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The Impacts of Nonnative Japanese Eelgrass (*Zostera japonica*) on Commercial Shellfish Production in Willapa Bay, WA

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Abstract

Eelgrass species worldwide are valued for the ecosystem service they provide to estuarine and marine habitats. One species, *Zostera japonica*, however, has some negative impacts outside its native range and is considered invasive. In Willapa Bay WA, USA, the nonnative eelgrass has expanded to the level where the shellfish industry is concerned about its potential impacts on its livelihood. Studies were conducted using paired plots, *Z. japonica* controlled with the herbicide imazamox vs. untreated controls, to assess the effects of *Z. japonica* on Manila clams (*Ruditapes philippinarum*) and Pacific oysters (*Crassostrea gigas*). Recruitment of new Manila clams was not affected by *Z. japonica*. The growth of young clams, total commercial clam harvests, clam quality and clam harvest efficiency, however, were greater on plots where *Z. japonica* was chemically controlled than where it was not treated. The response of oysters to *Z. japonica* control varied by site; there was no effect at one site, while the other sites had a 15% increase in shucked meat with *Z. japonica* control. The potential economic impact of a *Z. japonica* infestation of a shellfish bed was ~\$47,000 ha⁻¹ for Manila clams and \$4000 ha⁻¹ for oysters for each crop harvest cycle.

Keywords

Shellfish Aquaculture, Invasive Eelgrass, Manila Clams, Pacific Oyster

1. Introduction

Worldwide, there are over 60 species of seagrass which provide many ecosystem functions, including supporting diverse benthic assemblages, providing carbon to the estuarine food web, structural support for other primary producers and habitat for juvenile salmonids and other fish species [1]-[3]. A few seagrass species, however,

are considered to be invasive outside their native range [3]. Zostera japonica, in particular, has been reported to have several negative ecological consequences [4]-[6]. In Willapa Bay, WA, USA, coverage of the upper intertidal zone by Z. japonica has increased to the level where it is affecting the livelihood of the shellfish industry [5] [7]. Studies to document the impact of Z. japonica on shellfish, however, have been limited and have not addressed the economic impact to the industry [2]. Regulatory agencies' biologists and environmental groups are reluctant to sanction an effort to control a nonnative plant that performs many of the same ecological functions as the native eelgrass, Z. marina, in order to culture the nonnative shellfish species, Pacific oysters (Crassostrea gigas) and Manila clams (Ruditapes philippinarum) [1] [2] [8]. These groups place a high value on the ecosystem services and are in conflict with stakeholders considering economic value [2] [7]. Without reliable data to validate the shellfish industry's concern about the detrimental effects of Z. japonica, their need for on-farm management options to sustain their livelihoods has met with skepticism [2]. The purpose of this study is to document the impacts of Z. japonica on several different production parameters of shellfish farming in Willapa Bay, WA.

2. Material and Methods

2.1. Site Location and Shellfish Industry Background

Research was conducted under tidal estuary conditions in Willapa Bay, Washington, between 2010 and 2013. The study sites were located along a 10 km by 0.15 km band, within the 0.5 m to 1.5 m tidal height range, between 46.5114°N, 124.0030°W and 46.6089°N, 124.0357°W. Willapa Bay is a large, shallow bar-built estuary with 347 km² in surface area at mean higher high water (MHHW) and 191 km² at mean lower low water (MLLW). The tidal range between MHHW and MLLW is 2.4 to 3.4 m. More than half of the estuary's surface area and volume is drained at low tide [9]. Willapa Bay produces 17% of US commercial oysters (calculated from reported data for 2012 from http://www.nmfs.noaa.gov/ and WA Dept. Fish and Wildlife Data Service). Approximately 20% of the intertidal area is utilized for commercial aquaculture of Pacific oysters and Manila clams [10]. In Willapa Bay, these intertidal benthic grazers rely on oceanic phytoplankton as their main food source, with the majority of their growth occurring between May to September [11]. Clam and oyster farmers utilize natural recruitment and hatchery-set seed; it takes three to five years to reach commercial harvest size. Clams are then raked and removed by hand. Oysters are both dredged and picked by hand.

2.2. Experimental Details

Experiments were conducted using adjacent paired plots on tide flats completely infested with moderate to thick stands of *Z. japonica*. Sites for Manila clam experiments were privately owned commercial clam beds with a 4 - 6 cm layer of screened gravel, <2 cm, on the surface that was placed on site by growers to facilitate clam recruitment and protect against predation by crab. Sites for Pacific oysters had layers of existing oyster shell + sediment. The size profile of the original surface sediment, 0 to 7.5 cm depth, was: 1% < 0.1 mm; 42% > 0.1 mm < 0.23 mm; 54% > 0.23 mm < 0.5 mm; 1% > 0.5 mm. Treatment comparisons were an uncontrolled *Z. japonica* plot vs. a plot where the *Z. japonica* was controlled with the herbicide imazamox. Imazamox was applied at 0.14 kg ai·ha⁻¹ in late spring after the majority of new seedlings had germinated. Applications were made with a backpack sprayer at 20 l·ha⁻¹, using estuarine water as a carrier without the addition of a surfactant. Treatments were applied during low tide on a dry exposed canopy. Plot size and replicate number varied by experiment and are detailed within each experiment.

2.2.1. Effects of Nonnative Eelgrass on Settlement of Manila Clam Larvae

Paired plots were established at four locations 0.5 to 2 km north of Nahcotta WA. Site 1 had 3 by 4 m plots with 7 replicates, and was treated 5/24/12. Site 2 had 5 by 15 m plots with 3 replicates, and was treated 6/24/13. Site 3 had 3 by 4 m plots with 5 replicates, and was treated 6/24/13. Site 4 was 4 by 4 m plots with 7 replicates, and was treated on 6/24/13. To assess for clam settlement, sediment cores 5 cm deep × 11 cm diameter were taken. Sediment was collected in May 2013 for Site 1, and November 2013 for Sites 2 to 4. Sediment samples were immediately sieved after collection and everything between 2 mm and 0.5 mm was frozen. Rose Bengal dye was used to stain clams to enhance visibility [12]. Samples were evaluated under a dissecting scope for number of Manila clams <8 mm in length.

2.2.2. Effects of Nonnative Eelgrass on Manila Clam Growth

Growth of new clam seed. A site 6 km north of Nahcotta WA was established with 4 replications of 12 by 12 m plots that were treated on 5/4/09. Within each plot, 30 small Manila clam seeds 4 to 12 mm in size, mean 7.5 mm, were placed in 284 cm² plastic mesh (12 mm) round screen cages that were buried 5 cm in the ground with 2 cm extending above the sediment and left open on the top. There were three cages per plot. Macroalgae that were collected over the tops of the cages were removed several times per month. On 10/19/10 clams were removed from the cages; shell length and width and fresh and dry weight were recorded for each clam. Mean shell area ($A = \pi ab$) and weight were pooled for each cage and used for subsamples for statistical analysis.

Growth by age class. A site was established 2 km north of Nahcotta WA with 10 replications of 4 by 5 m plots that were treated on 6/24/13. To assess for clam growth by age class, sediment cores 8 cm deep by 20 cm diameter were taken on 11/11/13. All Manila clams within the core were removed and segregated by age class based on growth rings [13]. If any plot from any replication did not have >5 clams per core for any age class, that replication was not used for statistical analysis. Because there were not enough 1 year-old clams to assess from the November sample, the site was resampled on 3/30/14 for 1-year-old age class clams. Shell length and width for each clam were recorded. Mean shell area for each age class for every replicate was used for statistical analysis

2.2.3. Effects of Nonnative Eelgrass on Manila Clam Site Productivity

Paired plots were established at six locations distributed along a 10 km band north of Nahcotta WA. Plot size was 3 by 4 m. The number of replications for Sites 1, 2, 3, 4, 5 and 6 was 20, 8, 10, 11, 12 and 20, respectively. Treatments were applied 5/27/10 for Sites 1 to 5, and mid-May 2013 for Site 6. All sites were harvested in mid-November in the year of treatment. Sites were harvested by taking 0.09 m² cores 20 cm deep and removing all Manila clams >30 mm length. The total weight of Manila clams >30 mm was recorded for each plot.

2.2.4. Effects of Nonnative Eelgrass on Shellfish Quality

Oyster. Two commercial oyster beds with moderate infestations of *Z. japonica* 8 km north of Nahcotta WA were treated on 6/28/13. Site 1 had 2 year-old seed that was placed on the site on March 2012. There were three replications of 15 by 15 m plots. Site 2 had 3 year-old oysters that were placed on the ground on June 2012. There were six replications of 15 by 15 m plots. Plots were harvested 11/14/13. Thirty single oysters per plots were harvested from Site 1 and 60 per plot from Site 2. Total oyster weight, shell + meat, and total meat weight were recorded for each batch. For Site 2, oyster condition index (CI) was determined for 25 oysters per rep on three replications using standard protocol [14]. Oysters were harvested on 11/15/13. Total weight, meat dry weight and shell dry weight were recorded for each oyster. The mean CI for each replication was calculated (CI = meat dry wt./(total oyster wt-shell dry wt) $^{-1} \times 100$).

Clams. A commercial clam bed 3 km north of Nahcotta WA was treated on 5/27 /2010. There were 12 replicated plots of 3 by 4 m. The plots were harvested 11/15/2010 taking 0.09 m² cores 20 cm deep and removing all Manila clams >30 mm length. For each clam shell length and diameter, clam volume by displacement, clam total fresh weight, meat fresh and dry weight, and shell dry weight were recorded. The mean clam CI and meat dry weight per clam volume for each replication was calculated.

2.3. Effects of Nonnative Eelgrass on Manila Clam Harvest Efficiency

A commercial clam bed was treated on 6/24/2013. There were 5 by 7 m replicated plots. A 1 m² quadrat from the middle of each plot was harvested on 11/1/13 for marketable clams by a commercial digger. The harvester was told to harvest at his normal pace, was paid per unit of clams obtained and was not made of aware of the experimental objective. The total weight and the time to harvest each plot were recorded. After commercial digging, the plots were carefully re-dug and the number and weight of unharvested commercial clams were recorded. The percentage of unharvested clams by weight and number and the harvest speed in seconds/clam were calculated.

2.4. Statistical Analysis

Results were analyzed from each experiment using plot mean in a paired T test, with a two-tailed P value. If data failed normality testing based on Shapiro-Wilk, then Kruskal-Wallis One Way Analysis of Variance on ranks was conducted.

3. Results

3.1. Effects of Nonnative Eelgrass on Recruitment of Manila Clam Larvae

Across all sites, and two years of assessment there was no effect of Japanese eelgrass removal on the density of Manila clams that settled into the treated area within that season of treatment (**Table 1**).

3.2. Effects of Nonnative Eelgrass on Manila Clam Growth

After 138 days of growth on site, juvenile clams were confined within screened cages in the treated areas. They had 12%, 16% and 9% greater fresh and dry weight and size, respectively, on plots where *Z. japonica* was removed than where it wasn't (Table 2). Treatment comparison in clam size by age class was made after one season of growth. One, 2, 3, 4 and 5 year-old clams on plots where *Z. japonica* was removed were 24%, 33%, 26%, 7% and 8% greater, respectively, than where it wasn't (Table 3). The growth increase for the 4 and 5 year-old clams, however, was not significantly different.

3.3. Effects of Nonnative Eelgrass on Manila Clam Site Productivity

The yield of commercial clams after one growing season was greater in 5 out of 6 commercial clam farms where Japanese eelgrass was removed than where it was left untreated (**Table 4**). The mean increase in yield across all sites for just one season of *Z. japonica* control was 45%.

Table 1. Effect of Z. japonica control with the herbicide imazamox on the settlement of Manila clams in Willapa Bay, WA.

Treatment -	# of newly recruited manila clams ^a 1000 cm ⁻³ of surface sediment				
	Site 1	Site 2	Site 3	Site 4	
Z. japonica	23	61	27	375	
No Z. japonica	19	48	24	442	
T test significance	0.18	0.17	0.48	0.62	

^aclams < 8 mm in size.

Table 2. Effect of one summer of *Z. japonica* control with the herbicide imazamox on the fresh and dry weight and size of seeded juvenile Manila clams in Willapa Bay, WA.

Treatment	Fresh wt (g·clam ⁻¹)	Dry wt (g·clam ⁻¹)	Clam size (cm ²) ^a
Z. japonica	0.88	0.027	0.61
No Z. japonica	1.00	0.032	0.67
T-test significance	0.009	0.002	0.001

 $^{^{}a}(A=\pi ab).$

Table 3. Effect of one summer of *Z. japonica* control with the herbicide imazamox on the size of different ages of Manila clams in Willapa Bay, WA.

Treatment	Clam shell area (cm ²) ^a				
	1 yr-old	2 yr-old	3 yr-old	4 yr-old	5 yr-old
Z. japonica	0.38	1.13	1.95	3.33	3.81
No Z. japonica	0.47	1.69	2.62	3.57	4.15
T test significance	0.001	0.04	0.006	0.1	0.1

 $^{^{}a}(A = \pi ab).$

3.4. Effects of Nonnative Eelgrass on Shellfish Quality

The response of oysters to *Z. japonica* control is varied by site. At Site 1, with younger smaller oysters and less coverage of Japanese eelgrass, there was no treatment effect (**Table 5**). At Site 2, total oyster weight, meat weight per oyster and their condition index was increased by 12%, 15% and 15% respectively by controlling *Z. japonica*. The quality of commercial size clams at harvest was also improved by removing *Z. japonica* from the plot. Clams growing without Japanese eelgrass had 19%, 14% and 15% greater meat per clam, meat per clam volume, and clam condition, respectively, than those grown in untreated plots (**Table 6**).

3.5. Effects of Nonnative Eelgrass on Manila Clam Harvest Efficiency

For commercially hand harvested clams, removal of *Z. japonica* reduced the percent weight and percent number of clams missed by the digger by 5% and 8% respectively (**Table 7**). There was a non-significant trend for 30% increase in harvest efficiency, 1 second less per clam, when there was no *Z. japonica* hindering the picker (one-tailed P-value = 0.06).

Table 4. Effect of one summer of *Z. japonica* control with the herbicide imazamox on the yield of commercial size Manila clams (>30 mm length) in Willapa Bay, WA.

Treatment			Yield of comme	rcial clams (kg·n	n ⁻²)	
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Z. japonica	2.2	0.7	1.0	1.2	0.5	5.1
No Z. japonica	3.9	1.3	1.5	2.6	1.2	7.6
T-test significance	0.02	0.04	0.3	0.03	0.007	0.05

Table 5. Effect of one summer of *Z. japonica* control with the herbicide imazamox on the quality of Pacific oysters in Willapa Bay, WA.

	Site 1		Site 2		
Treatment	Shell + meat (g fw·oyster ⁻¹)	Meat (g fw·oyster ⁻¹)	Shell + meat (g fw·oyster ⁻¹)	Meat (g fw·oyster ⁻¹)	Oyster condition index ^a
Z. japonica	189	24	211	33	5.7
No Z. japonica	192	27	241	39	6.7
T test significance	0.9	0.2	0.05	0.04	0.02

^ameat fresh weight/(total shell + meat fresh weight shell dry wt.) $^{-1} \times 100$.

Table 6. Effect of one summer of *Z. japonica* control with the herbicide imazamox on the quality of Manila clams in Willapa Bay, WA.

Treatment	Meat yield (g dw·clam ⁻¹)	Meat/clam volume (g dw·clam·cm ⁻³)	Clam condition index ^a
Z. japonica	0.46	0.031	4.4
No Z. japonica	0.56	0.036	5.2
T test significance	0.0001	0.001	0.007

^ameat fresh weight/(total shell+ meat fresh weight shell dry wt.)⁻¹ × 100.

Table 7. Effect of one summer of *Z. japonica* control with the herbicide imazamox on the commercial harvest efficiency of Manila clams.

Treatment	% of total # of commercial clams ^a harvested	% of total wt of commercial clams harvested	Harvest efficiency (seconds clam ⁻¹)
Z. japonica	88	87	3.4
No Z. japonica	93	95	2.6
T-test significance	0.04	0.05	0.1

aclam length >30 cm length.

4. Discussion

Submerged aquatic vegetation dampens water currents, and is reported to enhance clam recruitment [15] [16]. Under the conditions of these studies, however, there was no observed effect of *Z. japonica* on Manila clam settlement. Two previous studies assessing the influence of *Z. japonica* on Manila clam settlement provide mixed results. Ruesink *et al.* [17] found no effect of *Z. japonica* on Manila clam recruitment, while Tsai *et al.* [6] showed the lowest recruitment numbers of Manila clams where *Z. japonica* was removed by hand; intermediate numbers were in *Z. japonica* plots, and highest numbers were where *Z. japonica* was removed by harrowing. They suggest that their finding could be an artifact of the process of physically removing *Z. japonica*.

In contrast to recruitment, *Z. japonica* had a very marked impact on Manila clam growth, production and quality. The most significant effect associated with *Z. japonica* control was total site commercial productivity. An average increased yield of commercial clams across six sites was 45%. Based on the differential results in growth rates between age classes of Manila clams, this degree of influence on commercial yield from just one season of *Z. japonica* removal was much greater than expected. For young, fast-growing clams, the beneficial effect of one season of *Z. japonica* removal ranged from 9% to 33% depending on the growth parameters assessed. In contrast, no significant differences were noted for fully mature clams (>4 years old). For the short time these paired plot studies were conducted, the 45% increase in commercial yield appears disproportionate and is likely an artifact of the study design. Manila clams are mobile and to a limited degree seek more favorable locations [18]. Because the yield response that occurred was largely the result of an increase in the number of commercial size clams, not the increase in size of mature clams, it is suspected that Manila clams moved out of the thick *Z. japonica* sites, into the more favored adjacent plots without *Z. japonica*. This movement of clams likely exaggerated the yield response demonstrated in short-term paired plot studies. A long-term study following the impact of colonization of a similar narrow-bladed nonnative eelgrass, *Z. notlii*, in France found that Manila clams all but disappeared from fully colonized sites after five years [19].

Overall, across an array of sites, years and experiments, the annual response for young clams and clam quality to seasonal removal of Z. japonica from commercial clam farms in Willapa Bay ranged from 15% to 25%. A similar effect was found at one site for Pacific oysters. These results concur with the one study previously done on the interaction between Z. japonica and Manila clams [6], but contrast with findings from other studies examining the relationship between eelgrass and other clam species. Mercenaria mercenaria, for example, had higher growth rates inside the seagrass environment than open sand [20]. This difference in clam growth response to eelgrass is less likely the result of species differences in either clams or eelgrass, but one of vegetation patch size. Eelgrass vegetation has a hydrodynamic baffling effect [6] [21] [22]. This can have either a positive or negative effect on the availability of particulate food for suspension feeding depending on patch size and site location [14]. In sites with large expansions of bare sediment and small patches of eelgrass, the slowing of water flow over the eelgrass would result in localized settling of food for suspension feeding and, consequently, greater growth in eelgrass compared to bare sediment [14] [22]. On the other hand, our studies were conducted under exact opposite conditions, small patches of bare sediment where the Z. japonica was removed in the midst of 100+ ha patches of solid Z. japonica. Under these conditions, the dense mats of Z. japonica reduced the flow of rich nutrient water flow over the surface sediment by up to 40% compared to nonvegetated mudflats [18]. Similar flow data was reported by Patten et al. [23] using a Sontek Argonaut Acoustic Doppler Velocimeter stationed 5 cm above the sediment floor. They reported a mean current over a 3-day period of 0.7 cm $\sec^{-1} \pm 7.7$ std. dev. and 3.8 cm $\sec^{-1} \pm 3.9$ std. dev. in 2009 and 2010, respectively, on clam beds covered with thick Z. japonica, compared to 3.3 cm $\sec^{-1} \pm 11.3$ std. dev. and 6.8 cm $\sec^{-1} \pm 4.4$ std. dev. for immediately adjacent 0.1 ha beds where the Z. japonica was controlled in those years. Boundary flow is an important determinant of growth rate of benthic suspension feeders, with faster bottom flow velocities leading to greater growth [24]. Ruesch and Williams [25] report that food resources and mussel growth over a 7 month period were lower 1 and 5 cm above the sea floor within eelgrass patches than open sand flat, and that the effect was linearly associated with patch size.

The long-term economic impact of *Z. japonica* on Manila clam farming is difficult to project based on these studies alone. Nevertheless, it can be estimated based on data from clam growth and harvest efficiency. The annual reduction in clam growth resulting from *Z. japonica* varies by clam age. For age class 1 to 3 years the mean reduction in growth was 27% per growing season. A conservative estimate of 15% decrease per year for these three age classes would result in a cumulative net difference in yield of 45%. On good production ground, after 3 to 5 years of growth the commercial yield of Manila clams in Willapa Bay ranges from 30,000 to 50,000 kg·ha⁻¹.

For the average yield, a 45% crop reduction would be 18,000 kg·ha⁻¹. At a wholesale price of \$5.50 kg⁻¹, less \$1.45 kg⁻¹ picking cost and \$1.45 kg⁻¹ for production, cleaning and marketing, there would be a net loss of \$46,000 ha⁻¹ per harvest cycle based just on growth reduction. Additional losses due to differences in harvest efficiency also need to be considered. The presence of *Z. japonica* resulted in an 8% increase in the amount of clams missed by a commercial harvester. Although these clams are not permanently lost, their entire \$4576 in market value (\$2.6 kg⁻¹ × 1760 kg·ha⁻¹ in unpicked clams) would not be captured until the next harvest cycle. For a three-year harvest cycle, there would be \$721 in lost interest if that capital was invested with a 5% annual rate of return, and \$686 in clams lost to natural attrition of ~5% yr⁻¹. This would result in a cumulative total net loss of ~\$47,407 ha⁻¹ for each harvest cycle of Manila clams on a *Z. japonica*-infested bed compared to a matched bed where the eelgrass was controlled.

The economic impact of *Z. japonica* on Pacific oyster production is more difficult to assess. The tidal zone for oyster production is normally deeper than the *Z. japonica* range, and thus potential for a negative impact is much less likely than for Manila clams. In the situations where this overlap occurs there could be up to a 15% loss in oyster meat harvested on that site. A typical oyster bed is seeded at ~ 2500 bushels ha⁻¹. When ready for harvest each bushel of oyster yields ~ 1.9 l of shucked meat, which wholesales for $\sim \$6.35$ l⁻¹. For 15% loss in meat this would be \$4222 ha⁻¹ in net returns to the grower for a 2 - 3 year harvest cycle. This value is conservative, as it does not include losses of nonsalable oysters that do not make grade because of poor condition. The commercial shucker of these oysters noted that oysters from the plots with nonnative eelgrass were not fat enough to make grade (Jolly Roger Oysters, personnel communication).

These studies indicate that, without some type of management, invasive eelgrass is economically disadvantageous to shellfish growers. Management of *Z. japonica* with the herbicide imazamox, however, is not without risks. Direct effects on shellfish, the inability to market shellfish from treated beds, or damage to non-target species, including native eelgrass and oceanic phytoplankton, which are the food sources for shellfish, are concerns. Imazamox is food tolerance-exempt and has no direct effect on shellfish growth, as has been noted [26] [27]. Conditions which result in damage to off-site native *Z. marina* have been studied [28], and treatment protocols have been put in place to minimize this risk [7]. Imazamox is in the water column that could potentially affect phytoplankton. The dose exposure duration that would result from a large-scale treatment of imazamox would be well below that required to have any lasting effect [7] [28].

5. Conclusion

The negative economic impact of *Z. japonica* to the shellfish farmer can be dramatic, particularly for Manila clams. Management of *Z. japonica* on shellfish beds through the use of the herbicide imazamox can help mitigate for the expected crop losses. The use of an herbicide in an estuary, however, is controversial. These results are not likely to moderate the conflict between stakeholders with divergent ecological or economic priorities, but they do provide the solid evidence that concerns by the shellfish industry are justified. Additional studies that assess large-scale economic and long-term environmental impacts of *Z. japonica* and its potential management are warranted.

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