Chapter 6

Fish abundance, growth, and $\delta^{15}N$ signatures as indicators of nitrogen loading in the Oyster Pond estuary

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Abstract

The purpose of this study were to examine the effects of land-derived nitrogen loads on fish within the Oyster Pond estuary, Falmouth, Massachusetts, we studied relative abundances, growth rates, and stable isotope signatures for several species of common estuarine fish. N-loads shifted fish species composition; *A. quadracus* were more abundant at low nitrogen levels, *A. pseudoharengus* were more abundant at high levels, and relative abundance of *Fundulus* species were unaffected. Nitrogen loads increased growth rates of year zero *Fundulus*, but not that of adults. $\delta^{15}N$ signatures identified the grazers, secondary consumers, and tertiary consumers among the fish of Oyster Pond. The $\delta^{15}N$ signatures of the consumers increased as nitrogen loads increased, suggesting that land-use differences in the watershed could be traced through the food web of Oyster Pond.

Introduction

Changes in fish populations, plant biomass, and increased nutrient loading over the past few decades (Emery 1997, Cermak et al. this volume) in Oyster Pond, an estuary in Falmouth, MA, have been cause for ecological concern. These changes could be examined to measure land-derived nitrogen inputs (Sand-Jensen and Borum 1991, Tober et al. 2000, Deegan 2002, Nixon 1988, Valiela 1995).

In Oyster Pond, nitrogen from human wastewater is the greatest contributor to nitrogen load, contributing 69% of the total (Good, this volume). Inputs of wastewater can be traced by measurements of stable isotopes of biota within an estuary (McClelland and Valiela 1997). The nitrogen stable isotope signature increases from one trophic level to the next. (Fig. 8). As nitrogen moves up the food web, a consumer shows a $\delta^{15}N$ signature that is 2‰ to 4‰ heavier than that of its diet (Minagawa and Wada 1984).

In this paper, we assessed the response of the fish assemblage in Oyster Pond to various nitrogen inputs by sampling population abundance, analyzing cohorts and measuring growth rates, as well as using stable isotope signatures to establish the linkage of fish populations to higher inputs from watersheds. In addition, we examined whether the response of fish growth was coupled with either the abundance of prey or abundance of plants in the macrophyte canopies fostered by the nutrient enrichment.

Methods

Study site

Fish were sampled at seven littoral zone locations in the Oyster Pond estuarine system, between September 4 and September 19, 2002 (Fig. 1).

Abundance and distribution of fishes

We determined fish abundance in five sites within the pond and the lagoon by towing a 5m seine for 10 meters. In three sites, we towed the seine by boat, covering an area equal to half of a full seine. We then compared the percent of total fish abundance with nitrogen loads. We also compared fish abundance with invertebrate abundance and plant biomass to see if a relationship existed.

Growth rates of fishes

To estimate growth rates we needed to define cohorts within the fish population. We used aggregate data from fish collected by the different methods of sampling (seines, traps, nets) to estimate frequency distribution sizes for each species by measuring the standard length of each fish caught, to the nearest 0.1 cm. We used MIX 3.1.3. (Ichthus Data Systems 1998) to identify cohorts and determine mean length for each cohort.

Monthly growth rates were then calculated by subtracting the mean length of one class size from the preceeding class size and dividing by the six month growing season (Tober et al. 2000). To calculate the monthly growth rate of year zero *F. heteroclitus* and *F. majalis*, we subtracted 0.6 cm, the average hatch length (Kneib 1993), and divided by two months, the average time the fish had to grow before we collected them. To calculate the monthly growth rate of *Alosa pseudoharengus*, we subtracted 0.4 cm, the average hatch length (Letcher et al. 1997), and divided the mean of the older cohort by three months, the time fish had to grow from birth before we collected them (Walton 1983). To calculate the time the second hatch occurred, we divided the smaller mean by the monthly growth rate and subtracted the number of months from the current date of the study.

Stable isotope analysis

To obtain the $\delta^{15}N$ value of fish present in each zone, fish were collected from each sampling site and washed with distilled water before heads and viscera were removed. All samples were then dried at 60°C for at least 24 hours. Dried samples of the same species and similar size from the same location were ground using a mortar and pestle to ensure that all parts of the body were represented in the final powder-like form. 4-5 mg of each sample was then packaged and sent to the Stable Isotope Facility at University of California, Davis for $\delta^{15}N$ analysis.

To understand the trophic positions of biota within the Oyster Pond food web, we compared the $\delta^{15}N$ values of sampled plants, invertebrates (data obtained by Cermak et al., this volume), and our $\delta^{15}N$ values for fish. To see if nitrogen loads resulting from differences in land use were reflected in the fish, we then compared $\delta^{15}N$ signals of fish from sites that receive different levels of nitrogen input (data from Good, this volume).

Results and Discussion

Fish distribution and composition

In Oyster Pond, we caught *Alosa pseudoharengus, Apeltes quadracus, Morone americana Menidia menidia,* as well as *Fundulus heteroclitus, F. majalis* and *Anguilla rostrata. Cyprinodon variegatus* were also found in the Lagoon and Trunk River. Four of these species were commonly found at all sampling sites (Table 1).

The abundance of the four common species responded differently to changes in land-derived nitrogen loads. *Fundulus heteroclitus* was abundant throughout the pond, but was not effected by nitrogen loading (Fig. 2). Abundance of *F. majalis* and *A. pseudoharengus* increased as nitrogen loads increased, but nitrogen loads decreased abundance of *A. quadracus* (Fig. 2).

The effects of nitrogen load increases on abundance of common species resulted in changed species composition (Fig. 3). *Fundulus* species are common regardless of loading rate. *A. quadracus* decreased dramatically before nitrogen loads reached 200 kg yr^{-1}. In contrast, *A. pseudoharengus* was most abundant in areas of high nitrogen loads (Fig. 3). These results indicate that nitrogen load altered taxonomic composition of fish populations within Oyster Pond.

The abundance of *Fundulus* spp. varied with prey density and plant biomass. *Fundulus* spp. abundance increased as prey density increased and decreased as plant biomass increased (Fig. 3). The abundance of *Fundulus* spp. also increased when invertebrate abundance increased per gram of plant biomass (Fig. 3). This finding supports previous research suggesting that predators have a more difficult time foraging for food with an increase in plant biomass (Deegan 2002).

Growth Rates of Fundulus heteroclitus, Fundulus majalis, and Alosa pseudoharengus

To estimate growth rates of the most common species, we first identified cohorts. The aggregate data for fish collected by seine, trap, and dip net were collated into one centimeter length bins (Fig. 5). From the frequency histogram, it appeared that there were considerate differences in the populations of *Fundulus heteroclitus* and *Fundulus majalis* collected in diferent sites within Oyster Pond. We applied Mix software to these frequency data and then identified cohorts as year 0, 1, 2, and 3 for each of the *Fundulus* species. Then, we used the mean length from these identified cohorts to calculate growth rates per year, and divided the results by twelve to determine the growth rate per month.

From our growth rate data, it appears that nitrogen loads have an effect on the number of cohorts found at each site. For *F. heteroclitus*, three cohorts were found in sites with areas of relatively low nitrogen, and two cohorts were found in areas with relatively high nitrogen (Table 2). There are differences in the number of cohorts of *F. majalis* at each site, although a direct correlation between number of cohorts and nitrogen load cannot be established. These results indicate there may be a negative impact of nitrogen on the longevity of *F. heteroclitus* within this system.

The frequency data of Fig. 4 and the growth rate data of Table 2 suggest that the growth rates of *Fundulus* species differ from site to site. To examine the relationship of growth and nitrogen load at each site, we plotted growth rates versus nitrogen loads (Fig. 6). Growth rates of the two *Fundulus* species older than one year were unaffected by nitrogen loads, but the young-of-the-year showed increased growth rates where there were higher inputs of nitrogen supply from land.

Growth rates of young-of-the-year *F. majalis* and *F. heteroclitus* were similar in range to measurements both at Oyster Pond in 2001 (Annett et al. 2001) and at other New England estuaries (Table 3). Although we used standard length and previous studies used total length, our growth rates are comparable.

Size frequency distributions and cohort analyses identified two cohorts within the *Alosa pseudoharengus* population of Oyster Pond (Figure 7). These are young-of-the-year cohorts preparing to return to sea (Bigelow and Schroeder 2002). The multiple cohort data suggest two different spawning events this season. From the calculated growth rates of *A. pseudoharengus* found both residing in and exiting the Oyster Pond system, we estimated that the two cohorts were born in early June and early July, respectively. Average growth rate for juvenile *A. pseudoharengus* within Oyster Pond was 1.8 ± 0.3cm per month, similar to the growth rate of 2.2 ± 0.7cm per month found in Damariscotta Lake, ME, a similar habitat for anadromous *A. pseudoharengus* (Walton, 1983).

Stable isotope analysis

In the Oyster Pond estuary, primary producers had the lowest $\delta^{15}N$ values, ranging from 3.72‰ to 6.70‰ (Cermak et al. this volume). Primary consumers included invertebrates and *Cypronidon variegatus*, with $\delta^{15}N$ signatures ranging from 5.20‰ to 6.73‰. All other fishes were higher order consumers. Those that are secondary consumers ranged in their $\delta^{15}N$ values from 8.86‰ for *Morone americana* to 9.51‰ for *Alosa pseudoharengus*. *Apeltes quadracus*, at the highest trophic level, had a $\delta^{15}N$ signature of 11.98‰. In general, a consumer's $\delta^{15}N$ signal is 2-4‰ heavier than that of its diet (Minagawa and Wada 1984). Our data show that in the food web of Oyster Pond, the nitrogen isotope signature increase from one trophic level to the next ranges from about 1.5‰ to about 4‰.

$\delta^{15}N$ signatures of fish species that were identified as secondary consumers in Fig. 8 could be compared across the different sites subject to different rates of nitrogen loading (Fig. 9). The results show that the fish, in spite of their mobility, did acquire a $\delta^{15}N$ signature of a relatively small area. This agrees with Butner and Brattstrom (1960), indicating that the home range of fish such as *Fundulus* spp. is fairly limited, but is a remarkable result in the case of the other, more active species such as *M. menidia* and *A. pseudoharengus*. In addition, there was a significant direct link between nitrogen loading from land and the $\delta^{15}N$ signatures of fish in the Oyster Pond estuary (Fig. 9). Secondary consumers in Oyster Pond had heavier $\delta^{15}N$ signatures associated with high nitrogen loading areas.

In conclusion, fish abundance, growth rates, and $\delta^{15}N$ signatures are indicators of wastewater-driven eutrophication. Nitrogen loads did not alter abundance of *Fundulus* species, but did increase abundance of *Alosa pseudoharengus* , and decreased abundance of *Apeltes quadracus*. Growth rates of juvenile fish increased with higher nitrogen loads, while growth rates for adults were unaffected. These data show that the human-derived wastewater nitrogen entering the estuarine waters, is being assimilated by primary producers, and affecting organisms throughout the food web as evidenced by the nitrogen isotope signatures of fish in Oyster Pond.

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Unpublished Materials

Cermak, M., E. Crandall, and J. Young. Unpublished data. Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

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Table 1. Abundance (± s.e.) mean of the four most common fish species found in Oyster Pond.

Species	Sampling area	Mean
Fundulus heteroclitus	Recharge1	1.41 ± 0.67
	Recharge 2	2.69 ± 1.20
	Recharge 3	1.29 ± 0.27
	Recharge 5	0.68 ± 0.20
	Recharge 6	
F. majalis	Recharge1	0.37 ± 0.07
	Recharge 2	1.82 ± 0.42
	Recharge 3	1.7 ± 0.46
	Recharge 5	1.05 ± 0.23
	Recharge 6	
Apeltes quadracus	Recharge1	0.38 ± 0.05
	Recharge 2	0.06 ± 0.06
	Recharge 3	0
	Recharge 5	1.28 ± 0.58
	Recharge 6	
Alosa pseudoharengus	Recharge1	0
	Recharge 2	0
	Recharge 3	0.89 ± 0.22
	Recharge 5	0.34 ± 0.05
	Recharge 6	

Table 2. Growth rates of year classes of *F. heteroclitus* and *F. majalis* collected from sites receiving different N loads from different recharge areas of Oyster Pond. Averages for all locations included in both species.

Species	Sampling site	Total N load (kg y^{-1})	Growth rate (cm/month)			
			Year 0	Year 1	Year 2	Year 3
Fundulus heteroclitus	3	240	1.95	0.34	-	-
	2	152	0.95	0.35	-	-
	1	136	0.95	0.42	-	-
	6	45	0.96	0.34	0.36	-
	7	7	0.71	0.42	0.34	-
						-
	Mean ± se		1.10 ± 0.22	0.37± 0.02	0.35 ± 0.01	-
						-
Fundulus majalis	3	240	1.80	0.38	-	-
	2	152	1.35	-	-	-
	1	136	1.20	0.50	-	-
	6	45	1.10	0.32	0.38	0.42
	7	7	1.20	0.39		
	Mean ± se		1.33 ± 0.12	0.40 ± 0.04		

Table 3. Growth rates of *F. heteroclitus* and *F. majalis* from various estuaries.

Species	Location	Growth rate (cm/month)				Reference
		Year 0	Year 1	Year 2	Year 3	
Fundulus heteroclitus	Oyster Pond 2002	1.10 ± 0.22	0.37± 0.02	0.35 ± 0.01	-	This study
	Oyster Pond 2001	1.50	0.28	0.25	0.23	Annett et al. (2001)
	Waquoit Bay	0.73	0.23	0.15	0.07	Tober et al. (2000)
	Great Sippewisset Marsh	0.84	0.24	0.16	0.18	Werme (1981), Valiela et al. (1977)
Fundulus majalis	Oyster Pond 2002	1.33 ± 0.12	0.40 ± 0.04	0.38	0.42	This study
	Oyster Pond 2001	1.50	0.25	0.22	-	Annett et al. (2001)
	Great Sippewisset Marsh	1.00	-	-	-	Werme (1981)

Figures

Fig. 1. Map of the Oyster Pond estuary. The large polygon around the pond represents the entire watershed. Numbered areas represent recharge zones within the watershed (Good, this volume). Fish were sampled along the coastline of each zone at sites that were also sampled for plant biomass by Cermak et al. (this volume).

Fig. 2. Abundance (mean ± s.e.) of four common fish seined from Oyster Pond, plotted versus nitrogen load estimated by S. Good (this volume) for each recharge zone. See Figure 1 for recharge zone locations. Linear regression and significance values are shown for each graph.

Fig. 3. Percent composition of *Fundulus heteroclitus* (Fh), *F. majalis* (Fm), *Alosa pseudoharengus* (Ap) and *Apeltes quadracus* (Aq) versus nitrogen load (Good, this volume) across the area of the pond. Abundance from Figure 2.

Fig.4. Percent (mean ± s.e.) of total fish abundance versus invertebrate abundance, plant biomass, and number of invertebrates per gram of plant biomass (Cermak et al. this volume). Diamonds represent small (1-2 cm) invertebrates and squares represent larger ones (3+ cm). Linear regression and significance values are shown for each graph.

Fig. 5. Size frequency of *Fundulus heteroclitus* and *Fundulus majalis* in different recharge zones within the Oyster Pond system. Number of fish included in each histogram indicated right side of each panel.

Fig. 6. Total nitrogen load vs. growth rate for *Fundulus heteroclitus* and *Fundulus majalis* year zero, one, and two individuals. Linear regression and significance values shown for *Fundulus heteroclitus* year zero (y=0.0003x + 0.3922, F= 21.10, P= 0.02) and year one (y= -0.0002x + 0.3943, F= 0.83, P=0.43) individuals; and for *Fundulus majalis* year zero (y=0.0017x + 0.689, F= 20.34, P= 0.02) and year one (y=0.0002x + 0.3759, F= 0.05, P= 0.84) individuals.

Fig. 7. Size frequency and cohort analysis results for *Alosa pseudoharengus* collected from the entire Oyster Pond system.

Fig. 8. $\delta^{15}N$ signatures of producers (Cermak et al. this volume) and consumers sampled in Oyster Pond. For species for which we had multiple values, mean signatures are represented and standard deviation bars are included. The dashed lines represent the mean $\delta^{15}N$ for each trophic level. Common names of consumers from top to bottom are: four-spined stickleback, alewife, silverside, striped killifish, mummichog, white perch, isopod, grass shrimp, sheepshead minnow, amphipod. *Sargassum was also collected in Oyster Pond, ($\delta^{15}N = 7.94$) but omitted here because it more likely grew in deeper offshore water and cannot be considered part of the food web.

Fig. 9. $\delta^{15}N$ signatures of fish versus total nitrogen load (Good this volume) in Oyster Pond and the lagoon. For species with multiple samples from a site, mean signatures are represented and standard error bars are included. Solid symbols represent secondary consumers; open symbols represent primary and tertiary consumers. A logarithmic regression line was fitted to species identified as secondary consumers in Figure 8. *Cyprinodon variegatus* and *Apeltes quadracus* were on different trophic levels (Fig. 8) and are not included in the regression.