

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

Steven R. Booth¹, Kim Patten² and Leslie New³

¹ Pacific Shellfish Institute, Olympia, WA 98501, ² Washington State University Long Beach Extension Unit, Long Beach WA 98631, ³ Washington State University Vancouver 98686

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 (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 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, acetylcholine (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 Bottrill 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 least 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 the 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.

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

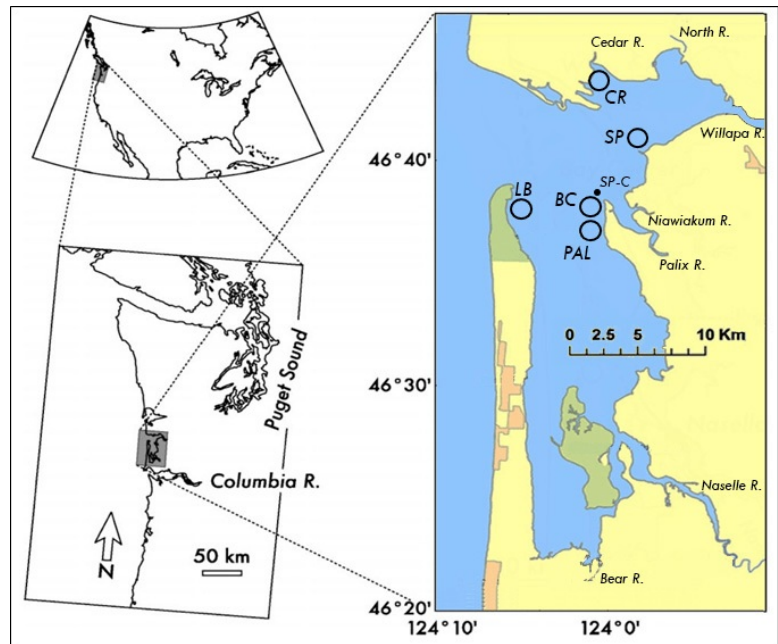


Figure 1. Willapa Bay, WA study sites: Cedar River (CR - 2011), Stony Pt. (SP - 2014), Stony Pt. Control (SP-C - 2014), Bay Center (BC - 2011), Leadbetter (LB - 2012), Palix (PAL - 2012).

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 \times 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 \times 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 \times 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.

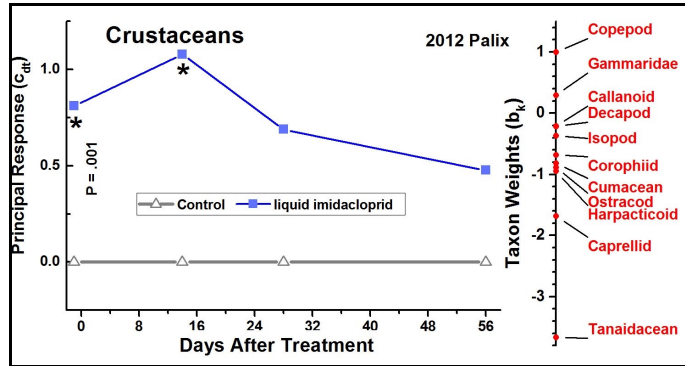


Figure 2. Principal Response Curve of crustaceans before and after treatment with liquid imidacloprid at Palix, 2012. P is probability that the primary axis (response) is significant. Asterisk (*) indicates the response at each sample date is significantly different from the control ($p < 0.05$). Weights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

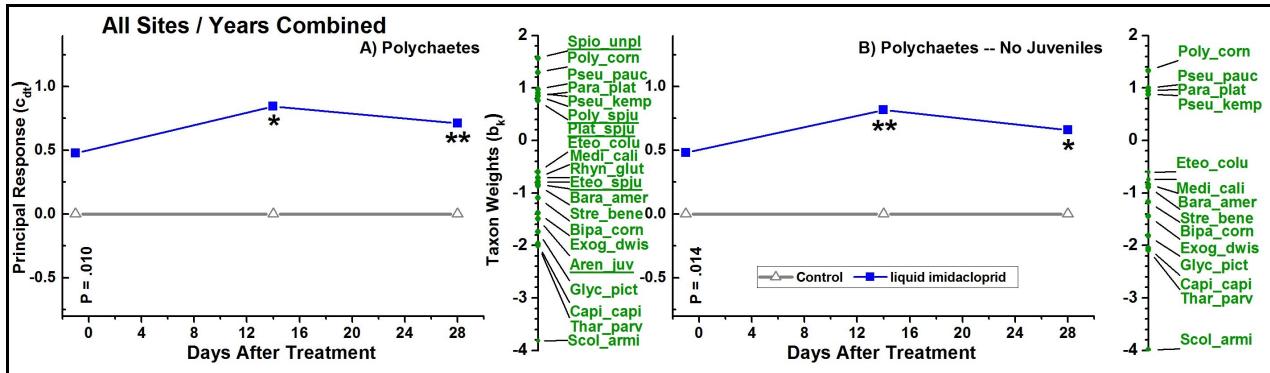


Figure 3. Principal Response Curve of A) all polychaetes (underlined taxa are juveniles) and B) non-juvenile polychaetes before and after treatment with liquid imidacloprid, pooled study sites and years. P is probability that axis 1 (Principal Response) is significant. Asterisks indicate the response 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 > -0.06 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

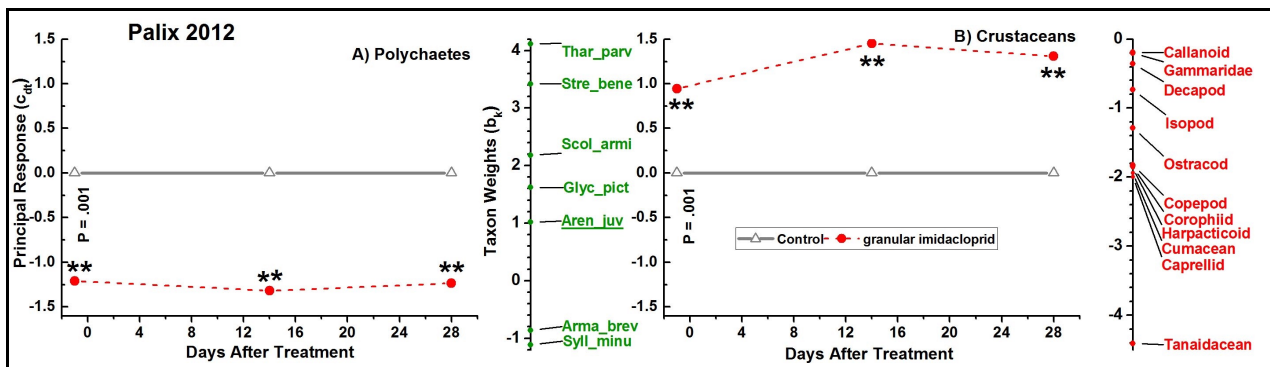


Figure 4. Principal Response Curves of A) Polychaetes (underlined taxa are juveniles) and B) Crustaceans at granular imidacloprid and control plots at Palix in 2012. P is probability that axis 1 (response) is significant. Asterisks (**) indicate the response at each sample date is significantly different from the control ($p < 0.01$). Weights indicate taxa that are positively or negatively correlated with the shape of the curve (polychaete weights > -0.06 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 LC₅₀ values of 10,440 ug/L for water flea (*Daphnia magna*) and 361,230 ug/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. LC₅₀ values were 10 ug/L and 1,112 ug/L for blue crab (*Callinectes sapidus*) megalope and juveniles, respectively (static 24 hr test, Osterberg et al 2012) and were 309 ug/L and 566 ug/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 ug/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 LC₅₀ of 137 ug/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 LC₅₀ of 43 ug/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). LC₅₀ values of carbaryl ranged between 5.6 and 16.4 ug/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 tsunamis 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 (Ferraro 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 <i>Z. japonica</i>	16
		control		4.1	sand	bare	16
	Cedar River	liquid IMI	July 14	2.0	silt	sparse <i>Z. marina</i>	16
		control	July 14	0.9	sand	bare	16
2012	Palix	liquid IMI	August 2	3.4	sand	sparse <i>Z. marina</i>	15
		granular IMI	August 2	3.4	sand /silt	bare	15
		control		3.4	sand	sparse <i>Z. marina</i>	15
	Leadbetter	liquid IMI	August 5	3.2	sand	bare	13
		granular IMI	August 5	2.0	sand	patchy <i>Z. japonica</i>	15
		control		2.4	sand	bare	16
2014	Stony Pt	liquid IMI	July 28	4.0	sand	patchy <i>Z. marina</i>	15
		control		3.6	sand	patchy <i>Z. marina</i>	21

¹ sparse, % cover < 20%; patchy, % cover > 20% and < 1 m² 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 Sedentaria	Order Capitellida
Sub-Class Errantia	Order Orbiniida	Family Arenicolidae (juv). 61
Order Eunicida	Family Orbiniidae	Family Capitellidae
Family Dorvilleidae	Leitoscololos pugettensis. 32	Barantoa nr. americana. 62
Dorvillea annulata. 01	Leitoscoloplos sp. 33	Capitella capitata - complex. 63
Order Phyllodocida	Paraonella platybranchia. 34	Magelona hobsonae 64
Family Polynoidea	Scoloplos armiger. 35	Heteromastus filiformis 65
Harmothoe imbricata. 02	Scoloplos sp. (juv). 36	Notomastus tenuis. 66
Family Goniadidae	Order Sabedellida	Notomastus sp. [juv]. 67
Glycinde picta. 03	Family Sabelidae	Mediomastus californiensis. 68
Glycinde sp. [juv]. 04	Unidentified Sabelid [juv]. 37	Family Maldaninidae
Family Chrysopetalidae	Family Oweniidae	Sabaco elongatus. 69
Paleanotus bellis. 05	Owenia sp. 38	
Family Hesionidae	Order Spionida	Phylum Mollusca
Micropodarke dubia. 06	Family Spionidae	Class Gastropoda
Microphthalmus sp. 07	Dipolydora quadrilobata 39	Unidentified [juv]. 70
Family Nereididae	Polydora cornuta. 40	Class Bivalvia
Neanthes limnicola. 08	Pseudopolydora kempfi. 41	Unidentified [adult]. 71
Neanthes virens. 09	Pseudopolydora pauci-	Unidentified [juv]. 72
Neanthes sp. [juv]. 10	branchiata. 42	Subclass Heterodonta
Nereis vexillosa 11	Pygospio californica. 43	Family Mytilidae
Nereis sp. [juv]. 12	Pygospio elegans. 44	Unidentified Mytilid [juv]. 73
Platynereis bicanaliculata. 13	Rhynchospio glutaea. 45	Family Cardiidae
Platynereis sp. [juv]. 14	Scolelepis squamata. 46	Clinocardium nuttali. 74
Family Syllidae	Scolelepis sp. [juv] 47	Family Myidae
Exogone dwisula. 15	Spionidae unident (post-	Sphenia ovoidea. 75
Exogone sp. 16	larval. 48	Cryptomya californica. 76
Sphaerosyllis californiensis. 17	Spiophanes norrisi 49	Unidentified Myid. 77
Sphaerosyllis sp. N-1. 18	Spiophanes bombyx 50	Unidentified Myid [juv]. 78
Syllides minutes. 19	Spiophanes sp. [juv] 51	Family Tellinidae
Syllides longocirrata. 20	Streblospio benedicti. 52	Macoma balthica. 79
Syllides sp. [juv]. 21	Order Terebellida	Macoma nasuta. 80
Family Nephtyidae	Family Terebellidae	Macoma sp. [juv]. 81
Nephtys caeca. 22	Poecyrrus sp. 53	Unidentified Tellinid. 82
Nephtys cornuta. 23	Unidentified Terebellid. 54	
Nephtys sp. unident. (juv). 24	Order Cirratulida	Phylum Arthropoda
Bipalponephtys cornuta. 25	Family Cirratulidae	Sub Phylum Crustacea
Family Phyllodocidae	Tharyx parvus. 55	Class Copepoda
Eumida longicornuta. 26	Order Opheliida	Order Calanoida. 83
Eteone californica. 27	Family Opheliidae	Order Harpacticoida. 84
Eteone fauchaldia. 28	Polycirrus sp. 56	Order Cyclopoida 85
Eteone sp. (juv). 29	Armandia brevis. 57	Unidentified copepod. 86
Phyllodoce hartmanae. 30	Ophelia limacina 58	Class Ostracoda
Phyllodoce sp. [juv]. 31	Thorocophelai mucronata. 59	Order Ostracoda. 87
	Unidentified Ophelid [juv] 60	Class Malacostraca
		Order Cumacea. 88
		Order Tanaidacea. 89
		Order Isopoda. 90
		Order Amphipoda
		Suborder Gammaridea. 91
		Suborder Corophidea
		Infraorder Caprellida. 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	Family Phyllodocidae	
Dorv_annu 01	Eumi_long 26	Spio_bomb 50
Order Phyllodocida	Eteo_cali 27	Spio_spju 51
Family Polynoidea	Eteo_fauc 28	Streb_bene 52
Harm_imbri 02	Eteo_spju 29	Order Terebellida
Family Goniadidae	Phyl_hart 30	Family Terebellidae
Glyc_pict 03	Phyl_spju 31	Poly_sp 53
Glyci_spju 04		Unid_Tere 54
Family Chrysopetalidae	Sub-Class Sedentaria	Order Cirratulida
Pale_bell 05	Order Orbiniida	Family Cirratulidae
Family Hesionidae	Family Orbiniidae	Thar_parv 55
Micro_dubi 06	Leit_puge 32	Order Opheliida
Micro_sp 07	Leit_sp 33	Family Opheliidae
Family Nereididae	Para_plat 34	Poly_sp 56
Nean_limn 08	Scol_armi 35	Arma_brev 57
Nean_spju 09	Scol_spju 36	Ophe_lima 58
Nere_vexl 11	Order Sabedellida	Thor_mucr 59
Nere_spju 12	Family Sabelidae	Unid_Ophe 60
Plat_bica 13	Unid_Sabe 37	Order Capitellida
Platy_sp 14	Family Oweniidae	Aren_juv 61
Family Syllidae	Owen_sp 38	Family Capitellidae
Exog_dwis 15	Order Spionida	Bara_amer 62
Exog_sp 16	Family Spionidae	Capit_capi 63
Spha_cali 17	Dipo_quad 39	Mage_hobs 64
Spha_N-1 18	Poly_corn 40	Hete_fili 65
Sylli_minu 19	Pseu_kemp 41	Noto_tenu 66
Sylli_long 20	Pseu_pauc 42	Noto_spju 67
Sylli_spju 21	Pygo_cali 43	Medi_cali 68
Family Nephtyidae	Pygo_eleg 44	Family Maldaninidae
Neph_caec 22	Rhyn_glut 45	Saba_elon 69
Neph_corn 23	Scol_squa 46	
Neph_unid 24	Scol_spju 47	
Bipa_corn 25	Spio_unid 48	
	Spio_norr 49	

Table A.3. Percentage variance partitioned by RDA and Monte-Carlo permutation F tests for significance of primary axis (axis 1).

Year	Site	Formulation	Metric	% Var. Attributed to:			% Trt. Var. Captured by axis 1	PRC Permutation Test Statistics		
				Time ¹	Treatment ²	Residual ³		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; partialled out of PRC diagram

² Constrained Variation; 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			
			2011	CR	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			
2012	PX	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 ($\bar{x} \pm S.E.$, 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 simultaneously 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 negative¹, positive², or neutral³ 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.

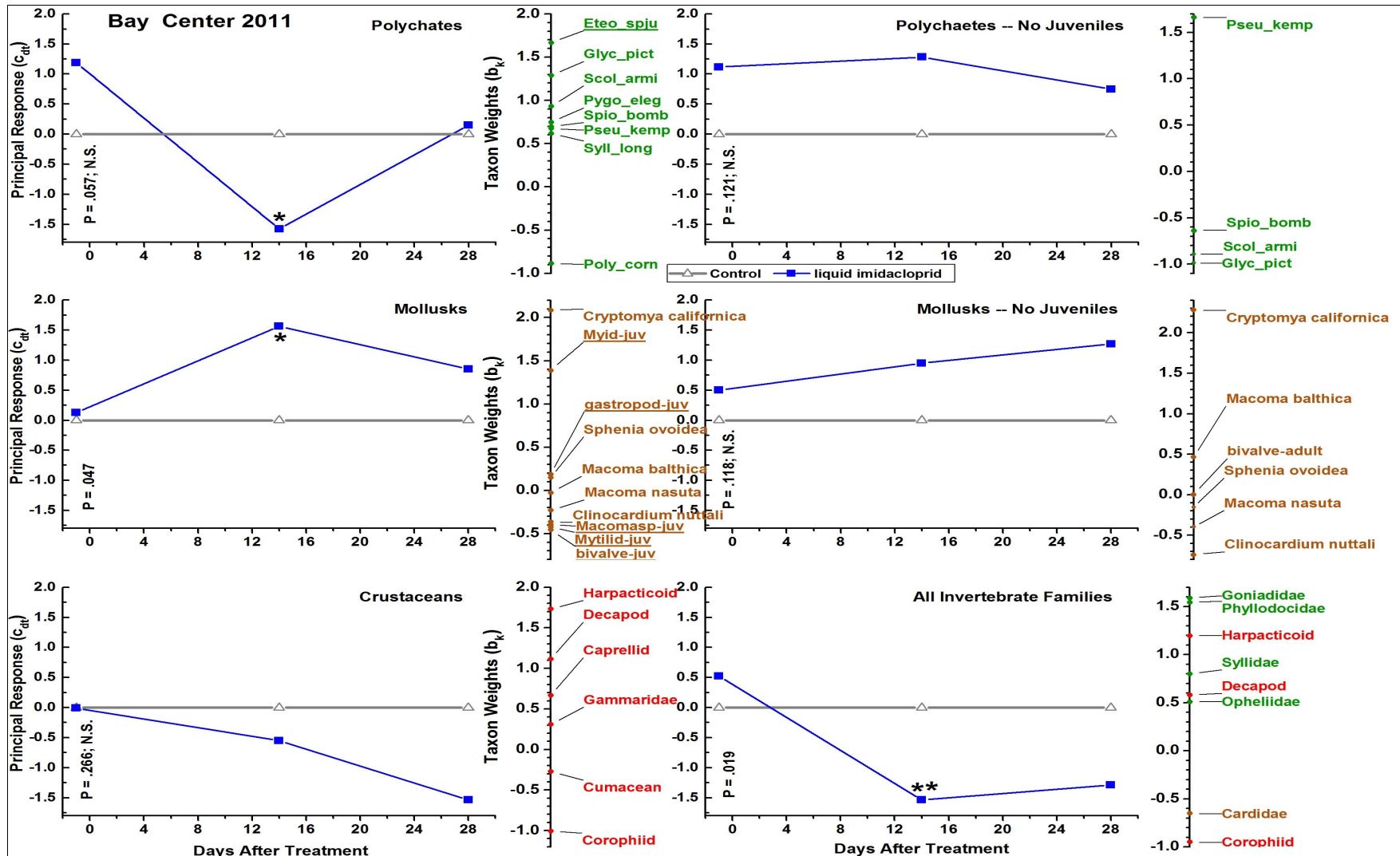


Figure A.5. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Bay Center in 2011. P is probability that the displayed primary axis is significant. Asterisks indicates the response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

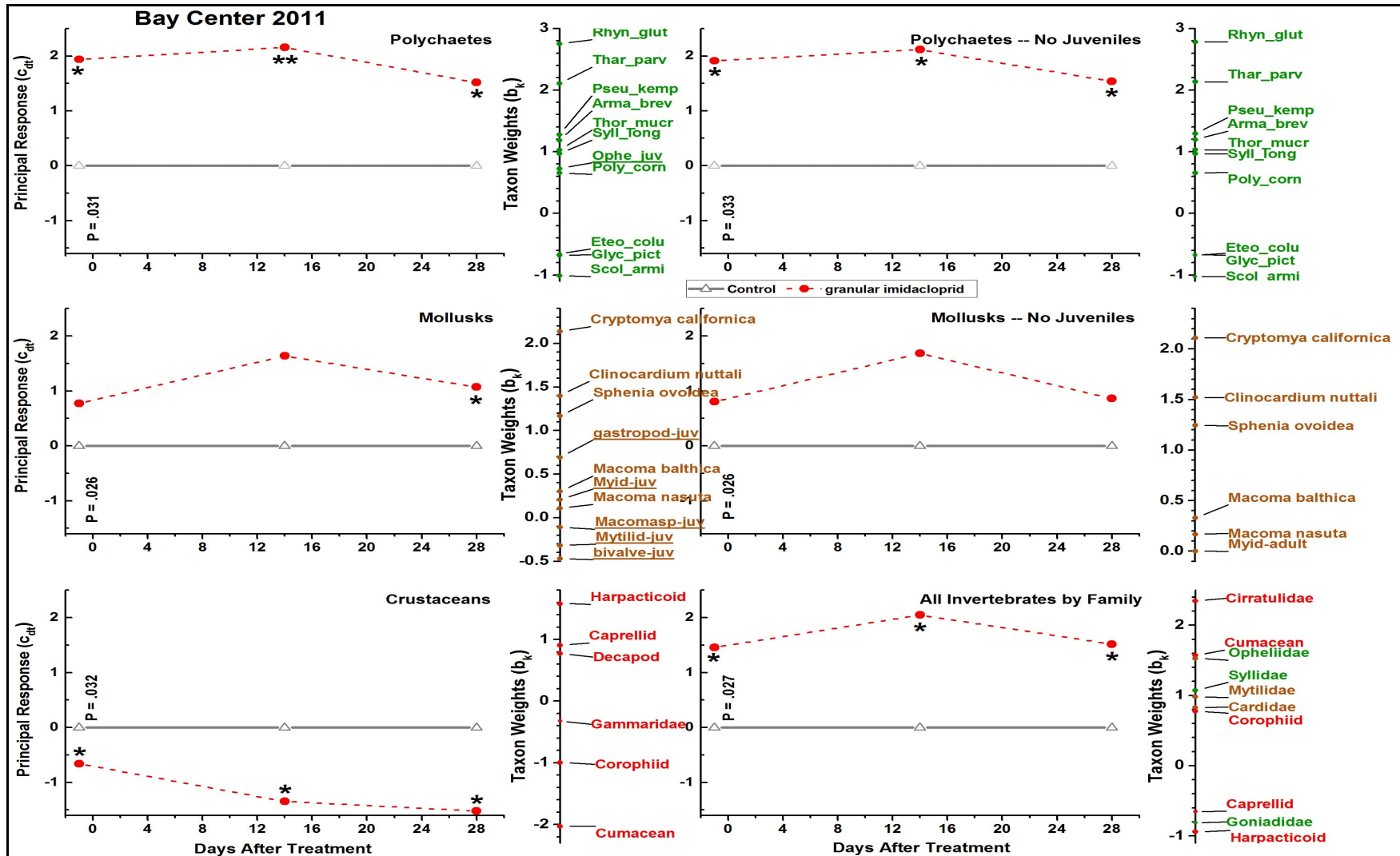


Figure A.6. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at granular imidacloprid and control plots at Bay Center in 2011. P is probability that the displayed primary axis is significant. Asterisks indicates the response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

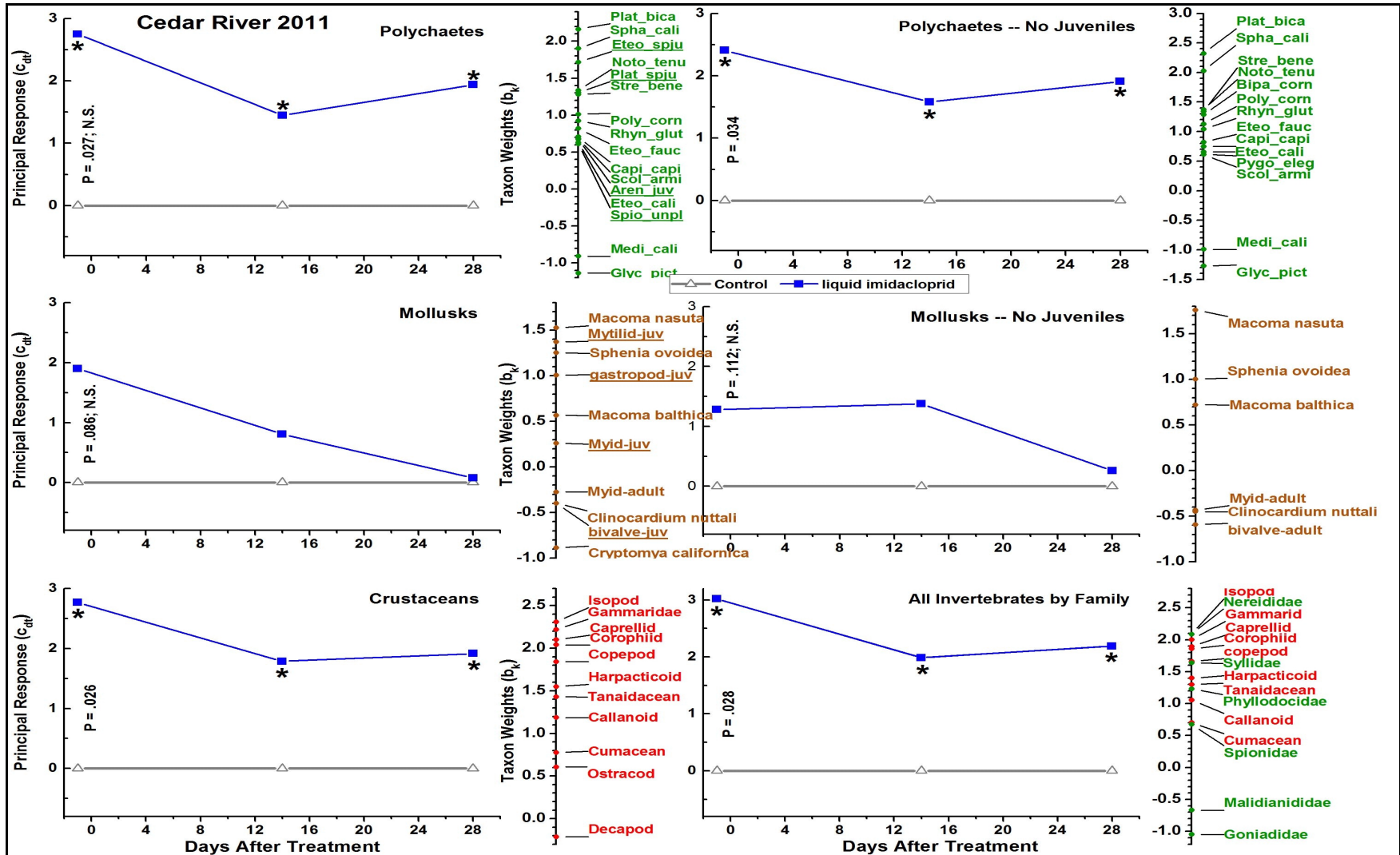


Figure A.7. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Cedar River in 2011. P is probability that the displayed primary axis is significant. Asterisks indicates the response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

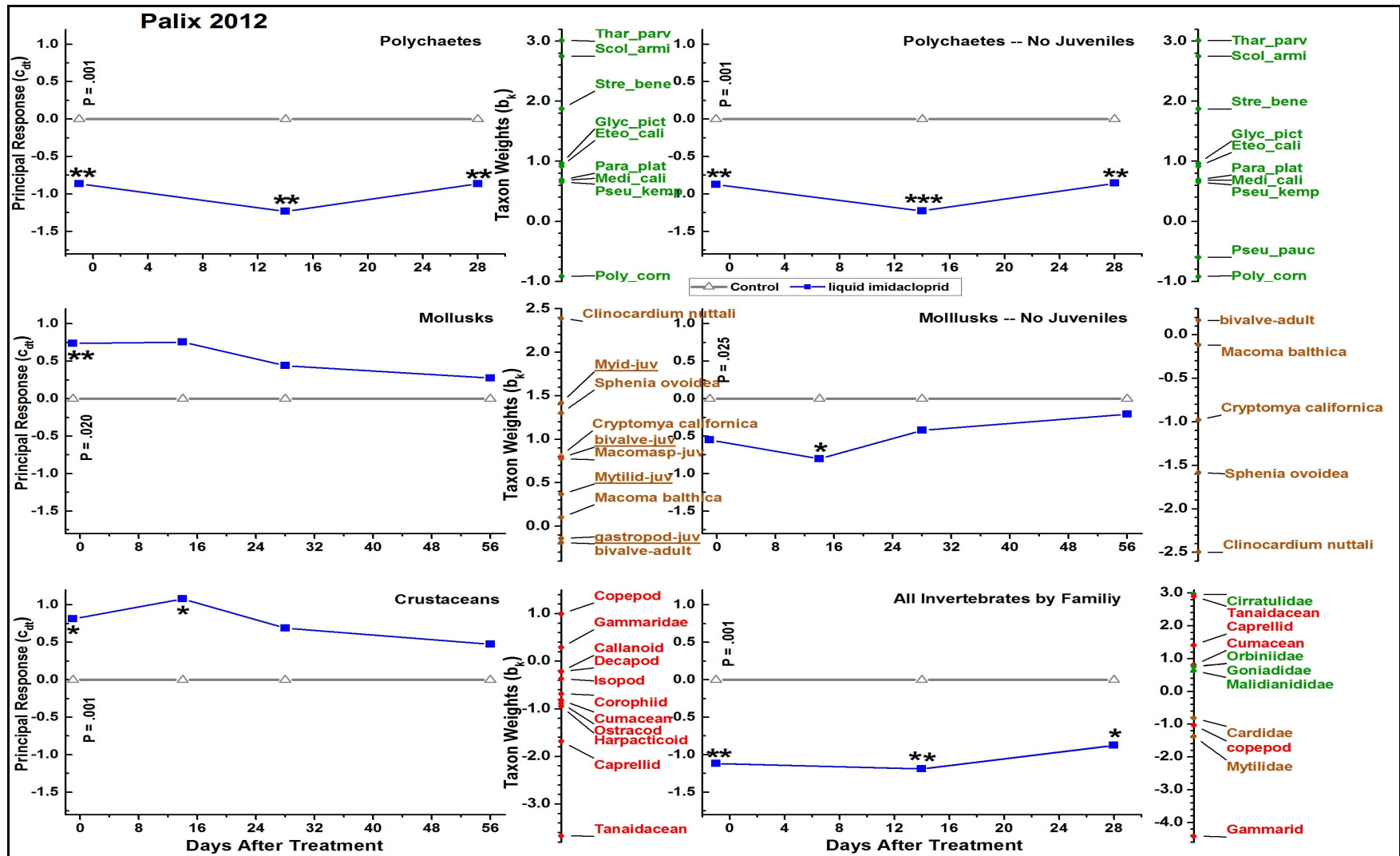


Figure A.8. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Palix in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response at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

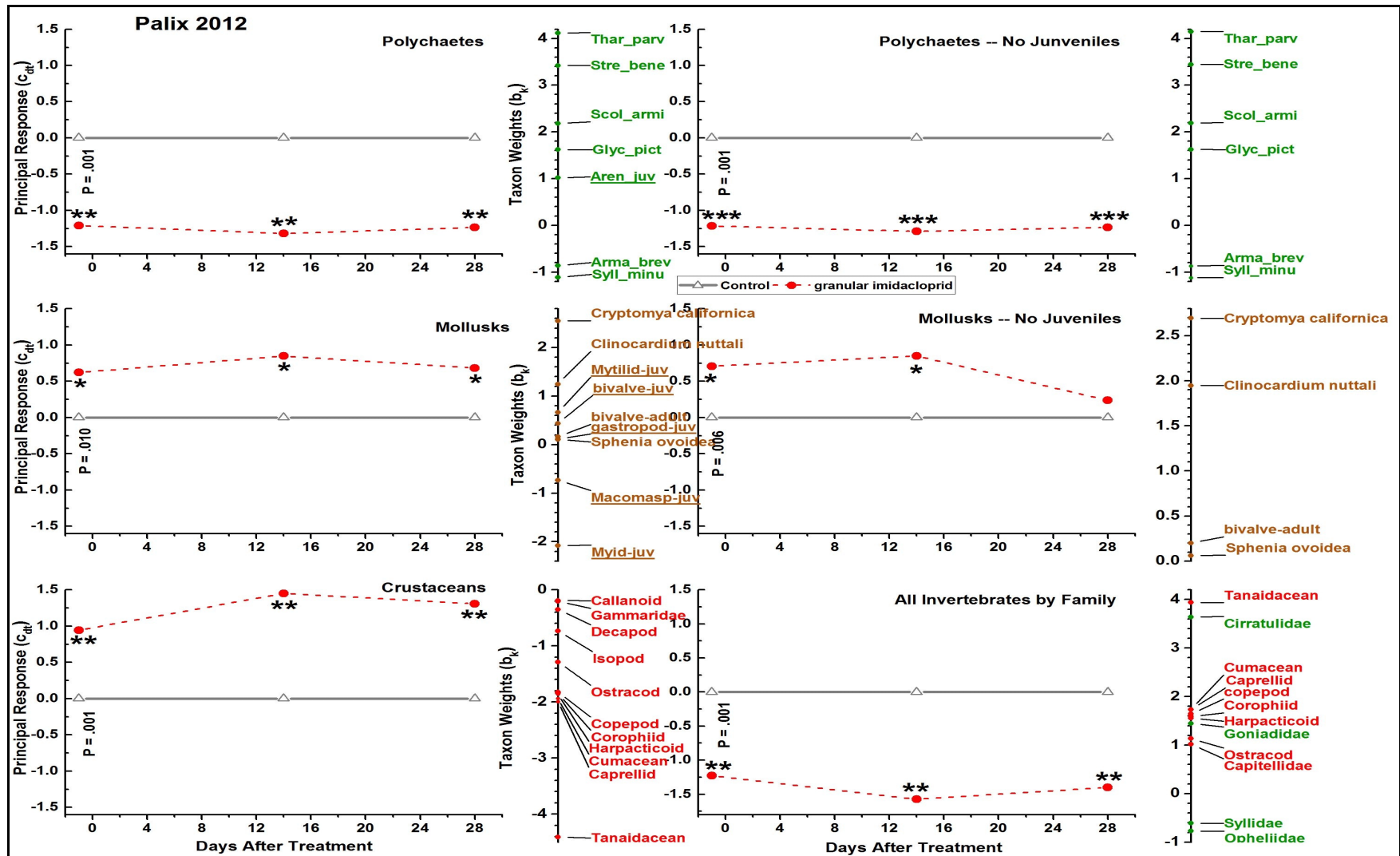


Figure A.9. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at granular imidacloprid and control plots at Palix in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response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

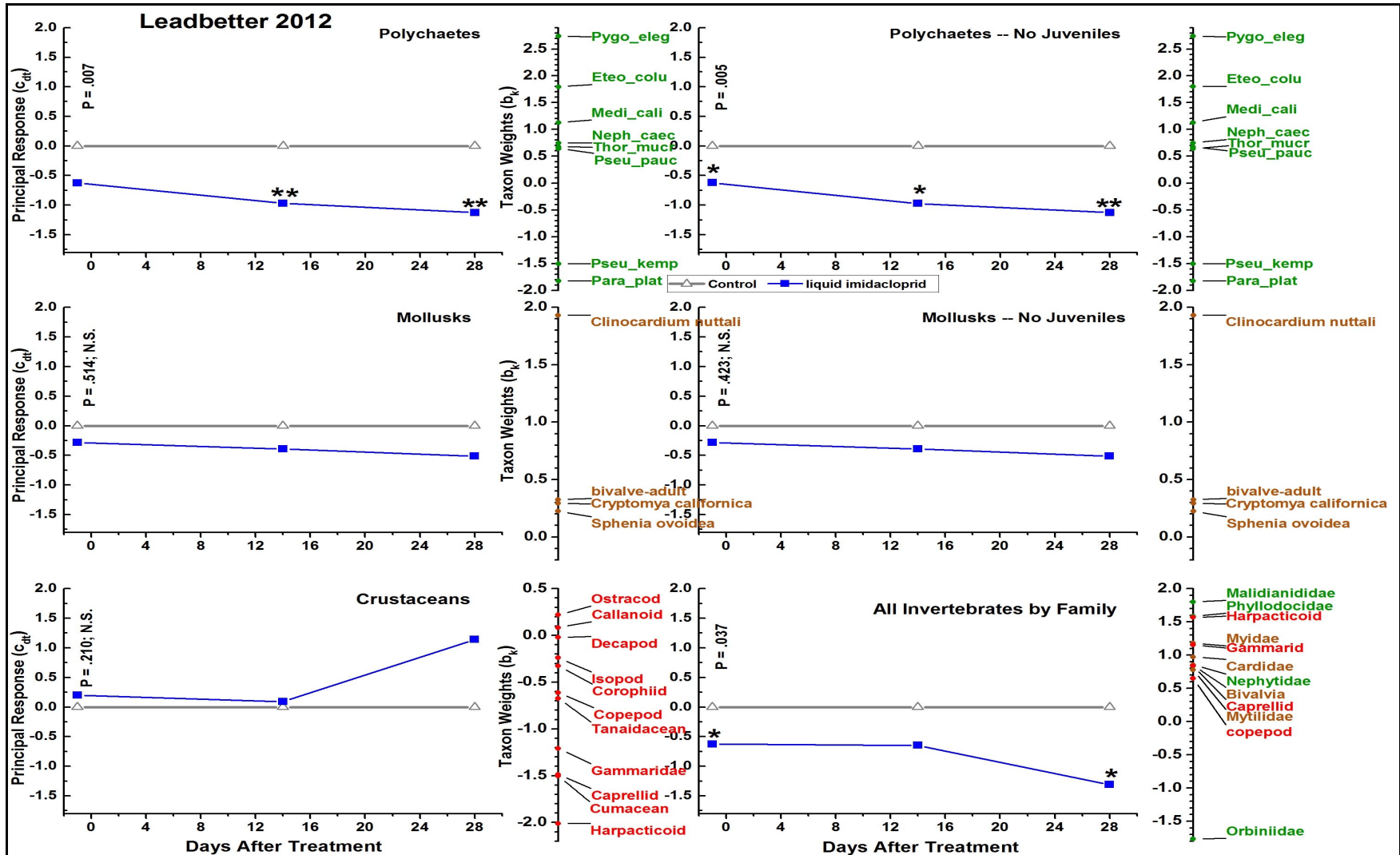


Figure A.10. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Lead Better in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response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

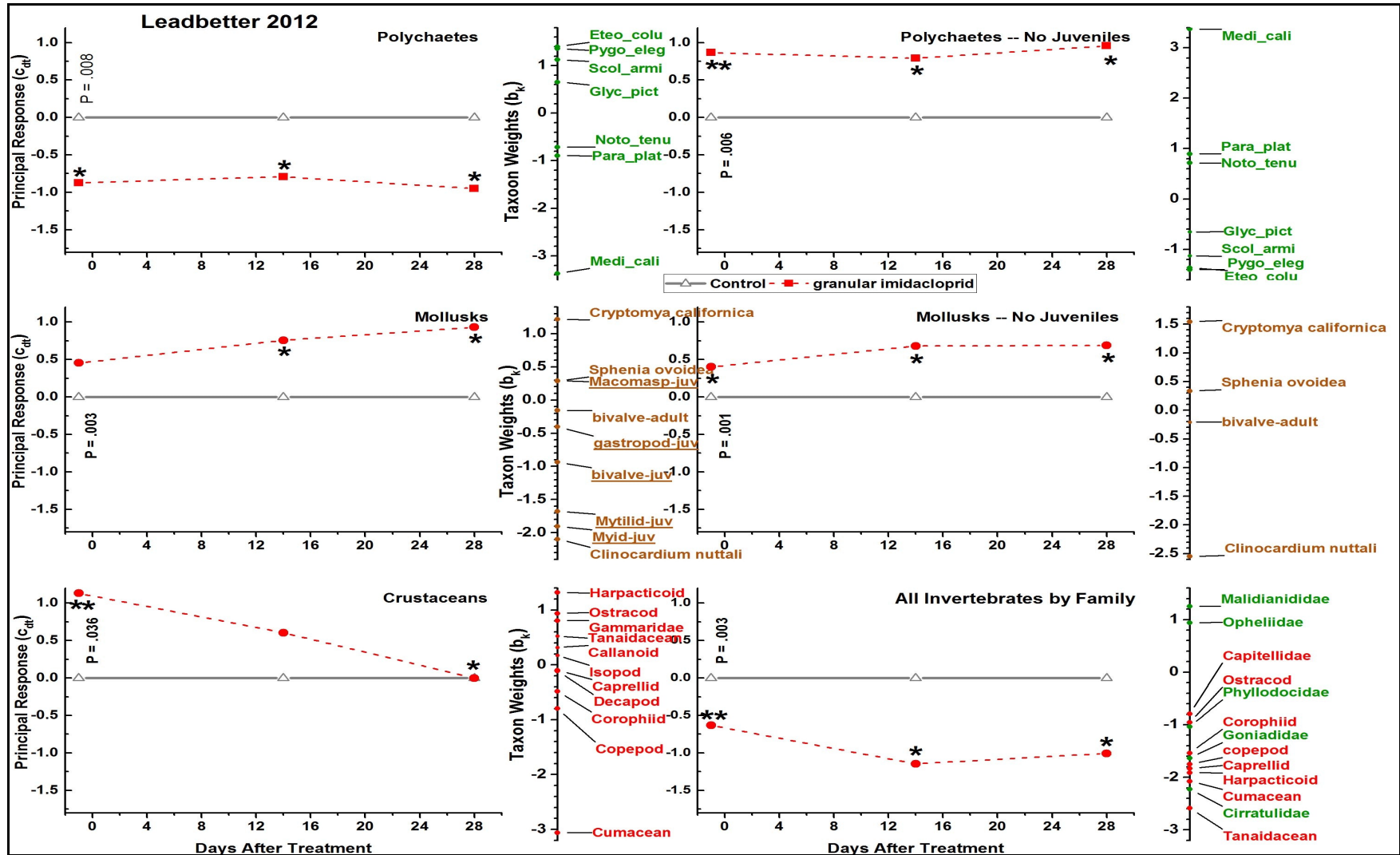


Figure A.11. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at granular imidacloprid and control plots at Leadbetter in 2012. P is probability that the displayed primary axis is significant. Asterisks indicates the response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

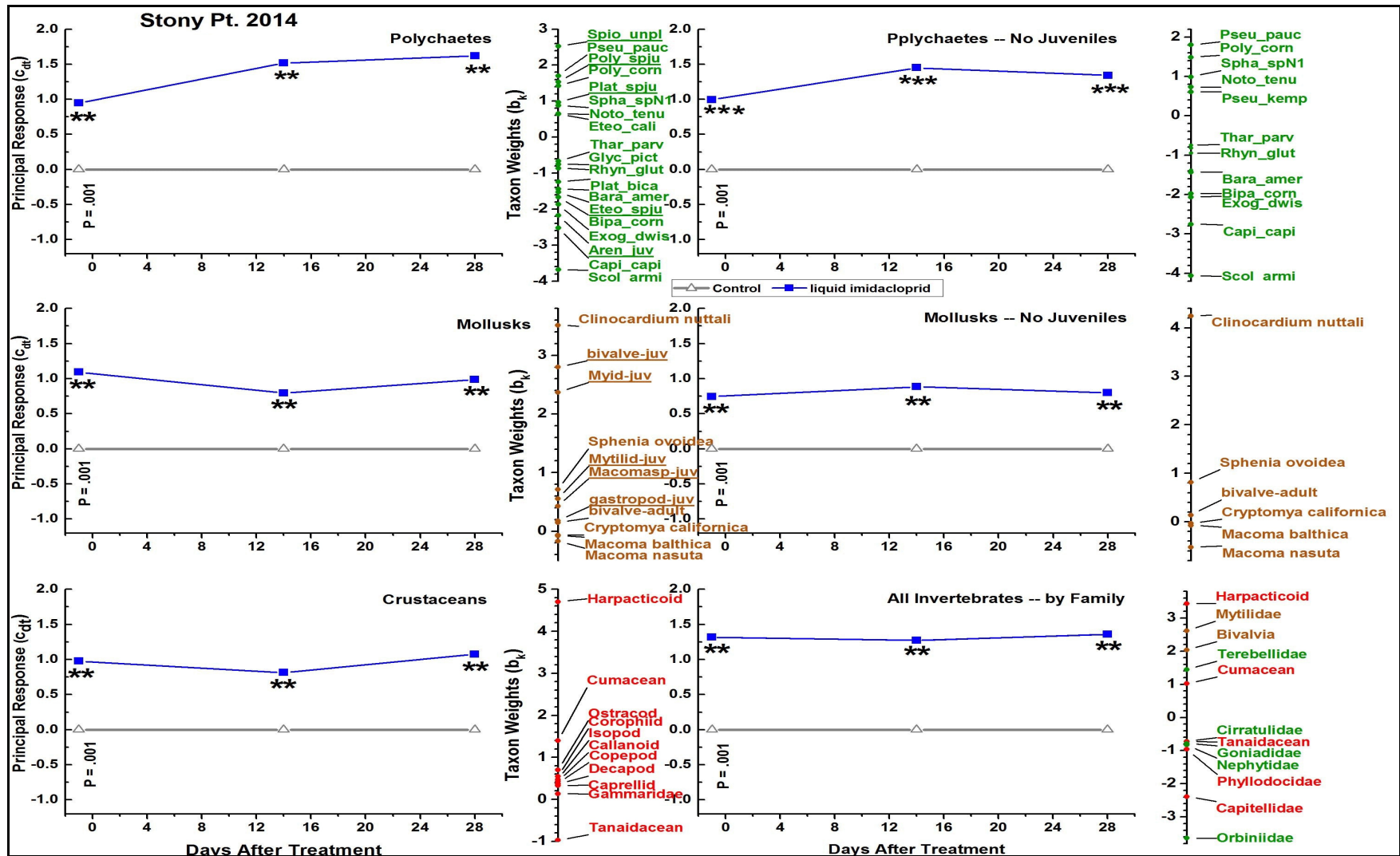


Figure A.12. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots at Stony Pt in 2014. P is probability that the displayed primary axis is significant. Asterisks indicates the response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

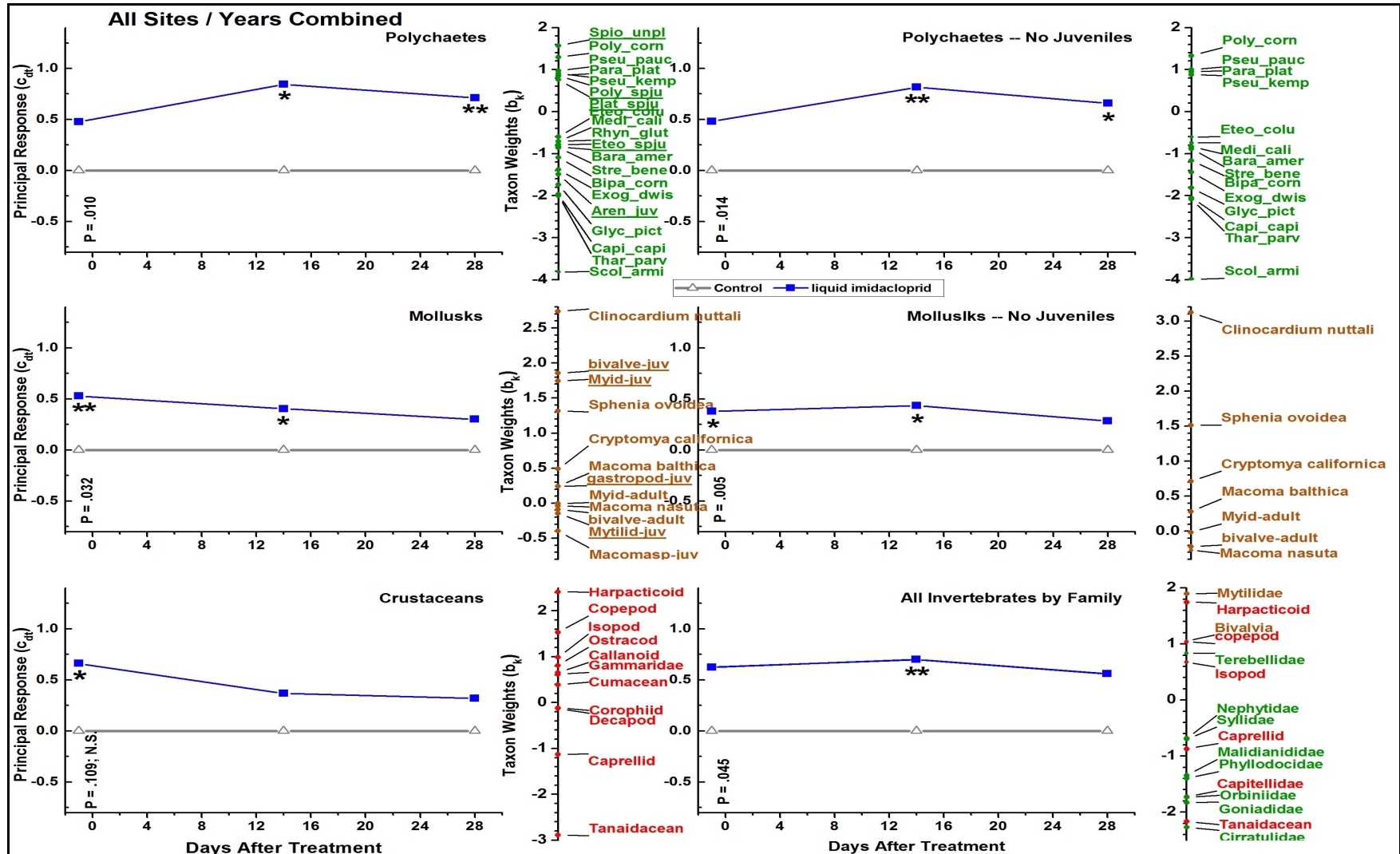


Figure A.13. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at liquid imidacloprid and control plots with all sites and years combined. P is probability that the displayed primary axis is significant. Asterisks indicates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

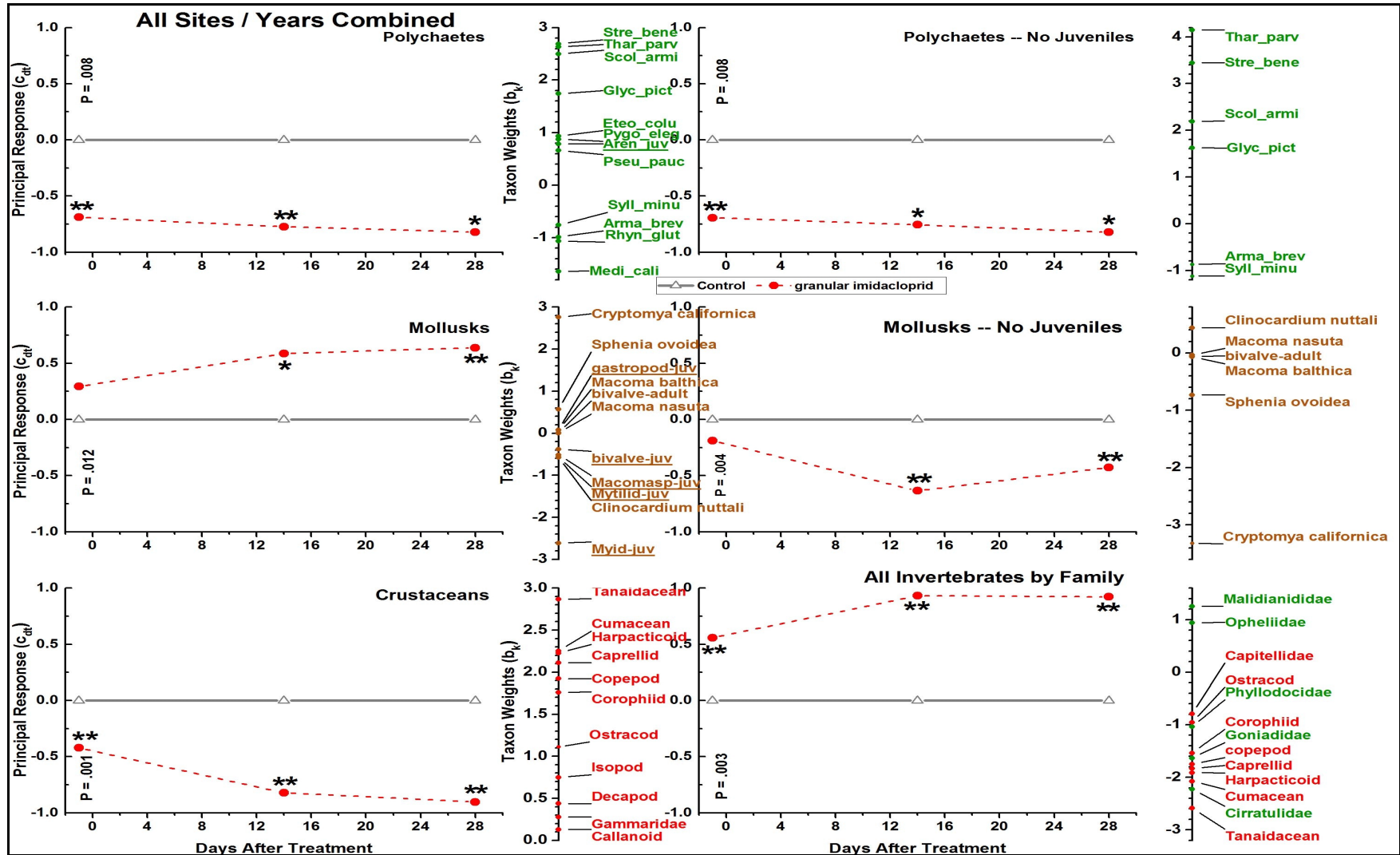


Figure A.14. Principal Response Curve of polychaetes (green labels), mollusks (brown labels), crustaceans (red labels) and all groups combined at granular imidacloprid and control plots with all sites and years combined. P is probability that the displayed primary axis is significant. Asterisks indicates 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 < -0.06 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.

References

- Anatra-Cordone, M. and P. Durkin. 2005. Imidacloprid - Human Health and Ecological Risk Assessment– Final Report. Prepared for USDA, Forest Service, Forest Health Protection, GSA Contract No. 10F-0082K, USDA Forest Service BPA: WO-01-3187-0150, USDA Purchase Order No.: 43-1387-4-3131, Task No. 24. Submitted by Syracuse Environmental Research Associates, Inc., 5100 Highbridge St., 42C, Fayetteville, New York 13066-0950.
- Barahona, M.V. and S. Sanchez-Fortun. 1999. Toxicity of carbamates to the brine shrimp *Artemia salina* and the effect of atropine, BW284c51, iso-OMPA and 2-PAM on carbaryl toxicity. *Environmental Pollution*, 104(3), pp.469-476.
- Bakalian, A. B. 1985. The use of Sevin on estuarine oyster beds in Tillamook Bay, Oregon. *Coastal Zone Management Journal* 13, pp.49–83.
- Banas, N.S., Hickey, B.M., MacCready, P. and J.A. Newton. 2004. Dynamics of Willapa Bay, Washington: A highly unsteady, partially mixed estuary. *Journal of Physical Oceanography*, 34(11), pp.2413-2427.
- Beukema, J.J., Cadée, G.C. and R. Dekker. 2002. Zoobenthic biomass limited by phytoplankton abundance: evidence from parallel changes in two long-term data series in the Wadden Sea. *Journal of Sea Research*, 48(2), pp.111-125.
- Booth, S.R., Patten, K. and A. Suhrbier. 2011. Field trials of imidacloprid on burrowing shrimp, 2009. Report from WGHOGA and PSI to Washington State Legislature 8 pp.
- Booth, S.R. and K. Rassmussen. 2011. Impact of imidacloprid on epi-benthic and benthic invertebrates: Initial studies to describe the Sediment Impact Zone (SIZ) related to imidacloprid treatments to manage burrowing shrimp. Report from PSI to WSU. 11 pp.
- Booth, S.R. and K. Rassmussen. 2013. Impact of imidacloprid on epi-benthic and benthic invertebrates: 2011 studies to describe the Sediment Impact Zone (SIZ) related to imidacloprid treatments to manage burrowing shrimp. Report from PSI to WSU. 27 pp.
- Booth, S.R., Patten, K., Hudson, B., and A. Suhrbier. 2015. Impact of imidacloprid on epi-benthic and benthic invertebrates: 2014 studies to describe the Sediment Impact Zone (SIZ) related to imidacloprid treatments to manage burrowing shrimp. Report to WDFW. 23 pp.
- Borcard, D., Legendre, P., and P. Drapeau, P. 1992. Partialling out the spatial component of ecological variation. *Ecology*, 73, pp.1045–1055.
- Brooks, K. M. 1993. Changes in arthropod and mollusk populations associated with the application of Sevin to control burrowing shrimp in Willapa Bay, July to September, 1992. Report prepared for Pacific County Economic Development Council, Aquatic Environmental Sciences, Port Townsend, Washington.
- Brooks, K.M. 1995. Long-term response of benthic invertebrate communities associated with the application of carbaryl (Sevin™) to control burrowing shrimp, and an assessment of the habitat value of cultivated Pacific oyster (*Crassostrea gigas*) beds in Willapa Bay, Washington to fulfill requirements of the EPA carbaryl data call in. US Environmental Protection Agency, Region X.
- California Rice Commission, 2014. List of Pesticides used on California Rice. (<http://www.omicnet.com/reports/rice/ListOfPesticidesUsedOnCaliforniaRice.pdf>).
- Chung, K.W., Chandler, A.R. and P.B. Key. 2008. Toxicity of carbaryl, diquat dibromide, and fluoranthene, individually and in mixture, to larval grass shrimp, *Palaemonetes pugio*. *Journal of Environmental Science and Health Part B*, 43(4), pp. 293-299.
- Colville, A., Jones, P., Pablo, F., Krasso, F., Hose, G. and R. Lim. 2008. Effects of chlorpyrifos on macroinvertebrate communities in coastal stream mesocosms. *Ecotoxicology*, 17(3), pp. 173-180.

- Cuppen, J.G., Van den Brink, P.J., Camps, E., Uil, K.F. and T.C. Brock. 2000. Impact of the fungicide carbendazim in freshwater microcosms. I. Water quality, breakdown of particulate organic matter and responses of macroinvertebrates. *Aquatic toxicology*, 48(2), pp.233-250.
- Dumbauld, B.R. 1994. Thalassinid shrimp ecology and the use of carbaryl to control populations on oyster ground in Washington Coastal Estuaries. Ph.D. Dissertation, School of Fisheries, Univ. of Wash., Seattle, WA pp 192.
- Dumbauld, B.R., Booth, S., Cheney, D., Suhrbier, A. and H. Beltran. 2006. An integrated pest management program for burrowing shrimp control in oyster aquaculture. *Aquaculture*, 261(3), pp. 976-992.
- Dumbauld, B.R., Ruesink, J.L., and S.S. Rumrill. 200r. Response of an estuarine benthic community to application of the pesticide carbaryl and cultivation of Pacific oysters (*Crassostrea gigas*) in Willapa Bay, Washington. *Aquaculture*, 290, 196-223.
- Dumbauld, B.R., Brooks, K.M. and M.H. Posey. 2001. The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries. *Marine Pollution Bulletin*, 10.42, pp. 826-844.
- Ehler, L.E. and D.G. Bottrell. 2000. The illusion of integrated pest management. *Issues in Science and Technology Online*. Spring, 2000. 6 pp.
- Ellis, J.D., Evans, J.D. and J. Pettis. 2010. Colony losses, managed colony population decline, and Colony Collapse Disorder in the United States. *Journal of Apicultural Research*, 49(1), pp.134-136.
- Emmett, R., Llansó, R., Newton, J., Thom, R., Hornberger, M., Morgan, C., Levings, C., Copping, A. and P. Fishman. 2000. Geographic signatures of North American west coast estuaries. *Estuaries*, 23(6), pp. 765-792.
- Feldman, K.L., Armstrong, D.A., Dumbauld, B.R., DeWitt, T.H. and D.C. Doty. 2000. Oysters, crabs, and burrowing shrimp: review of an environmental conflict over aquatic resources and pesticide use in Washington State's (USA) coastal estuaries. *Estuaries*, 23(2), pp.141-176.
- Ferraro, S.P. and F.A. Cole. 2007. Benthic macrofauna–habitat associations in Willapa Bay, Washington, USA. *Estuarine, Coastal and Shelf Science*, 71(3), pp.491-507.
- Ferraro, S.P. and F.A. Cole. 2012. Ecological periodic tables for benthic macrofaunal usage of estuarine habitats: insights from a case study in Tillamook Bay, Oregon, USA. *Estuarine, Coastal and Shelf Science*, 102, pp.70-83.
- Gauch, H.G., Jr. 1982. *Multivariate analysis in community structure*. Cambridge University Press, Cambridge.
- Gill, R.J., Ramos-Rodriguez, O. and N.E. Raine. 2012. Combined pesticide exposure severely affects individual-and colony-level traits in bees. *Nature*, 491(7422), pp.105-108.
- Goulson, D. 2013. An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology*. 50, pp. 977–987.
- Green, R.H., 1979. *Sampling design and statistical methods for environmental biologists*. John Wiley & Sons.
- Grue, C., Grassley, J.M., and J.A. Frew. 2011. Concentrations of imidacloprid in sediment pore water following application of imidacloprid in Willapa Bay, Washington. Report to WGHOGA. 20 pp.
- Grue, C.E., Grassley, J.M., Frew, J.A., and A. Troiano. 2012. Use of an enzyme--linked immunosorbent assay (ELISA) to quantify imidacloprid in sediment pore water following application of imidacloprid in Willapa Bay, Washington – matrix effects and cross-reactivity. Report to WGHOGA. 13 pp.
- Grue, C. 2013. Survival of ghost shrimp in sediments exposed to imidacloprid in the laboratory: Implications for control of shrimp on oyster beds in Willapa Bay, WA. Presentation to Pacific Coast Shellfish Grower's Association Annual Conference, Sun River, OR. Oct 2, 2013.
- Grue, C. and M. Grassley 2013. 2012 Final report: Environmental fate and persistence of imidacloprid

- following applications to control burrowing shrimp on commercial oyster beds in Willapa Bay, Washington. Report to Willapa Grays Harbor Oyster Growers Association and the Washington Department of Fish and Wildlife in partial fulfillment of WDFW Contract No. 12-1113.
- Jones, C.G., Lawton, J.H., and M. Shachak. 1994. Organisms as ecosystem engineers. *Oikos* 69, pp. 373-386.
- Key, P., Chung, K., Siewicki, T. and M. Fulton. 2007. Toxicity of three pesticides individually and in mixture to larval grass shrimp (*Palaemonetes pugio*). *Ecotoxicology and Environmental Safety*, 68(2), pp.272-277.
- Kuivila, K.M. and M.L. Hladik. 2008. Understanding the occurrence and transport of current-use pesticides in the San Francisco Estuary, Watershed. *Estuary & Watershed*, v. 6, article 2.
- Leonard, A.W., Hyne, R.V., Lim, R.P., Pablo, F. and P.J. Van den Brink. 2000. Riverine endosulfan concentrations in the Namoi River, Australia: Link to cotton field runoff and macroinvertebrate population densities. *Environmental toxicology and chemistry*, 19(6), pp.1540-1551.
- López-Mancisidor, P., Carbonell, G., Fernández, C. and J.V. Tarazona. 2008. Ecological impact of repeated applications of chlorpyrifos on zooplankton community in mesocosms under Mediterranean conditions. *Ecotoxicology*, 17(8), pp.811-825.
- MacGinitie, G. E. 1930. The natural history of the mud shrimp *Upogebia pugettensis* (Dana). *Annals and Magazine of Natural History* 6, pp.37-45.
- MacGinitie, G. E. 1934. The natural history of *Callinassa californiensis* (Dana). *American Midland Naturalist* 15, pp.166-177.
- Maud, S., Biggs, J., Williams, P., Whitfield, M., Sherratt, T., Powley, W., Heneghan, P., Jepson, P. and N. Shillabeer. 2009. The influence of simulated immigration and chemical persistence on recovery of macroinvertebrates from cypermethrin and 3, 4 dichloroaniline exposure in aquatic microcosms. *Pest management science*, 65(6), pp.678-687.
- Mohr, S., Berghahn, R., Schmiediche, R., Hübner, V., Loth, S., Feibicke, M., Mailahn, W. and J. Wogram. 2012. Macroinvertebrate community response to repeated short-term pulses of the insecticide imidacloprid. *Aquatic Toxicology*, 110, pp.25-36.
- Morrissey, C.A., Mineau, P., Devries, J.H., Sanchez-Bayo, F., Liess, M., Cavallaro, M.C. and K. Liber. 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. *Environment International*, 74, pp.291-303.
- Norkko, J., Norkko, A., Thrush, S.F., Valanko, S. and H. Suurkuukka. 2010. Conditional responses to increasing scales of disturbance, and potential implications for threshold dynamics in soft-sediment communities. *Marine Ecology Progress Series*, 413, pp.253-266.

- Osterberg, J.S., Darnell, K.M., Blickley, T.M., Romano, J.A. and D. Rittschof. 2012. Acute toxicity and sub-lethal effects of common pesticides in post-larval and juvenile blue crabs, *Callinectes sapidus*. *Journal of Experimental Marine Biology and Ecology*, 424, pp.5-14.
- Palmer, M. 2016. The ordination web page. Botany Department, Oklahoma State University, Stillwater, OK. <http://ordination.okstate.edu>
- Patten, K. 2006. Screening of alternative methods to manage burrowing shrimp infestations on bivalve shellfish grounds. Report from WSU to Washington State Commission for Pesticide Registration. 6 pp.
- Patten, K. 2015. Efficacy of Imidacloprid against burrowing shrimp, 2014. Report to the Washington Department of Fish and Wildlife.
- Pilditch, C.A., Valanko, S., Norkko, J. and A. Norkko. 2015. Post-settlement dispersal: the neglected link in maintenance of soft-sediment biodiversity. *Biology letters*, 11(2), p.20140795.
- Pisa, L.W., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Downs, C.A., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., McField, M. and C.A. Morrissey. 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. *Environmental Science and Pollution Research*, 22(1), pp.68-102.
- Posey, M.H., Dumbauld, B.R., and D.A. Armstrong. 1991. Effects of a burrowing mud shrimp, *Upogebia pugettensis* (Dana), on abundances of macro-infauna. *Journal of Experimental Marine Biology and Ecology* 148(2), pp. 283-294.
- R Core Team. 2016. A language and environment of statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL <http://www.R-project.org/>.
- Sardo, A.M. and A.M.V.M. Soares. 2010. Assessment of the effects of the pesticide imidacloprid on the behaviour of the aquatic oligochaete *Lumbriculus variegatus*. *Archives of environmental contamination and toxicology*, 58(3), pp.648-656.
- Schäfers, C., Hommen, U., Dembinski, M. and J.F. Gonzalez Valero. 2006. Aquatic macroinvertebrates in the Altes Land, an intensely used orchard region in Germany: correlation between community structure and potential for pesticide exposure. *Environmental toxicology and chemistry*, 25(12), pp.3275-3288.
- Simenstad, C.A. and K.L. Fresh. 1995. Influence of intertidal aquaculture on benthic communities in Pacific Northwest estuaries: scales of disturbance. *Estuaries*, 18(1), pp.43-70.
- Song, M.Y., Stark, J.D. and J.J. Brown. 1997. Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods. *Environmental Toxicology and Chemistry*, 16(12), pp.2494-2500.
- Tomizawa, M. and J.E. Casida. 2003. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annual Review Entomology*. 48, pp.339-64.
- Toumi, H., Burga-Perez, K.F. and J.F. Ferard. 2016. Acute and chronic ecotoxicity of carbaryl with a battery of aquatic bioassays. *Journal of Environmental Science and Health, Part B*, 51(1), pp.57-62.
- Vanblaricom, G.R., Eccles, J.L., Olden, J.D. and P.S. McDonald. 2015. Ecological effects of the harvest phase of geoduck (*Panopea generosa* Gould, 1850) aquaculture on infaunal communities in southern Puget Sound, Washington. *Journal of Shellfish Research*, 34(1), pp.171-187.
- Van den Brink, P.J. and C.J. Ter Braak. 1999. Principal response curves: Analysis of time dependent multivariate responses of biological community to stress. *Environmental Toxicology and Chemistry*, 18(2), pp.138-148.

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

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

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

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