

Final Technical Report

Period Covered by the Report: April 1, 2001 - March 31, 2004
Date of Final Report: April 30, 2004
EPA Agreement Number: R 82-7072-010
Title: Predicting Seagrass Survival in Nutrient Enriched Waters: Toward a New View of an Existing Paradigm
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Research Category: Estuarine Studies
Project Period: April 1, 2001 - March 31, 2004

Introduction

Nutrient enrichment and overfishing are two of the most common man-induced perturbations of coastal systems. Eutrophication can produce many undesirable effects in coastal systems. Among them is a decline in submerged aquatic vegetation (SAV), resulting from the overgrowth of SAV leaves by algae, which have outstripped the ability of grazers (e.g., amphipods, snails, and isopods) to control them (Twilley et al. 1985, Howard and Short 1986, Tomasko and LaPointe 1991, Duarte 1995). This can result in large reductions in primary and secondary production in near-coastal waters, which often include reductions in commercially important finfish and shellfish populations that rely on SAV as juvenile "nursery" habitat. Over the past two decades these consequences have led to major Federal, state and local research efforts to understand the causes and consequences of seagrass loss, in areas as varied as Cape Cod, Chesapeake Bay and Florida Bay.

Reductions in the abundance and composition of predator and herbivore populations can also produce profound effects in aquatic systems. Documented examples include the loss of kelp forests in the Pacific Northwest where overharvesting sea otters

led to dramatic increases in their sea urchin prey, which subsequently overgrazed and eliminated the kelps (Estes and Palmisano 1974, Duggins 1980). Similarly, the removal of herbivorous fishes from coral reefs by trap fishing has been related to the proliferation of algae with subsequent loss of coral cover and demise of the entire reef community (Hughes 1994; Done 1992).

A review of the literature on predator/prey interactions in SAV-dominated systems in both freshwater and marine ecosystems suggests that losses of top predators can indirectly lead to the disappearance of SAV. Mechanistically, removing top, or apex, predators might result in the following sequence of events: 1) increased small fish densities, with a subsequent decrease in their prey (i.e. epibenthic algal grazers such as amphipods and snails); 2) increased fouling on SAV after decreases in grazer populations; and 3) loss of SAV due to overgrowth by algal epiphytes. Interestingly, these are the same results that one would expect in systems exposed solely to nutrient enrichment. This "top down" alternative to the "bottom up" nutrient enrichment hypothesis could account for reductions in SAV biomass in heavily fished areas, but to date remains inadequately tested.

Important differences are to be expected in the degree of top-down susceptibility to cascading trophic effects among riverine, estuarine, marine and freshwater ponds and lakes (Heck and Crowder 1991; Strong 1992; Powers 1992; Rosemond et al. 1993; Polis and Strong 1996). This is because the cumulative effects of shifts in large predator abundance are likely to be more profound, in small, "closed" systems such as ponds and less important in larger, "open" systems such as rivers and estuaries. In addition, in open systems, where epiphyte grazers are recruited from drift or the plankton, large settlement

events may swamp the effects of their small fish predators that have been released from predator control by overfishing larger piscivores.

It has also been suggested that marine communities, with many omnivorous taxa and high levels of food-web redundancy, may be less susceptible to "top-down" effects than simpler fresh water communities (Strong 1992). In this scenario, systems with relatively simple linear food webs and few omnivores would be most likely to show strong evidence for trophic cascades.

Here we examine the cascading trophic effects of greatly reducing the influence of large fish predators, thereby simulating the effects of overfishing, in two SAV-dominated ecosystems along the northern Gulf of Mexico coast. Our objectives were to develop a mechanistic understanding of the indirect effects that may result from overharvesting large predators in two different, but common, types of SAV-dominated aquatic ecosystems, and, ultimately, to predict when these systems might be expected to shift between macrophyte - and phytoplankton-dominated states. In particular, we evaluated the degree to which the degree of "openness" and the degree of "omnivory" of the Delta and the Perdido Bay ecosystems influenced their susceptibility to top down effects. This was done with the use of replicated enclosures at both sites with different sized mesh that either allowed or prevented small invertebrate immigration and emigration from the enclosures. This effectively made one group of enclosures "open" and the other group "closed".

We were able to evaluate the degree to which omnivory influenced the susceptibility of both systems to top down control by enclosing both omnivorous and strictly carnivorous fish in replicate "open" and "closed" enclosures. We simulated the

effects of eliminating large, piscivorous predators through overfishing by increasing small fish density in each enclosure to 10X that of ambient densities. We also were able to test the hypotheses that cascading trophic effects are most likely to be seen in those enclosures that are "closed" to immigration and emigration with carnivorous fish, least likely to be seen in "open" enclosures with high degrees of omnivory, with intermediate top down effects observable in enclosures of the other two treatments.

Materials and Methods

Study Sites

Our first study site was a freshwater/oligohaline environment in the Mobile Bay Delta. The Delta is approximately 16 kilometers wide and 72 kilometers long (Crance 1971) with the southern portion characterized by many bays, marshes, bayous and submerged aquatic vegetation (Zolczynski and Eubanks 1990). Our site was just south of the "bayway", Interstate 10 (30° 40N, 87 ° 58W) on a broad shallow flat that supports a mixed stand of SAV species that is dominated by wild celery (*Valisneria americana*). Salinity generally ranges from 0-7 PSU annually (personal observation) and water temperature varies seasonally from approximately 18 °C to 30°C (personal observation) during the experimental period.

Our second site, Big Lagoon, Florida, is a semi-enclosed lagoon characterized by low energy regimes (cf. Gallagher 2001) located in the Perdido Bay system in the north central Gulf of Mexico (30 ° 23N, 87 ° 24W). It contains seagrass meadows dominated by turtlegrass, *Thalassia testudinum* and shoalgrass, *Halodule wrightii*, along with unvegetated sand flats in the shallow (< 2m) areas. Salinity generally ranges from mesohaline to polyhaline (13-30 PSU annually, Wear et al.1999; Spitzer et al. 2000).

Water temperature varies annually from approximately 7 to 30°C (Wear et al. 1999; personal observation) and the mean tidal range is 0.5 m (Spitzer et al. 2000).

Study Organisms

Bluegill

One of the most abundant small fish in the Delta is the omnivorous bluegill sunfish (*Lepomis macrochirus*), hereafter referred to as bluegills. Bluegills are distributed from the Great Lakes to the Gulf of Mexico, and the diet of fish < 80mm in TL (total length) consists mainly of plant material (submerged aquatic vegetation (SAV), SAV detritus and algae) as well as lesser amounts of invertebrates (e.g. gammarid amphipods)(Chaplin, 2001).

Bass

The carnivore used in the Delta experiment was the large mouth bass, *Micropterus salmoides*, hereafter referred to as bass. Bass are distributed throughout the United States east of the Rockies, with limited populations in southeastern Canada and northeastern Mexico (<http://www.tpwd.state.tx.us/fish/infish/species/lmb/lmb.htm>). Juvenile bass (<120mm) feed primarily on invertebrates and fish (Chaplin, 2001)

Pinfish

The dominant small fish in Perdido Bay is the omnivorous pinfish (*Lagodon rhomboides*). Pinfish are distributed from Massachusetts to Florida and throughout the Gulf of Mexico (Hoose and Moore 1977). Juvenile pinfish (<100 mm) typically inhabit vegetated estuarine habitats (Reid 1954, Hansen 1970, Coen et al. 1980). As juveniles, pinfish are omnivorous and feed on amphipods and shrimp (Carr and Adams 1973) as well as plant material and detritus (Darnell 1961, Carr and Adams 1973, Adams 1976).

Redfish

The carnivore used in the Perdido experiments was the redfish, *Sciaenops ocellatus*. Redfish are distributed from the Gulf of Maine to Mexico and are present in abundance along the Gulf States (Reagan 1985). As juveniles, redfish inhabit estuaries (Perett et al. 1980) and are major predators of crustaceans (mostly shrimp and crabs) and smaller fish (Reagan 1985).

Experimental Design

Twenty-four 2 m² square enclosures were placed at each of the study sites within dense stands of either *Valisneria americana* (Delta) or *Thalassia testudinum* (Perdido), to test the effects of removing the top predators on ecosystem structure and function in systems that differed in both the degree of openness and the degree of omnivory. The experimental design was a two by two factorial, with one factor being the degree of openness (“open” and “closed”), and the second factor being trophic category (omnivorous fish and strictly carnivorous fish), resulting in four treatments: 1) open/ omnivorous, 2) open/ carnivorous, 3) closed/ omnivorous and 4) closed/ carnivorous with 6 replicates per treatment. Six replicates was similar to the number used by Williams and Ruckelshaus (1993, n=7) and Neckles et al. (1992, n=5) who found significant results in their experimental studies of the relative effects of nutrient enrichment and mesograzers on seagrass and epiphytes. Identical experiments were conducted simultaneously in Perdido and Mobile Delta during the spring and fall of 1999, 2000 and 2001.

“Open” enclosures were constructed of 5.1 x 10⁻⁴ m diameter PVC frames covered with .019m bird netting, while the “closed” enclosures were constructed of a similar PVC frame covered with 1mm mesh reinforced on the exterior with .019m Vexar. Once

constructed, the cages were placed within the experimental grass bed at each site in a haphazard manner.

Prior to stocking the enclosures, time zero samples of SAV biomass and epiphyte and faunal abundance were taken from a predetermined region of each enclosure using standard methods (see below).

Following time zero sampling, the enclosures were stocked with omnivorous or carnivorous fish at elevated (10x ambient) densities. We estimated this to be approximately 30 pinfish/m² based on reported semi-demersal fish predator densities of around 3.0/ m² from Texas SAV- dominated sites (Huh, 1984) and 5 redfish /m² based on data from research conducted in the Perdido area (Nadeau, 1991). We also used 30 bluegills and 30 bass as our estimated 10x densities in the Delta (DeVries, personal communication).

Pinfish were collected using two minute trawls with a 4.88m otter trawl in surrounding grass beds in Big Lagoon. The redfish, bluegills and largemouth bass were obtained from aquaculture centers in Alabama and Texas. Once the enclosures were stocked and sealed (by securing a top constructed in the same manner as the enclosure with cable ties) the experiment was run for 4 weeks (short term) during 1999 and 8 weeks (short term) 2000. Following the experimental period, the enclosures were pulled up, cleaned and repositioned prior to the beginning of the next experiment. During 2001, a 16 week (long term) experiment was conducted by leaving half of the enclosures in place for an additional 8 weeks at the end of the 8 week (short term) experiment. Experiments were conducted from July 1 – August 9 (Perdido) and June 16- July 19 (Delta) for Spring 1999; from September 9- October 11 (Perdido) and September 13- October 14 (Delta) for

Fall 1999. In 2000, experiments were conducted in the spring from June 8- August 9 (Perdido) and June 27- August 25 (Delta) and in the fall from August 30- October 30 (Perdido) and September 6- November 13 (Delta). Finally, experiments were conducted in Perdido in 2001 from June 27- August 28 (short term) and June 27- November 12 (long term).

Sampling Regime

SAV Biomass

At the beginning (time zero) and end of the experimental period, SAV biomass samples were taken from a predetermined region within the enclosure (to prevent sampling the same area twice). A known area (.01-.06 m²) within each enclosure was harvested for aboveground biomass and all collected material was placed in a 1 mm sieve to remove excess sediment. The contents of the sieve were placed in labeled Ziploc bags and returned to the lab, where the samples were frozen until they were processed. In the lab, all intact shoots were counted and all aboveground material was placed into a pre-weighed aluminum weighing pan. The samples were then placed into a drying oven (80°C) for at least 24 hours (dried to a constant weight) and then weighed to the nearest 0.01g to determine aboveground biomass dry weight (DW). The biomass samples were then placed into a muffle furnace (500°C) for four hours and then weighed again to determine ash free dry weight (AFDW). DW and AFDW for each sample was converted to a per m² basis for statistical analysis.

Epiphyte Abundance

Epiphyte samples were also taken at time zero and end of each experimental period. Ten shoots were haphazardly selected from a predetermined region within the

enclosure and harvested near the sediment-water interface (ensuring that all above ground biomass was collected). The ten shoots were placed in labeled Ziploc bags, put on ice and returned to the lab for processing. At the lab, the oldest blade from each shoot was set aside and the rest of the shoot was discarded. Both sides of the blade were scraped with a razor blade to remove the epiphytes. The ten scraped blades were placed into a labeled and preweighed pan, which was put into a drying oven (80°C) for at least 24 hours and then weighed to the nearest 0.0001g to determine the DW of the scraped blades. The epiphytes were placed into a labeled vial with Qwater, and the contents of the vial were then run through a vacuum filtration system onto a GF/C filter. The filter was then placed in a labeled foil packet and frozen in an Ultralow freezer (-80°C) until being processed. Epiphyte samples were processed using standard wet chemical methods (Strickland and Parsons, 1972) using a Turner Designs Fluorometer to determine the amount of total Chlorophyll present. The chlorophyll amounts were standardized using the DW of the corresponding scraped blades.

Mesograzer Abundance

Time zero and final samples of SAV- associated epifauna were collected from each enclosure using a 0.07 m² plastic cylinder, whose lower edge was embedded in the sediment. The contents of the cylinder were collected for 2 minutes by a gasoline powered suction pump (Orth and van Montfrans 1987; Williams et al. 1990; Valentine and Heck 1993) and all the material was strained through a 0.5mm mesh collecting bag. Samples were rinsed into a labeled, Ziploc bag and frozen until they were sorted. Samples were later thawed and all macrofauna was removed using a magnifying lamp.

Animals were identified to family and classified as either a mesograzer or non-mesograzer according to published information.

Results

There were no significant differences between treatments at time zero for any of the experimental variables, (mesograzer abundance, epiphyte biomass, and SAV biomass) therefore, all results will be presented as the change in each variable (Final –To) that occurred during the experiment.

Perdido

Results from the first year of this study (1999) will not be presented for the Perdido Bay system since there were difficulties with enclosure construction, and we were not able to maintain enclosure integrity or the desired treatments. Thus we treat this experiment as a pilot study only. Below we describe the results of 2000 and 2001.

Fish Recovery

During 2000, pinfish recovery from enclosures ranged from 7- 100% with a mean of $69.2\% \pm 7.8$ s.e., while redfish recovery ranged from 0-100% with a mean of $54.5\% \pm 8.8$ s.e. In 2001, recovery of pinfish from the enclosures still ranged from 7- 100% but the mean increased to $75.8\% \pm 6.8$ s.e.. The range of redfish recovery in 2001 also remained the same as 2000(0- 100%), but the mean decreased to $41.3\% \pm 9.0$ s.e.

Mesograzer Abundance

The degree of openness never showed significant effects on mesograzer abundance in any of the experiments. However, trophic category was significant for mesograzer abundance during the long term experiment in 2001 (2ANOVA: $p= 0.013$, $df= 1,8$), with more mesograzers present in the carnivore treatment. In addition, there

was a significant interaction (2ANOVA: $p=0.04$, $df=1,16$) between openness and trophic category for mesograzer abundance during Fall 2000.

Gastropod abundance was affected by the degree of openness during Fall of 2000 with significantly (2ANOVA: $p<0.001$, $df=1,16$) more gastropods present in the closed treatment. Trophic category had a significant effect (2ANOVA: $p<0.012$, $df=1,8$) on gastropod abundance during the long term experiment in 2001, with more gastropods found in the carnivore treatment. There were no significant interactions between treatments for gastropod abundance.

Gastropod abundance patterns differed when the dominant gastropod, *Diastoma varium*, was removed. *Diastoma varium* was removed from analysis since it is not a major prey item of redfish or pinfish; therefore, *D. varium* abundance was not expected to be affected by an increase in fish density. Following the removal of *Diastoma*, the degree of openness had a significant (2ANOVA: $p=0.051$, $df=1,16$) effect on gastropod abundance in Fall 2000 with fewer gastropods present in the open treatment. Gastropod abundance without *Diastoma* was not significantly affected by trophic category, and there were no significant interactions between treatments for gastropod abundance without *Diastoma*.

Mesograzer abundance patterns also differed when *Diastoma* was removed. Mesograzer abundance without *Diastoma* showed no significant treatment effects for the degree of openness or for trophic category. However, there was a significant interaction (2ANOVA: $p=0.029$, $df=1,17$) between treatments for mesograzer abundance without *Diastoma* during Fall 2000.

The degree of openness had a significant (2ANOVA: $p=0.022$, $df = 1,20$) effect on the abundance of grazing crustaceans during Spring 2001, as there were significantly more grazing crustaceans in the open treatment. Grazing crustacean abundance showed no significant effects of trophic category. There was a significant interaction (2ANOVA: $p= 0.045$, $df= 1, 17$) between the degree of openness and the degree of omnivory for grazing crustacean abundance during Fall 2000.

Amphipod abundance showed significant degree of openness effects during Fall 2000 (2ANOVA: $p =0.003$, $df = 1, 17$) and during Spring 2001 (2ANOVA: $p =0.048$, $df = 1, 20$). In Fall 2000 there were more amphipods in the closed treatment, while in Spring 2001 there were more amphipods in the open treatment. Amphipod abundance also was significantly affected by trophic category during Spring 2001 (2ANOVA: $p =0.048$, $df = 1, 20$), with a greater abundance of amphipods in the carnivore treatment. There were no significant interactions between treatments for amphipod abundance.

Epiphyte Abundance

Epiphyte abundance showed no significant effects for the degree of openness. However, there was a significant trophic category effect on epiphyte abundance during Spring 2000 (2ANOVA: $p= 0.0014$, $df = 1, 17$), with greater epiphyte abundance in the carnivore treatment. There were no significant interactions between the degree of openness and the degree of omnivory for epiphyte abundance.

SAV Biomass

SAV biomass (DW and AFDW) showed no significant treatment effects for either of the treatments, nor were there any significant interactions between them for SAV biomass (DW and AFDW).

Delta

There were no results from the fall of year 2 (2000) and year 3 (2001) since 2000 was a drought year and salinities in the Delta exceeded 15 PSU for an extended period of time, causing all of the *V. americana* to die prior to the fall experimental period.

Vallisneria americana did not reappear in the Delta study area until after the completion of this study. Below we describe the results of 1999 and Spring 2000.

Fish Recovery

During 1999, bluegill recovery from enclosures ranged from 0- 93% with a mean of $17.5\% \pm 5.6$ s.e., while bass recovery ranged from 0-30% with a mean of $5.5\% \pm 1.6$ s.e. In 2000, recovery of bluegills from the enclosures ranged from 47- 53% and the mean increased to $51.3\% \pm 0.58$ s.e.. The range of bass recovery in 2000 remained the same as 1999(0- 30%), but the mean increased to $15.5\% \pm 3.1$ s.e.

Mesograzer Abundance

Mesograzer abundance showed no significant treatment effects. However, there was a significant interaction (2ANOVA: $p=0.043$, $df=1, 11$) between treatments for mesograzer abundance during Spring 1999.

Gastropod abundance was affected by the degree of openness during Spring of 1999 with a marginally significant (2ANOVA: $p=0.070$, $df=1, 8$) increase in gastropod abundance in the open treatment. Gastropod abundance showed no significant trophic category effects, and there were no significant interactions between treatments for gastropod abundance.

Neither treatment showed significant effects for grazing crustacean abundance. There was a significant interaction (2ANOVA: $p = 0.05$, $df = 1, 8$) between the degree of openness and the trophic category for grazing crustacean abundance during Spring 1999.

Amphipod abundance showed the exact same trends as the grazing crustaceans with no significant treatment effects. Again, there was a significant interaction between degree of openness and trophic category (2ANOVA: $p = 0.038$, $df = 1, 8$) during the spring of 1999.

Epiphyte Abundance

Epiphyte abundance showed no treatment effects for the degree of openness or for trophic category. Nor were there any significant interactions between treatments.

SAV Biomass

SAV biomass (DW and AFDW) showed no significant treatment effects. Nor were there any significant interactions between treatments for SAV biomass (DW and AFDW).

Discussion

The overall goals of our study were to test whether the degree of openness to immigration and varying food web complexity, either singly or in combination, affected the response of vegetated coastal habitats to the simulated effects of reducing the abundance of large predators via overfishing. This work was motivated by the predictions of Strong (1992; see also Polis 1994 and Polis and Strong 1997) that trophic cascades should occur primarily in aquatic systems that are closed and that have simple food web structure with low degrees of omnivory and redundancy. Our experiments were carried out in field mesocosms in conditions that were meant to simulate SAV habitats both open

and closed to immigration of mobile invertebrates, and that reflected the results of greatly reducing the abundance of large predatory fishes. Below we summarize the conclusions from this work, and evaluate alternative explanations for our results, with special attention given to the possibility of experimental artifacts.

There were no important cascading effects in any of the Perdido experiments. Although there were some effects on mesograzers associated with both the degree of openness and trophic category, these effects were not consistent across the four experiments. In addition, they were associated with significant effects on epiphytes only once, and we never observed cascading effects on SAV biomass. And even though SAV declines occurred in both the fall experiments, they were not associated with large epiphyte biomasses. Rather, they simply reflected the annual decline in SAV biomass that occurs in the fall of the year (Spitzer et al. 2001).

One explanation for the lack of cascading effects is the diversity of mesograzers. For example, although amphipod abundance was reduced by the omnivorous pinfish, increases in gastropod grazers, which are not often consumed by pinfish (Stoner and Livingston 1984), along with herbivory by the omnivorous pinfish themselves, appear to have compensated for the effects of reduced amphipod density on epiphyte biomass. Regarding the lack of significant effects of the degree of openness, we suggest that recruitment by the grazer *D. varium*, which do not have planktonic larvae and are not preyed upon to any large extent by either pinfish or redfish (Stoner and Livingston 1984; Overstreet and Heard 1978), prevented the occurrence any clear effects of closing off immigration of prey into the fine-meshed enclosures.

The two Delta experiments were similar in that neither manipulating the degree of openness nor trophic category produced strong effects on mesograzers, epiphytes or SAV. One issue that requires explanation is the large decline in SAV biomass in each of the experiments. This decline simply reflects the growth pattern of *V. americana* in our climatic regime, which grows rapidly in spring to maximum biomass in early summer, and subsequently declines over the late summer and fall until reaching very low standing crop in the coldest months (Stout and Heck 1990).

In any experimental manipulation, especially one employing caging, the possibility of artifacts biasing results must be seriously considered. In our work, two primary issues deserve discussion: caging artifacts of several types, and whether the duration of the experiment was sufficient to detect treatment effects if they existed. We address these in turn.

Caging artifacts can be manifold, but of primary concern here is whether we had truly open and closed immigration of potential mesograzers prey of the fishes in the enclosures. Since the integrity of the cages was maintained during the experiments, it seems certain that immigration of animals larger than the 1 mm mesh on the closed treatments did not occur. As to whether mesograzers were able to enter the open cages, the study by Gallagher (2001) in the same area is relevant. She found that in enclosures with the same 19 mm mesh we used in the open cages, immigration balanced emigration in cages both with and without pinfish inside. Although no experiments have been done with the other three fish species used in our experiments, we believe that is likely that prey taxa could and did enter the open enclosures in both the Delta and Perdido Bay, and that we did have both open and closed mesocosms with respect to mesograzers.

Our ability to maintain omnivore and carnivore densities is less certain. We did a good job of maintaining omnivorous pinfish densities in Perdido (average recoveries of 69% and 75%) and a fair job of maintaining redfish densities (average of 54% and 41% recoveries). Clearly, however, we did not recover most of the animals stocked in the Delta mesocosms at the start of the experiments. Instead we had recoveries of omnivorous bluegills that averaged 17.5% and 51.5%, while carnivorous bass averaged only 5.5% and 15.5% recovery. However, the degree to which reductions of fishes reflects our inability to capture all fish from the enclosures, or mortality that took place during the experiment is uncertain. Our feeling is that most of the loss of fishes was primarily due to mortality, although it is impossible to determine when mortality took place. It is important to recognize that realized densities at the end of the experiments were still well above average densities.

The paper by Menge (1997) provides help in determining whether the duration of our experiments was adequate to detect treatment effects if they existed. In his review of 23 studies done in rocky intertidal habitats, he found that experimental duration ranged from 0.5 to 156 months, but there was no significant relationship between experimental duration and the appearance of indirect (cascading) effects. Although caution is due in extrapolating from the rocky intertidal to SAV habitats, our two to four month experimental durations seem adequate to assess effects if they existed. In addition, Ruesink (2001) found significant mesograzer effects on algal epiphytes in only 2-4 weeks. Since her work was carried out in the much colder water of Washington state, it seems likely that we should have been able to detect mesograzer effects on epiphytes if they existed.

Overall, the biggest issue relating to experimental artifacts was our inability to verify that we maintained high densities of omnivores and carnivores in the Delta and in some of the Perdido enclosures. However, we saw no evidence that those enclosures with more fish had different outcomes than those with fewer fish. Also, our target fish densities were very high, and even with partial success in keeping them alive in the enclosures, our densities were much higher than ambient.

Conclusions

We found no substantial evidence that the degree of openness influenced the outcome of our manipulations, contrary to the opinion of Strong (1992) that closed systems should be most likely to exhibit cascading trophic effects. Nor did we see evidence that the presence or absence of dominant omnivores, either the blue gill or the pinfish, had significant impacts on cascading trophic effects. However, the relatively high faunal diversity, and food web complexity in our study areas would be expected to counter the effects due to the lack of immigration by mesograzers into fine mesh enclosures, and the high diversity and presumed food web redundancy should forestall the effects of manipulating blue gill and pinfish density (even if they are the most common demersal, mobile fishes in local SAV habitats).

Of course, we now know that trophic cascades exist in all types of ecosystems (Pace et al. 1999; Shurin et al. 2002), and that they are not confined to closed, aquatic systems of low species richness, as suggested by Strong (1992). Nevertheless, our conclusions that high species richness, omnivory and food web redundancy all seem to reduce the expression of cascading trophic effects, are consistent with a number of Strong's (1992) predictions.

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Appendix 1.

The quality assurance for this project was outlined in a plan according to ACES guidelines. During the course of this project there were a few changes made to the project that affected the QA plan. This section will address these changes and verify that the QA plan was utilized.

According to the QA plan, the following parameters were to be measured: 1) length measurements of fish used to stock the enclosures and stomach content of the fish following the experiment; 2) Primary production during and after experimentation; 3) epiphyte quantification; 4) enumeration and identification of mesograzers before and after the experiment; and 5) temperature and salinity measurements at the site. Length measurements of the fish were to be used to estimate growth rates within the enclosures and stomach contents were to be used to verify the diet of the enclosed fish. The fish portion of the project was the responsibility of Dr. Jim Cowan and his lab. Unfortunately, Dr. Cowan left the Dauphin Island Sea Lab before all the samples were collected and processed; therefore, the fish data is unavailable.

Primary production during (PPD) and after (PPA) the experiments was eliminated from the project for several reasons. In the Delta, visibility made it impossible to accurately mark the shoots for production and *Valisneria americana* grows differently than *Thalassia testudinum* with all of its growth occurring at the beginning of the season. Also, marking the shoots for production both in Perdido and the Delta required climbing into the enclosures disturbing the treatments by potentially knocking epiphytes, a response variable, from the seagrass. Due to the disturbance that would be caused by

climbing into the enclosures and the inability to compare production between the Delta and Perdido, it was decided that primary production be eliminated from the project.

It was also determined that epiphyte abundance could be determined more accurately by using fluorometric procedures (CHL a extraction) than by biomass; since biomass may include epiphytic, calcareous tube building worms which could bias the biomass results. Therefore, chlorophyll a extractions were used as a surrogate for epiphyte biomass in order to remove this bias. The point intercept method for determining epiphyte composition was eliminated from the plan since epiphyte composition was not a response variable that we felt provided pertinent data, because we were more concerned with algal overgrowth (epiphyte abundance) than with the species present.

The first 10 faunal samples from each site during 1999 were picked twice to ensure that all mesograzers were removed from the samples. Since only the occasional mesograzer was found during the second pass of these samples, the rest of the samples were only picked once.

Faunal identification was verified by having a second person identify the mesograzers present and samples were considered in agreement if they agreed at least 95%. We verified 3 of the 24 (~13%) time zero samples at each of the sites. All of the samples that were verified agreed with greater than 95% agreement, therefore no other samples were verified.

Faunal samples were to be preserved following identification; however, it was decided that faunal samples would be separated into grazer groups, dried and weighed to

determine an average size of individuals within enclosures. Therefore, none of the faunal samples are preserved.

All data was entered into Microsoft Excel and then printed and proofed for entry error by Patricia Spitzer. Once data entry was verified and corrected, if necessary, data quality tests (Grubbs testing, tested for normality and heteroscedasity) were conducted prior to analysis.

Data was also subjected to a Grubbs test for outliers and any data points that were greater than the critical Z were removed from the data set prior to data analysis. During 2000 in Perdido 13 of 198 (6.6%) of the spring short term data points were removed from analysis, while 6 of 189 (3%) of the fall short term data points were removed from analysis. During 2001, 1 of 236 (<0.1%) of the spring short term data points and 4 of 127 (3%) of the long term data points were removed from the data sets prior to data analysis. In the Delta, 3 of 138 (2%) and 0 of 84 (0%) points were removed from the 1999 spring short term and fall short term data sets , respectively. The 2000 spring short term experiment had 1 of 80 (1%) of the data points removed prior to analysis.

All data was also checked for normality and heteroscedasity prior to interpreting the results of the 2ANOVA's. 4 of the 36 2ANOVA's conducted on Perdido data over the two years (2000 and 2001) failed normality and transformations did not help since many of the data points were negative. However, ANOVA's are robust with regards to deviations from normality, therefore we did not feel it necessary to use non-parametric tests to analyze these data. All data from the Delta during 1999 and 2000 passed normality and heteroscedasity.

Power tests were conducted on the degree of openness, trophic category and the interaction between “openness” and trophic category for each of the parameters tested in Perdido each year: Chlorophyll a, SAV Biomass DW, SAV Biomass AFDW, Fauna # / g DW Vegetation, Amphipod #/ g DW Vegetation, Gastropod #/ g DW vegetation, Gastropod # w/o *Diastoma* / g DW vegetation, Grazing crustacean # / g DW vegetation, and Fauna # w/ o *Diastoma* / g DW vegetation. This resulted in a total of 36 power tests (18 per year) being conducted. Of these 36 power tests conducted on the Perdido data, none of them failed ($p < 0.05$). Similar power tests were conducted on the Delta data for each year for the following parameters: Chlorophyll a, SAV Biomass DW, SAV Biomass AFDW, Fauna # / g DW Vegetation, Amphipod #/ g DW Vegetation, Gastropod #/ g DW vegetation, and Grazing crustacean # / g DW vegetation. Of the 21 power tests conducted on the Delta data, none of them failed ($p < 0.05$).