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Assessing impacts of neonicotinoids on aquatic systems in the California central coast region

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Executive Summary

The unintentional transport of pesticides into aquatic habitats has weakened the functioning of freshwater ecosystems along California's central coast. Pesticides can affect non-target taxa, causing disruptions to natural trophic level responses and ecosystem-wide degradation. Neonicotinoids are the most commonly used class of insecticides globally but not much is known about their potential impacts on the form and function of freshwater ecosystems. Additionally, their high environmental mobility and persistence in aquatic environments may make them more of a threat to nontarget ecosystems than originally thought.

Prolonged exposure to neonicotinoids may be highly toxic to benthic macroinvertebrates (BMIs) but how these impacts vary among specific taxa, feeding groups, and across entire BMI assemblages is not well established. Differences in physiological adaptations and feeding strategies may make some BMIs more sensitive than others to prolonged neonicotinoid exposure. BMIs are used statewide as bioindicators of stream health, therefore data are abundant and may allow us to assess the relationship between neonicotinoid concentration and changes in BMI assemblages across many watersheds.

We aimed to assess the impacts of neonicotinoids on aquatic ecosystem health by 1) modelling the relationship between neonicotinoids and BMI assemblage structure across the California central coast and 2) investigating lethal and sublethal toxicity of a common neonicotinoid on BMIs in a controlled environment.

We assessed the relationship between stream health and neonicotinoid concentration using random forest (RF) modelling. We used the California Stream Condition Index (CSCI) as a proxy for stream health and predicted changes in CSCI scores in response to neonicotinoid concentrations while accounting for other watershed stressors (e.g. alteration of hydrology, water chemistry, and water temperature) along California's central coast. We were able to explain nearly 50% of the variation in CSCI scores at 43 sites using seven predictors including neonicotinoid concentrations (mean square error [MSE] = 0.023). We were able to improve model performance with a larger sample size using additional sites where BMI data were available but lacked corresponding neonicotinoid data; to use these data, we developed a separate model predicting

neonicotinoid concentrations at each site using 2,203 neonicotinoid observations. By including additional sites, we obtained a sample size of 110 sites and the model using estimated neonicotinoid concentrations was able to explain approximately 10% more of the variation in CSCI scores (60.4% variance explained; MSE = 0.024) than the model using measured neonicotinoid concentrations alone. Increasing neonicotinoid concentrations were related to a decrease in CSCI scores of up to 4% but had a minor impact on stream health compared with alterations to hydrology and water chemistry.

We explored lethal and sublethal toxicity of a common neonicotinoid (imidacloprid) on a variety of BMI taxa belonging to five functional feeding groups. We observed survival and behavior of BMIs exposed to four concentrations of imidacloprid (0 μg/L, 0.275 μg/L, 13 μg/L, and 35 μg/L) over 28 days in controlled microcosms. We found evidence suggesting BMIs were less likely to survive in the presence of imidacloprid. Filterers, collectors, and predators were less likely to survive than grazers and scrapers after prolonged exposure to imidacloprid. Sensitive taxa and soft-bodied taxa were less likely to survive than tolerant and hard-bodied taxa. Behavioral abnormalities appeared to positively correlate with increasing concentrations and longevity of exposure to imidacloprid. New Zealand mud snails survived the duration of the study period but showed an inability to adhere to surfaces after 17 days of exposure to imidacloprid concentrations of 35 μg/L. These findings were consistent with past studies and were supported by the absence of these same taxa at sites where they were expected but were exposed to neonicotinoids and were not observed.

This project is intended to add to the current understanding of neonicotinoid impacts on freshwater ecosystems by exploring the utility of RF modelling for relatively large-scale assessments and investigating lethal and sublethal toxicity of imidacloprid on a variety of BMIs. This work should be used as a pilot study from which to improve and expand upon with future work.

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1 Introduction

Freshwater ecosystems are one of the most threatened ecosystems worldwide (Barmentlo et al. 2019). California's central coast freshwater ecosystems have weakened in function as a result of pesticides being unintentionally transported into aquatic habitats and surface waters through runoff and irrigation from agricultural and urban areas (Smalling et al. 2013). Pesticides can negatively affect species outside of their targeted taxa through direct exposure and environmental degradation (Larsen et al. 2020). These effects can degrade ecosystem functionality by disrupting natural trophic level processes. Studying the potential effects of specific pesticides on aquatic ecosystems is imperative for informing management decisions surrounding California's coastal freshwater ecosystems.

Globally, neonicotinoids are the most commonly used insecticide; their use has increased dramatically in the past two decades (Barmentlo et al. 2019), yet not much is known about the potential consequences of this pesticide group on the form and function of freshwater ecosystems at the watershed scale (Sánchez-Bayo et al. 2016). Neonicotinoids are a class of neurotoxins commonly used for fruit and vegetable crops, structural pest control, professional landscaping, home garden care, and pet flea and tick treatments (Berghahn et al. 2012; Murray 2015; Rico et al. 2018). Neonicotinoid compounds target the post-synaptic nicotinic acetylcholine receptor in the central nervous system of certain insects, causing paralysis and death (Barmentlo et al. 2019; Lekvongphiboon and Praphairaksit 2020). Neonicotinoids are water-soluble to allow uptake through plant roots but only a small fraction of the active ingredient in the neonicotinoid is taken up by a plant, resulting in generally high environmental mobility. Additionally, neonicotinoids are moderately persistent in aquatic environments, allowing prolonged exposure to nontarget organisms (Wood and Goulson 2017; Rico et al. 2018). These compounds may therefore have a greater negative impact on non-target organisms and ecosystems than originally thought (Murray 2015).

Measuring benthic macroinvertebrate (BMI) species diversity and abundance in rivers and streams is a relatively common method for determining functionality of freshwater ecosystems because BMIs act as indicators of environmental stressors (Dudgeon 2010; Stribling and Dressing 2015; Larras et al. 2017). Prolonged exposure to neonicotinoids may be highly toxic to aquatic invertebrate larvae but the impacts on invertebrates vary widely depending on the taxa being affected and the specific neonicotinoid being used (Miles et al. 2017; Rico et al. 2018). Additionally, literature on the adverse impacts of neonicotinoids on freshwater insects is lacking, as most studies focus on terrestrial insect pollinators rather than aquatic invertebrates. This variability in insect responses to neonicotinoids and the lack of research focused on aquatic insects makes setting regulatory standards to protect the integrity of entire freshwater ecosystems difficult. Continuing to add to the current body of literature by studying the lethal and sublethal effects of specific neonicotinoids on a wide variety of taxa may provide a greater understanding of how certain neonicotinoids impact BMIs of various niches and thus how these pesticides may affect the greater functionality of freshwater ecosystems.

In addition to controlled experiments, watershed-scale assessments of neonicotinoid impacts may provide information on the comparative importance of neonicotinoids as a stressor in the presence of other natural and anthropogenic variables such as water temperature, precipitation, and urbanization. Continuous field monitoring of water quality and invertebrate communities is not feasible on the watershed scale, therefore modelling may help inform stakeholders and land managers of neonicotinoid impacts on water bodies at larger scales (Ouyang et al. 2017).

1.1 Project Objectives

The project goals comprised:

1. Modelling the effects of neonicotinoids on stream health across the California central coast.
2. Assessing lethal and sublethal toxicity of a common neonicotinoid (imidacloprid) on benthic macroinvertebrates in a controlled environment.

2 Modelling the effects of neonicotinoids on stream health

We explored the relationship between stream health and neonicotinoid concentration in a 64,485 km^2 area along California's central coast using Random Forest (RF) modelling. We utilized the California Stream Condition Index (CSCI) as a proxy for stream health and predicted changes in CSCI scores in relation to neonicotinoid concentrations while accounting for other important anthropogenic stressors (e.g. alterations to water temperature, water chemistry, or hydrology). We used three separate RF models: 1) predicting CSCI scores from neonicotinoid concentrations measured coincident to BMIs, 2) predicting neonicotinoid concentrations from natural and anthropogenic watershed variables, and 3) predicting CSCI scores using the predicted neonicotinoid concentrations. The first model was able to explain nearly 50% of the variance in CSCI scores using 43 neonicotinoid observations and six indices of watershed function (MSE = 0.023). The second model explained approximately 23% of variation in neonicotinoid concentrations using 30 predictors (MSE = 0.35). Longitude, latitude, and clay content of soils were the most influential factors for predicting neonicotinoid concentrations, consistent with past studies. By adding additional sites, the third model explained approximately 10% more of the variation in CSCI scores than the first model (60.4% variance explained; MSE = 0.024). Neonicotinoids had a minor impact on stream health (percent increase in MSE = 15.5) compared to alterations in hydrology and water chemistry (percent increase in MSE ≈ 28 and 30, respectively) but may have an impact comparable with sediment flux. Additionally, increasing concentrations of neonicotinoids related to a decrease in CSCI scores of up to 4%, which should be considered when establishing stream restoration goals.

2.1 Study Area

The study area comprised Monterey, Santa Cruz, San Luis Obispo, San Benito, Santa Clara, San Francisco, San Mateo, Santa Barbara, and Alameda counties, encompassing an area of 64,485 km^2 (24,898 mi^2) (Fig. 1). Approximately 8.3% of the total area is developed to some capacity and approximately 4.6% is agricultural land (Table 1).

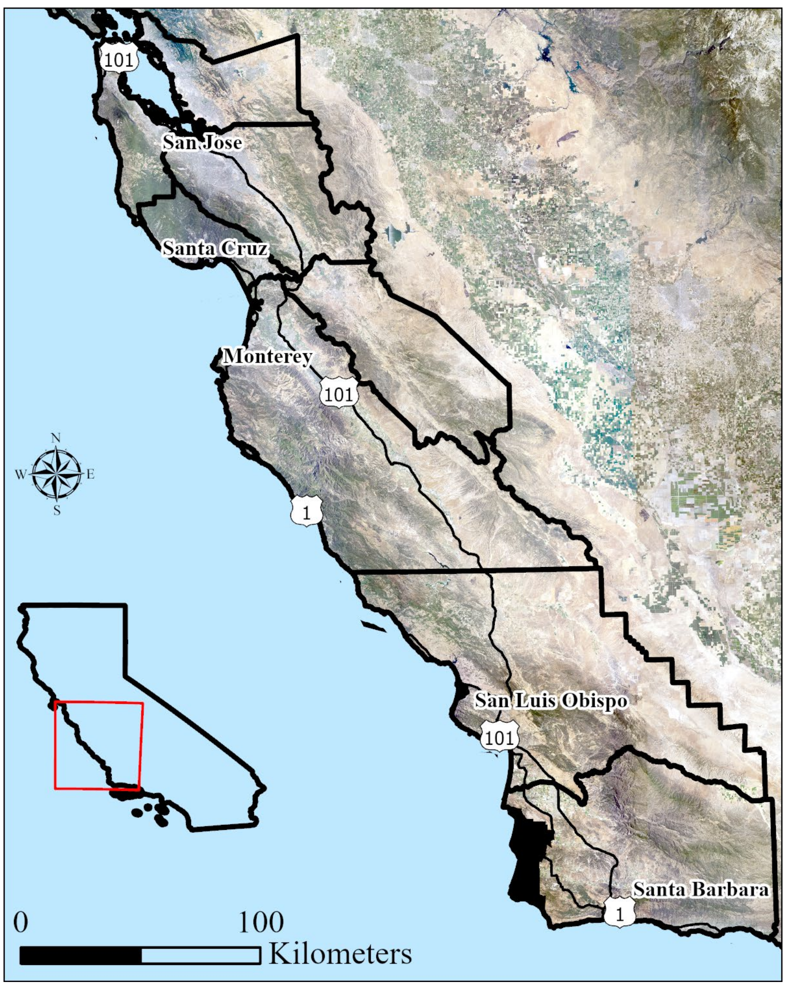

Figure 1. Study area encompassing 64,485 km^2 (24,898 mi^2) of Monterey, Santa Cruz, San Luis Obispo, San Benito, Santa Clara, San Francisco, San Mateo, Santa Barbara, and Alameda counties.

Table 1. Percent cover of various land cover classes within the study area calculated from the 2011 National Land Cover Dataset.

NLCD Landcover Classification	% Cover
Shrub/Scrub	27.6
Herbaceuous	26.0
Open Water	15.9
Mixed Forest	8.1
Evergreen Forest	7.9
Cultivated Crops	4.6
Developed, Open Space	3.3
Developed, Medium Intensity	2.5
Developed, Low Intensity	1.8
Developed, High Intensity	0.8
Emergent Herbaceuous Wetlands	0.7
Hay/Pasture	0.4
Woody Wetlands	0.3
Barren Land	0.2

2.2 Modelling Methods

We used a model-based approach to predict aquatic ecosystem responses to neonicotinoid concentration along California's central coast region. We utilized the California Stream Condition Index (CSCI) as a proxy for stream health (Mazor et al. 2016) and used measured and estimated neonicotinoid concentrations to predict changes in CSCI scores within the study area while accounting for important watershed variables such as water temperature, hydrology, land cover, and runoff using random forest (RF) models. RF is a machine learning algorithm used to model interactions between response and predictor variables based on features of a given observation. This modelling technique aggregates predictions from several uncorrelated decision trees using bootstrap aggregating (bagging) to produce robust models. Model accuracy and precision are assessed using random samples of data not used in creating individual trees (i.e., out-of-bag samples), similar to cross-validation (Olson and Cormier 2019; Yiu 2019). We used pseudo R^2 (percent variance explained) and mean square error (MSE) to assess overall model performance. This nonparametric modelling approach often provides results with equal or greater accuracy than other modelling methods (Cutler et al. 2007; Olson and Hawkins 2012) therefore we determined it was an appropriate method to use for this study.

2.2.1 Predicting Stream Health Based on Measured Neonicotinoid Concentrations

We sought to predict stream health based on measured neonicotinoid concentrations while accounting for other anthropogenic watershed stressors likely to affect stream health. We obtained datasets of benthic macroinvertebrates (BMIs), neonicotinoids, and 6 indices of watershed function (Table 2) (Thornbrugh et al. 2018) from the California Environmental Data Exchange Network (CEDEN), Surface Water Database (SURF), and StreamCat Metric Database. For this model, data were only used if they were spatially and temporally related (i.e., if data were collected within the same watershed and within the same water-year). Given this restriction, we obtained data for 43 sites to use in a RF model.

Table 2. Six indices of watershed function as defined by Thornbrugh et al. (2018).

Abbreviation	Definition
WHYD	Hydrologic Regulation
WCHEM	Water Chemistry Regulation
WSED	Sediment Regulation
WCONN	Hydrologic Connectivity
WTEMP	Temperature Regulation
WHABT	Habitat Provision

CSCI scores were used as a proxy for stream health. The CSCI uses information about environmental conditions and BMI data from over 2,000 unimpaired streams throughout California to model reference conditions of California streams (Rhen et al. 2015; Mazor et al. 2018). The CSCI combines observed-to-expected (O/E) and multimetric index (MMI) methods for assessing BMI species diversity and richness. The O/E score is a measure of taxonomic completeness calculated as the number of taxa observed at a site relative to model predictions of the number of taxa expected if the site were in reference condition. The MMI aggregates BMI metrics (e.g. number of pollution-tolerant species and taxonomic richness and diversity) into a single value representing ecological structure and function of a given stream (Rehn et al. 2015). Reference streams should have a CSCI score of one; a CSCI score of $\geq$0.92 represents a likely intact stream and a score $\leq$0.62 suggests a very likely altered stream (Rhen et al. 2015). We extracted watershed metrics from ArcGIS to use as predictors for estimating the expected CSCI scores for each site and calculated CSCI scores using the BMI data for each site, following methods from Mazor et al. (2018).

We ran a RF model with 1500 trees using the calculated CSCI scores as the response variable and the neonicotinoid concentrations and 6 indices of watershed function outlined by Thornbrugh et al. (2018) as predictor variables. We assessed performance of individual predictors by calculating the percent increase in mean square error (MSE) that results when each parameter is permuted.

Given the limited sample size, we also explored whether the relationship between stream health and neonicotinoid concentration could be more accurately modelled using generalized linear models (GLMs). We ran two GLMs based on a stepwise AIC model selection. GLM results were inferior to the RF model results and can be found in Appendix A.

2.2.2 Estimating Neonicotinoid Concentrations Based on Environmental Factors

We sought to improve model performance for predicting stream health as a function of neonicotinoid concentration by increasing sample size. Additional sites with BMI data were available but did not have coincident neonicotinoid data; to use these additional sites in RF models we used model estimates of neonicotinoid concentrations instead of using measured neonicotinoid concentrations for all sites. To model neonicotinoid concentrations, we used 2,203 measured neonicotinoid observations as the response variable and StreamCat watershed metrics as predictors in a RF model, yielding a sample size of 110 sites. We initially included 69 watershed parameters obtained from the StreamCat database. We assessed the performance of individual predictors by calculating the percent increase in MSE that results when each predictor is permuted. We removed predictors that produced a decrease in MSE when permuted (i.e., predictors with information that contributed noise to the model). Land cover and percent forest loss were the only two datasets we processed prior to running the model; all land cover classes representing developed land (low, medium and high-intensity and open development) were aggregated into a single class and we used a ten-year average of percent forest loss rather than the individual values for each year.

2.2.3 Predicting Stream Health from Estimated Neonicotinoid Concentrations

We aimed to create a more robust RF model to predict stream health by including additional sites using the estimated neonicotinoid concentrations as the predictor variable and CSCI scores as the response variable. We accounted for other watershed stressors likely to impact CSCI scores by including 6 indices of watershed function (Thornbrugh et al. 2018) in the model and calculated the percent increase in MSE for each parameter. We used the 43 calculated CSCI scores from the first RF model and included 64 additional CSCI scores from the Surface Water Ambient Monitoring Program (SWAMP) bioassessment scores webapp to use as a proxy for stream health and ran a model with 1500 trees.

2.3 Modelling Results and Discussion

RF modelling was effective for assessing stream health responses to neonicotinoid stress. Our findings suggest there is a weak negative correlation between neonicotinoid concentration and stream health, which underscores findings from Stone et al. (2014) that neonicotinoids were increasingly found in US streams at concentrations that exceed aquatic life benchmarks, but the adverse impacts of these pesticides on aquatic life are likely understated in assessments due to the inability to account for potential synergistic effects of multiple stressors.

The RF model predicting CSCI scores using measured neonicotinoid concentrations explained 48.6% of model variance using seven predictors (MSE = 0.023). Alterations to hydrology and water chemistry were the two top predictors followed by habitat provision and sediment regulation. Neonicotinoid concentration was the fifth strongest predictor (percent increase in MSE = 13.5) (Fig. 2).

The RF model predicting neonicotinoid concentrations suggests approximately 23.3% of the variance could be explained using 30 predictors (MSE = 0.35). Longitude and latitude were the two most important predictors followed by clay content of the soil (Fig. 3). The importance of clay content in soil is consistent with findings that neonicotinoids are retained in clay and loam (Mörtl et al. 2016). Neonicotinoid concentrations were highest in areas with urban or agricultural impacts (Fig. 4). These findings suggest neonicotinoids may be more prevalent in urban areas than expected. Additionally, latitude and longitude as well as an area's lithology should be considered when creating pesticide application guidelines. California spans approximately 10 degrees of latitudinal and longitude and has a very heterogeneous geological structure, therefore state or county-wide guidelines may not be most effective for protecting California coastal streams because they do not account for such variation in lithology, latitude, or longitude across the state. Although the model explains only a small proportion of the variance in neonicotinoid concentrations, it may be useful for understanding the likely range of concentrations throughout the region and the broad-scale spatial patterns driving variation in neonicotinoid concentrations (e.g. land cover) which may assist with prioritization of areas for future monitoring.

By including additional sites, the model using estimated neonicotinoids explained approximately 10% more variation in CSCI scores (60.4% variance explained, MSE = 0.024) than the model using measured neonicotinoid concentrations alone. Consistent with the first RF model, neonicotinoid concentrations had a minor impact on stream health compared to hydrology and water chemistry, which were the top two predictors of stream health. However, neonicotinoids interacted with both hydrology and water chemistry resulting in large decreases in CSCI scores at relatively low neonicotinoid concentrations when a site experienced the combined effects of altered hydrology or water chemistry and neonicotinoid stress (Fig. 5). All predictors contributed a greater percent increase in MSE than in the RF model using only measured neonicotinoid concentrations however neonicotinoid concentration fell from being the fifth most important predictor to having the lowest impact on stream health (percent increase in MSE = 15.5) (Fig. 6). Increasing neonicotinoid concentrations were related to decreases in CSCI scores 1 – 4% (Fig. 7). Although minor, this decrease is enough to push a stream over established CSCI thresholds (e.g. from a possibly altered stream to a likely altered stream).

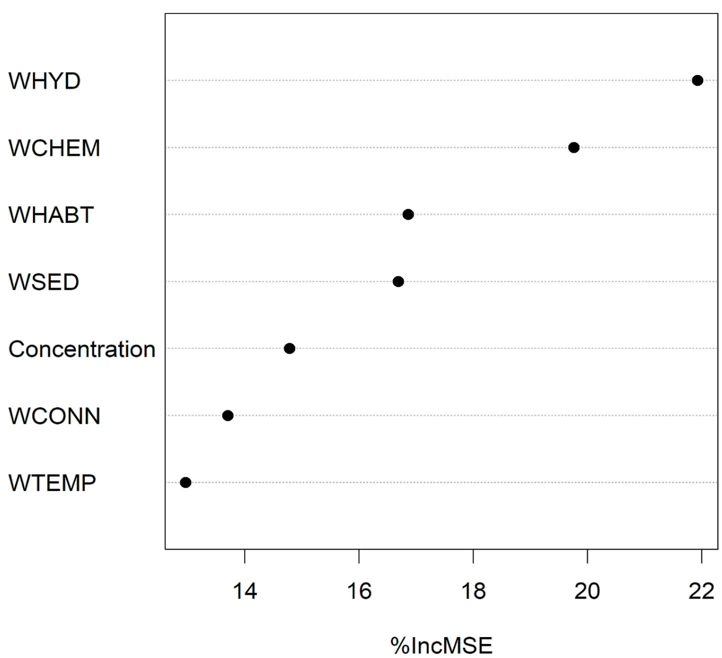

Figure 2. Variable importance of measured neonicotinoid concentrations and six watershed metrics used to predict changes in CSCI scores, measured as percent increase in MSE when variable permuted. See Table 2 for abbreviation definitions.

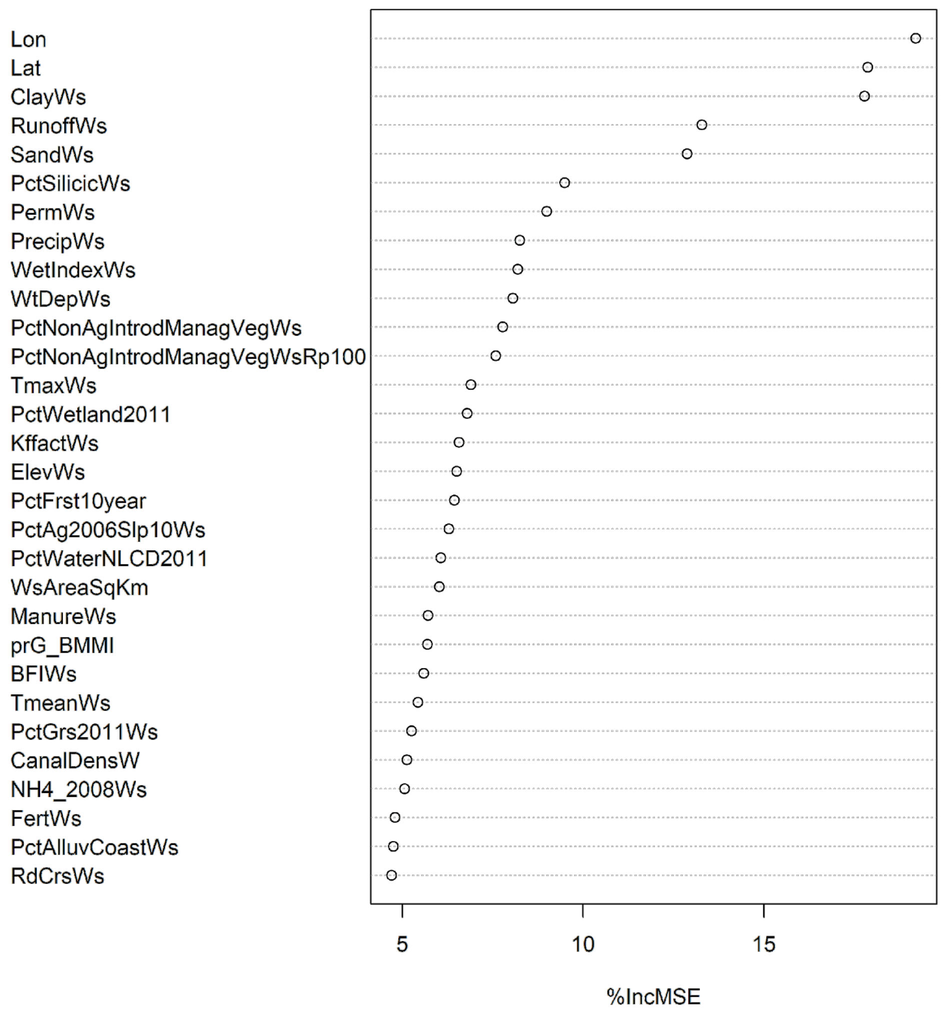

Figure 3. Variable importance of watershed metrics used to predict neonicotinoid concentrations in descending order of importance, measured as percent increase in MSE when variable permuted. Predictor codes and definitions are available on the StreamCat Metrics and Definitions wepage.

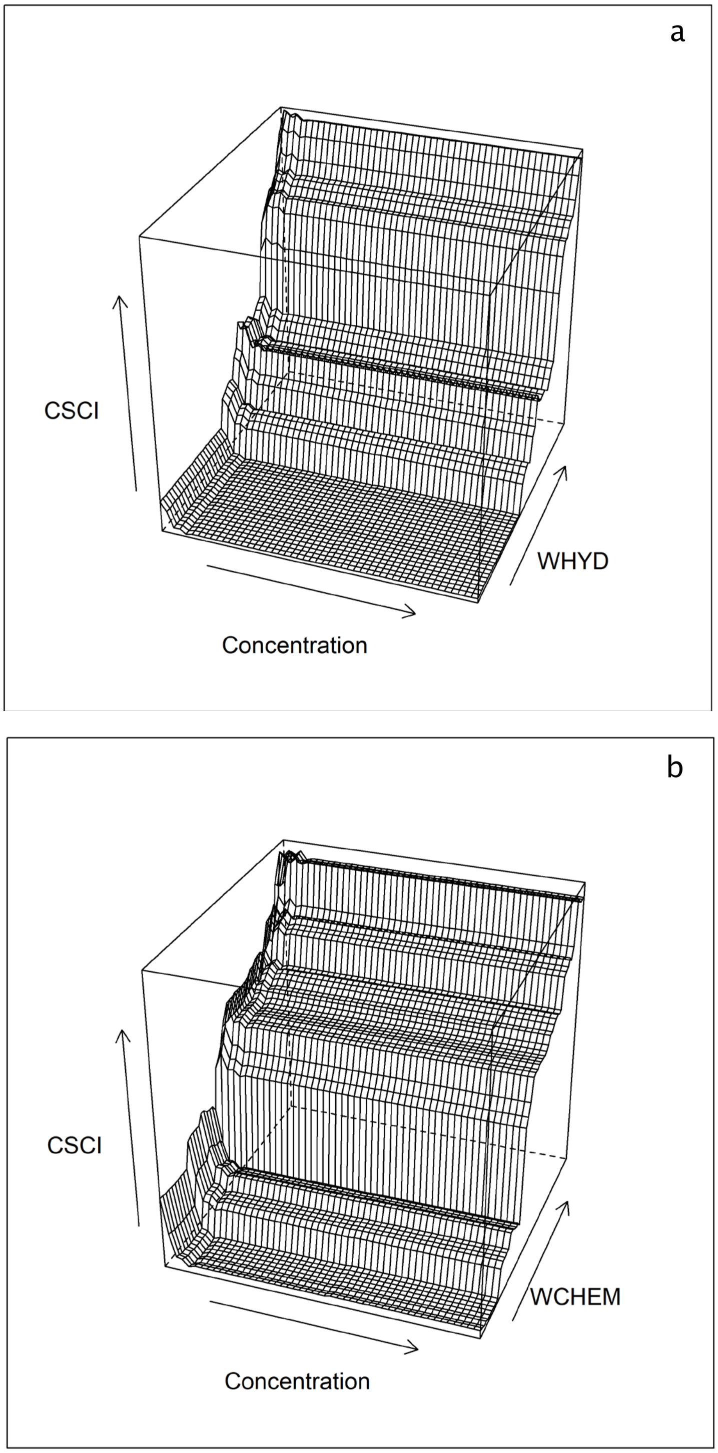

Figure 4. Comparative effects of neonicotinoid concentration and (a) alterations to hydrology and (b) alterations to water chemistry on stream health. Neonicotinoid concentration has a minor effect on stream health compared to hydrology and water chemistry. However, neonicotinoids interact with both hydrology and water chemistry resulting in large decreases in CSCI scores when hydrology and water chemistry are altered (i.e., when the stress indices are low). CSCI scores were used as a proxy for stream health.

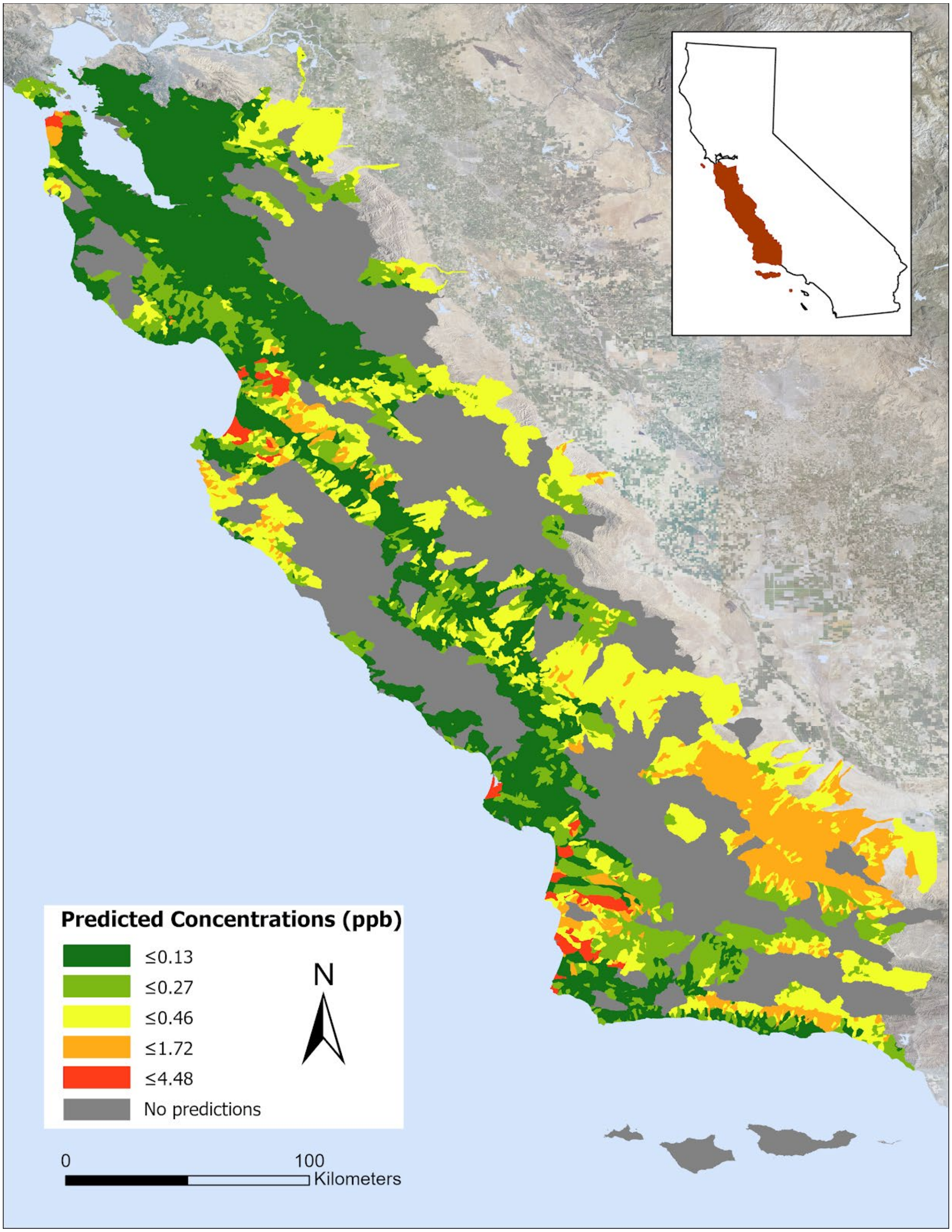

Figure 5. Predicted neonicotinoid concentrations produced from a Random Forest model using 2,203 measured neonicotinoid observations and 30 watershed metrics (Fig. 3) along the California central coast. Neonicotinoid concentrations are highest in areas heavily impacted by urbanization or agriculture. Areas with no predictions were not modelled because they did not meet thresholds of >10% urban or >5% agriculture within the study area.

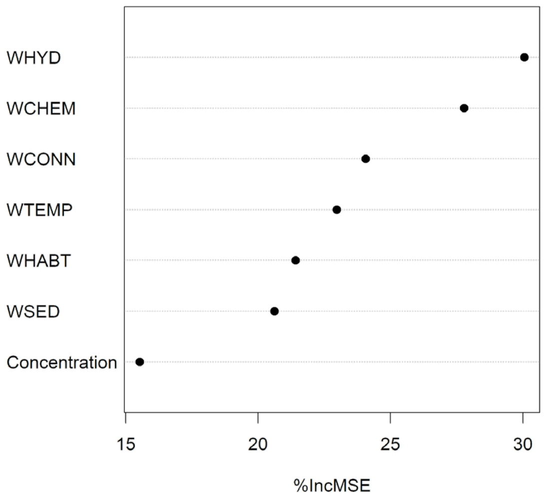

Figure 6. Variable importance of predicted neonicotinoid concentrations and six watershed metrics used to predict changes in CSCI scores, measured as percent increase in MSE when variable permuted. See Table 2 for abbreviation definitions.

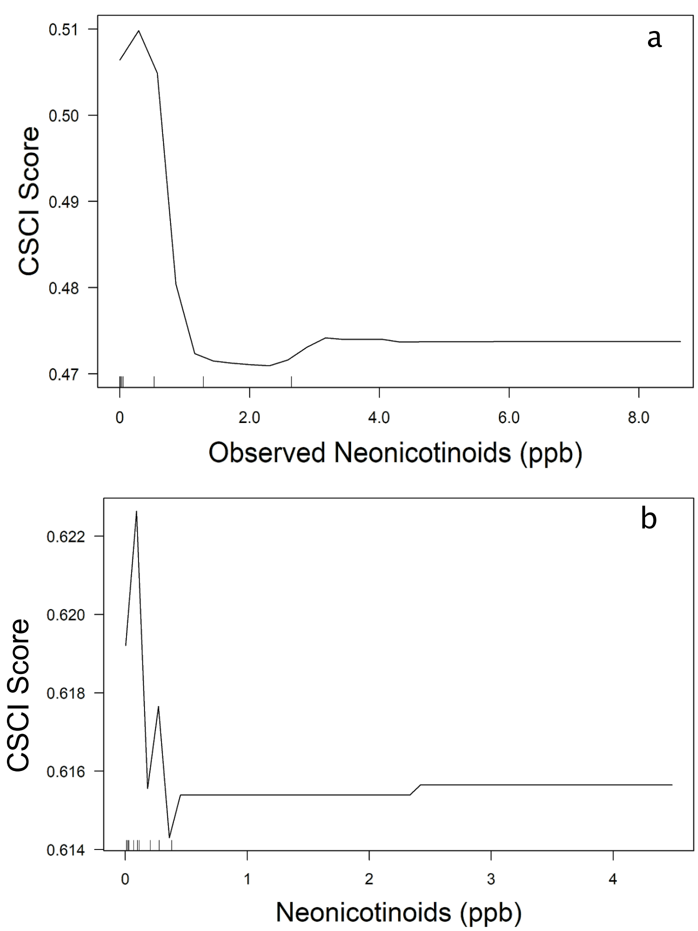

Figure 7. Partial dependence plots showing impacts of (a) measured neonicotinoid observations (n = 43) and (b) predicted neonicotinoid concentrations (n = 110) on CSCI score, after accounting for the effects of all other variables in the models.

Although random forest models are powerful, there were many limitations in this study due to data scarcity and time constraints. We could not include temporal variables in the neonicotinoid predictor model because we only had a few years where neonicotinoid data were collected. Additionally, available datasets that included both CSCI scores and pesticide measurements were often taken at different times in the year therefore time-dependent variables such as seasonality may have also affected CSCI scores but were not accounted for in the RF models. We also modelled the effects of neonicotinoids as a class rather than separating out specific neonicotinoids, therefore any differences in impacts to stream health between specific neonicotinoids were not explored in this study. The models using measured neonicotinoid concentrations and predicting neonicotinoid concentrations relied on available neonicotinoid data, which tends to be biased toward areas where neonicotinoids are expected; this may have biased the model for predicting neonicotinoid concentrations toward urban and agricultural areas although neonicotinoids may be present in minimally impacted streams.

3 Assessing Lethal and Sublethal Toxicity of Imidacloprid on BMIs

We aimed to add to the current understanding of neonicotinoid impacts on aquatic insects by studying toxicity of a common neonicotinoid (imidacloprid) on a variety of taxa. We explored lethal and sublethal effects of imidacloprid on benthic macroinvertebrates (BMIs) belonging to five functional feeding groups in controlled microcosms. We recorded survival and behavior of BMIs over a 28-day observation period and found marginal evidence that BMIs were less likely to survive in the presence of imidacloprid. Relatively sensitive taxa and soft-bodied organisms were less likely to survive than hard-bodied and tolerant taxa in the presence of imidacloprid. These findings were supported by observations from sites used in modelling stream health responses to neonicotinoids, where expected taxa were not found in streams exposed to neonicotinoids. Collectors, filterers, and predators were less likely to survive than grazers and scrapers and abnormal behavior appeared to positively correlate with increasing concentrations of imidacloprid. New Zealand mud snails survived the duration of the study period but showed an inability to adhere to surfaces after 17 days of exposure to imidacloprid concentrations of 35 μg/L. These findings were consistent with past findings of significant deterioration of foot muscle fibers of freshwater snails when exposed to pesticides not targeted at the order Gastropoda.

3.1 Experiment Methods

We assessed the lethal and sublethal effects of imidacloprid on BMIs collected from minimally impacted coastal streams using a microcosm experimental design. Survival and behavioral changes were recorded over a 28-day observation period and data were analyzed using Kaplan-Meier survival estimates and calculated proportions of behavioral differences between treatment groups.

3.1.1 Test Organism Collection

BMIs were opportunistically collected from streams with minimal human impact. Collection occurred over a period of two days at six sites located along the Big Sur River, Soberanes Creek, Aptos Creek, and Soquel Creek located in Monterey and Santa Cruz counties (Fig. 8). Collection sites were chosen based on proximity to the lab and CSCI score (≥ 0.8). Moving from downstream to upstream, we used D-frame and kick nets to collect organisms from riffles, pools, and along banks. Once captured, individuals were sorted by trophic level into capped jars containing stream water and placed on ice to reduce temperature and oxygen stress.

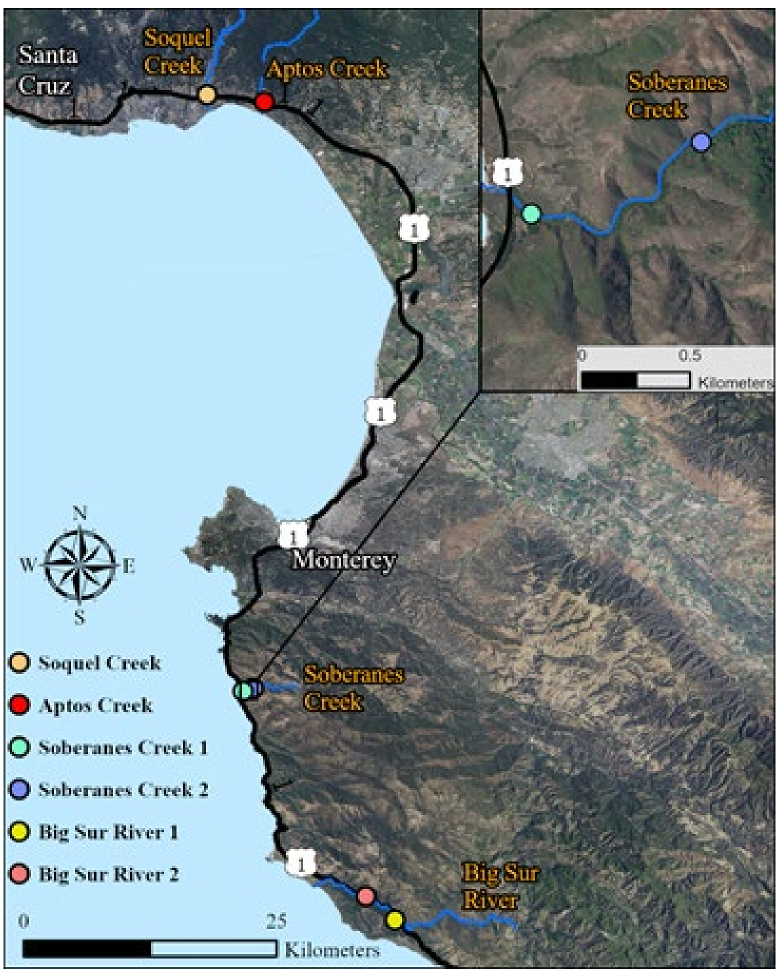

Figure 8. BMI collection sites located along the Big Sur River, Soberanes Creek, Aptos Creek, and Soquel Creek located in Monterey and Santa Cruz counties.

3.1.2 Microcosm Structure, Imidacloprid Application, and Data Collection

To explore the lethal and sublethal effects of imidacloprid exposure on BMIs we exposed a variety of taxa to increasing concentrations of imidacloprid in controlled microcosms and observed survival and behavioral changes over 28 days. We identified individuals to genus and counted individuals within each taxon. Taxa were sorted by their respective functional feeding groups (FFGs): grazers, predators, filter feeders, collectors/gatherers, and scrapers (Table 3). Individuals belonging to a given FFG were divided into five separate microcosms. Each microcosm comprised a 50-mL beaker covered with fine mesh containing oxygenated dechlorinated water, an adequate supply of food, and mesh substrate for BMIs to cling to. Microcosms were placed in four treatment units of increasing imidacloprid concentrations (0 μg/L, 0.275 μg/L, 13 μg/L, 35 μg/L) where the 0 μg/L group was the control. We replicated this setup, resulting in two identical and independent stations (Fig. 9). Each station was confined into a 10-L bin packed with ice to maintain a water temperature range between 4 – 15 °C.

Test organisms were monitored each morning for 28 days. Dead individuals were removed, food was replenished as needed, and any abnormal behavior of alive individuals, such as altered swimming behavior, attempts to drift (i.e., not clinging to substrate), or emergence from a casing were recorded (Appendix B). We replenished the ice at each station twice daily and recorded temperature before and after replenishing ice to monitor temperature fluctuations and ensure microcosm temperatures stayed between a nonlethal range of 4 – 15°C that reflected the range between winter and annual mean stream temperatures (Hill et al. 2015). To accommodate for the half-life of imidacloprid (33 – 44 days) (Fossen 2006), treatment solutions were replaced once per week. Microcosms were topped off with the appropriate solution if water level decreased.

Table 3. Functional feeding groups used in experiment with associated taxa and number of individuals.

Functional Feeding Group	Taxon	Count
Grazers	Amphipoda	22
	Ephemeroptera	5
	Trichoptera	10
Predators	Odonata	8
	Plecoptera	28
Filter feeders	Trichoptera	66
	Diptera	1
Collectors	Ephemeroptera	26
	Oligochaeta	8
Scrapers	Gastropoda	47

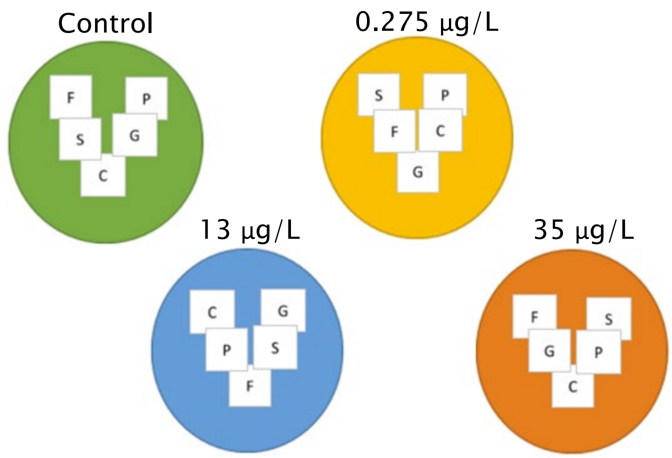

Figure 9. Functional feeding groups (FFGs) divided into microcosms and placed in four treatment units of increasing imidacloprid concentrations (0 µg/L [control], 0.275 µg/L, 13 µg/L, 35 µg/L). FFG abbreviations are defined as follows: F = filterers, P = predators, G = grazers, S = scrapers, and C = Collectors/gatherers.

3.1.3 Data Analysis

We calculated Kaplan-Meier survival estimates and plotted survival curves to assess survival probability of individuals over time. We estimated differences in survival probability between untreated and treated individuals, between each treatment group (control, 0.275 µg/L, 13 µg/L, 35 µg/L), and between FFGs within each treatment group. We calculated and plotted the proportion of BMIs exhibiting abnormal behavior in each treatment group over the four weeks of observation. All analyses were conducted using R software (R Core Team 2020).

3.2 Experiment Results and Discussion

We found marginal evidence that neonicotinoid presence was negatively correlated with survival probability when abiotic factors were controlled for. We found marginal evidence of a difference in survival probability between treated and untreated individuals in Station 2 and no evidence of a difference in survival probability between treated and untreated individuals in Station 1 (Fig. 10). Similarly, when assessing differences between treatment groups in Station 1, there was no evidence of a difference in survival probability between the control and the 0.275 µg/L or 13 µg/L groups and the survival probability may have been higher in the 35 µg/L group than the control (Fig. 11 a-c). Station 1 typically experienced daily temperatures 0.5 – 2 degrees warmer than those in Station 2, which may have affected survival rates more than exposure to different imidacloprid treatments.

When temperature was controlled for, there was faster die-off of individuals in any treatment group compared with the control group and survival probability in the control was consistently higher than survival probability of treated groups. In the 0.275 μg/L and 13 μg/L groups, there was early die-off but survival probability remained fairly constant after about day 12 whereas the group exposed to the highest concentration of imidacloprid (35 μg/L) experienced a greater die-off than the other two treated groups early on and survival probability continued to decline for the entirety of the study period (28 days) (Fig. 11 d-f).

In Station 2 collectors, filterers, and predators treated with imidacloprid generally experienced a greater decline in survival probability compared to the control group (Fig. 12 f, g, i). These FFGs comprised relatively sensitive taxa (mayflies, stoneflies, and caddisflies in the orders Ephemeroptera, Plecoptera, and Trichoptera respectively), which may in part explain the greater difference in survival between the control and treated groups in these FFGs compared to individuals in the grazer and scraper FFGs. Additionally, filterers which comprised 99% caddisflies (n = 66), experienced a particularly sharp decline in survival, reaching an estimated 50% survival probability within the first few days of the study in all three treated groups (Fig. 12 g). In contrast, predators which comprised 22% stoneflies and 78% damselflies (n = 18) showed more resilience to imidacloprid, reaching an estimated 50% survival probability at the end of the first week of observations only in the 35 μg/L group; individuals exposed to 0.275 μg/L and 13 μg/L of imidacloprid never reached a survival probability below approximately 75% during the study period (Fig. 12 i). This may suggest soft-bodied taxa are more likely to experience the negative effects of imidacloprid at lower concentrations while the exoskeleton of hard-bodied taxa may provide some protection from sublethal concentrations. These findings were supported by taxa that were expected by the O/E index (i.e., probabilities of capture > 0.5) but were not present at six of the study sites we used in modelling neonicotinoid effects; sites with higher neonicotinoid concentrations were missing soft-bodied organisms such as *Ceratopsyche hydropsyche* (net-spinning caddisflies), *Simulium* (black flies), and *Baetis* (minnow mayflies) that would be expected in a less impacted or intact stream.

The scraper FFG consisted entirely of New Zealand mud snails (NZMS) (n = 24), which maintained 100% survival for the duration of the study in both stations (Fig. 12 e, j). This is consistent with other findings that show snail species surviving neonicotinoid concentrations of up to 327 μg/L (Miles et al. 2016). NZMS resilience is likely a result of the species' physiological adaptations (i.e., a hard shell and operculum) that protect them from external stressors as well as neonicotinoids being specifically targeted at insects not mollusks (Weir and Salice 2011). Despite their resilience, NZMS appeared to exhibit abnormal behavior in the 35 μg/L concentration (i.e., not adhering to a surface) (Fig. 13). Such behavioral changes underscore findings from Jonnalagadda and Rao (1996) of significant muscle fiber deterioration in the foot of freshwater snails when exposed to pesticides not targeted at the order Gastropoda.

Over the course of the observation period, a greater percentage of treated individuals appeared to exhibit irregular behavior compared to untreated individuals in Station 2. Behavioral differences between treated and untreated individuals were most noticeable at the end of weeks two and three. By the end of week four behavioral differences became more discernible between each treated group, where abnormal behavior increased as imidacloprid concentration increased (Fig. 14).

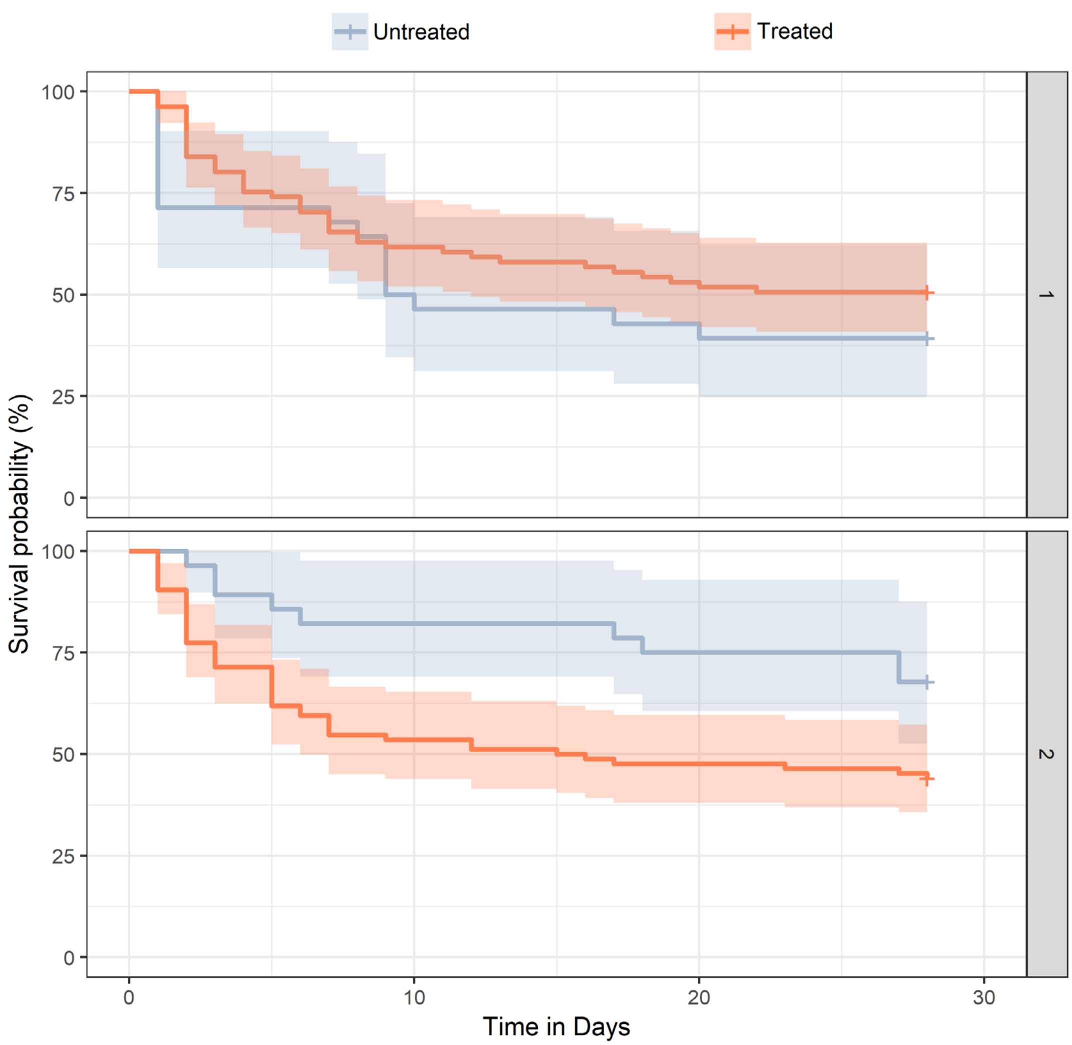

Figure 10. Kaplan-Meier survival curves of untreated and treated individuals for stations 1 and 2. Impacts from neonicotinoid exposure may be masked by temperature stress in Station 1. Station 2 results show marginal evidence that survival probability is lower for treated individuals than for untreated individuals.

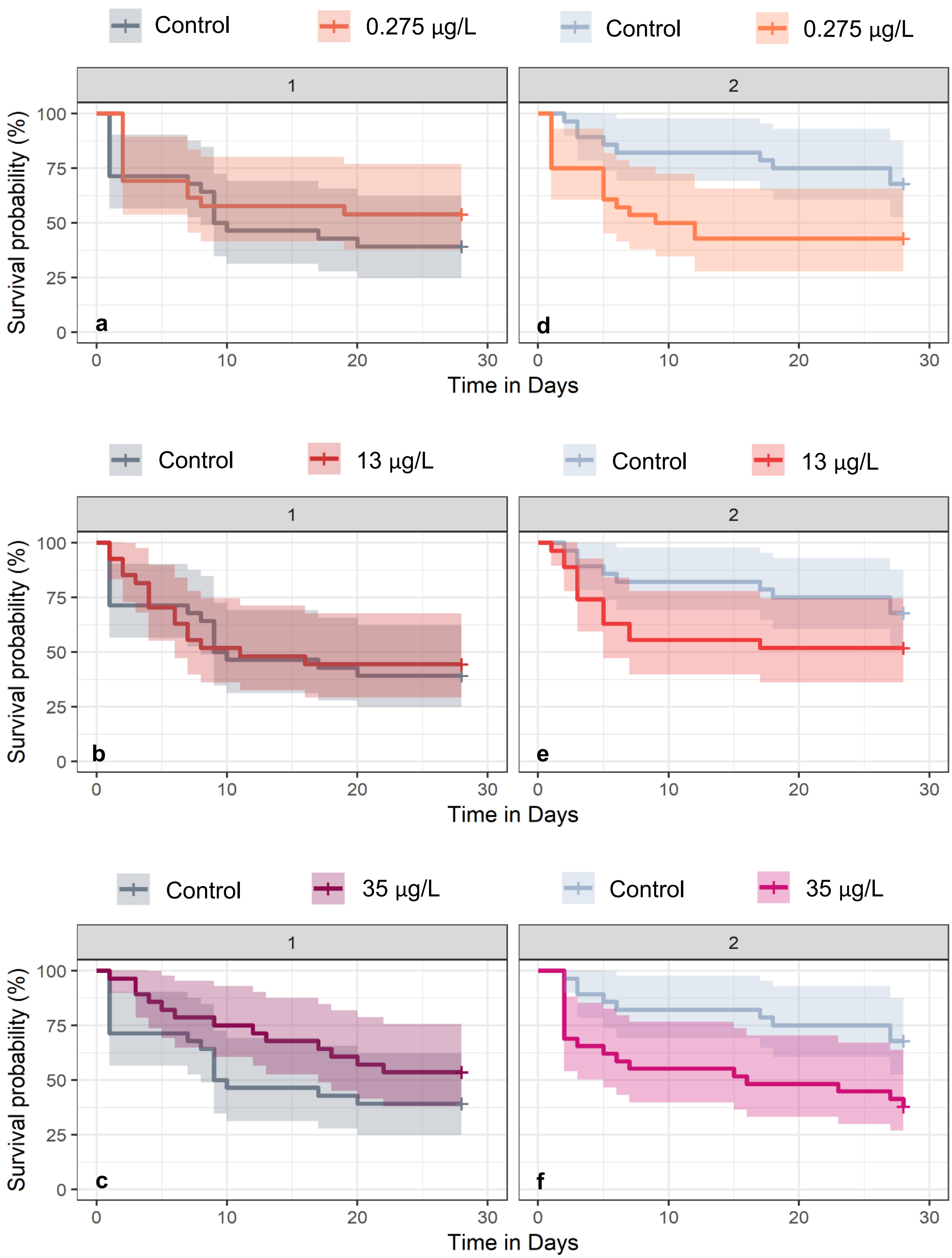

Figure 11. Kaplan-Meier survival curves of all four treatment groups (0 µg/L [control], 0.275 µg/L, 13 µg/L, 35 µg/L) for stations 1 and 2. When temperature is controlled, negative impacts to BMI survival appear to be more persistent in the highest concentration of imidacloprid (35 µg/L).

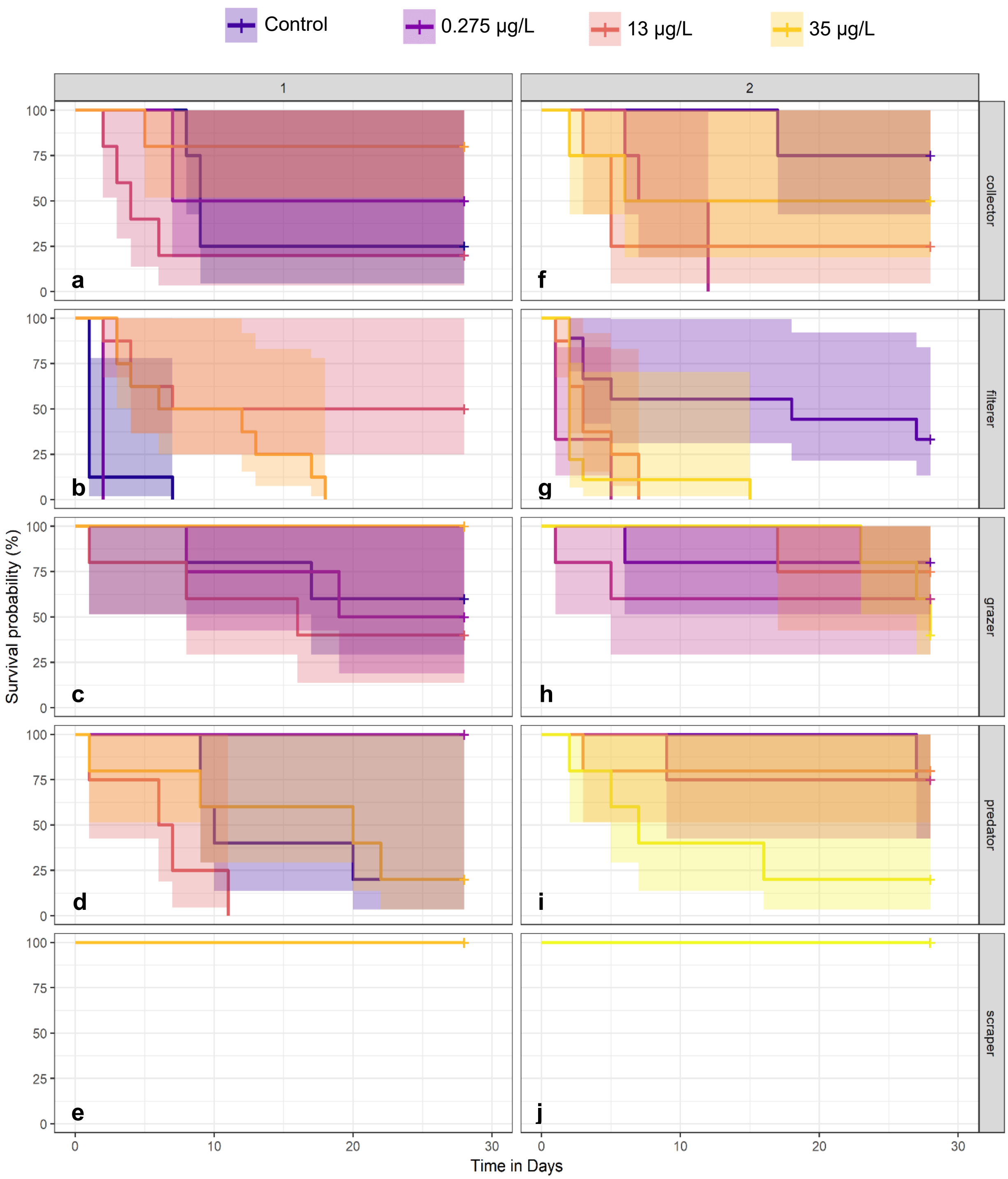

Figure 12. Kaplan-Meier survival curves of five functional feeding groups (FFGs) exposed to all four treatment groups (0 µg/L [control], 0.275 µg/L, 13 µg/L, 35 µg/L) for stations 1 and 2. Collectors, filterers, and predators were less likely to survive than grazers and scrapers and soft-bodied and sensitive taxa were less likely to survive than hard-bodied and tolerant taxa. The scraper FFG comprised all New Zealand mud snails, which survived the duration of the observation period.

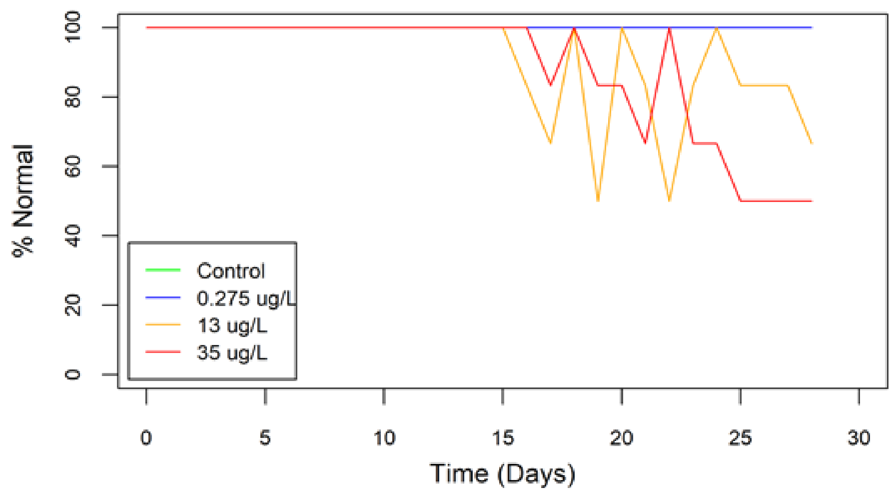

Figure 13. Behavioral changes in New Zealand mud snails for each treatment group in Station 2. Snails showed an inability to adhere to surfaces after about 17 days of exposure to imidacloprid concentrations of 35 µg/L.

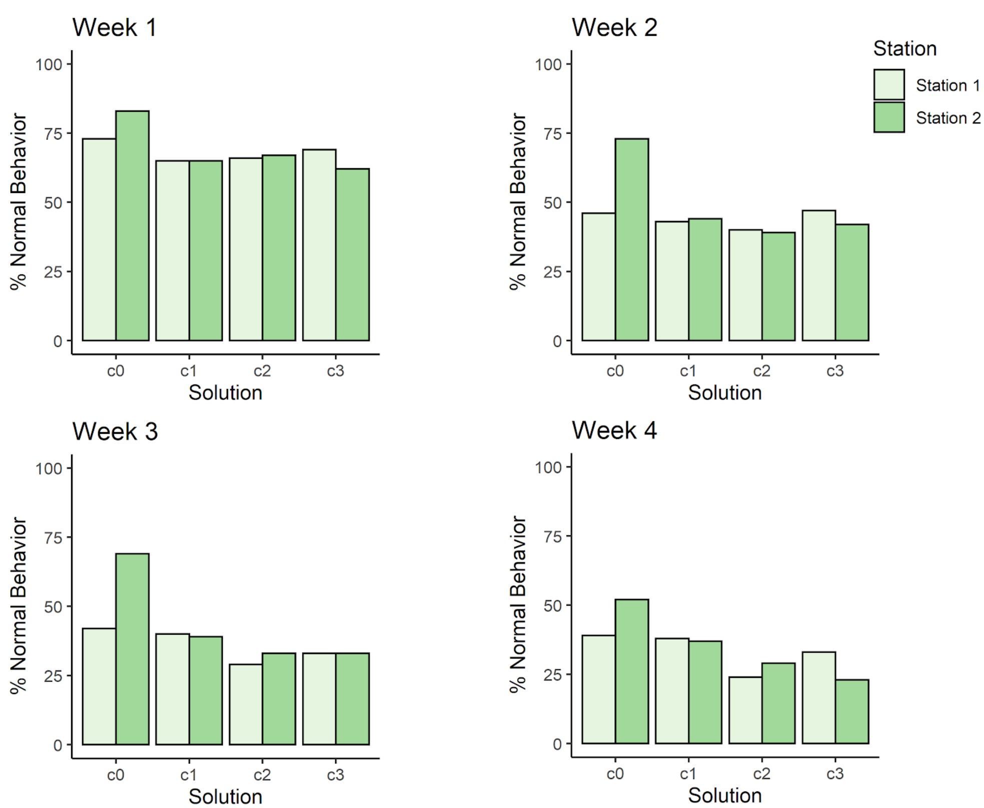

Figure 14. Percentage of individuals in each treatment group (0 µg/L [control], 0.275 µg/L, 13 µg/L, 35 µg/L) acting normal for each week of observation in stations 1 and 2. Abnormal behavior appears to positively correlate with increasing concentrations of imidacloprid. Treatment group abbreviations are as follows: c0 = control, c1 = 0.275 µg/L, c2 = 13 µg/L, c3 = 35 µg/L.

4 Conclusions and Future Work

Major findings from this study are summarized respective to the order in which they appear in the body of the report:

- Latitude, longitude, and clay content in soils were the top three predictors for modelling neonicotinoid concentrations. These findings suggest statewide policy may not be most effective for protecting California coastal streams because they do not account for variability in latitude, longitude, or lithology across the California.
- Neonicotinoids had a minor impact on stream health compared with alterations to hydrology and water chemistry, but neonicotinoids interacted with both hydrologic and chemical stressors, resulting in sharp declines in CSCI scores when a site experienced the combined effects of altered hydrology or water chemistry and neonicotinoid stress.
- Increases in neonicotinoid concentrations were related to approximately 1 – 4% decreases in CSCI scores. This increase is enough to push a stream over established CSCI thresholds (e.g. from a possibly altered stream to a likely altered stream).
- There was marginal evidence that neonicotinoid presence was negatively correlated with survival probability when abiotic factors were controlled for and negative impacts to BMI survival appeared to be more persistent in the highest concentration of imidacloprid (35 μg/L).
- Collectors, filterers, and predators were less likely to survive than grazers and scrapers and soft-bodied and sensitive taxa were less likely to survive than hard-bodied and tolerant taxa.
- Abnormal behavior appeared to positively correlate with increasing concentrations of imidacloprid.
- New Zealand mud snails survived the duration of the observation period but showed an inability to adhere to surfaces after 17 days of exposure to imidacloprid concentrations of 35 μg/L, consistent with past findings of significant muscle fiber deterioration in the foot of freshwater snails when exposed to pesticides not targeted at the order Gastropoda.

This work is intended to expand upon the current understanding of neonicotinoid impacts on freshwater ecosystems by exploring the utility of RF modelling for relatively large-scale assessments and investigating lethal and sublethal toxicity of imidacloprid on a variety of BMIs. We found freshwater ecosystem structure may be impacted by disruptions to BMI community composition through the loss of sensitive taxa and greatly reduced survival probability of entire FFGs after prolonged exposure to neonicotinoids. We found that RF modelling is an effective method for conducting watershed-scale assessments of stream health in relation to neonicotinoid stress. Although neonicotinoids had only a minor impact on stream health compared to hydrologic and chemical stressors at the watershed scale, the slight increase in stream health when neonicotinoid concentrations are relatively low suggests this minor impact may have a practical significance when establishing restoration goals. We recommend that future work focus on addressing some of the limitations of this study by doing the following:

- Explore if impacts to stream health differ depending on which specific neonicotinoid is present by modelling the impacts of specific neonicotinoids (as opposed to neonicotinoids as a class) on stream health.
- Attempt to isolate the effects of soil content to better predict neonicotinoid concentrations along California's central coast.
- Assess toxicity of neonicotinoids on a wider variety and greater number of BMI taxa.
- Better understand the effects of neonicotinoids on specific BMI taxa by identifying to species.
- Assess the impacts of other common neonicotinoids on BMIs.

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6 Appendices

Appendix A – Results of GLM produced using stepwise AIC model selection.

Deviance Residuals

Minimum	1st Quartile	Median	3rd Quartile	Maximum
-0.26804	-0.10504	-0.02174	0.12400	0.2585

Parameters[I]	Coefficent	Estimate Std.	Error t value	P value
(Intercept)	0.604464	0.076245	7.928	5.01E-08
Pct Urb2016	0.006284	0.002711	2.318	0.0297
Pct Ag2016	-0.002041	0.001185	-1.722	0.09842
Pct Imperv2011Cat	-0.012009	0.004129	-2.908	0.00791
Concentration[II]	-0.014839	0.017826	-0.832	0.41373

Null Deviance	1.11766	27 Degrees of Freedom
Residual Deviance	0.55724	23 Degrees of Freedom
AIC	-18.215	
Number of Fisher Scoring Iterations		2

[I]Parameter abbreviations: Pct Urb2016 = percent urban cover from NLCD (2016), Pct Ag2016 = percent agricultural cover (consolidated cropland and pasture) from NLCD (2016), Pct Imperv2011Cat = percent of impervious cover from StreamCat metrics (2011), Concentration = measured neonicotinoid concentrations from CEDEN.

[II]Neonicotinoid concentrations added post-hoc.

Appendix B – Standards used for assessing normal and abnormal behavior of BMI taxa.

FFG	Common Name	Normal Behavior	Abnormal Behavior
Grazer	Scud	• Swimming well • Responsive to stimuli • Clinging to food or netting	• Trouble swimming • Lying on its side in the bottom of the beaker
Grazer	Cased Caddisfly	• Clinging to side, netting, food • Retracts into case when stimulated • Crawls around when taken out of cold water	• Not clinging • Takes a long time to retract or crawl around
Grazer	Cookie-headed mayfly	• Clings to side, netting, instruments • Swimming well	• Trouble swimming • Cannot cling
Predator	Damselfly	• Responsive to stimuli • Clings to netting • Can right itself	• Cannot cling • Will fall to bottom, cannot move itself from being belly up
Predator	Common stonefly	• Responsive to stimuli • Clings to netting • Can right itself	• Cannot cling • Will fall to bottom, cannot move itself from being belly up
Filter feeder	Caddisfly	• Responsive to stimuli • Clings to netting • Crawls around when taken out of water	• Unresponsive • Does not uncoil when stimulated • Takes a long time to move
Filter feeder	Black fly	• Responds to stimuli • Clings to bottom	• Unresponsive • Cannot cling
Collector	Minnow mayfly	• Swimming well • Clings to netting	• Trouble swimming • Cannot cling
Collector	Worm	• Responsive to stimuli • Wrapped around netting	• Unresponsive • Cannot wrap itself in netting
Scraper	Mud snail	• Responsive to stimuli • Retracts into shell when prodded • Stuck to side	• Unresponsive • Lays on bottom of beaker with foot out