroni[A], *Néstor Mazzeo*[A], *Juan Pablo Pacheco*[A], *Juan Clemente*[A] *and Carlos Iglesias*[A,B]

[A]Grupo de Ecología y Rehabilitación de Sistemas Acuáticos, Departamento de Ecología Teórica y
  Aplicada, Centro Universitario de la Región Este-Facultad de Ciencias, Universidad de la
  República, Tacuarembó s/n, CP 20000, Maldonado, Uruguay.
[B]Corresponding author. Email: caif@cure.edu.uy

Abstract.   Zooplankton and bivalves are the main consumers of phytoplankton in shallow lakes; however, knowledge regarding trophic interactions between them is scarce. Competition for resources appears to be an obvious direct interaction, but the scheme may be more complex. Bivalves can consume all or only part of the zooplankton, constituting an intraguild predation module. In the present study we investigated the interaction between bivalves and zooplankton and its effects on phytoplankton grazing and community structure using an experimental approach in an aquarium. Two bivalve species were considered, namely *Diplodon parallelopipedon* (native) and *Corbicula fluminea* (non-native), in addition to a natural zooplankton community dominated by small-sized zooplankton. The findings indicate that phytoplankton consumption by the zooplankton is substantially less than that by the bivalves. Under the experimental conditions, the bivalves actively consumed small-sized zooplankton (rotifers and nauplii), but no consumption of medium-sized individuals (cladocerans and copepods) was observed. The differential consumption may have implications on the size and structure of zooplankton, favouring an average larger-sized community. Thus, phytoplankton may be negatively affected both directly by consumption and indirectly because of the resulting structure of the zooplankton community. The main results of the study are also discussed from the perspective of biomanipulation techniques in tropical and subtropical regions.

Additional keywords: experimental conditions, filtration rate, grazing, trophic interactions.

Received 7 April 2015, accepted 10 June 2016, published online 5 August 2016

Introduction

Phytoplankton, macrophytes and periphyton are the main primary producers in aquatic ecosystems providing energy support to the entire system (Lindeman 1942; Carpenter *et al.* 1985, 1987; Vadeboncoeur and Steinman 2002). Several factors are involved in the regulation of primary productivity, such as basin morphometry, nutrient input, water residence time and flushing (Carpenter *et al.* 2009). Moreover, direct competition for limiting resources (nutrients or light) between primary producers, grazing pressure from herbivores or indirect mechanisms through trophic cascades are also relevant factors controlling primary productivity (Phillips *et al.* 1978; Søndergaard and Moss 1998).

Top-down and bottom-up processes are important in determining which primary producer may dominate, and condition different ecosystem regimens in terms of structure and function (Scheffer *et al.* 1993, 1997; Fleeger *et al.* 2003; Scheffer and Carpenter 2003; Scheffer and Jeppesen 2007). In particular, grazing by large-sized zooplankton (Stephen *et al.* 2004) or filtration by bivalves (Caraco *et al.* 1997; Prins and Escaravage 2005; Newell *et al.* 2007) are important drivers of phytoplankton growth.

Zooplankton plays an important role in energy and matter transfer in food webs (Winder and Jassaby 2011), being highly dependent on the size of the organism (Burns 1968). However, marked reductions in phytoplankton biomass by small-sized zooplankton have been reported (Jeppesen *et al.* 1990), even in communities dominated by cyanobacteria (Kâ *et al.* 2012). The relevant organisms capable of markedly decreasing phytoplankton biomass (promoting the so-called clear-water state or regimen) in shallow lakes are medium- and large-sized cladocerans, typically *Daphnia* spp. (Scheffer 1998). Grazing by *Daphnia* sp. has been reported to be responsible for spring clearing in temperate lakes (Meijer *et al.* 1999). *Daphnia* spp. have the ability to feed on bacteria, protozoa, phytoplankton and even some small zooplankton, highlighting their important role in freshwater food webs (Yin *et al.* 2010). However, tropical and subtropical lakes are frequently dominated by small-sized zooplankton: cladocerans (*Diaphanosoma*, *Ceriodaphnia* and *Bosmina*), rotifers, juveniles and small copepodites (Dumont 1994; Lewis 1996; Branco *et al.* 2002; Ramirez García *et al.* 2002). This structural pattern was explained by the high predation exerted by small-sized fish over zooplankton (Sarma *et al.*

2005; Jeppesen *et al.* 2007; Meerhoff *et al.* 2007; Havens *et al.* 2009; Lacerot 2010; Sosnovsky *et al.* 2010; Havens and Beaver 2011; Iglesias *et al.* 2011).

Conversely, bivalves, which are capable of filtering large volumes of water, also play an important role in the transfer of energy to higher trophic levels in lake food chains (Strayer *et al.* 1999; Elliott *et al.* 2008; Vaughn *et al.* 2008). They can have an important role in coupling the pelagic and benthic food webs of a lake by importing a large portion of phytoplankton primary production from the water column into secondary production of sediments (Dame and Dankers 1988).

Numerous studies into the biology of freshwater bivalves have been performed in exotic and invasive species, due primarily to the economic effect of species like *Dreissena polymorpha* or *Limnoperna fortunei*. In contrast, the biology and ecological features of native bivalves, like *Diplodon* spp., are less known.

The invasion of a system by exotic bivalves like *Corbicula fluminea* or *D. polymorpha* may lead to an increase in water transparency and a reduction in phytoplankton biomass (Mayer *et al.* 2002; Vanderploeg *et al.* 2002; Zhu *et al.* 2006; Carroll *et al.* 2008; Wall *et al.* 2008; Strayer 2009). Bivalves are usually reported to be omnivores that can potentially consume detritus, bacteria, phytoplankton and zooplankton (Wong and Levinton 2006; Davenport *et al.* 2011; Peharda *et al.* 2012). In fact, some freshwater bivalve species have been reported to consume zooplankton (e.g. *Corbicula leana* and *L. fortunei*; Hwang *et al.* 2004; Molina *et al.* 2012). Bivalves and zooplankton may be competitors (Dame 1996), so an intraguild predation dynamic may arise (Polis *et al.* 1989; Polis and Holt 1992; Wong and Levinton 2006; Davenport *et al.* 2011; Peharda *et al.* 2012). Moreover, if the potential predation pressure exerted by bivalves over zooplankton does not affect the different size classes equally, predation may also affect the size structure of the resulting zooplankton community (Molina and José de Paggi 2008; Molina *et al.* 2012).

In the present study we analysed the interactions between bivalves and zooplankton experimentally. First, we compared the consumption of phytoplankton by bivalves (*D. parallelopipedon* and *C. fluminea*) compared with that of a natural subtropical zooplankton community (small-sized zooplankton). The first hypothesis tested was that bivalves exert a higher grazing pressure on the phytoplankton, thus outcompeting a typical subtropical zooplankton community. Second, we tested the ability of bivalves to consume zooplankton and consequently the presence of an intraguild predation module. The second hypothesis tested whether bivalves exert a greater predation pressure over small-sized zooplankton. Finally, we discuss the experimental evidence from the perspective of biomanipulation techniques for controlling eutrophic systems.

Materials and methods

The bivalves used in the experiments described below were collected by free diving at Laguna del Sauce (34°43′S, 55°13′W), Maldonado, Uruguay, and transported to the laboratory, where they were kept for 1 week in oxygenated aquariums and fed with algal cultures (principally composed of *Ankistrodesmus* sp.). Natural communities of phytoplankton and zooplankton were

collected from Laguna Blanca Lake (34°54′S, 54°50′W). Both systems are usually classified as eutrophic (Mazzeo *et al.* 2010*a*; Pacheco *et al.* 2010). In Laguna de Sauce, both native and exotic bivalves co-occur, particularly *Diplodon parallelopipedon* (Lea, 1834) (Bivalvia: Hyriidae), together with the non-native species *Corbicula fluminea* (Müller, 1774) (Bivalvia: Corbiculidae) and *Limnoperna fortunei* (Dunker, 1857) (Bivalvia: Mytilidae) (Mazzeo *et al.* 2010*b*). Laguna Blanca's plankton community is among the best studied in the region, being characterised by the recurrence of cyanobacterial blooms (*Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*) and the dominance of relatively small-sized zooplankters (Iglesias 2011).

Experimental design

Filtration rates estimation of *D. parallelopipedon*, *C. fluminea* and a natural zooplankton community were calculated in an experimental set-up in 2-L cylindrical aquaria (diameter 14 cm, height 50 cm; filled with 1 L water) with a layer of sediment (without organic matter) for bivalve settlement. The aquaria were filled with water from Laguna Blanca with its natural phytoplankton density (including *C. raciborskii* and edible species) obtained by filtering 1 L water through a 50-μm mesh (to avoid zooplankton). Tests were performed over a period of 24 h under controlled temperature (20°C) and light (12-h light–dark cycle) conditions. Phytoplankton control aquaria consisted in water filtered through a 50-μm mesh without bivalves. The length of bivalves was measured to the closest 0.1 mm: 2–3.5 cm for *C. fluminea* and 7–9 cm for *D. parallelopipedon*. Zooplankters retained in the 50-μm mesh were collected and used in subsequent experiments with zooplankton. The different treatments evaluated in the present study were as follows: (1) *D. parallelopipedon* (D); (2) *C. fluminea* (C); (3) natural zooplankton community (ZOO); (4) both bivalves species plus zooplankton (D+C+ZOO); and (5) a natural phytoplankton community (PHYTO). Treatments 3 and 5 were used as controls for the growth of zooplankton and phytoplankton respectively, without the filtration effect of bivalves. A detailed description of the experimental design is given in Table 1.

In vivo, chlorophyll-*a* (Chl-*a*) was measured using a fluorometer (Aquafluor Handheld Fluorometer; Turner Designs) and filtration rates were estimated from the decline in Chl-*a* concentration in the water from the start to the end of the

Table 1.   Experimental set-up used to test filtration rates over phytoplankton and interactions between bivalves and a natural zooplankton community

Herbivorous: *Diplodon parallelopipedon* (D), *Corbicula fluminea* (C) and zooplankton (ZOO). A detailed description of the natural community phytoplankton and zooplankton is given in the text. DW, dry weight; PHYTO, phytoplankton

Treatments	Abundance (individuals L^{-1})	Biomass (g DW)	Replicates	Time (h)
D. parallelopipedon	1	0.9	3	24
C. fluminea	1	0.2	3	24
D+C+ZOO	1D+1C+ZOO	1.6	3	24
ZOO	1050.7	0.5	3	24
PHYTO	–	–	3	24

experiment. This method has the disadvantage of particle decline during the experiment (Riisgård 2001). According to the recommendations of Coughlan (1969), to avoid this issue the following equation was used to estimate filtration rate (*F*, mL individual^{-1} h^{-1}):

$$F = \frac{V}{nt} \left(\frac{\ln C_0}{\ln C_t} - \frac{\ln C_0'}{\ln C_t'} \right)$$

where *V* is the volume of water in the aquarium, *n* is the number of individuals used, *t* is the duration of experiment (h), C_0 and C_t indicate initial Chl-*a* concentrations (µg L^{-1}) in the control and treatment groups respectively and C_0' and C_t' are the final Chl-*a* concentrations (µg L^{-1}) in the control and treatment groups respectively. The filtration rate of bivalves and zooplankton was expressed in term of biomass (g dry weight (DW)).

At the beginning and end of the experiment, 100-mL samples were taken from each cubitainer and fixed using acidified lugol for phytoplankton analysis. A 10-mL subsample was counted in sample in random fields at 10× and 40× magnification under an inverted microscope using the settling technique (Ütermöhl 1958). Counting was performed reaching at least 100 individuals of the most frequent species (Lund *et al.* 1958), considering the organism as the unit (cell, colony or filament). Biovolume was estimated according to Hillebrand *et al.* (1999) and population biomass was estimated as biovolume (mm^3 L^{-1}) and calculated as the individual volume of the species multiplied by the number of individuals. Moreover, to simplify the analysis, we divided the phytoplankton in two groups: edible (<30 µm) and non-edible (>30 µm). Non-edible phytoplankton was only integrated by *C. raciborskii*.

To determinate whether bivalves can consume zooplankton, the initial and final abundance of zooplankton was quantified in the D+C+ZOO treatment, with cubitainers containing only zooplankton (ZOO) used as a control treatment. At the beginning, three replicates of the field zooplankton community were obtained by heavy mixing of the water and filtering 15 L of water through a 50-µm mesh. At the end of the experiment, zooplankton samples were obtained by filtering the entire 1-L volume of each cubitainer through a 50-µm mesh; organisms were fixed with lugol and analysed later in the laboratory using Sedgwick–Rafter chambers (authors' own) with a binocular microscope at 10× and 40× magnification (Paggi and José de Paggi 1974). Counts were performed until we reached a count of 100 for the most common microzooplankton and the entire sample was then analysed for mesozooplankton. Thus, zooplankton abundance (individuals L^{-1}) was calculated. Micro- and mesozooplankton biomass was estimated by using average species biomass (DW) published for Laguna Blanca (Iglesias 2011) multiplied by its abundance in the sample. The DW of bivalves was estimated using available length–weight relationships (Marroni *et al.* 2014).

Data analysis

One-way analysis of variance (ANOVA) was used to detect significant differences among filtration rates, phytoplankton biovolume, zooplankton abundance, biomass and meso/microzooplankton ratios. In addition, two-way ANOVA was

used to compare filtration rates of bivalves and the natural zooplankton community considering: (1) the type of herbivore (three levels: *D. parallelopipedon*, *C. fluminea* and ZOO); and (2) phytoplankton (two levels: edible, non-edible). Homoscedasticity and normality of distribution were tested using Levenne and Shapiro–Wilk tests respectively. Tukey's post hoc comparisons among treatments and controls were also performed.

Results

The natural phytoplankton community was characterised by middle-sized species, such as *Euglena* sp., *Chlamydomonas* sp. and *Monoraphidium* sp. Moreover, *C. raciborskii* (filamentous cyanobacteria of a relatively larger size, >200 µm) was observed and represented 7% of total abundance; in terms of biomass (estimated as biovolume), *C. raciborskii* represented 50% of the community.

The natural zooplankton community consisted of small to medium-sized individuals and was dominated by the rotifer *Polyarthra* sp., copepod nauplii and the cladoceran *Bosmina* sp. Initial zooplankton total abundance was 1050.7 individuals L^{-1}. Filter feeding individuals were classified as microzooplankton (87.7%; rotifers and copepods nauplii), mesozooplankton (12.3%; *Bosmina* sp. and other cladocerans) and omnivore predators (3.5%; cyclopoid copepods); a detailed composition of the natural community used in the experiment is provided in Table 2. The meso/micro zooplankton ratio was 0.14 in terms of abundance and 0.86 in terms of biomass.

A significant decrease in phytoplankton biovolume in both bivalve treatments compared with the natural zooplankton community was confirmed ($F_{1,8} = 165$; $P = 5.67 \times 10^{-6}$; Fig. 1). Both bivalves exhibited a higher filtration rate than the natural zooplankton community ($F_{1,8} = 17.9$; $P < 0.05$). Thus, an efficient feeding of bivalves over both phytoplankton groups

Table 2. Composition of the natural subtropical zooplankton community used in the experiment
Abundance and biomass per taxa and the functional group assigned are presented

| Taxa	Abundance (individuals L^{-1})	Biomass (mg L^{-1})	Group
Rotifera			
Brachionus sp.	3.3	1	Microzooplankton
Filinia sp.	91.7	27.5	Microzooplankton
Hexarthra sp.	50	15	Microzooplankton
Keratella sp.	85	25.5	Microzooplankton
Tricocercha sp.	93.3	28	Microzooplankton
Polyarthra sp.	393.3	118	Microzooplankton
Crustacea			
Copepoda			
Ciclopoida	36.7	88	Omnivore predator
Calanoida	11.7	31	Mesozooplankton
Nauplius	205	32.8	Microzooplankton
Cladocera			
Bosmina sp.	71.7	71.7	Mesozooplankton
Diaphanosoma sp.	8.3	20.3	Mesozooplankton
Moina sp.	1.7	2.5	Mesozooplankton
Total	1050.7	462.8	
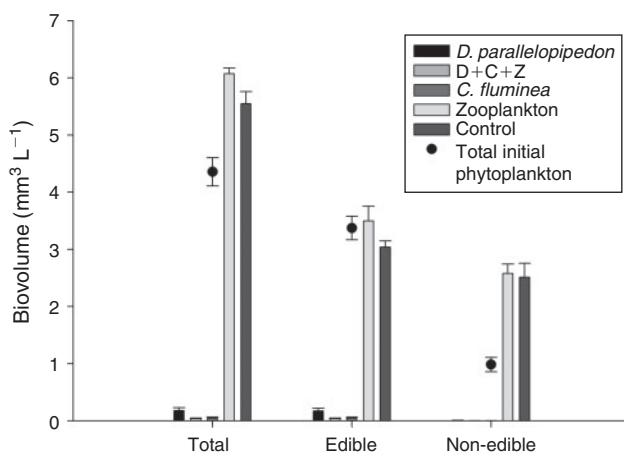
Fig. 1. Phytoplankton consumed by bivalves and zooplankton during the experiment. Total initial and final phytoplankton biovolume for all treatments is shown. Phytoplankton was assigned to two groups: edible and non-edible (*Cylindrospermopsis raciborskii*). D, *Diplodon parallelopipedon*; C, *Corbicula fluminea*; Z, zooplankton. Data show the mean ± s.e.m.


Fig. 2. Interactions between bivalves (*Diplodon parallelopipedon* (D) and *Corbicula fluminea* (C)) and zooplankton (ZOO). Effects of bivalve predation on total zooplankton abundance and on the abundance of each zooplankton functional group are shown (D+C+ZOO) compared with control treatment without bivalves (ZOO). Data show the mean ± s.e.m.

(edible and non-edible) used was observed. *C. fluminea* had a higher filtration rate than *D. parallelopipedon*. Mean (±s.e.m.) filtration rates for *D. parallelopipedon* and *C. fluminea* were 25.8 ± 7.5 and 106.8 ± 33.5 mL g DW h^{-1} respectively ($F_{1,8} = 27.2$; $P < 0.05$). Although there was a tendency for *C. fluminea* to graze more efficiently over both phytoplankton groups than *D. parallelopipedon*, the difference was not statistically significant ($P < 0.06$). Moreover, the natural zooplankton community could not efficiently graze any of the phytoplankton groups. Although an increase in *C. raciborskii* (non-edible) was found, the edible phytoplankton fraction was not affected (no differences between treatment and control were detected; $F_{1,8} = 2.657$; $P > 0.05$). The increase in the total phytoplankton biomass in the ZOO treatment indicated that growth rate exceeded the consumption by zooplankton (Fig. 1).

The abundance of zooplankton was affected by the presence of bivalves, with a significant decrease found in D+C+ZOO treatments compared with the control ZOO treatment ($F_{1,5} = 20.43$; $P < 0.05$). However, the observed effect was affected by zooplankton size, with the small-sized fraction (microzooplankton) being significantly consumed by the bivalves ($F_{1,5} = 22.4$; $P < 0.05$), whereas mesozooplankton and omnivore predators were not significantly affected by bivalve activity ($F_{1,5} = 7.35$; $P > 0.05$; Fig. 2).

Discussion

Filtration rates found in the present study concur with those reported in previous studies, both for *C. fluminea* (Way *et al.* 1990; Marroni *et al.* 2014) and *D. parallelopipedon* (Marroni *et al.* 2014). With regard to the first hypothesis tested in the present study, bivalves exhibited a higher grazing pressure over the phytoplankton than the natural zooplankton community. A zooplankton community with a few or even no large crustacean individuals feeding on phytoplankton and dominated by microzooplankton may canalise carbon and nutrient transfer to higher trophic levels because of preferential grazing on microbes (Sanders *et al.* 1992; Arndt 1993; Hambright *et al.* 2007).
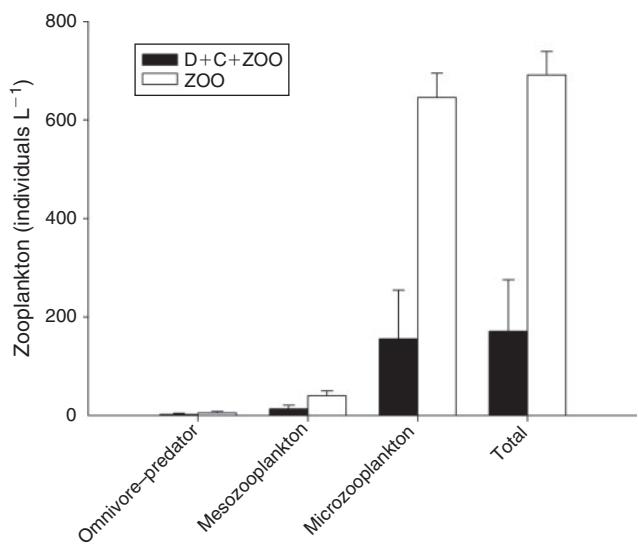
The results of the present study agree with those of previous studies, in which bivalves were shown to be capable of filtering a wide range of particles with different sizes and palatability (Alldredge and Gotschalk 1989; Passow *et al.* 1994; Crocker and Passow 1995). Bivalves can consume phytoplankton and resuspend materials from the sediment, including other kinds of bigger aggregates, such as detritus, faecal pellets and micro-organisms. However, bivalves are not simple passive filters feeders because they can sort and reject non-edible particles (Owen 1974; Ward and Shumway 2004; Lopes-Lima *et al.* 2014). Several studies suggest that the size of the food items ingested by some bivalves species (e.g. *Mytilus edulis*) is typically smaller than 40 μm (Widdows *et al.* 1979), whereas other studies found a wide range of particle sizes in gut contents, as big as 200 μm (Newell *et al.* 1989; Lehane and Davenport 2002).

These observations support the second hypothesis evaluated in the present study: bivalves, regardless of their native or non-native status, had a negative effect not only on phytoplankton biomass, but also on the zooplankton community, particularly microzooplankton. Thus, bivalves not only have indirect effects as a result of competing for limiting food resources, but also direct effects as a result of consuming both phytoplankton and zooplankton, as demonstrated experimentally. In fact, similar evidence of the consumption of rotifers and nauplii has been reported for other bivalve species, such as *M. edilus* (Wong and Levinton 2006), *D. polymorpha* (Wong *et al.* 2003), *C. leana* (Hwang *et al.* 2004) and *L. fortunei* (Molina *et al.* 2012). In contrast, some previous studies confirmed the consumption of cladocerans and copepods by bivalves after analysis of gut contents (Zeldis *et al.* 2004; Wong and Levinton 2006). In the present study we demonstrated a size-dependent effect of bivalves over zooplankton, constituting an intraguild predation module with small-sized individuals. In fact, no direct predation was found in the case of cladocerans, adult calanoid copepods
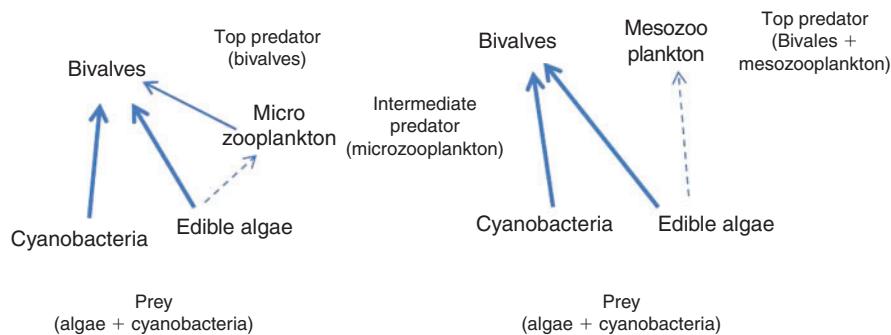
Fig. 3. Schematic structure of the biological interactions between the three players as determined in the present experimental study. Arrows indicate the consumption of potential prey, namely edible algae and cyanobacteria by intermediate (microzooplankton) and top (bivalves and mesozooplankton) predators. An intraguild predation module was only established between bivalves and microzooplankton, shown on the right. Edible algae is shared prey. Between bivalves and mesozooplankton, the intraguild module was not present under the present experimental conditions. Cyanobacteria were apparently only preyed upon by bivalves.

and adult cyclopoid copepods, for which bivalves are simply competitors (Fig. 3). This size-related effect has already been demonstrated experimentally in theorical (Ellner and Becks 2011) and experimental (Hiltunen *et al.* 2013; Wilken *et al.* 2014) plankton dynamics. It can be explained by the higher mobility of these zooplankton species, which allows them to escape (Peharda *et al.* 2012).

The evidence provided herein has several implications for the design of biomanipulation techniques. The results showed that *D. parallelopipedon* and *C. fluminea* have a very interesting potential to be used as alternative biomanipulation tools, because bivalves, by consuming a wider range of particles, can potentially exert a relatively stronger control over phytoplankton than a natural tropical or subtropical zooplankton community (without meso- and macrozooplankton). In the classical biomanipulation approach, once the phytoplankton biomass is diminished by medium- and large-sized zooplankton, like *Daphnia*, they tend to disappear from the water column (following the classic predator–prey cycle), thus allowing phytoplankton to recolonise the system (Scheffer 1998). Conversely, bivalves had two strategies that allow them to persist in the environment not providing a window of opportunity for phytoplankton, they can feed on other food resources, such as sediment organic matter, by pedal feeding behaviour (Haken-kamp and Palmer 1999; Marroni *et al.* 2014) and they can reduce their physiological activity to reduce energy costs (Winter 1978; Bayne *et al.* 1993; Navarro and Velasco 2003). Moreover, bivalves can consume microzooplankton; thus, the introduction of bivalves would not only have direct effects on phytoplankton removal, but could also lead to an increase in the size of zooplankton, which may indirectly or negatively affect phytoplankton (Wong and Levinton 2006).

The introduction of bivalves using sediment boxes may be an interesting perspective to mitigate the excessive growth of the phytoplankton community and cyanobacteria.

Acknowledgements

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Effects of temperature and food availability on the filtration and excretion rates of Diplodon parallelopipedon (Unionidae). Implicances for biomanipulation techniques in subtropical shallow lakes

Artículo 2. Sometido

Effects of temperature and food availability on the filtration and excretion rates of *Diplodon parallelopipedon* (Unionidae). Implicances for biomanipulation techniques in subtropical shallow lakes

Soledad Marroni [1*]**, Néstor Mazzeo**[1]**and Carlos Iglesias** [1*]

[1] Departamento de Ecología y Gestión Ambiental, Centro Universitario Regional del Este, Universidad de la República, Maldonado 20000, Uruguay. soledad.marroni@gmail.com (S.M.); mazzeobeyhaut@yahoo.com (N.M.); caif@cure.edu.uy (C.I.)

* Correspondence: soledad.marroni@gmail.com; caif@cure.edu.uy

Abstract: One of the ecological services provided by bivalves is water filtration. They can consume a wide particle range from water column, e.g. detritus, bacteria, phytoplankton and zooplankton. Use native Unionids to control phytoplankton crops in subtropical lakes can be an alternative solution to traditional biomanipulation techniques. However, how this potential solution varies under different environmental conditions must be known before up scalling to the ecosystem manipulation level. Here we aimed to elucidate the filtration and excretion rates (FR, ER) displayed by *Diplodon parallelopiepdon* under different food availability and under a wide range of water temperatures. We run a five times replicated laboratory experiment with different temperatures (10-15-10-25-30 °C) and three different phytoplankton biomass levels (low, middle, high). *Diplodon parallelopipedon* exerted a potential strong control over phytoplankton, with an optimum at 20°C. Altogether bivalve's activity might reduce or facilitate an increase of phytoplankton development depending of its grazing capacity and the nutrient remineralization released. Moreover, some bloom forming with floating capacity cyanobacteria, can actively reduce grazing and advantage their competitors using the released nutrients available in the water column. Our results suggests that the implementation of biomanipulation techniques using filter feeding bivalves might be very cautiously applied and more data at laboratory and mesocoms scales is needed to further understand resultant potential ecosystem responses.

1. Introduction

Bivalves are a key component of ecosystem, because they can influence whole ecosystemic functioning [1-5]. By filter feeding from the water column they can consume a wide range of food sources, e.g. phytoplankton, zooplankton, bacteria, detritus [6-9] moreover, basically based on their pedal feeding capacity, they can also fed directly on the organic matter of the sediments [10-11]. Bivalves can directly reduce phytoplankton biomass through filtration [11-14], but may also have an

40 impact through excretion of nutrients like ammonium (NH_3) and phosphate (PO_4), which are
41 immediately available for new primary production [15-19].

42 While native bivalves like *Diplodon spp.* are poorly studied, many studies have focused in exotic
43 species like *Dreissena polymorpha*, *Limnoperna fortunei* or *Corbicula fluminea*, principally because once
44 they infest a new system they usually produce drastic changes. When, these invasive bivalves attain
45 high densities, they have been shown to exert a strong top-down control of phytoplankton biomass
46 [1, 20-23], resulting in an increased water transparency comparable with the traditional zooplankton
47 grazing effect [24-26]. Moreover, indirect effects on macrophyte coverage are also reported since
48 they can take advantage of the clearer conditions to grow and expand along the lake basin [27-30].
49 Altogether suggesting that novel biomanipulación techniques might be implemented by the
50 facilitation of bivalves development, particularly in those systems where the development of
51 zooplankton based measures can be very difficult to implement, for example, as a consequence of
52 fish predation in subtropical shallow lakes [31-38].

53 In order to implement a biomanipulación solution to control phytoplankton crops in subtropical
54 lakes based on native Unionids, it is a must to understand how these bivalves respond to certain
55 environmental variables. Filtration rate of bivalves can be negative or positively affected by certain
56 environmental conditions e.g.: temperature and particle size and concentration, flow regimen and
57 bivalve size and gill morphology [39-44].

58 Regarding temperature, bivalves, as ectotherm organisms, on a certain range of values exhibit a
59 filtration rate positively correlated with temperature. It increases until an optimum is reached; above
60 which it start decreasing [45-51]. Other works show only a direct relationship between temperature
61 and filtration rate, perhaps because the range of temperature studied is too short [44, 52]. To know
62 how filtration rate varies with temperature can provide an insight of their performance along the
63 year, particularly if their maximums are in coincidence with those periods when phytoplankton
64 crops develops.

65 Food availability is also pointed to affect bivalve's filtration rate, generally increasing with particle
66 concentration up to a threshold, after which the rate may decline [40, 53-57]. How bivalves cope with
67 food availability can provide information of the range of phytoplankton biomass they can take count
68 of for example during a bloom or a food shortage period.

69 Through filtration activity bivalves can capture seston from water column and convert these
70 nutrients into bivalve tissue, particulate biodeposit (feces and pseudofeces) and dissolved nutrients
71 [58]. Bivalves can both remineralising and recirculating nutrients back to the primary producers.
72 They produce hypo-osmotic urine consisting primarily of ammonia [59]. Some's factors who
73 affected excretion rate are: individual size, temperature, stage in reproductive cycle and food
74 availability [15-18, 60].

75 Aiming to estimate *Diplodon parallelopipedon* filtration and excretion rates under different
76 temperature and food availability conditions we conducted a laboratory experiment with five
77 different temperatures (mimicking the year-round temperatures of a subtropical lake) and three
78 different phytoplankton concentration. We hypothesized that *D. parallelopipedon* filtration rates will
79 exhibit a dumped curve response with an optimum close to 20-25 °C and a positive response to food
80 concentration regardless of temperature. Secondly, we postulate that the excretion rate displayed by
81 *D. parallelopipedon* would be positively affected by temperature and food availability.

82 **2. Materials and Methods**

83 **2.1. *Specimens collection.***

84 Native bivalve *Diplodon parallelopipedon* specimens were collected by free diving from Laguna del
85 Sauce (34º 43'S, 55º 13'W), Maldonado-Uruguay, and transported to the lab facilities where they
86 were kept in oxygenated aquariums and fed with algal cultures, mainly *Ankistrodesmus spp*.

87 **2.2. *Experimental Design***

88 We run a five times replicated laboratory experiment with five different temperatures
89 (10-15-10-25-30 ºC) and with three different phytoplankton biomass levels (low, medium, high)
90 *Diplodon parallelopipedon* filtration rates were measured as the response variable according to [61]. Six
91 litters cylindrical aquariums filled up to 4 L of culture of mainly dominated by *Ankistrodesmus sp.*
92 with 4 bivalves per aquarium attaining a final density of 1 individual per litter were used. Bivalves
93 were introduced in a plastic basket filled with sediments without organic matter into the aquariums.
94 Random assigned treatments were: low (L, Chlo a= 2, 5-5 µgl^{-1}), middle (M, Chlo *a*= 10-15 µgl^{-1}) and
95 high (H, Chlo a= 20-30 µgl^{-1}) for phytoplankton biomass and for temperature. Three aquariums with
96 phytoplankton but without bivalves were used as control (C) for each phytoplankton biomass
97 treatment. Prior to each temperature test bivalves were acclimatized during two weeks under the
98 following experimental temperature. All along the experiment and acclimation periods a
99 phytoplankton culture of the highly palatable needle-shaped green algae *Ankistrodesmus sp.* were
100 used to fed the experimental bivalves.

101 **2.3. *Filtration rate measurements***

102 Filtration rate was estimated using the clearance method, as the volume of water cleared by unit of
103 time [61]. As this method was usually criticized because particles decline during the run might
104 condition measured filtration rates, [61] recommendations were followed, thus algal concentration
105 was kept within a certain range by periodically additioning a fix volume of *Ankistrodesmus sp.*
106 culture. In pilot runs of the experiment the phytoplankton exponential decrease for each used
107 temperature were determined. Consequently, the phytoplankton culture addition took place two
108 times, every 45 minutes for 20, 25, 30 ºC and every 90 minutes for 10 and 15 ºC.

109 Filtration rate (FR) (1) was deduced from the exponential decrease in the phytoplankton
110 concentration, measured as Chlorophyll-*a in vivo* (Chl-*a*) using a fluorometer (Aquafluor Handheld
111 Fluorometer, Turner Designs). Phytoplankton decrease in each treatment was measured every 30
112 minutes for 10-15ºC treatments and every 15 minutes for 20, 25 and 30ºC treatments.

$$FR = a \text{ V/n 1,} \tag{1}$$

113 Where V=volume of water, n=number of filtering bivalves, and *a* slope of the regression line in a
114 semi-ln plot of algal concentration with the time [44, 61].

115 Filtration rate of bivalves was expressed in terms of biomass (g DW, dry weight), using available
116 bivalve's length-weight relationships [14].

117 **2.4. *Excretion rate measurements***

118 Experiments were performed in 500 ml containers filled with commercial water to which bivalves
119 were added. Excretion rate tests were run immediately after each temperature filtration rate run. We
120 added 3 bivalves to each container, thus applied treatments were: phytoplankton (low, middle and
121 high) and temperature (10-15-20-25-30ºC). Five replicates and five controls for each treatment
122 (temperature x phytoplankton) were considered, controls consisted in the containers with
123 commercial water without bivalves. Containers were not artificially aerated and kept in the dark
124 thus simulating benthic conditions, runs lasted 6h, and for each temperature and food availability
125 being tested [18]. Water samples from all experimental containers were taken at the beginning and at

126 the end of the 6 h period. Water samples were rapidly frozen, total Phosphorous (TP) and total
127 Nitrogen (NT) were later determined in the lab according to [62].

128 Excretion rate of bivalves was expressed in term of biomass as microgram of N or P per milligram of
129 dry weight per day (mg g DW day^{-1}). Bivalve's dry weight was estimated using the available
130 length-weight relationships [11].

131 ***2.5. Statistical analysis.***

132 Two-way analyses of variance (2 way-ANOVA) was used to compare filtration and excretion rates
133 of *D. parallelopipedon*, considering: 1) Temperature (5 levels: 10-15-20-25-30^{0}C) and 2) phytoplankton
134 concentration (3 levels: low, middle, high). The homoscedasticity and normality distribution were
135 tested using Levenne and Shapiro-Wilk tests, respectively. Tuckey post-hoc comparisons among
136 treatments and controls were also performed.

137 **3. Results**

138 ***3.1. Filtration rate of D. parallelopipedon***

139

140 During the experiments, a reduction of phytoplankton biomass occurred in all treatments,
141 regardless temperature and food availability (Figure 1). Filtration rates (FR) of *D. parallelopipedon*
142 ranged from 0.27 to 0.78 L g DW h^{-1} (Figure 2). Moreover, FR was significantly affected by
143 temperature ($F_{(4,38)}$=16.56, p=0.002; post hoc test: 10=15<25=30<20) and a dumped relationship of FR
144 with temperature was evidenced. The minimum FR measured occurred at the two lower
145 temperatures (10-15 ^{0}C, p=0.99), an increase of FR occurred at 20 ^{0}C (p=0.002), and after that,
146 filtration rate decreased again showing similar values at the two hotter temperatures (25-30 ^{0}C,
147 p=0.99; Fig 2). By the other hand, FR was not significantly affected by food availability as similar
148 values were measured for all the phytoplankton biomass tested ($F_{(2,38)}$=1.29, p=0.28; post hoc test:
149 low=middle=high: Figure 2).

150

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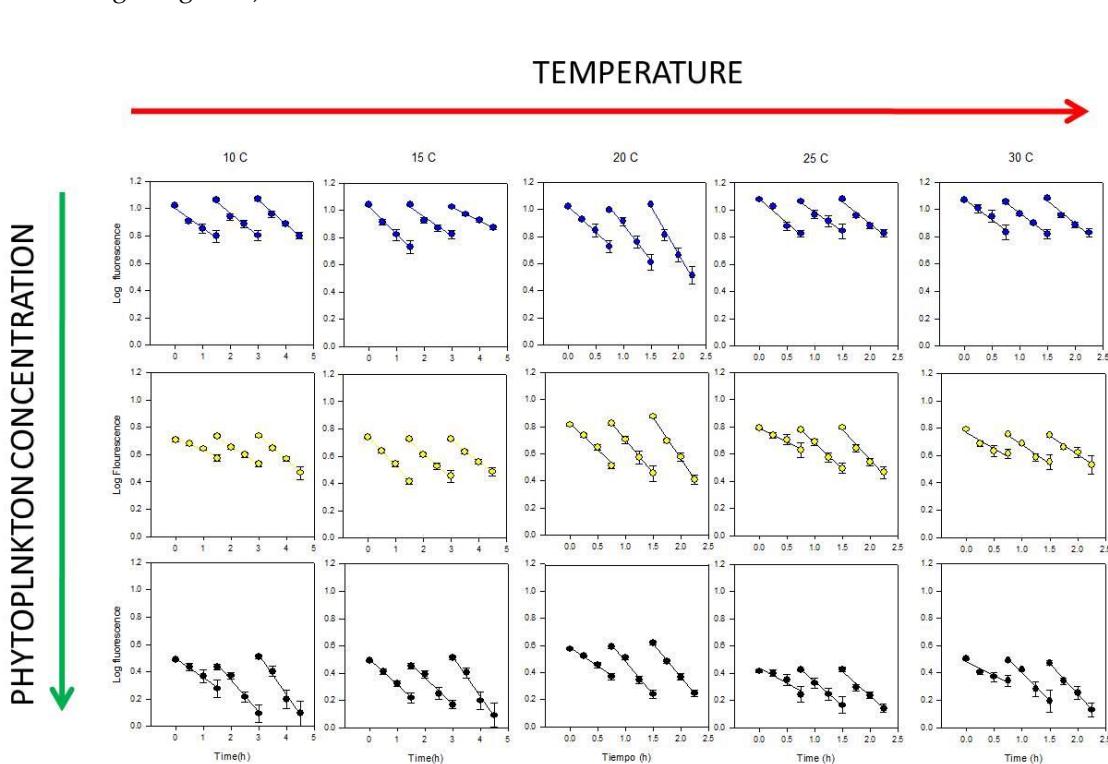
153   **Figure 1.** Exponential reduction in algal concentration due to grazing by *D. parallelopipedon* for
154   different food concentration (Low, middle, high) and temperature. Arrows indicate additions
155   of new algal suspension. Error bars correspond to Standard Error.

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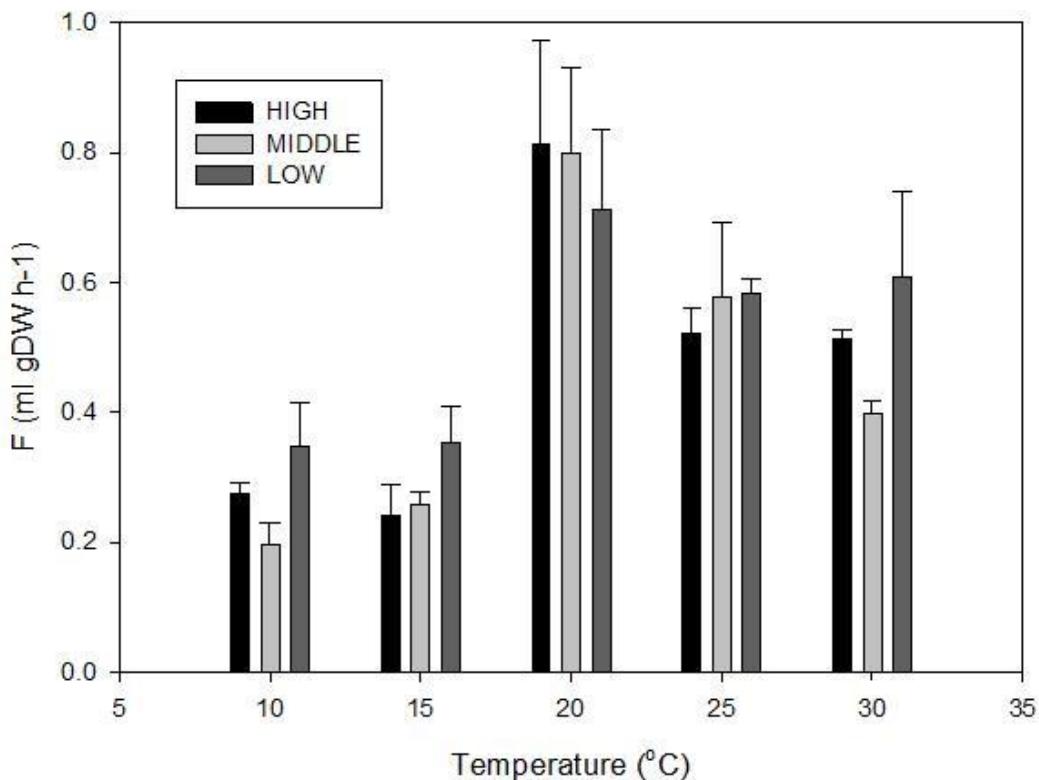
157   **Figure 2.** Filtration rate of *D. parallelopipedon* (ml g DW h^{-1}) vs. Temperature and Phytoplankton
158   biomass. Food concentration (Low, middle, high) and Temperature (10, 15, 20, 25 and 30 °C). Error
159   bars correspond to Standard Error.

160   ***3.2. Excretion rate of D. parallelopipedon***

161

162   The range of excretion rate of TP was 0.98-2.37 μg P g DW^{-1} h^{-1}. Temperature significantly
163 influenced bivalves excretion rate of TP ($F_{(4,68)}$=10.84, p=0.00). However, no significant differences on
164 the excretion rate between the two lower temperature values were detected (p=0.84), excretion rate
165 of P increased and the highest value occurs at 20°C. From this temperature excretion rate of P rested
166 constant, no significant differences between 20-30°C (p=0.54) (Figure 3a). Regarding the amount of
167 food offered (phytoplankton biomass treatments) no significant differences on the excretion rate of
168 bivalves were detected ($F_{(2,68)}$=0.29, p=0.6) (Figure 3b). A similar pattern was observed on the TN
169 excretion rate by bivalves with a significate effect of temperature ($F_{(4,68)}$=10.31, p=0.000). The lower
170 values occurred at 10°C (5.44μgN g DW^{-1}h^{-1}) there were no statistical differences for 10 and 15°C
171 (p=0.38). The highest excretion rate occurred at 30°C (28μgN gDW^{-1}h^{-1}), but there were no statistical
172 differences among 20, 25 and 30°C temperature treatments (p=0.34) (Figure 3c). The amount of food
173 also had not effect on the bivalves TN excretion rates ($F_{(2,68)}$=0.93, p=0.39) (Figure 3d).

174
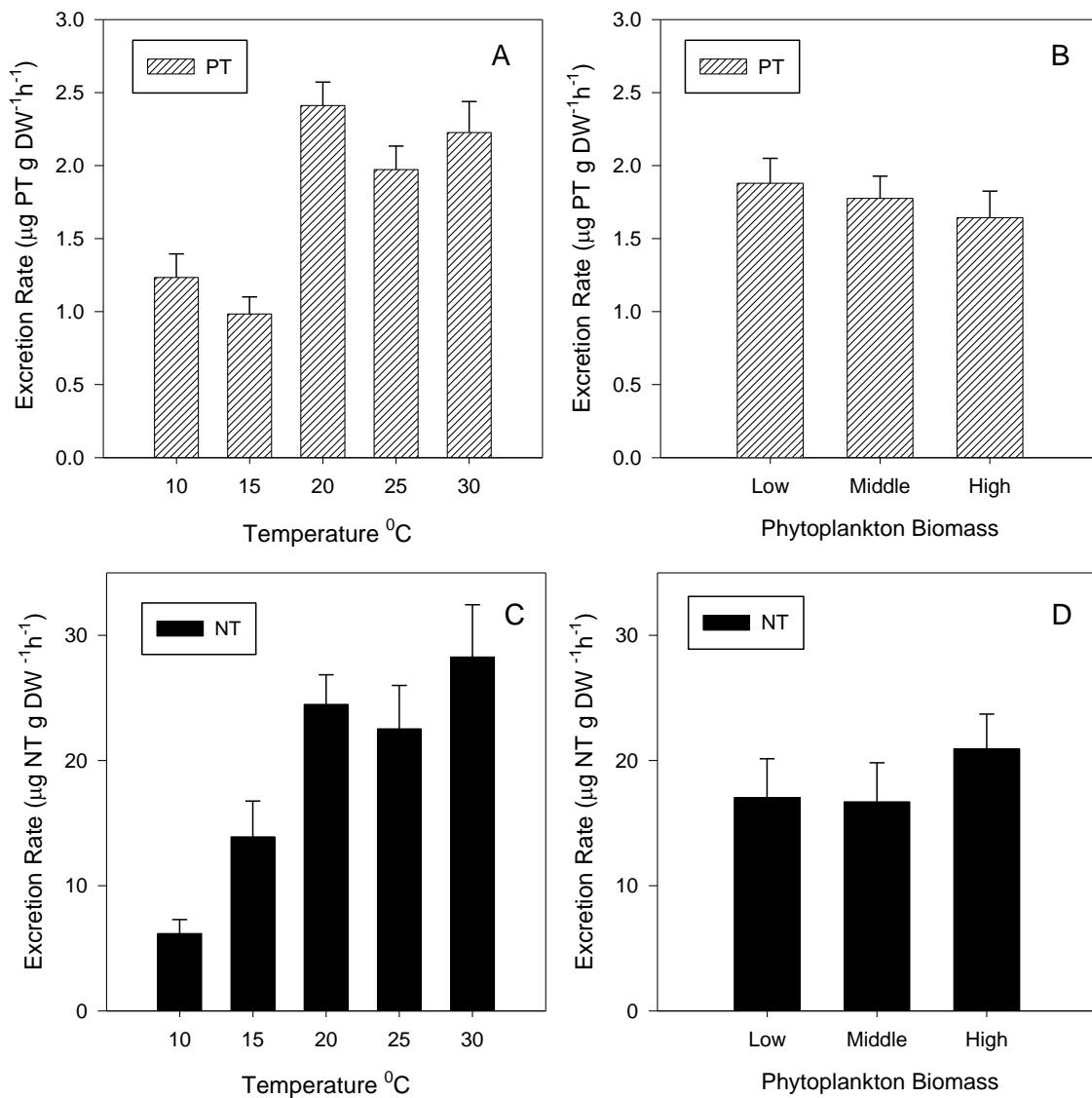
175
176
177 **Figure 3.** Excretion rate (ER) Phosphorous Total (PT) and Nitrogen Total (NT) (μg g DW^{-1}h^{-1} of
178 PT/NT) of *D. parallelopipedon.* (**A**) ER of PT vs. temperature; (**B**) ER of PT vs phytoplankton
179 biomass; (**C**) ER of NT vs temperature; (**C**) ER of NT vs. phytoplankton biomass. Excretion
180 rate for both nutrients increased with temperature; it is not different between treatment of
181 phytoplankton (low, middle, high). Standard error is showed.

182

183 **4. Discussion**

184 Filtration and nutrient excretions rates reported in literature for bivalves have been shown to vary
185 significantly due to experimental condition, methodology and algal feed. In these work, we reported
186 filtration and excretion rates of *Diplodon parallelopiepdon* in the same condition with different algal
187 concentration and for a wide range of temperature.

188 According to our first hypothesis the measured filtration rate exhibited by *D. parallelopipedon* varied
189 non-linearly with temperature, with a clear dumped response in the studied range of 10-30⁰C. This
190 contradict previous reported linear responses of bivalves [44, 52, 63] however, these works tested a
191 short temperature range, not enough to evidence a decrease on the observed filtration rate.

192 Moreover, our results concurs with patterns exhibited by *Dreissena polymopha* and *Corbicula fluminea*
193 with filtration optima at 22.9ºC and 25ºC, respectively [49,64,65]. In the present work, *D.*
194 *parallelopipedon* maximum filtration rate occurred also at mid temperatures (20-25ºC). When moving
195 up and down, filtration rates diminished which is in concordance with previous reported
196 experimental results [46,66] and with bivalves' filtration rate models which predicts a decrease of
197 filtration rates when moving apart from certain optimum temperature, particularly when
198 temperature is too high [50,67]. On the other hand, filtration rates for *D. parallelopipedon* found in the
199 present work is higher when compared with other bivalves like *Dreissena polymorpha* 3.8-6.94 mL mg
200 $DW^{-1}h^{-1}$ [68], *C. fluminea* 2-20 mL mg $DW^{-1}h^{-1}$ [69,70], or *L. fortunei* 1.5-3 mL mg $DW^{-1}h^{-1}$ [71], this
201 probably as consequence of the high palatability of the phytoplankton offered in our experiment.

202 Our results showed that bivalves can exert a high grazing pressure over phytoplankton on a wide
203 temperature range, particularly in the middle of the year round temperature range. On the hand,
204 when temperature overcome a certain threshold (between 25 and 30ºC) filtration rate decreased,
205 supporting the idea of a physiological stress being experienced by *D. parallelopipedon* individuals
206 faced to high temperatures (here, >25 ºC). By the other hand, the lowest filtration rates were
207 registered with the lowest temperatures, probably because of bivalves have generally a low-energy
208 life-style, with long periods of water filtering inactivity, these behavior might allow to adopt energy
209 saving strategies [71].

210 Moreover, several previous work showed that food concentration (and quality) might directly affect
211 the filtration activity displayed by bivalves [72-76], particularly an increase of the observed filtration
212 rate appears as a response of the increase of available phytoplankton, ensuring a maximum
213 ingestion rate [45,53]. When maximum ingestion rate is reached, it stop growing and is kept constant
214 [77,78], however, in these work we do not found differences in filtration rate related with food
215 concentration in the used range of 5-30 chl *a* μg l $^{-1}$. Perhaps the used range of algal concentration
216 (representative of oligo, meso and eutrophic ecosystems) was too narrow to reach the maximum
217 ingestion rate, which could have been attained if higher values had been tested.

218 Bivalves as well as can excreted a top down control over phytoplankton; they can also promote its
219 development by the remineralization of nutrients via their feces and pseudo feces excretion. In this
220 sense, bivalves are capable of releasing high quantities of nutrients to the water, particularly the bio
221 available forms of the common limiting Nitrogen and Phosphorus, like NH_3 and PO_4 that could be
222 quickly assimilated by phytoplankton. In our work we considered only dissolved TP and TN
223 excreted by *D. parallelopipedon*, and not accounted particulate forms like feces and pseudo feces. As
224 far as we investigated there were no previous reports on the excretion rate of *D. parallelopipedon*.
225 Measured values were higher when compared with exotic bivalve *D. polymorpha* [18]. On the other
226 hand, TP and TN excretion rates were lower compared with those reported for *L. fortuneii* in a
227 mesocosm experiment [74]. Moreover, we found an increase of the excretion rate simultaneously
228 with a decrease of the filtration rate exhibited for the highest applied temperature (Figure 1). This
229 observation again suggests that at high temperature bivalves might be stressed, and they may break
230 down proteins, excreting ammonia [79] not coming from the remineralization of ingested food.

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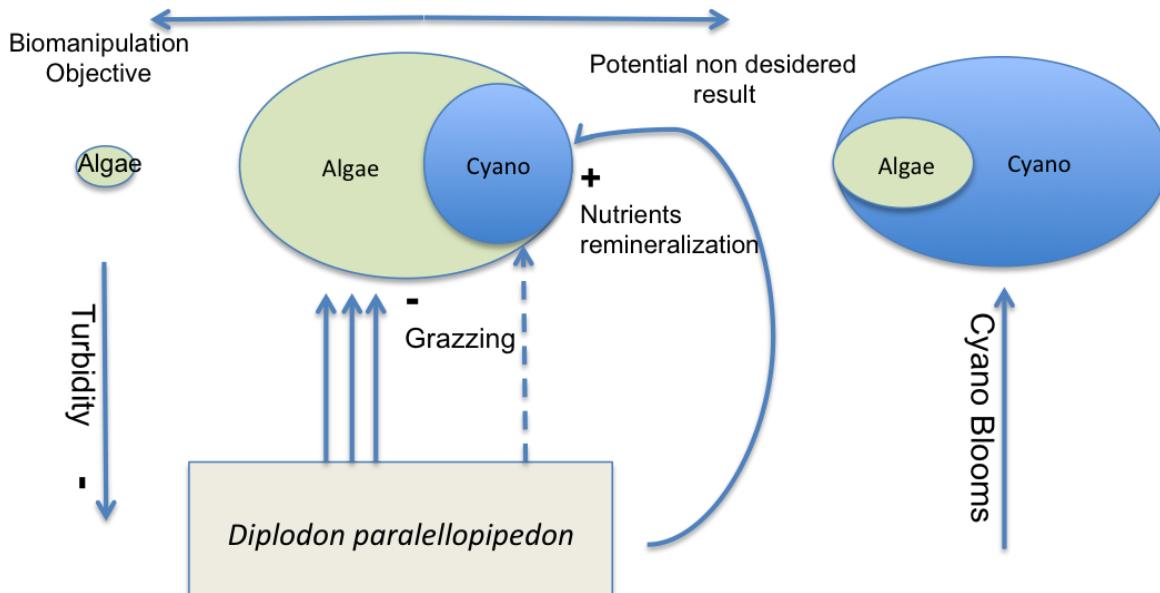
Figure 4. Schematic response of the biomanipulación using native Unionids in a subtropical system. *Diplodon parallelopipedon* can simultaneously affect directly over palatable algae and a at the same time release nutrients that can be used, for example, by bloom forming cyanobacterias. If the grazing exherted cannot overcome phytoplankton growing under the new nutrient availability conditions, then the overall effect of the bivalves will be the opposite of the original objective of lowering phytoplankton biomass and increasing water transparency. The potential non desired result would be the facilitation of bloom forming cyanobacteria to take advantage of competition release, a temporarily low turbidity conditions, being busted by the reminaralized nutrients. Temperature effect is not shown in the diagram but the experimental results presented here suggest that its rise enhances the non desired response.

242

It is widely recognized that water filtration is one of the ecological services provided by benthic filter feeders [50, 80]. Moreover, considering that some strategies to control eutrophication consequences included the introduction of bivalves in boxes into waterbodies [81, 82], knowing how rate filtration is affected by temperature and food concentration is of key importance to design effective plans. By one hand, *D. parallelopipedon* is able to consume a wide range of particles, thus exerting a potential strong control over phytoplankton [83]. Nonetheless, at higher temperatures bivalves are stressed and its filtration rate is significantly decreased, while simultaneously nutrient remineralization is increased (Figure 4). In this prospective scenario, that reflects summer time condition; higher temperature promotes phytoplankton biomass development being potentially busted by the nutrient released by the bivalve's community, which grazing pressure is reduced, altogether leading to an enhancement of phytoplankton development. Moreover, some bloom forming with floating capacity cyanobacteria, like *Microcystis aeruginosa*, could find a facilitated way to use the nutrients remineralized by *D. parallelopipedon* as they avoid grazing keeping in superficial layers, they take advantage of competitors remotion by filtration and using the released nutrients available in the water column (Figure 4).

On the other hand, mesocosm experiments with *L. fortuneii* showed that the impact of filtration rate can overcome the busting effect caused by nutrient release [74], In any case our knowledge is still scarce and more research will be needed to elucidate if this novel biomanipulación techniques using native unionids will move the system to the desired state or to the opposite.

262

263 **Aknowledgments**

270 **References**

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Can nutrient release and/or consumption by D. parallelopipedon bust phytoplankton cyanobacterial development? (Unionidae). Implicances for biomanipulation techniques in subtropical shallow lakes

Manuscrito 1

Autores: Soledad Marroni, Carlos Iglesias and Néstor Mazzeo

Can nutrient release and/or consumption by *D. parallelopipedon* bust phytoplankton cyanobacterial development?

Soledad Marroni [1*], Néstor Mazzeo[1] and Carlos Iglesias [1*]

[1]Departamento de Ecología y Gestión Ambiental, Centro Universitario Regional del Este, Universidad de la República, Maldonado 20000, Uruguay. soledad.marroni@gmail.com (S.M.); mazzeobeyhaut@yahoo.com (N.M.); caif@cure.edu.uy (C.I.)

*Correspondence: soledad.marroni@gmail.com; caif@cure.edu.uy

Abstract

Cyanobacterial blooms are common in freshwater ecosystem. Toxins released by cyanobacteria are highly toxic and affect both animals and humans. In our country, it is the main problem of drinking water systems. Some strategies of biomanipulación has been development by induce a change in food web. Bivalves are key component in the ecosystem because they can directly reduce phytoplankton biomass through filtration but may also have an impact through excretion of nutrients. The aim of this study is understand the roll of *D. parallelopipedon* in phytoplankton dynamics related with feeding/ nutrient excretion. First, we run experiment to compared feeding of *D. parallelopipedon* over 1- phytoplankton assemblages: dominated by *Cryptomonas sp.* and 2-wild population of *Microcystis aeruginosa.* Secondly, we studied nutrient released of *D. parallelopipedon* nitrogen and phosphorus. We verify our hypothesis that *D. parallelopipedon* favor presence of *M. aeruginosa* because it is not able to consume when it is forming colonies due to the capacity of flotation of the same; also it favors its growth through the release of nutrients. In summer time, higher temperature promotes phytoplankton biomass development being potentially busted by the nutrient released by the bivalve's community, which grazing pressure is reduced, altogether leading to an enhancement of phytoplankton development. Moreover, some bloom forming with floating capacity cyanobacteria, like *Microcystis aeruginosa*, could find a facilitated way to use the nutrients reminelralized by *D. parallelopipedon* as they avoid grazing keeping in superficial layers, they take advantage of competitors remotion by filtration and using the released nutrients available in the water

Keywords: Bivalves, Nutrient released, Temperature, Cyanobacteria,

Introduccion

Cyanobacteria blooms are frequently stable and resilient in lakes (Scheffer et al 2007; Bonilla et al 2012; Wilson & Chislock 2013). Cyanobacteria have developed competitive strategies for greater efficiency in capturing resources such as light and nutrients. They tend to dominate phytoplankton under particular conditions, such as in conditions of low N/P ratio, high temperatures, periods of

stratification of the water column, or in the presence of zooplankton communities dominated by small organisms (Leflaive & Ten- Hage 2007; UNESCO 2009). In our country, cyanobacteria blooms have been recorded in diverse ecosystems, they occur mainly in summer (Bonilla et al. 1995, 1997; Kruk & De León 2002).

Moreover, the release of toxins is an adaptive advantage of cyanobacteria that allows them to avoid predators (Wolfe 2000; UNESCO 2009). The toxins released by cyanobacteria are highly toxic and affect both animals and humans (Leflaive & Ten-Hage 2007; Azevedo et al. 2002). These substances called cyanotoxins are grouped into hepatotoxins, neurotoxins and cytotoxins according to their mechanism of action (Carmichael 1992). Microcystin-LR is a widely distributed hepatotoxin that inhibits protein phosphatase, generating cell deformation, necrosis, intrahepatic hemorrhage and cell death. The prolonged explosion of these toxins generates cumulative damage (Carmichael 1992, 1994, 2001; Harada et al. 1996; Codd et al. 1999; Zaccaroni & Scaravelli 2008).

Bivalves are a key component of ecosystem, because they can influence whole ecosystemic functioning (Vaugh et al. 2001; Newell 2004; Strayer et al. 2008; Dame 2012; Vaugh & Hoellein 2018). By filter feeding from the water column they can consume a wide range of food sources, e.g. phytoplankton, zooplankton, bacteria, detritus (Strayer et al. 1999; Pestana et al. 2009; Zhang et al. 2010; Peharda et al. 2012, Marroni et al. 2016). Moreover, some species basically based on their pedal feeding capacity, they can also feed directly on the organic matter of the sediments (Hakenkamp & Palmer 1999; Marroni et al. 2014). Bivalves can directly reduce phytoplankton biomass through filtration (Caraco et al. 1997; Prins et al. 2005; Newell et al. 2007; Marroni et al 2014), but may also have an impact through excretion of nutrients like NH_3 ammonium and PO_4 phosphate, which are immediately available for new primary production. The remineralization of nutrients i.e. the transformation of particulate nutrients that have settled out the water column into soluble forms that can be transported through the water column and made available to growing phytoplankton (Williams & McMahon 1989; Davis et al. 2009; Conroy et al. 2005; Vaughn et al. 2008).

In several cases the appearance of blooms of cyanobacteria has been related to the introduction into the system of exotic species of bivalves (Vanderploeg et al. 2002; Raikow et al. 2004). In this sense, recent studies indicate that *Limnoperna fortunei* is capable of modifying the proportion and concentration of nutrients, promoting the aggregation of *Microcystis spp*. and favoring the appearance of toxic blooms of cyanobacteria (Cataldo et al. 2012). *Dreissena polymorpha*, another invasive bivalve very studied, presents a differential consumption of phytoplankton, which produces a change in the composition and abundance of the planktonic communities (Holland 1993). It has been suggested that this differential consumption is what causes the appearance of toxic algal

blooms of *Microsystis spp*. (Vanderploeg et al. 2002; Raikow et al. 2004; Sarnelle et al. 2005). On the other hand, studies indicate that *Dreissena sp*. is able to coexist and consume *Microsystis spp*. (Caraco et al. 1997; Baker et al. 1998; Dionisio Pires & Van Donk 2002; Dionisio Pires et al. 2004).

Native bivalves like *D. parallelopipedon* are poorly studied. The relative effect of filtration and nutrient excretion by *D. parallelopipedon* on phytoplankton dynamics is not known, although in short-term experiments filtration has been done (Marroni et al 2014, 2016). The aim of this study is understand the roll of *D. parallelopipedon* in phytoplankton dynamics related with feeding/ nutrient excretion. First, we compared feeding of *D. parallelopipedon* over 1- phytoplankton assemblages: dominated by *Cryptomonas sp.* and 2-wild population of *Microcystis aeruginosa.* Secondly, we studied nutrient liberation of *D. parallelopipedon* and their fraction of nitrogen and phosphorus. Our hypothesis *D. parallelopipedon* favor presence of *M. aeruginosa* because it is not able to consume when it is forming colonies due to the capacity of flotation of the same; also it favors its growth through the release of nutrients.

Materials and Methods

Specimen collections

Native bivalve *Diplodon parallelopipedon* were collected by free diving at in the system Laguna Laguna del Sauce (34º 43'S, 55º 13'W), Maldonado-Uruguay, and transported to the lab facilities where they were kept in oxygenated aquariums and fed with algal cultures.

Experiment 1. Differential consumption of phytoplankton by D. parallelopipedon

To determinate consumption differential of *D. parallelopipedon* we run lab experiments at controlled conditions of temperature (20°C) and dark condition simulating natural conditions, during tests of 1h. We use an aquarium of 1 liter of capacity and we fill it with 500ml of mix of food sources. For each aquarium we put 1 individual of *D. parallelopipedon* (60-80 cm). Bivalves selected were starved 24h prior to begging the test. We offer to different food source: 1-phytoplankton assemblages: dominated by *Cryptomonas sp.* (F) and 2-wild population of *Microcystis aeruginosa* (MC). We mix both food source to make a gradient of relative concentration (%) between (MC) and (F). Treatment were: 1-100% MC, 2: 75%MC-25% F, 3 50%MC-50%F, 4: 25%MC-75%F y 5: 100%F. Treatment and controls were 3 time replicated. Controls consisted on treatment without bivalves. At the start and end of experiment, we took samples for counting with a light inverted microscopy, and these samples were taken to determinate which fractions were consumed. It was done in sedimentation chambers, random fields were counted until counting 100 individuals or colonies of the most frequent species (Uthermohl 1953). Wild population of *Microcystis aeruginosa* was

collected to Salto Grande. It reservoir is an artificial ecosystem created for hydroelectric power generation.

Experiment 2. Nutrient Excretion of D. parallelopipedon.

Experiments were performed in a series of containers with 500 ml de commercial water to which bivalves were added. For each container we added 3 bivalves between 60-80 mm representatives of Sistema Laguna del Sauce. Treatments of temperature were 15, 20 and 25 ^{o}C. We realized 5 replicates and 5 controls for each treatment. Controls were the containers with commercial water without bivalves we repeated it for different temperature. For each temperature bivalves were acclimatized bivalves during two week. For treatments of phytoplankton we used cultivate of lab dominated by needle-shaped cultured green algae (*Ankistrodesmus sp.*). Bivalves were first starved for 24h and then feeding for 2 hours. Phytoplankton concentration used was Chlo *a*= 10-15 µgl^{-1}. Containers were unaerated and were maintained for 6h in the dark, thus simulating benthic conditions (Conroy *et* al., 2005). At the end of the 6h, we took a sample of the water from all experimental containers. We analyzed concentration of Phosphorous and Nitrogen (SRP, PTD, PT and N0$_3$, NH$_4$, NTD and NT).

Ammonia and phosphate excretion rates were determined according to Conroy et al. 2005. Excretion rate of bivalves was expressed in term of biomass as microgram N or P per milligram dry weight per hour (µg g DW^{-1} h^{-1}). Bivalve's dry weight was estimated using the available length-weight relationships (Marroni et al. 2014).

Data analysis

One-way analysis of variance (ANOVA) was used to detect significant differences among different consumption of both food source MC and F. Homoscedasticity and normality of distribution were tested using Levenne and Shapiro–Wilk tests respectively. One-way analysis of variance (One way-ANOVA) was used to compare excretion rates of *D. parallelopipedon*, considering: Temperature levels: 15-20-25°C.

Results

Differential consumption of phytoplankton by D. parallelopipedon

In all treatment with *M. aeruginosa* we do not found consumption by *D. parallelopipedon* (Figure 1, above). We do not found significant differences (p=0.89) between control final and treatment with *D. parallelopipedon*. On the other hand, *D. parallelopipedon* in all treatments was capable of

consume *Cryptomonas sp.* In these case the differences were significant between treatments and controls (p=0.0001).
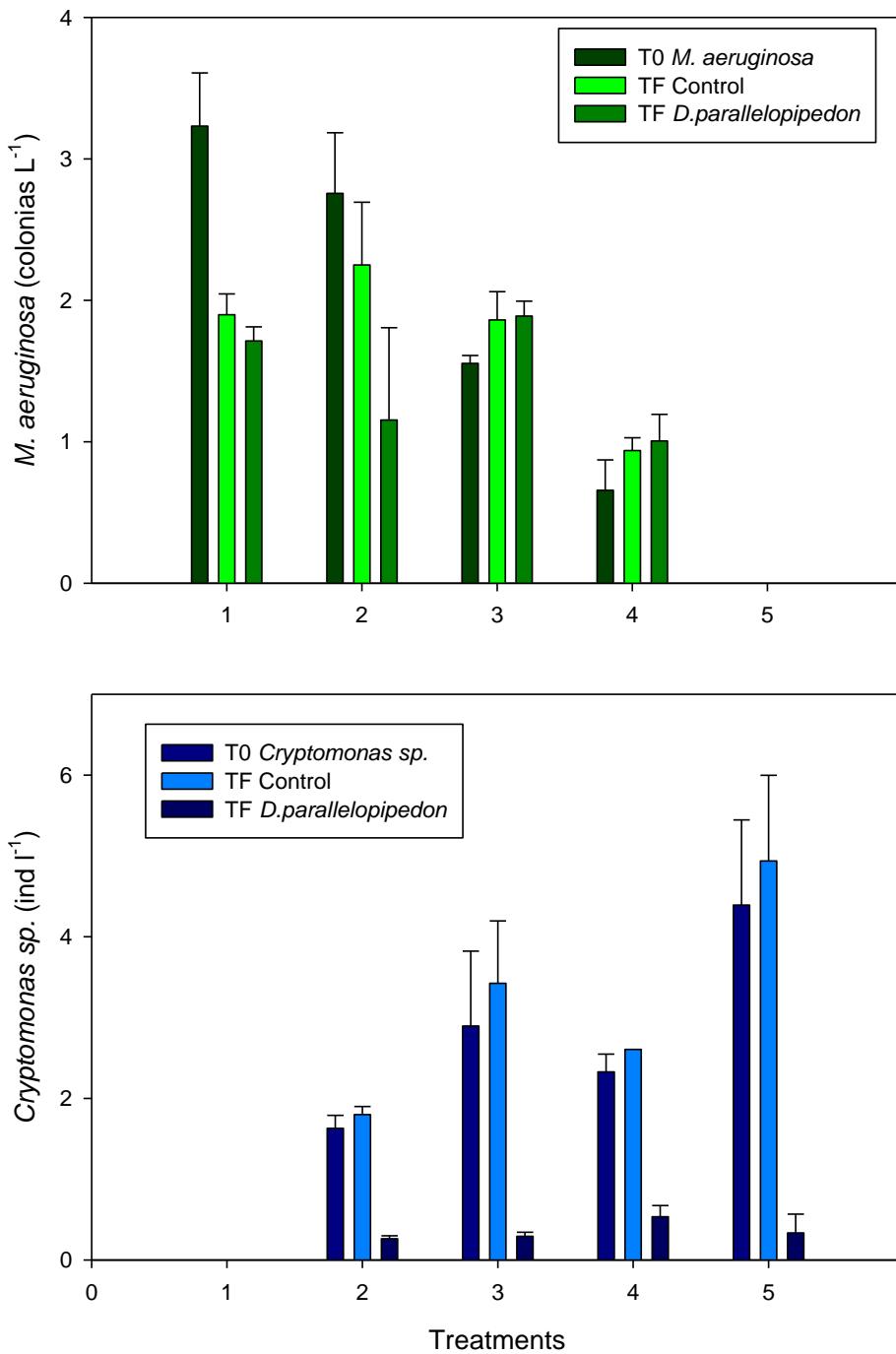
Figure 1. Experiment of Differential Consumption of *D. parallelopipedon, M. aeruginosa* (MC, above) and *Cryptomonas sp.* (C, below). Treatments 1: 100% MC, 2: 5% MC-25% C, 3: 50% MC-50% C, 4: 25% MC-75% C 5: 100% C. *D. parallelopiepdon* is not able to consume *M. aeruginosa* when it is forming colonies. On the other hand, it is able to consume and control palatable algae such as *Cryptomonas sp*.

Nutrient Excretion of D. parallelopipedon.

Phosphorous.

At the end of experiment for all temperatures there was an increase of PT, these increased is showed of dissolvent fraction like SRP (Figure 2). The higher increased of all fraction occurred at 20^0C, it differences were significate for all fractions and temperatures (PDT, $F_{(2,12)}$=16,p=0.004; PT $F_{(2,12)}$=9.04,p=0.04; SRP, $F_{(2,12)}$=54, p=0.000). The excretion rate of SRP was between 0.9-1.5 µg gr DW^{-1}h^{-1} (Table 1).

Nitrogen

There was an increase of NT at end of experiment in all temperatures (Figure 3). It was not significate different between temperatures ($F_{(2,12)}$=1.5,p=0.25). Other fraction like NO_3 showed a decreased at 20-25 ^{0}C, Figure 3, but these differences were not significant between temperatures ($F_{(2,12)}$=2.07,p=0.18). In case of NTD concentration were similar between temperatures, ($F_{(2,12)}$=0.2,p=0.78). NH_4 was the fraction with the highest increased at the end of experiment for all temperatures (Figure 4). There was an increased with temperature but it not was significate ($F_{(2,12)}$=1.8,p=0.19). The excretion rate of NH_4 was between 5.2-12.1 µg gr DW^{-1} h^{-1} (Table 1).

Table 1. Excretion rate (µg gr DW h^{-1}) of different fraction of Phosphorous (SRP, PTD, PT) and nitrogen (NO_3, NH_4, NTD and NT) at different temperature (15, 20, 25 ^{0}C).

|	SRP	PTD	PT	NO$_3$	NH$_4$	NT
15	0.9	1.1	0.8	0.3	8.4	15.9
20	1.5	1.6	1.4	-1.9	5.2	11.4
25	1.4	1.6	1.0	-8.4	12.1	17.7
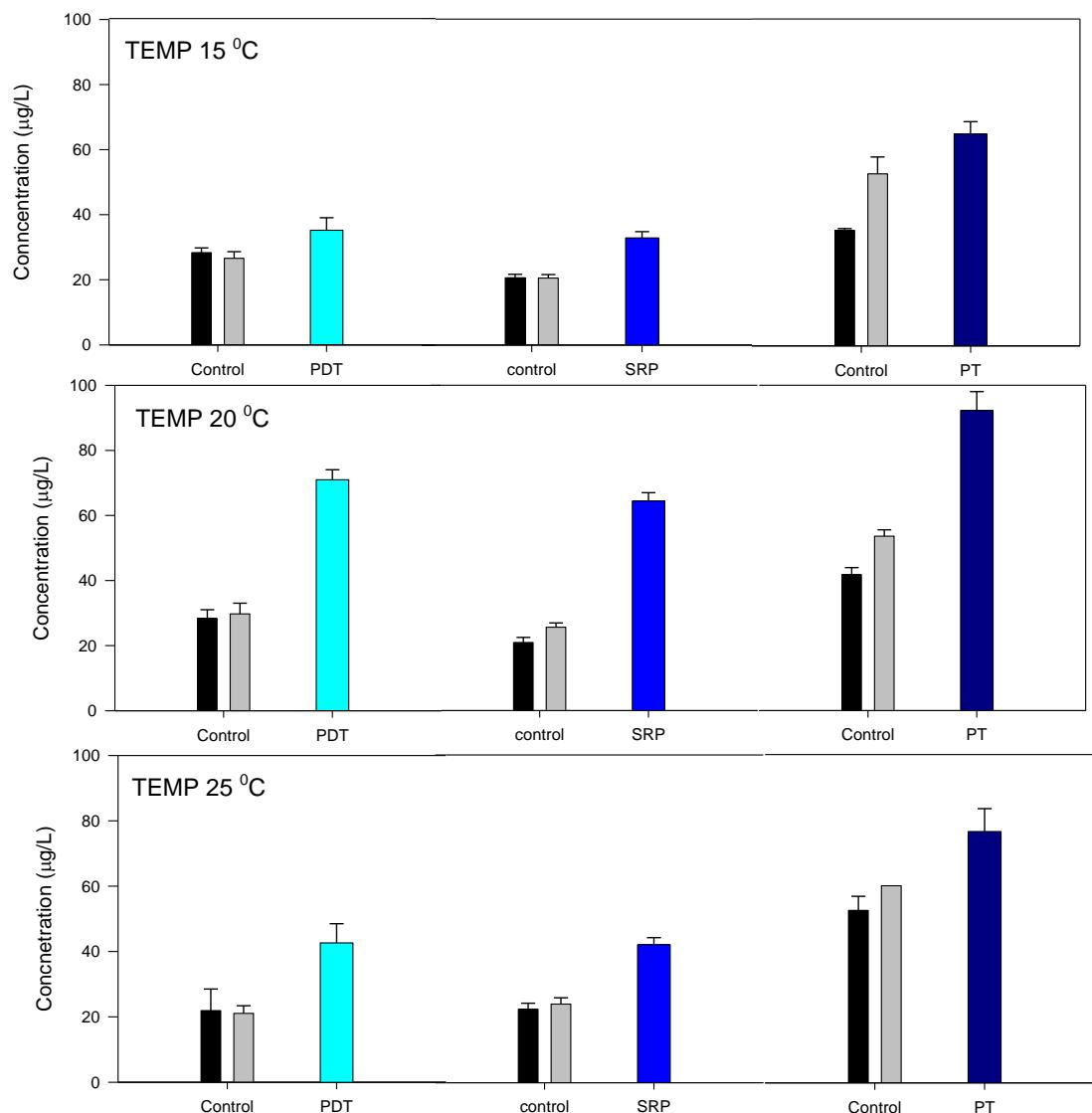
Figure 2. Nutrient Excretion (μg/L) of Phosphorous: PDT, SRP and PT, by *D. parallelopipedon.* Treatments: Concentration Initial (black bars); Final Control (grey bars) and Final Fraction (shade blue). The main increased was of all fractions PDT, SRP and PT at 20^0C.
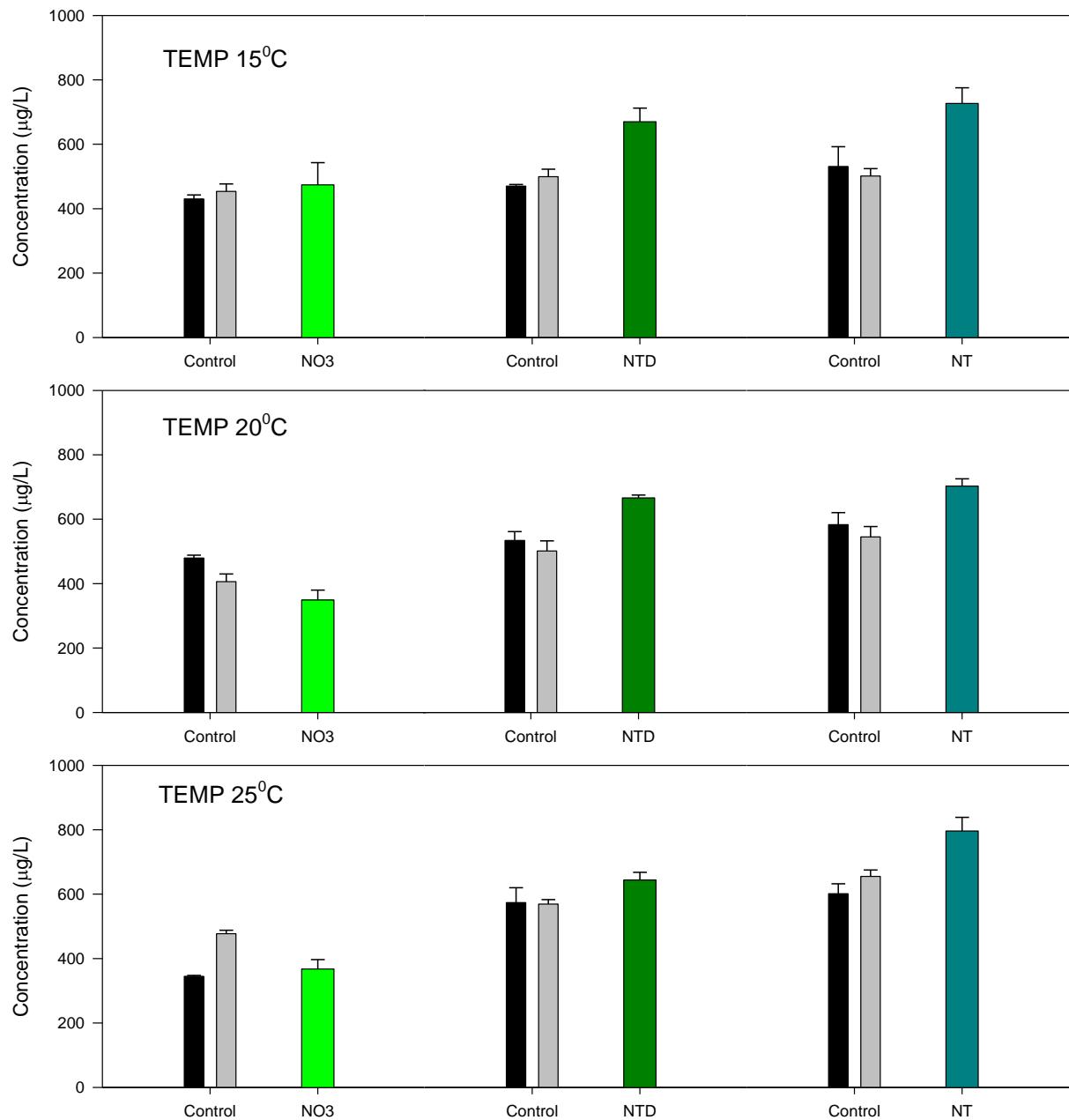
Figure 3. Nutrient Excretion of Nitrogen: NO_3, NTD and NT, by *D. parallelopipedon.* Treatments: Concentration Initial (black bars); Final Control (grey bars) and Final Fraction (shade green). For all temperatures there are an increase of NT and decreased of NO_3 concentration.
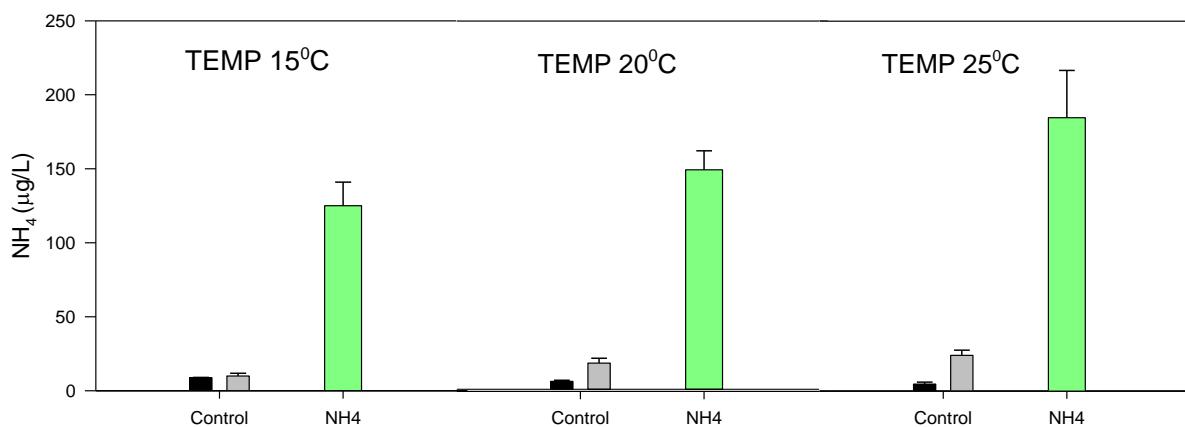
Figure 4. Excretion of NH_4 (μg/L) by *D. parallelopipedon.* Treatments: Concentration Initial (black bars); Final Control (grey bars) and Final Fraction (green) for temperatures (15, 20, 25 ^{0}C). The main increased for all temperatures and fraction was of NH_4.

Discussion

Our results indicate that *D. parallelopipedon* show a differential consumption of phytoplankton. *D. parallelopiepdon* is not able to consume *M. aeruginosa* when it is forming colonies. Due to the ability to float of the cyanobacteria and remain on the surface *D. parallelopipedon* cannot consume them. On the other hand, it is able to consume and control a palatable alga such as *Cryptomonas sp*. Moreover, *D. parallelopipedon* is capable of consume *Planktothrix agardhii,* filament cyanobacterial distributed in the water column (Marroni et al. 2014).

Some research show that some bivalves like *D. polymorpha* are capable of consumes *M. aeruginosa*, (Vanderploeg et al. 2002; Raikow et al. 2004; Sarnelle et al. 2005). However, these experiments are performed with laboratory cultures with single cells, where the cells are not able to form colonies, because they have not mucilage. Our grazing studies used the effect size colonies to understand feeding behavior of *D. parallelopipedon.*

Excretion rates of mussels vary with temperature and species. In these sense, *D. parallelopipedon* showed a positive effect of temperature with nutrient released (Spooner 2007; Marroni et al. 2019 submitted). Excretion rate measured in the experiments was higher when compared with the exotic bivalve *D. polymorpha* (Conroy et al. 2005) and lower compared to those reported for *L. fortunei* in a mesocosm experiment (Cataldo et al. 2012). The main forms of excretion were NH_3 and PO_4 these are soluble forms who are quickly incorporated by organisms like phytoplankton.

We verify our hypothesis that *D. parallelopipedon* favor presence of *M. aeruginosa* because it is not able to consume when it is forming colonies due to the capacity of flotation of the same; also it favors its growth through the release of nutrients.

In summer time, higher temperature promotes phytoplankton biomass development being potentially busted by the nutrient released by the bivalve's community, which grazing pressure is reduced, altogether leading to an enhancement of phytoplankton development. Moreover, some bloom forming with floating capacity cyanobacteria, like *Microcystis aeruginosa*, could find a facilitated way to use the nutrients reminelralized by *D. parallelopipedon* as they avoid grazing keeping in superficial layers, they take advantage of competitors remotion by filtration and using the released nutrients available in the water.

In these sense we have to possible scenarios 1- phytoplankton palatable or with dispersal on water column 2-dominance of *M. aeruginosa* forming colonial. In the first scenario *D. parallelopipedon* can exert both a control top-down (Through filtration rate) and bottom up (nutrient realized). Other possibility is that *D. parallelopipedon* cannot exert a top-down control of phytoplankton and released nutrient that can favor *M. aeruginosa* (scenario 2). In both scenarios *D. parallelopipedon* is capable of consume organic matter of sediments, so in case of phytoplankton cannot be palatable, they can survive because they have a second food source (Marroni et al. 2014).

On the other hand, if there are a great abundance of bivalves (ind/m^2) is enough, they can exert stronger top-down control over phytoplankton than bottom-up control; however we needed a new research to determinate the quantity of bivalves needed to achieve these effects in the field.

D. polymorpha an exotic bivalves are used in the Netherlands like a useful tool in the restoration of shallow eutrophic lakes (Reeders and Bij de Vaate 1990; Reeders et al. 1993). In the case of prolonged exposure to Microcystis-LR, *Dreissena polymorpha* does not present variations in its filtration rate (Vanderploeg et al. 2001) and can bioaccumulate these substances in its tissues, without causing the death of the organism (Dionisio Pires et al. 2004). In the case of *D. parallelopipedon,* new research is needed. We do not known if *D. parallelopipedon* is capable of bioaccumulated substances toxic like cyanotoxin, or if it cause damage in bivalve.

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Pedal vs. Filtration feeding contribution to bivalve´s biomass, an example using Stable Isotopes analysis and Diplodon parallelopipedon

Manuscrito 2

Autores: Soledad Marroni, Carlos Iglesias and Néstor Mazzeo

Pedal vs. Filtration feeding contribution to bivalve´s biomass, an example using Stable Isotopes analysis and *Diplodon parallelopipedon*

Soledad Marroni [1*], Néstor Mazzeo[1] and Carlos Iglesias [1*]

[1]Departamento de Ecología y Gestión Ambiental, Centro Universitario Regional del Este, Universidad de la República, Maldonado 20000, Uruguay. soledad.marroni@gmail.com (S.M.); mazzeobeyhaut@yahoo.com (N.M.); caif@cure.edu.uy (C.I.)

*Correspondence: soledad.marroni@gmail.com; caif@cure.edu.uy

Abstract

The native neotropical bivalve *Diplodon parallelopipedon*, naturally inhabits Laguna del Sauce lake (Maldonado, Uruguay). This animal can both obtain their food by filter and pedal feeding. While filter feeding consists on the removal of suspended particles including phytoplankton, zooplankton and bacteria from the water column, Pedal feeding is the direct consumption of organic matter from the surrounding sediment. Bivalves are considered mainly as filter-feeders, nonetheless the potential contribution of the pedal feeding pathway remains less studied. In the present study we aimed to determinate the relative contribution of the alternative food sources to *D. parallelopipedon* biomass, particularly, organic matter from the sediment (pedal feeding) and seston of the water column (filter feeding). Sampling was performed for analysis of stable isotopes: sediment, seston and bivalves (*D. parallelopipedon*, *L. fortunei*) of Laguna del Sauce were taken. We collected sediments organic matter (OM) as bulked samples from 2 different locations of Laguna del Sauce: one with little organic matter in the sediment (Low 0-10% OM) and another with greater concentration (High 20-30% OM). We use the mixing model to determinate importance relative of two sources. Our results suggest that organic matter of sediments is the main constituent of *D. parallelopipedon* biomass.

Keyword: Pedal feeding, filter-feeding, mixing model, *D. parallelopipedon*

Introduction

Freshwater mussels are key components in aquatic ecosystems (Strayer 2008; Vaughn et al. 2008; Haag 2012). In the classical view, unionid bivalves are considered only by their capacity of filter-feeding and their most studied impacts on freshwater systems are related to this characteristic (Vaughn & Hakenkamp 2001; Strayer 1999). In these sense, by feeding on suspended materials from the water column, bivalves are reported to couple pelagic and benthic process. Through their

filtration activity, they remove suspended particles, depositing them to the sediment as feces and pseudofeces (Yamamuro & Koike 1993; Newell 2005; Strayer 2014). As these are omnivorous organisms, they have the ability to feed on a wide variety of suspended materials in the water column: bacteria, phytoplankton and even zooplankton (Zangh et al. 2010; Davenport et al. 2011; Peharda et al. 2012; Marroni et al. 2016). Bivalves feeding process consists in the filtration of large water volumes, causing a continuous flow of particulate material from the water column to the sediments (Jörgensen 1990). Moreover, they are also reported as key components on ecosystemic functioning because while they transfer organic matter from water to the sediments, they remineralized nutrients into the water column, potentially stimulating simultaneously primary and secondary production in both compartments (Howard & Cuffey 2006; Spooner & Vaughn 2006; Vaughn et al. 2007).

On the other hand, less known for science some bivalves are able to feed themselves directly on the organic matter of the sediment through their pedal feeding capability (Vaughn & Hakenkamp 2001; Nicholson et al. 2005; Marroni et al. 2014). This feeding strategy consists in taking organic matter particles from the sediment, and transport them to their digestive tract. For a detailed description of the different strategies used to capture and transport particles please see Reid et al. 1992 and Nicholson et al. 2005. Other studies showed that pedal-feeding is size selective; larger particles are taken up with much higher efficiency than smaller ones (Brendelberger & Klauke 2009). Moreover, this feeding strategy was reported for small freshwater bivalves like Corbicula (Way et al. 1990; McMahon 1991; Reid et al. 1992), but also for Unionid adults like *D. parallelopipedon* (Marroni et al. 2014).

Understand the diet of bivalves is important to elucidate their role on ecosystem functioning and its potential application on biomanipulation of freshwater systems. Methodologically the diet of bivalves can be studied by direct observation of their gut content or indirectly by using Carbon and Nitrogen stable isotopes analysis (SIA). Following the SIA approach organic matter within the animal

tissue can be tracked back to the potential food sources available for them in the environment (Post 2002; Jardine et al. 2012, Incze et al. 1982; Peterson et al. 1985; Thorp et al. 1998; Raikow & Hamilton 2001; Jardin et al 2012; Kristensen et al. 2017). The ^{13}C/^{12}C and ^{15}N/^{14}N ratios on target animal tissue directly reflect the relative food sources contribution, assimilated and incorporated over a period of time with relative success in freshwater invertebrates (Kasai & Nakata 2005). Mathematical mixing models are commonly used to estimate the proportional contributions of food sources to the isotopic composition of the tissues of a consumer, which reflect the assimilated diet (Phillips 2001; Phillips and Gregg, 2003; Phillips et al. 2012; 2014).

Diplodon parallelopipedon, is a native Neothropical bivalve that naturally inhabits Laguna del Sauce lake (34º 43'S, 55º 13'W); Maldonado, Uruguay), it have been shown that it is able to obtain their food both by filter or pedal feeding (Marroni et al. 2014). High filtration rates from the water column have been reported; with maximum values reported at 20 $^{\circ}$C of 0.780 L g DW^{-1} h^{-1} (Marroni et al 2019, *submitted*). In the present work we used stable isotopes analyses and Mixing Models to estimate the relative importance of two potential food sources available for the bivalves, acquired by filtration or by pedal feeding. Our a priori working hypothesis postulate that as being principally filter feeders, Seston originated bivalves biomass will be higher than Organic matter´s contribution, which serves as an alternative food source in the system.

Materials and Methods.

Laguna del Sauce (34º 43'S, 55º 13'W)) is the second water source for human water supply of Uruguay (South America) (Fig 1). It was a coastal lagoon until 1947 when the connection with the Río de la Plata river through an emissary (del Potrero stream) was interrupted by the construction of a dam. Nowadays, it is a shallow reservoir without inputs of salt or brackish water. It is composed by three interconnected shallow systems (maximum depth 5 m): del Sauce (4045 ha), de los Cisnes (205 ha), and del Potrero (411 ha). According to its nutrient level and chlorophyll a concentration, Laguna del Sauce has been classified as eutrophic and it presents periodical phytoplankton blooms, mainly

cyanobacteria, particularly during summer (Mazzeo et al., 2010). In Laguna del Sauce coexists several filter feeding bivalves, the native *Diplodon parallelopipedon* (Lea, 1834, Hyriidae), the exotic Asian clam *Corbicula fluminea* (Müller, 1774, Corbiculidae), and the exotic golden mussel *Limnoperna fortunei* (Dunker, 1857, Mytilidae).
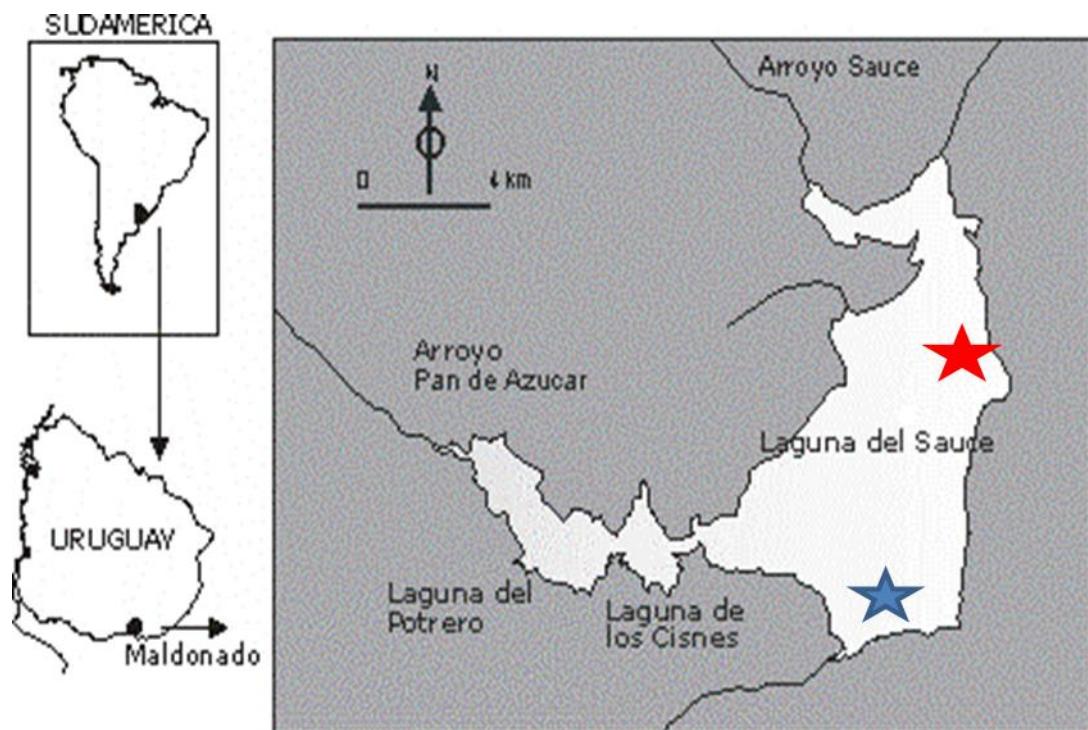

Figure 1. Laguna del Sauce (34_430S, 55_130W) of Uruguay (South America). Samples were taken from 2 different locations of Laguna del Sauce: one with little organic matter in the sediment (LOW-10%/blue) and another with greater concentration (High 20-30%OM/red).

Field Sampling

Stable isotopes samples of sediment, seston and soft body of bivalves (*D. parallelopipedon*, *L. fortunei*) from Laguna del Sauce were taken aiming to determine the relative importance of two main food sources, organic matter of the sediment (pedal feeding) and seston filtered from the water column (filter feeding). Organic matter samples from the sediment (OM) were collected as bulked samples from 2 different locations of Laguna del Sauce (Figure 1), one with low OM content (LOW 0-10% OM) and one with high concentration (High 20-30% OM).

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Immediately after returning from the field all samples were kept frozen until further processing. After freeze-drying, we homogenized the samples, weighed them into Sn capsules, and send them to determination of isotopes composition at UC Davis Stable Isotope Facility (Davis, California). The ratios of heavy to light N isotopes are expressed with δ notation as $\delta^{15}N$ ($^{15}N/^{14}N$) and defined as per mil (‰) deviation from a standard material:

$$\delta^{15}N = [(^{15}N/^{14}N_{sample}/^{15}N/^{14}N_{standard}) - 1] \times 1000; \text{(Eq. 1)}$$

where: N_{sample} is the ratio of heavy and light isotopes in the analyzed element, and $N_{standard}$ is the ratio of heavy and light isotopes in standard material, which for δ15N is atmospheric N.

Mixing Models

To determine the relative contribution of food items to the nutrition of aquatic organisms mixing models as a statistical tool were used (Phillips et al 2014). Stable Isotope Mixing Models in R with simmr (MixSIAR, Parnell and Inger 2019) software was used to run the analysis. Before analysis the results of stable isotopes were lipid corrected according to Post 2002.

Results

Our SIA main results are summarized in Table 1, moreover, useful information about the transport pathways of organic matter is depicted by the $\delta^{13}C$-$\delta^{15}N$ biplot map that shows of the system presented in Figure 2.

Table 1. Carbon and Nitrogen stable isotope ratios (%) and C/N ratios for the bivalves (*D. parallelopipedon* and *L. fortunei*), seston, and sediment. Values are mean $\pm$ SD. *n*, number of samples analyzed; place where collected samples with [OM], organic matter (low/high)**.** Data of Bivalves was corrected by lipid according to Post 2002.

| Sample	N	OM	δ^{14}C (%)	δ^{15}N (%)	C/N
D. parallelopipedon	10	High	-25.09 ±0.27	5.49 ±0.49	4.0 ±0.3
	6	Low	-26.39 ±0.27	6.02 ±0.28	4.4 ±0.4
L. fortunei	3	High	-26.10 ±0.2	4.01 ±0.42	5.1 ±0.1
	3	Low	-27.29 ±0.1	4.86 ±0.12	3.9 ±0.2
Seston	5	High	-22.11 ±1.28	4.18 ±0.55	9.9 ±0.8
	5	Low	-25.11 ±0.30	3.94 ±0.29	10.9 ±0.4
Sediment	3	High	-26.06 ±0.22	3.07 ±0.29	12.7 ±0.5
	3	Low	-25.61 ±0.32	3.37 ±0.20	11.6 ±0.4
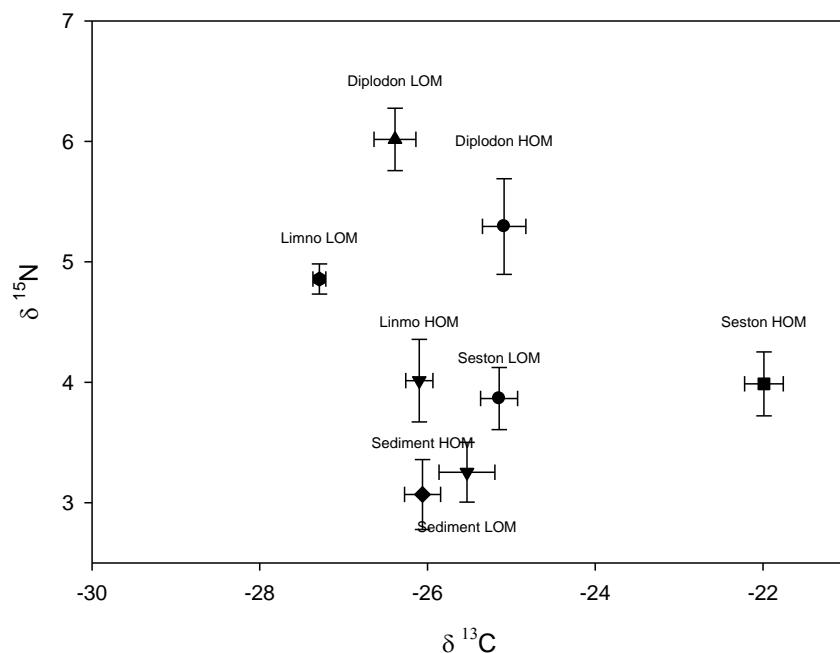
Figure 2. δ^{13}C and δ^{15}N plot of *D. parallelopipedon*, potential food sources: Seston and Sediment. *L. fortunei* (Limno) was collected like integrated signal of seston. We collected samples of two places with different level of organic matter OM of Laguna del Sauce (High /Low) (HOM/LOM). Data of Bivalves was corrected by lipid according to Post 2002. Bars indicate standard deviations.

As the organic matter of sediment and seston were considered as *D. parallelopipedon* food sources in the system, they were used as inputs for the Mixing Model analysis. In these sense, the output model showed that both sectors of the Laguna del Sauce, with low/high content of organic matter sediment, exhibited similar responses (Fig. 3). MO sediment with 75-98% constituted the main contribution to *D. parallelopipedon* biomass. While seston contribution to *D. parallelopipedon* biomass achieved a maximum of 25% along the system.

Some of the problems that arise in the analysis of stable isotopes are the stability over time of the isotopic signals of phytoplankton and their contamination with detritus (Jardin et al. 2012). One solution proposed by Jardin et al 2012, to the problem is the use of signals integrated in other organisms that consume the food source of our interest. In this sense we consider alternative signs of food sources of *D. parallelopipedon;* we use *L. fortunei* like signal of filter feeding. And for signal of organic matter sediments we kept the same since it is stable over time.

Then we analyzed data of *D. parallelopipedon,* considering an integrated signal of seston like, *L. fortunei* (Fig. 4). Again, it is observed that the greatest contribution to the biomass of *D. parallelopipedon* comes from organic matter in the sediment.
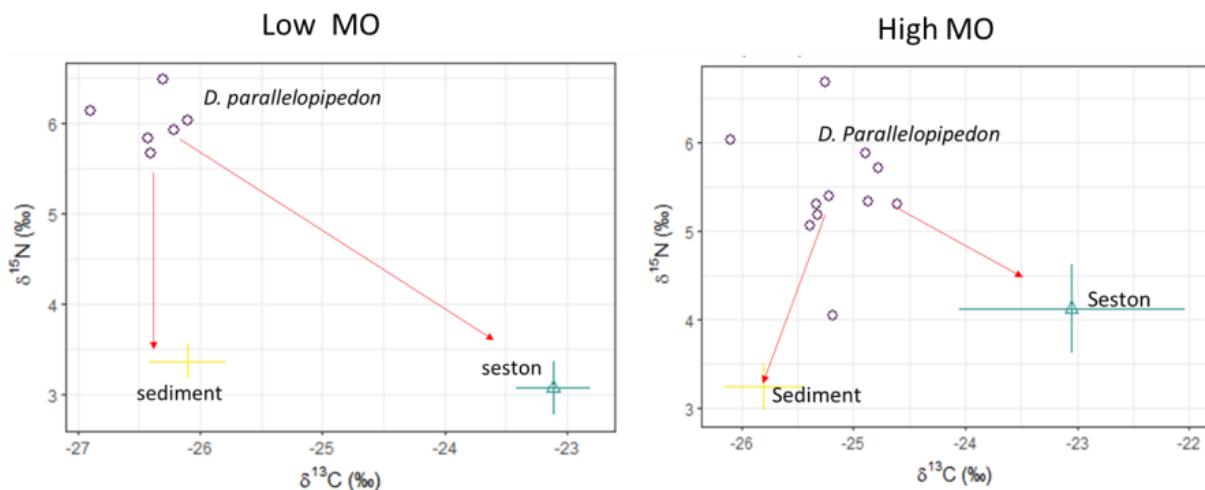
Figure 3. Analysis of stable isotopes of δ^{15}N and δ^{13}C values (mean ‰ ± SD), consumer or mixtures (*D. parallelopipedon*) and food sources (seston and sediment). In two sectors of the Laguna del Sauce with low (left, LOM) and high (right, HOM) organic matter content. The results in both cases indicate a greater contribution of organic matter from the sediment in the bivalve biomass composition.
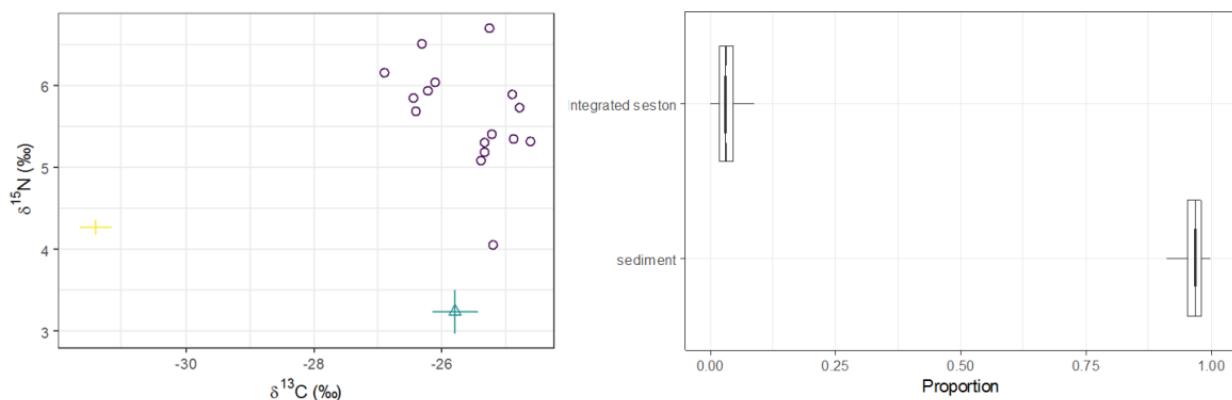

Figure 4. Left: Analysis of stable isotopes of δ^{15}N and δ^{13}C values (mean ‰ ± SD), consumer (*D. parallelopipedon, circles*) and food sources: integrated signal of seston (*L. fortunei*) sediment. Right: Mixing Model: Boxplot of the result of the relative importance of the 2 variables in the contribution of *D. parallelopipedon* biomass.

Discussion

Contrarily to our a priori hypothesis our results showed that organic matter was the principal contributor to *D. parallelopipedon* biomass in the system. In general, the role of Unionid in aquatic ecosystems emphasizes the suspension mode of feeding (Vaughn & Hakenkamp 2001) defining filter feeding as the removal of suspended particles from the water column, in the other hand, deposit

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feeding is the consumption of OM directly from the sediment. Previous results obtained by Raikow and Hamilton (2001) showed that freshwater mussels can feed on benthic food contributing up to 80% of the animals' metabolism and 20% to seston. Moreover, the patchy distribution of bivalves could be related to the preference for places of fine particulate material from which they are able to bury themselves and obtain food (Brendelberger & Klauke 2009).

In this sense, according to Stábile (2017) in Laguna del Sauce, phytoplankton primary production exhibited lower importance in the herbivores, both benthic and nektonic, isotopic signals. Moreover, Laguna del Sauce has been reported as a turbid by sediment resuspended system. Thus, light limitation in the water column for long periods occurs, and the contribution of organic matter from the wetlands associated with the main tributaries, as well as the litter of the dominant coastal tree vegetation (Willow, deciduous species) condition the observed patterns.

In conclusion, our approach allowed us to determine a high contribution of OM to *D. parallelopipedon* biomass, however it was not possible for us to be conclusive about the acquisition pathway. OM is commonly suspended in the water column and thus can be being taken by filtering it from the water column, and not by acquiring it directly from the sediments by pedal feeding.

Considering bivalves not only as consumers of phytoplankton but also consumers of OM, regardless it is acquired from sediments of filtered from the water, can have serious implications for both conservation and biomanipulación processes. Conservation process includes translocation of individuals, if sediment is rich in organic matter and allowing them to bury, translocation could be successful (Dunn & Sietman 1997). How the introduction of bivalves into OM suspended systems promotes water transparency, giving a light reach environment to phytoplankton development and also remineralizing nutrients into the water column, far from promotion the desire water improvements it might act facilitating better light and nutrient environment for certain groups able to take advantages of this improved conditions. Caution and a better comprehension of the impacts

promoted by bivalves are needed before upscaling to ecosystem level any biomanipulation program based on this kind of organisms.

Moreover, considering that some strategies to control eutrophication consequences included the introduction of bivalves in boxes into waterbodies (Olivie & Mitchell 1995; Dionisio Pires et al. 2007; Waajen et al. 2016). If it incorporation included boxes with sediment it could be more successful for better survival and adaptation. The bivalve pedal feeding allows them to survive in conditions where phytoplankton is poor or of poor quality or that is floating on the surface like cyanobacterial blooms

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