Effects of estuarine freshening on predator-prey interactions in plankton food webs

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Abstract

The highly productive waters of Louisiana coastal ecosystem are home to several economically- and ecologically important fisheries in the Gulf of Mexico, including oyster, shrimp, menhaden and blue crab. These systems are also perturbed by storms, hurricanes, floods, and other events on an annual basis. Such events are projected to increase as warming temperatures and changing precipitation patterns support more intense and wet hurricanes and flashier, more severe river flooding. Both river floods and hurricane events result in freshening of estuarine waters in Louisiana lasting days, weeks, or months. Changes in these ecosystems, which support the robust fisheries that Louisiana coastal waters are known for, are also important for understanding the effects of such events on ecosystem functions. Heterotrophic bacteria, autotrophic cyanobacteria, and the microbes that consume them are important members of microbial food webs in coastal waters. To understand the effects of freshening on microbial food web interactions, we conducted a series of feeding experiments with a cosmopolitan protistan grazer (*Paraphysomonas imperforata*) and cyanobacteria prey (*Synechococcus sp*.) at three different salinities (15, 25, and 35). Ingestion rates of *P. imperforata* were calculated based on disappearance of prey over 24-hour incubations, as measured by flow cytometry and converted to carbon. Results of the experiments show *Synechococcus sp.* experienced reduced growth and photosynthetic health (as F_V/F_M) with decreasing salinity. In addition, abundance, ingestion rate, and cell size of *P. imperforata* decreased with decreasing salinity, while *P. imperforata* growth rate increased. The results from this project suggest that combined effects of reduced salinity on both consumer and prey are likely responsible for the changes observed. These results contribute to our understanding of how the smallest members of the plankton contribute to productivity at higher trophic levels in freshwater-impacted coastal waters.

Introduction

Coastal ecosystems are perturbed by hurricanes, floods and other events which are projected to increase with ongoing climate change. Changing precipitation patterns support development of more intense and wet hurricanes (Collins et al. 2019) and more severe river floods (Seneviratne et al 2021). Microorganisms can be reliable indicators of coastal ecosystem responses to climate change and extreme events (Cavicchioli et al 2019), and changes in the amount or composition of microbial communities – especially autotrophic microbes –affect productivity of higher trophic levels, including important fisheries (Friedland et al 2012).

Microbial communities are made up of many species that span domains of life, sizes, and trophic roles. To study the effects of environmental change or extreme events on microbial food web interactions, grouping of microbes based on functions helps to identify broader patterns in ecosystem function and biogeochemical processes that wouldn't otherwise be possible (Gasol & Kirchman, 2018). In the planktonic microbes, size is an important cellular characteristic that scales with function. Planktonic microbes can be sorted into size-based classifications including: femtoplankton (<0.2 μm), picoplankton (0.2-2 μm), nanoplankton (2-20 μm), and microplankton (20-200 μm) Sieburth et al., 1978).

Studies on climate change and microbial food webs suggest different outcomes. Increasing temperature has been shown to enhance the flow of carbon from small picoplankton towards higher trophic levels (Šolić et al., 2018). Increasing pCO_2 led to a community shift from mesozooplankton (200 μm-2 mm in size) to smaller nanoplankton as dominant consumers in mesocosms deployed in temperate waters of Gullmafjord, Sweden (Taucher et al., 2017). However, the responses of microbial food webs to rapid changes like those caused by flood and/or hurricane events are less understood. The research described here sought to quantify how

hyposaline (i.e., decreasing salinity) conditions affect estuarine microbial food webs. We focused on a cosmopolitan nanoplankton predator (*Paraphysomonas imperforata)* and picoplankton prey (*Synechococcus* sp.) that are known to co-occur in coastal waters and with reasonable tolerances to different salinities. We hypothesized that effects of changing salinity would affect the consumer through changes in quality of the prey species. More specifically, we predicted that the ingestion rate of *P. imperforata* would increase as salinity decreased due to deleterious effects of low salinity on the prey (*Synechococcus* sp.) and, thus, the consumer's need to consume more prey.

Methods

Three 24-hour experiments were performed at three different salinities - 15, 25, and 35 – based on preliminary experiments that found growth of *Synechococcus* sp. at all three salinities but reduced photosynthetic yield at 15. Each experiment included three different concentrations of *Synechococcus* sp. (Christaki et al 2002). Each prey concentration was run with three control bottles without the consumer and three treatment bottles to which *P. imperforata* were added at the start of the experiment (T_0). Prior to addition to the treatment bottles, *P. imperforata* were concentrated on 3µm filter to separate them from the bacteria they are cultured in and starved for 2 hours prior to experiment. Flow cytometry samples were taken at T_0 and T_{24} and fixed with formalin. *Synechococcus* sp. cells were counted on the flow cytometer according to their size (forward scatter) and autofluorescence (chlorophyll, phycobiliproteins; Campbell 2001). *P. imperforata* were counted based on size and following staining with SYBR Green1 (Christaki et al., 2011). *P. imperforata (P)* growth (μ; Eq. 1) and ingestion rates *(*IR; Eq. 2*)* were calculated

based on disappearance of prey *(B)* using Frost equations (1972) with Heinbokel (1978) modification for rapidly growing protistan consumers (Eq.1, 2).

$$\mu = \frac{\ln (PTF/PT0)}{(TF-T0)} \quad (1)$$

$$IR = BT0 - BTF(\frac{PTF-PT0}{(lnPTF-\ln(PT0))})(TF - T0) \quad (2)$$

Statistical and graphical analyses were conducted using JMP Pro 16, Excel (Microsoft), and R (R Core Team 2016) software programs. Ingestion rates were $\log_{10}(x+1)$ transformed to better approximate normal distributions for statistical analyses and for effective graphing. A one-way ANOVA was used to determine if the ingestion rates varied significantly between salinity treatments.

Results

Initial experiments showed positive growth of *Synechoccus* sp. at all three experimental salinities; however, photosynthetic yield (as Fv/Fm) was significantly reduced at the lowest salinity (15; data not shown). When both the prey (*Synechococcus* sp.) and consumer (*P. imperforata*) were grown together, *P. imperforata* showed significantly lower ingestion rates at 15, the lowest salinity tested (ANOVA $F = 13.1$, $p < 0.010$; Fig.1). *P imperforata* ingestion rates at salinity 25 were significantly higher than rates at either 15 or 35 (Tukey-Kramer post-hoc test, $p = < 0.01$; Fig. 1).

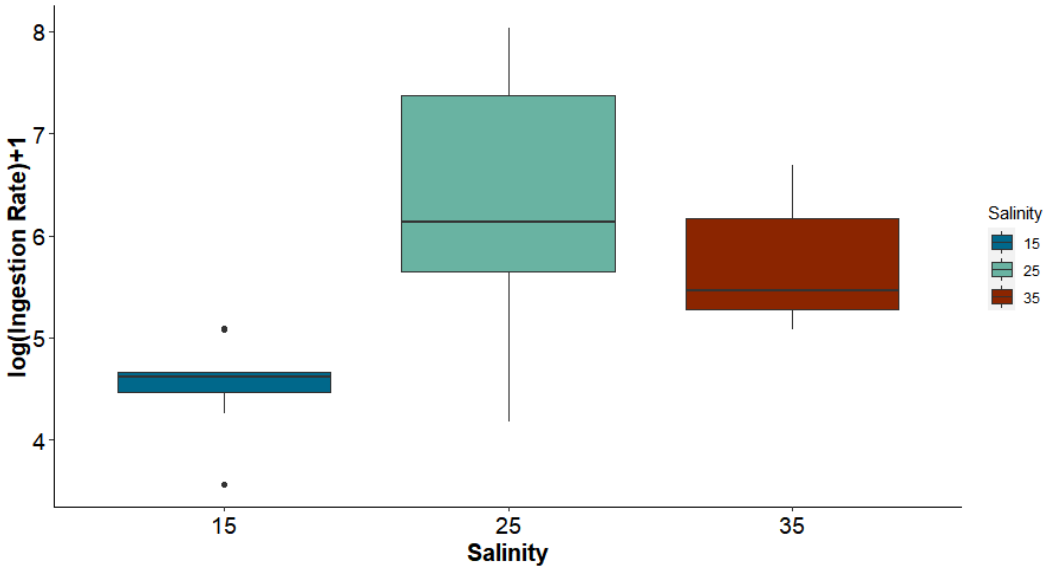

Figure 1. Box- whisker plot of *P. imperforata* ingestion rates when grown on *Synechococcus* sp. prey at three salinities (15, 25, and 35). Ingestion rates were log(x+1) transformed. Each box shows median, two hinges, and two whiskers. The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles, respectively).

Growth rates of *P. imperforata* also showed differences across the three salinities, with growth rate significantly higher in the salinity 15 treatment than in the others (ANOVA $F = 34.7$, $p < 0.05$; Fig. 2). Additionally, size of *P. imperforata* cells (as estimated length in μm) was significantly smaller in the lowest salinity treatment (ANOVA, $F = 51.6$, $p < 0.010$). Size of *P. imperforata* was otherwise consistent in the higher salinities of 25 and 35 ($p > 0.05$; Fig. 3).

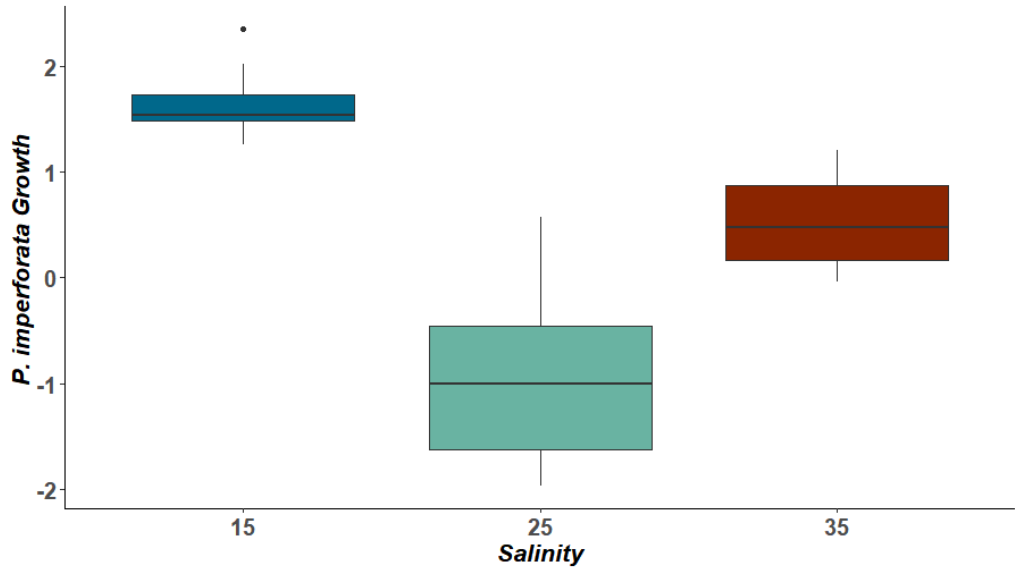

Figure 2 Box- whisker plot of *P. imperforata* growth rate in different salinities. Growth rate was calculated based on the change in abundance of cells – enumerated using flow cytometry – between T_0 and T_{24}. Each box shows median, two hinges, and two whiskers. The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles, respectively).

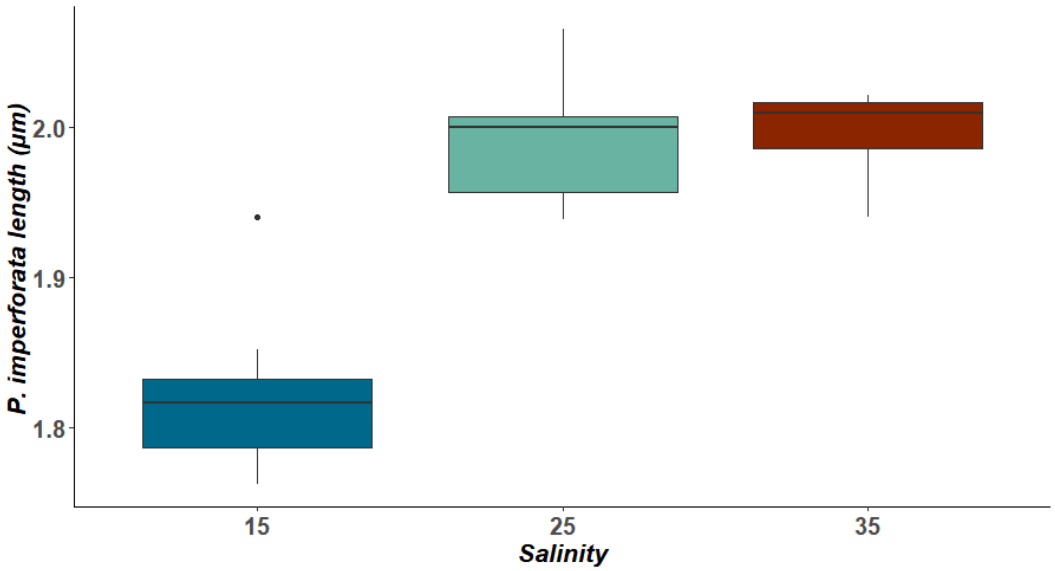

Figure 3. Box-whisker plot of *P. imperforata* size, as length (µm), at three salinities. Growth was estimated from flow cytometry forward scatter measurements calibrated with sized beads. Each box shows median, two hinges, and two whiskers. The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles, respectively).

Discussion & Conclusions

P. imperforata ingestion rates were reduced at the lowest salinity (15) and highest at salinity of 25. These results did not fit with the prediction that *P. imperforata* would make up for a lower quality prey at low salinity by consuming more, per unit time. However, the other results provide more evidence for the trade-offs in growth and feeding in this consumer-prey pair. Specifically, the combination of decreased ingestion rate, increased growth rate, but reduced size of *P. imperforata* indicate that the consumer was prioritizing cell division over cellular growth at the lowest salinity. These results suggest that the stresses of low salinity may be on both the prey and the consumer.

An interaction between the salinity tolerances of both consumer and prey is further supported by maximal ingestion rates of *P. imperforata* observed at the mid-salinity treatment (25). Preliminary results indicated that *Synechococcus* sp. became stressed at salinities < 20, and abundances of *P. imperforata* were also reduced with lower salinity conditions in this experiment. The salinity of 25 used in this set of experiments may therefore represent conditions where both *P. imperforata* and *Synechococcus sp.* are healthy and prey is abundant enough for ingestion and growth to occur. *Paraphysomonas* is a ubiquitous, opportunistic protistan consumer that is widely found in coastal environments (Lim et al 1999). Despite this natural estuarine history, results from this study show that environmentally-induced changes in ingestion rates by consumers like *P. imperforata* has the potential to affect transfer of carbon to higher trophic levels.

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