Douglas Steding, Ph.D.
dsteding@nwresourcelaw.com
(206) 971-1567

NORTHWEST RESOURCE
LAW PLLC

November 1, 2017

VIA E-MAIL AND ELECTRONIC
SUBMITTAL

Derek Rockett
Ecology Water Quality Program
P.O. Box 47775
Olympia, WA 98504-7775
Email: burrowingshrimp@ecy.wa.gov

Dear Mr. Rockett:

The Willapa/Grays Harbor Oyster Growers Association ("WGHOGA") submits these comments in support of the Draft Supplemental Environmental Impact Statement for Control of Burrowing Shrimp using Imidacloprid on Commercial Oyster and Clam Beds in Willapa Bay and Grays Harbor, Washington (the "Draft SEIS") issued by the Washington Department of Ecology for public comment on September 15, 2017. WGHOGA is supportive of issuance of a National Pollution Discharge Elimination Permit ("NPDES") as analyzed in the preferred alternative of the Draft SEIS, and offers the following comments that (1) emphasize the economic importance of the shellfish industry in Pacific and Grays Harbor County; (2) stresses the importance of a burrowing shrimp control program as part of the continued economic viability of the shellfish industry in those two counties; and (3) clarifies, corrects, or provides additional details relevant to the analysis undertaken by Ecology in the Draft SEIS.

A. Economic Importance of the Shellfish Industry and the Need to Control Burrowing Shrimp

Willapa Bay is the largest producer of farmed oysters in the United States. Combined with Grays Harbor, this area along the southwest Washington coast produces approximately 25 percent of all oysters in the United States. Willapa Bay is also a crucial component of the shellfish economy in Washington State, producing approximately 65 percent of the oysters and 13 percent of the clams harvested in Washington State. Shellfish aquaculture is the largest private employer in Pacific

County and a significant private employer in Grays Harbor County. It is one of the major industries in southwest Washington, and has increased in relative importance following declines in the timber and fishing industries.

Since at least the 1940s, two native species of burrowing shrimp (ghost shrimp, *Neotrypaea californiensis* and mud shrimp, *Upogebia pugettensis*) have caused impacts to Pacific Coast commercial clam and oyster production by disrupting the structure and composition of the substrate, causing these shellfish to sink and suffocate and eelgrass and crab habitat to disappear. Until recently, commercial shellfish growers in Willapa Bay and Grays Harbor, Washington, have successfully used the N-methyl carbamate insecticide "carbaryl" to control burrowing shrimp on culture beds receiving young oysters. The use of that chemical was phased out in favor of developing the least impactful method of burrowing shrimp control possible. WGHOGA is now seeking permit approval from the Department of Ecology to use the insecticide "imidacloprid" as a replacement for carbaryl for burrowing shrimp control in the aquatic environment of these two estuaries. The current proposal is to treat approximately 1.1 percent of total tideland area in Willapa Bay and 0.02 percent of total tideland area in Grays Harbor annually.

Without the ability to treat the tidelands, not only will there be loss of ecological value within these benthic habitats, there will be significant economic impacts to the region. In 2013, Northern Economics prepared an economic impact assessment of aquaculture in Washington, Oregon, and California for the Pacific Shellfish Institute ("PSI"). The input-output analysis determined that for every dollar spent by shellfish growers, a total of $1.82 worth of economic activity is generated in Washington. In addition, every dollar spent by shellfish growers generates approximately $0.76 in wages, and for every $1 million spent by the industry, nearly 27 jobs are created. Based on these calculations, the PSI study estimated that shellfish farmers in Washington spent approximately $101.4 million in the Washington economy in 2010, which in turn generated approximately $184 million. Shellfish farmers generated 1,900 direct jobs and paid $37 million in labor income in 2010, and they generated 810 additional jobs through indirect or induced activity. Further, the PSI study found that shellfish aquaculture in Pacific County in 2010 generated more than $90 million in total economic output, 1,580 jobs, and more than $45 million in labor income. In Grays Harbor, shellfish aquaculture generated almost $12 million in total economic output, 210 jobs, and almost $6 million in labor income in 2010. Not captured in the PSI study are the economic benefits from shellfish aquaculture in the form of "upstream" jobs.

If burrowing shrimp are not effectively controlled with pesticide treatments, then commercial shellfish production in Willapa Bay and Grays Harbor will likely be reduced 80 to 90 percent. In 2016, WGHOGA surveyed the members seeking permit coverage under the current application, and asked them to project bed losses over the next five years. The results of that survey indicate that cumulative losses will result in almost 500 acres of seed or nursery ground, 575 acres of fattening beds and more than 530 acres of clam beds lost by the end of year five. The resulting economic loss to WGHOGA members is estimated at an annual production value of $9600 per acre for oyster beds, and $13,000 per acre for clam beds. Cumulative losses by year five would total just under $50 million. This loss is production loss only, and does not include indirect economic impacts to the communities that surround Willapa Bay and Grays Harbor or the economic value of the lost habitat associated with the conversion of rich oyster or clam beds into benthic barrens. The direct economic loss number also excludes the losses already experienced by the growers due to not being able to control burrowing shrimp over the past three years and does not consider the real possibility of these growers having to close multi-generational farms due to escalating shrimp infestation.

B. WGHOGA's specific comments on the Draft SEIS

1. Integrated Pest Management Plan from WGHOGA

The Draft SEIS indicates that an Integrated Pest Management Plan (IPM) had not been submitted by WGHOGA by time of publication of the SEIS. WGHOGA notes here that the IPM plan has subsequently been submitted to Ecology, and a copy of that proposed plan is attached as Exhibit A. The plan has five elements, which collectively strive to increase the efficacy of imidacloprid applications, continue efforts to test and develop non-chemical controls of burrowing shrimp, and, ultimately, to reduce the use of imidacloprid over time. In summary the plan includes the following:

- Burrow Monitoring - Accurate monitoring of the population densities of burrowing shrimp are fundamental to all aspects of decision making in the IPM plan. WGHOGA will continue to monitor burrow counts on all beds covered under the NPDES permit on a yearly basis. Yearly monitoring will include date of survey, bed name, location, burrow counts, sediment characteristics, and native seagrass presence.
- Recruitment Research - Current research suggests that the detection and monitoring of newly settled juvenile burrowing shrimp recruits may be useful to predict bay wide population trends, and could be used to develop an annual

recruitment index. WGHOGA will incorporate young of the year (YOY) monitoring in locations where recruitment is likely to be observed. Data from these monitoring efforts will be useful as WGHOGA develops improved methods to quantify rates of new burrowing shrimp recruitment. Studies are planned to determine whether application of imidacloprid at seasons other than mid-summer would be more effective at controlling shrimp, while still protecting the environment. Improved control may help reduce the need for imidacloprid to treat or retreat shellfish beds to reduce burrowing shrimp.

- Efficacy Studies - Efficacy monitoring (i.e., counts of burrowing shrimp burrows before and after imidacloprid treatments) will be conducted during early spring, mid-summer and late summer to help select formulation type and timing for the delivery of imidacloprid to increase efficacy. Continued testing of non-chemical approaches such harrowing of new recruits, disking and dredging between crop rotations will be implemented to slow the establishment of adult populations. The overall goal of these efficacy studies is to determine ways to obtain sufficient burrowing shrimp control while continuing to reduce dependence on chemical use.
- Damage/Density Thresholds - WGHOGA will work to quantify the relationship between the density of burrowing shrimp populations and damage to oyster yield. To determine damage/density functions, studies will be undertaken to measure survival, growth, and harvest yield of oysters on beds with different densities of burrowing shrimp, considering the effects of habitat, season, culture technique, and other environmental variables. Initial efforts will focus on developing damage/density functions for the cultural practices suffering the greatest economic loss from burrowing shrimp. The data from this monitoring may help WGHOGA to reduce the need to control burrowing shrimp when they are at densities that, based on current knowledge and experience, require treatment.
- Continued Research - WGHOGA will continue to seek alternative physical, biological or chemical control methods that can be more species specific, economical, reliable and environmental responsible and will work with partner organizations to facilitate these activities. This ongoing monitoring will help growers determine the success of their shrimp control program and to aid them in making better management decisions in the future. For example, closely monitoring the beds will help identify dense groupings of shrimp that can be treated with precision, small-scale spot treatments or alert growers when recruitment events have occurred. The end goal is to manage the burrowing

shrimp populations on actively farmed beds, reduce the need for large scale treatments and to achieve efficacy sufficient to reduce the adult shrimp as indicated by burrows counts that are below the damage threshold.

2. Ecological Benefits of Burrowing Shrimp Control

Dr. Richard Wilson, a WGHOGA member, has spent decades sampling and documenting invertebrates and plankton associated with Willapa Bay, and in particular, the effects of burrowing shrimp on the ecology and primary productivity of the bay. Much of his work has focused on the benthic diatoms, which are important primary producers that form the base of the food web in these shallow estuarine systems. By forming a "biofilm" on the sediment surface, benthic diatoms can be a major food source for some birds (e.g., western sandpipers and dunlin, Mathot et al 2010), and indirectly for many other birds by supporting invertebrates they feed on (e.g., amphipods and benthic copepods), that, in turn, feed on the diatoms. Dr. Wilson's work on this subject is detailed in Attachment A. This Attachment constitutes a part of WGHOGA's comments on the SEIS. A summary of this work includes the following:

- The primary productivity of the intertidal areas of Willapa Bay is driven by the benthic habitat, in particular benthic diatoms that form a dense biofilm on surficial sediments.
- The burrowing ghost shrimp, *Neotrypaea californiensis,* through bioturbation, deleteriously disturbs and continually modifies the intertidal sediments. These actions thereby reduce or eliminate the benthic diatoms and their biofilm habitat, disrupting primary productivity essential to create and maintain the food web in the estuary.
- It is estimated around 9,000 publicly owned intertidal acres of Willapa Bay are subject to this disruption by ghost shrimp and have significantly lost ecological productivity. (Map Attachment A, Fig. 14).
- If ghost shrimp encroachment is not controlled by shellfish growers on their privately-owned lands, another estimated 6,000 - 9,000 acres of productive intertidal habitat that sustains many of the most important estuary species could be lost in the next decade.

WGHOGA strongly believes the final SEIS needs to include a discussion of the positive benefits of burrowing shrimp control. Although the SEIS, in Section 2.11, has a brief discussion of benefits, the majority of this is through a reference to fuller discussions in the FEIS. And, acknowledging that the FEIS is incorporated by reference into the SEIS, and therefore constitutes part of the official record and background for that document, the subject is too important not to be covered in some detail in the SEIS. This is particularly important because the SEIS is notable for its full and consistently conservative discussion of potential negative effects (i.e., erring on the side of concluding impacts will occur) of imidacloprid treatments by WGHOGA. Scientific objectivity, and a commitment to fully informing the public and decision makers in meeting its mandate under SEPA, make it imperative for Ecology to discuss the potential positive environmental effects of the proposed permit in the SEIS itself.

More specifically, WGHOGA notes that the FEIS included numerous discussions of the possible ecological and food web benefits of burrowing shrimp control, but then any such discussion is largely absent from the SEIS. For example, as noted in the FEIS, "Burrowing shrimp control using pesticides under either Alternative 2 (Carbaryl with IPM) or Alternative 3 (Imidacloprid with IPM) would have beneficial environmental effects in the form of preserving the substrate and biodiversity of commercial shellfish beds and promoting native eelgrass density and coverage, thereby improving foraging habitat and prey diversity for birds and fish, and cover for juvenile fish including listed species of salmonids." (FEIS page 2-59). WGHOGA notes again, to meet Ecology's mandate under SEPA, this should be quoted verbatim in the SEIS. The final SEIS should include the following points:

- As ghost shrimp recruit to intertidal areas damage from their bioturbation increases, with resulting loss of the benthic fauna and flora (Attachment A, Fig. 16) through continual disturbance and reductions in primary productivity (as described above). First of the critical intertidal biological elements to be displaced would be the benthic diatoms and their biofilm, followed by decreases in grazer invertebrates due to lack of food or inability to survive on the shifting sediments. Sediment instability with loss of finer sediment fractions would damage or destroy existing eelgrass, plus prevent any new seeds from sprouting.
- With decline and then loss of their food sources and habitats, including oyster bed and eelgrass habitats, higher trophic level forms such as birds and fish will experience reduced food resources. In more extreme cases the burrowing shrimp dominated areas may become unsuitable as foraging habitat for these vertebrates.
- Available evidence is that burrowing shrimp competitively exclude many other types of sediment associated organisms once they successfully recruit to an area. The burrowing shrimp tend to limit the occurrence of other species through constant sediment disturbance and create monocultures.

- Given these impacts, controlling ghost shrimp abundance through imidacloprid treatments via the proposed permit will help to preserve the food web from primary producers to higher-level predators. And by supporting the survival of oyster beds and eelgrass, these imidacloprid treatments will enhance habitat diversity when compared to the uniform mudflat habitats dominated by burrowing shrimp.
- Published scientific studies and reports support the conclusion that burrowing shrimp have negative effects on other species, and that their control provides food web benefits. For example, Ferraro and Cole (2007) examined benthic invertebrates associated with various habitat types (e.g. oyster beds, eelgrass, ghost shrimp mudflats) to help identify high value, critical habitat within Willapa Bay. They showed that shrimp dominated habitat had disproportionately lower macrofaunal biomass (other than of the shrimp themselves) and species diversity than the other habitat types. Other focused studies demonstrate that constant disturbance from burrowing shrimp can limit and exclude eelgrass (Dumbauld and Wyllie-Echeverria 2003; Hosack et al. 2006). These authors note that improvements to native eelgrass density and coverage could also improve recruitment of Dungeness crab and foraging habitat for fish and migratory birds (e.g. black brant geese).
- There are similar improvements from expanding oyster habitat (Hosack et al. 2006) and promoting recruitment of macoma clams (a species foraged on by medium to large migratory shorebirds such as red knot and curlew) that are discussed in the FEIS that are not fully referenced in the SEIS.
- Shellfish growers have pointed out the obvious lack of benthic organisms associated with burrowing shrimp dominated intertidal areas. One example can be seen in Attachment A, Fig. 15 (page 14), which compares two similar oyster growing areas with one having a dominant ghost shrimp population and the other without due to periodic chemical control of burrowing shrimp. The difference in diversity of habitats and species present is plain to the naked eye. WGHOGA members have taken members of the press, scientists, Ecology staff, even Washington Governor Jay Inslee on field tours of Willapa Bay to see just such impacts of burrowing shrimp on habitat and ecosystem diversity and conditions.

More information on these subjects can be found in the following references:

> Dumbauld, B.R. and S. Wyllie-Echeverria. 2003. The influence of burrowing thalassinid shrimps on the distribution of intertidal seagrasses in Willapa Bay, Washington, USA. Aquatic Botany 77:27–42.
>
> Ferraro, S. P., and F. A. Cole. 2007. Benthic macrofauna–habitat associations in Willapa Bay, Washington, USA. Estuarine, Coastal and Shelf Science 71:491-507.
>
> Hosack, G.R., B.R. Dumbauld, J.L. Ruesink, and D.A. Armstrong. 2006. Habitat associations of estuarine species: Comparisons of intertidal mudflat, seagrass (Zostera marina), and oyster (Crassostrea gigas) habitats. Estuaries and Coasts 29(6B): 1150–1160.
>
> Mathot, K.J., D.R. Lund, R.W. Elner. 2010. Sediment in stomach contents of Western Sandpipers and Dunlin provide evidence of biofilm feeding. Waterbirds 33 (3), 300-306.

3. Potential Effects on Dungeness Crab

WGHOGA appreciates the detailed assessment in the SEIS of the potential effects of the proposed permit on Dungeness crab. WGHOGA agrees with the SEIS's overall conclusions about potential effects to this species:

> *"most impacts to juvenile crab would be limited to on-plot, and immediately adjacent areas directly sprayed with imidacloprid during low tide conditions. Planktonic forms of Dungeness crab off-plot may be impacted by rising tidewaters carrying imidacloprid. Given the small area that would receive imidacloprid applications each year (if the permit is issued), compared to the total size of Willapa Bay and Grays Harbor, and the small number of animals that would be affected compared to the total number of animals present in these estuaries and surrounding areas, imidacloprid effects are not expected to impact bay-wide populations of Dungeness crab in these estuaries."* (Page 3-28)

WGHOGA, nonetheless, would like to put any localized and short-term impacts to Dungeness crab from imidacloprid treatments into perspective. First, off-plot impacts are unlikely except immediately adjacent to treated areas due to the rapid transport and dilution of imidacloprid by rising tide waters. This limits mortality of planktonic/juvenile recruits to a very small portion of the overall crab population.

This localized loss of planktonic/juvenile Dungeness crab recruits is dwarfed by the natural variability of natural larval recruitment and population sizes for the species. For example, as the SEIS notes, a single female Dungeness crab can produce one to two million eggs during each reproductive cycle (page 2-29). This guarantees that larval forms of this species are not limited by the availability of individuals in the plankton. Instead, recruitment of juvenile crabs from the plankton is limited by food and predation conditions experienced by the plankton, and ultimately by the physical habitat space available when they settle to the substrate. Thus, the reproductive biology of Dungeness crab ensures that the species can survive even large-scale die-offs of its planktonic forms.

For larger crabs, the size of the commercial fishery gives some idea of just how abundant Dungeness crab are in the Willapa Bay and Grays Harbor estuaries. The Washington Department of Fish and Game management strategy for Dungeness crab is a male only fishery with strict size limits over a controlled pot trap program. Timing of the fishery is controlled as well as the amount that can be harvested (approximately 50% of the total allowable harvest). Historically, Washington coastal Dungeness crab landing data back to 1950 show a large fluctuation in harvest, ranging from a low of 2.5 million pounds in 1981 to a high of 25 million pounds in 2004-05, with an average of 9.8 million pounds (Reed 2009). These commercial catches can be used to estimate the population of large Dungeness crab as follows: 9.8 million pounds crab/1-1.5 pounds per crab is 6.53 to 9.8 million crabs captured. But this represents only half of the population (females are excluded), and only half of the total legal sized male crabs that are available (i.e., due to limits on the allowable harvest). Therefore, a rough estimate of the total number of commercially sized Dungeness crabs is therefore 6.53 to 9.8 million times (2) times (2), which equals 26.1 to 39.2 million individual crab. This large number does not include juvenile Dungeness crab that are too small to be captured or retained in the fishery. A reasonable estimate of Dungeness crab given these data is 50 million or more, which strongly corroborates the SEIS estimate for Pacific County of 10-20 million crab. With a population this size, the loss of 2 or 4 or even 20 juvenile crab/acre treated with imidacloprid is obviously trivial to the overall Dungeness crab population, as the SEIS correctly concludes.

The wide swings in commercial landings of Dungeness crab reveal another important perspective on any localized, short-term effects from the proposed permit. Populations of Dungeness crab are obviously experiencing highly variable recruitment and survival over time. It is believed that this large fluctuation in landings is not a result of harvest patterns, but instead is due to varying ocean conditions including water temperature, food availability, and ocean currents (WDFW 2017) outside of the bays and estuaries where they recruit. In simple terms, the conditions outside of Willapa Bay and Grays Harbor have a much more profound effect on recruitment and population size than anything within these estuaries themselves, including the localized, short-term impacts that might result from imidacloprid treatments under the proposed permit. WGHOGA also notes that the previous burrowing shrimp management technique of using the pesticide carbaryl occurred during this time frame with no discernable effect of commercial landings on Washington's coast.

Under the proposed permit, WGHOGA's plots will be treated and then oysters will be introduced and cultivated. Once that occurs the plots then become a refuge for newly settled crab recruits (Armstrong and Gunderson 1985), a valuable nursery habitat for the species. Predation of these new recruits is likely the largest determinant of whether Dungeness crab survive to reach maturity. Of all the predators, other Dungeness crab seem to be the most effective. Cannibalism among Dungeness crabs has been noted by various studies dating back to the 40's (Pauly et al 1986). Cannibalism is cited as a possible cause of the dramatic population cycles characteristic of the Dungeness crab fishery (Botsford and Wickham 1978). This makes the refuge of the physically and spatially complex oyster beds very valuable to juvenile crabs, as this habitat offers far greater opportunities to hide and forage without being eaten than does any area of simplified mud flat that results when high numbers of burrowing shrimp are present. The same accords to development of eelgrass beds following chemical treatments to control shrimp control, a benefit noted repeatedly in the FEIS (e.g., page 1-21). Accordingly, the net effect of treating with imidacloprid to control burrowing shrimp is likely to be a net positive for Dungeness crab because of the enhanced nursery conditions for juvenile crab that will develop on the treated ground.

Given this information, WGHOGA believes the SEIS needs to more clearly state that the proposed permit will likely have net positive effects on Dungeness crab recruitment and survival that would more than offset any impacts to animals present on the plots during treatment. In addition, the SEIS should note that if the permit is denied, the acreage of oyster beds is expected to decline significantly over time due to the expansion of burrowing shrimp, and that this would constitute a long-term and likely permanent impact to Dungeness crab recruitment and survival in Willapa Bay and Grays Harbor.

4. Effects on non-target invertebrates – Dr. Steve Booth's Meta-Analysis of Prior Imidacloprid Trials

The SEIS includes extensive discussion of the observed effects of imidacloprid on non-target invertebrates, referencing both analysis of prior field trials in Willapa Bay in the FEIS, and, for the first time, the 2014 field trials in Willapa Bay. The SEIS also includes an extensive analysis of recent scientific research and papers on the effects of imidacloprid on invertebrates (e.g., EPA 2017). This amounts to a very substantial amount of empirical and research science to support the SEIS's conclusion that impacts to non-target invertebrates from imidacloprid treatments under the proposed permit will be "localized and short-term" (SEIS page 3-30). WGHOGA strongly agrees with that conclusion.

Very recently, Dr. Steven Booth of the Pacific Shellfish Institute led a group of researchers in drafting a scientific paper synthesizing the results from 8 critical empirical trials of imidacloprid that have been conducted in Willapa Bay (Booth et al. 2017). Dr. Booth has conducted all previous analyses of imidacloprid effects on invertebrates during the empirical trials, and is therefore in an unparalleled position to conduct such a follow up analysis. Results from individual trials have been reported previously but, until now, a comprehensive analysis of all data combined has been neither conducted nor published. Sixty analyses were conducted to examine the response to imidacloprid treatment by 6 taxonomic invertebrate groups. WGHOGA obtained permission from Dr. Booth to review his group's paper, which he expects to submit for publication in a scientific journal shortly. Further, Dr. Booth agreed to allow WGHOGA to submit this paper as part of its SEIS comments (as Attachment B), and to provide a summary of its analytical approach and main findings as follows:

Approach:

- A before-after-control-impact (BACI) design was initially applied to all trials.
- Principal Response Curve (PRC) analysis was used to capture and visually represent the change in abundance of each species group on the treated plots relative to the untreated control plots. Variability across trials due to site effects, replicate effects, unexplained effects (i.e., unconstrained variation) and time (conditioned variance) are removed or compensated for in PRC analysis. The

model thus enables a focus on the treatment vs control, and treatment versus time interactions to explain the response of invertebrate species groups, as well as the relative importance of individual species within those groups.

- Concentrations of imidacloprid in surface water, sediment pore water, and in whole sediment that were measured during the field trials are also presented.

Results:

- Only 6 of the 60 PRC analyses showed a significant negative effect from imidacloprid application. Five of these 6 PRCs represented mollusks, which represented < 2% of all organisms sampled among all sites and years.
- Crustaceans were negatively affected in only one of the 8 studies.
- Polychaetes were never negatively affected.
- The large majority of PRCs showed either no significant effect from imidacloprid application (control and treatment plots remained similar), a neutral treatment effect (variation between treatment and control plots without a clear direction positively or negatively), or ostensibly a “positive” treatment effect (treatment plots exceeded control plots).

Conclusions:

- The overall minimal response was likely due to the low concentrations of imidacloprid invertebrates were exposed to and for limited times, physiological tolerance to imidacloprid for some species, and multiple life-history strategies to rebound from natural disturbance and adaptation to a highly variable environment. These strategies include high mobility and dispersal behaviors, high intrinsic rates of reproduction, and rapid development.
- Dr. Booth concluded that long term effects of imidacloprid to manage burrowing shrimp and culture bivalves is expected to lead to a more diverse community of benthic invertebrates compared to otherwise similar estuarine ground with high densities of burrowing shrimp. He notes that burrowing shrimp are ecosystem engineers and control the structure of the immediate benthic community by limiting the survival and recruitment of other invertebrate species. (WGHOGA note - this is a finding also presented in the FEIS based on that document’s review of the scientific literature (page 3-4).

WGHOGA believes that Dr. Booth's paper is an extremely important contribution to the evaluation of whether the proposed permit will adversely affect non-target invertebrates. As the lead invertebrate researcher for all previous empirical trials of imidacloprid in Willapa Bay he is uniquely qualified to analyze the effects of those trials on non-target invertebrates. His PRC analysis of all existing trials is a more robust examination of imidacloprid effects than either results for individual trials, or extrapolation of expected effects based on research papers that examined imidacloprid toxicity in laboratory experiments on one or two species of aquatic invertebrates. WAGHOGA requests that the final SEIS include a citation and discussion of Dr. Booth's paper. That discussion should acknowledge the conclusions of that study, particularly the conclusion that the use of imidacloprid to treat burrowing shrimp does not result in the reduction of non-target species. Finally, the SEIS should acknowledge that these results corroborate findings in the FEIS that reduction of burrowing shrimp numbers can have positive effects on non-target invertebrates.

5. Location of Annual Treatments Under the Permit

WGHOGA noted with interest the discussion on page 2-14 discussing the potential spatial arrangement of individual plots that would be treated under the proposed permit, and the footnote of that page indicating that the density of the treated plots could influence the magnitude of off-plot environmental effects. WGHOGA wishes to reiterate that the location of plots to be treated will be determined on a year-to-year basis based on the density of burrowing shrimp, the status of individual beds (e.g., which have oysters, which are being prepared for seeding with oysters, etc.), the efficacy of prior treatments, and the business plans of the individual WGHOGA growers. Because of the inherent spatial variability that results from these variables, WGHOGA believe that it is extremely unlikely that proposed treatments will result in a high density of treatments plots in any given area in any single year. WGHOGA growers that will be covered by this permit have farms that are widely distributed in Willapa Bay and Grays Harbor; these farms are not all clustered in one or two areas. Thus, although WGHOGA does not object to this analysis in the SEIS, it wants to make clear that high density treatments are very unlikely under the permit.

The SEIS correctly states that WGHOGA each year must decide where they wish to treat, and then to submit that plan ("Annual Operating Plan" or AOP) to Ecology for its review and approval. This is appropriate given that the SEIS is not an appropriate venue for reviewing the details of which plots will be treated since this is largely dependent on annual variables. Reviewing which plots require treatment within the AOP allows for more targeted treatment (i.e. only treat areas that require it) that will still be subject to the constraints of the discharge permit.

As the SEIS notes, Ecology is retaining to itself authority to approve or disapprove of each year's AOP, or to request changes in the AOP as a condition of approval. Thus, practically, WGHOGA cannot have a higher density of treatment sites in any given year than Ecology agrees to. In practice, should any AOP propose a high density of treatment plots in any given area, WGHOGA expects that it would agree to sequence the treating of those plots with imidacloprid over the allowable treatment window of April 15 to December 15 to avoid any concerns about the collective effects of such treatments.

6. Size of Plots to be Treated Under the Permit

The SEIS discussed the size of plots to be treated under the permit as follows:

> *"Given the reduced acreage, and the elimination of aerial spraying from helicopters from the 2016 WGHOGA application, treated plots are now expected to be 10 acres or less in size, consistent with most of the prior field studies."* (page 1-36)

This is an important point because all previous field trials of imidacloprid treatments in Willapa Bay, except the 2014 trials, were tests on plots of about 10 acres or less. Thus, the proposed permit would be applied to areas comparable to those for which scientific results of the experimental trials are most applicable. And, these previous trials have demonstrated that the effects of imidacloprid on sediments, water quality, and animals are both localized and temporary, with most trials showing that conditions on treatment and control plots are comparable 14 to 28 days after treatment. This result was also observed in the 2014 trial on which a 90-acre plot was treated, demonstrating that recovery on treatment plots was not significantly impaired even on very large plots. Thus, the proposed permit has solid scientific evidence to support the conclusion that significant adverse effects will not occur.

WGHOGA reaffirms that it expects to treat plots of 10 acres or less in size under the proposed permit. As noted for Comment 5, under the Annual Operations Plan WGHOGA may propose that plots adjacent to or near one another will be treated in the same year. In such cases it will work with Ecology to determine the timing of such treatments.

7a. Annual Treatment Timing – Clarification and Need for An Extended Treatment Window

The SEIS correctly states that the proposed permit would allow imidacloprid applications during the period of April 15 to December 15, each year. Past treatments of shellfish beds to control burrowing shrimp have occurred almost exclusively in the period of mid-May to early September, primarily because this is the period with large magnitude low tides (i.e., very low or negative) that occur during daylight hours. WGHOGA anticipates that most treatments under the proposed permit will continue to occur during this "window".

Although most imidacloprid treatments are expected in the window from mid-May to early September, it is important that the permit allow treatments in the entire period from April 15 to December 15. This will allow WGHOGA to test different treatment timing to try and increase efficacy, and ultimately to reduce use of imidacloprid as part of the permit's IPM approach. Two approaches have been discussed by WGHOGA with Ecology. First are early season treatments before most annual eelgrass growth. Past work by Dr. Kim Patten of Washington State University cited in the SEIS (i.e., Section 2.8.4.2) has documented that efficacy of imidacloprid treatments can be hindered when eelgrass is too thick. If early treatments avoid this problem in areas of heavy eelgrass growth then efficacy could improve, and the need for future imidacloprid treatments of such beds could be reduced. Second, fall and early winter applications of imidacloprid would offer an opportunity to treat each year's new recruits of burrowing shrimp (e.g., planktonic forms that settle and burrow into the sediment). These very young shrimp may be particularly vulnerable to imidacloprid treatment, in part because they are found in the surface layers of the sediment, as opposed to being in deep burrows. Again, efficacy may be increased by such treatments, potentially reducing the need for future imidacloprid treatments, which could ultimately achieve the WGHOGA goal of reducing the amount of imidacloprid application needed in the future.

7b. Annual Treatment Timing – Effects on Birds

As discussed in the FEIS, which was incorporated by reference in the SEIS, large migrations of shorebirds and waterfowl migrate through Willapa Bay and Grays Harbor each year. These migrations have specific timing. As stated in the FEIS:

> *"The overall numbers of waterfowl and shorebirds are lowest in summer, highest in spring and fall, but remain relatively high throughout the winter (USDI/USFWS 1997). Peak migration*

> *through Willapa Bay occurs between mid-April and early May."*
> (page 3-37)

Given that most imidacloprid applications by WGHOGA will occur in the window of mid-May to early September, the great majority of the spring and fall-winter migrations of shorebirds and waterfowl that pass through Willapa Bay and Grays Harbor have a low potential exposure to imidacloprid applications by WGHOGA. And, as the SEIS correctly concludes based on its review of the scientific literature (e.g., page 3-24 of the SEIS), imidacloprid has extremely low toxicity to vertebrates, including birds. Thus, through avoidance of exposure, and low toxicity, WGHOGA believes there is no potential to impact migrating or resident birds in Grays Harbor and Willapa Bay.

The FEIS (pages 1-21 - 22) and SEIS (pages 1-9, 3-24 - 25), appropriately, also conclude that the potential for direct toxicity to birds is not significant, including for birds listed under the Endangered Species Act. However, the SEIS raises the prospect of possible food chain effects due to the temporary reduction in invertebrate prey on treated plots. WGHOGA believes this is incorrect. The SEIS repeatedly notes that no more than 1.1% of the intertidal area of Willapa Bay, and 0.04% of the intertidal area of Grays Harbor would be treated with imidacloprid (e.g., page 1-3 of the SEIS). Given that 98.9% or more of the intertidal area of these estuaries will be untreated, arguing for food chain effects is scientifically spurious. This is especially true given that the SEIS, in reviewing past experimental trials of imidacloprid in Willapa Bay, concludes that the invertebrates on treatment and control plots are statistically indistinguishable within 14-28 days after treatment in almost all cases. In addition, the FEIS noted that control of burrowing shrimp with imidacloprid could have positive food chain effects (e.g., promote the growth of eelgrass, support existence of oyster bed habitat), but these benefits were not discussed in the SEIS. The SEIS should therefore clarify that negative food chain effects, while theoretically possible, are extremely unlikely. And the SEIS should state that control of burrowing shrimp with imidacloprid could have positive food chain effects as discussed within the FEIS.

8. Planned treatment of shellfish beds with imidacloprid

The SEIS is not explicit in stating that, under the proposed permit, WGHOGA will not apply imidacloprid to any crop of oysters. Instead growers will treat the sediment on shellfish growing ground prior to planting a crop of oysters. WGHOGA members are committed to this approach to the use of imidacloprid, and believe it is important for Ecology in the SEIS, and in its communications about the proposed permit, to make clear that imidacloprid will not be sprayed on crops of oysters. No future consumer of oysters from WGHOGA farms needs ever worry that their product has been treated with imidacloprid.

9. Clarification of bed elevations that will be treated

The SEIS in numerous places states that the proposed permit will be used to treat shellfish beds at elevations from -2 feet to + 4 feet relative to mean lower low water (MLLW). The shellfish growing beds that WGHOGA members own do fall almost entirely within this elevation range, and so the SEIS is correct in concluding that treatments will occur within this elevation band. However, all farm plots have micro-topographical features that are either higher or lower than the surrounding bed. For example, drainage channels can be a foot or more deeper than the beds that they drain. WGHOGA therefore wants to clarify that small portions of their beds treated with imidacloprid may fall outside the -2 to +4 feet MLLW elevation range. In almost all cases, WGHOGA believe such areas will fall within plus or minus 0.5 feet of the elevation range stated in the SEIS.

10. Use of EPA (2017) to Establish a Toxicity Threshold[1] for the SEIS Analysis

WGHOGA commends Ecology for its comprehensive review of the scientific literature in the SEIS. When combined with the review of an even larger number of scientific papers in the FEIS, it is clear that a majority of the relevant scientific literature has been used to inform the analysis of potential effects of the proposed permit on sediments, water quality, and animals, including invertebrates. While a comprehensive survey of the literature is important, WGHOGA understands that some scientific papers or reports are more valuable or informative than others. The 2017 EPA Risk Assessment of Imidacloprid, referred to in the SEIS as "EPA (2017)," is clearly an especially important reference for evaluating the potential effects of imidacloprid treatments that would be conducted under the proposed permit. This is so because: 1) EPA 2017 is itself a review of more than 100 scientific studies of the effects of imidacloprid, and is therefore comprehensive, 2) it offers the scientific conclusions and opinions of the federal Environmental Protection Agency, the lead agency for implementation of the Clean Water Act and the associated National Pollution Discharge Elimination System

---

[1] As used here toxicity refers to the concentrations of imidacloprid, usually expressed in parts per billion, that have been observed to cause death or other adverse effects in invertebrates. Toxicity threshold is the toxicity value selected as the minimum known or suspected to cause adverse effects. All imidacloprid concentrations above the threshold are assumed to result in adverse effects.

("NPDES") permit system under which WHGOGA is requesting permit authorization, and 3) its analysis of imidacloprid toxicity includes specific evaluation of effects on marine invertebrates, and 4) EPA (2017) bases its analysis of imidacloprid toxicity on results actually observed in research and experiments, rather than on a statistical modeling of such results.

This last element is important if confusing. Other studies of imidacloprid toxicity, for example the Health Canada (2016) report reviewed in the SEIS, often extrapolate from toxicity levels actually observed in experimental trials using statistical modeling to guess what the lowest possible toxicity might be if more data were available. These results are projections of toxicity that are lower, and often much lower, than anything ever actually observed in scientific studies. These hypothetical toxicity levels are not an appropriate measure for evaluating the potential effects of imidacloprid treatments of the proposed WGHOGA permit. EPA (2017), very appropriately, bases its evaluation of imidacloprid toxicity on effects that have been observed in prior studies.

Even so, EPA (2017) is very conservative in its analysis. It started by selecting the lowest observed toxicity level for any study of marine invertebrates it reviewed that met its data validation and quality control criteria. That value is 33 parts per billion (ppb, equivalent to the microgram/liter or µg/l referred to in the SEIS) for mysid shrimp exposed to imidacloprid for 96 hours. Despite the 96-hour test of this study, EPA assumed that these results would apply to any duration of "acute" exposure (i.e., exposures lasting from minutes up to 96 hours), a very conservative assumption. Also, the study's value of 33 ppb was the estimated concentration that resulted in 50 percent mortality of the shrimp tested, yet EPA effectively treats the value as if it killed all test organisms. Finally, EPA divided this value of 33 ppb by two in order to build in a factor of safety. The result was a toxicity threshold in EPA (2017) of 16.5 ppb.

The SEIS adopts this EPA derived value of 16.5 ppb as its toxicity threshold (page 3-20). Understanding its origins in EPA (2017), WGHOGA supports Ecology's use of this toxicity threshold (referred to as "toxicity criterion" in the SEIS). But it is important to note just how conservative this threshold is when evaluating potential effects of the proposed permit. Most importantly, imidacloprid concentrations in water under the proposed permit will be diluted immediately upon inundation of the treatment plots by the rising tide. And given that tidal amplitude in Willapa Bay and Grays Harbor exceeds 10 feet (i.e., treatment plots will be covered with 6-10 feet of water, or more, at high tide depending on the bed elevation), and that the period from low tide to high tide is 6-7 hours, meaning that there is zero possibility of a 96-hour direct exposure to imidacloprid in water, either on-plot, or off-plot. In fact, work by Patten and Norelius (2017) cited in the SEIS estimates “dilution by approximately 50% every 4 minutes” during the incoming tide (page A-11). Thus, actual exposures by invertebrates to imidacloprid in water are likely to be on the order of a few hours on-plot (i.e., for the duration of low tide following application, plus the time for the rising tide to cover the plot), and less than an hour off-plot. Further evidence for how conservative the SEIS’s toxicity threshold is comes from a full review of the work of Patten and Norelius (reviewed on pages A-10 – 11 of the SEIS). In their trials with Dungeness crab they observed no mortality or tetany (paralysis) in imidacloprid concentrations of 100 ppb for 2 hours in megalopae, in 200 ppb for 6 hours in juveniles, or in 500 ppb in juveniles when the water was diluted by 50% every 4 minutes to mimic conditions during a rising tide. Clearly use of the 16.5 ppb toxicity threshold in the SEIS leads to a significant overestimate of the actual effects to invertebrates that would occur under the proposed permit.

11. SEIS Modeling of Off-Plot Effects

The SEIS recognizes that rising tidal waters will carry imidacloprid from treated plots to off-plot areas, and that this movement could result in off-plot impacts to invertebrates and water quality. WGHOGA noted that the SEIS’s references to potential off-plot effects consistently emphasizes “adjacent areas” or “adjacent off-plot areas.” WGHOGA agrees with this characterization. If off-plot effects occur due to imidacloprid being carried off the treatment plots, those effects are most likely immediately adjacent to the plots, or in features like drainage channels flowing off the plots, because in such cases the imidacloprid being carried by the first flush of the rising tide will have experienced very little dilution. In addition, adjacent areas are more likely to share the same tidal waters as those that inundated the treatment plots (i.e., water that has crossed the treatment plots before moving to off-plot areas. As distance from the treatment plots increases significant dilution is expected, both from the volume of tidal water present (i.e., that has flowed from the treatment plot to the more distant location), and because much of the tidal water arriving at any individual location will have come from areas other than the treatment plot. For water, this dilution with distance has been verified in field trials in Willapa Bay (SEIS page 3-12). By extension, diluted imidacloprid levels would be expected to have less and less chance of affecting invertebrate populations as distance from the treatment plots increases. WGHOGA notes that researchers that have done empirical trials of imidacloprid in Willapa Bay consistently report that off-plot impacts to invertebrates are either not evident, or are limited to areas immediately adjacent to the treated plots (Dr. Kim Patten and Dr. Steve Booth, pers. comm.).

Given the above, WGHOGA was surprised and disappointed by the modeling of potential off-site impacts conducted in the SEIS (page 3-21). While we recognize that Ecology acknowledged that "this modeling was 'worst case' due to incorporation of several assumptions," (which were overly conservative), the modeling nonetheless presents a false picture of off-plot impacts that contradicts the rest of the SEIS's more scientifically defensible analysis of such effects. WGHOGA believes that this modeling compromises the scientific quality of the SEIS's analysis of off-plot effects. And it gives an unfair and inflammatory talking point to opponents of the proposed permit, that the area experiencing off-plot impacts may be greater than the area of the treated plots.

Although the SEIS, in analyzing the modeling results, concludes that "[a]ctual toxicity to off-plot invertebrates is expected to be less," WGHOGA still feels that the entire modeling analysis should be eliminated. It is inaccurate, misleading, and unhelpful in assessing the potential effects of the proposed permit.

12. Ground Based Versus Aerial Treatment

The SEIS repeatedly and correctly notes that under the proposed permit WGHOGA would not use aerial spraying techniques, including spraying by helicopter. And, the SEIS clearly states that given only ground-based application methods will be used, that the required buffer between treated areas and active oyster beds is 25 feet (page 3-30). Unfortunately, in Chapter 1 there is a repeated discussion of FIFRA registration requirements that mentions aerial spraying, and 100-foot buffers required as part of that aerial spraying (e.g., page 1-16). To avoid confusion by the public and reviewing agencies, WGHOGA suggests that the Final SEIS clearly state that 25-foot buffers will be required under the proposed permit. It is not necessary to insert this in every instance in the SEIS discussing that only ground-based methods will be used. It would be helpful to at least include this clarification in the Fact Sheet, and in Section 2.8.4 which summarizes WGHOGA's proposed permit (Alternative 4).

13. Treating on Weekends

The SEIS states that imidacloprid treatments would not be allowed on "Federal holiday weekends" (e.g., page 1-29). WGHOGA wishes to acknowledge this temporal constraint on the proposed permit, but also to state that imidacloprid applications on weekends other than federal holiday weekends will occur. Such weekend treatments are necessary because there are a limited number of low tides suitable for imidacloprid treatments, and many such low tides occur on weekend days. Thus, logistically, WGHOGA needs the flexibility to treat on such days. All required public and agency notifications discussed elsewhere in the permit would obviously also be complied with for any weekend imidacloprid applications.

14. Factors Controlling Burrowing Shrimp Populations

The SEIS notes that there is some uncertainty about what controls burrowing shrimp populations, then lists several possible anthropogenic factors that may have led to increases in shrimp numbers over time (page 2-5). One potentially important anthropogenic effect is the significant decrease in Columbia River floods due to development of an extensive system of flood control dams. WGHOGA is aware of past work indicating that during large Columbia River floods, a large plume of freshwater or low salinity water traveled north along the coast, likely causing extensive periods of low salinity conditions in Willapa Bay and Grays Harbor. Such an event would be expected to negatively impact burrowing shrimp, as evidenced by their widespread absence or low population numbers in areas where freshwater rivers enter Willapa Bay and Grays Harbor. The timing of the onset of these diminished Columbia River flows (1930's-1950's) corresponds well with observed increases in burrowing shrimp populations. WGHOGA requests that Ecology include some discussion of this anthropogenic impact in its discussion of factors that may have affected burrowing shrimp populations.

15. Off-Bottom Culture:

The SEIS includes a useful summary of efforts to use off-bottom culture in areas containing burrowing shrimp, and discusses the many market and processing differences between off-bottom and ground culture of oysters (page 2-8 – 2-9). WGHOGA appreciates both this discussion, and the willingness of Taylor Shellfish to share some of its experiences and perspectives on these issues. Nonetheless, WGHOGA expects that some reviewers of the SEIS will submit comments claiming that off-bottom culture is a viable alternative to the purpose and objectives of the proposed permit and the alternatives analyzed in the SEIS. Accordingly, WGHOGA believes Ecology needs to have a more thorough discussion of this topic in the final SEIS. WGHOGA believes the following points should be emphasized:

- Section 2.2 of the SEIS states the purpose and objective of the proposed action: to preserve, restore, and maintain the viability of clams and oysters on commercial shellfish beds. Off-bottom culture was not considered as an alternative in the SEIS because it would not meet the purpose and objectives of the proposed action.

- Off-bottom culture in areas with burrowing shrimp is experimental. Past areas of off-bottom culture have failed when shrimp are present because the substrate is too soft to support the poles, ropes, bags and wires associated with such culture. Often these failures occur slowly over several years so that initial reports of success are ultimately deemed to be failures. In short, off-bottom culture is not a viable alternative for areas containing moderate or high densities of burrowing shrimp.
- WGHOGA confirms what Ecology was told by Taylor Shellfish: the shucked meat market associated with bottom oyster culture and the off-bottom shellfish market "are entirely different products, culture systems, processing, and markets" (SEIS page 2-8). It would be very difficult, and expensive, for WGHOGA members that have applied for the proposed permit to make a shift away from ground-based culture. Furthermore, this would result in large disruptions in the shellfish market, to on-shore processing and support services, and to the local economy of communities surrounding Willapa Bay and Grays Harbor.

16. Clarification on the Pellet Form of Imidacloprid

The SEIS in numerous places discusses that the pelletized version of imidacloprid "dissolve[s] on contact with water from the incoming tide" which would act to limit or prevent accidental ingestion of these pellets by birds or other animals (e.g., SEIS page 1-23). WGHOGA agrees with this assessment within the SEIS, but nonetheless wishes to provide two clarifications based on their collective experience using the pelletized version. First, even when the tide is completely out the surface of the sediment where pellets are contains enough water to result in dissolution of the pellet within a few seconds. Second, the commercial formulation used by WGHOGA (i.e., Mallet) is composed of small particles that have an appearance like coarse salt. Most of these particles are smaller than the visual image generated by the word "pellet," which helps to explain their rapid dissolution on contact with water. WGHOGA will continue to work with the supplier of this material to refine the breakdown characteristics as part of its IPM plan to deliver the maximum efficacy with the minimum level of treatment. Related to refining chemical treatment methods to maximize efficacy, WGHOGA incorporates by reference the analysis performed by Dr. Kim Patten as part of the applications submitted in support of the Sediment Impact Zone Authorization where he analyzed various methods of treatment and resulting efficacy to further this IPM goal of maximum efficacy with minimum treatment amount.

The SEIS in numerous places also discusses that the pelletized version of imidacloprid could be spread by boat. This is correct, but WGHOGA may use a variety of methods to apply the pelletized version, including by hand, or using motorized ground equipment. The discussion of application techniques on page 2-6 of the SEIS is correct in listing a variety of techniques will be used to apply imidacloprid under the proposed permit, whether using the granular or liquid form of this pesticide.

WGHOGA also notes that the SEIS, on page 2-14, indicates the granular form of imidacloprid "would be applied to shallow standing water over commercial clam and oyster beds." WGHOGA wishes to clarify that the pelletized version of imidacloprid may be applied to beds with a wide range of shallow water depths, although most are expected when water is 2 feet or less.

17. Clarification on Partial Treatment of Plots

The SEIS correctly notes that WGHOGA members "request flexibility in being able to only partially spray some plots" (SEIS page 1-3). Although likely obvious, especially given the discussion concerning WGHOGA's IPM plan (Comment 1), WGHOGA wants to clarify that this means than on any given legal parcel, the growers may wish to treat only a subset of that parcel. For example, portions of a parcel may not have high densities of burrowing shrimp, and thus would not need treatment. This flexibility will allow growers to evaluate each parcel based on its site-specific characteristics, and to adopt a range of management approaches based on those characteristics consistent with the goals of IPM plan.

18. Clarification on Use of Personal Protective Equipment by Applicators

The SEIS includes many references to the use of personal protective equipment (PPE) by personnel involved in the application and handling of imidacloprid. To avoid confusion WGHOGA wishes to clarify that the PPE requirements that legally apply are those associated with the pesticide label and registration documents. Page 3-8 of the SEIS is an example where this is correctly referenced. Although applicators may choose to use more PPE than that specified by the label, WGHOGA wants to ensure that the SEIS does not imply that the proposed permit will impose new or different PPE requirements than those on the label and registration documents.

19. Impacts of No Action Alternative

The SEIS (page 2-24) includes a useful summary of efforts to use off-bottom culture in areas containing burrowing shrimp, and discusses the many market and processing differences between off-bottom and ground culture of oysters (page 2-8 – 2-9). WGHOGA appreciates both this discussion, and the willingness of Taylor Shellfish to share some of its experiences and perspectives on these issues. Nonetheless, WGHOGA expects that some reviewers of the SEIS will submit comments claiming that off-bottom culture is a viable alternative to the purpose and objectives of the proposed permit and the alternatives analyzed in the SEIS. Accordingly, WGHOGA believes Ecology needs to have a more thorough discussion of this topic in the final SEIS. WGHOGA believes the following points should be emphasized:

- Off bottom techniques used in Willapa Bay and Grays Harbor were developed to utilize areas of the bay where bottom culture was not feasible, for instance in high-current areas, or areas otherwise not suitable for bottom culture.
- Areas of the bay heavily infested by shrimp will not support any type of oyster culture because both bottom culture and the equipment associated with off-bottom culture will both eventually sink into the shrimp-infested mud.
- There are areas of the bay where long-line or other off-bottom techniques are already sinking due to shrimp infestations, reinforcing the conclusion that off-bottom culture techniques are not a viable alternative to a shrimp control program.

20. Resubmission of FEIS Comments

WGHOGA is aware that the National Ocean and Atmospheric Administration (NOAA), and US Fish and Wildlife Service (USFWS) intend to submit comments on the SEIS. Ecology will recall that NOAA and USFWS also submitted comments on the FEIS. These comments were extensive, and in WGHOGA's view, contained many inaccurate statements and conclusions that were not supported by either the details of the proposed permit at the time, or the information and analyses in the FEIS. If Ecology recalls those comments, it may not remember that WGHOGA submitted responses to the NOAA and USFWS comments in time for them to be included in the official record for the FEIS. Although WGHOGA does not know what NOAA and USFWS intend to submit in the way of comments on the current permit and SEIS, it is not unreasonable to expect that some of those comments will be the same or like those they submitted previously. Accordingly, WGHOGA has included as Attachments C and D to these comments unedited copies of the responses it prepared and submitted for the FEIS. We hope that these responses provide useful information to Ecology as it works to address the new NOAA and USFWS comments on the SEIS.

Again, WGHOGA greatly appreciates the opportunity to submit these comments on the Draft SEIS, and looks forward to continuing to work Ecology during this permitting process.

Sincerely,

Douglas Steding, Ph.D.

Attachments (4)

Bay Center Mariculture Co.
PO Box 356, Bay Center, Wa. 98527
Ph. 360-875-6172 Fax 360-875-5937
bcfarms@baycenterfarms.com

ATTACHMENT A

Comments on Draft SEIS on the use of the nicotine based pesticide imidacloprid to control burrowing ghost shrimp (*Neotrypaea californiensis*) in Willapa Bay and Grays Harbor.

To: Derek Rockett, Permit Writer
Washington State Department of Ecology
Water Quality Program
PO Box 47775
Olympia, WA 98504-7775
http://ws.ecology.commentinput.com/?id-aelUM

Commenter: Richard Wilson, Ph.D.

November 1, 2017

BURROWING GHOST SHRIMP DISRUPTION AND DESTRUCTION OF THE INTERTIDAL AREA OF BIOTIC PRODUCTIVITY IN WILLAPA BAY

Willapa Bay: A unique shallow intertidal marine sedimentary basin
where the combination of geological and biological aspects
unite to create a bountiful sustainable food web
until reduced or eliminated
by the burrowing ghost shrimp, *Neotrypaea californiensis*

Introduction: The Draft SEIS fails to inform the readers of the extent of physical and biological damage the burrowing decapod, *Neotrypaea californiensis,* aka ghost shrimp, are imparting on the intertidal benthic habitats of Willapa Bay and Grays Harbor. Their damage effects many more estuarial species besides the oysters and clams. The ghost shrimp have a negative impact on the basic food web so vital to the health of these two important near shore marine areas. The detail of why this benthic area is important and how it operates is left unexplained. It does not mention the important role of how the silicate mineral sediments are converted and which biological groups are important to build the food web. This in turn requires knowledge of these interacting physical and biological aspects and how we must recognize and manage for biotic productivity. Especially important is recognizing the importance of the micro benthos and the dependence of the estuary biota on those populations. What should be proposed in the draft SEIS with the studied use of imidaclopid is recognition of these important relationships and attempting to apply a management strategy to benefit the entire benthic biota. Destruction by ghost shrimp expansion is far greater and widespread than oysters sinking into a sedimentary colloidal hydrogel of fine sand.

General Benthic habitats: Mud, Oysters and Eelgrass: Many research papers have used specific benthic characteristics to define and then evaluate various intertidal estuary habitats for important biotic factors such as productivity. Following from Hosack, et al., 2006, and their sampling study this comment paper will also discuss the Willapa intertidal as; 1) open **Mud**, 2) **Eelgrass** dominated areas and 3) areas with **Oyster** crops on the mudflat (Fig. 1) and the ghost shrimp impact on each.

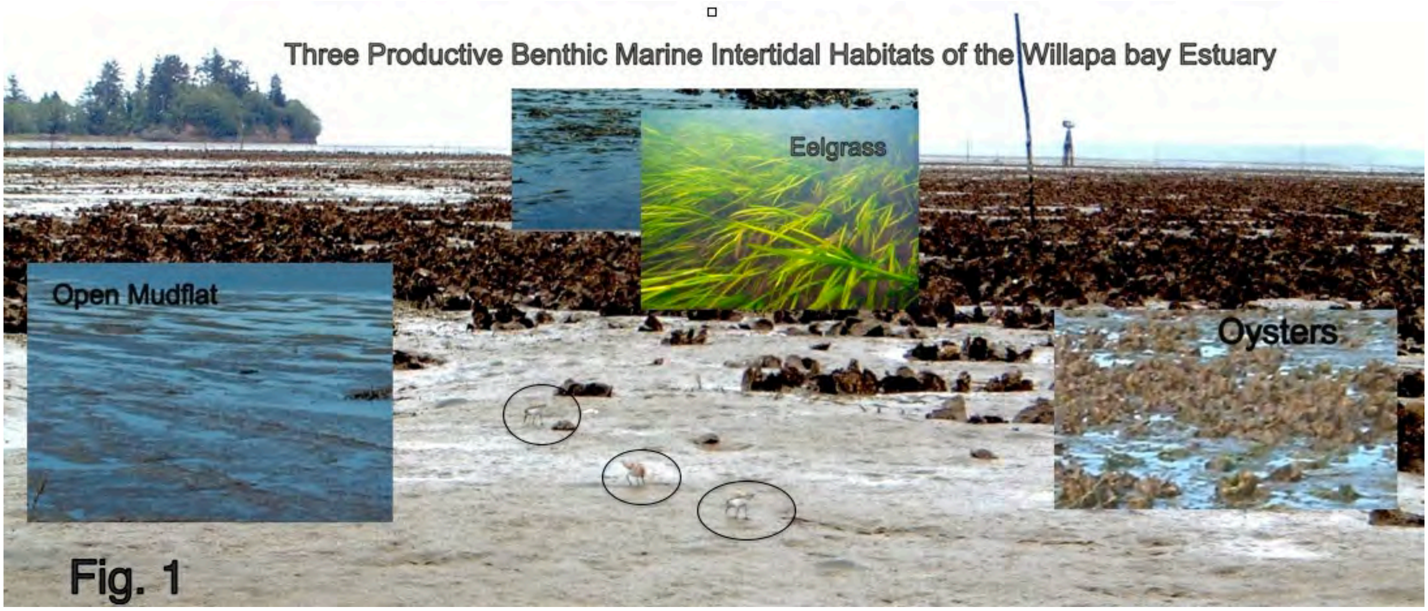

Three Productive Benthic Marine Intertidal Habitats of the Willapa bay Estuary

Fig. 1

First we need to realize the diversity and biomass of the species utilizing the intertidal benthic habitats in the two coastal estuaries. Even without shellfish the numbers of species and their abundance, with most being microscopic, is extraordinary. The benthic habitat is where a unique set of physical and environmental aspects come together to provide the conditions to allow basic primary productivity. It is the setting where igneous silicate minerals and fresh water (rain) with atmospheric carbon dioxide react to extract necessary nutrients for the photosynthesizing benthic diatoms to make the carbohydrates and fatty acids (lipoproteins) upon which to build the food web so essential to all the trophic levels such as the more familiar and visible like fish, birds, shellfish and crabs. Starting with the chemical change known as weathering, which results in freeing soluble materials essential for diatoms to create nutrients and form their frustules (shells,tests). The SEIS seems to point only to damage of bivalve shellfish sinking in the bioturbated sand made soft by ghost shrimp. Those are true impacts on growing shellfish on those areas but is only one relatively small effect. It seems misleading in the negative impact to most other members of the benthos (flora and fauna), which most owe their existence to the benthic contribution the sediment surface provides. We need to understand primary productivity as is instigated by microscopic single celled photosynthesizing benthic diatoms. These are the key combiners and converters of solar and nutrients into essential carbohydrates and lipoproteins. It is important to note the important sediment interface to the marine water in the intertidal presents the closest and best position to combine essential nutrients, solar and soluble silica for the diatoms. This unique combination provides the base of the food web. These benthic diatoms and the biofilm they create are reduced then removed by the activities of a burrowing decapod, the ghost shrimp (*Neotrypaea californiensis*). The important habitat for healthy productivity is the stable sediment surface, which ghost shrimp over time can reduce and then eliminate. The diatoms need those important nutrients that are derived from the igneous silicate mineral sands to produce and package for passage up to the higher trophic levels.. thus sand to shorebirds. Diatoms are key to nearshore marine productivity.

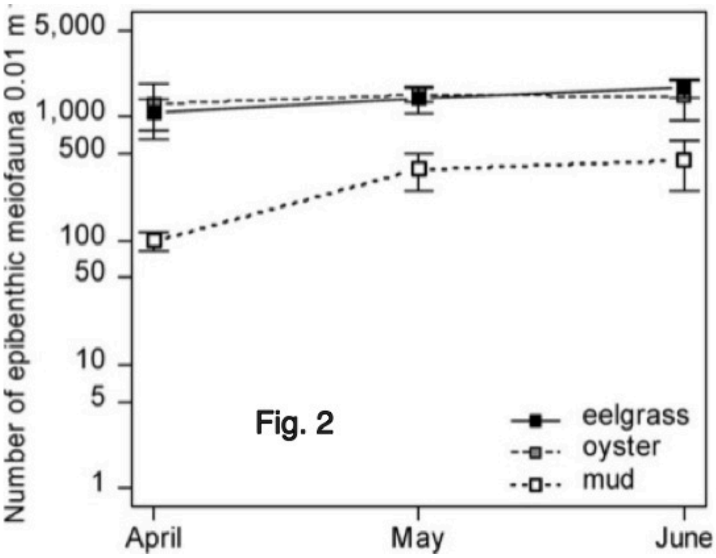


Fig. 2

The three habitat types by Hosack, et al., are based on what the sediments are supporting which in turn can depend on tidal elevation, currents, etc. In general, all require a relatively stable sediment surface made such with adequate proportions of smaller components to stabilize the fine sand (Fig. 2 - meio - between micro and macro). It did not account for the important benthic microscopic assemblage.

The important aspect of the nearshore intertidal is what builds the food web, especially the more microscopic benthic components as they form the base of the nutritional sequence. How this compares among the general habitat types will be noted. The ghost shrimp reduce or eliminate both the micro and macro components. The focus is to call attention to the extensive benthic phytoplankton (primarily diatoms) and their significant presence on the nutrient rich silicate mineral sands. It must be noted that often the more highly productive intertidal areas are combinations of mud, eelgrass and shellfish (Fig. 3). The object is to maintain this balance. The clean sediment surface of the intertidal is normally coated by the abundant benthic diatoms and their biofilm. In this regard there are over 80 species, not seen without microscope, identified from the intertidal mudflats of Willapa Bay (Hemphill-Haley, 1995). This productive primary level of the food web is adapted to the daily tidal changes between aquatic and atmospheric and the corresponding fluctuations in salinity, temperature, etc. As will be noted the combination of rain and CO_2 (carbonic acid) on the exposed igneous minerals creates critical components, which will carry through the various levels of the food web. Although even with extremes of changing from aquatic to atmospheric critical exposure to aspects such as solar and temperature at times probably benefit primary productivity.

Oysters and Eelgrass habitats exist due to the stable nutrient rich silicate intertidal sediments. The basis seems to be the open mud or mudflat that appears barren with respect to larger biotic elements, but retains surface areas open to the rain and sun. Overall the key importance of the intertidal sediments and the phytoplankton adaptation to it must be understood and that action or protection be carefully undertaken should this cease to be the case. The burrowing ghost shrimp through bioturbation can change the intertidal sediments and reduce or eliminate the benthic diatom productivity essential to initiate the food web.

Mud Habitat: What the currents transported**.** The mudflat**,** basically a fine grained igneous silicate mineral sand and silt is reflective of the change in gravity from stream transport when reaching

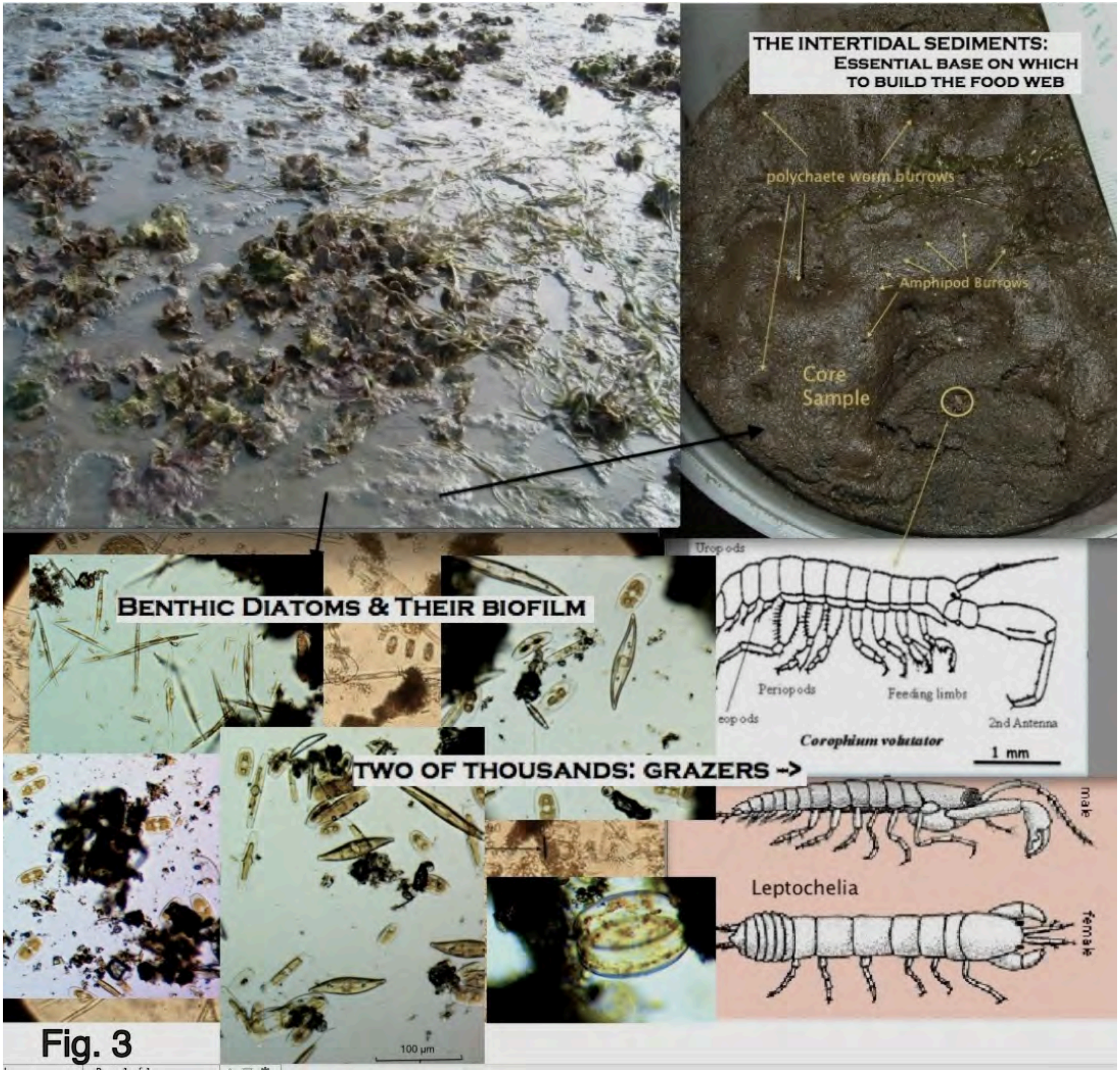


Fig. 3

sea level in the estuaries. Depending upon relative position some organics and another product of weathering, a clay mineral, will add and help stabilize the mix. The tenet here is that a major basis of productivity in both the eelgrass and shellfish (oyster) habitats is the underlying fine grained clastic sediment - the Mud. They each depend on the other but the biotic productivity of the open mud seems to be key to initiate a productivity role for eelgrass and oysters on the sediment surface. Sampling was done on each and will be discussed as such but are integrally connected to the geology and chemical breakdown of the silicate minerals. The following pages will consider all three habitats (Mud, Eelgrass and Shellfish) as dynamic and interchangeable areas of the mudflat and when occupied often take on different but interconnected roles.

Eelgrass Habitat: A closer look on a blade**:** Eelgrass, a seagrass, is used to define a specific type of intertidal area and often is held in high esteem as to habitat value for the biota of the estuary (Fig. 2). However, this rooted seagrass seems to play conflicting roles. Thick eelgrass growth shades the benthic sediments and decreases mudflat productivity that would benefit the other biota. It can block or divert vital tidal currents and allow a composting deleterious layer to form over the silicate sediments eliminating diatoms and over time elevate the intertidal area. However, the long fast growing blades of eelgrass seasonally provide an amazing microbiotic habitat as they become coated with diatoms, their biofilm and microfauna. Eelgrass in its growing season, then serves to increase benthic habitat with diatom attachment areas. Where diatoms are present, consumers will collect and as on the sediment surface, become the prey. The individual members of this epiphytic coating probably also resuspend as do those on the open benthic surface.

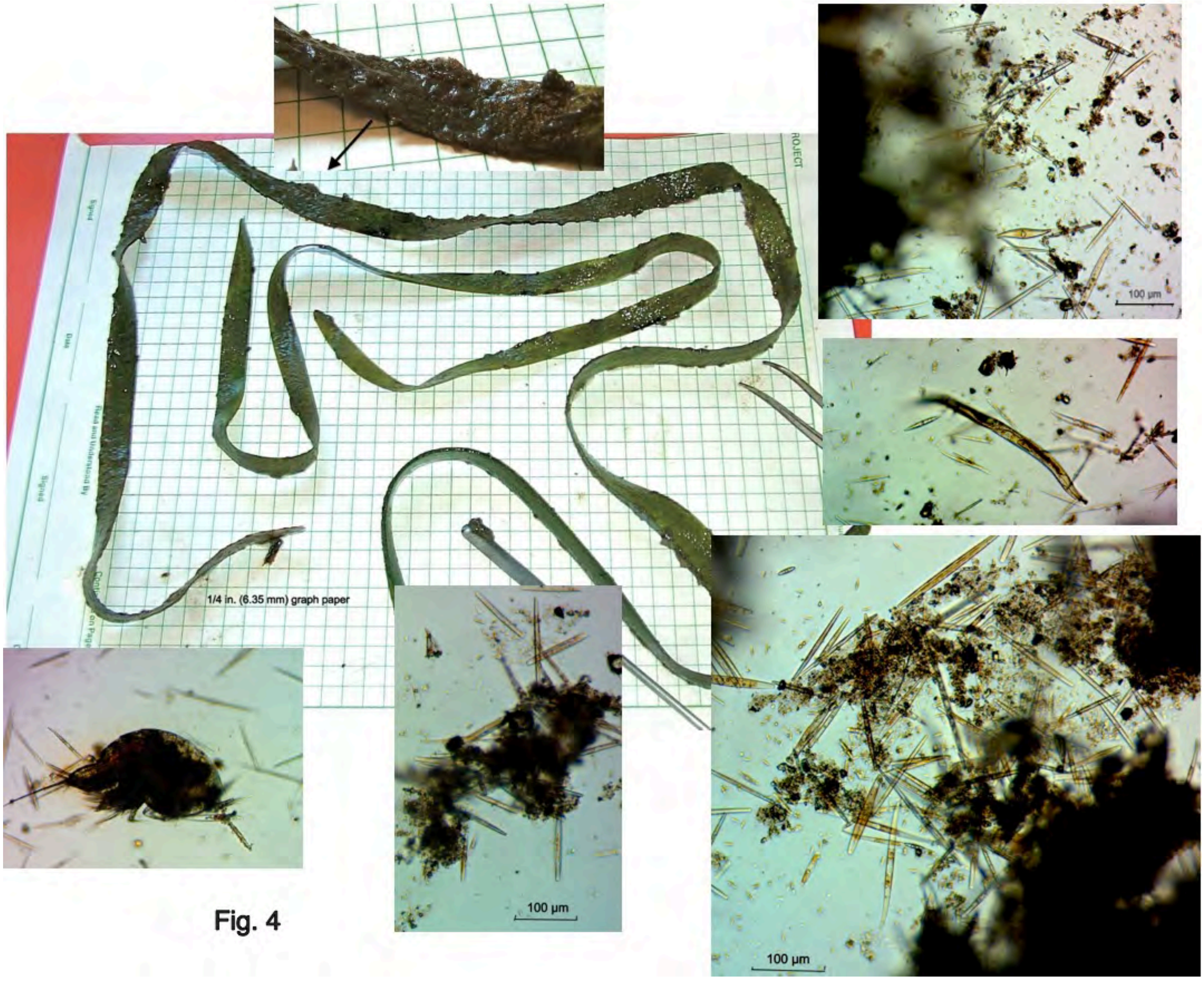


Fig. 4

Samples of surface coating were scraped from eelgrass blades and diluted with seawater to free diatoms and invertebrates from the biofilm coating. Using the eelgrass blade (Fig. 4) an array of the larger mobile pennate (pointed) diatoms with numerous smaller diatoms in the 5-20 µm range were noted. Invertebrates were intermixed within the biofilm and likely were important prey for higher trophic levels such as fish and crabs or birds at low tide. Since this biotic assemblage especially diatom morphologies, on the blade is very similar, if not identical, to that observed on the open benthic sediments, it is assumed it was inoculated from the adjacent mudflat. Since benthic diatoms on the mudflat seem to remain active all year, those moving with the tide would then be available to catch and grow on eelgrass blades when they are seasonally available.

O**yster Cluster -** A Place of Attachment**:** Following are photomicrograph images of surface samples from the pictured oyster cluster. Fairly large three year old cluster with five live oysters. All eight areas sampled for microscope examination, including the underside shell areas, had fine sediment and micro organisms, especially diatoms. Again, it seems the organic biofilm is key to the adhesiveness of this coating of diatoms, sediment particles and invertebrates. It also seemed

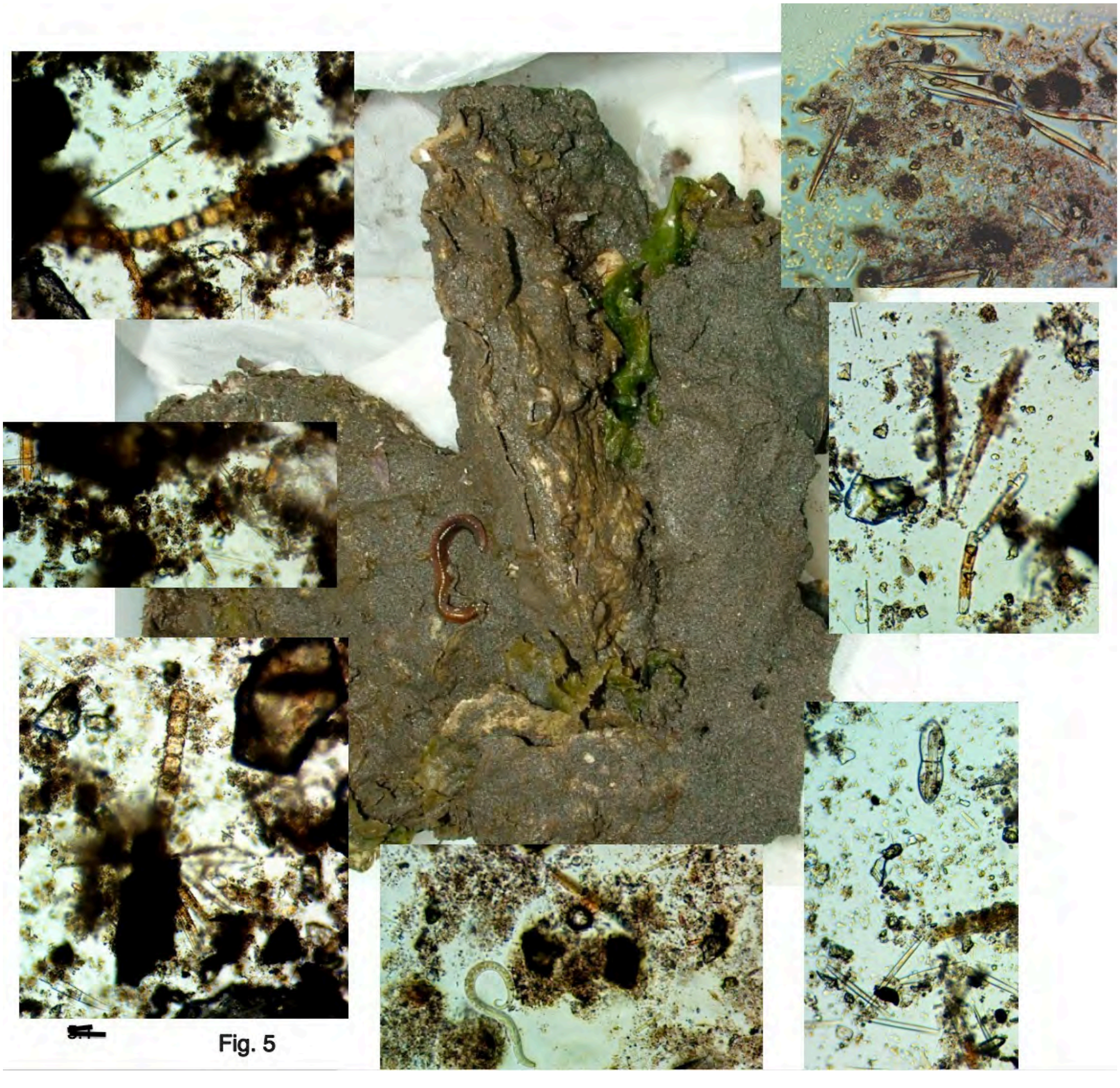


Fig. 5

different diatoms types were on different sampled areas. Although the oyster cluster covered some measure of area of the benthic sediment surface the additional attachment surfaces created was probably at least three times greater. The oyster clusters allow diatom and biofilm access to nutrients and sunlight. Note worm that had evacuated its burrow between two oysters, plus the barnacles and macroalgae (*Ulva*?). The microscope image dimensions are generally within a 200-300 µm range.

Igneous Silicate Mineral Sand: When the igneous structures or deposits being uplifted become exposed the silicate minerals crystalized under high pressure and heat that comprise these andesite/basalt igneous rock types begin to undergo a chemical change, e.g. they weather. Those minerals are unstable under normal atmospheric pressure and temperature. Some with iron oxidize but the important chemical change is with freshwater (rain) and carbon dioxide to make available the essential components to support diatoms on which to build the food web (Fig.6). This needs to be understood to realize what the burrowing ghost shrimp modify thus reducing the estuary productivity. First the history of this region plays a big role.

The west coast of North America from California to Alaska holds a geologic history dominated by volcanism ranging in age from Recent back to over 60 million years. For most of that history what is now the western portions of Oregon and Washington were subsiding and were covered by marine waters. There was neither Cascade mountains or Coast Range until later in this sequence. The global crust under this region sink by as much as 3 km (± 10,000 ft.) below sea level allowing it to fill with igneous rocks and sediments derived by underwater volcanism and island volcanoes. When the subsiding of the crust stopped about 20 million years ago, uplift and volcanic activity; in response to the the crustal fracturing would provide for the beginnings of the Coast Range (includes Willapa Hills bordering Willapa Bay). A north-south line of volcanism which continues today is the Cascade Range running from northern California to Canada.

The millions of years of volcanic deposition and now by uplift allowed the various watersheds to transport and accumulate igneous silicate mineral sands and silt as deposits in Willapa Bay. Weathering of these igneous rocks upland keep a rich supply of nutrients in the streams and ground water into the bay. Perhaps the most important process in primary marine productivity is the chemical change (a process of weathering) for exposed igneous origin silicate minerals. The igneous minerals, which crystallized under extreme heat and pressure are unstable at surface temperature and pressure. They can be altered by oxidation or more commonly, the process of hydrolysis. Fresh water (rain) and CO_2 (carbonic acid) will chemically breakdown the igneous silicate minerals when exposed (Fig. 6). The millions of years of igneous intrusions, features and deposition of fine grained igneous silicate rock pieces (clastics) allow this important relationship. Our igneous rock types with relationship to silica content ranged between andesite and basalt (chart) over the 60 plus million years of activity.

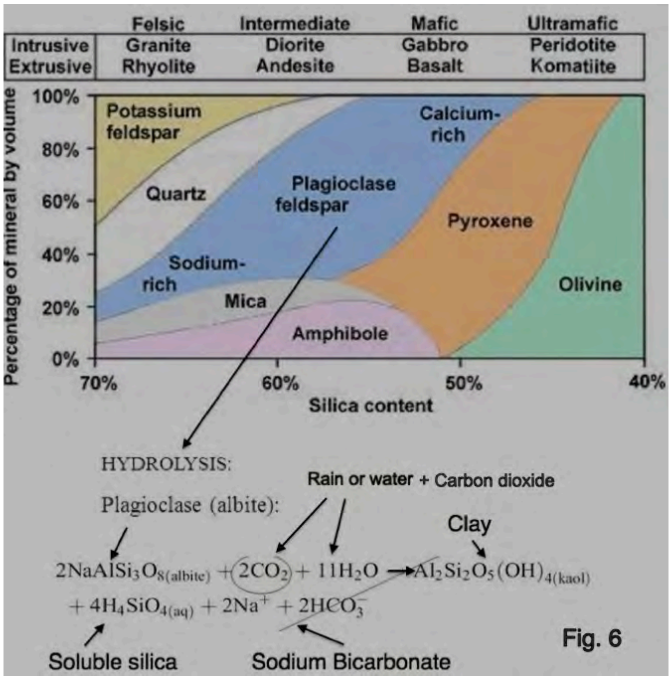


Fig. 6

The mafic silicate minerals, those higher in iron and magnesium and lower in silica, are generally most easily weathered by the process of hydrolysis as shown (Fig. 6) This is the same for the other igneous silicate minerals with exception of quartz. As example, a plagioclase, which from the chart can be seen as a dominant igneous mineral group provides an example. Important here are the stable end categories of most silicate minerals; a clay, soluble silicate (silicic acid) and important soluble cations, e.g. Ca, Mg. Fe, Al, Na, Mn, Li, Ti, Zn, Cr, etc. These then are critical for and will be available to the benthic diatoms and thus the marine food web.

The rivers and creeks transport the partially eroded igneous silicate minerals as clastics (sand and silt) plus some weathering products like clay and soluble silicate out on to the mudflat. Unique for Willapa Bay are the numerous relatively small watersheds cut into the Coastal range, which feed out onto the intertidal. This is unlike most west coast harbors or bays where one larger river enters the ocean and after long transport many of the minerals abrade away leaving primarily quartz sand. When transported sedimentary material reaches sea level where a loss of gravitational energy causes the larger clastics to deposit out first with the smaller and lighter fraction depositing further out into the mudflat. The igneous silicate fine sand, silt and clay deposit make up the mudflat. It will become the important base for the benthos to establish. Fig. 7 is of a mid Willapa Bay benthic mudflat sediment sample mixed in water and allowed to settle by grain size, shape and specific gravity in an Imhoff cone (left). The microscope sample (right) was taken midway to show the size and physical diversity of the different silicate minerals. Note the angularity indicating little wear by water transport in the short distance from watershed to the bay.

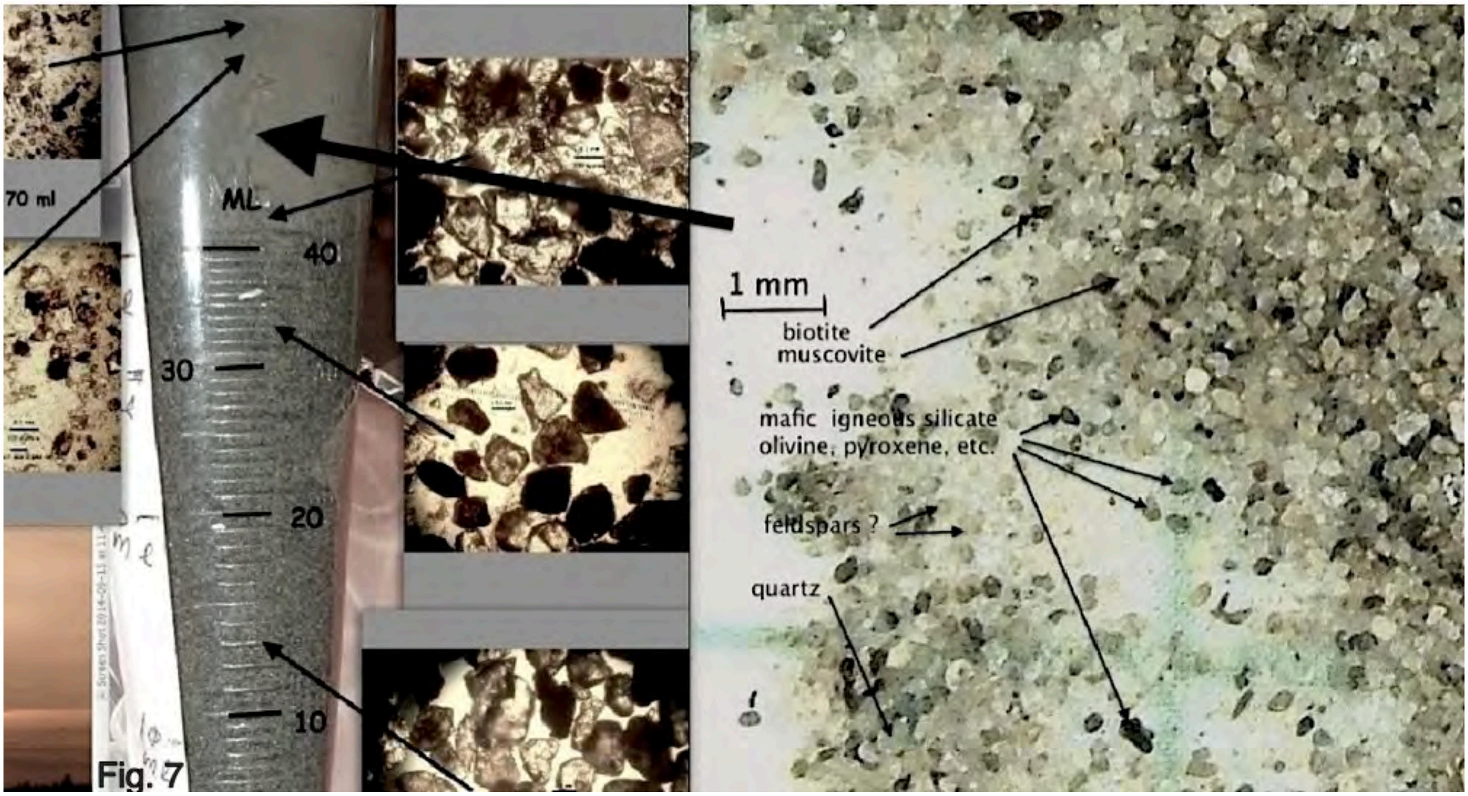


Fig. 7

Of special note is the formation of silicic acid from the hydrological chemical weathering process under atmospheric conditions. Silicic acid, a soluble silicon, is required by the diatoms to form their frustules (tests/shells). This important source of soluble silica from the watershed and probably the intertidal surface silicate minerals when exposed to rain at low tide. The ground water and streams from the Willapa Hills are rich in soluble silicate. Oceanic upwelling is often touted as the source of silicic acid and other nutrients to build the phytoplanktonic flora. Banas, et al., 2007, credits ocean upwelling to diatom abundance within Willapa Bay, which probably does at times contribute nutrients. Their testing strategy and report did not consider the abundant benthic diatoms as critical to the Willapa Bay food web. One problem with this ocean model for total diatom supply is that it does not account for nutrient loss (e.g. soluble silica) and replacement from upland streams and ground water through the process of hydrolysis to remain at stable levels. The huge abundance of diatoms on the sediment surface would indicate a closer source. Thus, an important input of useful minerals, elements and soluble silicate for benthic diatoms are the by products from the constant surface weathering of the uplifted adjacent Coast Range.

Benthic Diatoms: A look among the surface sand grains: Epipelic or benthic diatoms grow on or hang out near the surface of the intertidal sediments at the water/sediment interface. A field study of diatoms by Eileen Hemphill-Haley, 1995, along with taxonomic assistance from Kathleen Sayce, listed over eighty species of benthic diatoms on the intertidal mudflat surface of Willapa Bay. Sampling the biofilm on the sediment surface over the years shows the amazing diatom abundance and diversity which I photographed and posted as; *Benthic Diatom & Biofilm Habitat*. With optimal conditions such as solar availability and necessary nutrients diatoms can divide and double within days, while producing abundant carbohydrates and lipids and by sheer mass provide the basic primary productivity making the shallow and extensive intertidal areas of Willapa Bay so important. They form the base of the food web. The two images (Fig. 8 & 9) of benthic diatoms from surface mud samples, show part of the abundance and diversity even though samples have been diluted to free diatoms out of the sand grains and biofilm. The images (Figs. 8 & 9) represent ± one cubic millimeter in volume as they are in a Rafter cell that is one millimeter deep. Both summer samples are from the same oyster growing bed and show some of the variation in diatom morphology and size. If Fig. 8 sample represents say 500 diatoms per mm³ - how many over a square meter one centimeter deep? The smaller 5-20 µm diatoms in Fig. 8 are at a size we would culture for shellfish larvae.

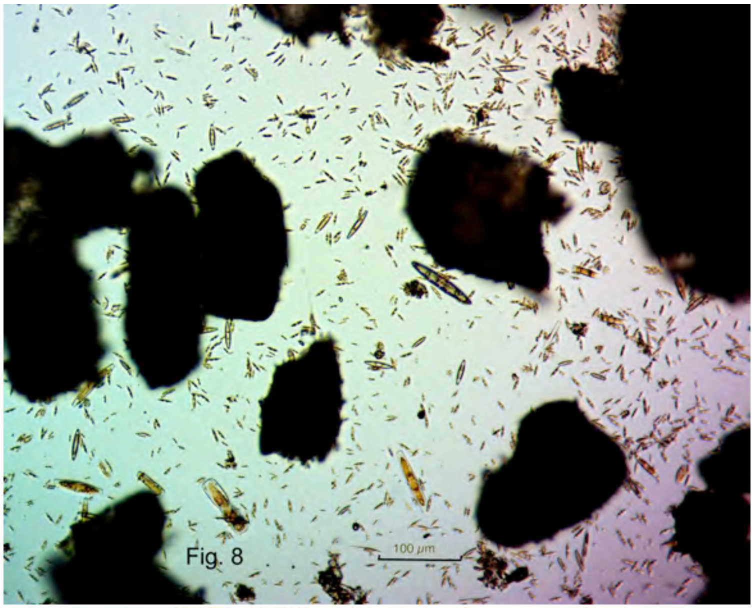


Fig. 8

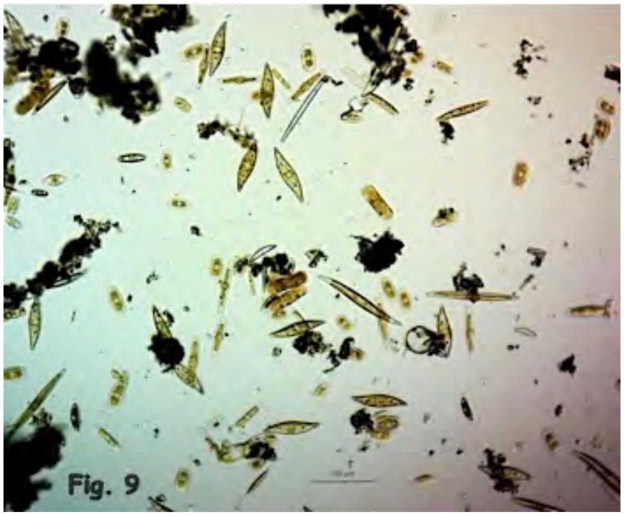


Fig. 9

Most benthic diatoms *are mobile* and can glide between sand grains and the biofilm they secrete to keep associated to the sediment surface or move across the sediment surface to new areas. They can re-suspend into the water column and can even move in mass over the sediment surface with currents or remain on the moist surface with biofilm during the low tide. With their biofilm the diatoms can coat objects or organisms including the blades of eelgrass or even ghost shrimp areas during winter months when the burrowing decapods are inactive. Diatom species have high tolerances for salinity and temperature differences with forms living in both fresh and marine waters or even on a wet surface. Marine shores with volcanic upland areas for nutrient supply (especially silicic acid) seem their forte. Mats of diatoms can remain on the moist sediment surface during the low tide and often are picked up by the flood tide and thus transported on the surface as organic slicks (Fig. 10). What is really interesting is how quickly these benthic diatom masses can establish on oyster growing areas which have been harvested and the silicate sand exposed. The fresh exposed sand within a few days or a week, will develop a rich new diatom and biofilm coating. Testing also confirmed that when the diatoms are numerous the zooplanktonic grazers are quick to find the food source. Recovery was rapid and with a stabilized sedimentary surface in spring and summer, new eelgrass sprouts within a few weeks from natural reseeding. This happens by reducing the adult burrowing shrimp which through bioturbation can reduce or prevent this whole renewal of productivity.


Fig. 10

Benthic Diatoms and their Biofilm: When a stable clean sediment composition is present the benthic diatoms drift in with the tide and utilize the surface. Many extrude quantities of an organic extracellular polymeric material (EPS). This organic mixture becomes the biofilm substance as it covers and creates an organic slime habitat over the sand and silt surface. The diatoms seem to use it for protective cover while according to research this sticky film holds in place finer sediments, provides organic media for a host of other forms such as bacteria plus provides an extra

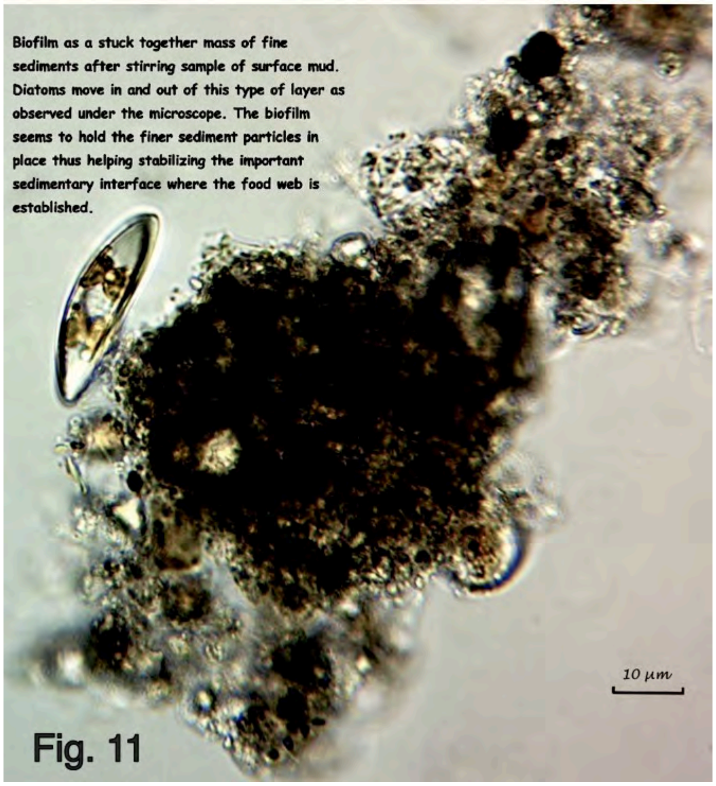

Biofilm as a stuck together mass of fine sediments after stirring sample of surface mud. Diatoms move in and out of this type of layer as observed under the microscope. The biofilm seems to hold the finer sediment particles in place thus helping stabilizing the important sedimentary interface where the food web is established.

Fig. 11

benthic nutrient source. Various research has EPS composed largely (e.g. ± 90%) of polysaccharides (carbohydrates) with the balance being lipoproteins. The polysaccharides vary and may be composed of neutral sugars, uronic acids, sulfonated sugars, or ketal-linked pyruvate groups. In short, this coating indicates stability of the tidal sediment surface with masses of diatoms using the habitat when ghost shrimp are not dominant.

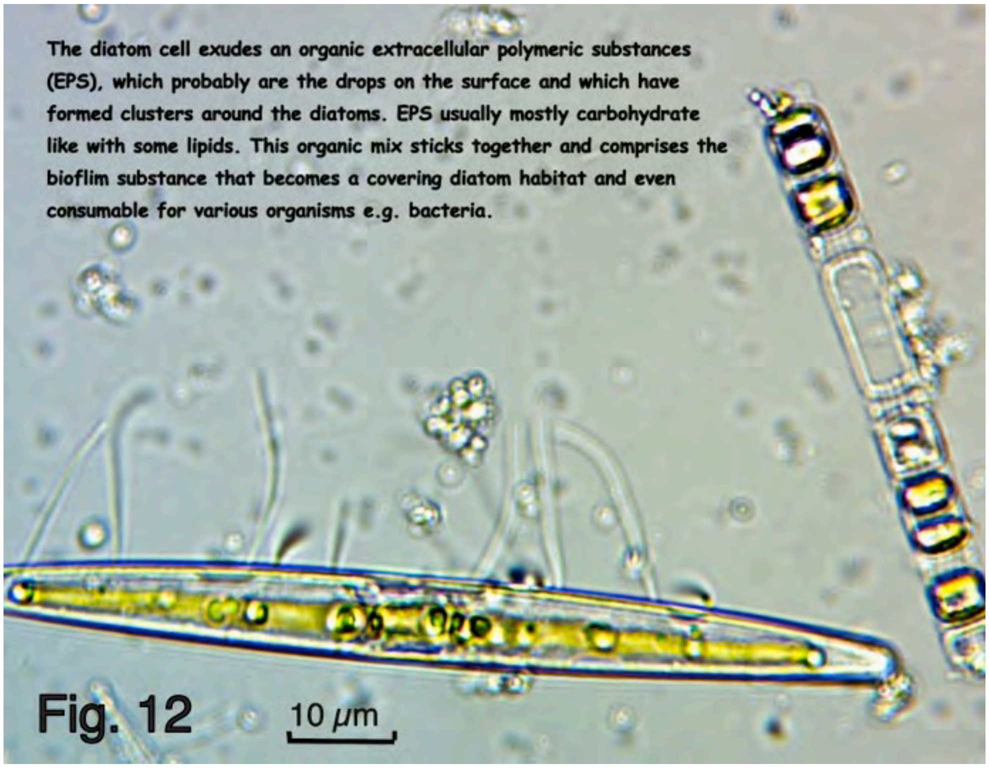

The diatom cell exudes an organic extracellular polymeric substances (EPS), which probably are the drops on the surface and which have formed clusters around the diatoms. EPS usually mostly carbohydrate like with some lipids. This organic mix sticks together and comprises the bioflim substance that becomes a covering diatom habitat and even consumable for various organisms e.g. bacteria.

Fig. 12

Why would the diatoms in general produce more carbohydrates and lipids than they can utilize or retain and end up extruding them out? Some reason they get rid of the more starchy carbohydrate material and retain the lipid faction while others suggest they jettison the carbs when nitrogen is more available for lipid formation. They might just produce more than needed when optimal conditions are present. Getting rid of some sticky carbs probably helps account for the high lipid content of diatoms. This surface slime (snot, film, etc.) provides habitat for other biotic elements such as microbial forms, worms, etc. Diatoms have an animal like urea cycle which allows efficient use of carbon and nitrogen from the environment thus opening pathways for producing high-energy fatty acids (lipids) along with carbohydrates. Some in the science world refer to them as metazoans which is interesting but for the estuary, their primary function is photosynthesis and food production. See L. J. Stal & J. F. C. de Brouwer, 2003 for discussion on the biofilm production by diatoms. Keep in mind ghost shrimp prevent or remove biofilm formation.

Diatom Consumers: Attraction to the intertidal surface**:** The next step in forming the food web are the primary consumers. Their role is to consume the lipoproteins and carbs in diatoms and in turn become the prey for higher trophic levels. There are dozens of different invertebrate groups which are attracted to the rich pickings of a stable tidal flat. Many invertebrates move onto the area (the grazers) with the tide. Some invertebrates filter out the benthic diatoms from the tidal currents e.g. shellfish and yet others have adapted to remaining on the intertidal surface during the tidal cycle.

Because of their abundance as shown by extensive sampling an informative example involving benthic diatoms and a crustacean, *Corophium*, will hopefully exemplify importance of diatoms for grazers, (drawing of this amphipod, Fig. 3). *Corophium* remains when the tide leaves the mudflat because it constructs a burrow, however, different in many respects from that of a ghost shrimp. First the abundant small *Corophium* is unlike the destructive burrowing of the ghost shrimp, with a lined burrow about 10 cm (± 4 inches) deep. They give us verification of the richness of the mudflat if ghost shrimp are not abundant. This amphipod at normally over 10,000 per m^2 is dependent upon the thousands of benthic diatoms they can reach from their burrow during a daily tide (Fig. 13).

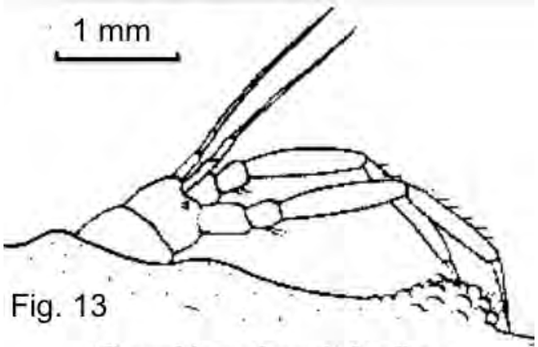


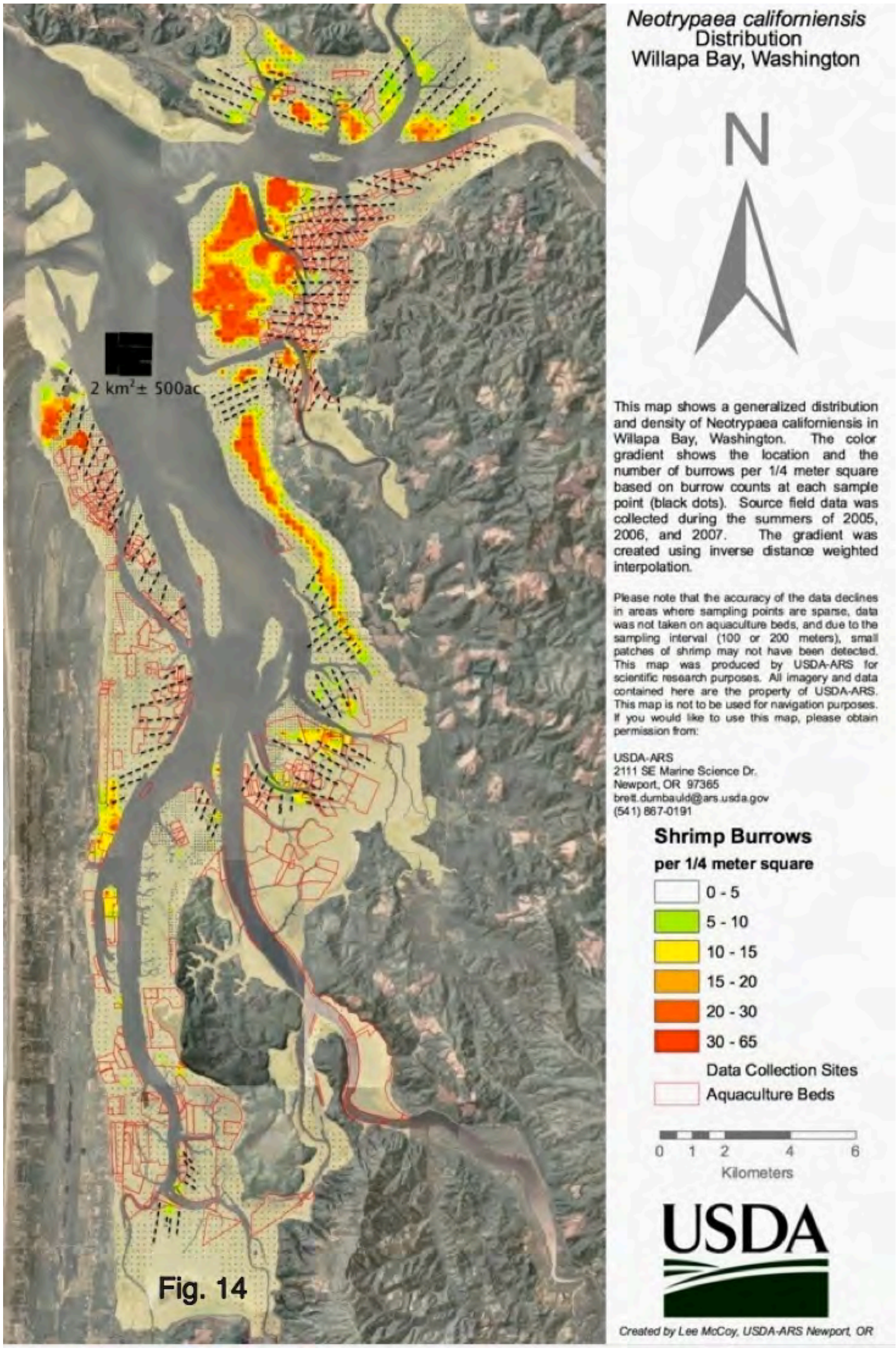
Fig. 13

***Corophium* deposit feeding**
(After Meadows and Reid, 1966)

Corophium, has been the object of extensive investigation due to their importance as a key food source for shorebirds. The decades of study of *Corophium* on the Bay of Fundy and relationship to shorebirds are summarized by the Bay of Fundy Ecosystem Partnership report, *Corophium*. They term this amphipod "Master of the Mudflats" and a Keystone species. Their report, is very interesting reading with many researched aspects of its life, such as each *Corophium* reportedly consumes up to 4,000 benthic diatoms per day, which have to be within reach of the burrow or within a few millimeters (Fig. 13). Illustrations have this amphipod feeding on deposits (detritus) from around the burrow opening but it was later found diatoms were the major nutrient source. This sand flea relative can masticate (chew up) the silica shells thus they could not be identified from stomach samples. Thus, the necessary daily diatom availability, abundance, movement and fecundity are further proved when *Corophium* is present. Also, this key crustacean would not be present in the surface sediments in that abundance if the sediment stability was not present for them to construct and keep a lined burrow. Then their importance as prey for higher trophic forms such as birds and fish. The report on the Bay of Fundy holds a single sandpiper to gain the body weight to migrate, would ingest 10,000 to 20,000 *Corophium* per tidal cycle. Thus, available exposed stable, tidal flat areas with few ghost shrimp are very important especially in the spring here in Willapa Bay when the lower daylight tides open up more feeding area. However, these lower elevation areas are also preferred by the ghost shrimp and if not controlled, will eliminate both diatoms and C*orophium*. This has happened on thousands of public intertidal mudflat acrea and now many oyster farming acres (Fig. 14).

Ken Brooks presented his extensive sampling data and analysis of changes in Arthropod and Mollusk populations before and after application of a pesticide to control ghost shrimp. His sampling protocol and data presents numbers for *Corophium* and *Leptochelia* along with many other invertebrates, on different oyster beds and a control area. His data sheets *Willapa Bay data*, show abundance declining as ghost shrimp numbers increase. They also show the fast recovery about seven weeks post treatment back to the greater than pre-treatment numbers when *Corophium* climbs to over >20,000 per m^2. Most likely *Corophium* is taking in a combination of diatoms and biofilm with the latter being basically a carbohydrate. Thus, the benthic diatom availability, abundance, movement and fecundity are firmly established by the numbers of this amphipod as reported by Ken Brooks. In fact the health of the mudflat might be judged by abundance of *Corophium* and the tube dwelling *Leptochelia*. Beside the diatoms being unable to exist on the ghost shrimp bioturbated mudflat *Corophium* cannot construct a lined burrow in the loose fine sand created by this burrowing decapod and a decrease or end to a key prey species for fish and sandpipers.

Numerous other invertebrate adults and larvae and whether grazing or filtering, including benthic shellfish, are dependent upon the benthic diatoms or their organic biofilm coating and a firm intertidal substrate. It is important to note, while using one well studied, crustacean as example, numerous other adult and larval invertebrate species make up a mass of epibenthic grazing or filtering predators on benthic diatoms. Examples are obvious from Brook's data sheets (see link above). If ghost shrimp numbers were low, the small benthic Arthropods and Mollusks would normally include about twenty species with often over 30,000 individuals/m² in or on the top sediment layer, biofilm or surface water when the tide is in. Many other invertebrate groups are also present, for example the various worms would contribute greatly to the biomass. Larger consumers of benthic diatoms, of course, includes shellfish. Point here is the benthic diatoms must be a major source of nutrients to a huge important segment of the estuary biota and thus provide the base of the food web. The ghost shrimp bioturbation will reduce or remove this important process from the intertidal sediment surface.



This map shows a generalized distribution and density of Neotrypaea californiensis in Willapa Bay, Washington. The color gradient shows the location and the number of burrows per 1/4 meter square based on burrow counts at each sample point (black dots). Source field data was collected during the summers of 2005, 2006, and 2007. The gradient was created using inverse distance weighted interpolation.

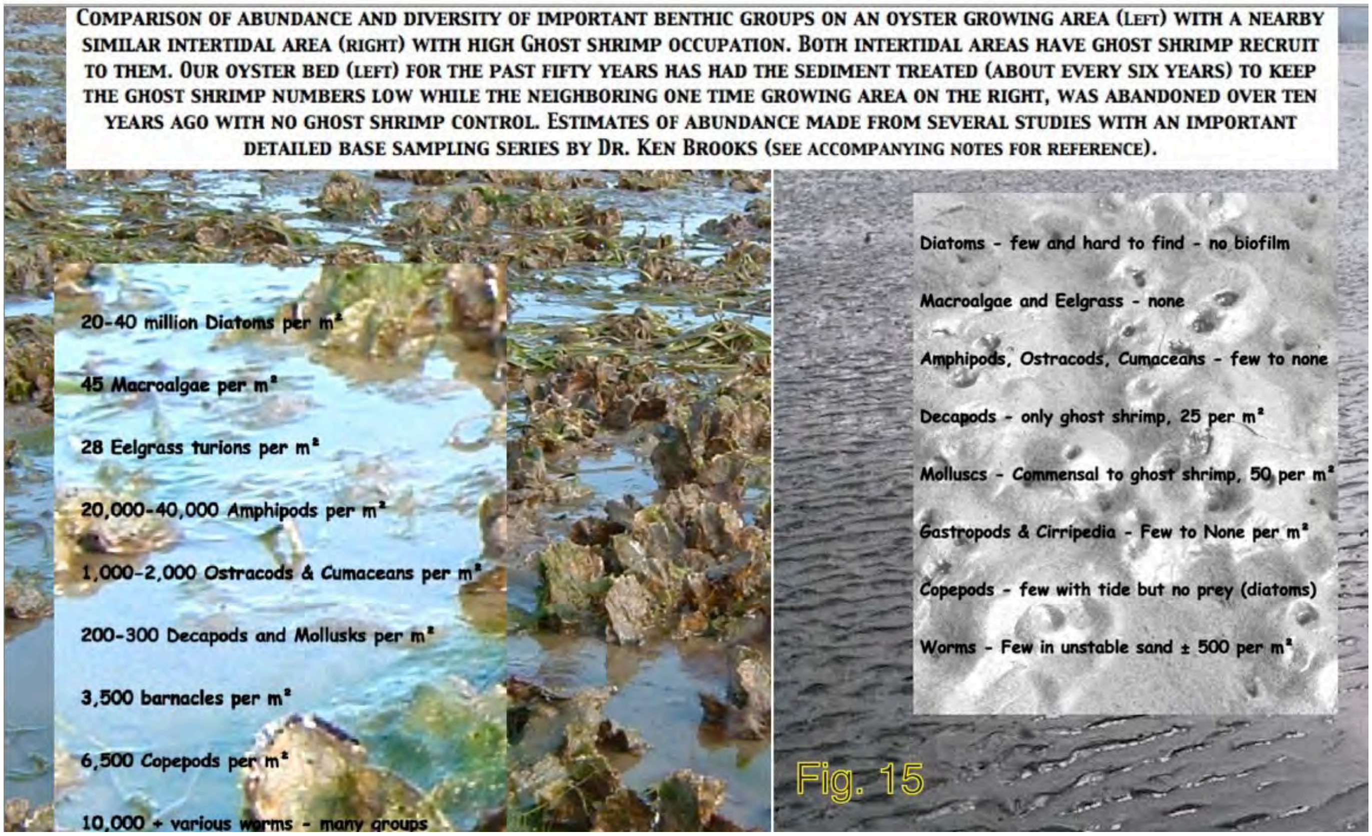
Please note that the accuracy of the data declines in areas where sampling points are sparse, data was not taken on aquaculture beds, and due to the sampling interval (100 or 200 meters), small patches of shrimp may not have been detected. This map was produced by USDA-ARS for scientific research purposes. All imagery and data contained here are the property of USDA-ARS. This map is not to be used for navigation purposes. If you would like to use this map, please obtain permission from:

USDA-ARS
2111 SE Marine Science Dr.
Newport, OR 97365
brett.dumbauld@ars.usda.gov
(541) 867-0191

Fig. 14

Ghost Shrimp: How extensive and where are they in Willapa Bay? Dr. Brett Dumbauld is one of most knowledgeable researchers on the burrowing scavenger decapod, *Neotrypaea californiensis*, aka, ghost shrimp. His decades of sampling and research allowed him to construct the map (Fig. 14) displaying the extent of their encroachment over the public intertidal ground in Willapa Bay. He did not survey privately owned oyster growing beds. I indicate areas as dotted lines where encroachment by ghost shrimp can and have generally occurred. They would contain those growing areas which would require periodic control to remain as shellfish growing areas. It would means a loss of most of the benthic fauna and flora on these areas unless treated. Also note his burrow numbers are for 1/4 of a square meter while in order to have a stable surface for oysters, diatoms, etc. a burrow density of less 10 burrows per m^2 is used. The map shows the area covered and represents basically the most productive intertidal benthic habitat areas of Willapa Bay in terms of salinity, sediment composition, currents and elevation. These are conditions which create the prime diatom and crustacean forage areas for shorebirds and fish if ghost shrimp have not taken them over. The map also illustrates that if growers do not keep up a treatment program as part of an IPM program on their own intertidal property more productive intertidal tideland of Willapa Bay will be lost to benthic productivity. Sadly, about half the growers have currently stopped the use of chemical treatment of the sediment and probably will lose this ground for any type of shellfish cultivation. Furthermore, and most important is the loss of the food web for the entire biota. Add to this the fact that no ghost shrimp treatments have been allowed for the past two summers with a resulting increase of ghost shrimp dominated mudflat with destabilization of the benthic sediments and the negative impact on the benthic biota. There are already thousands of public intertidal acres which fall into this category indicated by the red and green areas on Dumbauld’s map (Fig. 14). The barren sandy public areas could be reclaimed by reduction of the ghost shrimp but it would take agencies and others to realize the possibility and advantage of increasing forage acreage for managed species like fish, crabs and shorebirds. There are few unique temperate marine intertidal areas like Willapa Bay that can sustain the valuable food web to support a oceanic nursery and rich near shore biota.

It takes several years for the ghost shrimp to achieve their destructive size and abundance to completely modify a heterogenous sand and silt area into a uniform unstable fine grained sand area (Fig. 16). However, when ghost shrimp achieve this and naturally they will, it turns the mudflat from biologically rich to a single dominant species. Sampling shows it starts with loss of benthic diatoms and the primary consumers. Comparison on my own farm of two oyster beds of similar elevation and location near Bay Center, demonstrate this (Fig. 15). One area has had periodic shrimp treatment on a 5-6 year (crop removal schedule) and the other, due to size and



COMPARISON OF ABUNDANCE AND DIVERSITY OF IMPORTANT BENTHIC GROUPS ON AN OYSTER GROWING AREA (LEFT) WITH A NEARBY SIMILAR INTERTIDAL AREA (RIGHT) WITH HIGH GHOST SHRIMP OCCUPATION. BOTH INTERTIDAL AREAS HAVE GHOST SHRIMP RECRUIT TO THEM. OUR OYSTER BED (LEFT) FOR THE PAST FIFTY YEARS HAS HAD THE SEDIMENT TREATED (ABOUT EVERY SIX YEARS) TO KEEP THE GHOST SHRIMP NUMBERS LOW WHILE THE NEIGHBORING ONE TIME GROWING AREA ON THE RIGHT, WAS ABANDONED OVER TEN YEARS AGO WITH NO GHOST SHRIMP CONTROL. ESTIMATES OF ABUNDANCE MADE FROM SEVERAL STUDIES WITH AN IMPORTANT DETAILED BASE SAMPLING SERIES BY DR. KEN BROOKS (SEE ACCOMPANYING NOTES FOR REFERENCE).

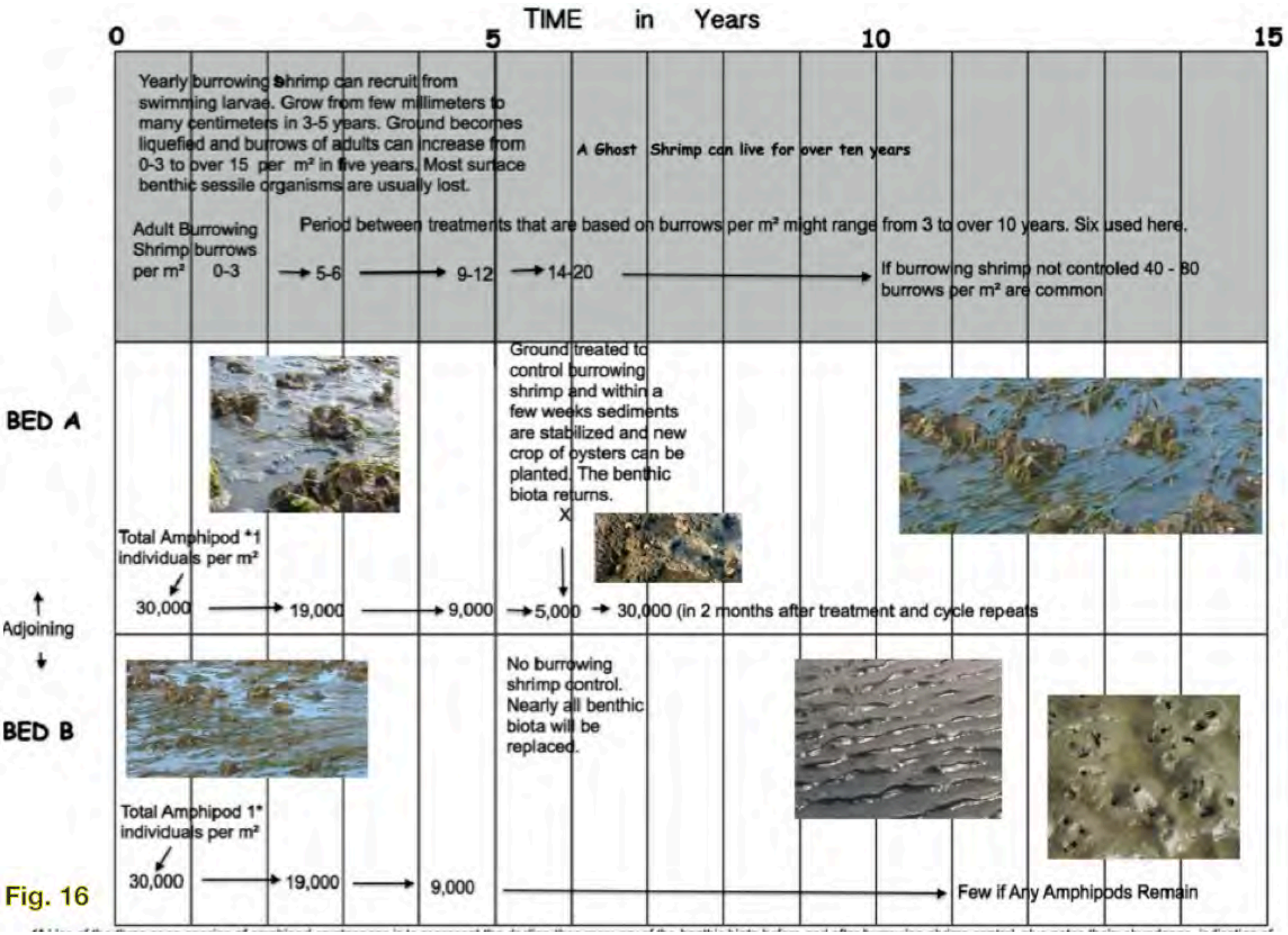
20-40 million Diatoms per m^2
45 Macroalgae per m^2
28 Eelgrass turions per m^2
20,000-40,000 Amphipods per m^2
1,000-2,000 Ostracods & Cumaceans per m^2
200-300 Decapods and Mollusks per m^2
3,500 barnacles per m^2
6,500 Copepods per m^2
10,000 + various worms - many groups

Diatoms - few and hard to find - no biofilm
Macroalgae and Eelgrass - none
Amphipods, Ostracods, Cumaceans - few to none
Decapods - only ghost shrimp, 25 per m^2
Molluscs - Commensal to ghost shrimp, 50 per m^2
Gastropods & Cirripedia - Few to None per m^2
Copepods - few with tide but no prey (diatoms)
Worms - Few in unstable sand ± 500 per m^2

Fig. 15

limits on treatment, has been without ghost shrimp control for over ten years. It now is barren of life with over 60 adult ghost shrimp burrows per square meter. The possible abundance figures for various taxa are from several studies (mainly Brooks, 1993) and actual counts.

Burrowing Ghost Shrimp Takeover: Ghost shrimp larvae go through a long swimming phase which includes ocean time offshore and then a return in late summer to an intertidal area to start a life within the nearshore sediment. At this stage they are just a few millimeters in size and part of the zooplankton. The following chart (Fig. 16) is based on two comparable oyster mudflat areas subject to ghost shrimp recruitment: Bed A was treated periodically, while Bed B was not treated. The following (Fig. 16) traces an expected time of settlement and treatment. There is variation among growing areas in this timing to reach adult numbers to cause sedimentary modification. Plus it depends on the year to year recruitment numbers along with the many physical factors such as weather, elevation, sediment composition, etc.



Yearly burrowing shrimp can recruit from swimming larvae. Grow from few millimeters to many centimeters in 3-5 years. Ground becomes liquefied and burrows of adults can increase from 0-3 to over 15 per m² in five years. Most surface benthic sessile organisms are usually lost.

A Ghost Shrimp can live for over ten years

Period between treatments that are based on burrows per m² might range from 3 to over 10 years. Six used here.

If burrowing shrimp not controled 40 - 80 burrows per m² are common

Ground treated to control burrowing shrimp and within a few weeks sediments are stabilized and new crop of oysters can be planted. The benthic biota returns.

30,000 (in 2 months after treatment and cycle repeats

No burrowing shrimp control. Nearly all benthic biota will be replaced.

Fig. 16

Fig. 16, 1* The example of abundance of amphipods, mainly *Corophium* and including *Leptochelia* a tanaid, as explained, is their dependence upon the sediment composition and stability to be able to remain when the tide leaves during the intertidal phase and to their importance to the food web.

Ghost shrimp pump water into their burrowing network to bioturbate (mix) with the intertidal fine sand and silt, a process which creates a homogeneous unstable mixture. The finer sediment components (silt, clay, organics, etc.) end up on the surface where tidal or wind/wave energy currents can transport eventually out to sea. Any diatoms and biofilm which might have been on the surface is dispersed also. This results in a lack of sand cohesiveness and compaction with the homogeneous unstable fine sand. It also prevents nearly all other species which are burrowing, rooted, sessile, planktonic or dependent upon the mudflat to vacate or avoid the sediment surface.

The Food Web: Diverted and prevented by Ghost Shrimp**.** In Willapa Bay, around 9,000 acres from Dumbauld''s estimate of over 8,000 acres about ten years ago, have become ghost shrimp dominated areas (map Fig. 14) to the exclusion of nearly all other estuary plants and animals. In short, all those species that once depended upon those intertidal sediment areas to graze, derive nutrients or prey on others are replaced. More acreage is being taken over (invaded) each year and if ghost shrimp are not controlled by shellfish growers another 6-9,000 acres (estimated) of productive prime intertidal land would likely be lost in the next decade. Again, many will ask so what? The simple answer is the primary productivity initiated by benthic diatoms, that if reduced or absent, means less carbohydrates and lipids for numerous diverse primary consumers. They in turn will short the next higher trophic levels. This is critical for the more familiar fish, birds, crabs etc. For example, it must be considered that the decreasing areal extent of forage area due to ghost shrimp will have a direct impact on juvenile fish or migrating shorebird abundance with the decrease in diatoms and key benthic invertebrates. The following (Fig. 17) attempts to illustrate this important function of the Willapa intertidal where the sand to shorebird connection happens.

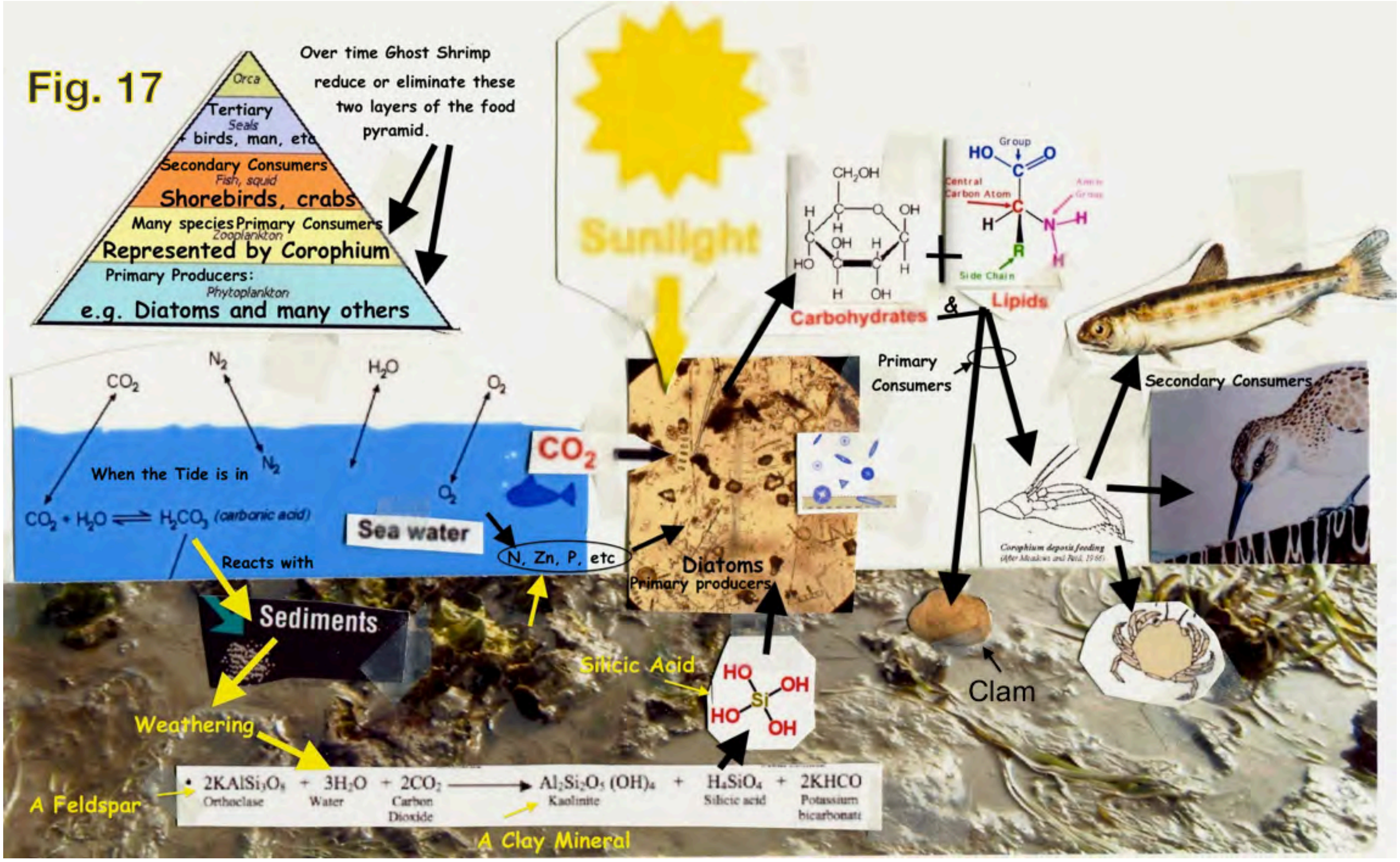


Fig. 17

One of many examples of a functioning food lineage within a healthy food web

Fig. 18

A few References:

For access to other research reports dealing with the intertidal benthic:

Banas, N. S., B.M. Hickey, J.A. Newton & J. Ruesink. 2007. Tidal exchange, bivalve grazing and patterns of primary production in Willapa Bay, Washington, USA. Mar. Ecol. Prog. Series 341: 123-139. http://coast.ocean.washington.edu/willapa/

Bay of Fundy Ecosystem Partnership, Keystone *Corophium*, Master of the Mudflats. Fundy Issue #13. Excellent reading. http://www.bofep.org/corophiu.htm

Brooks, Kenneth M., 1993. Changes in Arthropod and Mollusk Populations Associated with the Application of Carbaryl (Sevin) to Control Burrowing Shrimp on Oyster Beds in Willapa Bay, Washington, to Fulfill Requirements of the EPA Carbaryl Data Call In. EPA CFR Chapter 1; Part 160, Good Laboratory Practices and conforms to the Puget Sound Estuary Protocols.

Clifton, H. E., Philipes, R. L., 1960. Lateral trends and vertical sequences in estuarine sediments, Willapa Bay, Washington. Economic Paleontologists and Mineralogists. Pacific Section. Symposium No. 4. pp. 55-71.

Ferraro, S.P. & Faith A. Cole, 2007. Benthic macrofauna - habitat associations in Willapa Bay, Washington, USA. Vol. 71, Issues 3-4. pp. 491-507,

Gerdo, Veronica and R. G. Huges, 1994. Feeding behavior and diet of *Corophium* in southeastern England. Marine Ecology Progress Series, oVl. 114; pp.103-108

Hemphill-Haley, Eileen, 1995, Intertidal diatoms from Willapa Bay, Washington. U.S. Geological Survey. Application to Studies of Small-Scale Sea Level Changes. Northwest Science, V. 69 No.1

Hosack, Geoffrey R., Brett R. Dumbauld, Jennifer L. Ruesink, and David A. Armstrong; 2006. Habitat Associations of Estuarine Species: Comparisons of Intertidal Mudflat, Seagrass (Zostera marina), and Oyster (Crassostrea gigas) Habitats. Estuaries and Coasts Vol. 29, No. 6B, pp. 1150–1160.

Jardine, Catherine B., Alexander L. Bond, Peter J. A. Davidson, Robert W. Butler, and Tomohiro Kuwae, David William Pond, Academic Editor. 2015; Biofilm Consumption and Variable Diet Composition of Western Sandpipers (Calidris mauri) during Migratory Stopover. PMC4397082 Published online 2015 Apr 14. doi: 10.1371/journal.pone.0124164.

Stal, L. J. & J. F. C. de Brouwer, 2003. Biofilm Formation by Benthic Diatoms and Their Influence on the Stabilization of Intertidal Mudflats. Breathe-Forschungszentrum Terramare, No. 12. pp 109-121 http://www.watt.icbm.de/ .

Wilson, R. L. Various comment albums spanning many years with the subject of Willapa Bay intertidal benthic with images. https://www.flickr.com/photos/76798465@N00/albums/

ATTACHMENT B to WGHOGA comments on the Draft Supplemental Environmental Impact Statement for Control of Burrowing Shrimp using Imidacloprid on Commercial Oyster and Clam Beds in Willapa Bay and Grays Harbor

Response of Estuarine Benthic Invertebrates to Large Scale Field Applications of Imidacloprid[*]

Steven R. Booth[1], Kim Patten[2], Leslie New[2], and Bobbi Hudson[1]
[1] Pacific Shellfish Institute, Olympia, WA 98501, [2]Washington State University Long Beach Extension Unit, Longbeach WA 98631, [3]Washington State University Vancouver 98686

Abstract

The response of estuarine benthic invertebrates to the neonicotinoid insecticide imidacloprid following large scale field applications in Willapa Bay, Washington (U.S.A.) was examined using Principal Response Curve Analysis. A total of 60 analyses were conducted to examine the response of 6 taxonomic assemblages (polychates, non-juvenile polychaetes only, mollusks, non-juvenile mollusks only, and crustaceans, and all invertebrates combined). The response was significant ($p < 0.05$) among 51 of the analysis, but interpretation was often confounded by significant difference between treated and control assemblages before treatment. In general, the response of the treated assemblages relative to the control assemblage usually did not change much over time, indicating a minimal treatment effect on the assemblage as a whole. Only 6 PRCs of 60 showed a significant negative effect from imidacloprid application. Five of the 6 PRCs represented mollusks, which represented < 2% of all organisms sampled among all sites and years. Crustaceans were negatively affected in one of 8 studies. Polychaetes, both with and without juveniles, were never negatively affected. The large majority of PRCs showed no significant effect from imidacloprid application, a neutral treatment effect, or ostensibly a "positive" treatment effect. The overall minimal response was likely due to exposure to low concentrations of imidacloprid for limited times, physiological tolerance to imidacloprid for some species, and multiple life-history strategies to rebound from natural disturbance and adaptation to a highly variable environment. These strategies include high mobility and dispersal behaviors, high intrinsic rates of reproduction, and rapid development. The highly variable environment was reflected in the response as variation among years, sites, replicates, and perhaps haphazard movements of individuals, particularly juvenile bivalves.

1. Introduction

The selective nature of neonicotinoid insecticides towards insects has helped make them the most widely used class of insecticide in the world. Neonicotinoids are agonists of the primary neurotransmitter of the cholinergic nervous system, acetocholine (Ach) (Tomizawa and Casida 2003). That is; they block the transmission of nerve impulses along the central nervous system. Because the molecular structure of the nicotinic receptor site differs between insects and other animals and because they are metabolized differently by insects and other animals, they are selectively more toxic to insects than other animals, particularly vertebrates. Neonicotinoids act systemically so are most effective against pests that feed directly on plant tissues, thus applications are usually foliar or seed dressings (Goulson 2013). Neonicotinoids are "reduced risk" insecticides (Ehler and Bottrll 2000) and are compatible with many integrated pest management programs in a variety of cropping systems.

The effects of neonicotinoid insecticides on terrestrial insects, including non-targets, have been comprehensively assessed and reported (e.g., Goulson 2013, Pisa et al 2014). The most controversial unintended effect of neonicotinoids has been on pollinators of agricultural crops, primarily honeybees (Pisa et al. 2014). Neonicotinoids can directly kill honeybees via spray drift during foliar applications against pest insects, or affect them indirectly when the bees forage for nectar and pollen from treated plants. Neonicotinoids have been implicated, along with Varroa mites and several pathogens (Ellis et al. 2010), as contributing to colony collapse disorder (Gill et al 2012).

---

[*] In final preparation for submission to "Estuarine, Coastal, and Shelf Science"

Reported effects on non-target aquatic invertebrates are much less common. Almost all data related to toxicity of neonicotinoids to aquatic invertebrates come from laboratory and mesocosm studies that feature freshwater. Exposure of estuarine invertebrates to any insecticide is almost always associated with run-off or leaching from upland agricultural use than from direct application (e.g., Kuivial and Hladik 2008, Morrisey et al. 2015). The authors of a recent comprehensive review of neonicotinoid impacts of non-target invertebrates reported, "There are no published works regarding the marine environmental contamination of neonicotinoids" (Pisa et al 2015).

The singular large scale insecticidal use in an estuary, worldwide, has featured applications of the broad spectrum carbamate insecticide, carbaryl, to control burrowing shrimp in coastal estuaries of Oregon and Washington in the U.S.A. (Feldman et al. 2000). Burrowing shrimp (*Neotrypaea californiensis*, *Neotrypaea gigas*, *Upogebia pugettensis* reside in burrows where they disrupt the structural integrity of sediments, causing surface dwelling organisms, including ground-cultivated oysters, to sink and die. Annual applications of carbaryl to mostly non-contiguous commercial oyster beds were begun in the early 1960s. Use was controversial since inception and a near 50 year search for alternative management tactics ultimately lead to the neonicotinoid compound, imidacloprid (Booth 2010).

We examined the response of epibenthic and benthic invertebrates to large scale field trials of the neonicotinoid imidacloprid ((2E)-1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) (IMI) that targeted burrowing shrimp. A total of 8 trials were conducted in 2011, 2012, and 2014 under state and federal experimental use permits in partial fulfillment of requirements for Federal labels and Washington state permits (Booth et al. 2011, Booth and Rassmussen 2011, Booth and Rassmussen 2013, and Booth et al. 2015). Here, we consolidated those studies to describe the response of 6 assemblages of benthic invertebrates at each study and when data from all studies were pooled. Results were interpreted in terms of the physiological susceptibility of particular taxa and the resilience of the taxonomic assemblages in light of adaption to a dynamic and highly variable environment. Relevant life history strategies include high mobility and dispersal behaviors, high reproductive rates, and rapid development. The results also reflected the highly variable environment in terms of differences among study years, sites, and replicates, but also the high variability among species life histories, and perhaps haphazard movement of individuals.

2. Methods

2.1. Experimental design

The experimental design comprised a "before-after-control-impact" (BACI) approach (Green 1979) that featured plots that were treated with liquid formulated IMI (Nuprid® 2F; NuFarm US or Protector ®), granular formulated IMI (Mallet ® 0.5G), or were left untreated to serve as a control plot. In general, a liquid IMI plot and a granular treated plot were compared to a single control plot within a study area. Plots were separated by at last 500m. Application rate for all imidacloprid treatments was 0.5 lb a.i./ac. Over the course of 3 years, a total of eight trials were conducted among 5 study areas (Figure 1). In 2011, the triple plot design was used at one study area (Bay Center), but only a liquid IMI plot was compared to a control plot at a second area (Cedar River). Triple plots were used at two study areas in 2012 (Leadbetter and Palix). In 2014, 36ha of contiguous tidelands were treated with liquid IMI but an internal 4 ha plot was compared to a 3.6ha control plot located 4 km distant. Imidacloprid treatments were applied in July or August. The liquid formulation was applied aerially using helicopters when plot surfaces were fully exposed during extreme low morning tides The granular formulation was applied using an ATV equipped with a granular spreader during ebb flow prior to full surface exposure during extreme low morning tides (water depth ~ 5 cm).

2.2. Imidacloprid sampling

Comprehensive descriptions of procedures to sample, handle, and analyze samples are presented elsewhere (Booth and Rassmussen 2013, Grue and Grassley 2013, Booth et al. 2015, Patten 2015). Briefly, concentrations of IMI and its breakdown product, olefin, were measured in surface waters, substrate pore water, and sediments before and after treatment according to protocols that were fairly well standardized among study sites and years. Briefly, samples were taken along each of 4 to 6 transects that radiated from plot center and extended up to 480 m off plot, primarily in the direction of tidal currents. Water was sampled at one or two hours after IMI application as the tide inundated the plot treated with the liquid formulation or as it flowed off of the plot treated with the granular formulation, then at 6, 12, and 24 hr later. Porewater and sediments were sampled at 1, 14, 28, and 56 days after treatment according to an iterative process that depended on the results of the previous sample. Seagrass, *Zostera marina*, was also sampled and analyzed for concentrations of IMI.

2.3. Invertebrate sampling

Treated and control plots were sampled at the day before and at 14 and 28 days after treatment (DAT). In 2012, the plot treated with liquid IMI and associated control were also sampled at 56 DAT at one of the two study sites, but only mussels and crustaceans were enumerated. Plot sizes, primary sediment composition, vegetation, treatment dates, and sample sizes characteristics are presented in th Appendix (Table A1).

Invertebrates were sampled using a 10.2 cm internal diameter corer to a depth of 10 cm. In 2011 and 2012, cores samples and identification labels were placed inside one gallon Ziploc® storage bags, transported in coolers from the study sites, and sieved one or two hours later in salt water through 0.5 mm mesh to save time during sampling. In 2014, cores were sieved on site immediately after sampling. Sieved samples were fixed in 10% buffered formalin.

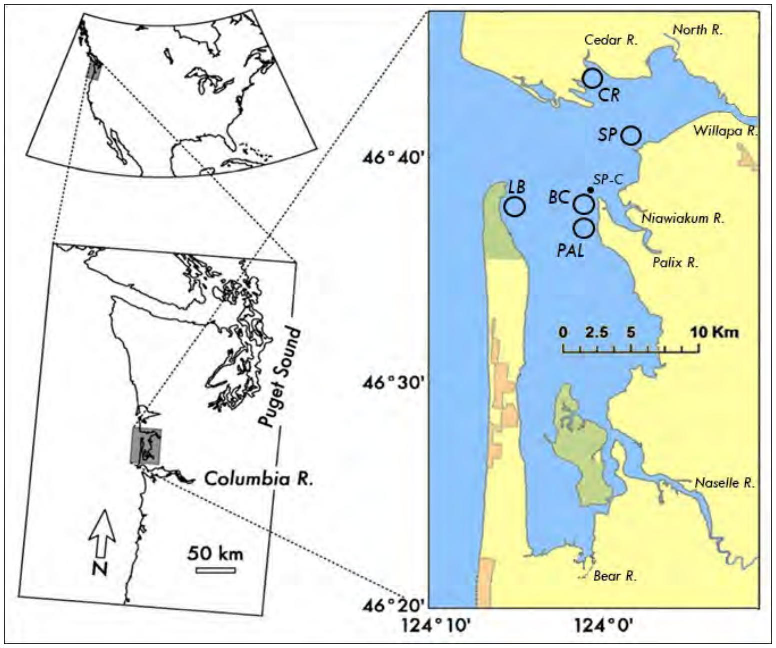


Figure 1. Willapa Bay, WA study sites: Cedar River (CR - 2011), Stony Pt. (SP - 2014), Stony Pt. Control (SP-C - 2014), Bay Center (BC - 2011), Leadbetter (LB - 2012), Palix (PAL - 2012).

2.4. Sample identification

After at least two weeks, samples were re-sieved through 100 µm mesh using freshwater, transferred to 70% isopropyl alcohol, stained with rose Bengal, and stored until further processing. Invertebrates were sorted from bits of algae, eelgrass, and debris. Polychaetes were identified, mostly to species, and enumerated by Ruff Systematics, Inc. Crustaceans and mollusks were identified and enumerated by PSI staff to the most specific taxonomic level possible (identifiable taxonomic unit (ITU)).

2.5. Data analysis

Principal Response Curve (PRC) analysis is a multivariate ordination technique that was derived from

Redundancy Analysis (RDA), primarily to simplify assessment of pesticide treatments on abundances of aquatic invertebrates in mesocosms (Van den Brink and Ter Braak 1999) and has since become fairly standard for such experimental systems (e.g., Colville et al. 2008, Lopez-Mancisidor et al. 2008, Mohr et al. 2012). PRC's have also been used to interpret biomonitoring data (e.g., Leonard et al. 2000, Cuppen et al. 2000) and has been favorably compared to other multivariate techniques (Van den Brink et al. 2009). In PRC analysis, effects due to time (conditioned variance) are partialled out, leaving treatment effects plus effects due to the treatment × time interaction (constrained variance) and remaining residual (unconstrained) variance. Removing time from the equation allows the response of a treated species assemblage to be compared to an untreated control assemblage along a horizontal time axis, greatly simplifying interpretation of results. As in RDA, the maximum constrained variance among a set of samples is extracted and projected onto a primary axis, the maximum constrained variance that is uncorrelated with the primary axis is projected onto a second axis, the maximum constrained variance that is uncorrelated with either primary or secondary axes is projected onto a third axis, and so forth, until all constrained variance has been projected. The Principal Response at each sample time is a canonical coefficient (c_{dt}) that represents the maximum variance of species abundances in the treated assemblage relative to the control assemblage that is explained by a single (usually the primary) RDA axis (axis 1). An increase in the canonical coefficient over time represents increasing abundance of the treated assemblage relative to a control assemblage; a decrease in the coefficient over time represents a decrease in abundance. The amount of total variation that is captured by axis 1 axis can be assessed for significance over the entire time series using a Monte Carlo permutation test. An additional Monte Carlo permutation test can be used to determine if the treatment effect (e.g., IMI application) and treatment × time interaction are significant at each sample time. Finally, PRC analysis presents a coefficient (b_k) that expresses the correlation of each species, or taxa, with the basic response pattern of the entire taxon assemblage. The relative abundance of a given ITU at a given sample time = $c_{dt} \times b_k$. Highly weighted taxa (high values of b_k) are highly positively correlated with the basic PRC pattern (e.g. abundances resembles the basic pattern) while taxa with negative taxonomic weights are negatively correlated (abundances resemble the opposite pattern of the entire assemblage).

Principal Response Curve analyses were conducted using the 'vegan' package (v 2.3-3) for the R programming language (v 3.2.2). PRCs were created and analyzed for a total of six metric assemblages of benthic invertebrates (polychaetes, mollusks, and crustaceans, non-juvenile polychaetes, non-juvenile mollusks, and assemblage of all invertebrates categorized by family as the most specific taxon. Studies of liquid and granular formulated IMI were analyzed separately. PRC analyses were conducted on log-transformed abundance data (ln (x) +1, where x = number of individuals per m^2 per taxa. Separate analyses were conducted for each individual test (year, study site, and formulation), and for all sites and years pooled. In addition to the curve, the analysis determined the amount and proportion of conditioned variance (time effects), constrained variance (explained by treatment plus treatment x time effects), or unconstrained (unexplained) variance. Monte Carlo permutation F-type ANOVA (number of permutations = 999) was used to test the significance of a) the amount of constrained variance (e.g., conditional variance was removed as part of the PRC analysis so was expressed in the ANOVA as 0), and b) the response of each treated assemblage relative to the control assemblage at each sample date. PRC analysis output included the amount of constrained variance displayed on PRC. A second Monte Carlo test determined the significance of the PRC diagram (null hypothesis: axis 1 does not represent a significant proportion of the total variance).

3. Results

3.1. Field concentrations of imidacloprid

Concentrations of IMI in surface waters, porewaters, sediments, eelgrass, and associated field and laboratory controls are detailed elsewhere (Booth and Rassmussen 2013, Grue and Grassley 2013, Booth et al. 2015, Patten 2015). A very general summary comparison was that IMI concentrations varied substantially among years and study areas, with a notable difference between formulations (Table A5).

Because on-plot surface waters were sampled on the first post-treatment inundation tide (10 cm deep, ~ 2 hours after treatment (HAT)), and because granular IMI was applied to shallow standing water near the end of the out-going tide, concentrations were generally lower than in samples from the plots treated with liquid IMI while the plot was fully exposed. Concentrations also varied substantially within plots. Concentrations in surface waters also rapidly dissipated. Imidacloprid was detected in only 1 of 10 surface water samples taken at 6 HAT in 2011 and never at any longer post-treatment intervals. Consequently, surface waters were not sampled past 6 HAT in 2012 or 2014.

Concentrations of IMI in porewater declined precipitously according to power functions from initial concentrations (1 hr post-treatment) of 12 ppb in 2010 and 2011 (combined) (Grue and Grassley 2012), ~100 ppb in 2012 (Grue and Grassley 2012), and ~ 150 ppb in 2014 (Booth et al. 2015) to ~ 1 ppb at 14 DAT and to barely or non-detectable (0.04 ppb) concentrations at 28 and 56 DAT (all studies). Concentrations of IMI in sediment sampled from 5 treated plots at 1 DAT in 2012 averaged 21.4 ppb (range was 6.3 to 89 ppb) (Grue and Grassley 2012) and 57.5 ppb (range was 57 – 64 ppb) among 4 sediment samples from the plot treated in 2014 (Booth et al. 2015). Concentrations of a primary metabolite of IMI, olefin, were orders of magnitude lower, if detected at all, in both water and sediment.

Based on an application rate of 0.5 lb a.i./ac, sample depth, specific gravity, and percent moisture, the theoretical maximum concentration of IMI in porewater was 1121 ppb (Grue and Grassley 2012), far higher than sampled here. Most of the difference was due to dissipation into surrounding waters during tidal exchange. Off-site water samples indicated that IMI was sometimes transported several hundred meters from the treated plot, but at extremely low concentrations and only in the first few days after treatment (Grue and Grassley 2012) (Booth et al. 2015). Imidacloprid concentrations were further reduced by molecular binding to the sediments (Grue and Grassley 2012). Binding rates approached 90% in sediments with high amounts of total organic carbon.

3.2. Identifiable taxonomic units

A total of 95 invertebrates were identified to species or the most specific identifiable taxonomic unit (ITU) (Appendix, Table A2).

3.3. Partitioned variances and treatment effects

The percentage of total variance that is conditioned (attributed to time effects), constrained (attributed to treatment effects plus treatment x time interaction effects), and unconstrained (attributed to replicate, site, or unexplained effects) is presented in the Appendix for each PRC analysis (Table A3). Analyses with lower percentages of unconstrained variance were those with lower diversity (i.e., all studies at Bay Center and Cedar River in 2011). Treatment effects were significant in 54 of the 60 analysis and axis 1 displayed a significant amount of the constrained variance In 51 of the 60 PRCs (also Table A3); 49 analysis had both a significant treatment effect and a significant axis 1.

The canonical coefficient (principal response) of the test assemblage was significantly different from the control assemblage before treatment in 40 of the 60 analyses. Hence, a significant treatment effect over all sample dates, as determined by Monte Carlo ANOVAs, was not always informative. Furthermore, the treatment effect was often significant even when the overall proportion of constrained variance (variance due to treatment effects plus treatment x time interaction effects) was low (< 10%). Low constrained variance may be an artifact of the ordination analysis (e.g., the "arch effect" (Gauch 1982)), and have "nothing to do with nature" (Palmer 2016), but analyses with higher proportions of constrained variation are intuitively more explanatory. The more informative analyses were those with a significant percentage of constrained variance and an axis 1 that displayed a significant proportion of the constrained variance. Forty-nine of the 60 PRCs meet these criteria. Unconstrained variance was >75% for 31 and < 50% for 12 of the 49 more informative PRCs.

3.4. Principal response curves

The 60 PRCs are presented in the Appendix (Figures A5 – A14), arranged by study site and year, as trajectories of the principal response were often consistent among the 6 taxonomic assemblages at each study site and year. Response trajectories were less consistent among studies within a given assemblage. Each of the more informative PRCs had one of 3 potential outcomes based on the position of the principal response at the final sample date relative to the pre-treatment sample date (the end response): 1) a negative end response, in which principal response of the test assemblage relative to the control assemblage was lower at the final sample date compared to before treatment (e.g. Figure 2), 2) a positive end response, in which the principal response of the test assemblage relative to the control assemblage was higher at the final sample date compared to before treatment (e.g., Figure 3), and 3) a neutral end point, in which the principal response of the test assemblage relative to the control assemblage was the same at the final sample date compared to before treatment (e.g., Figure 4). Another potential scenario, indicative of a severe negative effect, with a response that is significantly higher than the control before treatment but is significantly lower than the control at both post-treatment sample dates was not realized in our studies.

The status of the end response (negative, positive, or neutral) of each of the 49 PRCs with both a significant percentage of constrained variance and an axis 1 that displayed a significant proportion of that variance is presented in the appendix as Table A6. The end responses of 6 significant PRCs were negative, 5 of which were either mollusks with or without juveniles included, while 1 of the 6 was the assemblage of crustaceans treated with granular IMI at Palix, 2012 (Figure 2). Four of the 6 were from studies of the liquid formulation of IMI. Two of the 5 PRCs with a positive end responses were polychaetes in the combined liquid IMI studies, with juveniles both included and excluded (Figure 3). Three of the 5 featured mollusks. Three of the 5 were from studies of the granular formulation of IMI. The end response of 38 of the 49 PRCs with both significant treatment effects and a significant axis 1 was neutral. The trajectories of 34 of the 38 PRCs were essentially flat. That is, the response was significantly lower for the treated assemblage than the control assemblage at all sample date (e.g., Figure 4), significantly greater for the treated than the control at all sample dates (also Figure 4), or not significantly different between the treated and control assemblage at all sample dates. The trajectories of 4 PRCs shifted either up or down at 14 DAT, but returned to pre-treatment status at 28 DAT. Nineteen of the 38 PRCs with a neutral end response were from studies of the liquid formulation of IMI and 19 were from studies of the granular formulation.

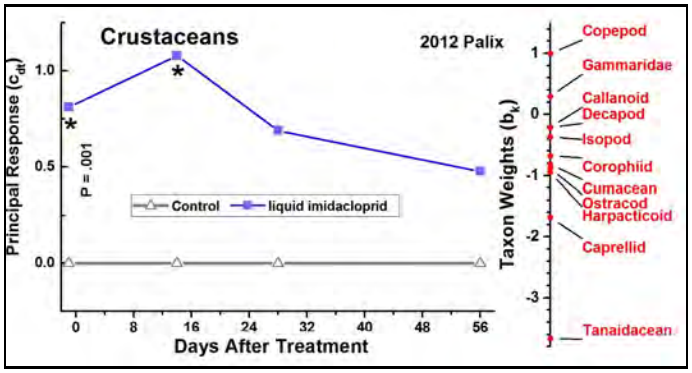


Figure 2. Principal Response Curve of crustaceans before and after treatment with liquid imidacloprid at Palix, 2012. P is probability that the primary axis (response) is significant. Asterisk (*) indicates the response at each sample date is significantly different from the control (p < 0.05). Weights indicate taxa that are positively or negatively correlated with the shape of the curve.

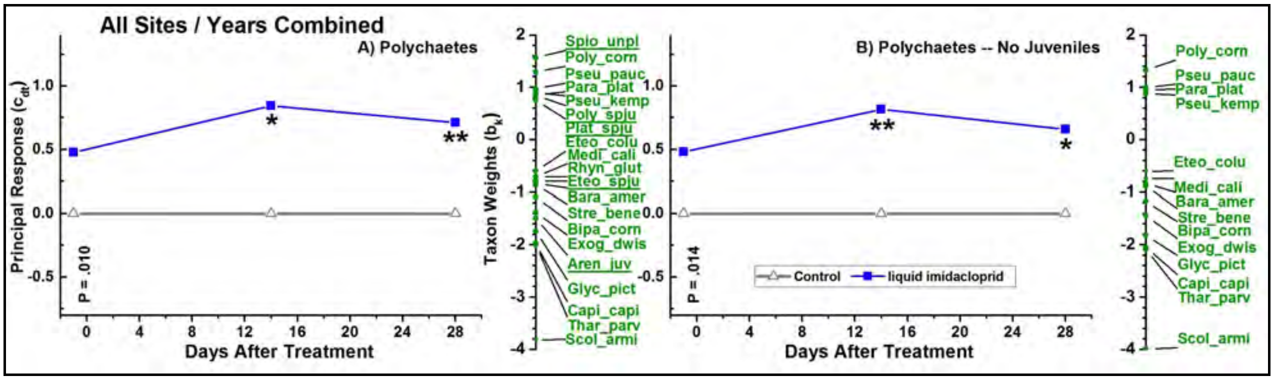


Figure 3. Principal Response Curve of A) all polychaetes (underlined taxa are juveniles) and B) non-juvenile polychaetes before and after treatment with liquid imidacloprid, pooled study sites and years. P is probability that axis 1 (Principal Response) is significant. Asterisks indicate the response at each sample date is significantly different from the control (*, p < 0.05; **, p < 0.01). Weights indicate taxa that are positively or negatively correlated with the shape of the curve (weights > -.06 and < 0.06 are not shown). Table A2 lists polychaete full names and abbreviations.

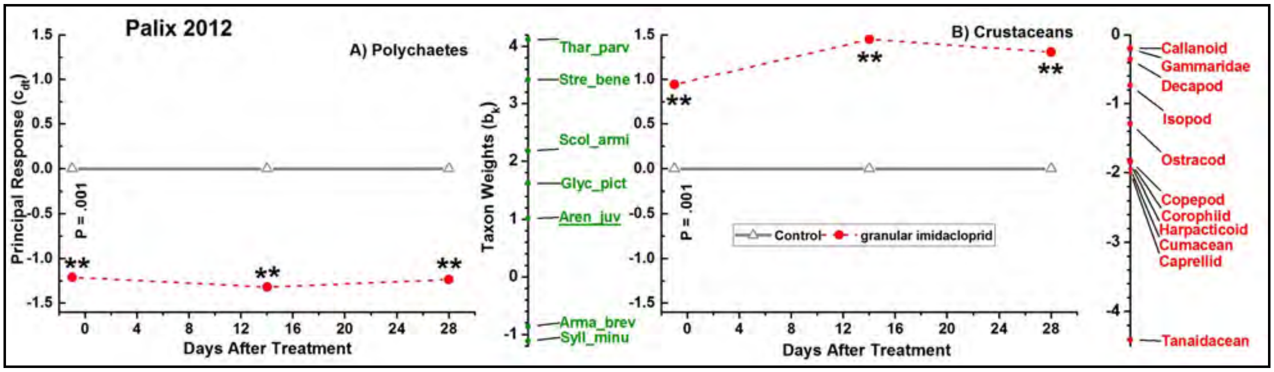


Figure 4. Principal Response Curves of A) Polychaetes (underlined taxa are juveniles) and B) Crustaceans at granular imidacloprid and control plots at Palix in 2012. P is probablility that axis 1 (response) is significant. Asterisks (**) indicate the response at each sample date is significantly different from the control (p < 0.01). Weights indicate taxa that are positively or negatively correlated with the shape of the curve (polychaete weights > -.06 and < 0.06 are not shown). Table A2 lists polychaete full names and abbreviations.

Both the trajectory and the end response of all non-juvenile polychaete PRCs were very similar to those that included juveniles. However, the flat trajectory of non-juvenile polychaetes treated with granular IMI at Leadbetter in 2011 was higher than the control, whereas the flat trajectory was lower than the control at all sample dates when juveniles were included in the analysis. The trajectory or end response of non-juvenile mollusks was different than mollusks with juveniles included in 6 of the 8 comparisons, perhaps most notably in the PRC of all studies combined; the end response was positive with juveniles included, but negative with juveniles excluded from analysis.

Weights of individual species or ITUs were generally not consistent among PRCs of the same taxonomic assemblage among different studies. For example, weights of harpacticoid crustaceans were positive at Bay Center and Cedar River in 2011 and at Stony Pt in 2014, but were negative at Palix and Leadbetter in 2012. Sedentary polychaetes (Sub Class Sedentaria) were not affected more than mobile polychaetes.

In summary, only 6 PRCs of 60 showed a significant negative effect from IMI application, representing studies of both granular and liquid formulations at the 2012 Palix study area and of each formulation when all studies across all years were combined. Five of the 6 PRCs represented mollusks, which represented < 2% of all organisms sampled among all sites and years. Crustaceans were negatively effected in one of 8 studies and polychaetes were never negatively effected. The large majority of PRCs showed no significant effect from IMI application, a neutral treatment effect, or ostensibly a “positive” treatment effect.

4. DISCUSSION

4.1. Toxicological susceptibility

The minor and transitory effects from IMI indicated by the PRC analyses were at least partly due to limited exposure to potentially toxic concentrations. Imidacloprid demonstrably affected estuarine aquatic benthic invertebrates in controlled arenas. Toxicity tests of standard saltwater test crustaceans report LC_{50} values of 361,230 *u*g/L for water flea (*Daphnia magna*) and 10,440 *u*g/L for brine shrimp (*Artemia* sp.) (static 48 hr test, Song et al. 1997). These values were substantially higher than the concentrations sampled in our studies. LC_{50} values of 10 *u*g/L and 1,112 *u*g/L for blue crab (*Callinectes sapidus*) megalope and juveniles, respectively (static 24 hr test, Osterberg et al 2012) and 309 *u*g/L and 566 *u*g/L for larval and adult grass shrimp (*Palaemonetes pugio*), respectively (static 96 hr test, Key et al. 2007). There are no published laboratory studies of IMI effects on polychaetes, but the freshwater oligochaete *Lumbriculus variegatus* suffered 35% mortality after 10 days of exposure to 500 *u*g/kg (ppb) IMI in spiked soil samples (Sardo and Sores 2010). These controlled tests feature exposure to concentrations for much longer time periods than those experienced by organisms in our field trials, as IMI quickly dissipated into surrounding waters or became bound to sediments.

As previously noted, IMI is less toxic to non-insect invertebrates than many other insecticides. Very few, if any studies have been published that directly compared the toxicities of IMI and carbaryl to non-insect invertebrates. An LC_{50} value of 43 *u*g/L was reported for the grass shrimp (*P. pugio*) (Chung et al. 2008). Field studies of large scale applications of carbaryl to manage burrowing shrimp likewise demonstrated that physiological tolerance by individuals is not the only factor determining the ability of assemblages of estuarine invertebrates to rebound from exposure to toxins (Brooks 1993, Brooks 1995, Dumbauld 1994, Dumbauld et al. 2001, Booth 2006). Brooks (1993) described impacts to the epibenthic meiofauna as extremely short-term (< 2 day). A study of the sediment impact zone related to the carbaryl applications similarly showed that minimal effects in terms of both distance from the treated plot (< 180 m) and time since treatment (< 1 yr) (Booth 2006).

4.2. Tolerance of disturbance

Although individuals survived carbaryl and IMI applications by virtue of limited exposure or physiological tolerance, assemblages of estuarine benthic invertebrates were able to withstand the applications due to adaptation to a variety of natural disturbances. Simenstad and Fresh (1995) assessed the effects of disturbance from 5 intertidal aquaculture practices, including carbaryl applications against burrowing shrimp in Willapa Bay, on the epibenthic and benthic communities in Pacific Northwest estuaries. They noted that individual species differ in their susceptibility to disturbance, especially short term (e.g., 2 days post disturbance) but that the epi-benthic and benthic infaunal assemblages are quite resilient long-term (51 days). They concluded that the ability of these communities to rebound from aquaculture related disturbances stems from the communities' natural adaptation to the highly dynamic estuarine environment. "Scant" or "moderate" effects of harvest activities associated with geoduck clam (*Panopea generosa*) aquaculture, which in Puget Sound, Washington (VanBlaricom et al. 2015). Cultured geoduck are harvested by liquifying the sediments that surround each clam within a radius of 15 – 30 cm and a depth of 30 cm or more. The authors noted strong seasonal trends in the structure of benthic communities and that organisms are adapted to not only normal seasonal events, but also more haphazard events such as floods, storms, and even small tsunami and submarine landslides.

The intertidal environment of Willapa Bay is particularly dynamic at both spatial and temporal scales. The estuary itself is relatively shallow, which leads to especially large maximum and minimum tides (Emmett et al. 2012). Velocities of receding and advancing tides can reach several meters/second where gradients are smooth (Patten and PSI pers. obs.). Associated laminar flow transports and distributes sediments across the tideflats (Wheatcroft et al 2013) to erodable channels that can transport "orders of magnitude" greater loads of suspended sediments during peak tidal flows (Wiberg et al 2013). Major drainage channels are often displaced by 100s of meters by the spring following a series of winter storms (Patten and PSI, pers. obs.). Small bivalves reside at shallow substrate depths and are easily dislodged and transported with sediments disturbed by storms or extreme tidal currents (Norkko et al. 2001, Beukema et al. 2002). The juvenile myids and mytillids in our studies were the size of large grains of sand so were particularly prone to dispersal by sediment transport.

Salinity is especially variable in Willapa Bay, and was characterized as "extremely unsteady" in salt balance at the scale of both between and within seasons (Banas et al. 2004). Because the mouth of the estuary and 5 of the 7 primary rivers are located in the northern portion of the estuary, currents generally circulate from north to south (reversible to south-north) so general gradients in sediment type, salinity, and productivity are also north-south (Banas et al. 2004). In the summer months, temperatures in shallow puddles left during low tides can reach 40°C within a few hours on a sunny day in Willapa Bay (Pacific Shellfish Institute monitoring data). These factors, as well as others (i.e., amount and type of vegetation and detritus), also vary at more local scales according to differences in tidal elevation, aspect, proximity to rivers and other upland inputs, and other factors. As noted above, and seconded in the VanBlaricom article, the highly variable estuarine habitat made it hard to identify suitable reference sites and replicate sample stations.

Estuarine epibenthic and benthic invertebrates are well adapted to both seasonal and abrupt environmental changes. They are highly prolific, fecund, and often produce multiple generations per year. Most are mobile, with pelagic juvenile life stages that move not only within an estuary, but among estuaries via ocean currents. In addition to dispersal during dedicated pelagic larval, post-larval, or juvenile life stages, frequent small scale movements over long time periods by settled benthic invertebrates lends resilience in soft-sediment communities at a much larger spatial scale (Pilditch et al.

2015). Immigration, albeit simulated, has been shown to greatly accelerate the ability of a freshwater aquatic macroinvertebrate community to recover after pesticide exposure (Maund et al. 2009).

The variable estuarine habitat was reflected in our PRC analyses as percentage unconstrained variance. Unconstrained variance represents differences among samples, replicates, or sites (e.g., Cuppen et al 2000). The percentage of unconstrained variance was usually higher than those reported in most controlled mesocosm studies, which ranged from ~20% (Cuppen et al. 2000) or more typically ~40% (Maund et al. 2009, Mohr et al. 2012, Van den Brink and Braak 1999) or ~55% (Colville et al. 2008, Lopez-Mancisidor et al. 2008). However, unconstrained variance was 75% and 70% in a study of pesticide runoff effects on aquatic arthropods near conventionally managed and organic orchards in Germany (Schafers et al. 2008), which is more in line with percentages in our analyses.

Percentage of unconstrained variance was greatest in the analyses of combined study sites and years, reflecting the inherent variability therein. Uncontrollable experimental conditions, particularly annual weather conditions and seasonal trends, varied among years and study areas. The inconsistent patterns of taxon weights across study years and sites also reflected both the variable estuarine environment and the various life history strategies among estuarine species (or ITUs). For example, species vary in response (break from diapause, developmental rate) to water temperature.

We suspect that dispersal, high reproductive rates, rapid growth, and perhaps haphazard movement likely accounted for the “positive” treatment effects of IMI. Movement or growth of juvenile bivalves, *Macoma* spp. in particular, onto the plots treated with granular IMI post-treatment may have accounted for the positive end point of the PRC of pooled studies and the negative end point in PRC when juveniles were discarded. Harpacticoid crustaceans were 4 times more abundant on the test plot than the control plot at Stony Pt. In 2014, perhaps due to slightly warmer water temperatures that could have accelerated development, reproduction, and aggregation. Slight differences in the density and development of vegetative cover could have also enhanced the production of meiofauna and associated small benthic infauna (Dumbauld et al. 2009)

4.3. Long-term effects of imidacloprid via burrowing shrimp

Long term effects of IMI used to manage burrowing shrimp and culture bivalves is expected to lead to a more diverse community of benthic invertebrates compared to otherwise similar estuarine ground with high densities of burrowing shrimp. Burrowing shrimp, via bioturbation, are ecosystem engineers (Jones et al. 1994), (alternatively termed bioengineers (Posey et al. 1991, Dumbauld et al. 2009)) of soft-sediment intertidal habitats in many northeastern Pacific estuaries (Dumbauld et al. 2009) and thus control the structure and development of the immediate benthic community. Species diversity was lowest in ghost shrimp dominated habitat compared to six other inter-tidal habitat types (Ferarro and Cole 2007, Ferraro and Cole 2012). The very low relative abundance of mollusks found in our studies also demonstrated the ability of burrowing shrimp to control the local habitat. Suppression of burrowing shrimp allows other benthic organisms, primarily bivalves, to establish, followed by meiofauna that adhere to the bivalve and associated small benthic infauna (Dumbauld et al. 2009). Cultured oysters provide habitat for benthic infauna and physical structure and cover for surface dwellers such as juvenile crab, further enhancing diversity (Dumbauld et al. 2009).

APPENDIX

Table A1. Study site / field plot characteristics.

Year	Site	Treatment	Application Date	Plot Size (ha)	Substrate	Vegetation[1]	Cores / Plot[2]
2011	Bay Center	liquid IMI	July 14	4.2	sand	bare	20
		granular IMI	July 14	4.1	sand	sparse *Z. japonica*	16
		control		4.1	sand	bare	16
	Cedar River	liquid IMI	July 14	2.0	silt	sparse *Z. marina*	16
		control	July 14	0.9	sand	bare	16
2012	Palix	liquid IMI	August 2	3.4	sand	sparse *Z. marina*	15
		granular IMI	August 2	3.4	sand /silt	bare	15
		control		3.4	sand	sparse *Z. marina*	15
	Leadbetter	liquid IMI	August 5	3.2	sand	bare	13
		granular IMI	August 5	2.0	sand	patchy *Z. japonica*	15
		control		2.4	sand	bare	16
2014	Stony Pt	liquid IMI	July 28	4.0	sand	patchy *Z. marina*	15
		control		3.6	sand	patchy *Z. marina*	21

[1] sparse, % cover < 20%; patchy, % cover > 20% and < 1 m^2 and > 5m apart.

[2] Sample sizes are smaller than previously reported due to time-series blocking requirements for permutation tests.

Table A2. List of 96 taxa identified and enumerated from all samples at all sites and years. Table A2b lists abbreviations.

Phylum Annelida	
Class Polychaeta	
Sub-Class Errantia	
Order Eunicida	
Family Dorvilleidea	
Dorvillea annulata.	01
Order Phyllodocida	
Family Polynoidea	
Harmothoe imbricata.	02
Family Goniadidae	
Glycinde picta.	03
Glycinde sp. [juv].	04
Family Chrysopetalidae	
Paleanotus bellis.	05
Family Hesionidae	
Micropodarke dubia.	06
Microphthalmus sp.	07
Family Nereididae	
Neanthes limnicola.	08
Neanthes virens.	09
Neanthes sp. [juv].	10
Nereis vexillosa	11
Nereis sp. [juv].	12
Platynereis bicanliculata.	13
Platynereis sp. [juv].	14
Family Syllidae	
Exogone dwisula.	15
Exogone sp..	16
Sphaerosyllis californiensis.	17
Sphaerosyllis sp. N-1.	18
Syllides minutes.	19
Syllides longocirrata.	20
Syllides sp. [juv].	21
Family Nephtyidae	
Nephtys caeca.	22
Nephtys cornuta.	23
Nephtys sp. unindent. (juv).	24
Bipalponephtys cornuta.	25
Family Phyllodocidae	
Eumida longicornuta.	26
Eteone californica.	27
Eteone fauchaldia.	28
Eeone sp. (juv).	29
Phyllodoce hartmanae.	30
Phyllodoce sp. [juv].	31
Sub-Class Sedentaria	
Order Orbiniida	
Family Orbiniidae	
Leitoscololos pugettensis.	32
Leitoscloplos sp.	33
Paraonella platybranchia.	34
Scoloplos armiger.	35
Scoloplos sp. (juv).	36
Order Sabedellida	
Family Sabelidae	
Unidentifed Sabelid [juv].	37
Family Oweniidae	
Owenia sp.	38
Order Spionida	
Family Spionidae	
Dipolydora quadrilobata	39
Polydora cornuta.	40
Pseudopolydora kempi.	41
Pseudopolydora paucibranchiata.	42
Pygospio californica.	43
Pygospio elegans.	44
Rhynchospio glutaea.	45
Scolelepis squamata.	46
Scolelepis sp. [juv]	47
Spionidae unident (post-larval.	48
Spiophanes norrisi	49
Spiophanes bombyx	50
Spiophanes sp. [juv]	51
Streblospio benedicti.	52
Order Terebellida	
Family Terebellidae	
Poycirrus sp.	53
Unidentified Terebelid.	54
Order Cirratulida	
Family Cirratulidae	
Tharyx parvus.	55
Order Opheliida	
Family Opheliidae	
Polycirrus sp..	56
Armandia brevis.	57
Ophelia limacina	58
Thorocophelai mucronata.	59
Unidentified Ophelid [juv]	60
Order Capitellida	
Family Arenicolidae (juv).	61
Family Capitellidae	
Barantoall nr. americana.	62
Capitella capitata - complex.	63
Magelona hobsonae	64
Heteromastus filiformis	65
Notomastus tenuis.	66
Notomastus sp. [juv].	67
Mediomastus californiensis.	68
Family Maldanindae	
Sabaco elongatus.	69
Phylum Mollusca	
Class Gastropoda	
Unidentifed [juv].	70
Class Bivalvia	
Unidentified [adult].	71
Unidentified [juv].	72
Subclass Heterodonta	
Family Mytilidae	
Unidentified Mytilid [juv].	73
Family Cardiidae	
Clinocardium nuttali.	74
Family Myidae	
Sphenia ovoidea.	75
Cryptomya californica.	76
Unidentifed Myid.	77
Unidentifed Myid [juv].	78
Family Tellinidae	
Macoma balthica.	79
Macoma nasuta.	80
Macoma sp. [juv].	81
Unidentified Telinid.	82
Pylum Arthropoda	
Sub Phylum Crustacea	
Class Copepoda	
Order Calanoida.	83
Order Harpacticoida.	84
Order Cyclopoida	85
Unidentified copepod.	86
Class Ostracoda	
Order Ostracoda.	87
Class Malacostraca	
Order Cumacea.	88
Order Tanaidacea.	89
Order Isopoda.	90
Order Amphipoda	
Suborder Gammaridea.	91
Suborder Corophidea	
Infraorder Capreillida.	92
Infraorder Corophida.	93
Unidentified amphipod [juv].	94
Order Decapoda.	95

Table A2b. Polychaete name abbreviations. Table A2a lists full name.

Sub-Class Errantia	
Order Eunicida	
Family Dorvilleidea	
Dorv_annu	01
Order Phyllodocida	
Family Polynoidea	
Harm_imbri	02
Family Goniadidae	
Glyc_pict	03
Glyci_spju	04
Family Chrysopetalidae	
Pale_bell	05
Family Hesionidae	
Micro_dubi	06
Micro_sp	07
Family Nereididae	
Nean_limn	08
Nean_vire	09
Nean_ spju	10
Nere_vexl	11
Nere_spju	12
Plat_bica	13
Platy_sp	14
Family Syllidae	
Exog_dwis	15
Exog_sp	16
Spha_cali	17
Spha_N-1	18
Sylli_minu	19
Sylli_long	20
Sylli_spju	21
Family Nephtyidae	
Neph_caec	22
Neph_corn	23
Neph_unid	24
Bipa_corn	25
Family Phyllodocidae	
Eumi_long	26
Eteo_cali	27
Eteo_fauc	28
Eteo_spju	29
Phyl_hart	30
Phyl_spju	31
Sub-Class Sedentaria	
Order Orbiniida	
Family Orbiniidae	
Leit_puge	32
Leit_sp	33
Para_plat	34
Scol_armi	35
Scol_spju	36
Order Sabedellida	
Family Sabelidae	
Unid_Sabe	37
Family Oweniidae	
Owen_sp	38
Order Spionida	
Family Spionidae	
Dipo_quad	39
Poly_corn	40
Pseu_kemp	41
Pseu_pauc	42
Pygo_cali	43
Pygo_eleg	44
Rhyn_glut	45
Scol_squa	46
Scol_spju	47
Spio_unid	48
Spio_norr	49
Spio_bomb	50
Spio_spju	51
Streb_bene	52
Order Terebellida	
Family Terebellidae	
Poly_sp	53
Unid_Tere	54
Order Cirratulida	
Family Cirratulidae	
Thar_parv	55
Order Opheliida	
Family Opheliidae	
Poly_sp	56
Arma_brev	57
Ophe_lima	58
Thor_mucr	59
Unid_Ophe	60
Order Capitellida	
Aren_juv	61
Family Capitellidae	
Bara_amer	62
Capit_capi	63
Mage_hobs	64
Hete_fili	65
Noto_tenu	66
Noto_spju	67
Medi_cali	68
Family Maldanindae	
Saba_elon	69

Table A3. Percentage variance partitioned by RDA and Monte-Carlo permutation F tests for significance of primary axis (axis 1).

				% Var. Attributed to:				PRC Permutation Test Statistics		
Year	Site	Formulation	Metric	Time[1]	Treatment[2]	Residual[3]	% Trt. Var. Captured by axis 1	F	Pr(>F)	Sig.[4]
2011	BC	liquid	All Polychaetes	22.6	16.0	61.4	43.3	2.36	.057	NS
			No juv Poly	24.7	15.4	59.9	41.1	2.21	.121	NS
			Mollusks	16.2	17.3	66.5	63.0	3.44	.047	*
			No juv Moll	17.1	14.9	68.0	75.3	3.46	.118	*
			Crustaceans	17.0	15.2	67.8	56.3	2.66	.266	NS
			All Invertebrates	20.3	14.3	65.4	61.2	2.81	.019	*
		granular	All Polychaetes	19.3	37.9	42.8	77.7	12.34	.031	*
			No juv Poly	20.2	41.6	38.2	80.6	15.80	.033	*
			Mollusks	14.2	24.3	61.5	65.9	4.69	.026	*
			No juv Moll	14.4	25.8	59.8	76.2	5.90	.026	*
			Crustaceans	9.2	33.5	57.3	69.6	7.33	.032	*
			All Invertebrates	13.5	36.4	50.1	73.6	9.34	.027	*
2011	CR	liquid	All Polychaetes	17.0	38.1	44.9	71.9	10.97	.027	*
			No juv Poly	13.0	40.2	46.8	74.8	11.60	.034	*
			Mollusks	38.0	12.0	50.0	62.4	2.69	.086	NS
			No juv Moll	33.4	13.5	53.1	69.7	3.19	.112	NS
			Crustaceans	15.5	56.6	27.9	91.3	33.40	.026	*
			All Invertebrates	14.5	52.5	33.0	88.3	25.31	.028	*
2012	LB	liquid	All Polychaetes	3.7	8.7	87.6	80.8	6.99	.007	**
			No juv Poly	3.7	8.9	87.4	81.3	7.20	.005	**
			Mollusks	2.2	2.8	95.0	69.5	1.83	.514	NS
			No juv Moll	1.7	3.2	95.1	84.4	2.56	.423	NS
			Crustaceans	4.2	3.6	92.2	71.2	2.57	.210	NS
			All Invertebrates	2.9	5.5	91.6	68.4	3.61	.037	*
		granular	All Polychaetes	3.7	7.6	88.7	70.1	5.60	.008	**
			No juv Poly	3.8	7.7	88.5	70.6	5.73	.006	**
			Mollusks	2.7	7.6	89.7	86.9	5.40	.003	**
			No juv Moll	1.8	11.4	86.8	90.7	11.12	.001	**
			Crustaceans	2.7	8.3	89	49.5	4.39	.036	*
			All Invertebrates	2.5	7.6	89.9	63.8	5.00	.003	**
2012	BC	liquid	All Polychaetes	10.3	8.4	81.3	83.8	8.29	.001	***
			No juv Poly	11.0	9.1	79.9	87.5	9.50	.001	***
			Mollusks	5.3	4.6	90.1	64.9	3.68	.020	*
			No juv Moll	5.5	5.6	88.9	71.1	5.16	.025	*
			Crustaceans	12.2	8.3	79.5	71.8	7.87	.001	***
			All Invertebrates	7.8	8.3	83.9	74.2	6.61	.001	***
		granular	All Polychaetes	11.8	17.4	70.8	90.8	21.45	.001	***
			No juv Poly	12.4	18.6	69.0	91.5	23.60	.001	***
			Mollusks	7.0	4.5	88.5	68.6	5.40	.010	**
			No juv Moll	3.7	8.9	87.4	74.8	7.56	.006	**
			Crustaceans	6.6	26.8	66.6	91.7	35.51	.001	***
			All Invertebrates	6.8	19.9	73.3	88.3	22.24	.001	***
2014	SP	liquid	All Polychaetes	5.8	20.9	73.3	82.7	26.84	.001	***
			No juv Poly	6.5	18.9	74.6	81.3	23.50	.001	***
			Mollusks	2.8	17.0	80.2	83.5	20.72	.001	***
			No juv Moll	1.5	1.9	96.6	84.7	22.57	.001	***
			Crustaceans	2.3	15.0	82.7	85.4	7.87	.001	***
			All Invertebrates	3.6	19.2	77.2	86.3	24.53	.001	***

All	All	liquid	All Polychaetes	1.3	2.8	95.9	84.9	9.21	.010	**
			No juv Poly	1.4	2.8	95.8	85.0	8.84	.014	**
			Mollusks	2.1	1.8	96.1	76.4	5.25	.032	*
			No juv Moll	1.3	2.5	96.2	82.1	8.14	.005	*
			Crustaceans	3.5	1.6	94.9	73.1	4.54	.109	NS
			All Invertebrates	1.1	2.0	96.9	79.6	5.78	.045	*
		granular	All Polychaetes	3.2	4.4	92.4	71.9	9.12	.008	**
			No juv Poly	3.3	4.6	92.1	88.5	9.57	.008	**
			Mollusks	1.6	3.7	94.7	77.8	6.70	.012	*
			No juv Moll	1.8	5.0	93.2	76.5	9.08	.004	*
			Crustaceans	2.6	8.2	89.2	81.4	16.59	.001	***
			All Invertebrates	2.1	5.6	92.3	77.4	10.05	.003	**

[1] Conditioned Variation; partialed out of PRC diagaram

[2] Constrained Variantion; includes treatment x time interaction

[3] Unconstrained Variation; due to site effects, replicate effects, and unexplained variation

[4] Significance of axis 1 relative to other axis: *, $p > 0.05$; **, $p > 0.01$; ***, $p > 0.001$

Table A4. Monte Carlo permutation tests for main treatment effects (IMI) and interaction effects (IMI x time).

Year	Site	Formulation	Group	Terms	F	Pr (>F)	Sig.[1]
2011	BC	liquid	All Polychaetes	IMI	1.81	.037	*
				IMI * Time	1.82	.023	*
			Non juv Polychaetes	IMI	2.16	.024	*
				IMI * Time	1.61	.038	*
			All Mollusks	IMI	2.76	.047	*
				IMI * Time	1.35	.124	NS
			Non juv Mollusks	IMI	3.09	.058	NS
				IMI * Time	0.75	.562	NS
			Crustaceans	IMI	2.05	.016	*
				IMI * Time	1.34	.193	NS
			All Invertebrates	IMI	1.69	.026	*
				IMI * Time	1.46	.052	NS
		granular	All Polychaetes	IMI	12.13	.030	*
				IMI * Time	1.91	0.03	*
			Non juv Polychaetes	IMI	15.57	.033	*
				IMI * Time	2.02	.033	*
			All Mollusks	IMI	4.33	.030	*
				IMI * Time	1.39	.064	NS
			Non juv Mollusks	IMI	5.29	.03	*
				IMI * Time	1.23	.217	NS
			Crustaceans	IMI	6.78	.028	*
				IMI * Time	1.87	0.28	*
			All Invertebrates	IMI	9.43	.032	*
				IMI * Time	1.84	.032	*
2011	CR	liquid	All Polychaetes	IMI	10.43	.031	*
				IMI * Time	2.41	.031	*
			Non juv Polychaetes	IMI	11.34	.027	*
				IMI * Time	2.08	.027	*
			All Mollusks	IMI	1.92	.030	*
				IMI * Time	1.20	.371	NS
			Non juv Mollusks	IMI	2.61	.030	*
				IMI * Time	0.98	.404	NS
			Crustaceans	IMI	32.15	.030	*
				IMI * Time	2.21	0.30	*
			All Invertebrates	IMI	24.53	.033	*
				IMI * Time	2.07	.033	*
2012	PX	liquid	All Polychaetes	IMI	8.07	.001	***
				IMI * Time	0.09	.313	NS
			Non juv Polychaetes	IMI	9.30	.001	***
				IMI * Time	0.81	.490	NS
			All Mollusks	IMI	3.58	.005	**
				IMI * Time	0.92	.512	NS
			Non juv Mollusks	IMI	4.88	.005	**
				IMI * Time	1.13	.296	NS
			Crustaceans	IMI	7.64	.001	***
				IMI * Time	1.37	.112	NS
			All Invertebrates	IMI	6.51	.001	***
				IMI * Time	1.20	.120	NS

		granular	All Polychaetes	IMI	21.42	.001	***
				IMI * Time	1.11	.018	*
			Non juv Polychaetes	IMI	23.59	.001	***
				IMI * Time	1.10	.022	*
			All Mollusks	IMI	5.31	.005	**
				IMI * Time	1.28	.170	NS
			Non juv Mollusks	IMI	6.48	.003	**
				IMI * Time	1.81	.065	NS
			Crustaceans	IMI	34.56	.001	***
				IMI * Time	2.10	.001	***
			All Invertebrates	IMI	22.03	.001	***
				IMI * Time	1.58	.001	***
2012	LB	liquid	All Polychaetes	IMI	6.69	.005	**
				IMI * Time	0.98	.112	NS
			Non juv Polychaetes	IMI	6.91	.003	**
				IMI * Time	0.98	.115	NS
			All Mollusks	IMI	1.40	.303	NS
				IMI * Time	0.61	.695	NS
			Non juv Mollusks	IMI	2.45	.158	NS
				IMI * Time	0.30	.827	NS
			Crustaceans	IMI	1.53	.289	NS
				IMI * Time	1.04	.224	NS
			All Invertebrates	IMI	3.27	.031	*
				IMI * Time	1.00	.203	NS
		granular	All Polychaetes	IMI	5.58	.008	**
				IMI * Time	1.21	.024	*
			Non juv Polychaetes	IMI	5.71	.006	**
				IMI * Time	1.21	.019	*
			All Mollusks	IMI	5.31	.003	**
				IMI * Time	1.28	.129	NS
			Non juv Mollusks	IMI	10.61	.002	**
				IMI * Time	0.82	.349	NS
			Crustaceans	IMI	4.27	.017	*
				IMI * Time	2.30	.002	**
			All Invertebrates	IMI	4.82	.001	***
				IMI * Time	1.50	.004	***
2014	SP	liquid	All Polychaetes	IMI	25.76	.001	***
				IMI * Time	3.36	.001	***
			Non juv Polychaetes	IMI	22.95	.001	***
				IMI * Time	2.95	.001	***
			All Mollusks	IMI	19.80	.001	***
				IMI * Time	2.12	.001	***
			Non juv Mollusks	IMI	22.48	.001	***
				IMI * Time	2.09	.012	*
			Crustaceans	IMI	7.66	.001	***
				IMI * Time	1.37	.116	NS
			All Invertebrates	IMI	24.51	.001	***
				IMI * Time	1.95	.001	***
All Years	All Sites	liquid	All Polychaetes	IMI	8.78	.014	**
				IMI * Time	1.03	.001	***
			Non juv Polychaetes	IMI	8.49	.018	*

		IMI * Time	0.96	.001	***
	All Mollusks	IMI	5.01	.021	*
		IMI * Time	0.78	.241	NS
	Non juv Mollusks	IMI	7.89	.002	**
		IMI * Time	0.86	.263	NS
	Crustaceans	IMI	4.14	.125	NS
		IMI * Time	0.70	.090	NS
	All Invertebrates	IMI	5.73	.061	NS
		IMI * Time	0.76	.006	**
granular	All Polychaetes	IMI	9.07	.010	**
		IMI * Time	0.65	.086	NS
	Non juv Polychaetes	IMI	9.53	.010	**
		IMI * Time	0.64	.093	NS
	All Mollusks	IMI	6.21	.007	**
		IMI * Time	1.20	.055	NS
	Non juv Mollusks	IMI	7.67	.006	**
		IMI * Time	2.10	.011	*
	Crustaceans	IMI	15.54	.002	***
		IMI * Time	2.42	.001	***
	All Invertebrates	IMI	9.70	.003	**
		IMI * Time	1.64	.001	***

[1] Significance of effect: *, $p > 0.05$; **, $p > 0.01$; ***, $p > 0.001$

Table A5. Concentrations of imidacloprid ($\bar{x}$ ± S.E., N), confidence intervals (C.I.), and ranges among sites of differing formulation during large scale field trials, 2011, 2012, and 2014.

Formulation	Site[1]	Concentration (ppb)	95 % C.I.	Range	Reference
liquid IMI	Bay Center	11 ± 3, 5	4 – 18	4 – 19	Patten 2011
	Cedar River	1250 ± 150, 2	-656 – 3156	1100 – 1400	Patten 2011
	Leadbetter	1500 ± 0, 1			Patten 2011
	Palix	2400 ± 0, 1			Grue and Grassly 2012
	Stony Pt	796 ± 260, 5	75 – 1715	180 – 1600	Booth et al. 2014
	Coast	230 ± 0, 1			Booth et al. 2014
	Nisbett	290 ± 0, 1			Booth et al. 2014
granular IMI	Bay Center	52 ± 9, 5	26 – 78	27 – 82	Patten 2011
	Cedar River	24 ± 8, 2	-72 – 119	16 – 32	Patten 2011
	Leadbetter	73 ± 0, 1			Patten 2011
	Palix	490 ± 0, 1			Grue and Grassly 2012
liquid IMI	All	685 ± 186, 16	288 – 1082	4 – 2400	
granular IMI	All	97 ± 50, 9	-18 – 211	16 – 490	

[1] Two treated sites not sampled for benthic invertebrates: Coast, adjacent to and treated simultaneoulsy with Stony Pt. with less vegetation and more uniform substrate; Nisbett (2014), N. Willapa near Cedar River, silty substrate.

Table A6. Number of PRCs with a negative[1] , positivie[2], or neutral[3] position of the principal response at the final sample date compared to pre-treatment (PRC end response) for each of 49 PRC analysis with both significant treatment effects and a significant axis 1.

PRC End Response	Year – Study Site – Formulation	No. of PRCs	Taxonomic Assemblage
Negative	2012 – Palix – Liquid	2	Mollusk
			Crustaceans
	All Years, Sites – Liquid	2	Mollusk
			Non-juvenile Mollusk
	2012 – Palix – Granular	1	Non-juvenile Mollusk
	All Years, Sites – Granular	1	Non-juvenile Mollusk
	Total	**6**	
Positive	All Years, Sites – Liquid	2	Polychaetes
			Non-juvenile Polychaetes
	2011 – Bay Center -- Granular	1	Mollusks
	2012 – Leadbetter – Granular	1	Mollusks
	All Years, Sites – Granular	1	Mollusks
	Total	**5**	
Neutral	2011 – Bay Center – Liquid	2	Mollusk
			All Families
	2011 Cedar River – Liquid	4	Polychaetes
			Non-juvenile Polychaetes
			Crustaceans
			All Families
	2012 – Palix – Liquid	4	Polychaetes
			Non-juvenile Polychaetes
			Non-juvenile Mollusks
			All Families
	2012 – Leadbetter – Liquid	3	Polychaetes
			Non-juvenile Polychaetes
			All Families
	2014 – Stony Pt – Liquid	6	Polychaetes
			Non-juvenile Polychaetes
			Mollusks
			Non-juvenile Mollusks
			Crustaceans
			All Families
	2011 – Bay Center – Granular	5	Polychaetes
			Non-juvenile Polychaetes
			Non-juvenile Mollusks
			Crustaceans
			All Families
	2012 – Palix – Granular	5	Polychaetes

		Non-juvenile Polychaetes
		Mollusks
		Crustaceans
		All Families
2012 – Leadbetter – Granular	5	Polychaetes
		Non-juvenile Polychaetes
		Non-juvenile Mollusks
		Crustaceans
		All Families
All Years, Sites – Granular	4	Polychaetes
		Non-juvenile Polychaetes
		Crustaceans
		All Families
Total	**38**	

[1] Response of the test assemblage relative to the control was lower at the final sample date compared to before.

[2] Response of the test assemblage relative to the control was higher at the final sample date compared to before.

[3] Response of the test assemblage relative to the control assemblage was the same at the final sample date compared to before.

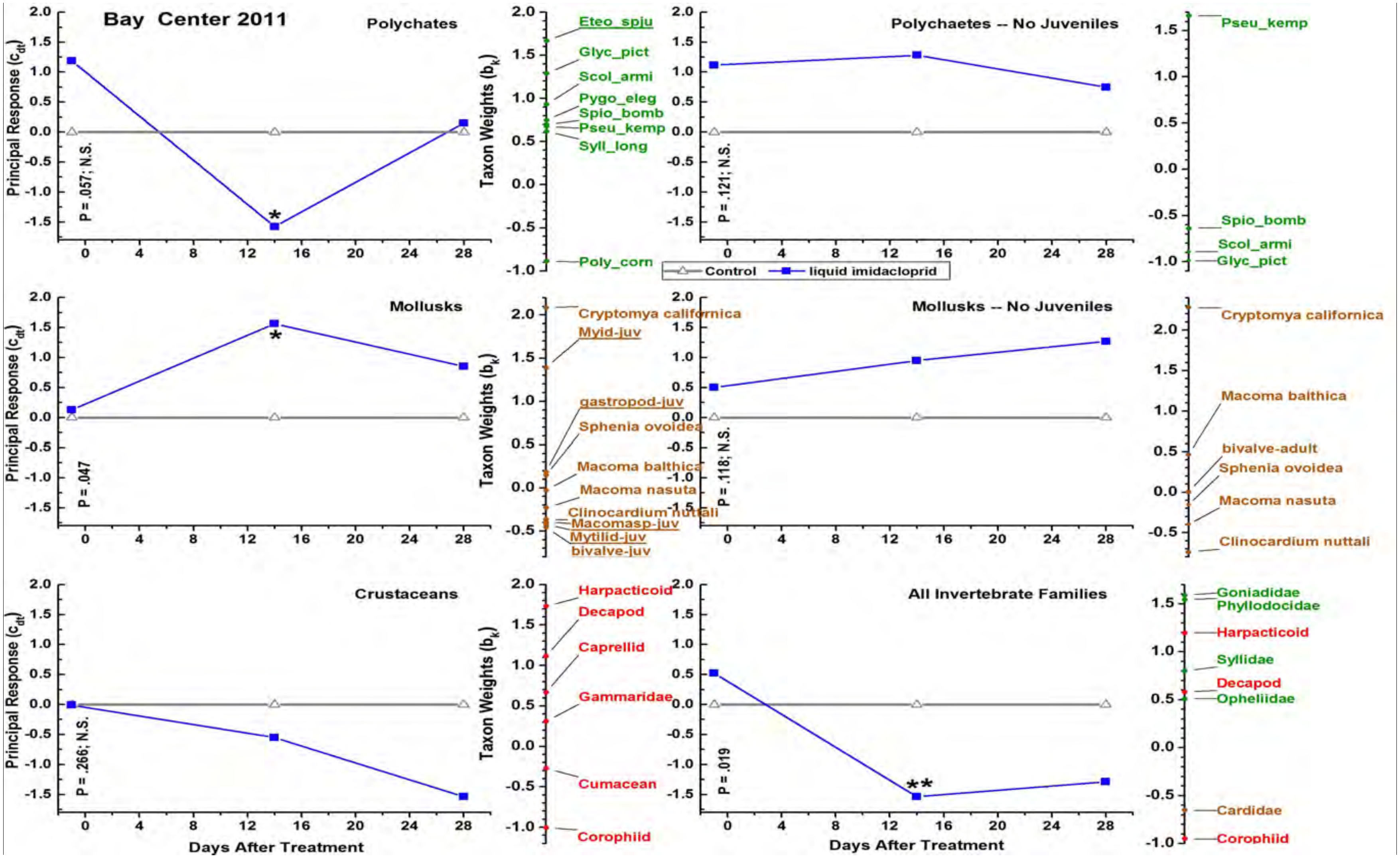


Figure A5. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Bay Center in 2011. P is probability that the displayed primary axis is significant. Asterisks indicates the response at each sample date is significantly different from the control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Taxon weights indicate taxa that are positively or negatively correlated with the shape of the curve (weights > -0.06 and < .06 for polychaetes are not shown). Underlined taxa are juveniles. Table A2 lists polychaete full names and abbreviations.

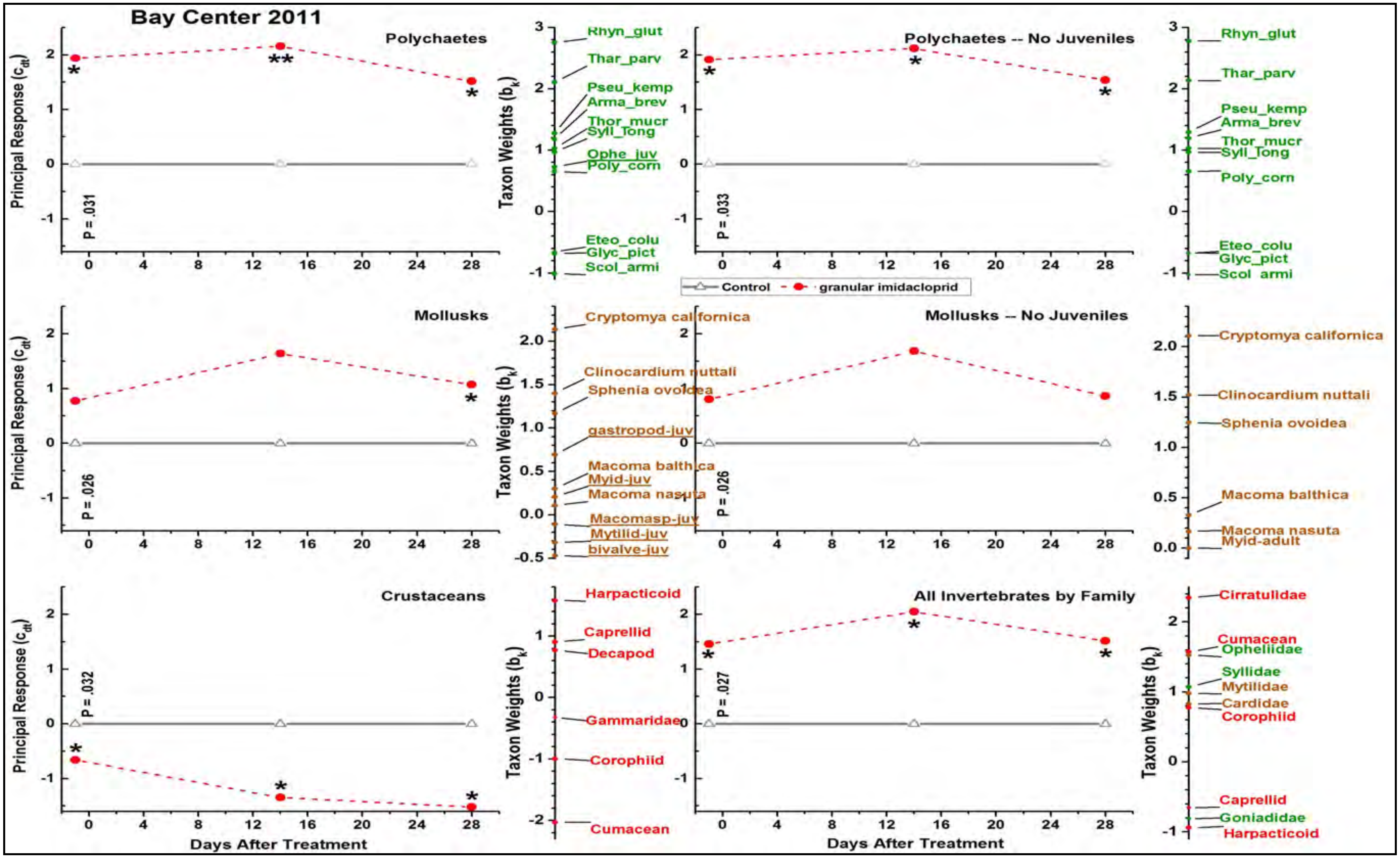


Figure A6. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at granular imidacloprid and control plots at Bay Center in 2011. P is probability that the displayed primary axis is significant. Asterisks indicates the response at each sample date is significantly different from the control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Taxon weights indicate taxa that are positively or negatively correlated with the shape of the curve (weights > -0.06 and < .06 for polychaetes are not shown). Underlined taxa are juveniles. Table A2 lists polychaete full names and abbreviations.

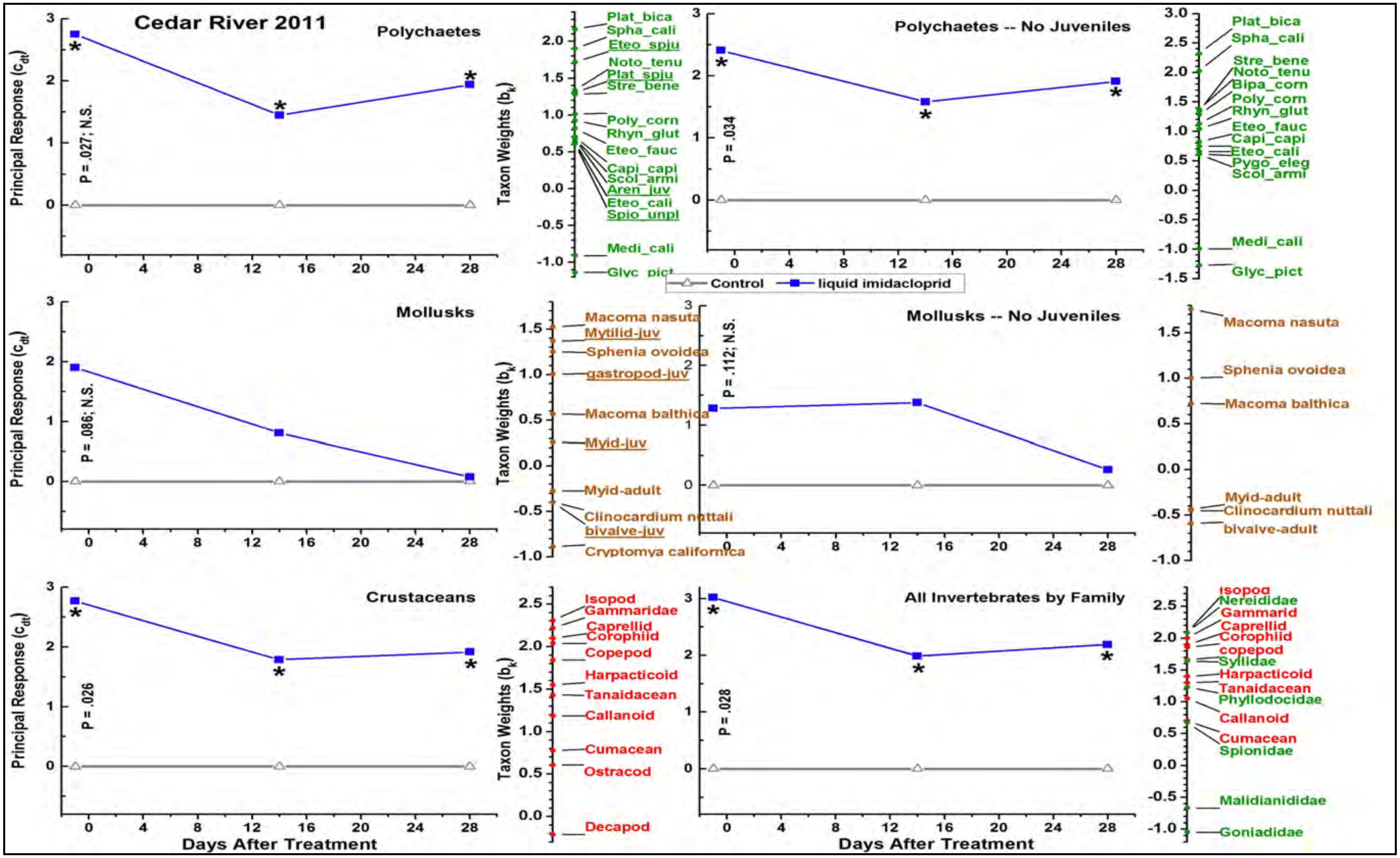


Figure A7. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Cedar River in 2011. P is probability that the displayed primary axis is significant. Asterisks indicates the response at each sample date is significantly different from the control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Taxon weights indicate taxa that are positively or negatively correlated with the shape of the curve (weights > -0.06 and < .06 for polychaetes are not shown). Underlined taxa are juveniles. Table A2 lists polychaete full names and abbreviations.

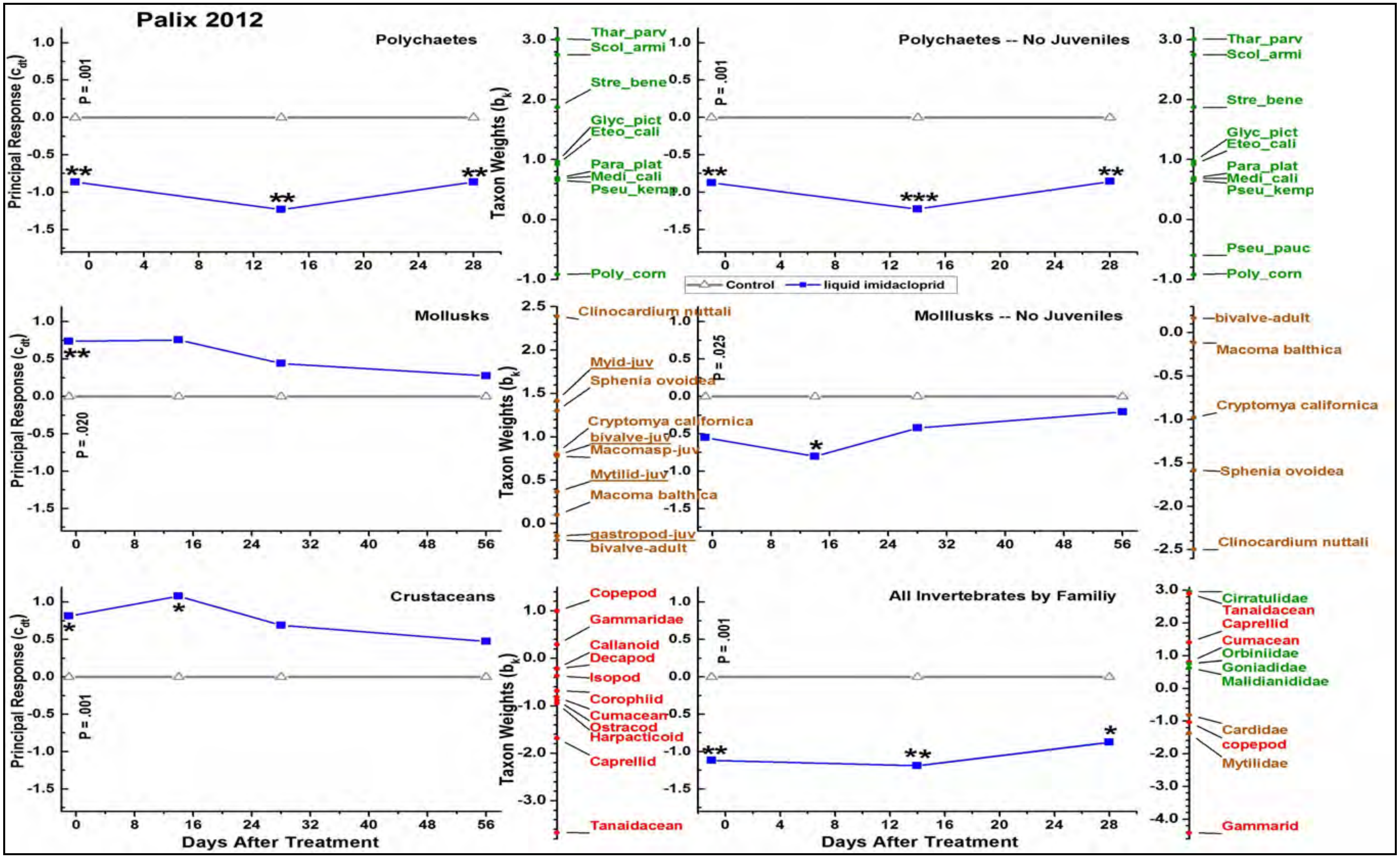


Figure A8. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Palix in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response at each sample date is significantly different from the control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Taxon weights indicate taxa that are positively or negatively correlated with the shape of the curve (weights > -0.06 and < .06 for polychaetes are not shown). Underlined taxa are juveniles. Table A2 lists polychaete full names and abbreviations.

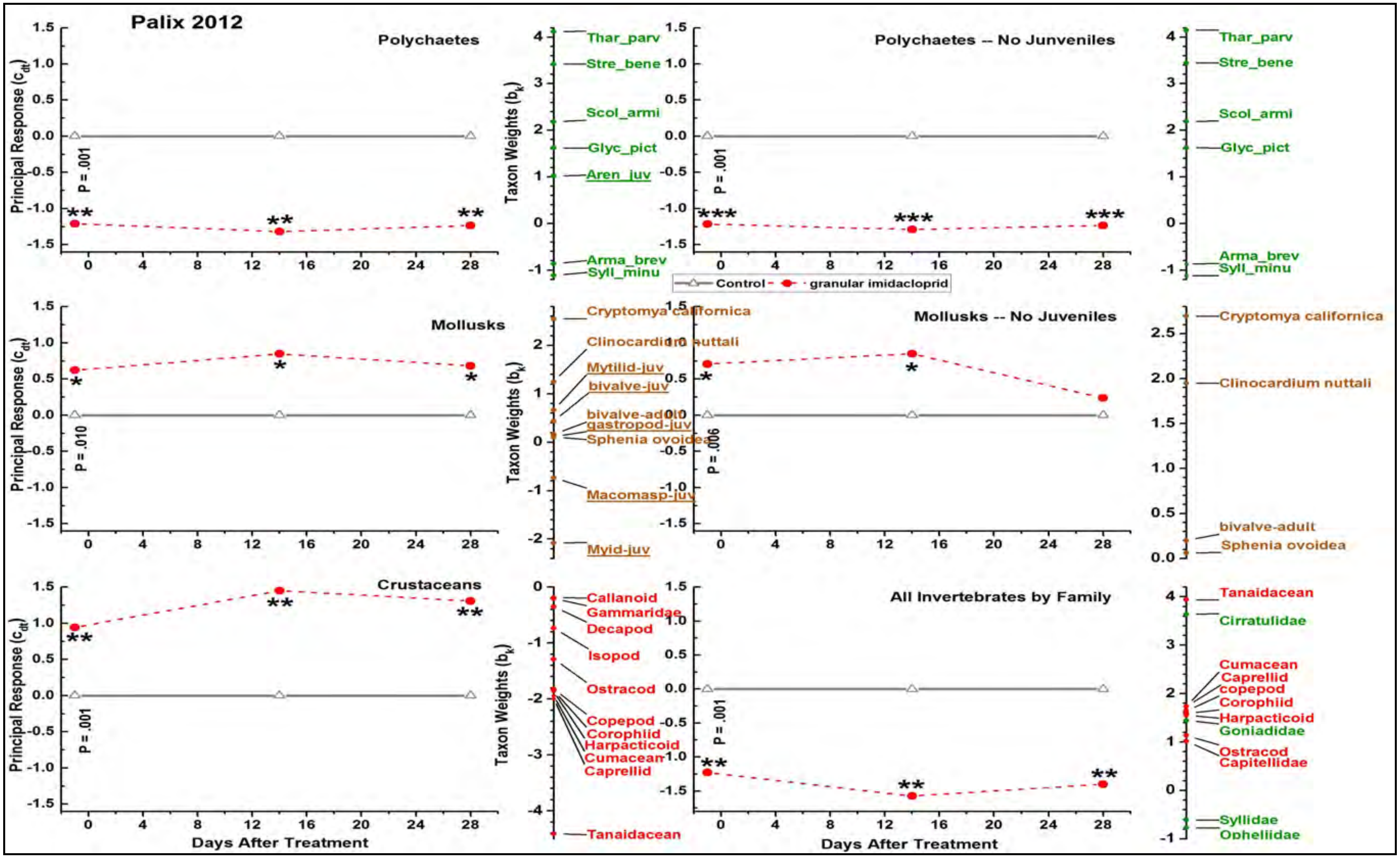


Figure A9. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at granular imidacloprid and control plots at Palix in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response at each sample date is significantly different from the control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Taxon weights indicate taxa that are positively or negatively correlated with the shape of the curve (weights > -0.06 and < .06 for polychaetes are not shown). Underlined taxa are juveniles. Table A2 lists polychaete full names and abbreviations.

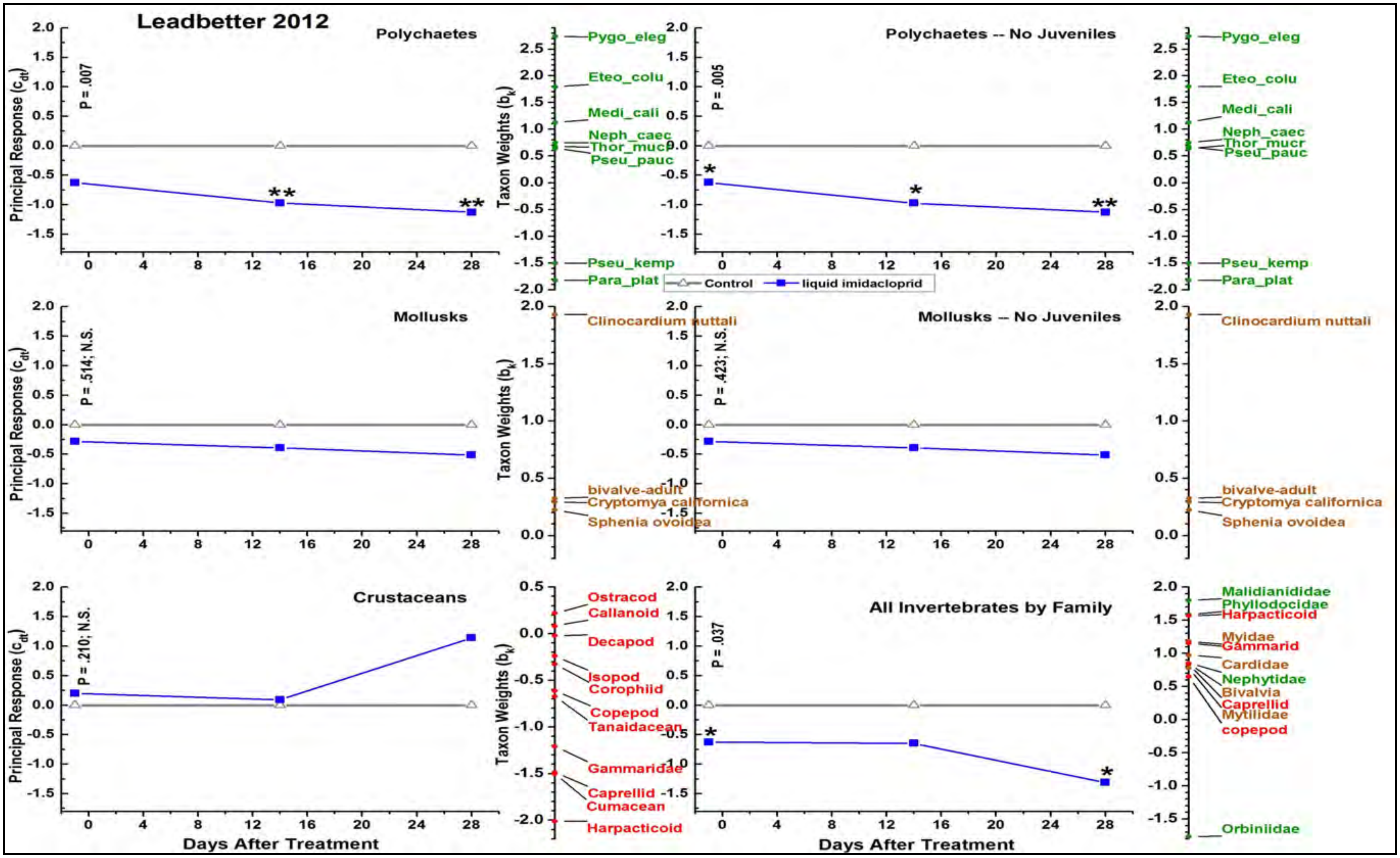


Figure A10. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Lead Better in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response at each sample date is significantly different from the control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Taxon weights indicate taxa that are positively or negatively correlated with the shape of the curve (weights > -0.06 and < .06 for polychaetes are not shown). Underlined taxa are juveniles. Table A2 lists polychaete full names and abbreviations.

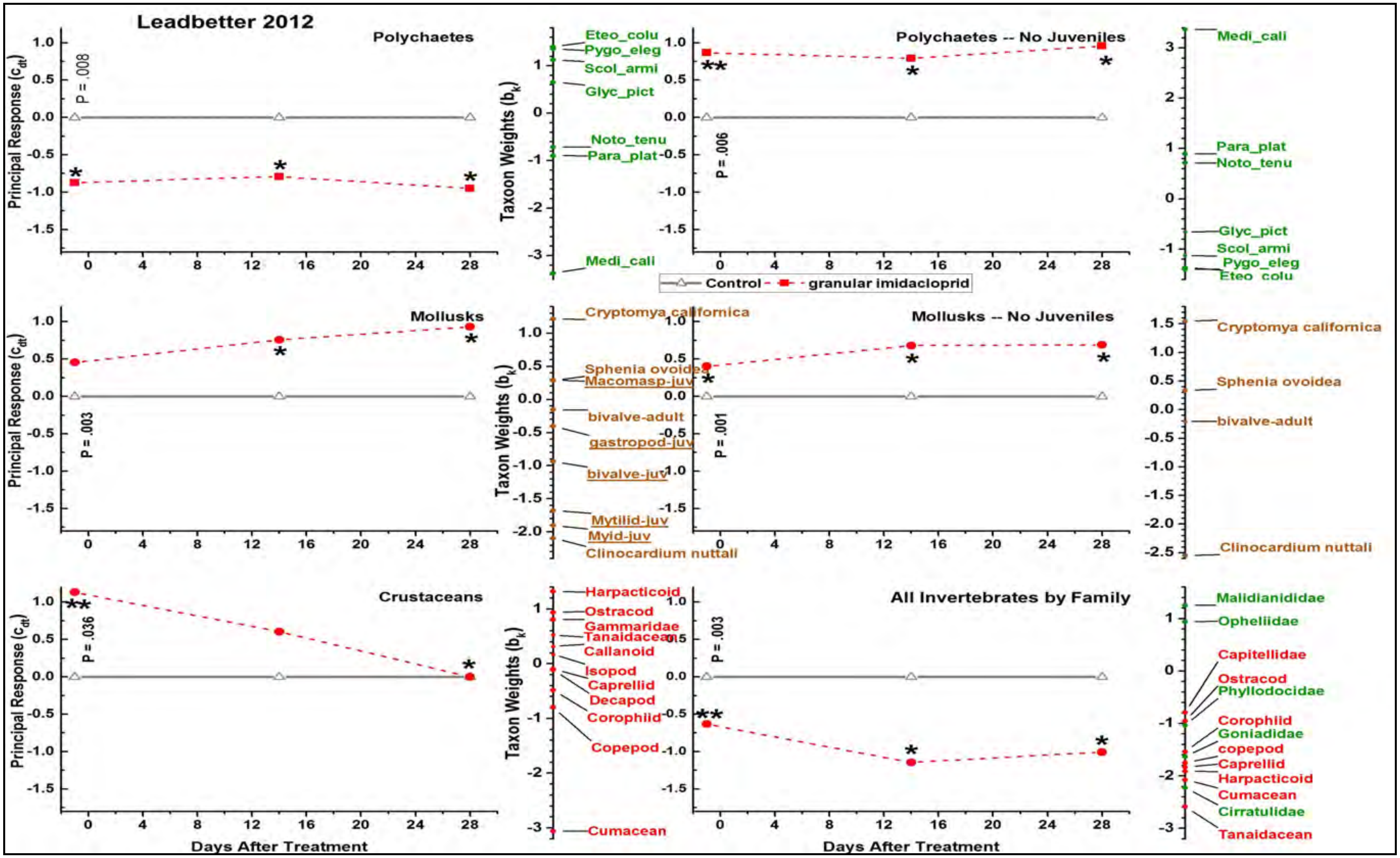


Figure A11. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at granular imidacloprid and control plots at Leadbetter in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response at each sample date is significantly different from the control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Taxon weights indicate taxa that are positively or negatively correlated with the shape of the curve (weights > -0.06 and < .06 for polychaetes are not shown). Underlined taxa are juveniles. Table A2 lists polychaete full names and abbreviations.

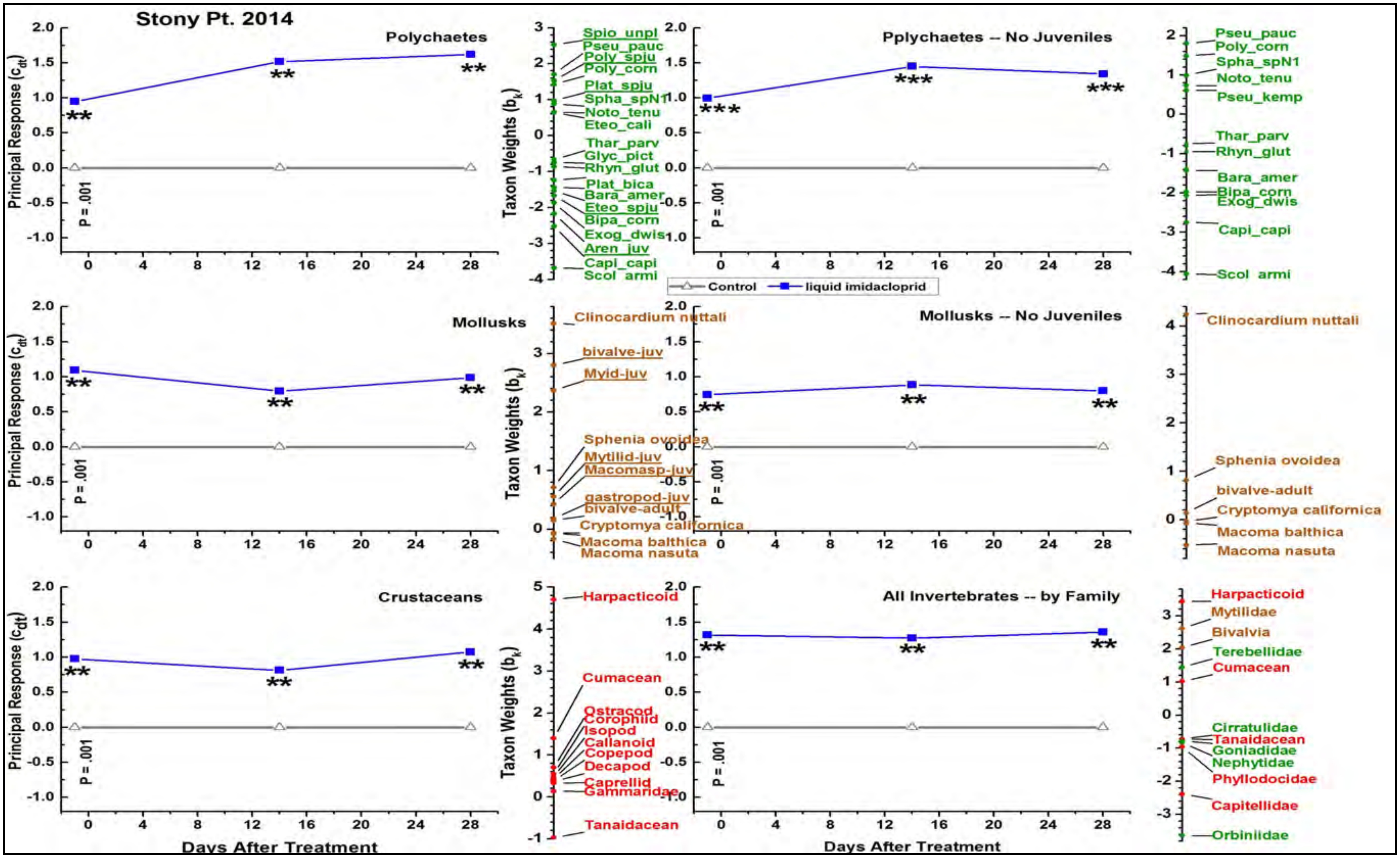


Figure A12. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Stony Pt in 2014. P is probability that the displayed primary axis is significant. Asterisks indicates the response at each sample date is significantly different from the control (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Taxon weights indicate taxa that are positively or negatively correlated with the shape of the curve (weights > -0.06 and < .06 for polychaetes are not shown). Underlined taxa are juveniles. Table A2 lists polychaete full names and abbreviations.

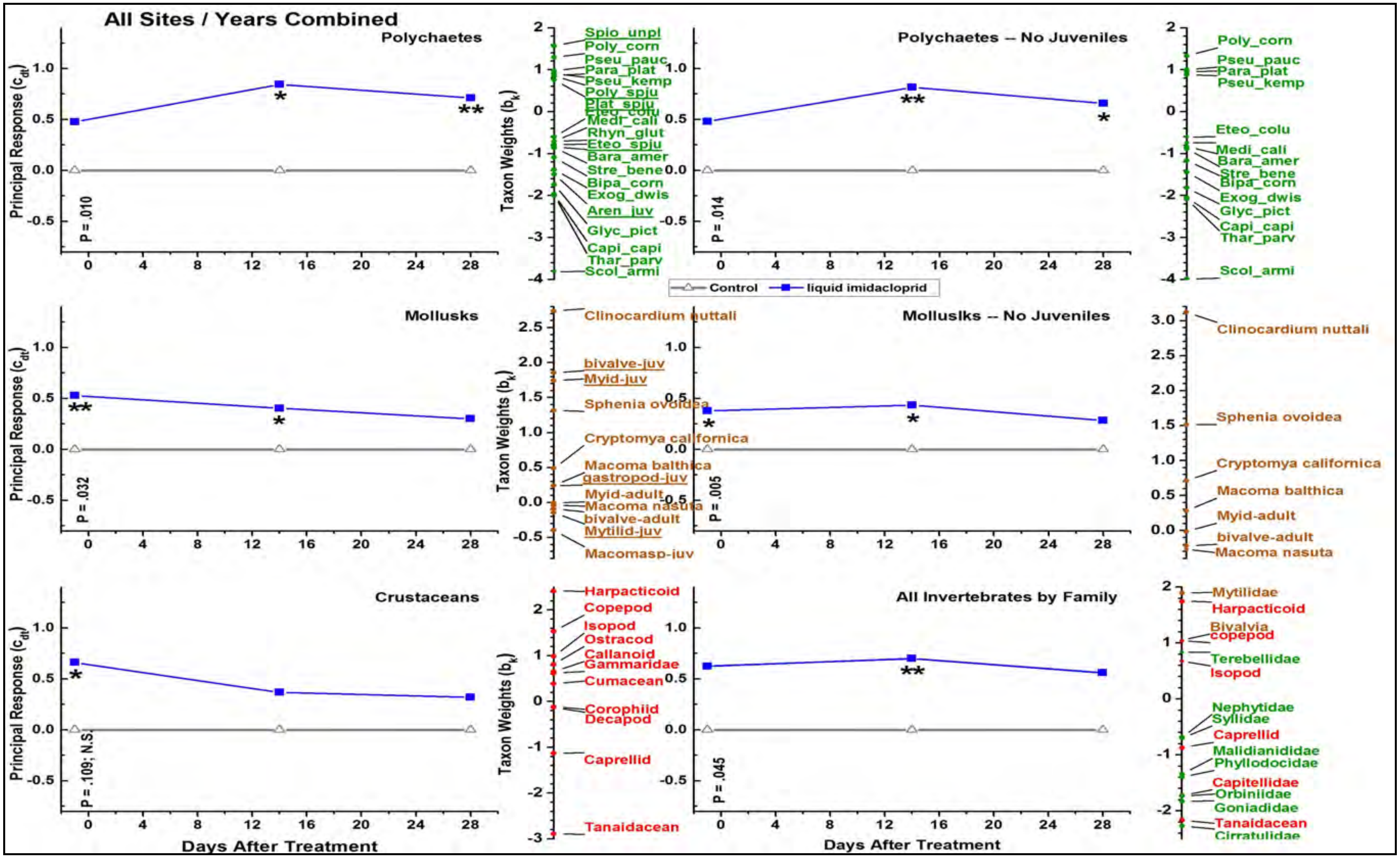


Figure A13. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots with all sites and years combined. P is probability that the displayed primary axis is significant. Asterisks indicates the response at each sample date is significantly different from the control (*, p < 0.05; **, p < 0.01; ***, p < 0.001). Taxon weights indicate taxa that are positively or negatively correlated with the shape of the curve (weights > -0.06 and < .06 for polychaetes are not shown). Underlined taxa are juveniles. Table A2 lists polychaete full names and abbreviations.

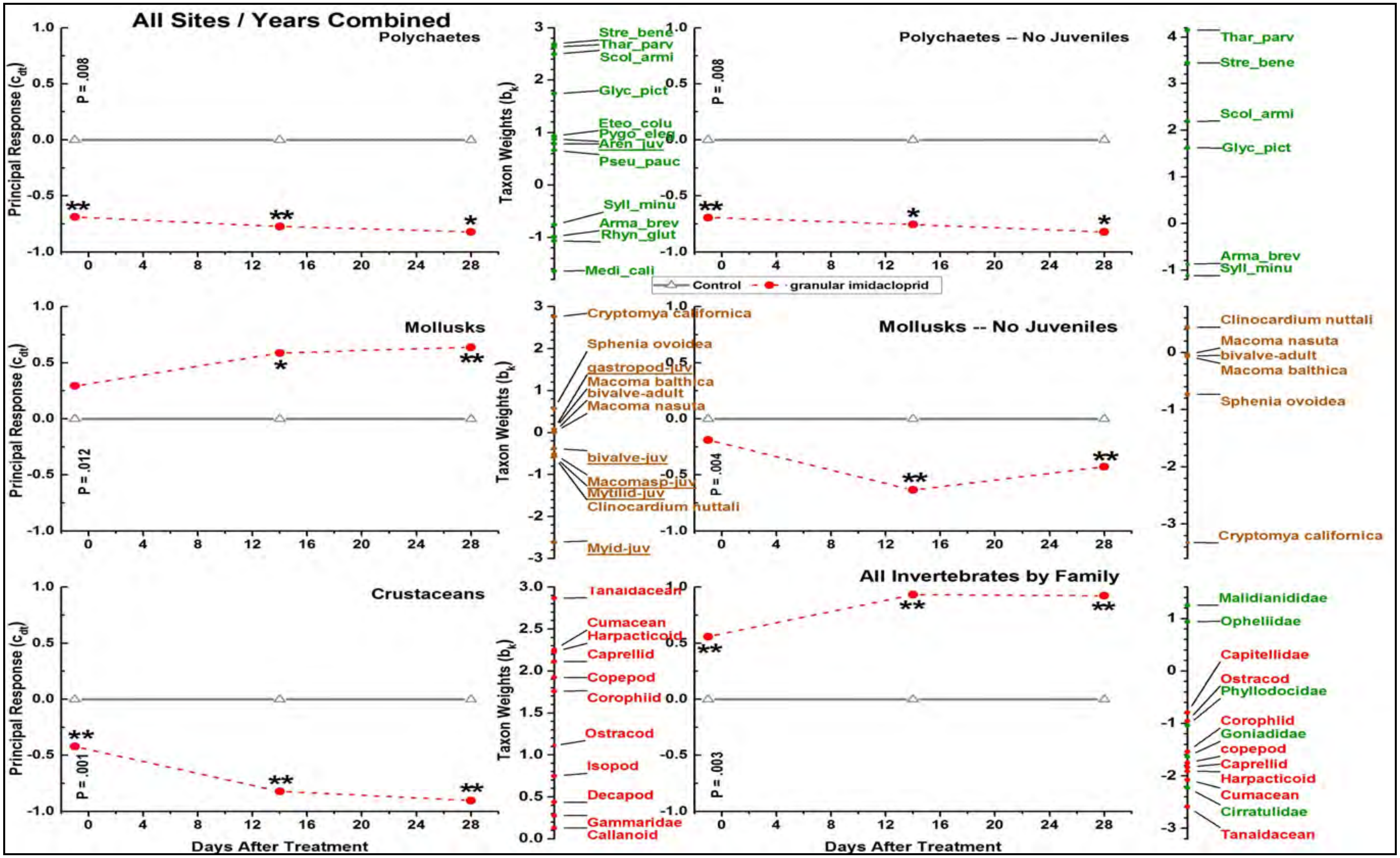


Figure A14. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at graunular imidacloprid and control plots with all sites and years combined. P is probability that the displayed primary axis is significant. Asterisks indicates the response at each sample date is significantly different from the control (*, p < 0.05; **, p < 0.01; ***, p < 0.001). Taxon weights indicate taxa that are positively or negatively correlated with the shape of the curve (weights > -0.06 and < .06 for polychaetes are not shown). Underlined taxa are juveniles. Table A2 lists polychaete full names and abbreviations.

References

Anatra-Cordone, M. and P. Durkin. 2005. Imidacloprid - Human Health and Ecological Risk Assessment– Final Report. Prepared for USDA, Forest Service, Forest Health Protection, GSA Contract No. 10F-0082K, USDA Forest Service BPA: WO-01-3187-0150, USDA Purchase Order No.: 43-1387-4-3131, Task No. 24. Submitted by Syracuse Environmental Research Associates, Inc., 5100 Highbridge St., 42C, Fayetteville, New York 13066-0950.

Bakalian, A. B. 1985. The use of Sevin on estuarine oyster beds in Tillamook Bay, Oregon. Coastal Zone Management Journal 13, pp.49–83.

Banas, N.S., Hickey, B.M., MacCready, P. and J.A. Newton. 2004. Dynamics of Willapa Bay, Washington: A highly unsteady, partially mixed estuary. Journal of Physical Oceanography, 34(11), pp.2413-2427.

Beukema, J.J., Cadée, G.C. and R. Dekker. 2002. Zoobenthic biomass limited by phytoplankton abundance: evidence from parallel changes in two long-term data series in the Wadden Sea. Journal of Sea Research, 48(2), pp.111-125.

Booth, S.R., Patten, K. and A. Suhrbier. 2011. Field trials of imidacloprid on burrowing shrimp, 2009. Report from WGHOGA and PSI to Washington State Legislature 8 pp.

Booth, S.R. and K. Rassmussen. 2011. Impact of imidacloprd on epi-benthic and benthic invertebrates: Initial studies to describe the Sediment Impact Zone (SIZ) related to imidacloprid treatments to manage burrowing shrimp. Report from PSI to WSU. 11 pp.

Booth, S.R. and K. Rassmussen. 2013. Impact of imidacloprd on epi-benthic and benthic invertebrates: 2011 studies to describe the Sediment Impact Zone (SIZ) related to imidacloprid treatments to manage burrowing shrimp. Report from PSI to WSU. 27 pp.

Booth, S.R., Patten, K., Hudson, B., and A. Suhrbier. 2015. Impact of imidacloprid on epi-benthic and benthic invertebrates: 2014 studies to describe the Sediment Impact Zone (SIZ) related to imidacloprid treatments to manage burrowing shrimp. Report to WDFW. 23 pp.

Borcard, D., Legendre, P., and P. Drapeau, P. 1992. Partialling out the spatial component of ecological variation. Ecology, 73, pp.1045–1055.

Brooks, K. M. 1993. Changes in arthropod and mollusk populations associated with the application of Sevin to control burrowing shrimp in Willapa Bay, July to September, 1992. Report prepared for Pacific County Economic Development Council, Aquatic Environmental Sciences, Port Townsend, Washington.

Brooks, K.M. 1995. Long-term response of benthic invertebrate communities associated with the application of carbaryl (Sevin™) to control burrowing shrimp, and an assessment of the habitat value of cultivated Pacific oyster (*Crassostrea gigas*) beds in Willapa Bay, Washington to fulfill requirements of the EPA carbaryl data call in. US Environmental Protection Agency, Region X.

California Rice Commision, 2014. List of Pesticides used on California Rice. (http://www.omicnet.com/reports/rice/ListOfPestcidesUsedOnCaliforniaRice.pdf).

Chung, K.W., Chandler, A.R. and P.B. Key. 2008. Toxicity of carbaryl, diquat dibromide, and fluoranthene, individually and in mixture, to larval grass shrimp, Palaemonetes pugio. Journal of Environmental Science and Health Part B, 43(4), pp. 293-299.

Colville, A., Jones, P., Pablo, F., Krassoi, F., Hose, G. and R. Lim. 2008. Effects of chlorpyrifos on macroinvertebrate communities in coastal stream mesocosms. Ecotoxicology, 17(3), pp. 173-180.

Cuppen, J.G., Van den Brink, P.J., Camps, E., Uil, K.F. and T.C. Brock. 2000. Impact of the fungicide carbendazim in freshwater microcosms. I. Water quality, breakdown of particulate organic matter and responses of macroinvertebrates. Aquatic toxicology, 48(2), pp.233-250.

Dumbauld, B.R. 1994. Thalassinid shrimp ecology and the use of carbaryl to control populations on oyster ground in Washington Coastal Estuaries. Ph.D. Dissertation, School of Fisheries, Univ. of Wash., Seattle, WA pp 192.

Dumbauld, B.R., Booth, S., Cheney, D., Suhrbier, A. and H. Beltran. 2006. An integrated pest management program for burrowing shrimp control in oyster aquaculture. Aquaculture, 261(3), pp. 976-992.

Dumbauld, B.R., K.M. Brooks, and M.H. Posey. 2001. Response of an estuarine benthic community to application of the pesticide carbaryl and cultivation of Pacific oysters (*Crassostrea gigas*) in Willapa Bay, Washington. Marine Pollution Bulletin, 10.42, pp. 826-844.

Ehler, L.E. and D.G. Bottrell. 2000. The illusion of integrated pest management. Issues in Science and Technology Online. Spring, 2000. 6 pp.

Ellis, J.D., Evans, J.D. and J. Pettis. 2010. Colony losses, managed colony population decline, and Colony Collapse Disorder in the United States. Journal of Apicultural Research, 49(1), pp.134-136.

Emmett, R., Llansó, R., Newton, J., Thom, R., Hornberger, M., Morgan, C., Levings, C., Copping, A. and P. Fishman. 2000. Geographic signatures of North American west coast estuaries. Estuaries, 23(6), pp. pp.765-792.

Feldman, K.L., Armstrong, D.A., Dumbauld, B.R., DeWitt, T.H. and D.C. Doty. 2000. Oysters, crabs, and burrowing shrimp: review of an environmental conflict over aquatic resources and pesticide use in Washington State's (USA) coastal estuaries. Estuaries, 23(2), pp.141-176.

Ferraro, S.P. and F.A. Cole. 2007. Benthic macrofauna–habitat associations in Willapa Bay, Washington, USA. Estuarine, Coastal and Shelf Science, 71(3), pp.491-507.

Ferraro, S.P. and F.A. Cole. 2012. Ecological periodic tables for benthic macrofaunal usage of estuarine habitats: insights from a case study in Tillamook Bay, Oregon, USA. Estuarine, Coastal and Shelf Science, 102, pp.70-83.

Gauch, H.G., Jr. 1982. Multivariate analysis in community structure. Cambridge University Press, Cambridge.

Gill, R.J., Ramos-Rodriguez, O. and N.E. Raine. 2012. Combined pesticide exposure severely affects individual-and colony-level traits in bees. Nature, 491(7422), pp.105-108.

Goulson, D. 2013. An overview of the environmental risks posed by neonicotinoid insecticides. J. Appl. Ecol. 50, pp. 977–987.

Green, R.H., 1979. Sampling design and statistical methods for environmental biologists. John Wiley & Sons.

Grue, C., Grassley, J.M., and J.A. Frew. 2011. Concentrations of imidacloprid in sediment pore water following application of imidacloprid in Willapa Bay, Washington. Report to WGHOGA. 20 pp.

Grue, C.E., Grassley, J.M., Frew, J.A., and A. Troiano. 2012. Use of an enzyme--linked immunosorbent assay (ELISA) to quantify imidacloprid in sediment pore water following application of imidacloprid in Willapa Bay, Washington – matrix effects and cross-reactivity. Report to WGHOGA. 13 pp.

Grue, C. 2013. Survival of ghost shrimp in sediments exposed to imidacloprid in the laboratory: Implications for control of shrimp on oyster beds in Willapa Bay, WA. Presentation to Pacific Coast Shellfish Grower's Association Annual Conference, Sun River, OR. Oct 2, 2013.

Grue, C. and M. Grassley 2013. 2012 Final report: Environmental fate and persistence of imidacloprid following applications to control burrowing shrimp on commercial oyster beds in Willapa Bay, Washington. Report to Willapa Grays Harbor Oyster Growers Association and the Washington Department of Fish and Wildlife in partial fulfillment of WDFW Contract No. 12-1113.

Jones, C.G., Lawton, J.H., and M. Shachak. 1994. Organisms as ecosystem engineers. Oikos 69, pp. 373-386.

Key, P., Chung, K., Siewicki, T. and M. Fulton. 2007. Toxicity of three pesticides individually and in mixture to larval grass shrimp (Palaemonetes pugio). Ecotoxicology and Environmental Safety, 68(2), pp.272-277.

Kuivila, K.M. and M.L. Hladik. 2008. Understanding the occurrence and transport of current-use pesticides in the San Francisco Estuary, Watershed. Estuary & Watershed, v. 6, article 2.

Leonard, A.W., Hyne, R.V., Lim, R.P., Pablo, F. and P.J. Van den Brink. 2000. Riverine endosulfan concentrations in the Namoi River, Australia: Link to cotton field runoff and macroinvertebrate population densities. Environmental toxicology and chemistry, 19(6), pp.1540-1551.

López-Mancisidor, P., Carbonell, G., Fernández, C. and J.V. Tarazona. 2008. Ecological impact of repeated applications of chlorpyrifos on zooplankton community in mesocosms under Mediterranean conditions. Ecotoxicology, 17(8), pp.811-825.

MacGinitie, G. E. 1930. The natural history of the mud shrimp *Upogebia pugettensis* (Dana). Annals and Magazine of Natural History 6, pp.37–45.

MacGinitie, G. E. 1934. The natural history of *Callianassa californiensis* (Dana). American Midland Naturalist 15, pp.166–177.

Maund, S., Biggs, J., Williams, P., Whitfield, M., Sherratt, T., Powley, W., Heneghan, P., Jepson, P. and N. Shillabeer. 2009. The influence of simulated immigration and chemical persistence on recovery of macroinvertebrates from cypermethrin and 3, 4 dichloroaniline exposure in aquatic microcosms. Pest management science, 65(6), pp.678-687.

Mohr, S., Berghahn, R., Schmiediche, R., Hübner, V., Loth, S., Feibicke, M., Mailahn, W. and J. Wogram. 2012. Macroinvertebrate community response to repeated short-term pulses of the insecticide imidacloprid. Aquatic Toxicology, 110, pp.25-36.

Morrissey, C.A., Mineau, P., Devries, J.H., Sanchez-Bayo, F., Liess, M., Cavallaro, M.C. and K. Liber. 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. Environment International, 74, pp.291-303.

Norkko, J., Norkko, A., Thrush, S.F., Valanko, S. and H. Suurkuukka. 2010. Conditional responses to increasing scales of disturbance, and potential implications for threshold dynamics in soft-sediment communities. Marine Ecology Progress Series, 413, pp.253-266.

Osterberg, J.S., Darnell, K.M., Blickley, T.M., Romano, J.A. and D. Rittschof. 2012. Acute toxicity and sub-lethal effects of common pesticides in post-larval and juvenile blue crabs, *Callinectes sapidus*. Journal of Experimental Marine Biology and Ecology, 424, pp.5-14.

Palmer, M. 2016. The ordination web page. Botany Department, Oklahoma State University, Stillwater, OK. http://ordination.okstate.edu

Patten, K. 2006. Screening of alternative methods to manage burrowing shrimp infestations on bivalve shellfish grounds. Report from WSU to Washington State Commission for Pesticide Registration. 6 pp.

Patten, K. 2015. Efficacy of Imidacloprid against burrowing shrimp, 2014. Report to the Washington Department of Fish and Wildlife.

Pilditch, C.A., Valanko, S., Norkko, J. and A. Norkko. 2015. Post-settlement dispersal: the neglected link in maintenance of soft-sediment biodiversity. Biology letters, 11(2), p.20140795.

Pisa, L.W., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Downs, C.A., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., McField, M. and C.A. Morrissey. 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. Environmental Science and Pollution Research, 22(1), pp.68-102.

Posey, M.H., Dumbauld, B.R., and D.A. Armstrong. 1991. Effects of a burrowing mud shrimp, Upogebia pugettensis (Dana), on abundances of macro-infauna. Journal of Experimental Marine Biology and Ecology 148(2), pp. 283-294.

Sardo, A.M. and A.M.V.M. Soares. 2010. Assessment of the effects of the pesticide imidacloprid on the behaviour of the aquatic oligochaete Lumbriculus variegatus. Archives of environmental contamination and toxicology, 58(3), pp.648-656.

Schäfers, C., Hommen, U., Dembinski, M. and J.F. Gonzalez Valero. 2006. Aquatic macroinvertebrates in the Altes Land, an intensely used orchard region in Germany: correlation between community structure and potential for pesticide exposure. Environmental toxicology and chemistry, 25(12), pp.3275-3288.

Simenstad, C.A. and K.L. Fresh. 1995. Influence of intertidal aquaculture on benthic communities in Pacific Northwest estuaries: scales of disturbance. Estuaries, 18(1), pp.43-70.

Song, M.Y., Stark, J.D. and J.J. Brown. 1997. Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods. Environmental Toxicology and Chemistry, 16(12), pp.2494-2500.

Tomizawa, M. and J.E. Casida. 2003. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annual Review Entomology. 48, pp.339-64.

Vanblaricom, G.R., Eccles, J.L., Olden, J.D. and P.S. McDonald. 2015. Ecological effects of the harvest phase of geoduck (Panopea generosa Gould, 1850) aquaculture on infaunal communities in southern Puget Sound, Washington. Journal of Shellfish Research, 34(1), pp.171-187.

Van den Brink, P.J. and C.J. Ter Braak. 1999. Principal response curves: Analysis of time dependent multivariate responses of biological community to stress. Environmental Toxicology and Chemistry, 18(2), pp.138-148.

Van den Brink, P.J., Den Besten, P.J., bij de Vaate, A. and C.J. Ter Braak, C.J., 2009. Principal response curves technique for the analysis of multivariate biomonitoring time series. Environmental monitoring and assessment, 152(1), pp.271-281.

Wheatcroft, R.A., Sanders, R.D. and B.A. Law. 2013. Seasonal variation in physical and biological factors that influence sediment porosity on a temperate mudflat: Willapa Bay, Washington, USA. Continental Shelf Research, 60, pp.S173-S184.

Wiberg, P.L., Law, B.A., Wheatcroft, R.A., Milligan, T.G. and Hill, P.S., 2013. Seasonal variations in erodibility and sediment transport potential in a mesotidal channel-flat complex, Willapa Bay, WA. Continental Shelf Research, 60, pp.S185-S197.

Zhenga, S., Chena, B., Qiua, X., Chenb, M., Maa, Z. and X. Yua. 2016. Distribution and risk assessment of 82 pesticides in Jiulong River and estuary in South China. Chemosphere 144, pp. 1177–1192.

Acknowledgments:
We thank the 1) State of Washington College of Agricultural, Human, and Natural Resource Sciences, and 2) Washington State Department of Fish and Wildlife for funding the original studies and this manuscript.

celebrating 40 YEARS
HARTCROWSER
www.hartcrowser.com

ATTACHMENT C to WGHOGA comments on the Draft Supplemental Environmental Impact Statement for Control of Burrowing Shrimp using Imidacloprid on Commercial Oyster and Clam Beds in Willapa Bay and Grays Harbor

DATE: April 3, 2015

TO: Jesse DeNike, Plauché & Carr

FROM: Jeff Barrett

RE: **NMFS Agency Comments**
12733-02-10

CC: Adrienne Stutes, James Selleck

The information provided within is in response to agency comments for the Willapa-Grays Harbor Oyster Growers Association project.

NMFS Comments

The following information is in response to comments from the National Marine Fisheries Service (NMFS), December 8, 2014.

Comment #1

The draft permit allows significant increases in acreages for shrimp control over previous levels. Specifically, the treatment in Grays Harbor would increase from 200 acres treated per calendar year to 500 acres, and treatment in WIllapa Bay would increase from 600 to 1,500 acres per calendar year for a total of 2,000 acres treated annually. This more than doubles the amount of area previously permitted for treatment with the carbamate insecticide, carbaryl.

Response

The total acreage being proposed does not necessarily represent an increase in the treated area. The higher acreage for imidacloprid is due in part to relative uncertainty on the efficacy of treatment, because imidacloprid is less toxic than carbaryl, and the ability to control burrowing shrimp may require more frequent spraying with imidacloprid. The increased acreage may represent re-spraying areas that have been previously treated, so that the total unique area exposed to imidacloprid in Willapa Bay and Grays Harbor could be much smaller than the acreage allowed. In addition, as growers learn how to best use imidacloprid, it is expected that the total acreage requested for spraying each year will decrease.

Even under the unlikely assumption that all permissible acres are sprayed, and that all acres are unique (i.e., not re-sprayed), 1,500 acres in Willapa Bay represents only 3.33 percent of the total tideland acreage (45,000 acres), and 500 acres in Grays Harbor represents only 1.45 percent (of 34,460 acres). Thus, the vast majority of both of these estuaries will not be sprayed in any given year, ensuring that any ecological benefits from unsprayed areas will be present and unimpaired. That includes any ecological benefits from the presence of burrowing shrimp, including as prey to other organisms.

There is also a significant difference in the concentration of chemical applied. The active ingredient for carbaryl was 8.0 pounds per acre, but imidicloprid is only applied at 0.5 pounds of active ingredient per acre, so the total applied active ingredient per year will be drastically reduced. Accordingly, concerns by NMFS of a significant impact to the estuary in general, or to fish in particular, are not supported given that the vast majority of both estuaries will not be affected by imidacloprid treatments and those acres that are sprayed will have much less active ingredient.

Comment #2

[p. 2] *NMFS strongly encourages a more cautious approach. There are far too many unknowns with imidacloprid's use; and issues to be worked out regarding impacts to other aquatic and terrestrial biota. We believe measured increases in acreage treated up to the proposed amount would allow for more effects information to be obtained. Ecology should begin by keeping the acreage as before.*

Response

As with Comment #1 above, the NMFS reviewer is incorrectly assuming that every acre being requested in the permit will be sprayed each year, and that none of the areas sprayed would be sprayed more than once. If re-spraying areas is needed, the total unique acreage sprayed would effectively be smaller than that sprayed with carbaryl. Ecology chose to consider the full 2,000 acres in the EIS to ensure that any possible impacts were reviewed. This decision also reflected the understanding that imidacloprid is not as effective as carbaryl, and in order to obtain sufficient efficacy of shrimp removal using imidacloprid, a more flexible treatment plan could be needed.

Data collected from Willapa Bay documented a large recruitment pulse of burrowing shrimp in the last few years, possibly due to a recruitment cycle (Dumbauld, USDA-ARS, data under review, personal communication). This pulse resulted in a need to treat an extensive area in order to facilitate oyster growing. If future burrowing shrimp recruitment is reduced to levels more normally observed in the past 20 years, then it is possible that the total acreage of uniquely treated areas may be reduced in time to match the previous acreage used for carbaryl application. The issuance of the new permit needs to allow for adequate levels of imidacloprid application in order to match the current recruitment cycle, and ensure the effectiveness of treatment. Without these increased areas, the required effects to control burrowing shrimp may not be achieved.

Extensive field studies conducted by the University of Washington, Washington State University, and the Pacific Shellfish Institute have documented that imidacloprid is much less toxic to non-target organisms than carbaryl. In addition, as mentioned above, the concentration of active ingredient proposed for imidicloprid is significantly lower than that used for carbaryl, and 2,000 acres is the greatest possible area for a potential treatment range. The NMFS reviewer's recommendation that acreage limits for imidacloprid mimic those used for carbaryl ignores the much lower toxicity of imidacloprid, and lower application rates of active ingredient. Accordingly, a direct link in acreages sprayed with each of these two chemicals is scientifically unsupported.

Comment #3

The rationale for these increases, by Ecology, are 1) the growers were concerned with the efficacy of imidacloprid, and 2) growers believed the number of burrowing shrimp have been increasing.

NMFS is not convinced that an increased area for imidacloprid application in necessary, based on the growers' concern with the efficacy of imidacloprid. Trials conducted from 2010 to 2012 indicated that granular and liquid forms of imidacloprid were moderately to highly effective in reducing densities of shrimp. A 500+ acre application at 0.5 lb imidacloprid study occurred in 2014, but the results of this application have not been made available to Ecology or NMFS. NMFS would like Ecology to review data regarding efficacy, water quality, sediment, and benthic results before making a final determination.

Regarding the suggestion by Ecology that growers believe the numbers of burrowing shrimp are increasing, The NMFS has not been provided data from Mr. Rockett of Ecology to support this claim. Sampling results from water chemistry studies conducted during test treatments is not available (at the time of this review).

Response

As above, NMFS makes the assumption that all acres contained in the acreage limits will be sprayed, and that the limits represent unique acres rather than re-spraying areas that have already been treated.

While efficacy studies have been conducted for several years, much of this work has been on small plots or on adult burrowing shrimp only. As noted above, a recruitment pulse of young burrowing shrimp was documented in Willapa Bay. In response, growers are experimenting with imidacloprid to determine the most effective treatment for burrowing shrimp that requires the least amount of chemical. Given the cost of spraying, growers are highly motivated to determine how to use as little imidacloprid as necessary. Prior studies have documented a number of interrelated variables that affect efficacy, including the seasonal timing of spraying (e.g., early versus late summer), the amount of eelgrass that is present, the frequency and depth of water that is retained on the plots during low tide, and the density of burrowing shrimp. The growers require higher acreage limits to ensure they can successfully treat burrowing shrimp, while resorting to a less effective approach of shrimp control. As previously discussed, over time the growers are expected to become more knowledgeable about the use of imidacloprid and therefore more effective at treatment, resulting in fewer acres being sprayed each year. This trend could be accelerated if burrowing shrimp recruitment drops to levels more representative of the past 20 years.

Results from the 2014 field studies have been provided to Ecology, and should be finalized and available to NMFS soon. The 2014 field studies include results of imidacloprid application to large plots on water quality, sediment data, benthic biota, and efficacy in reducing shrimp burrows. Separate scientific data are also in review (Dumbauld, USDA-ARS, personal communication) regarding the recent increases in burrowing shrimp recruitment. The results from the 2014 field studies arrived at the same conclusions as previous work; in particular, that areas treated with imidacloprid have invertebrate communities that are not statistically different than non-sprayed control areas at 14 days following treatment. Thus, the 2014 studies provide additional support for the conclusion that imidacloprid is not producing a significant negative impact on invertebrate communities where it has been sprayed.

Comment #4

[p. 3] *Burrowing shrimp play an important role in the ecosystem. Habitat modifications include beneficial and adverse effects. Shrimp are prey, and an important link in estuarine trophic pathways. Dungeness crab and cutthroat trout feed on shrimp, and control of shrimp is likely to reduce the quality of EFH for ESA fish, salmon, groundfish, and coastal pelagic species.*

Response

The agency's concerns over habitat impacts resulting from the total acreage treated again make incorrect assumptions about the total acres that will be sprayed. For argument's sake, assuming 2,000 acres (of 79,460 acres) will be sprayed every year, this represents only 2.52 percent of the total tidelands acreage in both Willapa Bay and Grays Harbor. With 97.48 percent of the two estuaries left untreated, any negative habitat impacts will be *de minimus*. This is particularly true given that field trials for imidacloprid have uniformly failed to find significant negative effects on non-target invertebrates, at both 14 and 28 days following treatment. Thus, even areas that are sprayed are likely to retain the majority of the invertebrate fauna that were present prior to treatment, or that are present on non-sprayed control areas.

Numerous studies have documented that burrowing shrimp typically reduce the biodiversity and density of other invertebrate species (Dumbauld and Wyllie-Echeverria 1997; Dumbauld and Wyllie-Echeverria 2003; Colin et al. 1986; Doty et al. 1990), and biodiversity on eelgrass and oyster habitats is often greater than on burrowing shrimp habitat (Ferraro and Cole 2007). The burrowing activities of the shrimp alter the habitat quality in a way that is deleterious to many other species, including those that are important prey for fish. Burrowing shrimp are prey for a variety of species, but no fish species listed by the NMFS reviewer feeds exclusively on burrowing shrimp. Most biologists view areas with high biodiversity as being more valuable ecologically, than areas with low biodiversity. We assume NMFS also supports higher biodiversity, and therefore should not object to control of burrowing shrimp on a small percentage of these estuaries on an annual basis. That still leaves an overwhelming amount of acreage for burrowing shrimp, including whatever ecological values in creating habitat or serving as prey organisms that come with their presence.

The DEIS discusses the history of burrowing shrimp control in detail, but it is worth noting that this is not an eradication proposal. The proposed use of imidacloprid is to help maintain the control of shrimp on oyster beds, as has occurred for over 50 years. While burrowing shrimp are native to the area, their populations in the bay expanded significantly in the 1940s. Imidacloprid is being proposed as a less environmentally impactful solution for selective control of burrowing shrimp.

Comment #5

Control of burrowing shrimp may reduce habitat quality for green sturgeon, and green sturgeon may suffer direct effects by ingesting imidacloprid bound sediments. Prey resource is a primary element of green sturgeon critical habitat.

Response

Green sturgeon feed opportunistically on burrowing shrimp, but as described above, imidacloprid is going to be applied to only a small percentage of Willapa Bay and Grays Harbor. The NMFS reviewer provides no evidence that reducing burrowing shrimp on less than three percent of these estuaries per year, which is a maximum level of treatment unlikely to be achieved as discussed in comments above, would have any deleterious effect on green sturgeon feeding. It is not credible to contend that green sturgeon having access to over 75,000 acres of untreated estuary for feeding would be impacted by any reduction in burrowing shrimp on such a small proportion of the remaining area. In addition, review of the NMFS website on green sturgeon[1] indicates a number of factors thought to be contributing to low green sturgeon numbers. None of those threats involve insufficient food. Instead, limits on spawning habitat are deemed "the principal factor in the decline…"

Imidacloprid will be primarily applied on existing oyster habitat. Green sturgeon do not prefer to feed directly in oyster habitat (Kim Patten, WSU, personal communication). No sturgeon feeding pits have been observed, and there is no scientific evidence of green sturgeon feeding in oyster beds.

To the extent that green sturgeon may feed in other areas immediately following imidacloprid treatment, they may encounter and ingest burrowing shrimp containing imidacloprid residues. This theoretical scenario is not scientifically concerning, because one of the advantages of imidacloprid is its extremely low toxicity to vertebrates. High doses of imidacloprid injected directly into white sturgeon and rainbow trout tissue resulted in persistence in the plasma of the fish 36 hours later, but there were

---

[1] Available at: http://www.nmfs.noaa.gov/pr/species/fish/ greensturgeon.htm#threats.

no discernable effects to the brain, liver, kidney, or muscle tissues (Frew 2013). Absence of impacts from direct injection of high imidacloprid doses provides strong support that incidental ingestion of exposed shrimp will have no effects on sturgeon or other fish. Another advantage of imidacloprid is that it dissolves rapidly in sediments, and is diluted quickly with the incoming tide. Frew (2013) found concentrations in the sediments following application around green sturgeon foraging habitat to be two orders of magnitude below the threshold value in which effects on sturgeon have been noted in laboratory studies.

As described in the Environmental Impact Statement (EIS), there is no evidence of reduced prey availability or of harmful exposure of imidacloprid to green sturgeon. Thus, there is no scientific basis for the NMFS reviewer's concern over potential effects on essential fish habitat for green sturgeon or any other fish species.

Comment #6

Experiments in imidacloprid-treated rice fields by Hayasaka et al. (2012) showed direct negative effects on the species abundance of the zooplankton community, leading to the indirect suppression of growth in fishes feeding on the zooplankton species. Sanchez-Bayo and Goka (2006) found indirect effects on algae growth in rice fields after changes of the arthropod communities induced by imidacloprid.

Response

This paragraph is a direct copy and paste from the publication "Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid" (Van Dijk et al., 2013). It is not the opinion of the reviewer, using the citations listed. The algae blooms in reference were *Spirogyra* sp., which developed specifically in the absence of *Chironomus yoshimatsui*, a freshwater midge. This study was conducted in a restricted area with no rapid tidal flushing or dispersal mechanisms that exist for imidacloprid in the estuary. More importantly, this study found that biodiversity was not impacted by the use of imidacloprid, and that differences between treatments could not be attributed to imidacloprid due to limited exposure.

More misleading is the study referenced from Hayasaka et al. (2012). That study examined the cumulative effects of two insecticides, imidacloprid and fipronil. The study determined that fipronil was more persistent in the soil than imidacloprid, and that ecological impacts on benthic species and associated fish was a result of the residual fipronil, not imidacloprid.

Thus, both references are inappropriately cited by NMFS. Field studies of imidacloprid in Willapa Bay and Grays Harbor are the best test for expected effects of imidacloprid spraying to support shellfish aquaculture in these estuaries, not obscure references based on freshwater ecosystems. Relevant studies have consistently shown that invertebrate communities are largely indistinguishable between treatment and control plots at 14 and 28 days following imidacloprid treatment in these estuaries. This is strong empirical support for issuance of the permit.

Comment #7

Ecology is clearly aware that imidacloprid is a persistent broad spectrum pesticide that will kill nearly all benthic organisms on the acreage directly treated. NMFS believes will impact benthic prey species in areas where the spray has drifted by tidal currents. Including prey for salmon, groundfish, coastal pelagic species, herring, sandlance, and smelt. Activities reducing prey directly affect the growth and survival of pacific salmon (NMFS 2009). NMFS encourages ecology to take impacts to fish into greater consideration.

Response

This statement is incorrect. It assumes that imidacloprid causes direct mortality in benthic organisms. In fact, nearly all of the field studies with imidacloprid in Willapa Bay have shown that invertebrate communities in treatment and control plots are statistically indistinguishable at 14 and 28 days following treatment. The experimental design of these studies generally makes it difficult to determine if the lack of a treatment effect is due to low indirect mortality or rapid recolonization from surrounding portions of the estuary. The continued presence of native shellfish and some large polychaetes that do not generally migrate supports the conclusion that some taxa suffer no mortality. Regardless, there is no scientific or experimental evidence supporting the reviewer's bold statement of widespread and lingering impacts from imidacloprid treatment.

With respect to vertebrate species, imidacloprid has limited effects except in extremely high concentrations. For example, the lethal toxicity (LC_{50}) of imidacloprid for juvenile white sturgeon was found to be 124 milligrams per liter ($mg\ L^{-1}$), and injected concentrations as high as 250 micrograms per kilogram ($\mu g\ kg^{-1}$) had no effect on kinetics of rainbow trout (Frew 2013). Also, these were laboratory studies using direct injections into the tissues, and do not account for rapid tidal dispersal of the chemical or low rates of absorption experienced in the environment. Imidacloprid does not even directly kill burrowing shrimp except at concentrations much higher than those allowed under the permit. Instead, it causes a temporary paralysis response in crustaceans (Gervais et al. 2010). Burrowing shrimp must continuously clear their burrows, and during the temporary paralysis the burrows collapse, suffocating the shrimp. Thus, imidacloprid is highly selective to burrowing shrimp, which makes it an ideal chemical control.

The list of fish species presented by the NMFS reviewer is overly broad. Imidacloprid is not expected to affect many of these species, as they either do not feed directly on the benthic tidelands where imidacloprid will be administered, or are highly mobile species that migrate between habitat types. Studies conducted in the estuaries found no significant difference in fish abundance between the three habitat types discussed here: eelgrass, shrimp-infested areas, and oyster beds (Dumbauld, USDA-ARS, data under review, personal communication). When these fish are found in the nearshore, examination of stomach content is not representative of the respective habitat where they were caught. For example, salmon diets in the estuary were found to be more closely dependent on pelagic insects from other associated habitats. The salmon fed on insects in freshwater prior to migrating through the benthic nearshore habitat. The likelihood of exposure is further reduced, as imidacloprid will only be administered during low tide when fish are not present due to the lack of water. Other proposed methods to reduce any potential for impacts of imidacloprid treatment include waiting until after seasonal out-migration of juvenile salmon completes, and application only during periods of low wind to prevent the accidental spread of imidacloprid beyond established buffers from estuary channels or sloughs.

Comment #8

[p. 4] *NMFS is concerned with delayed, lingering, and latent effects. Experimental studies have found significant effects from persistent, toxic levels below no effects levels. Cumulative effects of neonicotinoids imply that even the lowest concentrations have toxic effects if sustained over a long period, which is especially relevant for species with a long life span such as sturgeon (Van Dijk, et al. 2012). The serious concern about the far-reaching consequences of abundant use of imidacloprid for aquatic ecosystems is justified.*

Response

Data submitted to Ecology for fieldwork in 2011, 2012, and the soon-to-be released data for 2014 all document that imidacloprid in surface water is rapidly diluted by the incoming tide following treatment. Given an approximately 10-foot tidal range and unimpeded mixing within Willapa Bay and Grays Harbor, imidacloprid in water can be expected to dilute to non-detectable levels within one or two tidal cycles at most, and to be below any possible biologically relevant concentration in that same timeframe. Previous work has similarly documented that imidacloprid concentrations in sediment pore water and whole sediments exhibit an approximately exponential decline over 28 days and 56 days respectively. Many sediment samples are below detection levels within 28 days. Given the exponential rate of decline following treatment, imidacloprid is expected to be below detection levels, and ultimately below any possible biologically relevant concentration, within a weeks to a few months at most. Monitoring in 2015 will be conducted to confirm that the areas sprayed with imidacloprid the year prior are below laboratory detection levels. Collectively, all of this empirically derived research information shows that there is no basis to conclude that imidacloprid is persistent in water, sediment pore water, or whole sediments.

Against this body of empirically derived evidence the NMFS reviewer again refers to the Van Dijk publication that was inappropriately applied above, and which does not scientifically support the argument against imidacloprid use in Willapa Bay and Grays Harbor. The reviewer also inappropriately cites the Hayasaka et al. (2012) paper, which in fact describes imidacloprid as not having residual effects.

In addition to the scientific evidence documenting that imidacloprid is not persistent, and therefore will not have chronic, sub-lethal effects, we note that comments by the NMFS reviewer were copied and pasted from the Van Dijk et al. (2012) paper, and are accordingly misleading. That was not an experimental study. The Van Dijk study was a comparison between separate databases from separate locations, which were not paired by direct spatial or temporal scales. The study states, "A significant negative relationship between imidacloprid concentration and macro-invertebrate abundance…does not necessarily imply that imidacloprid is the main cause for lower species abundance." The paper used correlation analysis to draw comparisons of sites with up to three kilometers between the locations where imidacloprid was used and the locations where invertebrate surveys unrelated to the imidacloprid application were conducted (the surveys were part of a separate national monitoring program). The work was also conducted on freshwater ecosystems, examined agricultural runoff of imidacloprid, and often included water bodies with limited flushing. All of these differences from the proposed use of imidacloprid in Willapa Bay and Grays Harbor indicate the reference to this study by NMFS is scientifically inappropriate.

The Van Dijk study acknowledged that imidacloprid does not bind with vertebrate receptors (e.g., green sturgeon), and specifically targets insects. Other studies have confirmed this as well, specifically in showing limited or no effect in sturgeon (Frew 2013). Cumulative effects cited by the NMFS reviewer were not experimentally tested in the Van Dijk paper. Van Dijk cites Hayasaka et al. (2012), as discussed above, which was again based on the simultaneous use of multiple insecticides (imidacloprid and fipronil) in fresh water systems, and is not scientifically relevant when discussing sturgeon in an estuary.

Comment #9

The Puget Sound toxic site recovery standard of 50% recovery to biotic richness and abundance is not sufficiently protective of aquatic resources and their habitats. Applying this standard to acres sprayed and off-site areas affected could represent a huge and continuing loss in biotic production for other valuable species. NMFS recommends the use of an 80% recovery to support listed species and other resources.

Response

This statement of expected impact is unsupported by any scientific or other information. The Department of Ecology is charged with implementing the Clean Water Act, including the NPDES permit under consideration here, in Washington State. As the responsible agency, Ecology developed criteria and standards for assessing the magnitude of impacts to water, sediments, and related biota for NPDES permitted discharges. That includes the entire SIZ program, which is an Ecology created and implemented system, not a component of the federal CWA. As noted above, the Department of Ecology has properly concluded the use of imidacloprid, as conditioned, will comply with the applicable regulations, and the NMFS has not provided concrete or specific evidence demonstrating Ecology's determination is erroneous.

Further, it is important to recognize that the threshold criterion referenced by the USFWS is only relevant when invertebrates on imidacloprid treated sediments show declines in number or types of organisms that approach 50 percent compared to control plots. The experimental results on imidacloprid show that this is rarely the case. Instead, treatment plots often have higher numbers and/or types of organisms than control plots, and in other cases show only small declines in selected taxa.

NMFS's recommended value of 80 percent appears entirely artificial and not based on scientific or regulatory standards. Willapa Bay and Grays Harbor are highly dynamic environments, with shifting species presence and abundance relative to seasonal and tidal fluctuations. Specific species readily migrate through habitats, which can result in shifts in the species complex present, while still maintaining high richness and abundance. The 2011, 2012, and 2014 studies consistently found that invertebrate biodiversity and abundance between treatment plots and control plots, following imidacloprid application, were not statistically distinguishable. As discussed above, the absence of differences could be due to limited effect of imidacloprid to non-target species, to recolonization of treatment beds by species moving back into treated plots from nearby untreated areas, or some combination of the two. Regardless, the lack of differences between treatment and control plots at 14 and 28 days following spraying demonstrates that the effects from imidacloprid treatment are relatively short-lived and site-specific. Also, an 80 percent standard would not be representative of recovery, considering that over 97 percent of the estuaries will be left untreated, and only a very limited number of relative acres will be sprayed.

Comment #10

[pp. 4–5] *NMFS recommends grant programs to research alternates to pesticide use.*

Response

We thank the NMFS for alternative recommendations. It's important to note that extensive alternative approaches have been tested over the years. These pilot studies have included mechanical removal of burrowing shrimp, covering plots with tarps, non-toxic liquid applications, and using above ground stakes for oyster attachment (see DEIS section 1.5). None of these alternatives were successful, however, and disruption of the sediments by burrowing shrimp make it difficult or impossible to anchor any above-ground oyster lines or other structures. There is also a specific market for the product that is produced with ground cultured oysters, and alternative methods may make the product unprofitable or limit production to levels that would not support that market (i.e., for shucked oysters).

It is important to note that the direct effects from mechanical control of burrowing shrimp (i.e., graveling or frosting) are considered more impactful to invertebrate biodiversity and non-target species than from the treatment using imidacloprid (Ferraro and Cole 2007). Physical disruption of the sediments, such as by adding gravel substitutes or compressing the sediments, has greater direct negative impacts to species diversity and habitat within the estuaries than chemical treatment, but requires a larger area of treatment to reach the same efficacy as imidacloprid in controlling burrowing shrimp.

Comment #11

[in the summary] *All required sampling should be conducted every year over the duration of the permit. This requirement would be informative. For example, if the data supported it, yearly sampling results could justify measured increases in acreage treated (up to the proposed limit) in subsequent issuances of the NPDES permit.*

Response

See Comment #4 for the National Pollution Discharge Elimination System (NPDES) permit below.

NMFS Appendix Comments – Draft NPDES Permit

The following information is in response to comments from the National Marine Fisheries Service, December 8, 2014. These address comments to the Draft NPDES Permit.

Comment #1

The permit requires that treatment not cause or contribute to further impairment for any parameter in these estuaries. There is no list of existing impairments provided to determine how the permittee (or the public) can ensure this requirement is attained.

Response

Section 3.2 of the Draft EIS (DEIS) addresses Elements of the Environment. Sediments, air, and surface water quality impairments for each estuary are outlined in Sections 3.2.1 through 3.2.3, respectively. Specific threshold and compliance monitoring is outlined in each section, and all results must be submitted to Ecology as part of the NPDES permit and regulations under the Washington State Water Quality Standards.

Comment #2

Section S2, item number 2 allows the permittee to apply other pesticides for experimental use to an area of one acre or less. What is the procedure the WSDA must go through prior to issuance of these permits?

What outcomes are monitored? Does this go through public process? NMFS requests notification and an opportunity to comment.

Response

Application of other pesticides for experimental use trials has not been proposed by the growers under this permit, nor is it expected at this time. Experimental use of non-listed chemicals is subject to review in the Annual Operations Plan, and must be conducted under a Washington State Experimental Use Permit (NPDES Permit special condition, Section S2.H).

Comment #3

For section S3A; other than water sampling, there are no requirements to sample sediments or benthic communities off-site of the SIZ boundaries.

Response

Survey results from the 2011, 2012, and 2014 field surveys have concluded that imidacloprid dilutes quickly in surface water. Previous work (Frew 2013) also concluded that imidacloprid does not bind to the sediments for extended periods, and that sediment concentrations of imidacloprid fall off quickly with distance from the treatment plot (see, for example, results submitted to Ecology for the 2012 field trials). Similarly, off-site benthic communities have displayed few or no effects from nearby imidacloprid treatments in previous field work. Accordingly, there is limited scientific value in sampling off-site sediments and benthic communities, and such sampling involves significant expense and logistical difficulties. Accordingly, monitoring during the life of the permit correctly focuses on assessment of on-plot sampling.

Comment #4

For section S4B; NMFS does not agree with the Sediment Monitoring Schedule, years should not be skipped given that the data from last summer's treatment are not available. What is the purpose to allow years to be skipped?

Response

Results from the 2014 field trials have been submitted to Ecology, and should be made available to NMFS shortly. These results indicate that imidacloprid is not persistent in the sediments past approximately 56 days. In fact, in 2014, 7 out of 8 whole sediment samples had concentrations of imidacloprid that were undetectable at 28 days following treatment. These results are consistent with those found in the 2011 and 2012 studies. The scientific evidence indicates that it is highly unlikely that imidacloprid will persist over multiple years. Hence, sediment sampling can be spaced over the length of the permit, in order to align costs and logistical difficulties with expected environmental impacts.

Comment #5

For section S4F; the term representative to the treatment plot is not clearly defined, and does not require sediment samples be randomly chosen. What are Ecology's criteria for selecting samples?

Response

Sediment sampling locations have been chosen using a gridded sample pattern, following recommendations from an Ecology hired statistician. Field scientists are not able to sample in all areas (e.g., areas with high concentrations of shell hash), as these areas do not allow for the proper sampling of sediment. However, if the plot has a mixture of sand and silt sediments, all efforts will be made to sample all sediment types, as has been done in prior year's work, to be representative of the whole bed.

Comment #6

For section S4G; How are 10-acre sample sub-plots selected to be representative of the entire acreage treated? Criteria should be in place and well understood. Random selection of sites is critical.

Response

Ten-acre sub-plots are necessary to carry out a monitoring plan. The sizes of sample sub-plots are based on the maximum area field crews can survey during the 2-3 hour sampling window of the low tide cycle. The sub-plots are generally chosen by the field sampling team based on the location of representative conditions for the entire plot, presence of shells that can interfere with sediment sampling, and the patterns of water flow onto the plots during the rising tide. Although sampling is done on sub-plots, the samples are taken from across the sub-plot area to help ensure representative coverage for the larger plot.

Comment #7

For section S6A1a; What criteria will Ecology use to approve treatment with imidacloprid on grounds that have less than the action threshold of ten burrows per square meter?

Response

Section 2.8.3.3 of the DEIS addresses requirements and restrictions related to the NPDES Permit. Specifically, a risk profile will be used to define a qualitative scale for burrowing shrimp presence. Sampling at specific locations in the estuaries will be used to determine shrimp recruitment, and draw comparisons from sediment samples taken from treatment sites. Ecology will evaluate the risk profile over time, and work with the growers to address the threshold and determine if adjustments are needed based the efficacy of imidacloprid treatments.

Comment #8

There is no requirement for the permittee to provide elevations of proposed treatment areas or control. Elevation is important to interpreting benthic data. What administrative steps will Ecology follow when it receives a non-compliance notification?

Response

Elevation data is collected by the science team when determining the location of treatment and control plots. These data are verified during the field trials. It is used as part of the criteria for determining if the control plots are truly representative of, or equivalent to, the treatment plots. Ecology will work with the growers, in compliance with the NPDES permit, to address non-compliance of any site parameters.

Comment #9

NMFS feels there are other aspects that are impossible to comment on at this time, because the reference documents are not yet provided. These include details in sampling and analysis, and the Annual Operations Plan. Will there be a public review process on these components of the proposed action?

Response

This statement from the NMFS reviewer is relatively vague. Compliance requirements for the NPDES permit have been well defined in the DEIS, and include a complete list of references for documents related to this proposal. The results from the 2014 field surveys are expected to be available to NMFS soon, and, as described above, they are consisted with previous studies conducted on smaller treatment plots.

R:\NOTEBOOKS\1273302_WGHOGA Ongoing Permit Support\Deliverables\Memos\NMFS Comments Memo 20150403\WGHOGA NMFS Responses 20150403.docx

celebrating 40 YEARS
HARTCROWSER
www.hartcrowser.com

ATTACHMENT D to WGHOGA comments on the Draft Supplemental Environmental Impact Statement for Control of Burrowing Shrimp using Imidacloprid on Commercial Oyster and Clam Beds in Willapa Bay and Grays Harbor

DATE: April 6, 2015

TO: Jesse DeNike, Plauché & Carr

FROM: Jeff Barrett

RE: **Agency Comments**
12733-02-10

CC: Adrienne Stutes, James Selleck

The information provided within is in response to agency comments for the Willapa-Grays Harbor Oyster Growers Association (WGHOGA) project.

USFWS Comments

The following information is in response to comments from the United States Fish and Wildlife Service (USFWS).

Comment #1

Page 3. (Cover Memo). *The stated primary objective is control of burrowing shrimp on commercial shellfish beds. With our previous letter to Ecology, when offering scoping comments (Letter to Donald A. Seeberger, dated February 14, 2014), the Service recommended that the EIS and permit framework should give fair and equal consideration to alternate culturing methods and practices. Control and removal of a native species that performs important ecological functions should not be the primary objective. Instead, this effort should be directed at developing and refining robust IPM methodologies that adaptively manage shellfish production systems to avoid harming ecological resources.*

Comment #2

Page 4. (Page vi). *"At the time of this writing ... there are no known alternatives to chemical applications to effectively control burrowing shrimp." COMMENT – The stated primary objective is flawed. Other alternatives should be given fair and equal consideration, including alternate culturing methods and practices, and a robust IPM methodology with stricter limits on the use of chemical control agents.*

Comment #3

Page 4. (Pages 1-3). *"With low burrowing shrimp recruitment over the past ten years or so, it has been possible to farm some beds without shrimp control. However, due to the recent large recruitments of burrowing shrimp in Willapa Bay and Grays Harbor, growers are now seeing high shrimp densities in substrate without distinction by crop." COMMENT – Ecology and the WGHOGA acknowledge that burrowing shrimp numbers and densities exhibit cyclical changes over time. There is little or no evidence to substantiate the claims that Willapa Bay and Grays Harbor are currently experiencing anything unusual related to burrowing shrimp recruitment, numbers, abundance, and densities.*

Response to Comments #1–#3

The USFWS is correct in saying that burrowing shrimp are native to Willapa Bay and Grays Harbor. However, as discussed in the Draft Environmental Impact Statement for the proposal (DEIS), burrowing shrimp rapidly expanded in these estuaries during the middle of the 20th century and caused a major decline in oyster production between 1950 and 1965. In recognition of the destructiveness of burrowing shrimp, Washington Department of Fisheries (now Washington Department of Fish and Wildlife [WDFW]) personnel began testing various methods of control during the 1950s, eventually resulting in the use of carbaryl (see DEIS Section 2.4). While burrowing shrimp have a limited ecological role, they have been consistently managed since the 1950s to prevent unlimited expansion throughout these estuaries, particularly on commercial shellfish beds. Further, the enhanced ecosystem functions granted by shellfish and eelgrass beds are far more important than those of burrowing shrimp beds. Shellfish beds provide refuge for juvenile fish and mobile crustaceans (Coen et al. 1999; Grabowski et al. 2005), and important habitat for epibenthic invertebrates, molluscs, polychaetes, and crustaceans (Lenihan et al. 2001; Rothschild et al. 1994). These habitats are lost completely when burrowing shrimp are allowed to take over. High densities of burrowing shrimp are known to significantly reduce both species composition and abundance of other types of invertebrates in this benthic community (see discussion in DEIS, Section 3.1). For example, burrowing shrimp cause significant sediment disturbance in which sedentary species such as deposit-feeding polychaetes, bivalves, tanaids, amphipods, and many other sedentary species are reduced in numbers in burrowing shrimp ecosystems. These invertebrate species are important components of the ecosystem and can be lost completely if burrowing shrimp numbers are allowed to increase unchecked.

USFWS errs in failing to recognize the significant ecological importance of shellfish beds, beyond the habitat functions listed above. In addition to these functions, shellfish beds provide important ecosystem services such as water filtration, which results in decreased suspended solids, turbidity, and increased denitrification. Accordingly, Washington State and Federal law recognize the ecological importance of shellfish beds. WAC 173-26-221 identifies commercial and recreational shellfish beds as critical saltwater habitats that "require a higher level of protection due to the important ecological functions they provide." Similarly, NMFS's Essential Fish Habitat (EFH) Consultation Guidance specifically identifies intertidal and subtidal shellfish beds as types of EFH. See p. 5.3 of the EFH Consultation Guidance, available at: http://www.habitat.noaa.gov/pdf/efh_consultation_guidance_v1_1.pdf. Although burrowing shrimp may partially provide similar ecosystem functions, the detrimental effects of sediment destabilization are far more deleterious than any positive functions they may provide. Furthermore, USFWS apparently interprets this as an eradication proposal. It is not. It is intended to provide shellfish growers in Willapa Bay and Grays Harbor a critical tool for managing burrowing shrimp on a limited number of tidelands similar to what they have been since the 1950s after burrowing shrimp populations dramatically increased.

USFWS expresses concern over the lack of robust Integrated Pest Management (IPM) methodologies for managing shellfish production and burrowing shrimp control. However, the DEIS contains a detailed discussion of the use of IPM methodologies (see DEIS, Section 2.8.4). An IPM plan has been in place since at least 2002 and the WGHOGA is dedicated to implementing this plan and looking for viable alternative or concurrent methods of controlling burrowing shrimp in their shellfish beds. Indeed, given the substantial expense of chemical applications to control burrowing shrimp, and the hundreds of thousands of dollars the growers have had to spend to obtain permits for such use, WGHOGA would happily forgo chemical control of shrimp if non-chemical methods provided sufficient support for their farms.

However, after many years of hard work, WGHOGA and their science advisors have determined that, to date, the non-chemical control and mechanical control methods tried have either failed completely to control burrowing shrimp, or have provided only very limited efficacy that is not sufficient to support oyster culture on WGHOGA farms. Non-chemical treatments investigated by the growers include harrowing, shallow rototilling, clay injection, electroshocking, raking, compaction, hypersaline solution, etc. (see discussion in DEIS, Section 2.8.4). These non-chemical treatments have not been successful for a variety of reasons:

- They have failed to control burrowing shrimp;
- They are impractical on a commercial scale;
- They significantly harm the shellfish crop and/or non-target species; and/or
- They have other negative environmental consequences.

Therefore, the USFWS Comment #1, that “this effort should be directed at developing and refining robust IPM methodologies that adaptively manage shellfish production systems to avoid harming ecological resources,” is in fact achieved through this proposal. We recommend that USFWS fully review the alternative shrimp control methods tried by WGHOGA, and the reasons why these methods have proven not to be feasible, rather than dismiss them out of hand. WGHOGA is very interested in constructive suggestions from USFWS (or any other agency) that would reduce the need for chemical control.

Alternative methods such as line culture or bag culture may work in other ecosystems, or in certain parts of Willapa Bay and Grays Harbor, but they are not ecologically or financially viable as the sole methods of raising shellfish. In areas heavily infested with burrowing shrimp, growers report that the stakes and poles used to support alternative cultural methods fall over rendering these systems ineffective and resulting in high mortality of oysters in the failed systems. In addition, the shellfish market served by WGHOGA includes a large component of shucked oysters, for which ground culture with its high production rates and cost efficiencies is the only viable method. A shellfish culturing method that is good for burrowing shrimp but not economically viable for WGHOGA, is ultimately not an appropriate method.

USFWS also questions claims that "there is little or no evidence to substantiate the claims that Willapa Bay and Grays Harbor are currently experiencing anything unusual related to burrowing shrimp recruitment, numbers, abundance, and densities." WGHOGA and the Washington State Department of Ecology (Ecology) agree that the recent increase in burrowing shrimp populations is not "unusual." However, that is not the relevant question. The fact is that the increase in shrimp density is occurring; and as a consequence, shellfish beds are becoming more inundated with burrowing shrimp. WGHOGA agrees that recruitment is cyclical; currently the cycle is pointing towards increased recruitment on shellfish beds. As a result, it is currently very important that the WGHOGA have a tool for controlling burrowing shrimp at their disposal. Imidacloprid would only be used on an as-needed basis.

WGHOGA's members are wholly committed to maintaining the health and sustainability of the Willapa Bay and Grays Harbor estuarine ecosystems. They are important advocates for water quality and ecosystem health as a whole. Without a healthy ecosystem, they would not be able to maintain a viable shellfish industry in Washington State.

Comment #4

Page 4. (Pages 1-6). *The documentation prepared by Ecology and the WGHOGA refers repeatedly to a single metric or measure of efficacy: Is the practice or treatment sufficient to reduce numbers below the "damage threshold" of ten burrows per square meter? The documentation provides little information to describe where this damage threshold originated, who developed the threshold, and how it is justified. The damage threshold is presented as a given and there is no effort to evaluate whether it is valid and appropriate for its intended purpose. In this sense, the proposed IPM methodology is arbitrary.*

Response

The burrowing shrimp IPM that has been in place since 2001 has consistently worked at developing appropriate methods of determining a damage/density threshold, as well as accurate shrimp population census methods. The existing criteria of 10 shrimp burrows per square meter is the best and most accurate method found to date. This was discussed thoroughly in Dumbauld et al. 2006. In addition, this threshold has been accepted by Ecology and was used in the NPDES permit for carbaryl. Determining burrowing shrimp densities is a difficult task because real densities are often higher than what is seen when identifying burrow numbers. In addition, it can be very difficult to distinguish between shrimp burrows and some polychaete burrows. Therefore, a "damage threshold" of 10 burrows per square meter has been a good measure of extent of the burrowing shrimp population in a given shellfish bed. WGHOGA has determined that they lose shellfish and beds cannot be adequately farmed at densities higher than this.

Comment #5

Page 4. (Pages 2-35). *"Additional field trials were conducted during summer 2014 ... If the results of these studies are available, they will be reported in the Final EIS." COMMENT – The 2014 field trials include the first treatment sites larger than 30 acres, target collection of information from sites where the substrate has a high organic content (influencing persistence), and address deficiencies stemming from earlier work conducted without an approved data sampling and analysis plan (D. Rockett, pers. comm. 2014). The National Marine Fisheries Service has requested that Ecology provide results from the 2014 field trials when they become available (T. Hooper, pers. comm. 2014); to date, Ecology has not provided this information.*

Response

The results of the 2014 field trials were not available at the time these comments were written. The Draft 2014 Field Report was submitted to Ecology on February 2, 2015. The results from the 2014 field trials corroborate the results from previous years' trials that were conducted on smaller plots (i.e. < 10 acres). Specifically, the 2014 trials found that plots treated with imidacloprid were not statistically distinguishable from unsprayed control plots for the majority of the invertebrate comparisons that were conducted. In addition, the 2014 results again documented rapid dilution of imidacloprid concentrations in water with the first rising tide, and approximately exponential declines in sediment concentrations, with non-detectable levels within 28 days (whole sediments) and concentrations below screening levels at 28 days (sediment porewater).

Comment #6

Page 4. (Pages 2-35). *Ecology should not advance a permit decision until more data is collected (during 2014 and 2015) and shared with the public. A decision to issue the permit and authorize SIZs while relevant and important data remain unavailable would be premature. Ecology should not advance the permit decision until they have fully addressed and can be responsive to science-based concerns regarding fate and transport, efficacy, persistence, and effects to non-target organisms. We recommend to Ecology that the work made possible by the Experimental Use Permit should continue.*

Response

WGHOGA agrees that, as a general rule, more information is better than less when making decisions that could affect the environment. However, the USFWS response ignores the many years of study that have already been conducted to investigate the effects of imidacloprid application to oyster beds. These results are summarized in the DEIS. Results of the 2014 field trials were submitted to Ecology on February 2, 2015. These results were very similar to those from previous years; therefore many of the outstanding questions regarding fate and transport, efficacy, persistence, and effects to non-target organisms can be answered based on multiple years' data.

In addition, the permit, if issued, will require a robust monitoring program, including water, sediment, and invertebrate sampling in both Grays Harbor and Willapa Bay to ensure unacceptable impacts are not occurring. Thus, the permit itself responds to USFWS's request for continued information gathering, including information gathered through an Experimental Use Permit.

Comment #7

Pages 4-5. (Pages 2-47 through 2-56). *Alternatives considered and Eliminated from Detailed Evaluation. Ecology and the WGHOGA document alternative mechanical, physical, and chemical control methods, and describe alternative culturing systems. Many of these practices are flawed in principle and have little or no merit. Others do have merit but were eliminated because they are not economically feasible on relevant spatial scales. However, graveling and frosting are established practices with the specific goal of firming substrates and fostering good conditions for larval attachment, maturity, and growth. Graveling and frosting should have a role in IPM methodologies directed at successful shellfish culturing on tidelands affected by burrowing shrimp. Long-line and stake culturing are also established practices, and are used successfully by some growers and farm operators in these same portions of Willapa Bay and Grays Harbor. Much of the information used to discredit these practices appears to be anecdotal and not based on either scientific studies or rigorous and comparative evaluation. Ecology and the WGHOGA should address more seriously and objectively whether methods of ground- based culturing and production require reevaluation in light of new science and the many concerns related to aquatic pesticide applications. Chemical control methods with lethal and biologically significant sub-lethal effects to non-target organisms should be a last resort and only implemented after a robust IPM methodology has exhausted all other alternatives at each specific location.*

Response

While USFWS alleges some of the Alternatives Considered and Eliminated from Detailed Evaluation are "flawed in principle and have little or no merit," it fails to specify which methods fall into that category. With all due respect to the USFWS reviewers, there is no indication that they have the expertise necessary to critique these alternative methodologies.

Graveling and frosting is an IPM methodology used by growers in those areas where it is financially feasible; however, that alternative is not economically feasible (or ecologically justified) as a sole control method, on the larger scale of oyster farming. WGHOGA is interested in the most ecologically sound AND economically viable methods of shellfish farming. Relying solely on alternative culturing methods such as graveling and frosting will potentially cause lethal and biologically significant sub-lethal effects to non-target organisms such as benthic and epibenthic invertebrates, as well as native and non-native eelgrass. The best scientific and technical information clearly demonstrates use of imidacloprid as part of an IPM program is the best option for effectively controlling burrowing shrimp while minimizing adverse environmental impacts.

USFWS is correct that long-line and stake culturing methods are used successfully in some parts of Willapa Bay and Grays Harbor. However, these methods are not feasible throughout Willapa Bay and Grays Harbor. First, the sediment in many parts of both bays is simply too soft and/or burrowing shrimp densities are too high to sustain these practices. In these areas, the long-lines and stakes sink into the substrate, causing the oysters to be on the ground where they are susceptible to sinking and suffocation due to burrowing shrimp. Second, it is not economically feasible to grow all oysters on long-line or stake culture. This practice, which requires significant capital investment and ongoing costs, is used for oysters that will be sold on the half-shell market, not the shucked market. The majority of oysters cultivated in Willapa Bay and Grays Harbor are destined for the shucked market, and the only economically feasible method for culturing significant quantities of shucked oysters is ground-culture.

At this point in the process, the IPM program has all but exhausted all alternatives as an exclusive method of controlling shrimp in Willapa Bay and Grays Harbor.

Comment #8

Page 5. (Pages 2-55). *A variety of native, biologically and economically important species prey on burrowing shrimp, including smelt (family Osmeridae), herring (family Clupeidae), chum salmon (Oncorhynchus keta), surfperch (family Embiotocidae), flounder (family Pleuronectidae), cutthroat trout (0. clarki), white and green sturgeon (Acipenser transmontanus, A. medirostris), and Dungeness crab (Metacarcinus magister). "Both the green and white sturgeon ... [feed] on burrowing shrimp ... 40 to 50 percent of the organisms by number and weight ... [found in green sturgeon stomach contents] were burrowing shrimp (Dumbauld et al. 2008)." As far as we know, there is no scientific information supporting Ecology's claim that"...sturgeon generally do not feed on shellfish beds."*

Response

Members of the WGHOGA and their Science Team have noted that although green sturgeon do obviously feed on burrowing shrimp, they are not often noticed in the shellfish beds themselves. There has also been a lack of visual observation of sturgeon pits in commercial shellfish beds. All scientific studies conducted on green sturgeon and burrowing shrimp have occurred on mudflats away from commercial shellfish beds (Frew 2013). Researchers working in these coastal estuaries have observed that sturgeon prefer to feed outside of commercial shellfish beds (K. Patten, WSU Extension, personal communication; B. Dumbauld, USDA-ARS, personal communication). In addition, there is no scientific evidence showing that green sturgeon do definitely feed on commercial shellfish beds.

Again, it is important to remember that this is not an eradication proposal, but rather to continue the existing practice of managing burrowing shrimp control activities on limited commercial shellfish beds. Even under the unlikely assumption that all permissible acres are sprayed, and that all acres are unique (i.e., not re-sprayed), 1,500 acres in Willapa Bay represents only 3.33 percent of the total tideland acreage (45,000 acres), and 500 acres in Grays Harbor represents only 1.45 percent (of 34,460 acres). Thus, the vast majority of both of these estuaries will not be sprayed in any given year, ensuring that any ecological benefits from unsprayed areas will be present and unimpaired. That includes any ecological benefits from the presence of burrowing shrimp, including their being prey to other organisms. Any contention of a significant impact to the estuary in general, or to fish in particular, is not scientifically credible given that the vast majority of both estuaries will not be affected by imidacloprid treatments.

Review of the NMFS website on green sturgeon[1] indicates a number of factors thought to be contributing to low green sturgeon numbers. None of those threats involve insufficient food. Instead, limits on spawning habitat are deemed “the principal factor in the decline…”.

Comment #9

Page 5. (Pages 2-57 and 2-58). *Here and elsewhere, Ecology and the WGHOGA have repeated claims that without chemical control of burrowing shrimp there will be “...increased burrowing shrimp activity; reduction in eelgrass growth and density; and reduced biodiversity, which could lead to a reduction in the presence of birds, fish, and other species that feed on organisms that inhabit eelgrass.” Ecology and the WGHOGA claim that Alternative 3 (Imidacloprid Applications with IPM) would “...have beneficial environmental effects in the form of preserving the substrate and biodiversity of commercial shellfish beds, and promoting native eelgrass density and coverage, thereby improving foraging habitat and prey diversity for birds and fish, and cover for juvenile fish including ... salmonids.” COMMENT – The Service does not agree that these claims are justified or established in fact. These claims are misleading, especially in light of the WGHOGA current practice of removing both native and non-native eelgrasses (Zostera marina and Z. japonica, respectively) where they complicate shellfish production.*

Response

USFWS offers no support or specific explanation for its broad statement of disagreement noted in the above comment. In contrast, the DEIS includes extensive information demonstrating the ecological

---

[1] Available at http://www.nmfs.noaa.gov/pr/species/fish/ greensturgeon.htm#threats

impacts of burrowing shrimp on other species, and of the benefits to many of these species where burrowing shrimp have been controlled. Chapter 3 of the DEIS discusses the results of several scientific papers that have looked at the effects of burrowing shrimp in the benthic communities in which they live. Species composition and invertebrate abundances are significantly reduced in areas with high densities of burrowing shrimp (Posey 1985). General sediment disturbance affects the composition of infaunal and epifaunal invertebrates (Dumbauld et al. 2001; Ferraro and Cole 2007; Posey 1986). There is a reduction in numbers of deposit-feeding polychaetes, bivalves, tube-dwelling invertebrates, and other sedentary species in areas with dense populations of ghost shrimp. The DEIS also includes reference to studies showing the many benefits of shellfish ecosystems, and the fact that these systems are indeed more beneficial habitats for juvenile fish and invertebrates (see DEIS Section 3.1, citing Coen et al. 1999 and Grabowski et al. 2005).

Eelgrass communities are also highly functional as nursery and feeding habitats, and WGHOGA has not proposed control to native eelgrass, only the invasive, non-native Japanese eelgrass. In addition, juvenile fish such as salmon feed very little within the eelgrass beds themselves; they are more likely to feed on insects and other invertebrates on the shellfish beds and use the eelgrass beds as a refuge habitat (Dumbauld and Wyllie-Escheverria 2003; B. Dumbauld, USDA-ARS, personal communication). Shellfish and eelgrass ecosystems are complimentary in supporting juvenile fish and invertebrates. Without the solid substrates that shellfish beds provide as foraging and refuge habitat, juvenile fish such as salmon would find reduced habitat diversity and quality within Willapa Bay and Grays Harbor. This would likely cause a decrease in salmon stocks as they attempt to adapt to an environment that is now largely devoid of food and structures. By extension, denying the permit would hurt fish populations because, over time, shellfish beds would be reduced in areal extent as burrowing shrimp made more and more commercial beds inhospitable to continued culture of oysters. Denying the permit would also likely damage native eelgrass, as native eelgrass is not able to grow in areas dominated by burrowing shrimp (see DEIS Section 3.1). In contrast, as USFWS has recently acknowledged, while eelgrass density and abundance can be reduced in the presence of shellfish aquaculture generally, this reduction is temporary and some impacts are likely offset by the increase in light penetration and fertilization provided by shellfish. Further, USFWS has recognized oyster bottom culture in particular can coexist with eelgrass beds (USFWS 2009).

Comment #10

Page 5. (Pages 2-58 through 2-60). *With our previous comment letter to Ecology (Letter to Donald A. Seeberger, dated February 14, 2014) the Service stated that we do not support large scale chemical treatment of mixed native and non-native eelgrass beds, and that permits proposed for issuance by Ecology do not adequately address mitigation for collateral damage to non-target vegetation. We expect that these chemical control practices will cause significant damage to native flora and fauna, including damage that extends off of the treated beds and sites.*

Response

Comment noted. This comment and reference comment letter (Letter to Donald A. Seeberger, dated February 14, 2014) are in reference to the NPDES permit for imazamox. This permit has already been issued and is separate from the NDPES application for imidacloprid.

Comment #11

Page 6. (Pages 2-61). *Ecology and the WGHOGA claim that if burrowing shrimp are not controlled they will "...proliferate unmanaged, with likely unrecoverable damage ... [causing] significant alterations to the bay-wide ecosystem." COMMENT – Burrowing shrimp are native and perform important ecological functions in these systems. As such, they do not represent an alteration of the bay-wide ecosystem. However, chemical control methods do represent an intrusive alteration, and may have unintended consequences.*

Response

As discussed above, this is not a burrowing shrimp eradication proposal. It is proposal to maintain burrowing shrimp populations at current and historic levels since they dramatically and unexpectedly increased beginning in the 1950s. The total area of these estuaries that may be treated with imidacloprid is quite small when compared to the total size of Willapa Bay and Grays Harbor. Assuming every possible acre is sprayed, and that none of the permitted spraying is reapplication to areas previously sprayed, a mere 3.3 percent of the total tidelands exposed at low tide in Willapa Bay and 1.5 percent in Grays Harbor may be treated under the proposed NPDES permit. There are good reasons to assume not all acres will be sprayed and many applications will be to previously treated areas, so these already small values are likely overestimates. Regardless, this basic analysis demonstrates that vast areas of both bays will be untreated and fully available for burrowing shrimp. By contrast, if imidacloprid use is not permitted, the amount of shellfish habitat will drastically decline over time, with attendant, negative impacts to fish and other species, as discussed above.

Comment #12

Page 6. (Pages 3-13). *"Based on currently available information and studies, and requirements to comply with the conditions of all applicable pesticide registrations, permits, and regulations (including the Washington State Water Quality Standards and SMS), no significant unavoidable adverse impacts to sediments would be expected with the proposed action (Alternative 3: imidacloprid applications with IPM), or with Alternative 2 (carbaryl applications with IPM)." COMMENT – The Service does not agree that this conclusion is accurate or justified.*

Response

The broad statement of disagreement noted in the above comment is wholly unsupported and USFWS has not shown any particular expertise with imidacloprid in sediments. This comment conflicts with the available scientific evidence that is thoroughly reviewed in the DEIS, and in the recently released results for field trials in 2014. The results of experimental field trials conducted to date show that, under Ecology's stringent requirements, there is little to no long term effect of imidacloprid on the sediments. Persistence time in sediments is low and benthic invertebrates recovery very quickly after treatment (Hart Crowser 2013 and 2015). Studies conducted on both small (< 10 acres) and large (> 40 acres) plots have shown very similar results that imply no significant unavoidable adverse impacts to sediments. Finally, the Washington State Department of Ecology is the sole regulatory agency with expertise in administering the Washington State Water Quality Standards and SMS, and it has concluded that the use of imidacloprid as conditioned in the permit would comply with these regulations.

Comment #13

Page 6. (Pages 3-24). *"A SIZ is the area where the applicable State sediment quality standards of WAC 173-204-320 through 173-204-340 are exceeded due to ongoing permitted or otherwise authorized wastewater, storm water, or nonpoint source discharges (WAC 173-204-200)." COMMENT – The threshold criterion for "minor" adverse effects to sediments and benthos are not adequately protective. The Service expects that the proposed permit and SIZs cannot be implemented without causing significant adverse impacts to sediments and native benthos.*

Response

This statement of expected impact is unsupported by any scientific or other information. Ecology is charged with implementing the Clean Water Act, including the NPDES permit under consideration here in Washington State. As the responsible agency, Ecology developed criteria and standards for assessing the magnitude of impacts to water, sediments, and related biota for NPDES-permitted discharges. That includes the entire SIZ program, which is an Ecology-created and -implemented system, not a component of the federal CWA. As noted above, Ecology has properly concluded the use of imidacloprid, as conditioned, will comply with the applicable regulations, and USFWS has not provided concrete or specific evidence demonstrating Ecology's determination is erroneous.

Further, it is important to recognize that the threshold criterion referenced by USFWS is only relevant when invertebrates on imidacloprid treated sediments show declines in number or types of organisms that approach 50 percent compared to control plots. The experimental results on imidacloprid show that this is rarely the case. Instead, treatment plots often have higher numbers and/or types of organisms than control plots, and in other cases show only small declines in selected taxa.

Comment #14

Page 6. (Pages 3-30, 3-31, 3-33). *"The degree of toxicity of carbaryl to marine vegetation varies considerably (WDF and ECY 1985). Some marine plants and algae are growth-inhibited by carbaryl, while others are not affected." "Imidacloprid ... is taken up ... by plants and is present in the foliage of plants. However, this is based on limited information regarding ... marine vegetation." "No studies were available to assess the toxicity of imidacloprid to marine algae." COMMENT – Imidacloprid treatments would overlap significantly with native eelgrass and would expose phytoplankton. If there is little or no information to assess potential effects to these important resources, we do not agree that a finding of no significant adverse impact can be justified for plants.*

Response

As explained in the DEIS, imidacloprid is an acetylcholinase inhibitor, and plants do not have a biochemical pathway involving acetylcholinase (see DEIS Sections 1.7 and 3.2.4). Therefore, plants are not vulnerable to imidacloprid toxicity. Further, any theoretical concern about impacts to plants is ameliorated by the low concentration of imidacloprid, and rapid dilution on incoming tides, that will characterize imidacloprid treatment under the proposed permit. Thus, there is no credible scientific basis for concluding that WGHOGA's proposed use of imidacloprid would have significant adverse impacts to plants.

Comment #15

Page 6. (Pages 3-31). *"While imidacloprid would be applied to areas with high populations of burrowing shrimp on commercial shellfish beds only, research indicates that imidacloprid can move off-site rapidly in surface water and can be detected at least 480 meters (1,575 feet) away from the application site." COMMENT - These findings clearly indicate that effects and damages will not be limited to the treatment sites. Neighboring owners will have their tidelands exposed and affected even if they choose to avoid the practice of using chemical control methods for burrowing shrimp.*

Response

While imidacloprid can move off-site rapidly in surface water and can be detected at least 480 meters away, the concentrations present in the surface water at these distances is generally minimal. Results from the 2014 field studies showed that off-plot concentrations of imidacloprid ranged from 0.054 to 0.55 micrograms per liter (µg/L). These concentrations are very low. A review of the toxicity literature on imidacloprid, required by Ecology as a condition for field trials in 2012, found the most sensitive taxon of invertebrates applicable to these estuaries, mysid shrimp, had an LC50 of 37 µg/L. Using EPA guidance that 10 percent of this value should be considered the threshold for biological impacts, the 2012 studies and documentation submitted to Ecology concluded that 3.7 µg/L could be considered a threshold of concern. The 0.054 to 0.55 µg/L values found in studies of off-site movement of imidacloprid are far below this threshold, despite use of a sampling methodology (collection of water at the front edge of the tidal prism) designed to maximize the amount of imidacloprid collected. Thus, the science shows that offsite movement is very unlikely to impact flora or fauna even on the first tidal flush. In addition, imidacloprid dilutes so quickly in surface water that it is not likely to impact flora or fauna at distances away from the application site. There is no indication that neighboring owners will have their tidelands exposed and affected, and USFWS's contention to the contrary is directly undermined by the actual scientific data.

Comment #16

Page 7. (Pages 3-37). *Statements referring to bull trout occurrences in Pacific Coast drainages is incorrect. Several rivers support local populations and spawning trout. Bull trout occur regularly in Grays Harbor, have been documented in low numbers in Willapa Bay, and represent the southernmost populations of bull trout in North America. The species is listed under the Endangered Species Act (ESA).*

Response

There has been only a single potential observation of bull trout in Willapa Bay, by a technician 1 mile downstream of the Willapa/Forks Creek hatchery (Berg 2002). Tellingly, there is no designated critical habitat for bull trout in Willapa Bay (USFWS 2009).

While bull trout are present in some Pacific coast drainages, they only spawn farther north, and the closest spawning population is in the Quinault River. Any bull trout found in Grays Harbor are migratory adults.

Comment #17

Page 7. (Pages 3-43). *Bull trout occurrence in Willapa Bay is infrequent and only in very low numbers, but it is incorrect to state that bulltrout are unlikely to use habitats on commercial shellfish beds. Bull trout migrate in water less than 10 meters and are opportunistic foragers, traveling to take advantage of seasonal food resources. Bull trout feed on marine forage fish and juvenile salmonids, within eelgrass meadows and other complex nearshore habitats.*

Response

Since there has only ever been a single potential observation of bull trout in Willapa Bay, and there is no designated critical habitat in Willapa Bay, it is unlikely that bull trout will use shellfish bed habitat for foraging in this bay.

The EIS addresses overlapping bull trout foraging habitat and shellfish beds in Grays Harbor. The full shoreline in Grays Harbor is designated critical habitat, based on adult foraging activity. There is no spawning habitat in the rivers that feed into Grays Harbor. Acoustic tagging and sampling of bull trout from 2001 to 2005 found that bull trout are present in the Chehalis River from late February to early July.

Imidacloprid would be unlikely to adversely affect adult bull trout. The area for imidacloprid application would be small in relation to the total tideland area of Grays Harbor. Imidacloprid will generally be applied at low tide when bull trout would not be on the shellfish beds, and thus they would not be directly exposed during spraying. Imidacloprid does not bioaccumulate in invertebrates, and uptake through contaminated prey would therefore be *de minimus*.

Comment #18

Page 7. (Pages 3-45, 3-46). *Grays Harbor and Willapa Bay support the only populations of snowy plover in Washington. Several beaches and sandy pits are currently or recently used, and are designated critical habitat. While nesting occurs at only a few locations, suitable foraging habitats extend to sand and mudflats, sand islands, and open beaches; including areas with the proposed SIZ, and are considered essential for recovery of the species. Graveyard Spit and Leadbetter Point are currently the most productive breeding sites in Washington, and impacts to prey could have significant adverse effects.*

Response

The best information available does not support the statement that Graveyard Spit and Leadbetter Point are the *most* productive breeding sites in Washington. Nesting only occurs at three locations in Washington: the Pacific Coast facing beach of Leadbetter Point, the northwest corner of Graveyard Spit, and Midway Beach. Nesting at Damon Point in Grays Harbor (a single nest) was last observed in 2006, and does not currently constitute a nesting location. Absence of shellfish aquaculture at all these locations means they are at no risk of being sprayed with imidacloprid. Data show that Graveyard Spit and Leadbetter Point actually have limited nesting. Nest success at Leadbetter Point is generally below 20 percent, and represents less than half of Washington nests (WDFW Survey Report, 2013). There were only three nests at Graveyard Spit in 2013. Instead, the majority of nests have generally been found along Midway Beach to the north, making this location the most productive breeding site in the state.

USFWS's claim of impacts to snowy plover foraging is also not supported by the best available information. In fact, the Biological Opinion from the USFWS office in Washington (2009) found that there are no records of snowy plovers foraging or nesting in the bay or along the eastern shore of the Long Beach Peninsula. Thus, according to USFWS itself, snowy plover do not feed in areas where imidacloprid will be used. And even if they did, there is no reason to believe that imidacloprid would reduce foraging success. Snowy plover have a short bill, and can only feed on the upper layer of the beach surface, foraging for small invertebrates. Preferred foraging habitats include undisturbed sparsely vegetated areas of wet or dry beach-sand, preferably above the high tide or the upper tidal area when water recedes (WDFW 1995). The studies cited above showing that control of burrowing shrimp results in increased numbers and biodiversity of other invertebrates give creditability to the argument that imidacloprid treatment would improve foraging for snowy plover, rather than producing any negative impact. In any case, there is no evidence that food is limiting this species. WDFW and USFWS both consider human modifications and disturbance to sand beaches, and nesting habitats, the greatest concern to recovery of the Snowy Plover.

Comment # 19

Page 8. (Pages 3-49). *"Alternative 3 (Imidacloprid Applications with IPM) would provide adequate burrowing shrimp control ... with potentially reduced environmental side effects, compared to carbaryl. Imidacloprid would be unlikely to adversely affect polychaete worms or molluscs (bivalves, snails), including oysters and clams (Hart Crowser 2013; Grue and Grassley 2013; CSI 2013). A potential exception is imidacloprid effects in sediments high in organic matter. The limited information available for such sediments suggests adverse effects to polychaete worms and crustaceans (see Draft EIS Chapter 2, Section 2.8.3.5). A study of imidacloprid effects in high organic soils is expected during the summer of 2015. Results from this trial may result in adjustments to permit conditions during the five-year term of the permit." COMMENT – Ecology should not advance a permit decision until more data is collected (during 2014 and 2015) and shared with the public. A decision to issue the permit and authorize SIZs while relevant and important data remain unavailable would be premature. Ecology should not advance the permit decision until they have fully addressed and can be responsive to legitimate scientific concerns regarding fate and transport, efficacy, persistence, and effects to non-target organisms, including several species listed under the ESA and their designated critical habitats. We recommend to Ecology that they should continue limited field trials under the Experimental Use Permit.*

Response

See comments above where USFWS also suggested scientific certainty as the standard prior to any permit decision being taken.

Results of the 2014 field trials were submitted to Ecology on February 2, 2015, and therefore were not available to USFWS at the time this comment was drafted. These results were very similar to those from previous years, and confirmed that application of imidacloprid to large commercial shellfish beds did not produce different outcomes than from trials on smaller treatment blocks (e.g., 10 acres). With publication of the 2014 data, the science regarding fate and transport, efficacy, persistence, and effects to non-target organisms can be answered based on multiple years' data. And these trials were on estuarine shellfish beds using application techniques and concentrations that are the same as those proposed in the NPDES permit, making these trials a very good indicator of future effects (or lack thereof).

In addition, most of the commercial shellfish beds in Willapa Bay and Grays Harbor are not located in sediments that are high in organic carbon. Such sediments are typically softer and less desirable to shellfish growers. Field trials are planned to continue in areas of high organic carbon (and in sandy sediments) as part of the required monitoring associated with the permit. Based on the substantial body of scientific evidence already collected, imidacloprid applications can be allowed in areas with sandy sediment (low organic carbon), with high scientific certainty that environmental impacts will not result.

Specific Comments for the Draft Permit

Comment #20

Page 8. (Page 5). *The threshold criterion for "minor" adverse effects to sediments and benthos are not adequately protective. They are not adequately protective of the natural ecosystems in Willapa Bay and Grays Harbor, or the ESA-listed species that occur there. The Service expects that the proposed permit and SIZs cannot be implemented without causing significant adverse impacts to sediments and native benthos, including prey resources on which several listed species depend. Ecology and the WGHOGA acknowledge that there are a number of outstanding issues and concerns regarding fate and transport, efficacy, persistence, and effects to non-target organisms (Ecology 2014, pp. 1-33 through 1-37). Therefore, the Service opposes the authorization of SIZs in Willapa Bay and Grays Harbor.*

Response

Similar allegations were raised in Comment #13 above, and the response to that comment applies with equal force here. USFWS's concerns regarding the threshold for adverse effects and environmental impact in this comment are not based on specific scientific or regulatory support. In contrast, fellow scientists and the regulators at Ecology have properly researched and relied on the accumulated scientific evidence documenting exponential declines in sediment concentrations of imidacloprid after treatment, inability to distinguish treatment and control plot invertebrate numbers or communities, and past research showing that control of burrowing shrimp actually increases the numbers and biodiversity of potential prey species to fish and birds. In keeping with the nature of scientific investigation and uncertainty, Ecology properly acknowledged that some questions remain, limited the scope of proposed imidacloprid applications, and required a robust yet focused monitoring program to run concurrently with permit implementation as a check on the program, and to decrease scientific uncertainty over time.

Comment #21

Page 8. (Page 6). *"This permit does not convey property rights of any sort, or any exclusive privileges, nor does it authorize any injury to private property or any invasion of personal rights." COMMENT – Imidacloprid can move off-site rapidly and might be detected at a distance of 1,000 or 2,000 feet from* *the application sites. This fact illustrates that effects and damages will not be limited to the treatment sites. Neighboring owners will have their tidelands exposed and affected even if they choose to avoid the practice of using pesticides to control burrowing shrimp.*

Response

As noted in more detail above, it is very unlikely that imidacloprid would be detected in the water column or sediments at distances greater than 1,000 feet from the application site. To date, only low concentrations of imidacloprid have been found at distances up to 800 feet from the application site, and these are concentrations at which there are no expected effects on non-target organisms. It is very unlikely that neighboring owners will have their tidelands affected significantly, if at all, by the application of imidacloprid on commercial shellfish beds.

Comment #22

Page 8. (Page 6). *The draft permit identifies and proposes to use the following action threshold: "'No oyster or clam bed may be treated with imidacloprid unless the mean burrow count exceeds the determined action threshold of ten burrows per square meter ... If the mean burrow count is less ... a bed may be treated ... provided [that] a justification is approved by Ecology." COMMENT – The documentation prepared by Ecology and the WGHOGA provides little information to describe where this threshold originated, who developed the threshold, and how it is justified. The damage threshold is presented as a given and there is no effort to evaluate whether it is valid and appropriate for its intended purpose. In this sense, the proposed IPM methodology is arbitrary. Ecology has acknowledged that a well-defined method for determining the treatment threshold has not yet been formulated.*

Response

See Response to Comment #4 above.

Comment #23

Page 9. (Page 7). *The draft permit proposes inadequate treatment buffers. Imidacloprid can move off-site rapidly and might be detected at a distance of 1,000 or 2,000 feet from the application sites.*

Response

Results of field trials indicate that imidacloprid dissolves and dissipates rapidly. It is unlikely that imidacloprid will be detectable at distances of 1,000 feet or more from the application sites. If it is detected, the concentrations will be below biologically relevant thresholds. Thus, there is no scientific or other basis for requiring treatment buffers.

Comment #24

Page 9. (Page 9). *"Minor effects, or the maximum allowable biological effects within the SIZ ... are exceeded if ... any one of the following ecological metrics is reduced by more than 50 percent, 14 days after imidacloprid application ... Class Polychaeta abundance and richness, Phylum Mollusca abundance and richness, and Class Crustacea abundance and richness." COMMENT – The threshold criterion for "minor" adverse effects to sediments and benthos are not adequately protective. The Service expects that the proposed permit and SIZs cannot be implemented without causing significant adverse impacts to sediments and native benthos, including prey resources on which several listed species depend. We oppose the authorization of SIZs in Willapa Bay and Grays Harbor.*

Response

See Responses to Comments #14 and #20 above.

Comment #25

Page 9. (Page 21). *"Nothing in this permit excuses a Permittee from compliance with any applicable federal, state, or local statutes, ordinances, or regulations." COMMENT – There has been no consultation under the ESA addressing the effects of aquatic application of imidacloprid, and there is no valid, current ESA coverage for the application of imidacloprid to control burrowing shrimp. To date, no federal action agency has requested consultation with the Services to address the practice and its potential effects to listed species. Without a valid, current incidental take permit or statement addressing the effects of this practice on listed species, parties engaging in aquatic application of imidacloprid lack ESA coverage.*

Response

Impacts to ESA listed species are extensively analyzed in the DEIS (see pages 1-23 through 1-25 and Section 3.2.5.3) and supporting literature. As summarized in the DEIS:

> Based on currently available information and studies, and requirements to comply with the conditions of all applicable pesticide registrations, permits and regulations (including Washington State Water Quality Standards), no significant unavoidable adverse impacts to threatened, endangered or protected species would be expected with the proposed action (Alternative 3: imidacloprid applications with IPM). With the exception of some salmonid life stages, it is unlikely that these species would be present on treatment sites at the time of imidacloprid applications. There is a low probability of adverse effect to birds or large vertebrates. Permit conditions protective of surface water quality would also be protective of salmonids. The requested Ecology NPDES Permit, if issued, would require discharge monitoring to be conducted to evaluate the effects of pesticide

> applications. Adjustments to permit conditions could be made throughout the five-year term of the permit.

USFWS has not provided any information demonstrating that WGHOGA's use of imidacloprid would adversely affect ESA-listed species of modify critical habitat. As discussed throughout the rest of this memorandum, its concerns are largely unsupported and directly undermined by the best technical and scientific information available.

Specific Comments for the SIZ Applications and Notices

Comment #26

Page 9. (SIZ Notice, Page 2). *The threshold criterion for "minor" adverse effects to sediments and benthos are not adequately protective. The Service expects that the proposed permit and SIZs cannot be implemented without causing significant adverse impacts to sediments and native benthos, including prey resources on which several listed species depend. Therefore, we oppose the authorization of SIZs in Willapa Bay and Grays Harbor.*

Response

See Responses to Comments #14 and #20 above.

Comment #27

Page 9. (SIZ Notice, Page 2). *"The names and addresses of other landowners affected by the proposed SIZ are listed in Attachment B." COMMENT – Attachment B fails to identify the U.S. Department of the Interior, U.S. Fish and Wildlife Service, as a landowner. The proposed SIZ for Willapa Bay extends onto tidelands located within the Leadbetter Point Unit of the Willapa National Wildlife Refuge (US Fish and Wildlife Service 2011, pp.2-57 through 2-61), and the SIZ for Grays Harbor extends into the Grays Harbor National Wildlife Refuge at Bowerman Basin. If Ecology issues the proposed permit and authorizes the proposed SIZs, we expect that there will be negative direct and indirect effects to the Service's trust resources. We do not support the issuance of an individual NPDES permit at this time and we oppose the authorization of SIZs in Willapa Bay and Grays Harbor, especially in light of the potential for adverse effects to several listed species.*

Response

Comment noted. We apologize for the oversight.

See responses to similar comments above. USFWS's claims of impacts to listed species and the environment are unsupported and undermined by the best technical and scientific information available.

Comment #28

Page 10. (SIZ Application, Pages 5, 11). *"Limited toxicity data are available to quantify the toxicity of degradation products or metabolites, as the majority of studies have focused on the parent compound imidacloprid ... Several studies conducted on insects found ... only the olefin derivative, which occurs as a metabolite in treated plants, has toxicity comparable to imidacloprid (Nauen et al. 1998; Suchail et al. 2001; Kagabu et al. 2004; SERA 2005; EFSA 2006; Tomalski et al. 2010)." "Seven out of 20 eelgrass samples had detectable concentrations of imidacloprid on the first day post-treatment." COMMENT – We can expect that detectable concentrations of imidacloprid and/or olefin will be present in eelgrass located both on and off of the treatment sites. Eelgrass will, in turn, represent a potentially significant exposure pathway for a variety of wildlife species, including waterfowl.*

Response

It is highly unlikely that eelgrass located off the treatment sites would have imidacloprid and/or olefin in their tissues. Imidacloprid and olefin were detected in a minority of eelgrass samples on the first day post-treatment in the 2012 field trials (reviewed in the DEIS), but not again after that, indicating that imidacloprid is not taken up by some eelgrass, and breaks down quickly in eelgrass that does. Patten et al. (2011) reported that eelgrass became established quickly on bare plots treated with imidacloprid, thus indicating that eelgrass is capable of rapid growth when burrowing shrimp are reduced, and is not adversely affected by imidacloprid. Because imidacloprid dilutes rapidly in surface water, it is highly unlikely that imidacloprid would be found in eelgrass off the treatment sites.

Comment #29

Page 10. (SIZ Application, Page 16). *All Known, Available, and Reasonable Methods of Prevention, Control, and Treatment (AKART). COMMENT – With our previous letter to Ecology (Letter to Donald A. Seeberger, dated February 14, 2014), the Service recommended that the EIS and permit framework should give fair and equal consideration to alternate culturing methods and practices. Control and removal of a native species that performs important ecological functions should not be the primary objective. Instead, this effort should be directed at developing and refining robust IPM methodologies, with stricter limits on the use of chemical control agents and an emphasis on adaptively managing shellfish production systems to avoid harming ecological resources. Graveling and frosting are established practices with the specific goal of firming substrates and fostering good conditions for larval attachment, maturity, and growth. Graveling and frosting should have a role in IPM methodologies directed at successful shellfish culturing on tidelands affected by burrowing shrimp. Long-line and stake culturing are also established practices, and are used successfully by some growers and farm operators in these same portions of Willapa Bay and Grays Harbor. Much of the information used to discredit these practices appears to be anecdotal and not based on either scientific studies or rigorous and comparative evaluation. Ecology and the WGHOGA should address more seriously and objectively whether methods of ground- based culturing and production require reevaluation in light of new science and the many* *concerns related to aquatic pesticide applications. Chemical control methods with lethal and biologically significant sub-lethal effects to non-target organisms should be a last resort and only implemented after a robust IPM methodology has exhausted all other alternatives at each specific location.*

Response

See responses to similar comments above (e.g., Comments #1–#3 and #7).

Comment #30

Page 10. (SIZ Application, Page 18). *Ecology and the WGHOGA acknowledge that burrowing shrimp numbers and densities exhibit cyclical changes over time. There is little or no evidence to substantiate the claims that Willapa Bay and Grays Harbor are currently experiencing anything unusual related to burrowing shrimp recruitment, numbers, abundance, and densities.*

Response

See Response to similar Comments #1–#3 above.

Specific Comments for the Fact Sheet

Comment #31

Page 11. (Pages 37, 38). *“Dungeness crab and fish were counted on the day of application and again 24 hours after treatment ... The average across all sites and treatments was two affected crab per acre ... The highest count was 3.4 affected crab per acre ... Bird predation of [paralyzed] crab ... appeared to be the main cause of crab mortality.” “Birds were observed foraging on and nearby the sites following treatments.” COMMENT – Willapa Bay and Grays Harbor support vitally important migratory and resident bird populations. If Ecology decides to issue the proposed permit, we expect that these waterfowl, raptor, and shorebird populations will be exposed to imidacloprid and its degradation products both on and off the treated sites. Birds that forage on the exposed tidelands will encounter and may ingest the granular pesticide product directly. Birds that forage on the exposed tidelands are also likely to ingest contaminated vegetation, sediments, and/or prey items. The western snowy plover, which is listed as threatened and uses sand and mudflats, sand islands, sand spits, and open beaches located in Grays Harbor and Willapa Bay, is likely to be exposed and affected.*

Response

The contention that snowy plover are “likely to be exposed and affected” is demonstrably false. As noted in the response to a similar comment above, the USFWS itself found that there are no records of snowy plovers foraging in Willapa Bay in its Biological Opinion for Snowy Plover (2009). Similarly, the

Service's designation of critical habitat for snowy plover[2] excludes nearly the entirety of both Willapa Bay and Grays Harbor, with the small exceptions being beach areas immediately adjacent to the mouth of these estuaries, which are not areas proposed for spraying with imidacloprid. USFWS is the federally designated lead agency for snowy plover, and therefore the data and information contained within the Biological Opinion for Snowy Plover demonstrate there should not be adverse impacts to this species.

USFWS's wide-ranging conclusion of impacts to other bird species that it does not have specific expertise over is also not scientifically credible. To recap the extensive scientific data reviewed in the DEIS, and results from the 2014 field studies:

- Imidacloprid is applied during a short, low-tide window. Following inundation by the first rising tide, imidacloprid is quickly diluted to levels below which biological effects on even the most sensitive invertebrate taxon (mysid shrimp) are not expected. Subsequent tidal cycles will continue the process of dilution and flushing of imidacloprid.

- Sediments exposed to imidacloprid experience an approximately exponential decline in concentrations, and are usually non-detectable within 28 days for whole sediment and are below biological effects levels within 14 days for sediment porewater. During the time when sediments have detectable concentrations of imidacloprid, these concentrations are very low relative to toxicity thresholds for invertebrates.

- Most eelgrass samples in treated plots have not tested positive for either imidacloprid or one of its primary breakdown products (imidacloprid olefin). No eelgrass sample has had detectable concentrations 14 days after treatment.

- Invertebrates collected from plots treated with imidacloprid are usually not statistically different, in numbers or types of invertebrates, than in control plots not so exposed. Where differences are found, sometimes treatment plots have more and sometimes less invertebrates than control plots. Thus, even where differences occur, they do not support a conclusion that imidacloprid is having a significant adverse impact.

- Imidacloprid has extremely low toxicity to vertebrates, including those bird species reviewed. In general, toxicity is associated with imidacloprid levels that are 2–4 orders of magnitude higher (i.e., 100 to 10,000 times) than levels being proposed for application under the NPDES permit.

- Megafauna that are either dead or in tetany have been observed following treatment, and some birds, notably gulls, have been observed feeding on them. No dead or impaired birds have been observed, however, among those seen feeding on affected megafauna.

---

[2] Available at http://www.fws.gov/arcata/es/birds/WSP/documents/WSPCH_June2012/6-19-2012_FR_rule.pdf

- Affected megafauna are only seen one day after treatment, indicating that any potential for feeding on such organisms by birds would be limited to a very short period following treatment.

Although not detailed in the DEIS, carbaryl, which has been sprayed on the two estuaries since the 1960s, and which is by all accounts more toxic than imidacloprid to both vertebrates and invertebrates, results in dead megafauna with some subsequent feeding by birds. Yet despite the 50^{+}-year record of carbaryl treatments, dead and impaired birds have not been observed associated with such feeding. This further supports the conclusion that the much less toxic chemical imidacloprid has no potential to directly affect foraging birds.

As to ingestion of pelletized imidacloprid (i.e., Protector 0.5G), this product is used in shellfish beds where extensive areas of standing water are present, even at low tide. Once applied, the pellets rapidly sink to the bottom and dissolve. Thus, direct ingestion of mallet by birds is unlikely both because the habitat, being flooded, is unsuitable for many shorebirds to feed, and because the pellets rapidly dissolve.

In summary, essentially all existing scientific data and analysis, which is extensively covered in the DEIS, supports the conclusion that birds in Willapa Bay and Grays Harbor have not been, and in the future will not be, negatively affected by imidacloprid treatments.

Comment #32

Page 11. (Pages 56-58). *There has been no consultation under the ESA addressing aquatic application of imidacloprid, and there is no valid, current ESA coverage for the application of imidacloprid to control burrowing shrimp. To date, no federal action agency has requested consultation with the Services to address the practice and its potential effects to listed species. Without a valid, current incidental take permit or statement addressing the effects of this practice on listed species, parties engaging in aquatic application of imidacloprid lack ESA coverage.*

Response

See response to Comment #25 above.

Comment #33

Page 11. (Page 59). *"Monitoring data will characterize the spatial extent, fate, and transport of imidacloprid following application, and help to determine if concentration are a concern for non-target organisms." COMMENT – Ecology, the WGHOGA, and their research partners acknowledge that the limited field trials performed to date have failed to meaningfully and adequately address a number of outstanding issues and concerns regarding fate and transport, efficacy, persistence, and effects to non-target organisms (Ecology 2014, pp. 1-33 through 1-37). Ecology should not advance a permit decision* *until more data is collected (during 2014 and 2015) and shared with the public. A decision to issue the permit and authorize SIZs while relevant and important data remain unavailable would be premature. Until field trials have adequately addressed the many unresolved questions, and to the satisfaction of all interested stakeholders, Ecology should not advance the permit decision. We recommend that Ecology should instead continue limited field trials under the Experimental Use Permit. We do not support the issuance of an individual NPDES permit at this time and we oppose the authorization of SIZs in Willapa Bay and Grays Harbor. The Service acknowledges that continuing a program of limited field trials would improve the state of our knowledge regarding imidacloprid applications and effects in the estuarine and marine environments.*

Response

See responses above to similar comments about the level of scientific certainty necessary to support permit issuance. The contention that "the satisfaction of all stakeholders" (however broadly "stakeholders" is defined) is not contained in any applicable regulatory standards of which we are aware and, if implemented, would allow any stakeholder to have veto power over the entire process.

See response above about availability of the 2014 field data, which adds tests of large plot spraying to the already existing body of scientific research on imidacloprid effects.

The statement "*Ecology, the WGHOGA, and their research partners acknowledge that the limited field trials performed to date have failed to meaningfully and adequately address a number of outstanding issues and concerns regarding fate and transport, efficacy, persistence, and effects to non-target organisms*" is a gross misrepresentation of what Ecology and the DEIS in fact conclude. Nowhere does the DEIS, draft permit, or fact sheet state or even imply the multiple years' worth of field trials fail to meaningfully and adequately address the listed issues. Rather, these documents present an honest discussion of these studies, along with other appropriate scientific and technical information, including limitations. A robust monitoring program is required in the draft permit to confirm that as the permit is implemented WGHOGA meets all its required conditions under that permit, and that environmental effects associated with that implementation continue to meet regulatory criteria and goals set by Ecology. In short, monitoring being proposed by Ecology focuses on compliance, and confirmation, not on a *post hoc* effort to gather information that is needed to justify issuing the permit, as USFWS contends. Notably, NPDES permits *regularly* require monitoring as a condition of permit issuance, so the monitoring being required here is not unique. Accordingly, additional limited field trials and experimental use permits are not scientifically justified, and needlessly delaying permit issuance for such studies could have extensive and adverse impacts on commercial shellfish beds and the broader environment of Willapa Bay and Grays Harbor.