Kim Patten

(Email Submission)

Please find attached my comments for the SEIS for imidacloprid. The first attachment are my comments on the SEIS, the second is supporting documentation.

Comment on: 'Supplemental Environmental Impact Statement for Control of Burrowing Shrimp Using Imidacloprid on Commercial Oyster and Clam Beds in Willapa Bay and Grays Harbor, Washington – Draft'

From: Kim Patten, Ph.D., Extension Professor, Washington State University Long Beach Research and Extension Unit.

Date: 11/1/2017

Thank you for the opportunity to comment on the Draft SEIS. It is a well prepared document. Below I have supplied comments in eight separate areas for your consideration.

 # 1. USE OF THE TERM 'AERIAL'

1.6.2 Summary of Impacts of and Mitigation Measures:
Alternatives 1, 3 and 4, pages 1-22 to 1-31.

The Draft SEIS, under Alternative 4, consistently uses the word "aerial application" as the application method used under this alternative. This is not correct. Aerial refers to application by air (airplane or helicopter). This is not allowed under Alternative 4. The wording should be replaced with ground-based broadcast boom or hand application for the 2F product, and hand, ground or boat spreader-based application for the 0.5 G product. The uses of "aerial" for Alternative 4 puts the growers in legal jeopardy with the label (See bolded relevant section of the 2F label below).

PROTECTOR 2F LABEL
"RESTRICTIONS: Do not harvest shellfish within thirty days after treatment. All ground must be properly staked and flagged to protect adjacent shellfish and water areas. **For aerial applications, the corners of each plot must be marked so the plot is visible from an altitude of at least 500 ft. Aerial applications must be on beds exposed at low tide.** A single application of imidacloprid per year is allowed. No adjuvants or surfactants are allowed with the use of this product. All applications must occur between April 15 and December 15. **A 100-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray;** a 25 foot buffer zone is required if treatment is by hand spray. Do NOT apply when winds are greater than 10 mph or during temperature inversions. **Do not apply aerially during Federal holiday weekends. During aerial applications, all public access areas within one quarter (1/4) mile and all public boat launches within a quarter (1/4) mile radius of any bed scheduled for treatment shall be posted**. Public access areas shall be posted at 500 feet intervals Draft Label at those access areas more than 500 feet wide. Signs shall be a minimum of 8 ½ x 11 inches in size, and be made of a durable weather-resistant, white material. The sign will say "Imidacloprid will be applied for burrowing shrimp control on [date] on commercial shellfish beds. Do not Fish, Crab or Clam within one-quarter mile of the treated area. The location of the treated area will be included on the sign ...."

By the SEIS using the word "aerial" for Alternative 4, it could be legally inferred from the label that the grower would need to comply with all the label requirements stated in the label for aerial application by helicopters, e.g. need 100' buffer, etc.

I don't think it was the intention of the Department of Ecology to use wording in the SEIS that would equate backpacking spraying to aerial application by helicopters; however, in a court of law, such an inference could be made. To avoid costly lawsuits defending the permit, I would suggest adjusting the wording so that there is no confusion in the application terminology and so that it is consistent with the label, and the intent with which it will be used. Consider replacing 'aerial' with 'ground-based broadcast

boom' or 'hand application' for the 2F product, and 'hand, ground or boat spreader-based application' for the 0.5 G product, or some other wording that won't legally compromise Ecology and the growers when the NPDES permit is issued.

 # 2. CAUSE OF THE PROBLEM

2.4 History and Background

This section of the SEIS states that the shrimp population dynamics in Willapa Bay and Grays Harbor are poorly studied and not known.

"The factors controlling burrowing shrimp populations are not well known, in part because long-term data on burrowing shrimp numbers in Willapa Bay and Grays Harbor are not available. Several authors (e.g., Stevens 1929, Feldman et al. 2000, Sanford 2012), have hypothesized that human-related impacts may have contributed to changes in Willapa Bay which led to increased burrowing shrimp populations. These potentially include excessive harvest of native Olympia oysters during the 1900s, land use changes in the watersheds (e.g. logging, farming), disturbance associated with current shellfish farming (including chemical and physical efforts to reduce burrowing shrimp), and other human activities. Changes in climate and oceanic conditions may also have altered conditions in ways that are favorable for burrowing shrimp."

While the purpose of the SEIS is not to provide a complete review of population dynamics of burrowing shrimp in SW coastal WA, it should at least reflect recent population trends reported by Dumbauld and others. There were major recruitment events in 1989, 1993 and 1994, followed by 17 years of little to no recruitment that continued until 2012. The past several years have all had consistent solid recruitment (see WSU 2017 data presented in the economic section below). The important aspect of this to consider is that, since ghost shrimp are long-lived as adults (>10 years), any major recruitment event will refresh the adult population. Consequently the upsurge in recent recruits will pose a significant long-term pest threat level not seen in the past 2 decades. This is germane as it relates to the economic section below.

The SEIS also speculates that overall shrimp population in the bay could be associated with historic and current shellfish harvesting and farming. This is a significant overreach. There is also no mention of over-fishing, or the damming of the Columbia River and its impact on fresh water purges of the bays. Both of these variables are mentioned frequently as causative in historic population trends, but are not mentioned in the SEIS.

 # 3. POTENTIAL IMPACT OF THE PROBLEM BASED ON 2017 RECRUITMENT DATA.

Section 2.6 Economics.

This section of the SEIS details estimated economic damage to the industry if chemical control is not an option. It states $50 million in cumulative losses by 2022. These estimates were made by the industry prior to knowing the population dynamics of shrimp on their beds over the next 5 years. That population is based on the number of recruits that have survived and grown into adults that can cause damage. WSU Long Beach and USDA have done extensive population monitoring for the past several years to try to understand what those populations will be in the future.

The need to control burrowing shrimp is based on the population of adult shrimp that is responsible for bioturbation. The standard economic threshold for treatment has been 10 burrows/m^2. This is an adult shrimp population of ~ 6 to 7 adults/m^2. An adult population of burrowing shrimp at any one time is based on natural mortality and recruitment rate of juvenile shrimp. Adults can live >10 years. Prior to

2014 there were many years with very low recruitment of new juvenile shrimp. This meant that the need to control burrowing shrimp in Willapa Bay was moderate and limited to sites with residual populations of adult shrimp. WSU sampling of recruitment populations over the last 4 years, however, has indicated that there have been significant new populations of juvenile shrimp settling across most of the tideflats in the bay. This has been especially noticeable on shellfish beds near the mouth of the bay. For example, on one bed we have been monitoring (bed A40), there were 140, 340 and 50 new recruits/m^2 in 2015, 2016 and 2017 respectively. Mean population of ghost shrimp by recruit age class for three growing areas in Willapa Bay, based on extensive sampling in September 2017, is provided in Table 1. These data indicate that recruitment numbers were slightly down for 2017 for the northern part of the bay but up for the southern part of the bay. The data also indicate that there was a decent survival rate of previous years' recruits. The sub-adult population of ghost shrimp is very high in all these regions and represents a very real threat to the future of the shellfish industry in Willapa Bay for 2018 to 2022. If these recruitment trends continue, it is likely that the economic impact stated in the SEIS could be a low estimate (Section 2-6, page 60). Furthermore, based on samples collected 10/31/17, there appeared to be continued episodes of significant recruitment during October 2017 (see footnote in Table 1).

Table 1. Mean density of ghost shrimp by age class in three shellfish growing regions in Willapa Bay based on sampling done in late September 2017*

	Ghost shrimp density (#/m^2) **				
Location	2014 recruits	2015 recruits	2016 recruits	2017 recruits	Total population of sub-adult shrimp***
Tokeland/Cedar River area	112	88	137	35	372
Stackpole area	16	28	54	50	148
Nahcotta Flats & Middle Is. Sands	41	16	21	104****	182

*Data are means from replicated coring over multiple locations within each region.

**Recruit age is approximate, based on carapace length: 2014 recruit ~ 7.65 mm to 12.5; 2015 recruit ~ 6.6 to 7.6 mm; 2016 recruit 4.5 to 6.5 mm; 2017 recruit <4.5 mm.

*** Total population of non-adult shrimp is the sum density of all shrimp <12.5 cm carapace sampled in September 2017.

**** Four sites off the Nahcotta Flats were resampled in 10/31/17 to assess if there was on-going recruitment occurring during the fall. At those sites, the mean density of 2017 recruits was 244 ± 21, n=13 with 95% of them having a carapace <2mm. Three locations that were sampled 10/7/17 were resampled on 10/31/17. The was a >60% increase in new recruit density during that time period (94/m^2 to 230/m^2).

4. 2017 DATA ON MECHANICAL CONTROL

2.8.5.1 Mechanical Control Methods

This section evaluates mechanical control options for the industry and suggests that they have limited options. At the time of its writing, however, there was no hard data on harrowing or dredging. Mechanical harrowing or dredging has been suggested by the public and others as a method to control young burrowing shrimp that are near the surface. It has been claimed that harrowing from a barge dislodges or destroys young- shallow- tender recruits and could, if practiced aggressively, be used by the industry as an alternative to chemical control. Prior attempts to gather data on efficacy of this method have been hampered due to the lack of juvenile shrimp populations in adequate density to conduct research. In recent years, populations of recruits have been high enough to allow that research to be conducted. WSU Long Beach conducted two studies in 2017 to assess efficacy of harrowing and clean-up dredging (see Studies 1 and 2 below). These studies indicate that these efforts slightly reduced the population of new/young shrimp compared to untreated sites, but those reductions were not statistically significant and did not reduce the populations to levels that would be consider of practical value.

Study 1: Deep harrowing

Site: Bed A40 Cedar River, sandy sediment, Goose Point Oyster bed, recruit population May 2017 ~200/ m^2 range.

Experiment design: Randomized complete block, 0.5 by 1.5 m plot size, 3 replications.

Treatments: Untreated control and hand harrowing. An aquatic weed rake with a set of six -25 cm long x 2.5 mm wide tines was pulled by hand through the treated plots down to the 20 cm depth in the sediment, 3 times in each direction. This was done in 0.3 to 0.5 m of water during an incoming tide. New recruits were noted as swimming off the disturbed treated plots.

Assessment: Sixteen days post-treatment the plots were cored (2 cores/plot, 10 cm diameter by 40 cm depth), and recruits collected by sieving (2 mm mesh) and measured to the nearest 0.01 mm carapace size. Data were analyzed by ANOVA for the total number (between 2 and 6 mm carapace, and within each mm size bracket of carapace). Data were also collected on recruit density and size by depth (0 to 10 cm, 10 to 20 cm, and 20 to 30 cm) within the plots.

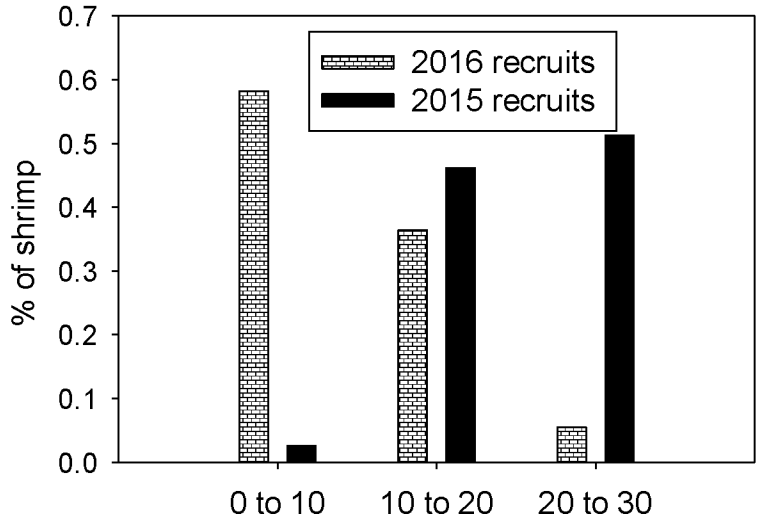
Results: There were no differences in shrimp densities due to treatment for all size brackets (Table 2). There was a slight trend for harrowing to numerically reduce the density of recruits, but these differences were not close to being statistically different or of practical relevancy. A significant portion (>40%) of new 2016 recruits were deeper than 10 cm and >95% of the 2015 recruits were deeper than 10 cm (Figure 1). Surface dredging or harrowing from a barge is unlikely to get much deeper than 10 cm.

Summary: Deep harrowing, far in excess of the depth that would be achieved by barge harrowing, provided no relevant control of new recruits.

Table 2. The efficacy of deep harrowing on the population density of young burrowing shrimp in May 2017.

	# burrowing shrimp by size class/m^2				
Treatment	2-3 mm	3-4 mm	4-5 mm	5-6mm	total 2-6 mm
Untreated control	62	68	68	5	203
20 cm deep harrowing	26	62	26	10	124
F test value	1.8	0.0	2.0	0.5	2.1
Probability of significance	0.3	0.9	0.2	0.5	0.2

Figure 1. Distribution of recruits by sediment depth (cm) on bed A40



Study 2: Barge dredging

Site: Bed A55 Cedar River, sandy/silty sediment Taylor bed, recruit population moderately high (May 2017 ~100 to 200/m^2 range).

Experimental design: Whole bed, pseudo-replicate, comparison of inside and outside a 20- acre bed that was dredged during winter 2016.

Treatment: The bed was dredged to remove transplanted oysters between 10/24/2016 and 12/15/2016. There were twelve 3-hour dredging sessions. The total cost to dredge the sites was estimated by the grower to be $24,000.

Assessment: Three transects (replications) that ran inside and outside the dredged bed were compared. Transects were sampled (4- 10 cm diameter cores 30 cm deep) for recruit density at 17 m and 33 m inside and outside the bed. Data were pooled (inside vs. outside along each transect (replication n=3)) to compared density of 2015 and 2016 recruits. Recruit density was analyzed by one-sample Wilcoxon signed rank test for non-parametric data; a Mann-Whitney Rank Sum Test for the data did not pass the Shapiro-Wilk normality test.

Results: There were no differences in shrimp densities due to treatment for all recruit ages (Table 3). There was a slight trend for dredging to numerically reduce the density of recruits, but these differences were not close to being statistically different or of practical relevancy. In addition, the study was neither truly randomized nor replicated. The dredged bed had a residual shell base and was siltier sediment than the comparison zones immediately outside the bed. The difference in treatment could have been due to site rather than dredging.

Summary: Cleanup dredging to remove transplant oysters left behind did not statistically reduce recruit densities. The nonsignificant difference between treatments could have been site difference. Regardless, the recruit density in the dredged beds was still too high to be of practical control value.

Table 3. The efficacy of cleanup dredging on the population density of young burrowing shrimp in May 2017.

	# burrowing shrimp by size class/m^2		
Treatment	2016	2015	2015+2016
Untreated control	129	75	163
20 cm deep harrowing	204	34	279
Probability of significance	0.24	0.15	0.10

 # 5. THE NEED FOR BETTER DATA RELATING TO SPATIAL & TEMPORAL EXPOSURE OF IMIDACLOPRID IN WATER.

3.0 Affected Environment, Potential Impacts, and Mitigation Measures

The draft SEIS uses water exposure data developed during commercial-size applications in Willapa Bay. That is a good data set that provides expected maximum exposure concentration for a risk assessment immediately following an application. This assessment is fine for species that are exposed in that first 5-10 cm of tidal inundation. However, it is not realistic for fauna, such as fish or Dungeness crab megalopae. These fauna would be exposed to the concentration of imidacloprid that is found in the actual water column, not the wetting front. Unfortunately, we have very little data on what those values are because the former SAPs and NPDES required data only from the first 10 cm of the wetting front. We have no idea about the extent of dilution of imidacloprid over time in the water column. While it is important to have a conservative approach to risk assessment, it is equally important to use realistic exposure data. This point may want to be addressed in the SEIS, and/or considered later when developing the SAP and NPDES for monitoring.

#6. A MAJOR IMPACT FACTOR NOT CONSIDERED FOR THE 'NO ACTION ALTERNATIVE'

The SEIS does a good job detailing the potential impacts of the four alternatives. One consideration that was not addressed with the No Action Alternative (#1) is that if this alternative is selected then there will be no future NPDES. Without an NPDES, there is no possibility for anyone to obtain an Experimental Use Permit (EUP) for future research. "A Washington State Experimental Use Permit is required for all experiments involving pesticides that are not registered, and for all experiments involving uses not allowed by the pesticide label". Coverage under a NPDES permit is required whenever an experimental pesticide is going to be applied to an aquatic environment. One of the conditions of the previous NPDES was to allow for new research to be conducted on alternative chemical control on a limited scale (<1 acre). Based on conversations with WSDA and Dept. of Ecology, there are no exceptions to this rule, regardless of how small the plot is or how environmentally benign the treatment may be. Since a pesticide is defined by EPA as "Any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest," then virtually all future burrowing shrimp control options would be considered pesticides and prohibited from being evaluated. By definition the following could be considered pesticides: subsurface injection with fresh water, ultra-sound and electro-shocking. These three methods have been tested in the lab with some marginal suppression of burrowing shrimp, but now could not be tested in the field. Any new chemistries with selective control in the lab and with the potential for minimal non-target impact, that might be found in the future, would also not be able to be evaluated in the field.

If Dept. of Ecology does not choose Alternative Four, the No Action Alternative is the default. One of the unintended consequences of the No Action Alternative would be to virtually eliminate any future research on burrowing shrimp control, other than mechanical control. Mechanical control has been well vetted over the past 70 years, and has been found to have very limited potential. In addition, due to potential impacts to eelgrass, it is unlikely it would even be allowed under the new restrictive Nationwide Permit imposed by the Army Corps of Engineers. Under the current burrowing shrimp recruitment conditions, and with few options for research on new control methods, the long-term consequences to the shellfish industry in Willapa Bay under the No Action Alternative would be grim.

I realize that there is serious opposition to Alternative Four by many stakeholders. These stakeholders insist that the No Action Alternative is the only sane choice and the industry should find other methods to control burrowing shrimp. Unfortunately, the No Action Alternative slams the door on the industry's ability to find alternative control methods, other than mechanical/cultural methods. The extensive research over 70 years has yet to even hint that there are any good mechanical methods to manage adult shrimp populations and the industry can not all convert to off-bottom culture.

In summary, a major research effort will be needed to find and test other options for control. However, it is impossible to make a valid inference on efficacy without field testing. You can't field test without an EUP. You can't get an EUP without an NPDES. Since you can not get an NPDES under the No Action Alternative, you virtually eliminate the ability to conduct research on alternative controls. Unfortunately the unintended consequences of the No Action Alternative will mean no future control for burrowing shrimp will likely ever be developed. To that end, the shellfish industry in Willapa Bay will go through a major decline over the next several decades.

#7. IMPACT ON BENTHIC INVERTEBRATES

Information on the potential impacts of imidacloprid on benthic invertebrates is presented in the 2015 FEIS (Chapter 3, Section 3.2.5, pages 3-48 through 3-49). Some new additional analysis is included in this SEIS. PSI and WSU recently reassessed the data sets obtained under previous SAP studies in Willapa Bay using Principal Response Curve Analysis (PRC). PRC analysis is a multivariate ordination technique that was derived from Redundancy Analysis, primarily to simplify assessment of pesticide treatments on abundances of aquatic invertebrates in mesocosms and has since become fairly standard for such experimental systems. We are in the process of submitting this analysis for publication to either Nature or Coastal Shelf and Estuary Science.

One of the major points of this analysis is to highlight the fact that the default response of estuarine epibenthic and benthic invertebrates to imidacloprid is neutral, rather than negative. In fact only 6 PRCs out of 60 showed a significant negative effect. The large majority of PRCs showed no significant effect from imidacloprid application, a neutral treatment effect, or ostensibly a "positive" treatment effect.

I've attached the current draft of that paper. Below is the title and abstract

Response of Estuarine Benthic Invertebrates to Large Scale Field Applications of Imidacloprid. Steven R. Booth[1], Kim Patten[2] and Leslie New[3]. Pacific Shellfish Institute[1], Olympia, WA 98501, Washington State University Long Beach Extension Unit[2], Long Beach WA 98631, Washington State University Vancouver[3] WA 98686

A total of 60 analyses were conducted to examine the response of 6 taxonomic assemblages (polychaetes, 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 analyses, but interpretation was often confounded by significant differences 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.

#8. EFFECTS OF BURROWING SHRIMP ON CLAMS

1.7 Areas of Controversy and Uncertainty, and Issues to be Resolved

"Research on the effects of burrowing shrimp on commercial shellfish beds has been done where oysters are the primary crop. Field research data are lacking regarding how burrowing shrimp affect clams, and the threshold for damage to clam beds."

The SEIS is correct in stating that there have been no studies showing the direct impact of burrowing shrimp on commercial clam production. We have attempted to collect economic threshold data several times, but have not been successful. The main reason for this failure is due to the fact that we could not maintain gravel on the surface long enough to conduct an experiment. Gravel is much denser than oysters, and rapidly sinks in areas infested with burrowing shrimp. If you don't have gravel, you don't have clams. We have also attempted to place mature clams on sites with different densities of burrowing shrimp and assess thresholds, but because clams are very mobile, we have never been able to find them at the conclusion of the study. In addition, the average harvest cycle for commercial clams in Willapa Bay is 3 to 4 years. Because population dynamics of burrowing shrimp are not steady, determining accurate

economic thresholds for burrowing shrimp over that 3 to 4 year duration is exceedingly difficult. It would be reasonably easy to design an experiment that examines the sinking rate of gravel as a function of burrowing shrimp density. From that, a threshold for treating burrowing shrimp could be developed. However, I would be uncertain as to what timeframe should be used to determine the threshold for sinking (6, 12 or 36 months), especially when shrimp populations are not constant.

The point I want to make is that what seems like a simple data request – "shrimp treatment threshold for clam production" – is exceedingly difficult to obtain. We don't even have an accurate method for quantifying burrowing shrimp density, other than excavating and sifting sediment down to a meter in depth. Because the total acreage for treatment is very limited (500 acres), I think it would be realistic to set the threshold similar to what has worked for oysters (10 burrows/m^2), and let the industry decide where their treatment priority areas are based on the economic impact it will have to their farms.

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Response of Estuarine Benthic Invertebrates to Large Scale Field Applications of Imidacloprid

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keywords
burrowing shrimp, estuarine benthic invertebrates, imidacloprid, principal response curve, Willapa Bay

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).

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 rather 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 was 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 A.1).

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.

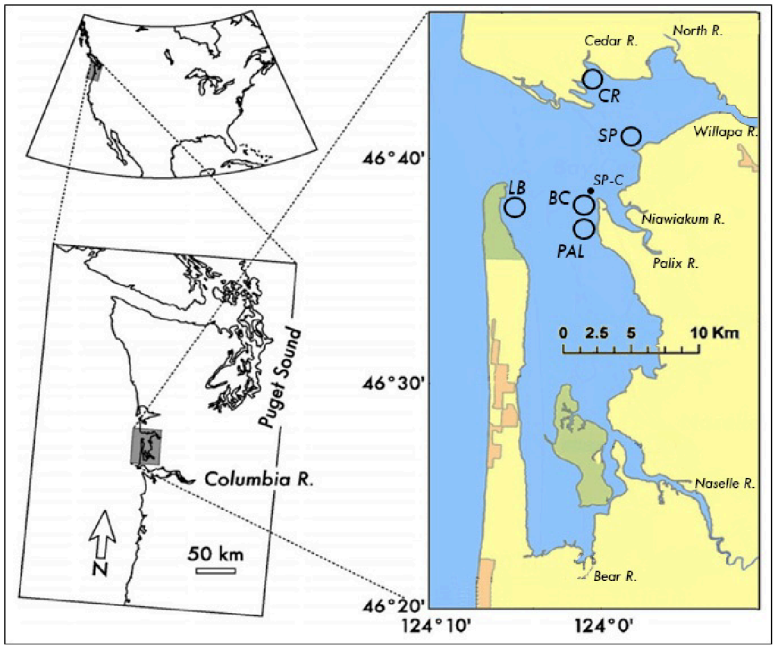

Figure 1. Willapa Bay, WA study sites: Cedar River (CR - 2011), Stony Pt. (SP - 2014), Stony Pt. Control (SP-C - 2014), Bay Center (BC - 2011), Leadbetter (LB - 2012), Palix (PAL - 2012).

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) (R Core Team 2016). 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 A.5).

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 A.2a).

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 A.3) and the significance of the treatment and treatment x time interaction effects are presented in Table 4. Axis 1 displayed a significant amount of the constrained variance in 51 of the 60 PRCs. Analyses with lower percentages of unconstrained variance were those that were less diverse (i.e., all studies at Bay Center and Cedar River in 2011). Treatment effects were significant in 54 of the 60 analysis (Table 4). Both treatment effects and axis 1 were significant in 49 of the 60 analysis.

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 A.5 – A.14), 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 A.6. 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.

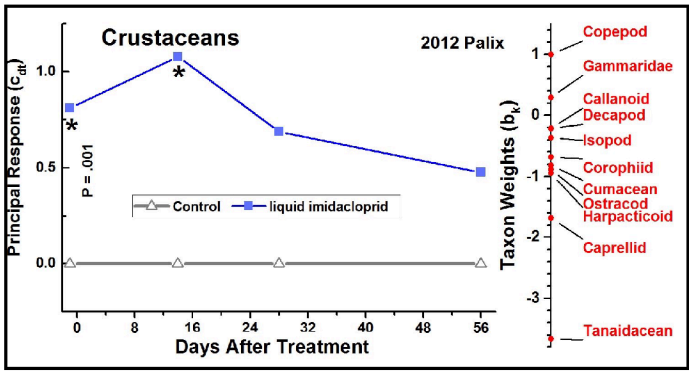

Figure 2. Principal Response Curve of crustaceans before and after treatment with liquid imidacloprid at Palix, 2012. P is probability that the primary axis (response) is significant. Asterisk (*) indicates the response at each sample date is significantly different from the control ($p < 0.05$). Weights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

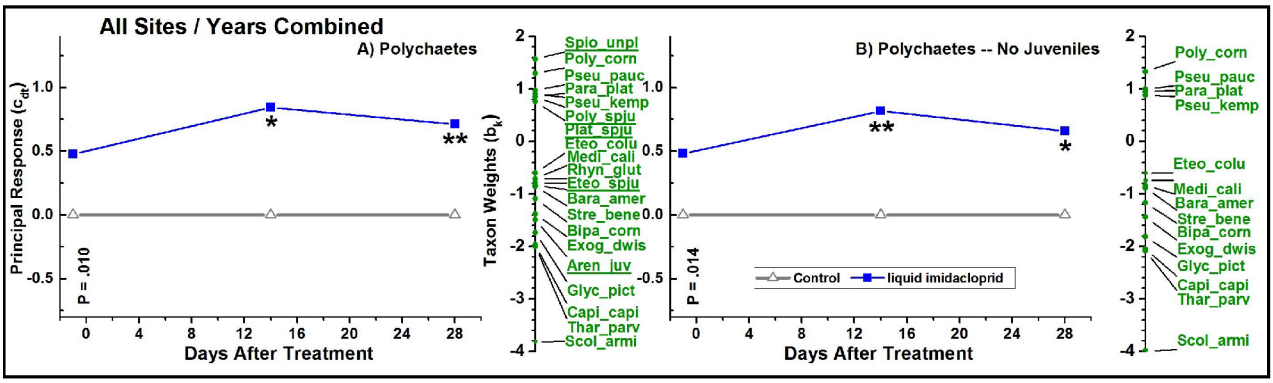

Figure 3. Principal Response Curve of A) all polychaetes (underlined taxa are juveniles) and B) non-juvenile polychaetes before and after treatment with liquid imidacloprid, pooled study sites and years. P is probability that axis 1 (Principal Response) is significant. Asterisks indicate the response 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 A.2 lists polychaete full names and abbreviations.

Both the trajectory and the end response of all non-juvenile polychaete PRCs were very similar to those

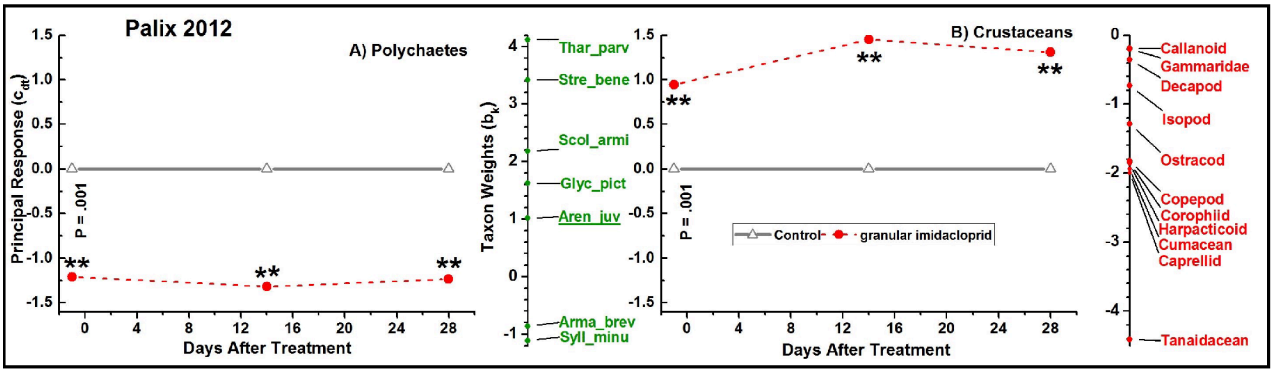

Figure 4. Principal Response Curves of A) Polychaetes (underlined taxa are juveniles) and B) Crustaceans at granular imidacloprid and control plots at Palix in 2012. P is probablility that axis 1 (response) is significant. Asterisks (**) indicate the response at each sample date is significantly different 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 A.2 lists polychaete full names and abbreviations.

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 effects

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 laboratory arenas. Toxicity tests of standard saltwater test crustaceans report LC_{50} values of 10,440 *u*g/L for water flea (*Daphnia magna*) and 361,230 *u*g/L for 4th naupliar stage brine shrimp (*Artemia* sp.) (static 48 hr tests, Song et al. 1997). These values were substantially higher than the field concentrations sampled in our studies. LC_{50} values were 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 were 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 bound to sediments.

Because carbaryl has been the only other insecticide applied to manage estuarine burrowing shrimp, it is a useful reference to assess for relative toxicity to non-insect invertebrates. Very few, if any, studies have been published that directly compared the toxicities of IMI and carbaryl to non-insect invertebrates, but comparisons between generally similar studies showed carbaryl to be much more toxic. An LC_{50} of 137 *u*g/L was reported for 24 hr old *Artemia salina* (Barahona and Sanchez-Fortun 1999) in an experimental system similar to that used by Song et al. (1997) and an LC_{50} of 43 *u*g/L of carbaryl was reported for the grass shrimp (*P. pugio*) (Chung et al. 2008) in an experimental system similar to that used by Key et al. (2007). LC_{50} values of carbaryl ranged between 5.6 and 16.4 *u*g/L among 9 studies of toxicity to *D. magna* (Toumi et al. 2016).

4.2. Disturbance effects

Although estuarine benthic invertebrates survived IMI applications by virtue of limited exposure or

physiological tolerance, they were also 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. 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). "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. As noted by Dumbauld et al. (2009), natural disturbance is essential to maintain community structure in many ecosystems, and that aquaculture is generally in the same scale.

The intertidal environment of Willapa Bay is particularly dynamic at both spatial and temporal scales. Salinity is especially variable in Willapa Bay and was characterized as "extremely unsteady" in salt balance, both between and within seasons (Banas et al. 2004). 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 flows transport and distribute sediments across the tideflats (Wheatcroft et al 2013) to erodable channels that carry "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.). Water temperatures also vary widely and can reach 40°C within a few hours in shallow puddles left during low tides on sunny summer days in Willapa Bay (Pacific Shellfish Institute monitoring data). Because the mouth of the estuary and 5 of the 7 primary rivers that flow into Willapa Bay 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). The amount and type of vegetation and detritus also vary at more local scales according to differences in tidal elevation, aspect, and proximity to rivers and other upland inputs. 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 in Willapa Bay and Puget Sound.

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.

Estuarine epibenthic and benthic invertebrates have evolved several life history strategies to deal with 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 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).

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. 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. 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. 2001).

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. 2001) 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. 2001). Cultured bivalves in North American West Coast estuaries, including oysters in Willapa Bay and Grays Harbor managed with carbaryl to suppress burrowing shrimp, did not reduce the capacity of the larger ecosystem to adapt to disturbance (Dumbauld et al. 2009). The same conclusion would hold given the smaller treatment area and lower toxicological impact from a burrowing shrimp management program using imidacloprid.

APPENDIX

Table A.1. 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 A.2a. List of 96 taxa identified and enumerated from all samples at all sites and years. Table A.2b lists polychaete 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 pauci-branchiata. 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 A.2b. Polychaete name abbreviations. Table A.2a 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 A.3. Percentage variance partitioned by RDA and Monte-Carlo permutation F tests for significance of primary axis (axis 1).

				% Var. Attributed to:			% Trt. Var. Captured	PRC Permutation Test Statistics		
Year	Site	Formulation	Metric	Time[1]	Treatment[2]	Residual[3]	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 A.4. 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 A.5. 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 A.6. 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.

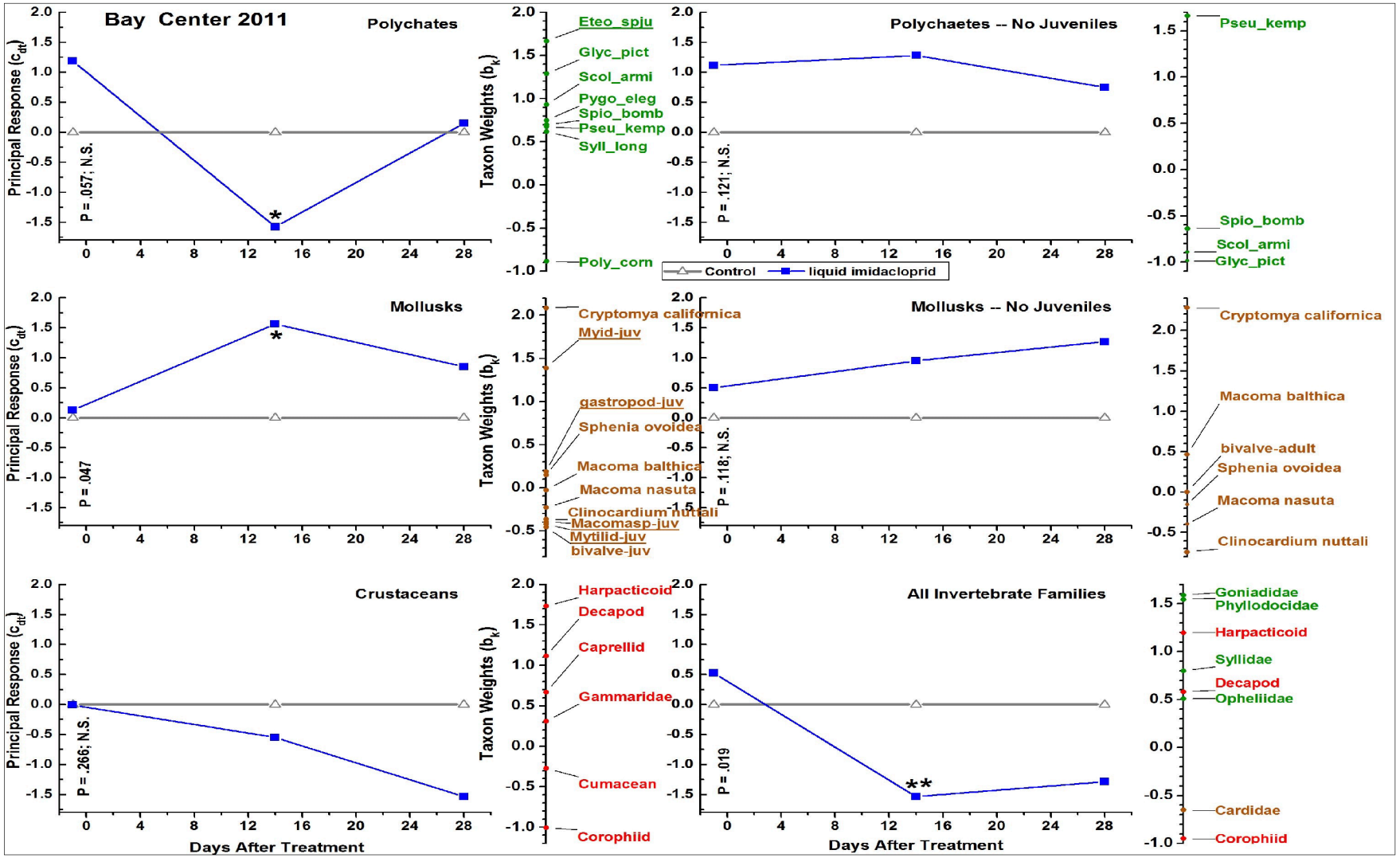

Figure A.5. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Bay Center in 2011. P is probability that the displayed primary axis is significant. Asterisks indicates the response 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 A.2 lists polychaete full names and abbreviations.

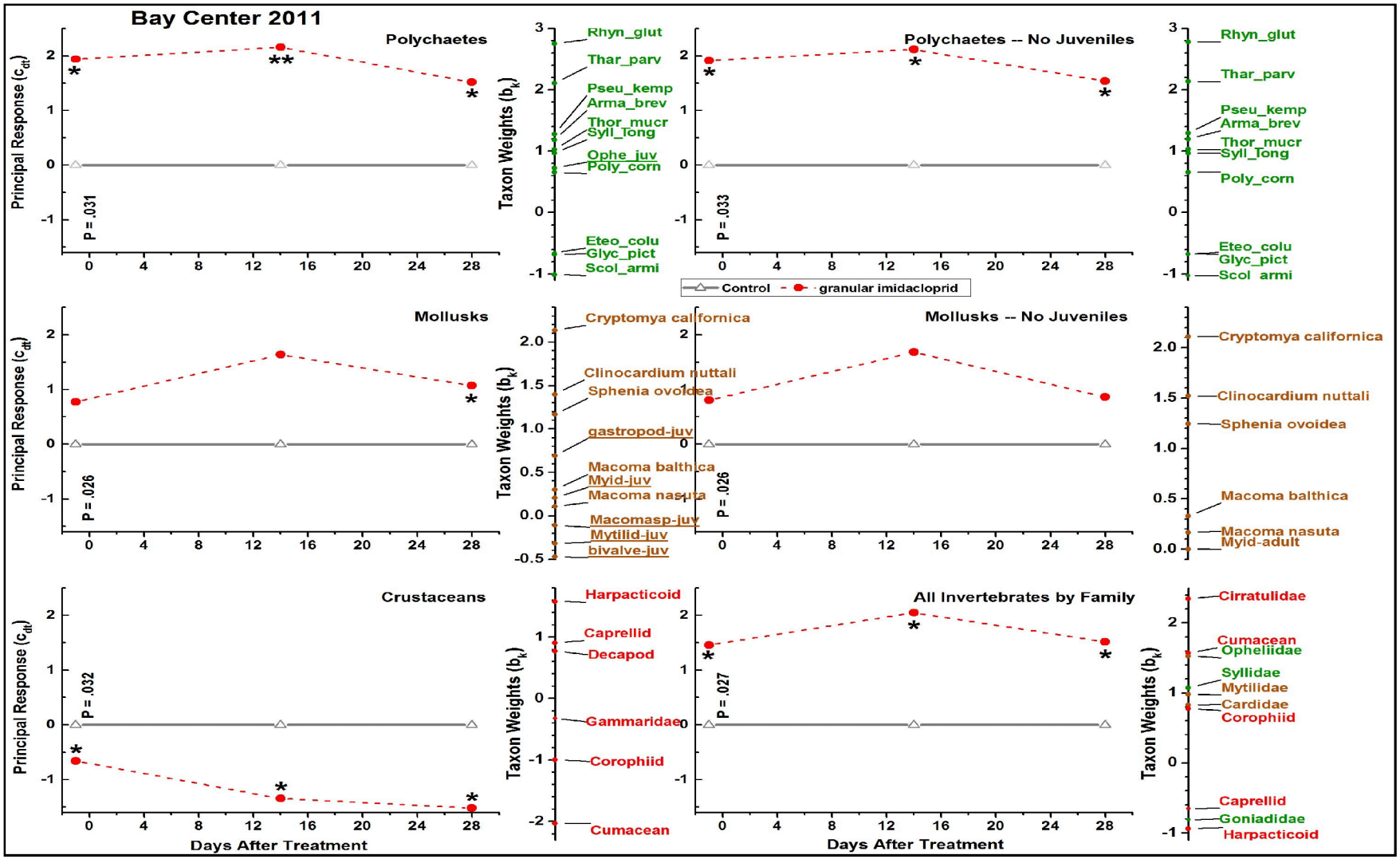

Figure A.6. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at granular imidacloprid and control plots at Bay Center in 2011. P is probability that the displayed primary axis is significant. Asterisks indicates the response 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 A.2 lists polychaete full names and abbreviations.

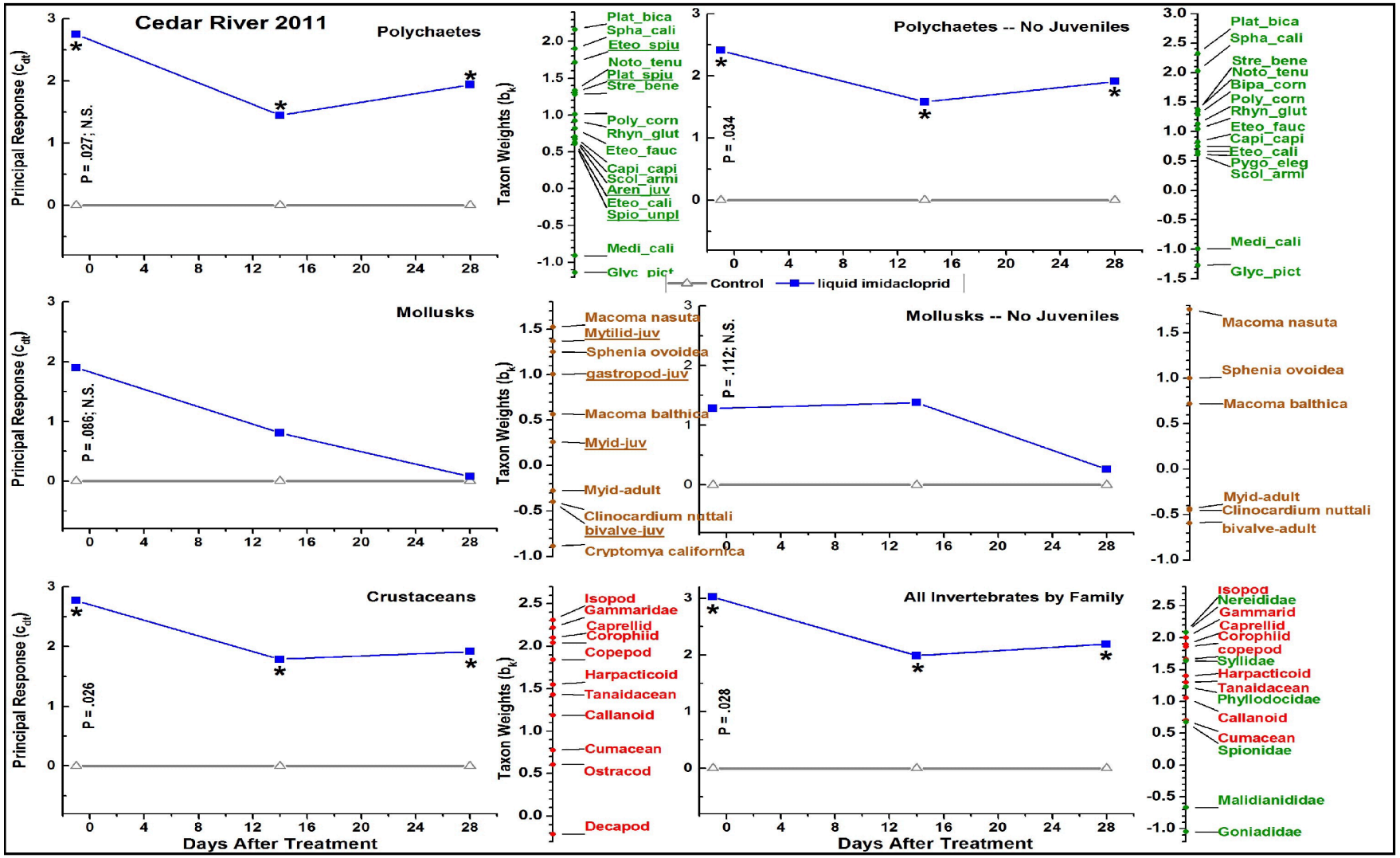

Figure A.7. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Cedar River in 2011. P is probability that the displayed primary axis is significant. Asterisks indicates the response 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 A.2 lists polychaete full names and abbreviations.

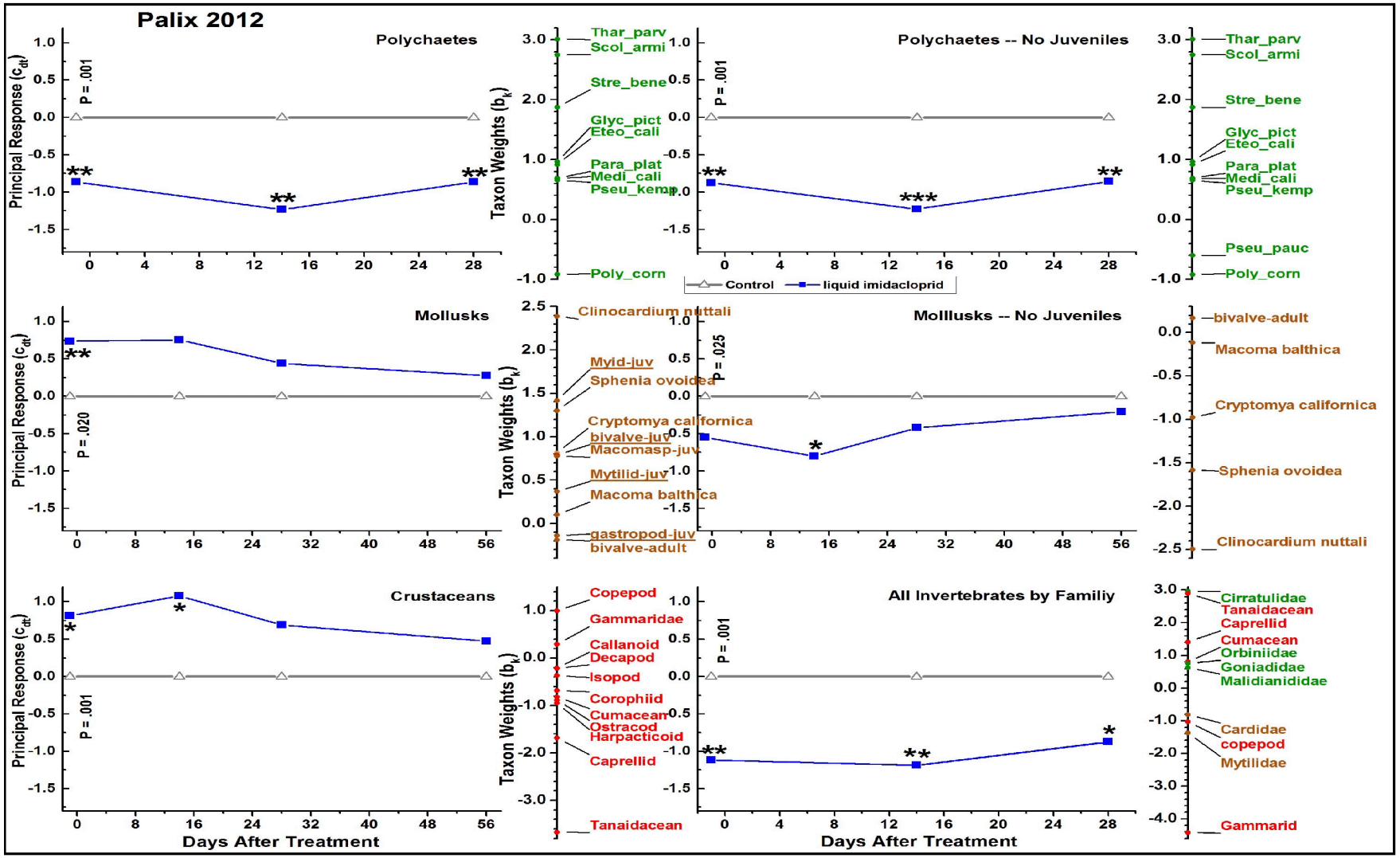

Figure A.8. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Palix in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response at 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 A.2 lists polychaete full names and abbreviations.

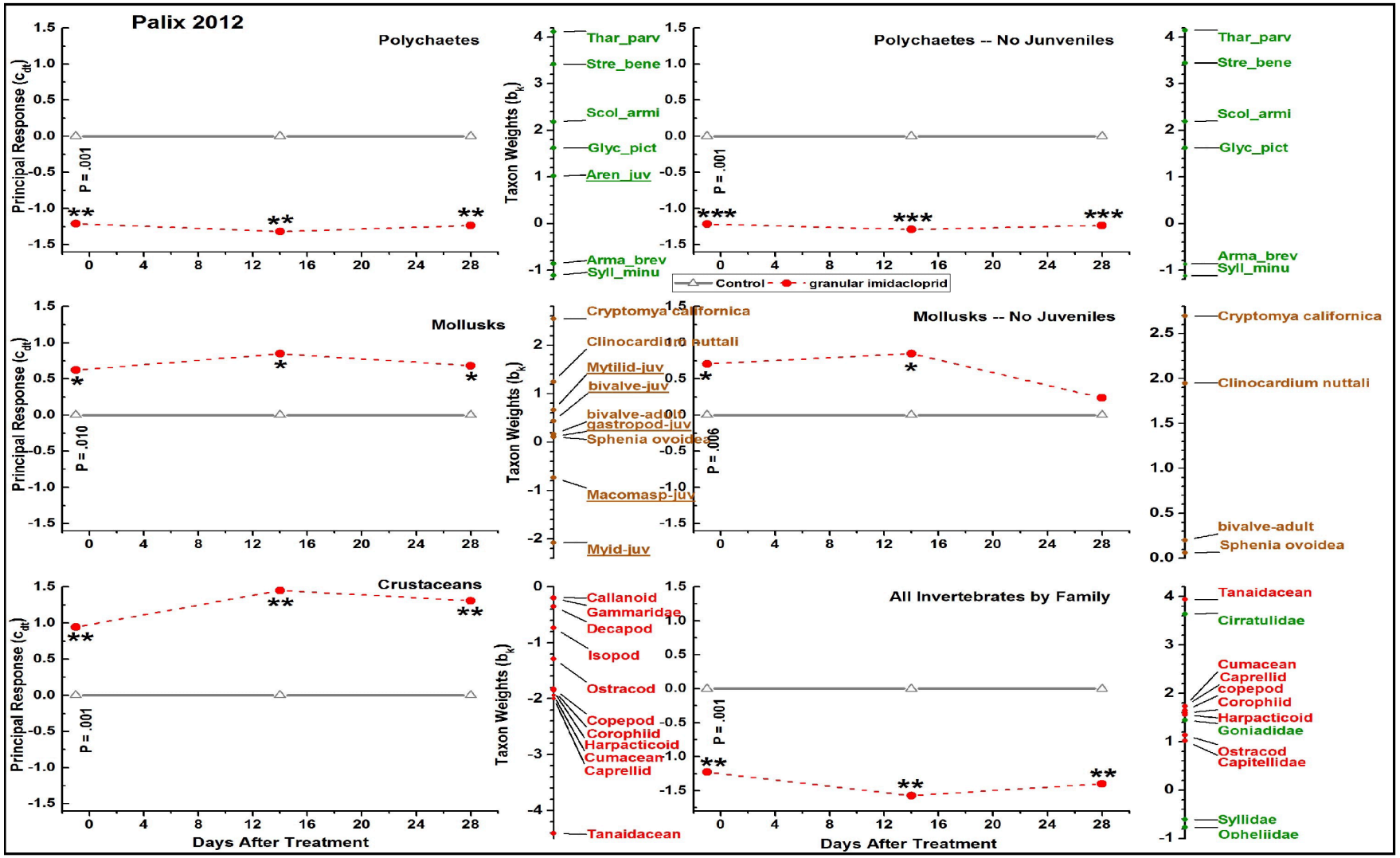

Figure A.9. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at granular imidacloprid and control plots at Palix in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response 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 A.2 lists polychaete full names and abbreviations.

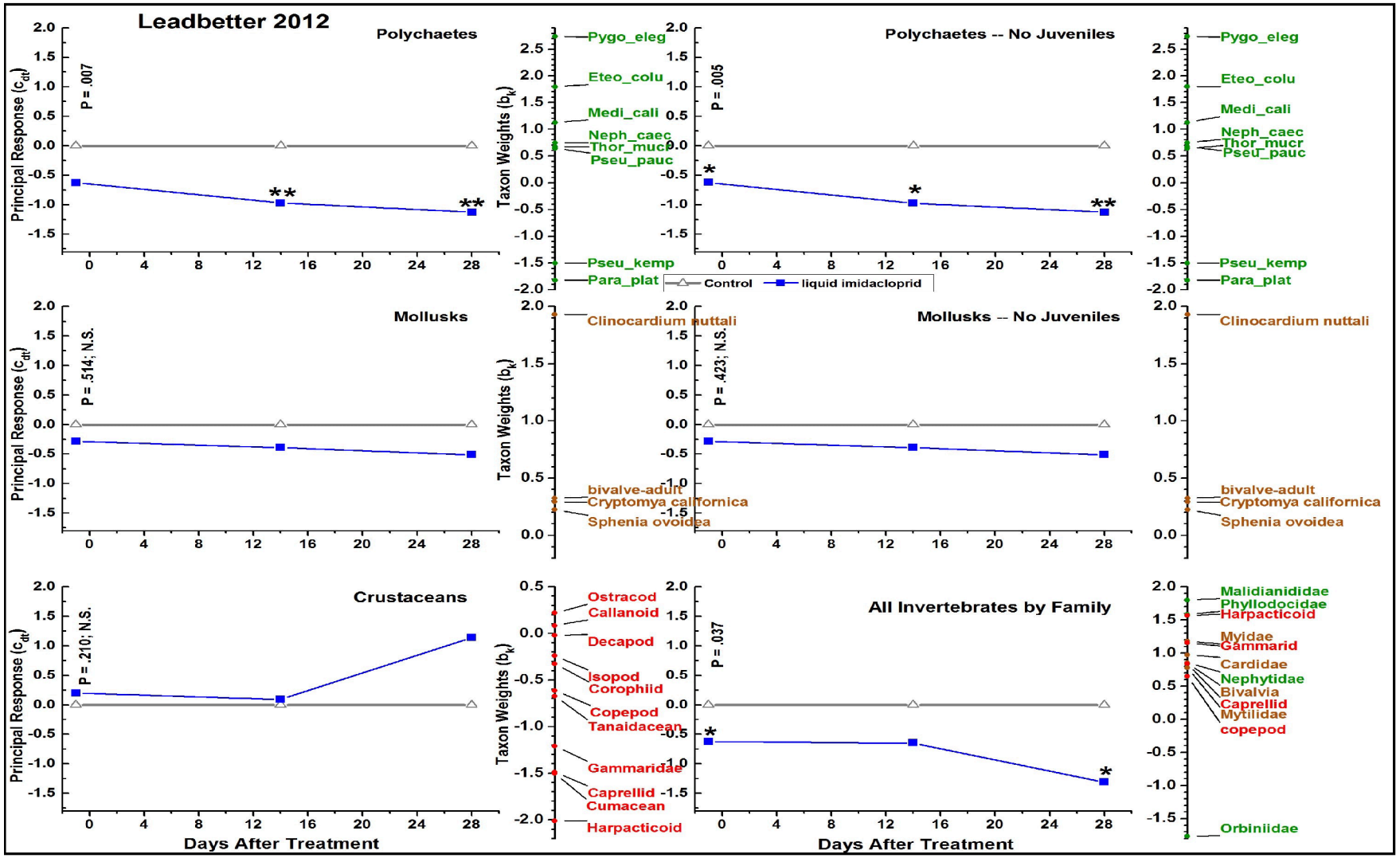

Figure A.10. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Lead Better in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response 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 A.2 lists polychaete full names and abbreviations.

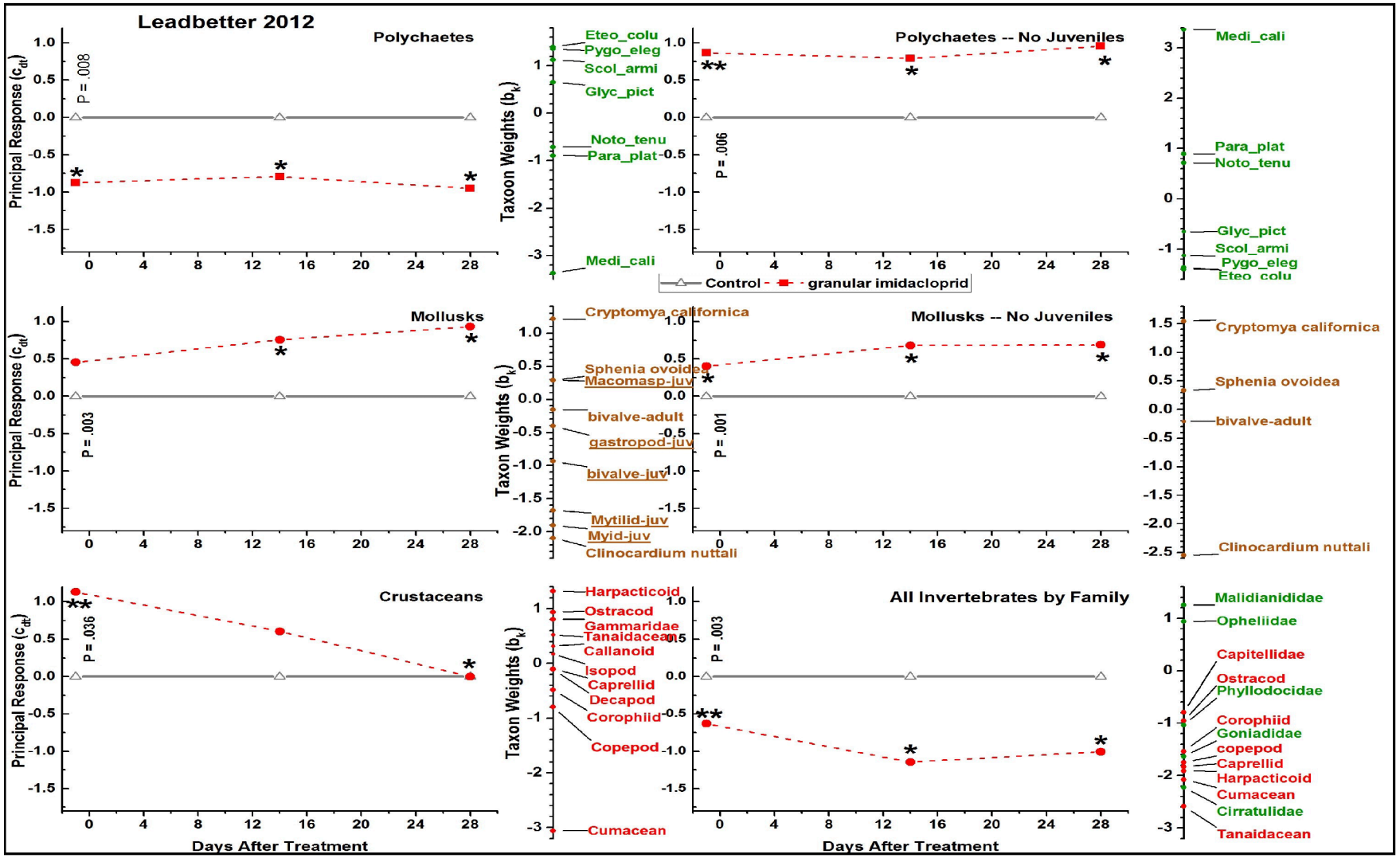

Figure A.11. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at granular imidacloprid and control plots at Leadbetter in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response 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 A.2 lists polychaete full names and abbreviations.

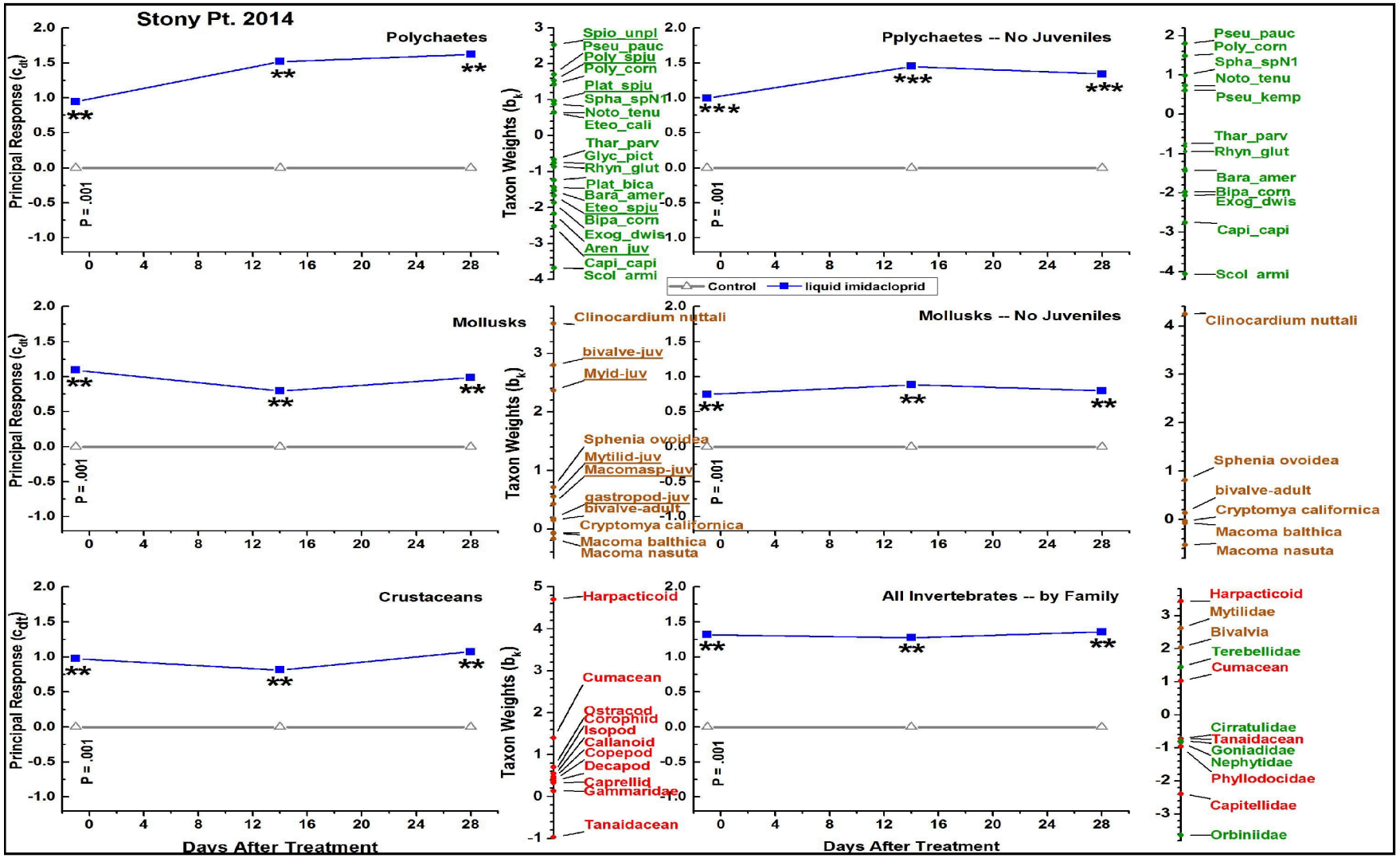

Figure A.12. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Stony Pt in 2014. P is probability that the displayed primary axis is significant. Asterisks indicates the response 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 A.2 lists polychaete full names and abbreviations.

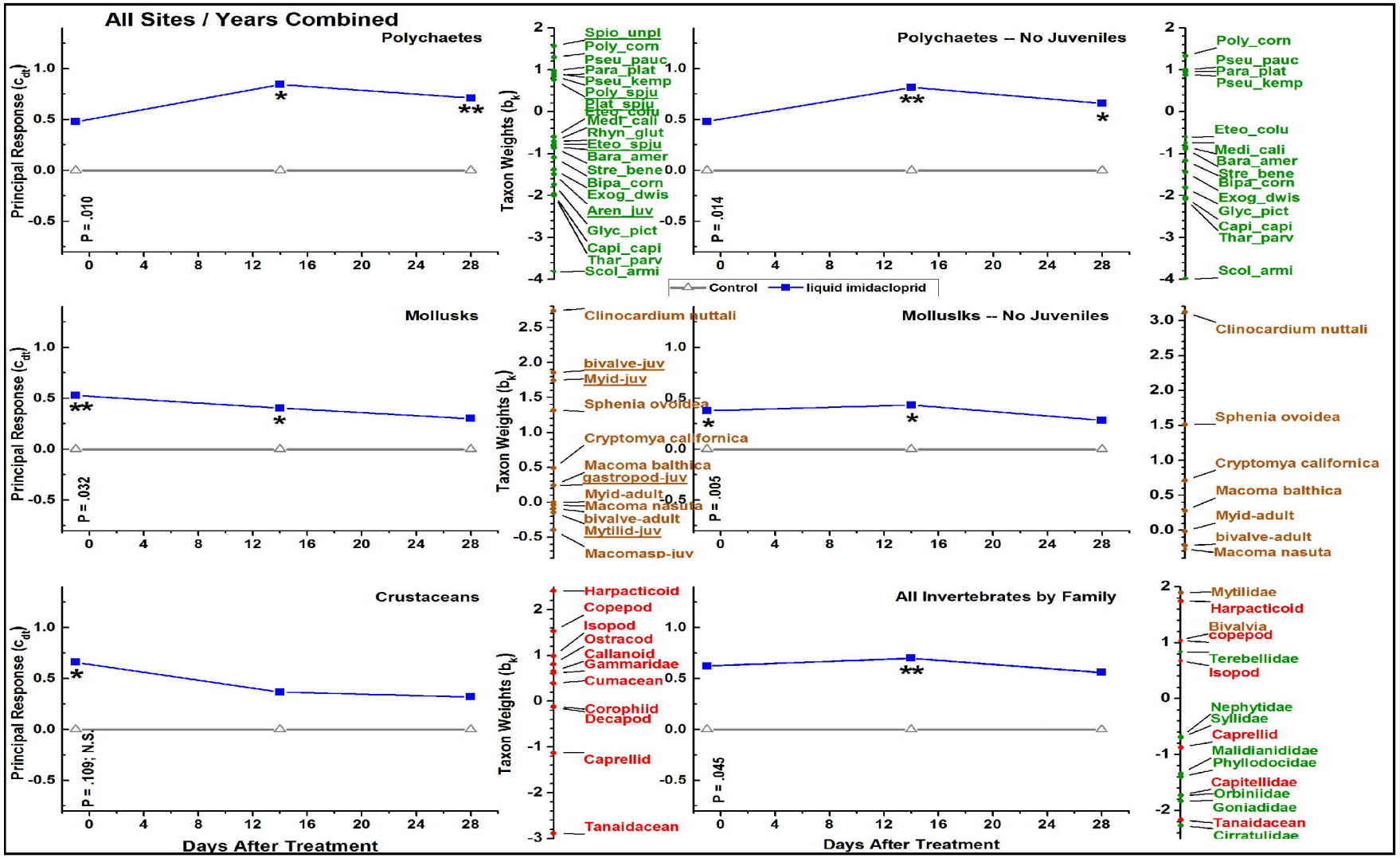

Figure A.13. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots with all sites and years combined. P is probability that the displayed primary axis is significant. Asterisks indicates 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 A.2 lists polychaete full names and abbreviations.

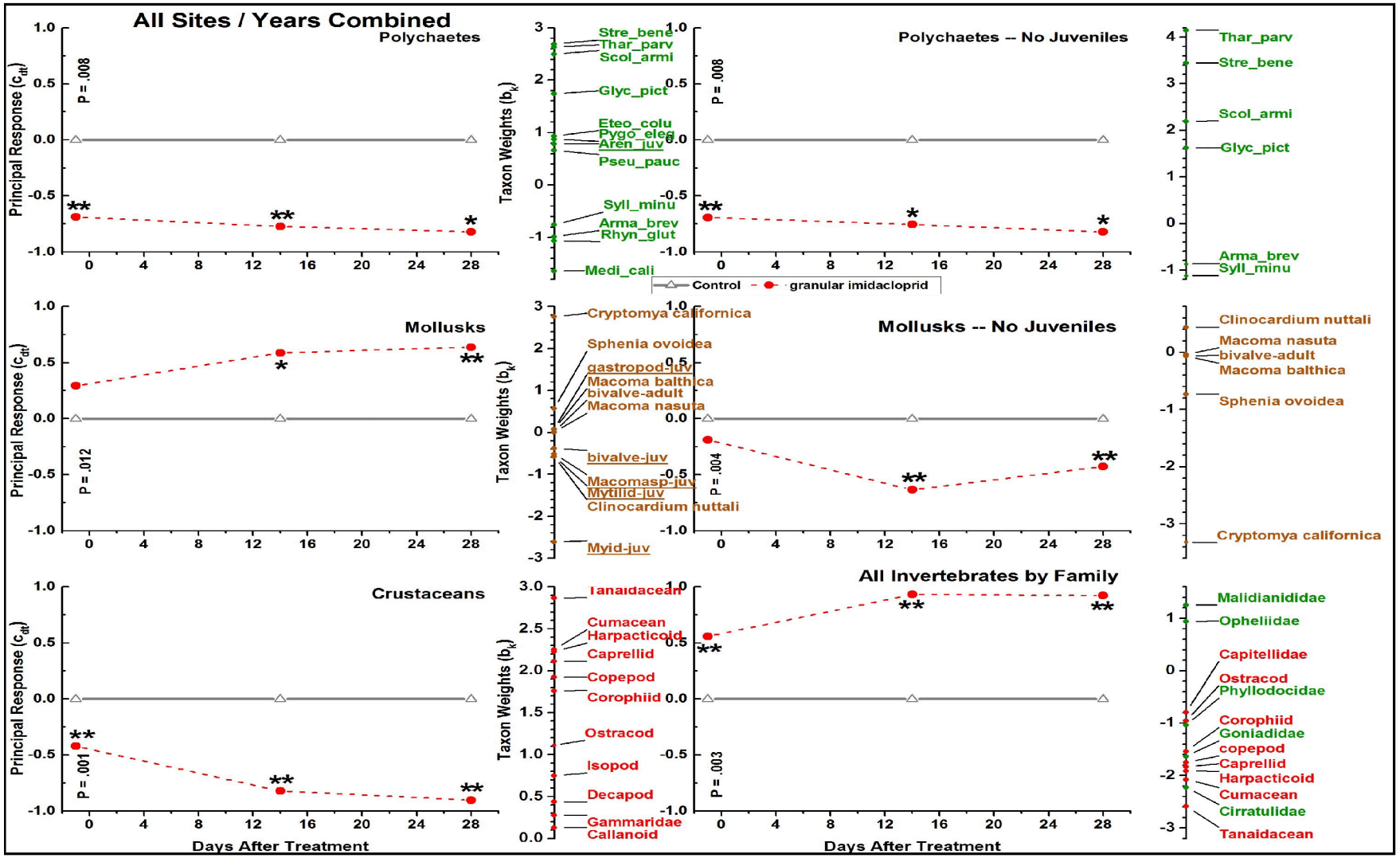

Figure A.14. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at graunular imidacloprid and control plots with all sites and years combined. P is probability that the displayed primary axis is significant. Asterisks indicates 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 A.2 lists polychaete full names and abbreviations.

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.

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