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I recently learned that the Washington Dept. of Ecology is soliciting comments on an update to permit for removing noxious weeds with a focus on controlling *Zostera japonica* on clam beds in Willapa Bay. Thank you for the opportunity to provide comments on this topic again. I continue to oppose the use of herbicides to control *Zostera japonica* in Willapa Bay, especially on clam beds. Back in 2012 I provided extensive comments against listing *Zostera japonica* as a noxious weed and against allowing herbicide control of *Zostera japonica* on clam beds (comments attached). After reviewing that document, most of those comments still have not been addressed by peer reviewed research and are as relevant today as they were 12 years ago.

In addition to those previous comments, I would like to point out new research that indicates the presence of seagrass in general provides a valuable and under appreciated service of reducing pathogenic bacteria in the water column and in shellfish (Lamb et al. 2017, Ascioti et al. 2022, Dawkins et al. 2024). Lamb et al. (2017) found that *Enterococcus* bacterial levels were 3-fold lower when seagrass were present. Ascioti et al. (2022) estimated that the seagrass sanitation effect resulted in about 8 million fewer gastrointestinal cases worldwide. The work of Lamb et al (2017) and Ascioti et al. (2022) was based on mixed species seagrass beds suggesting that the deactivation or removal of pathogens was not dependent on the type of seagrass present. In a German study, eelgrass (*Zostera marina*) the locally dominant seagrass in Washington and the Pacific Northwest was found to suppress pathogens in seawater (Tasdemir et al. 2024). The wieght of evidence is that the presence of seagrass, regardless of species is associated with lower pathogen loads in the environment and fewer cases of gastroenteritis. Work in Puget Sound (Dawkins et al. 2024) found that not only does the presence of seagrass reduce pathogens in the water column, but they also showed a 65% reduction in human bacterial pathogens in marine bivalves in locations with seagrass. Taken together, these publications suggest that seagrass presence, regardless of species, provides a beneficial service by removing pathogenic bacteria from the environment and from shellfish growing in seagrass beds. Unfortunately, none of these studies explain the mechanism of pathogen removal, and additional mechanistic work is required. Actively removing seagrass from clam beds may reduce or limit the efficacy of this important, under-recognized service and could have implications for human health.

I would be interested to learn about how the requirement that “prohibited in drainages that are flowing to areas containing the native eelgrass *Zostera marina*.” is evaluated and enforced? Are there records audits and fines associated with inappropriate applications? This seems to rely on the goodwill and self-regulation. Further, I wonder if the application on ~100 acres of clam beds actually justify the “need” for this chemical eradication tool? If this tool is only being used on a small fraction of clam beds in Willapa Bay, is it really needed and do the environmental impacts really justify its continued usage.

Ascioti et al. 2022. The sanitation service of seagrasses – dependencies and implications for the estimation of avoided costs. Ecosystem Services 54: 101418. Doi: 10.1016/j.ecoser.2022.101418

Dawkins et al. 2024. Seagrass ecosystems as green urban infrastructure to mediate human pathogens in seafood. Nature Sustainability. Doi: 10.1038/s41893-024-01408-5

Lamb et al. 2017. Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes and invertebrates. Science 355: 731-733.

Tasdemir et al. 2024. Epiphytic and endophytic microbiome of the seagrass Zostera marina: Do the contribute to pathogen reduction in seawater? Science of Total Environment 908: 168422. Doi: 10.1016/j.scitotenv.2023.168422

I recently learned that the Washington State Noxious Weed Board would be considering the listing of dwarf Japanese eelgrass (Zostera japonica Ascher. et Graebn.) as a noxious weed in all Washington State Waters. I would like to make 4 points against the listing of Z. japonica as a "Noxious weed species" in Washington State.

First, it is my opinion that much of the "information" on the environmental and economic impacts of Z japonica colonization is based on unpublished reports. For example, the Weed Board "written findings" with regard to Zostera japonica draw extensively upon unpublished reports, particularly Mach et al. 2010 and Fisher et al. 2011 and anecdotal information. Unpublished reports have not been peer reviewed, do not provide adequate descriptions of methods and assumptions and should not be taken as "definitive" sources. For example, the Fisher et al. (2011) "white paper" presents experiments and socioeconomic analysis but provide insufficient details (e.g. plot sizes, replication, sampling methods, statistical methods, assumptions) to evaluate the data quality and has not been peer reviewed or published. Consequently, it is difficult to evaluate the validity of conclusions reached by these authors. Likewise, the Mach et al. 2010 document was developed as a brief synopsis of ongoing scientific studies (again with insufficient details to critically evaluate the data) to identify data gaps and most of this work has not been published in the peer reviewed literature. As a result, these documents cannot be considered on par with a published scientific study that has undergone peer review.

A thorough evaluation of the ecological and economic impacts associated with Z japonica colonization where methods and assumptions are clearly stated would go a long way toward clarifying the impacts of these interactions. I am not aware of a thorough economic or ecological evaluation of the positive and negatives associated with Z. japonica colonization. There are 2 published studies on interactions between Manila clams and Zostera japonica. Tsai et al. (2010) concluded that Manila clam condition (measured as meat dry weight) was reduced in the presence of *Z. japonica*. Although this decreased condition was statistically significant, the decrease in clam meat weight was about 0.4 mg (Tsai et al. 2010). Assuming a 40 mm adult Manila clam weighs about 600 mg (Tsai et al. 2010) this is less than a 0.1% decrease in meat weight. Just because a difference is "statistically significant" does not mean that it is biologically or economically meaningful. Clam shell growth was not affected by *Z. japonica* presence and plots with *Z. japonica* had increased clam recruitment relative to removal plots (Tsai et al. 2010). This paper suggests that Z. japonica really doesn't have much of a negative effect on clam production. The second study, from Korea, concluded that intensive mechanical Manila clam harvest stimulated *Z. japonica* sexual reproduction and that the seagrass beds recovered within about 1 year of disturbance (Park et al. 2011). I think that these 2 published studies taken together suggest that Manila clam production and Z. japonica can co-exist the way they do in Asia, without the need for mechanical or chemical control of Z. japonica.

Second, the impacts of Z. japonica on estuarine health and ecosystem services have not been identified or quantified and even more importantly the impacts of mechanical and chemical control measures have not been identified or quantified. There is a fair amount of information available on the biology and ecology of Z. japonica. With the exceptions of Willapa and Padilla Bays, Z. japonica and Z. marina distributions are generally separate, with little chance for competitive interaction between the two species. The presence of Z japonica likely increases the primary production, benthic microalgae colonize seagrasses and in many cases these epiphytes are actually the dominant primary producers (Moncreiff and Sullivan 2001). The leaf surface area of a Z japonica bed provides much more epiphyte substrate than a comparable Z marina bed. One of the critical pieces of information missing in the assessment of Z japonica is a critical evaluation of the species that utilize this habitat relative to Z marina. In a recent peer reviewed report, Lamberson et al. (2011) recently observed bird foraging in seagrass habitat and concluded there was no evidence to suggest that birds are negatively impacted by the presence of Z. japonica. Other recent work concluded that benthic macrofaunal species richness, abundance and biomass in *Z. japonica* habitat was greater than or equal to that in oyster, mud shrimp or *Z. marina* habitat (Ferraro and Cole 2012). Benthic invertebrate community composition, abundance, species richness, and diversity associated with patches of *Z. japonica* and *Z. marina* in Washington were similar (Hahn 2003). Although anecdotal reports suggest Z japonica utilization, I am not aware of any published studies that have critically evaluated fisheries species (e.g. salmonids, herring, Dungeness crab, perch, etc.) utilization of Z japonica in comparison to Z. marina. However, work in Europe with the ecologically similar Z. noltti has found that a variety of species utilize this habitat when flooded including spawning herring (Polte and Asmus 2006a, b). Semmens (2008) concluded that salmonids had a preference for Z. marina over other intertidal habitats but was based on a limited sample size of 17 fish. This brief review of peer reviewed publications suggests that Z. japonica may be an important contributor to estuarine ecosystem services.

Although there has been a fair amount of work on how to kill Z japonica (e.g. mechanical removal, herbicide applications, thermal disruption, etc.), there has been little or no scientific evaluation of the collateral impacts associated with control methods. Mechanical removal by digging clearly has a negative impact on the macrofauna, but this has not been quantified. Additionally, there is little evidence to suggest the long term success of mechanical removal. Despite intensive eradication efforts which have been successful at some sites, Z. japonica continues to increase patch numbers and colonize Humboldt Bay (Ramey et al. 2011). Consequently, when eradication efforts cease, the plant will rebound. Herbicides are usually not target specific and will likely negatively impact other macrophytes (Z marina and algae) as well as benthic and planktonic microalgae. The impacts of herbicide applications in estuarine waters are very poorly studied (see below). One effective method of controlling Z japonica in Humboldt Bay was to pump hot water into the sediments to kill the plants (Ramey et al. 2011). This likely has a negative impact on all of flora and fauna in the sediments but again the impact has not yet been quantified (Ramey et al. 2011). Additionally, there has been no quantification of the economic costs associated with the California control research. Even more difficult to quantify but equally important are the biogeochemical impacts associated with Z japonica control. Killing all of the animals in the sediments with hot water may turn areas from being a nutrient sinks to nutrient sources which may have its own unique set of issues. For example, the 10 y Brown Tide bloom in Laguna Madre TX is believed to have been triggered by ammonium released from decomposing fish and sediment invertebrates killed in a severe winter freeze (Buskey et al. 1997). It seems prudent to understand the effects that the control measures have not only on the target but also on other components of the system.

Third, listing Z japonica habitat as a noxious weed allows the use of commercial herbicide applications to estuarine areas, despite inadequate testing and quantification of the ecological effects. Because *Z. japonica* is currently listed as a Class C Noxious weed on shellfish beds in Washington, commercial shellfish growers can use industrial methods to control the plant. Washington Department of Ecology is actively working on developing a National Pollutant Discharge Elimination System (NPDES) permit for the use of the herbicide Imazamox to control *Z. japonica* on commercial shellfish beds in estuarine waters[1]. Imazamox is registered for use in the aquatic environment by the US Environmental Protection Agency (US EPA 2008), despite the lack of evidence for efficacy on estuarine plants and major data gaps with regard to effects on estuarine/marine fish, shrimp and mollusks (US EPA 1997). Imazamox inhibits production of acetolactate synthetase, which prevents the formation of the essential amino acids valine, leucine, and isoleucine (Mallory-Smith and Retzinger 2003). This mechanism of action is not specific to *Z. japonica* and may have a negative effect on other photosynthetic organisms (e.g., native eelgrass, macroalgae, phytoplankton, and microphytobenthos). Toxicity tests of Imazamox on a marine diatom species (S*keletonema costatum*) showed an 11% reduction at 40 ppb, the test concentration was below the labeled use application rates of 50 to 500 ppb for aquatic plants (US EPA 1997, 2008, 2012). This report concludes "*Additional aquatic plant growth studies needed to be done to determine if the unicellular species such as diatoms and algae are sensitive to imazamox up to 500 ppb. If they are sensitive, then an EC50 will need to be determined.*" (US EPA 2008; italics added for emphasis). To date, an EC50 has not been determined for diatoms. Recently, Seattle Shellfish LLC., filed a letter report[1] by Dr. Richard Wilson on the importance of marine diatoms to the Willapa Bay food web in general and more specifically for shellfish. Given the sensitivity of diatoms to the herbicide imazamox described above, it seems likely that chemical control of *Z. japonica* will also have adverse impact this important food resource. It is ironic that carbaryl pesticide applications historically used to suppress burrowing shrimp in commercial shellfish grounds may have favored the expansion of

Z. japonica beds in these same areas (Dumbauld and Wyllie-Echeverria 2003). Consequently, the unforeseen impacts of burrowing shrimp control may have exacerbated Z japonica colonization. Interestingly, carbaryl pesticide applications are scheduled to be phased out by the end of 2012 (Schreder 2003). Federal and state resource agencies and citizen groups have expressed concerns about the potential for impacts of Imazamox to non target organisms such as *Z. marina* (considered essential fish habitat by NOAA National Marine Fisheries Service) and listed endangered species (ESA) such as salmonids[1]. Again, it seems prudent to understand the effects and impacts of any management actions taken to control Z. japonica, so that the cure isn't worse than the disease.

Finally, it is important to recognize that this action is diametrically opposed to existing national and international seagrass conservation efforts (Orth et al. 2006) and that control of *Z. japonica* is likely to be an expensive endeavor with limited potential for success. In Humboldt Bay, Cal F&G has had a program to eradicate a small population of Z. japonica; despite almost a decade of intensive effort Zostera japonica continues to persist and expand in the system.

[1] http://www.ecy.wa.gov/programs/wq/pesticides/comments.html

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