The Transport of Chemicals and Biota into Coastal Rivers and Marine Ecosystems

By

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Abstract

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Coastal rivers link terrestrial and freshwater systems to oceans. River networks drain watersheds, delivering freshwater, nutrients, sediment, chemicals, and biota to estuaries and coastal ecosystems. These influences can negatively and positively affect downstream receiving water bodies. Effects of rivers on oceans depend on rates of transport and export of river-borne materials from channels versus rates of in-channel processing: degradation, storage, or biological uptake of those materials. In California, under a Mediterranean climate, biotic transformations slow and fluxes increase during the winter months, which coincide with higher river flows.

I studied effects of two river-borne substances on coastal oceans: pyrethroid insecticides and riverine algae. Pyrethroids are in widespread use in both agricultural and urban environments in California. In upstream reaches of the Salinas River, in Central California, sediment samples collected in agricultural and urban creeks were found to be toxic to a common test species, and the mass of pyrethroids present in all these sediments could explain the measured toxicity. While compositional differences in sediment pyrethroid mixtures between the agricultural an urban land uses were not dramatic, movement of pyrethroids short distances from the areas of terrestrial application to downstream waterways was confirmed.

Suspended sediments sampled from three coastal rivers during storm events showed that pyrethroids were routinely discharged from these rivers. Sediments in estuaries and downstream reaches of the rivers contained concentrations of pyrethroids above those expected to be toxic. However pyrethroid residues were not detected in bed sediments of the continental shelf or in the deep sea, presumably due to dilution or degradation.

Coastal rivers can also transport nutrients, organic matter, or biota. We collected algal drift in the Eel River as it entered the estuary of the Eel in Northern California. The major component of this drift during summer base flows was benthic macroalgae which had detached from upstream aquatic habitats, while a winter storm delivered more terrestrial material. Marine and freshwater macroalgae were fed to interstitial estuarine invertebrates, both in an experiment examining preferences of free-swimming benthic isopods and amphipods, and in an experiment examining consumption rates of enclosed amphipods on various types of algae. These experiments showed the quick consumption and preference for freshwater algae. The flux of freshwater algae into the

estuary and its quick consumption suggest that fluxes of riverine algae into marine ecosystems are likely to disappear rapidly due to preferential grazing, and would be easy to overlook as potentially important trophic subsidies for estuarine or coastal marine ecosystems.

Dedication:

I dedicate this thesis to my advisors, Mary Power and Donald Weston; thank you for the support and advice. Second, thank you to all the field assistants who braved the mud and water, including Buddy Betts, this work would not have been possible without your help.

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Patterns of Pyrethroid Contamination and Toxicity in Agricultural and Urban Stream Segments

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Abstract:

Pyrethroid insecticides are in widespread use in both agricultural and urban environments. In order to understand if there are systematic differences in the composition of pyrethroid mixtures found in sediment arising from runoff from these two land uses, and to compare their toxicological effects, sediment samples were collected from three creeks in and around Salinas, California. Pyrethroids were present in sediments from both agricultural and urban reaches of all three creeks. Sediment from all sampling locations in both agricultural and urban areas was toxic to *Hyalella azteca*, an amphipod commonly used for sediment testing, and, in all cases there was sufficient mass of pyrethroid present in the sediment to explain the measured toxicity. The organophosphate chlorpyrifos likely contributed to toxicity in one instance. While the compositional differences in sediment pyrethroid mixtures between the land uses were not dramatic, there was a tendency for cyfluthrin and cypermethrin to be typical of urban areas, and lambda-cyhalothrin to be found in agricultural reaches. Bifenthrin and permethrin were somewhat characteristic of urban and agricultural areas, respectively, though either land use could be a potential source.

Introduction:

Pesticides are used widely in urban areas, where insects are both nuisances and, in some cases, vectors for disease. Due to the withdrawal of some of the most widely used organophosphates, pyrethroid pesticides are now used extensively in urban environments, whether applied by homeowners or professional pest controllers. Over 327,000 kg of pyrethroids were used by professional applicators for structural pest control and landscape maintenance in California in 2005 (www.cdpr.ca.gov/docs/pur/purmain.htm), and although data are not publicly reported, retail sales to homeowners can be assumed to be considerable. Recent research points to bifenthrin, cyfluthrin and cypermethrin, as the greatest cause for concern in creeks within residential areas, being the most frequent contributors to aquatic toxicity in streams in and around Sacramento, California (Weston et al. 2005, Amweg 2006).

While a dramatic increase in commercial and home use of pyrethroids has been reported, agricultural use of pyrethroids has been relatively steady in California over the past decade, ranging from a low of 105,000 kg in 1999 up to 142,000 kg in 2005 (the most current data available). However, some industry segments, like almond and stone fruit production, have reported a reduction in organophosphate use with an increased use of pyrethroids (Epstein et al.

2000). As a result of this widespread use, agriculture-affected water bodies may contain pyrethroid residues in the sediments, with permethrin the most commonly found (Weston et al. 2004, Weston et al. 2008). However, since permethrin is among the least toxic of the pyrethroids to aquatic life (Solomon et al. 2001), the pyrethroids bifenthrin, lambda-cyhalothrin and esfenvalerate are more frequently found at concentrations associated with toxicity. Pyrethroids are believed to be responsible for toxicity in agricultural-affected sediment samples in about 60% of the instances when toxicity to the standard toxicity testing species, *Hyalella azteca*, is observed (Weston et al. 2008).

Thus, pyrethroids are widely used in both agriculture and urban areas, and both uses have resulted in sediment contamination of creeks within the watersheds. However, when both agricultural and urban areas are in close proximity to one another, it may be difficult to distinguish the sources of the pesticides. Downstream toxicity may not be traceable to a single well-defined source since both urban and agricultural subwatersheds can deliver runoff into the same waterbody, and thus contribute pyrethroids and aquatic toxicity to that water body. In order to make informed management decisions, take regulatory action, or inititate mitigation, it is necessary to be able to discriminate among the potential pyrethroid sources.

While research has been conducted on pyrethroids from agricultural and urban land uses, comparisons between the two different uses have not been made. This study explores the relative toxicological impact and compositional differences in the pyrethroid mixtures of urban and agricultural areas. If such differences exist, it may be possible to develop characteristic "fingerprints" of pyrethroids from both land uses in order to guide management actions.

Materials and Methods:

Study area:

Salinas, California was chosen as the study site because of the close juxtaposition of urban and agricultural land uses. Salinas is the county seat of Monterey County, and a major urban center with a population of approximately 150,000 people. In addition to residential housing, the city includes associated commercial and industrial development, much of which supports the agricultural industry. The farmland surrounding the city produces salad vegetables (e.g., lettuce, spinach) as well as many other fruits and vegetables (e.g., strawberries, broccoli, cauliflower, celery, artichokes, and wine grapes). Agricultural production is heavily dependent on irrigation, for annual rainfall is approximately 33 cm, and largely limited to November through April. The city itself is surrounded by agricultural lands, but is unique in that is has a flood control basin in the center of the city, Carr Lake Regional Park, which is also used for agriculture (http://www.ci.salinas.ca.us).

Three creeks run through the city: Gabilan Creek, Natividad Creek, and Alisal Creek, the later being renamed Reclamation Ditch at a point southeast of Salinas where the water course turns to the northwest (Figure 1). All three creeks originate in undeveloped land in hills northeast of the city, flow through agricultural lands, through the city, and then back in to agricultural lands. The three water courses join in the Carr Lake area. The combined flow from all three water courses leaves Carr Lake via the Reclamation Ditch, which flows northwest and finally empties into Tembladero Slough and ultimately into the Pacific Ocean in Monterey Bay. Flow into the creeks varies dramatically with season. During the winter, large storm events produce the greatest amount of flow through the creeks (e.g., up to about 200 cfs at US Geological

Survey gage in Reclamation Ditch; http://waterdata.usgs.gov/ca/nwis). During the summer, flow rates are very low (about 3 cfs in Reclamation Ditch) and the minimal water present is return flow from irrigated agricultural fields or urban runoff from landscape irrigation.

Sampling Procedures:

Background samples, intended to have little or no pesticide residue, were taken from Gabilan and Alisal Creeks upstream of any urban or agricultural development (Table 1; Stations SG1 and SA1). There was no comparable background site accessible on Natividad Creek. Two to three additional sampling sites were established along each watercourse as they passed through agricultural lands, and then through the city of Salinas. When possible, particular effort was made to establish sites just upstream of transition points between agricultural and urban land uses, so that those sites would be indicative of the integrated effects of the upstream land use (e.g., agricultural) and just prior to the inputs from the downstream land use (e.g., urban). Urban portions of Natividad and Gabilan creeks consisted largely of single-family residential development, with only minor commercial influence. Urban sites along Reclamation Ditch were a mix of residences, commercial establishments, and industry.

Samples were collected on September 23, 2005, prior to the onset of the winter rains. At each site, the upper one centimeter of the surface sediment in the creek beds was skimmed off with a stainless steel scoop and transferred into solvent-cleaned glass jars. The finest-grained sediments (silts and clays) available at each site were collected since pyrethroids are strongly hydrophobic and associate with the organic fractions of the sediment. In the lab, sediment was homogenized by hand mixing, and then held at 4ºC for toxicity samples, and -20ºC for chemistry samples.

Analytical Methods:

Chemical analysis of the sediment was done using the methods outlined in You et al. (2004). Briefly, the sediment sample was sonicated with 50 ml of a 50:50 mixture of acetone and methylene chloride. Three extractions were done, with the extracts combined and solvent exchanged to hexane. Clean-up was performed using Florisil (Thermo Fisher Scientific, Waltham, MA), deactivated with distilled water, and elution from the column with 30% diethyl ether in hexane. Florisil extracts were solvent exchanged to hexane, reduced to 1 ml, 25 mg of primary/secondary amine (PSA) was added, and the samples shaken for 2 min. Following centrifugation, the supernatant was analyzed on an Agilent 6890 series gas chromatograph with an Agilent 7683 autosampler and an electron capture detector (Agilent Techologies, Palo Alto, CA). Two columns from Agilent, a HP-5MS, and a DB-608 were used. The seven pyrethroids quantified were: bifenthrin, lambda-cyhalothrin, esfenvalerate, deltamethrin, permethrin, cyfluthrin, and cypermethrin. Analytes also included one organophosphate, chlorpyrifos, and 21 organochlorines, including: alpha-, beta-, delta-, and gamma-BHC, heptachlor, heptachlor epoxide, alpha- and gamma-chlordane, alpha- and beta-endosulfane, endosulfan sulfate, p,p'-DDE, p,p'-DDD, p,p'-DDT, aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone, and methoxychlor.

Grain size was determined using wet sieving, and total organic carbon was measured using a CE-440 Elemental Analyzer from Exeter Analytical (Chelmsford, MA), following acid vapor treatment to remove inorganic carbon.

Toxicity Testing:

Ten-day toxicity tests were performed using 7-10 day old freshwater amphipods, *H. azteca*, according to standard U.S. Environmental Protection Agency protocols (EPA 2000). Using 8 replicates for each sediment sample, about 50-75 mL of sediment, and about 250 mL of overlying water were added to 400 ml glass beakers. Tests were conducted at 23ºC, with a 16 h light: 8 h dark cycle, with feeding of 1 ml of yeast/cerophyll/trout chow per beaker per day. Fresh water was delivered with an automatic water delivery system that provided two volume additions (500 ml) daily using Milli-Q purified water, made moderately hard by added salts. Water samples for pH, conductivity, alkalinity, hardness, and ammonia were taken at the beginning and end of the test; dissolved oxygen and temperature were monitored regularly. Mortality of amphipods was determined by sieving sediment on a 425 µm screen, and determining the proportion of the initial 10 amphipods per beaker that survived the 10-d exposure.

Toxicity data were analyzed using ToxCalc 5.0 software (Tidepool Scientific Software, McKinleyville, CA). Each batch of test sediments tested included a control sediment from San Pablo Dam Reservoir (Orinda, CA), and survival in test sediments was statistically compared to the control using a t-test with arcsine transformation. Control survival ranged from 86-95%.

The concentrations of each pyrethroid in the sediments were used to calculate toxic units (TU) with respect to *H. azteca* as:

$$TU = \frac{\text{Actual concentration of pyrethroid in sediment}}{\text{Known 10-d LC50 for } \textit{H. azteca}}$$

Since pyrethroids are strongly hydrophobic, both the actual concentration and the LC50 were organic carbon (oc) normalized. The reported 10-d sediment LC50 values were as follows: cypermethrin = 0.38 µg/g oc, lambda-cyhalothrin = 0.45 µg/g oc, bifenthrin = 0.52 µg/g oc, deltamethrin = 0.79 µg/g oc, cyfluthrin = 1.08 µg/g oc, esfenvalerate = 1.54 µg/g oc, permethrin = 10.83 µg/g oc (*9, 10*). Pyrethroid TUs were assumed to be additive due to the common mode of action of compounds within the class.

Results:

The sediment samples consisted of fine-grained material ranging from 20-85% fines (silts and clays combined) with a median of 41% fines. The percent total organic carbon of the sediment samples ranged from 0.6 – 4.4% with a median of 2.0%.

All sediments were tested for acute toxicity to *H. azteca*, and only minimal mortality was seen in the designated background sites, prior to the creeks entering agricultural lands (Table 1: SA1 and SG1). SA1 had only 4% mortality; SG1 had 14% mortality. While the later value was statistically different (probability < 0.05) from the concurrent control sample with 5% mortality, the mortality rate in a later control test was comparable to the SG1 sample, and the 14% mortality seen at SG1 is not considered to be a meaningful difference.

The remaining 11 other sediment samples collected in the study were significantly toxic, with mortality rates ranging from 31-100% (Figure 2). The highest mortality was seen in two urban sites; the mixed use urban site of SR2 (100% mortality) and the residential area of SN3

(96%). Substantial toxicity was seen in agricultural sites as well, with 90% mortality at SN1, 84% mortality at SA2, and 84% mortality at SG2. All three of these sites were in agricultural reaches of their respective creeks, prior to the creeks entering any urban development. There was little overall difference in the toxicity of agricultural and urban reaches, with a median mortality of 68% among the urban sites and 75% among the agricultural sites.

The two background sites contained no detectable pyrethroids (Table 2). However, pyrethroids were present at every other site, whether in areas of agricultural or urban land use. Permethrin was the dominant pyrethroid, and generally typified the agricultural reaches of the creeks. However, it was also found in some urban areas (e.g. SR2, SN2, SN3). It can not be conclusively determined from the existing data whether the permethrin residues in urban areas represent input from the surrounding urban landscape, or transport from more upstream agricultural areas. At only one site (SA2) was the permethrin concentration above the estimated 10-d sediment LC50 for *H. azteca*. At several sites concentrations were about one-third that threshold.

Bifenthrin was present at most sites, and its concentration reached at least half the *H. azteca* LC50 at five sites (SR1, SR5, SN2, SN3, SG3). On Natividad and Gabilan Creeks, the compound was clearly associated with urban land uses, with no measurable bifenthrin in sediments from agricultural regions, but then increasing to over 10 ng/g in urban areas. In Reclamation Ditch the data suggest both urban and agricultural bifenthrin sources.

Among the other pyrethroids, lambda-cyhalothrin tended to be associated with agricultural reaches, and attained concentrations at least half the LC50 at three sites (SA2, SR1, SR2). Cypermethrin and cyfluthrin attained their highest concentrations in urban reaches. Esfenvalerate concentrations were far below the LC50, and the compound was not clearly associated with one particular land use.

Sediment concentration data for the non-pyrethroid analytes are not shown, but concentrations were generally not toxicologically significant at least with respect to explaining *H. azteca* mortality results. Chlorpyrifos was nearly always below 20 ng/g, which would represent about one-third of a TU given the median organic carbon content in the samples (2.0%) and the reported chlorpyrifos LC50 to *H. azteca* (2.97 µg/g oc; (Amweg and Weston 2007)). The sole exception was SR5 where chlorpyrifos reached 68 ng/g, or 1.6 TU given the organic carbon content at this site (1.4%). The organochlorine pesticides or their degradation products were frequently detected but well below acutely toxic concentrations to *H. azteca*. The most commonly detected were DDE (maximum 254 ng/g), DDD (max. 234 ng/g), DDT (max. 152 ng/g), dieldrin (max. 40 ng/g), endrin (max. 14.9 ng/g), alpha-chlordane (max. 8.5 ng/g) and gamma-chlordane (max. 7.0 ng/g). These concentrations were all below 0.1 TU given the LC50 estimates of Weston et al. (2004).

The pyrethroid concentrations alone showed a strong relationship to *H. azteca* mortality as observed in the sediment toxicity tests (Figure 3). Not only did mortality show a significant increase concurrently with increasing pyrethroid TUs, but the overall pattern suggested 50% mortality occurred at about one TU (0.6-1.4 depending on sample), precisely the relationship that would be expected if pyrethroids were the dominant contributor to toxicity. Assuming additive toxicity among the pyrethroids such that the compound-specific TUs could be added to derive a total pyrethroid TU, every site, excluding the two background locations, contained at least 0.5 TU. Six of the eleven sites reached or exceeded one TU. Even without the additivity assumption, a strong pyrethroid contribution to toxicity is still suggested with eight of the eleven sites reaching at least 0.5 TU and three reaching one TU.

There is also limited evidence from toxicity identification evaluation (TIE) procedures for a contributing role of pyrethroids. Sample SR2, which contained potentially toxic concentrations of lambda-cyhalothrin and cypermethrin, was tested with addition of an esterase enzyme to the overlying water (Weston et al. 2008). The enzyme is intended to cleave the ester bond present in pyrethroids, substantially reducing the toxicity. Without esterase, the SR2 sediment caused near complete mortality; with esterase 38% of the *H. azteca* survived (Weston and Amweg 2007), supporting the suspected role of pyrethroids in explaining the toxicity. Results from TIE manipulation of a second sample containing lambda-cyhalothrin at probable toxic concentrations are less conclusive. Sediment SN1 was tested in a dilution series with addition of piperonyl butoxide (PBO) to the overlying water, a procedure which makes pyrethroids more toxic. Without PBO in the overlying water the LC50 of SN1 sediment was 29.8% (expressed as percent original sediment when diluted with control sediment; 95% confidence interval of 23.3-38.2%) (Amweg and Weston 2007). With PBO, the LC50 was reduced to 19.8% (16.2-24.3%). While the PBO did increase the toxicity (decreasing the LC50) as expected if lambda-cyhalothrin were the toxicant, the decrease was not as dramatic as usually seen with PBO, and the LC50 confidence intervals did slightly overlap. Thus, the results from the SN1 sample were inconclusive and the presence of another unidentified toxicant in the sample remains a possibility.

Discussion:

It is clear that pyrethroids from both agricultural and urban uses are reaching the creeks of Salinas, and that they are usually present in the fine sediment in these creeks at concentrations acutely toxic to *H. azteca*, a species widely used for sediment toxicity assessment. This observation is consistent with prior studies in agricultural areas of California (Weston et al. 2004, Weseton et al. 2008, Anderson et al. 2006, Phillips et al. 2006) and work in urban creeks of the Sacramento, California and San Francisco Bay areas (Weston et al. 2005, Amweg et al. 2006). Toxicity testing with *H. azteca* of both agricultural and urban reaches of Salinas creeks commonly showed acute mortality, and there is evidence from both toxic unit analysis and TIE procedures that pyrethroids were the major contributor to this toxicity. The organophosphate chlorpyrifos was also likely a contributor at one agricultural site. Although this compound no longer has appreciable use in urban environments, it is still widely used in agriculture.

This study indicates that the differences between an agricultural pyrethroid ‘fingerprint’ and an urban one are not dramatic, but yet some distinctions could be made. Cyfluthrin and cypermethrin were characteristic of urban-affected stream reaches. Bifenthrin was also commonly found and attained highest concentrations in sediments located in urban areas, though it has agricultural sources and uses as well. On the other hand, lambda-cyhalothrin was distinctly found in agriculture-affected samples. Permethrin was characteristic of sediments found in areas with both land uses.

California is unique in that commercial use of pesticides requires reporting of that use to the California Department of Pesticide Regulation, including the compound applied and the amount used. For the most part, the pesticide-related land use distinctions made on the basis of the Salinas creek data are supported by reported use data from California as a whole, and specifically from Monterey County in which Salinas is located (Table 3). The usage data as well as the environmental monitoring both support the primarily urban sources of cyfluthrin, the agricultural sources of lambda-cyhalothrin, and the dual sources of permethrin and bifenthrin.

The only significant difference between the Salinas findings and pesticide use data is cypermethrin, for which dominant urban use is suggested by the creek sediment data and from statewide use statistics, but in Monterey County use is primarily agricultural.

The use data (Table 3) also suggests that some other pyrethroids are distinctly urban or agricultural, though those distinctions could not be made with the Salinas creek data set. The presence of deltamethrin would be a clear marker of urban sources, since its agricultural use is negligible. Similarly esfenvalerate and fenpropathrin are likely to be from agricultural sources because of very limited non-agricultural use. It should, however, be recognized that Table 3 excludes retail sales, as that data are not tracked by California agencies with the level of detail available for commercial pesticide applications. For example, esfenvalerate can be found in some retail products sold for home and garden use. Finally, it should be recognized that these distinctions apply only to pyrethroid use in California. There are likely to be regional differences in crops produced and pesticides applied which prevent broad national generalizations. A pyrethroid that may have only non-agricultural uses in one area of the country could be a significant agricultural insecticide in another, and thus the distinctions made here would have to be reassessed in other locations.

The very fact that there were differences in pyrethroid composition among the sampling sites suggests that the sediments on which the pyrethroids are adsorbed may be transported fairly limited distances. Water-soluble pesticides can travel considerable distances (Kuivila and Foe 1995), but being particle-associated, pyrethroid dispersal may be more limited. Our most downstream site, SR5, located approximately 4 km downstream of Salinas contained only esfenvalerate and bifenthrin, with no evidence of the lambda-cyhalothrin, permethrin, cypermethrin and cyfluthrin known to be present in more upstream locations. This conclusion may be a consequence of the timing of sampling. Sediments were collected in September, near the end of the dry season when flow is very low and limited to irrigation runoff. Major winter storm events, typically beginning in December in the Salinas area, may be important in promoting sediment transport over greater distances, and blurring the land use distinctions evident in dry season sampling.

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Figure 1: Map of Salinas (a) illustrating the creeks sampled, Alisal Creek (SA1, SA2) and Reclamation Ditch (SR1, SR2, SR3, SR4, SR5), Gabilan Creek (SG1, SG2, SG3), and Natividad Creek (SN1, SN2, SN3). Flow is generally from the east to the west. The urban areas are shaded gray. The white agricultural area in the center of the city represents Carr Lake.

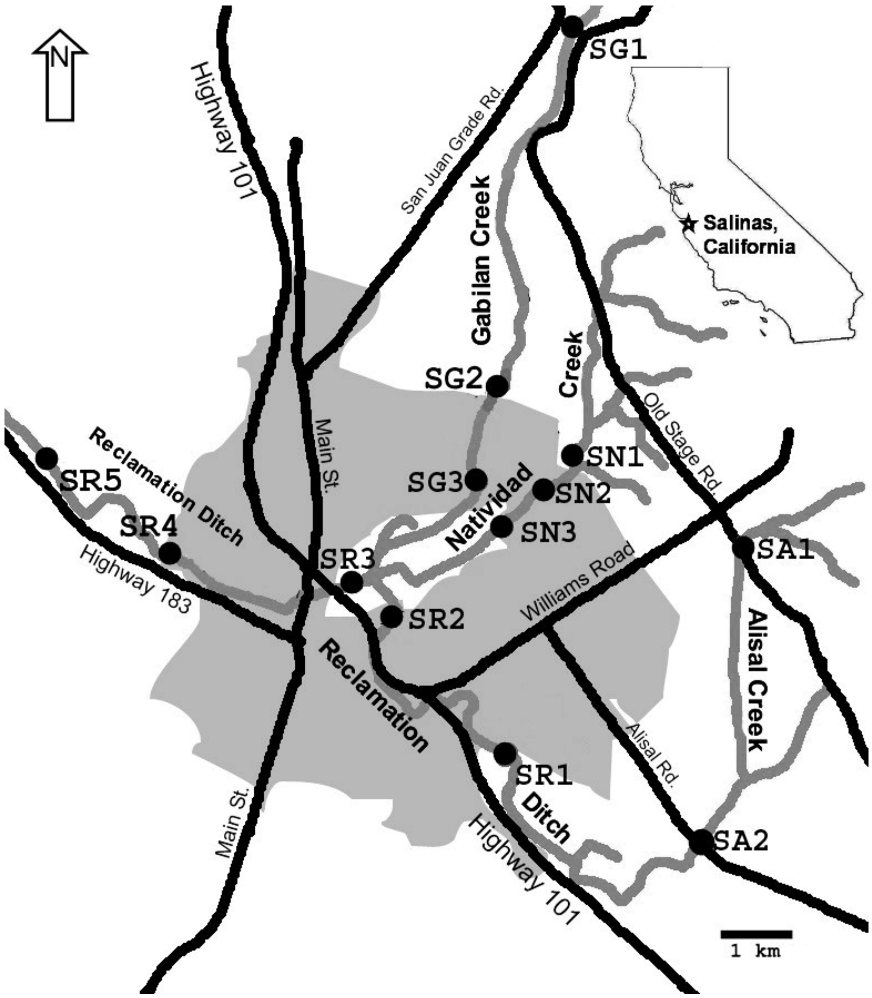

Figure 2: Percent mortality of Hyalella azteca when exposed to sediment from the sampling sites. Shading indicates the classification of each site into background, agricultural, mixed urban (residential/commercial/industrial), or residential.

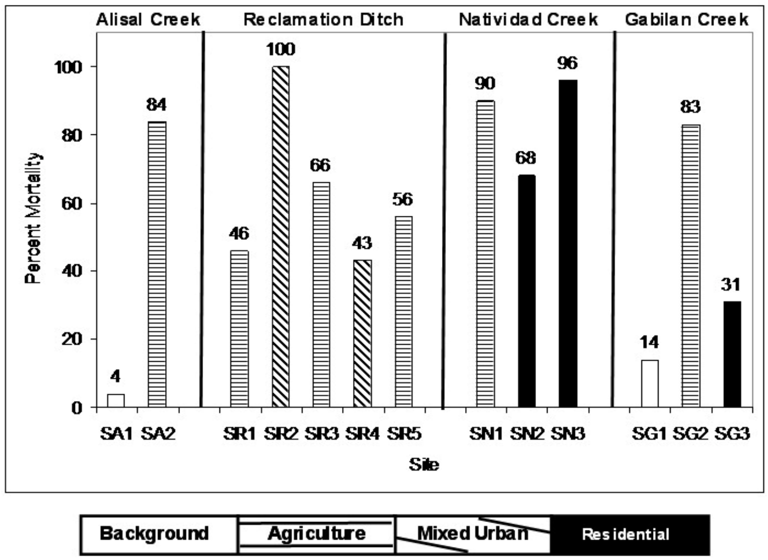

Figure 3: Graph of the percent mortality of Hyalella azteca at each site in Salinas, CA in relation to the sum of pyrethroid toxic units (TU).

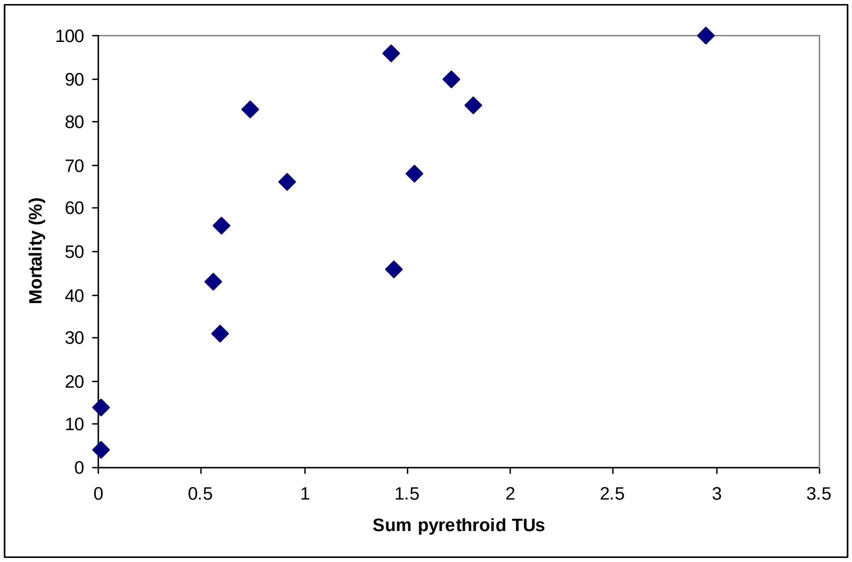

Table 1: Locations and descriptions of sampling sites along the three water courses in and around Salinas

Site name	**N Latitude W Longitude**	**Site description**	**Surrounding land use**
Alisal Creek/Reclamation Ditch			
SA1	36.69238 121.56915	Alisal Creek @ Old Stage Rd.	Point where creek transitions from undeveloped land to agricultural land
SA2	36.64567 121.57698	Alisal Creek @ Alisal Rd.	Agriculture
SR1	36.65858 121.61379	Reclamation Ditch @ Moffett St.	Agricultural area, just prior to creek entering commercial district
SR2	36.67978 121.63735	Reclamation Ditch @ Cesar Chavez Park	Mixed commercial and residential
SR3	36.68507 121.64772	Reclamation Ditch @ Sherwood Dr.	Edge of agricultural Carr Lake, just prior to creek entering commercial district
SR4	36.68426 121.66735	Reclamation Ditch @ Victor St. and Victor Way	Commercial district, just prior to creek entering agricultural lands
SR5	36.70475 121.70525	Reclamation Ditch @ San Jon Rd.	Agriculture
Natividad Creek			
SN1	36.70202 121.60262	Natividad Creek @ Boronda Rd.	Agriculture just prior to creek entering residential area
SN2	36.69887 121.61067	Natividad Creek @ Freedom Pkwy.	Residential
SN3	36.69020 121.62151	Natividad Creek @ Gee St.	Residential
Gabilan Creek			
SG1	36.78040 121.58541	Gabilan Creek @ Old Stage Rd.	Undeveloped land
SG2	36.71553 121.61643	Gabilan Creek @ Boronda Rd.	Agriculture just prior to creek entering residential area
SG3	36.70030 121.62196	Gabilan Creek @ Independence Blvd. and Lexington Dr.	Residential

Table 2: Pyrethroid concentrations (ng/g, dry weight basis) in the sediments at the sampling sites, with sites shaded based upon surrounding land use. ND indicates not detected (<1 ng/g). Deltamethrin was among the analytes but was never detected at any site. The number of Hyalella azteca toxic units (TU) each concentration value represents, given the sediment organic carbon content, is shown in parentheses. Bifenthrin = Bif, Cyfluthrin = Cyf, Cypermethrin = Cyp, Esfenvalerate = Esf, Lambda-cyhalothrin= Lam, Permethrin = Per

Background	Agricultural	Mixed urban	Residential

Site and land-use	**Total organic carbon (%)**	**Bif**	**Cyf**	**Cyp**	**Esf**	**Lam**	**Per**
Alisal Creek/Reclamation Ditch							
SA1	3.57	ND	ND	ND	ND	ND	ND
SA2	0.56	ND	ND	ND	ND	1.6 (0.6)	72.0 (1.2)
SR1	2.51	7.4 (0.6)	ND	2.1 (0.2)	4.3 (0.1)	5.5 (0.5)	14.1 (0.1)
SR2	1.84	4.0 (0.4)	3.5 (0.2)	7.0 (1.0)	3.4 (0.1)	6.8 (0.8)	82.5 (0.4)
SR3	1.99	3.4 (0.3)	ND	ND	1.6 (0.1)	2.0 (0.2)	67.8 (0.3)
SR4	2.00	1.2 (0.1)	1.1 (0.1)	2.7 (0.4)	ND	ND	8.1 (<0.1)
SR5	1.39	4.0 (0.6)	ND	ND	1.0 (0.1)	ND	ND
Natividad Creek							
SN1	0.93	ND	ND	ND	ND	6.8 (1.6)	9.0 (0.1)
SN2	2.99	10.5 (0.7)	3.7 (0.1)	5.1 (0.5)	1.4 (<0.1)	3.0 (0.2)	11.3 (<0.1)
SN3	2.15	8.8 (0.8)	ND	4.6 (0.6)	1.0 (<0.1)	ND	9.3 (<0.1)
Gabilan Creek							
SG1	4.40	ND	ND	ND	ND	ND	ND
SG2	1.87	ND	ND	2.0 (0.3)	ND	1.0 (0.1)	68.8 (0.3)
SG3	4.02	10.7 (0.5)	ND	1.0 (0.1)	ND	ND	5.4 (<0.1)

Table 3: Relative agricultural and non-agricultural commercial use of pyrethroids in California as a whole and in Monterey County in which Salinas is located (2005 data; www.cdpr.ca.gov/docs/pur/purmain.htm). Non-agricultural use consists largely of applications by professional pest control firms, and figures do not include retail sales to homeowners for which comparable data are not available. The table also shows whether the compound was characteristic of urban or agricultural stream segments in the current study.

Pyrethroid	Statewide agricultural use (kg)	Statewide non-agricultural use (kg)	Monterey County agricultural use (kg) and primary crop	Monterey County non-agricultural use (kg)	Finding in current Salinas study
Bifenthrin	9,439	18,748	297 Strawberries	175	Largely urban but some agricultural
Cyfluthrin	7,810	14,526	11 Lettuce	63	Urban
Cypermethrin (including S-cypermethrin	14,070	92,068	3138 Lettuce	140	Urban
Deltamethrin	38	6,238	0	19	Not detected
Esfenvalerate	14,780	118	1555 Artichokes, lettuce, broccoli	0	Undetermined
Fenpropathrin	17,940	3	2295 Grapes, strawberries	0	Not measured
Lambda-cyhalothrin	10,296	6,298	1390 Lettuce	2	Agricultural
Permethrin	67,796	183,110	9900 Lettuce, spinach, celery	519	Both urban and agric.

Pyrethroid insecticide transport into Monterey Bay through riverine suspended solids

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Abstract:

Pyrethroid pesticides are used widely in both agricultural and urban landscapes. Toxicity has been recorded in creeks and rivers throughout California, confirming pyrethroids move at least short distances from the areas of terrestrial application into downstream waterways. However, further downstream transport into the marine ecosystem has received little study. The Monterey Bay was chosen as the study system in the current project due to the close proximity of both urban centers and intense agriculture. Suspended sediments were sampled from three major rivers during storm events and showed that pyrethroids were routinely discharged from these coastal rivers, with concentrations of bifenthrin and permethrin in suspended solids as high as 22 and 83 ng/g, respectively. These suspended solids deposited in estuaries and downstream reaches of rivers as they approached the coast, where concentrations of pyrethroids in the sediment were above those expected to be toxic. However, despite transport onto the continental shelf, pyrethroid residues were not detected in bed sediments of the shelf or in the nearby deep sea canyon, presumably due to dilution and degradation.

Introduction:

Pyrethroids are current-use insecticides, applied for agricultural purposes and urban pest management. They are a synthetic form of pyrethrin, which is a natural insecticide produced by some chrysanthemums. Acting as a neurotoxin, pyrethroids prolong the time that the sodium channels of neurons remain open, causing hyper-excitation of the cells and paralysis of the organism. Due to the withdrawal of some of the most widely used organophosphate insecticides from urban uses, pyrethroid use has been on the rise, for example, with nearly 422,000 kg used by professional applicators in California in 2010 (http://www.cdpr.ca.gov/docs/pur/purmain.htm). This number is limited to commercial applications, and does not include retail sales that are likely to be quite significant, as pyrethroids are the most widely available insecticide sold to homeowners.

Unlike organophosphates, pyrethroids are highly hydrophobic, with log octanol water partition coefficients in the range of 4-7 (Laskowski 2002). This hydrophobicity results in association of the chemicals with the organic carbon in sediment. The major routes of degradation are hydrolysis (particularly under alkaline conditions), photodegradation, or biodegradation. Depending on conditions and the specific compound, degradation can take months in soils (Laskowski 2002) or years in sediments (Gan et al. 2005). If pyrethroids have not degraded at their site of application, they can be transported from the treated lands to surface waters via suspended solids carried by irrigation or stormwater runoff (Gan et al. 2005). These pyrethroid-contaminated sediment particles can then affect downstream aquatic organisms (Amweg et al 2005; Anderson et al. 2006; Holmes et al. 2008).

The Salinas River watershed is one of the richest agricultural areas in California. Several major urban areas are in the watershed, with over 65% of the area as rangeland and pastureland, and 20% in agriculture. However, it is the intense agricultural lands found in the Salinas Valley and the wide variety of crops grown there that have earned it the name, "America's Salad Bowl." Strawberries, broccoli, spinach, lettuce, celery, and artichokes are some of the dominant crops, all of which are treated with pyrethroids. Monterey County, in which the Salinas Valley lies, used 3,958,629 kg of pesticides in 2010, including 17,674 kg of pyrethroids.

Previous work has highlighted the fact that agricultural pyrethroids used in the area have been found in river sediments and can cause toxicity to freshwater invertebrates (Anderson et al. 2003, Anderson et al. 2006). Urban use of pyrethroid pesticides has led to their appearance in Salinas River tributaries. These compounds have been identified as the cause of toxicity in 80% of samples taken in and around the city of Salinas (Ng et al. 2008). While these studies have confirmed transport over short distances from areas of application into nearby creeks and rivers, further transport downstream into the marine environment has been studied only in highly urbanized southern California embayments (Lao et al. 2010, 2012; Anderson et al. 2010).

The Salinas River is the largest of three major rivers that flow into Monterey Bay. It provides approximately 1,400,000 tons of sediment to Monterey Bay annually (Griggs and Hein 1980). During infrequent winter high flow events, the Salinas River breaches the coastal dunes, and discharges directly to Monterey Bay. However, during more typical low to moderate flow conditions, the Salinas River flows to the landward side of the coastal dunes, then turns north to become the Old Salinas River for 7 km, eventually reaching Monterey Bay via Elkhorn Slough. While the Salinas River is probably the cause for most concern with regards to pesticide use, due to the amount of agriculture and urban development within its watershed, two other rivers may also be of significance. The Pajaro River, with a watershed much smaller than that of the Salinas River, contributes 220,000 tons/yr of sediment to the Monterey Bay (Griggs and Hein 1980). Agriculture comprises about 25% of the watershed area, but pockets of coastal urban areas in the Pajaro watershed could also prove to be important inputs of residential use pyrethroids. On the other hand, the San Lorenzo River's watershed is mostly forested, with areas of urban development and residential use, including the city of Santa Cruz, California. This river contributes 140,000 tons/yr of sediment to the Monterey Bay (Griggs and Hein 1980) and provides a good contrast to the previous two, more agricultural, watersheds. These three major watersheds that flow into Monterey Bay are of special interest due to their discharge into the coastal waters of the federally-protected Monterey Bay National Marine Sanctuary off the central coast of California.

For both urban and agricultural applications of pyrethroids and other pesticides, summer irrigation or winter rains can transport residues into creeks and rivers. The main routes of riverine transport to Monterey Bay are the Salinas River and, secondarily, the San Lorenzo and Pajaro Rivers. As stated above, the sediment loads from each river are considerable, and that sediment potentially carries with it hydrophobic pyrethroid pesticides. Sediment from the rivers first enters the Bay and then deposits on the continental shelf, where the major accumulation of fine-grained, organic-rich sediments is found along the 80-m isobath (Eittriem et al. 2002). These soft sediment deposits at the head of Monterey Canyon may occasionally fail, transporting sediments and any associated pesticides into the deep-sea environment through episodic turbidity flows down submarine canyons.

The Monterey Bay and its three major rivers may be an ideal location with which to study agricultural and urban pyrethroid pesticide transport via suspended solids, and their eventual incorporation into benthic marine sediments. The objectives of the current study were to determine the concentrations of pyrethroids in suspended solids in three major rivers whose watersheds drain into the Monterey Bay and determine the concentrations of pyrethroids incorporated in the bedded marine sediments from the Monterey Bay and Canyon.

Materials and Methods:

Suspended sediment sampling:

River water samples were taken for analysis of suspended solids for pyrethroid pesticides at bridges on the San Lorenzo (36°58'32.41"N, 122° 1'23.32"W), Pajaro (36°52'47.68"N, 121°47'35.40"W), and Salinas (36°43'53.56"N, 121°46'56.95"W) Rivers during major winter rain events in 2008 and 2009. It was thought that these major storms would transport the highest amounts of pyrethroids via suspended solids into the Monterey Bay. All bridges were about 2.5 to 3.3 km upstream from the mouths of the rivers. These three sites were chosen for easy bridge access and close proximity to the mouth of the river to allow for an integration of the whole watershed just before entrance of water and riverine sediments into the Bay. While water at the sites was minimally influenced by tidal action, sampling was performed only after confirmation that the water was not saline. Water samples were collected using a solvent-cleaned stainless steel bailer, and then transferred into stainless steel kegs for transport.

Significant rain events in the region are largely limited to the winter months, from December through March. The heaviest rain events for the 2007/2008 rain year and the bulk of the sampling occurred on January 4 and January 25-27, 2008 at all three rivers. On February 27, 2008 only the San Lorenzo and Pajaro Rivers were sampled due to low rainfall in the Salinas River watershed. Winter storm events for the 2008/2009 rain year were sampled on November 2, 2008 (San Lorenzo); February 15-16, 2009 (San Lorenzo and Pajaro); and March 3-5, 2009 (all three rivers). Samples were a composite of multiple days when a rain event lasted for more than one day. Water temperature was recorded and a subsample of water was taken for total suspended solid analysis at each sample event. Water samples were returned to the laboratory and stored at 4°C. Within 24 hours, the suspended solids were extracted from the water samples using a continuous flow centrifuge (Whisperfuge, GEA Westfalia Separator, Oelde, Germany; 9,000 x G; approximately 10,500 rpm) processing approximately 1.5 L/min. The particle-free discharge of the centrifuge was discarded, and the sediment recovered from the centrifuge bowl was stored in solvent-cleaned amber glass bottles at -20°C.

Bed sediment sampling:

The finest-grained sediments and highest amounts of sediment deposition in Monterey Bay are known to occur along the 80 m isobath of the continental shelf (Eittriem et al. 2002), so a transect along the 80 m isobath was sampled to make sure the most recently deposited sediment was sampled for the analysis (Figure 1; Table 1). Additional samples were taken between this isobath and each of the river mouths. Benthic grab samples were taken with a modified Van Veen grab sampler on April 15-18, and May 16, 2008. The upper 2 cm of sediment were skimmed from each grab using a stainless steel spatula, transferred into a solvent-cleaned glass jar, and stored at 4°C until they were returned to the laboratory. In the lab, the samples were homogenized by hand mixing and subsampled for chemical analysis. Sediment for chemical analysis was transferred into solvent-cleaned amber glass jars and frozen.

Our primary benthic sampling in early 2008 happened to occur after a period of relatively low rainfall, with an average of 45 cm of rain falling throughout the watersheds of the three rivers in the winter of 2007-2008. In order to determine if results may have been different in years with greater runoff, historic bed sediment samples were obtained from the Central Coast Long-term Environmental Assessment Network (http:/www.cclean.org). This group had archived samples spanning the years of 2000 to 2006, taken at sites along the 80 meter isobath, and stored at -20°C. Six samples were chosen for chemical analysis from October 2005 and November 2006, all from the 80 m isobath off the mouths of the three rivers (Figure 1). The archived samples had been taken after heavy rain years, when the potential for pyrethroid transport into Monterey Bay would have been greater. In the winters of 2005/2006 and 2006/2007, there was an average of 92 and 73 cm of rain throughout the watersheds, respectively.

To determine if pyrethroids were reaching the deep sea, sediments from Monterey Canyon were taken, sampling the finest-grained material available. Nine sediment cores were taken with plastic push cores about 40 cm long, and 8 cm in diameter using the ROV Tiberon and Ventana on three different cruises (Figure 1; Table 1). Only cores with minimal biotic disturbance visible were used for chemical analysis. Sediment was extruded from the cores, and the upper 1-2 cm of sediment were skimmed off for pyrethroid analysis. This sediment was transferred to solvent-cleaned amber glass jars using a metal spatula, and stored at -20°C.

To test for estuarine impacts of pyrethroids, samples were taken at sites in the Elkhorn Slough Research Reserve on June 5, 2008 (Figure 2). The Old Salinas River and Elkhorn Slough receive inputs from other agriculture-affected tributaries, so they may contain pesticide residues from sources other than the Salinas River. Six bed sediment samples were taken along the Old Salinas River and throughout Elkhorn Slough. The top 1 cm of sediment was collected with a stainless steel spatula and stored in a solvent-cleaned glass jar. In the laboratory, the sediment was homogenized by hand mixing and subsampled for chemical analysis.

Chemical analyses:

Frozen sediment samples were thawed, homogenized, and freeze-dried to remove excess water (You et al. 2008). Approximately 3-5 g of dry sediment was mixed with 2 g of treated copper, 5 g of silica, and 30 g of diatomaceous earth. Sediment samples were extracted using a Dionex 200 Accelerated Solvent Extractor (Dionex, Sunnyvale, CA, USA). Extractions were completed using a 1:1 (v/v) methylene chloride-acetone mixture at 100°C and 200 psi for two static cycles of 5 min.. Extracts were then dried with 12 g of anhydrous Na_2SO_4 and concentrated to 1 ml in a TurboVap II evaporator (Zymark, Hopkinton, MA, USA) under a constant stream of N_2 gas, 50°C, and 15 psi. Further clean-up was performed by elution through an Envi-Carb II/PSA Solid Phase Extraction column. After solvent exchange into hexane, the eluent was then concentrated to 1 ml under a gentle stream of N_2 using a Pierce Model 1878 Reactivap^{TM} (Rockford, IL, USA). Further clean-up with copper was necessary for a number of samples due to residual sulfur. Analysis of final extracts was performed on an Agilent 6890 series gas chromatograph with an Agilent Technologies 7683 autosampler and micro-electron capture detector (GC-ECD, Agilent Technologies, Palo Alto, CA, USA) using both HP-5MS and

DB-608 columns. Quantification of the analytes was performed on the column with the least amount of background interference. Samples were analyzed for eight pyrethroids, including bifenthrin, lambda-cyhalothrin, esfenvalerate, deltamethrin, permethrin, cyfluthrin, cypermethrin, and fenpropathrin. The method detection limits ranged from 0.2-0.6 ng/g and a uniform 1 ng/g reporting limit was used for all analytes.

Sediments were analyzed for organic carbon content on a CE-440 elemental analyzer from Exeter Analytical (Chelmsford, MA, USA) following acid-vapor treatment to remove inorganic carbon.

Results and Discussion:

Grain size and organic carbon:

Grain size was analyzed for the bed sediment samples collected in the Elkhorn Slough estuary and on the Monterey Bay continental shelf (Table 1). Samples in the estuary were primarily muddy (25-97% silt and clay) with the exception of two sandy sites at the mouth of Elkhorn Slough. Continental shelf samples were, as expected, muddiest along the 80 m isobath, with 39-95% silt and clay. Shelf samples in shallower water, closer to the river mouths, tended to be sandier, with 2-65% silt and clay.

Organic carbon was determined for three bed sediment samples in Elkhorn Slough and the Old Salinas River (ESK, ESP, and ESM). The organic carbon content was 2.97, 3.06, and 2.32%, respectively.

River suspended solids:

Pyrethroids were found in all but one sample (Pajaro River; February 15-17, 2009). Bifenthrin was the most commonly found pyrethroid with detection in nearly all samples (Table 2). Concentrations commonly exceeded 10 ng/g, and reached a maximum of 21.6 ng/g. Permethrin was the second most common pyrethroid, found in all but two suspended solid samples in 2007/2008, though not at all in 2008/2009. The reason for the absence of permethrin in 2008/2009 is unknown, since its use in Monterey County only declined 20% from the prior year. Permethrin was also the pyrethroid having the highest concentration, with 83.0 ng/g in the Salinas River suspended sediment sample of January 4, 2008. In fact, the same sample also showed the highest amount of cypermethrin (23.4 ng/g) and esfenvalerate (42.0 ng/g). It is helpful to put these concentrations in toxicological context by comparing them to LC_{50}s for sensitive benthic invertebrates, though it is recognized that such organisms would not be exposed until the suspended material was deposited as bed sediments. Assuming an organic carbon content of about 2%, some of these concentrations, particularly those of bifenthrin, are well over the LC_{50} for two common amphipod toxicity test organisms: *Hyalella azteca* (a freshwater amphipod) has LC_{50}s ranging from 8-216 ng/g dry sediment and *Eohaustorius estuarius* (an estuarine amphipod) has LC_{50}s ranging from 16-280 ng/g (Table 2). However, no samples had concentrations above the LC_{50}s for *Ampelisca abdita*, a common marine amphipod testing species. These LC_{50} concentrations were determined at temperatures standard for these species, between 15-23˚C depending on the organism. However, temperatures more typical of the Monterey Bay benthic environment are closer to 10˚C for depths ranging from 30 to 80 m, and closer to 5˚C for the deep-sea canyon. Since pyrethroids are more toxic at lower temperatures,

LC_{50} values *in situ* are expected to be one-half to one-third the values listed in Table 2 (Weston et al. 2009).

There were no apparent trends in amounts or types of pyrethroids found from one storm to the next during a rain season. There were relatively minor differences in pyrethroid composition and concentration in the suspended sediments of the three rivers, presumably due to varying land uses between the three watersheds. Lambda-cyhalothrin, fenpropathrin, and esfenvalerate were found in the Salinas and Pajaro watersheds, but were rare or absent in the San Lorenzo watershed. These three pyrethroids are mainly agricultural pesticides and the Salinas and Pajaro watersheds have a large agricultural influence, while the San Lorenzo River watershed is primarily undeveloped land or residential, with only a minor agricultural contribution. The broad uses of the remaining pesticides, encompassing both urban and agricultural applications, make the dominant sources hard to distinguish.

The present study addressed only the particle-associated pyrethroids, and did not consider the potential for water column toxicity due to the dissolved fraction. It has been shown that pyrethroids can be found in sufficient concentrations in the water column to cause toxicity to sensitive invertebrates (Weston and Jackson 2009, Weston and Lydy 2010; 2012). If such toxicity occurs in the Monterey area, it would probably be limited to the freshwater river environments, comparable to locations where it has been previously documented, and would not likely be a concern in the marine environment following dilution with coastal waters.

Estuarine sediments:

No pyrethroids were found in the Elkhorn Slough estuary. However, just upstream of the estuary, on the Old Salinas River, two sites had detectable amounts of pyrethroids. At ESP all pyrethroids were found except for cyfluthrin. The highest concentration was 19.7 ng/g of esfenvalerate, a pyrethroid typically of agricultural sources. However, bifenthrin and cypermethrin were the pyrethroids of greatest toxicological concern, when concentrations are viewed in the context of known amphipod toxicity thresholds. The other Old Salinas River site, ESM, contained bifenthrin and esfenvalerate, with the compound of greatest toxicological concern being esfenvalerate at 35.6 ng/g. While bed sediments in Elkhorn Slough and seaward are unlikely to represent a risk of acute lethality to aquatic life due to pyrethroids, concentrations in the Old Salinas River are sufficient to cause toxicity to sensitive benthic invertebrates.

Marine sediments:

In Spring 2008, 25 samples were taken throughout the Monterey Bay continental shelf, from the 80 m isobath and shallower. None of these samples contained detectable amounts of pyrethroids (< 1 ng/g). Six archived bed sediment samples from previous years with greater rainfall (2005 and 2006) from the 80 m isobath were analyzed, and also showed no detectable pyrethroids. Finally, all seven of the deep-sea samples from Monterey Canyon, collected by a remotely operated vehicle from depths of 167-3165 m, also contained no detectable pyrethroids.

While transport of pyrethroids into the bay is occurring, as evidenced by the suspended sediment data, pyrethroids were not detected in bed sediments of the coastal bay or in the deep marine canyon, most probably due to dilution or degradation. The three rivers combined provide 2.7 million tons/yr of silt and clay to Monterey Bay (Eittreim et al. 2002; bulk density conversion of Griggs and Hein 1980). Given a median total pyrethroid concentration from suspended sediments in the current study (23 ng/g), Monterey Bay receives approximately 62 kg/yr of pyrethroids from the three rivers, or about 0.4% of the pyrethroids applied annually in Monterey County. Yet dilution with uncontaminated suspended material in coastal waters and with existing bed sediments upon deposition would make it difficult to detect pyrethroids in the bed sediments of the shelf given the methods used. The pyrethroid with the highest median concentration in suspended sediment was bifenthrin, at 10.6 ng/g. With an analytical detection limit of 1 ng/g, about a 10-fold dilution would be sufficient to reduce concentrations to below thresholds of both acute toxicity and analytical detection. While collection of the most freshly deposited material (upper 1-2 mm) might find measurable bifenthrin, it is difficult to collect this material by grab sampling, and the upper 2-cm stratum used probably diluted the most recent material with sediment that had been on the seabed for many years. These findings are consistent with that of a previous study on DDT on the Monterey Bay shelf (Paull et al. 2002). Shelf sediments contained only 10% of the concentration of DDT and its degradates typical of terrestrial drainages in the region, indicating a 90% dilution with cleaner sediments from the ocean or coastal erosion.

In situ degradation of pyrethroids probably also contributed to the lack of measurable residues in marine sediments. Concentrations of DDT have been found in Monterey Bay sediments both on the continental shelf and in the marine canyon (Hartwell 2004, Paull et al. 2002), 30 years after the use of DDT was banned. However, DDT has a half-life of a decade or more and its degradates, DDE and DDD, are similarly persistent. Bifenthrin and permethrin, the dominant pyrethroids in our riverine suspended samples, have sediment half-lives of 0.5-5 years under temperature conditions typical of Canyon benthic habitats (4ºC; Gan et al. 2005). Typical deposition rates in the Canyon range from 2-6 mm/yr (Paull et al. 2002). Given that our canyon samples utilized approximately the upper 15 mm of the sediment column, the material analyzed represented deposition over about 3-8 years, plus the time required for the particles to move into the deep sea.

The absence of measurable pyrethroids in coastal sediments of the present study contrasts with the findings of other studies examining pyrethroids in coastal sediments of Southern California (Lao et al. 2010,2012), in which they were detected in 34% of the areas sampled. Yet this difference can be explained by closer examination of the Lao et al. study sites. Pyrethroids were most commonly detected and at the highest concentrations in the mouths of urban watersheds (e.g., Ballona Creek, Los Angeles River, San Diego Creek) and in the marinas near their points of discharge. The more strictly marine locations (Los Angeles/Long Beach Harbor, San Diego Bay) had the lowest frequency of pyrethroid detection and concentrations. In addition, the fact that the Lao et al. sites were in a highly urbanized area, whereas the Monterey Bay is primarily affected by agriculture, may also be significant, as pyrethroids can be present in urban runoff far more frequently than in agricultural discharges (Weston and Lydy, 2010).

Conclusions:

Terrestrial application of pyrethroid insecticides, in both urban and agricultural environments, are reaching the coastal waters of Monterey Bay through riverine transport. Nearly every sample collected over multiple storm events spanning two years contained pyrethroid residues in suspended sediment at concentrations that, if deposited without dilution, would be acutely toxic to sensitive benthic invertebrates. These sediments do appear to deposit within the downstream reaches of at least some of the rivers as they approach the coast, creating potentially toxic bed sediments. However, no pyrethroid residues could be found on the continental shelf or in the deep-sea environment of Monterey Canyon, from depths of 40 m to over 3000 m. Apparently once the riverine suspended sediments are in shelf waters, dilution with cleaner material (e.g., ocean-derived particles and sediments from coastal erosion) and degradation of the pyrethroids in the coastal environment prevents accumulation of toxic residues in marine bed sediments.

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Figure 1: Map of Monterey Bay and Canyon. Land is gray, with the San Lorenzo, Pajaro, and Salinas Rivers shaded darker to show where they enter the ocean. River sample stations are shown as black dots near river mouths. Marine sample stations are indicated with black dots, with stations deeper than 100 meters labeled. Isobaths are shown at 100-meter intervals, with the 200-m isobath shaded darker to highlight the continental shelf edge.

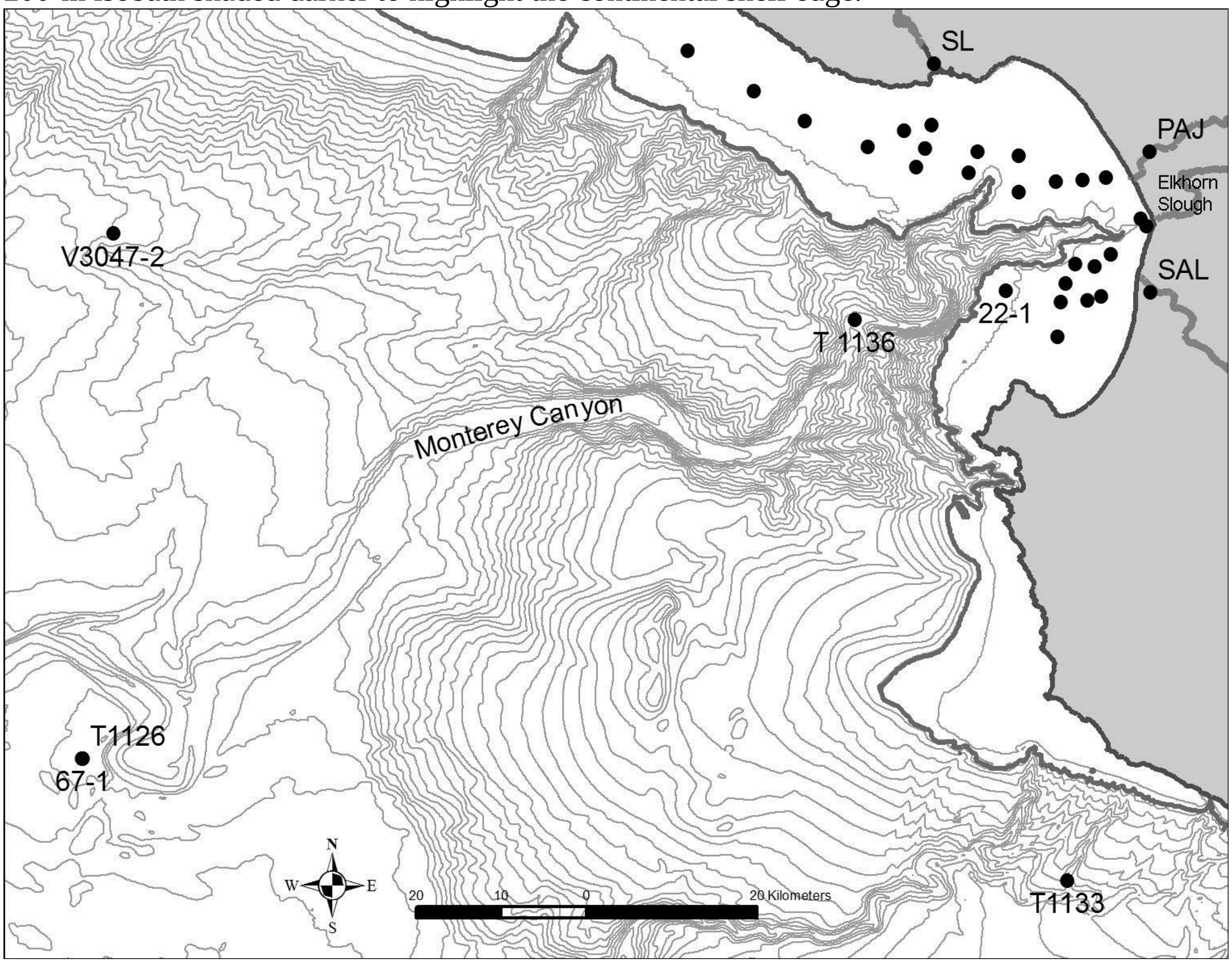

Figure 2: Map of Elkhorn Slough, Pajaro and Salinas Rivers. Land is shaded gray, while water is white. Black dots are labeled with sample station names.

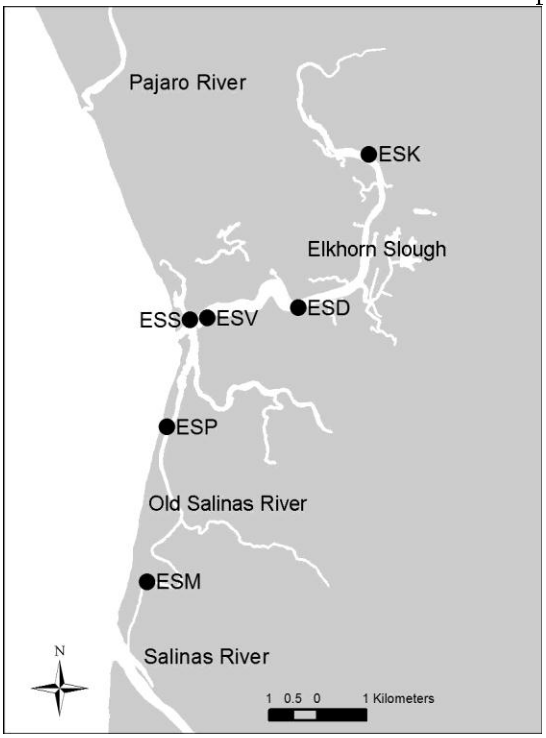

Table 1: Bed sediment samples used for pyrethroid analysis. Including collection date, water depth, latitude, longitude, and percent silt and clay.

Site	Collection date	Water depth (m)	Latitude	Longitude	silt and clay (%)
ELKHORN SLOUGH					
ESK	6/5/08	intertidal	36° 50.430’	-121° 44.636’	92.1
ESD	6/5/08	intertidal	36° 48.730’	-121° 45.648’	66.3
ESV	6/5/08	intertidal	36° 48.635’	-121° 46.911’	2.7
ESS	6/5/08	intertidal	36° 48.610’	-121° 47.150’	15.1
ESP	6/5/08	intertidal	36° 47.427’	-121° 47.485’	97.4
ESM	6/5/08	intertidal	36° 45.703’	-121° 47.789’	25.7
CONTINENTAL SHELF					
SedDep05	4/17/08	83	36° 41.063’	-121° 53.372’	39.1
SedDep06	4/17/08	81	36° 44.437’	-121° 52.882’	86.7
SedDep07	4/15/08	83	36° 51.449’	-121° 59.047’	75.7
SedDep08	4/16/08	81	36° 53.084’	-122° 05.438’	91.9
SAL30	4/16/08	33	36° 46.263’	-121° 50.004’	2.2
SAL50	4/16/08	52	36° 45.503’	-121° 51.039’	13.4
SSAL40	4/17/08	41	36° 43.616’	-121° 50.632’	3.8
SSAL60	4/17/08	61	36° 43.371’	-121° 51.496’	33.4
PAJ20	4/16/08	20	36° 51.156’	-121° 50.316’	2.6
PAJ40	4/16/08	41	36° 50.979’	-121° 51.810’	18.2
PAJ60	4/15/08	61	36° 50.872’	-121° 53.489’	62.3
SL40	4/16/08	42	36° 54.457’	-122° 01.357’	39.2
SL60	4/16/08	59	36° 52.968’	-122° 01.801’	65.2
NSAL	4/17/08	27	36° 48.084’	-121° 47.756’	1.6
SPAJ	4/15/08	30	36° 48.52’	-121° 48.151’	NA
NPAJ	4/15/08	49	36° 52.504’	-121° 55.860’	50.1
SSL	4/16/08	51	36° 52.770’	-121° 58.472’	46.1
NSL	4/16/08	54	36° 54.090’	-122° 03.117’	54.1
SedRef1	4/18/08	82	36° 59.155’	-122° 16.800’	39.4
SedRef2	4/18/08	81	36° 56.615’	-122° 12.610’	81.1
SedRef4	5/16/08	83	36° 54.746’	-122° 09.380’	88.8
SedDep1	4/18/08	81	36° 51.800’	-122° 02.366’	88.0
SedDep2	5/16/08	80	36° 50.245’	-121° 55.871’	95.5
SedDep3	5/16/08	80	36° 45.670’	-121° 52.283’	82.8
SedDep4	4/17/08	80	36° 43.251’	-121° 53.214’	94.0
PREVIOUSLY ARCHIVED SHELF SAMPLES					
SedDep1	10/30/05	81	36° 59.155’	-122° 02.366’	32
SedDep2	10/30/05	80	36° 50.245’	-121° 55.910’	89
SedDep3	10/30/05	80	36° 45.670’	-121° 52.290’	92
SedDep4	10/30/05	80	36° 43.145’	-121° 53.225’	94
SedDep1	10/24/06	81	36° 59.155’	-122° 02.366’	93
SedDep3	10/24/06	80	36° 45.670’	-121° 52.290’	90

Table 1 (continued)

MONTEREY CANYON					
22-1	9/12/07	167	36° 44.000’	121° 56.667’	no data
T1126	8/5/07	3165	36° 14.381’	122° 55.152’	no data
T1133	9/9/07	1133	36° 06.663’	121° 52.789’	no data
T 1136 62-1	9/10/07	1856	36° 42.135’	122° 06.226’	no data
V3047-2	06/28/07	372	36° 47.599’	122° 53.163’	no data
T1136 62-2	9/10/07	1856	36° 42.135’	122° 06.226’	no data
67-1	9/9/07	3165	36° 14.380’	122° 55.150’	no data

Table 2: Ten-day sediment LC_{50}s for *Hyalella azteca*, *Ampelisca abdita*, and *Eohaustorius estuarius* (ng/g sediment assuming 2% organic carbon) provided for comparison with the actual pyrethroid content in suspended sediments from the river mouths and bed sediment in Elkhorn Slough and Old Salinas River. Concentrations of pesticides in dry sediment samples in ng/g. The reporting limit (RL) is 1 ng/g dry weight sediment. nd = not detected, n/a = not available.

Sample	Date	Suspend-ed solids (mg/L)	bifen-thrin	fenpropa-thrin	lambda-cyhalo-thrin	perme-thrin	cyflu-thrin	cyperme-thrin	esfenva-lerate	deltame-thrin
Hyalella azteca	Amweg 2007 Ding 2010		10.4	31.3	9	216.6	21.6	7.6	30.8	15.8
Ampelisca abdita	Anderson 2008		1896	n/a	n/a	17826	n/a	938	n/a	n/a
Eohaustorius estuarius	Anderson 2008		15.8	n/a	n/a	280	n/a	22	n/a	n/a
SUSPENDED SEDIMENT 2007/2008										
SL1	1/4/2008	268.3	1.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SL2	1/26/2008	n/a	4.1	n.d.	3.7	36.0	n.d.	n.d.	<RL	n.d.
SL3	2/24/2008	n/a	10.6	n.d.	n.d.	30.1	n.d.	n.d.	n.d.	n.d.
PAJ1	1/4/2008	53.3	5.3	3.6	n.d.	30.4	n.d.	6.8	n.d.	n.d.
PAJ2	1/28/2008	n/a	21.6	5.3	1.1	33.1	n.d.	n.d.	n.d.	n.d.
PAJ3	2/24/2008	n/a	12.1	n.d.	4.6	n.d.	n.d.	n.d.	<RL	n.d.
SAL1	1/4/2008	37.1	14.0	n.d.	7.6	83.0	n.d.	23.4	42.0	n.d.
SAL2	1/27/2008	n/a	1.1	n.d.	n.d.	24.9	n.d.	n.d.	n.d.	n.d.
SUSPENDED SEDIMENT 2008/2009										
SL	11/2/2008	46.3[a]	10.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SL	2/15-16/09	60.9[b]	12.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SL	3/3-5/09	30.1	6.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PAJ	2/15-17/09	81.3[a]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PAJ	3/3-5/09	89.4[b]	11.2	7.6	4.1	n.d.	n.d.	n.d.	n.d.	n.d.
SAL	3/5/2009	324	3.8	n.d.	3.0	n.d.	n.d.	n.d.	2.1	n.d.
ELKHORN SLOUGH AND OLD SALINAS RIVER										
ESS	6/5/2008		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ESK	6/5/2008		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ESD	6/5/2008		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ESV	6/5/2008		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ESP	6/5/2008		6.4	4.5	2. 0	17.8	n.d.	4.5	19.7	1.8
ESM	6/5/2008		1.7	n.d.	n.d.	n.d.	n.d.	n.d.	35.6	n.d.

The importance of riverine algae in organic matter export to the Eel River estuary Northern California

Charlene M. Ng, Mary E. Power

Introduction:

Coastal rivers deliver freshwater and transport solutes, sediments, organic matter, and organisms to nearshore communities. River discharge transports minerals and nutrients that, if limiting and biologically available, can spur coastal marine productivity. In addition, rivers export particulate organic matter to estuaries and coastal oceans. Organic matter enters drift in rivers from two sources: terrestrial plant detritus (e.g. leaves, wood, seeds, fruits, and stems) and *in situ* primary production of algae, cyanobacteria, aquatic bryophytes, and macrophytes. High in the drainage basin, terrestrial plant detritus generally makes up more than 90% of the standing stock of river carbon in small headwater channels (Mayer and Likens 1983, Vannote et al. 1980). However, in wider, sunlit channels downstream, organic standing stocks and fluxes are often dominated by aquatic primary producers (Vannote et al. 1980, Stevenson et al. 1993). Attached algae often make up most of the primary producer biomass (Stevenson et al. 1993, Power and Stewart 1987). Attached algae enter drift when they detach during bed-scouring flood flows. Algal growths can also slough from substrates at lower base flows when the drag of their biomass exceeds their tensile strength. Drag increases as biomass accrues, while tensile strength declines with senescence and rotting. In addition, grazing or “bulldozing” (Dayton 1971) could detach and export algal biomass (Lamberti et al. 1987). Growth-related sloughing may happen under favorable flow and temperature conditions, while decay-related sloughing may occur during stressful periods, as occur in drought seasons when flows decline and water temperatures become warmer than optimal for algae.

Drifting organic matter, whether terrestrial or riverine in origin, may decay or be consumed near its point of original growth, or it may travel downstream. In rivers draining to the ocean, riverine drift algae could be a potentially important trophic subsidy for nearshore communities (Polis et al. 1997). In general, carbon exports from rivers to oceans have been assumed to be derived primarily from terrestrial vegetation or soil carbon (Meybeck 1982, Onstad et al. 2000). In part, this is because the refractory “fingerprints” of organic matter from terrestrial woody vegetation (lignins and lignin-derived phenols, celluloses and their derivatives) are relatively easy to distinguish and trace (Opsahl and Benner 1997). In contrast, the contributions from riverine algae to organic fluxes from rivers to coastal oceans are poorly quantified and their contributions to coastal productivity are not yet well understood. These contributions may be underestimated if riverine algae exported to coastal ecosystems are chemically labile, or rapidly and preferentially consumed because they are more edible and nutritious than terrestrial plant detritus or marine algae and vascular plants.

We quantified and characterized the types of organic matter in riverine drift just upstream of its entry into the brackish reaches of the Eel River from July through November 2011. We also quantified the feeding preferences and consumption rates of the most conspicuous benthic estuarine primary consumers in the Eel River estuary, interstitial amphipods, on riverine algae versus common marine macroalgae in the Eel estuary.

Site description:

The Eel River Watershed:

The Eel River watershed (Figure 1) is the third largest watershed entirely contained within the geographic boundary of California, draining a total of 9,540 km^2. The river has four major tributaries including the North Fork, Middle Fork, South Fork, and Van Duzen River. It flows northwest, though the California Coastal Ranges, eventually reaching the Pacific Ocean just south of Humboldt Bay, at the Eel River Estuary. Near the mainstem headwaters, two small dams and a diversion divert flow to the south into the Russian River. Logging and road construction have greatly impacted the Eel River, roughly doubling the loading of fine sediments into the channel (TMDL, CDFG 2010). Other human impacts include small towns, limited agriculture, gravel and sand mining, and ranching. However, much of the basin is still forested, and human impacts are relatively low compared with those in most other moderately large river basins.

Under California's Mediterranean climate, the watershed experiences warm, dry summers and cool, wet winters, with bed-scouring floods if rainfall is sufficient. Tectonically-induced coastal uplift and river incision into soft marine sedimentary rocks has produced a steep, canyon-bound channel with fragile, steep walls. This geology, along with modern logging and road construction, has led to very high erosion rates. The Eel River has the highest average annual suspended-sediment yield, normalized to basin area, of all rivers in the United States (Brown and Ritter 1971).

Long-term studies have linked hydrology and food webs to algal dynamics throughout a season and from year to year (Power et al. 1996, Wootton et al. 1996, Marks et al. 2003, Power et al. 2008). These local studies, in combination with basin-wide surveillance and mapping, are being used in efforts to predict algal productivity and food web dynamics in the larger river basin (National Center for Earth Surface Dynamics, NSF-STC annual report 2011). More knowledge of algal productivity, taxonomic composition, phenology, nutritional value, stoichiometry and fate of upstream algae would help explain and predict downstream fluxes of algal-dervied nutrients and organic matter, and their roles in mediating riverine influences on the marine environment.

The Eel River Estuary:

The Eel River Estuary (Figure 2) covers a total of 62 km^2, and is channeled by 64 km of tidally driven sloughs. Tidal changes of up to two vertical meters occur daily, with two low and two high tides within 24 hours. The saltwater influence can reach as far upstream as Fernbridge (40°36'35.14"N, 124°12'04.97"W), 11 km from the ocean (Monroe et al. 1974, CDFG 2010).

In the early 1900s, the Eel River estuary was large enough to be navigable by large ships, but deposition near the mouth of excessive sediment from upstream erosion has left the current estuary shallow with a greatly decreased tidal prism, consisting mainly of sand bars and coastal marshland. Historically, the watershed near the mouth of the Eel River was forested riparian habitat and wetlands, but is now sparsely settled residential and agricultural land, with dairy farms dominant (Williams 1988, Monroe et al 1974). Boles (1977) described the river bed at the mouth of the estuary as sandy, with substrates coarsening to silty sands and fine gravels north of Cock Robin Island. East of the island, Boles reported a bed of sand and coarse gravel, and at

Fernbridge, a coarse gravel bed with cobbles (Boles 1977). With changes in river outflows (discharge shifting towards the northern outlet channel), the entire estuary and river mouth is now gravel bedded, and the bed at Fernbridge remains dominated by pebbles, cobbles, and gravel (C. Ng, personal observations 2008-2012).

Oceanographic context:

Northern California experiences large coastal summer upwelling, bringing nutrients from the deep ocean to the western coast of North America. This upwelling supports considerable marine productivity. Upwelling ceases or reverses to downwelling during fall, winter, and early spring months. During these months, organic material, nutrients, and micronutrients in riverine flow may have particular importance in the marine ecosystem. Under Mediterranean seasonality, the winter months also coincide with higher river flows that could increase these fluxes.

<u>Biota:</u>

Algae:

The dominant macroalga in the Eel River (and in many circumpolar rivers and lakes worldwide (Whitton 1970)) is the green alga, *Cladophora glomerata* (L.) Kutz. 1843. This filamentous alga branches and can grow to lengths of several meters, vastly increasing the ecological surface area available in the river for ecological production. Rough cell walls and the absence of mucilage on surfaces of *Cladophora* make filaments easy to colonize by epiphytes (Stevenson and Stoermer 1982). As *Cladophora* growth peaks in mid-summer, filaments become covered with epiphytic diatoms. These epiphytes change the color of *Cladophora* proliferations from an initial bright green of clean growth, to a yellowish color mid-summer, and finally to a rusty red in the late summer months. Changes in color correspond to changes in the thickness of the carotenoid-laden epiphytic diatoms encrusting the host filaments and in diatom species dominance (Power et al. 2009, Furey et al. 2012). Earlier diatom assemblages dominated by monolayers of *Cocconeis* spp. are eventually replaced by later successional multi-story assemblages dominated by other diatoms (*Epithemia* spp.) that host endosymbiotic nitrogen-fixing cyanobacteria. Freshwater diatom frustules have been found in marine cores taken from the deep marine canyons off the continental shelf 10 km west of the mouth of the Eel River (Sculley et al. 2009), confirming the transport of freshwater algae, at least of diatom frustules, to the coastal ocean. *Epithemia*-covered *Cladophora* proliferations mediate two important riverine-aerial exchanges: 1) They increase areal rates of nitrogen fixation by 50-60 fold (Welter et al. in preparation), introducing a limiting nutrient, nitrogen, from the atmosphere into the riverine ecosystem; 2) They elevate rates of insect emergence from the river up to 25-fold, suggesting that the nitrogen fixing *Epithemia* and their cyanobacterial endosymbionts are highly nutritious (Power et al. 2009). After midsummer, massive *Cladophora* proliferations decline due to grazing, senescence, or sloughing, and persist as low residual stubble 5-10 cm high. These residues are overgrown by late successional stages of green algae (typically *Mougeotia* sp., *Spirogyra* spp. and *Oedogonium* spp.) that resist epiphytization due to their smooth cell walls and mucopolysaccharide extracellular exudates. Similar seasonal succession occurs in southwestern prairie-margin streams (Power and Stewart 1987) and in the Great Lakes (Stevenson and Stoemer 1982).

Marine invertebrates:

The most abundant invertebrates in the marine substrate are two amphipods (*Ansigammarus confervicolus* and *Corophium stimpsoni*) and one isopod (*Dynamenella dilatata*) (Boles et al. 1977). All three eat algae and detritus (Boles et al. 1977). *Ansigammarus confervicolus* is widely distributed throughout the North American Pacific Coast (Bousfield 1979), and can reach up to 1 cm in length (C. Ng, personal observations 2009-2012). It is adapted to a wide range of salinities, between 5 and 25 parts per thousand, with an optimum between 5 and 10 parts per thousand (Sharp 1980). While usually found under substrates, they can be found drifting during high tides (Levings 1976), and found in the water column at night (Davis 1978). *Corophium stimpsoni* is commonly found in areas with high freshwater influence (Smith 1953), and can reach up to 1 cm in length. *Corophium spp.* build tubes of mucilage and silt which they attach to hard surfaces. *Dynamenella dilatata* has a high tolerance for freshwater, and is found firmly attached to hard surfaces, including rocks and woody debris, but can be found swimming in the water column in the evening (C. Ng, personal observations 2012).

Methods:

Drift sampling:

At Fernbridge, CA where drift samples were collected (Figure 2, Table 1), the entire (9.5 – 84 m wide, depending on discharge) cross-section could be waded until higher flows occurred in late fall. On July 18 and August 15, 2011, seines were set all the way across the river. Ten-meter long seines with six-mm mesh were deployed in a series (1-2 nets at a time) across the river and held in stream 10 to 63 minutes, with longer sampling periods for slower velocity sections (Figure 3). The ends of nets were wrapped around 2.5 cm diameter PVC pipes, and placed over rebar pounded into the riverbed for stabilization. Nets were allowed to bow slightly downstream, so that each net sampled a total of nine meters of the river cross channel. Two to four persons held nets in place, using large cobble or boulders and our feet to press bottom edges of nets on to the river bed so that the whole water column was sampled. At one 8-meter interval near the thalweg, the flow was too fast (> 1.5 m/sec) to hold seine nets in position. To sample this reach, we used large framed drift nets, 75 cm by 100 cm, and 2 meters long, with 6 mm mesh, supported by a 1.9 cm diameter PVC frame (Figure 4). These nets were deployed for 10 minutes by two individuals, who positioned them to completely sample these fast flowing sections. Nets were collected carefully to retain all material captured, and algae and detritus were removed and stored on ice in large plastic bins. On September 25, the sample date when the total discharge was lowest, the river was only 9.5 meters across, so only the 75 cm by 100 cm framed drift nets were deployed in a series across the entire river. All algae were collected in the cod ends, put into plastic bags, and stored in a cooler with ice.

After nets were removed, a water sample (2 L) was collected from each sampled cross-stream interval, 10 cm below the water surface and near where the cross-stream midpoint of each net had been. Flow velocity (using a Marsh McBirney Flo-Mate Model 2000 Flowmeter) and depth measurements were taken at 1 to 3 meter cross-stream intervals to calculate the amount of water sampled by each seine and to determine discharge at Fernbridge (see Appendix Table 1). On six other dates (Table 1), we subsampled drift using the framed drift nets at selected intervals across the river. The number of samples and their spatial deployment on given dates are detailed in Appendix Table 1. During winter storm events (October 8, November 2, November 24, 2011), only one or two framed drift net samples, taken < 10 m from the water's edge, could be safely collected.

To estimate the entire organic matter flux from river to estuary from these subsamples, we assumed that organic matter was relatively uniformly mixed throughout the flow that the proportion of the flux of drifting organic matter captured (Drift sampled/Total Drift) was proportional to the amount of the total discharge we filtered (Q sampled/Total Q) during a given sampling period (Table 1). The total discharge was estimated by adding the discharges recorded at the USGS gaging stations at Bridgeville (on the Van Duzen) and at Scotia (on the mainstem) which accounts for over 90% of the flow of the Eel River mouth (Geyer et al. 2000).

After the sampling period, the seines were carefully lifted to retain the sample, carried to shore, and rolled out on the gravel bar. All organic material was picked out of the nets and divided into freshwater aquatic versus terrestrial plant litter. Samples were put into 33 cm x 23 cm aluminum pans and filled with water to a depth of 3 cm. A 13 x 10 grid was placed over the material and the type of dominant macroalgae under each cell was recorded. This percent cover technique allowed us to roughly characterize the composition of macroalgae and terrestrial detritus in each sample. Freshwater aquatic material was categorized as "green" (relatively lightly epiphytized filamentous green macro-algae, predominantly *Cladophora*, *Mougeotia, Spirogyra*, *Oedogonium,* and *Enteromorpha*); "yellow" (mostly *Cladophora* covered with a monolayer of epiphytes, largely diatoms), "red" (mostly *Cladophora* covered with a deeper multilayered epiphyte assemblage) and *Nostoc* (an easily recognized free-living cyanobacteria with gelatinous colonies often well represented in the drift). Small algal samples of each category were preserved with 3% formalin for microscopic evaluation. These samples were examined under a light microscope using 100x magnification. Ten fields of view were randomly chosen, and filament composition was approximated using a whipple grid. Terrestrial plant litter was characterized as brown alder leaves, other brown leaves, green alder leaves, other green leaves, or twigs (alders were the only nitrogen-fixing riparian plants represented in the terrestrial drift). Samples were then processed for dry weights (oven-dried for over 96 hours at 60 ^{o}C) and ash-free dry mass (combusted for 4 hours at 500^{o}C).

<u>Preference experiments:</u>

Preference experiments were performed south of Cock Robin island (Figure 2). Feeding preferences of free-living, benthic estuarine crustacea for various freshwater and marine algae were compared by observing their recruitment to four types of algal "baits": *Cladophora* that was generally clean or lightly epiphytized (green) and moderately epiphytized with a monolayer of epiphytic cells (yellow) (supplies of rusty (heavily epiphytized) *Cladophora* were inadequate for use in these experiments); and *Ulva* spp. and *Enteromorpha* spp., the two dominant marine primary producers at the site. Similar biomasses (0.5-1.5 g damp weight, n = 5 replicates of each type) of freshwater and marine algae into the Eel River estuary were clamped in a split in plastic pipette bulbs, which were then driven into the gravel bed of the estuary (Figure 5). Colonization and consumption rates of invertebrates on *Ulva* versus green (non-epiphytized or lightly epiphytized) and yellow (moderately epiphytized) *Cladophora* were quantified in 45-90 minute field trials. Algae were partially dried (10 spins in salad spinners lined with 0.3 mm nylon mesh) and damp weighed before and after deployment into the gravel bed.

Consumption rate experiments:

Freshwater algae that drift from a riverine to a marine system may lose mass due to dehydration, consumption by grazers, or fragmentation that is not consumed by grazers. Algae may also lose mass during experimental handling or transport. It was impossible to evaluate biomass disappearance rates from the algae deployed in the open estuary in pipettes for preference experiments, because of unknown weight changes due to sloughing and osmosis (e.g. dehydration by freshwater algae placed in sea water). Consumption rates were therefore evaluated in closed containers (Figure 6), and three controlled experiments were performed on August 1, August 14, and August 30, 2011, to determine the different causes of algal weight loss during incubation in the estuary.

Ansigammarus confervicolus, which recruited quickly by swimming to the algal baits, was chosen for the feeding rate experiments. *Ansigammarus confervicolus* were held in in clear plastic polypropylene cups (9 cm diameter, 8 cm depth) fitted with a "false floor" of 1-mm mesh screen set 1 cm above the bottom of the plastic cup. The screen served as a substrate for the animals and also to isolate them from feces and small algal fragments, to prevent ingestion of particles that would likely be lost during feeding in open environments.

Forty cups were each filled with 200 mL of filtered (SFCA membrane 0.2 µm) natural seawater. Ten cups with similar biomass (see Appendix Table 2 and Appendix Figure 1) of each algal type: *Ulva spp.*, *Enteromorpha spp.*, lightly epiphytized *Cladophora spp.*, and moderately epiphytized *Cladophora spp.* were tested. For each algal type, five cups lacked amphipods (controls) and five cups were each stocked with 40 amphipods on August 1 and August 14, and with 20 amphipods on August 30 (experimental treatments).

Amphipods were starved in experimental cups for 24 hours, after which feces were collected and the seawater replaced. One gram of algae, damp weight was placed into each cup. After 1.5 to 4 hours (3 hours on Aug.1, 1.5-3 hours on Aug. 4, and 4 hours on Aug. 30) algal weight was recorded to determine the amount of algae consumed by the amphipods. In the cups, the loss due to fragmentation was low, with most weight changes due to feeding, dehydration, or rehydration.

After the feeding trial, small algal fragments that fell through the one mm mesh false floor were collected and weighed to estimate losses from fragmentation during feeding. Algal fragments remaining above the 1-mm floor were collected and partially dried in a salad spinner and re-weighed. The amount of algal dehydration was determined by taking the algal weights before introduction to seawater and subtracting algal weights of material retained on the mesh in control cups without grazers after one hour. The amount of algae consumed by the amphipods was calculated as the amount of algae initially added, minus the fragmented algae and the algae retained on the mesh after one hour, after correcting for losses due to dehydration estimated from controls.

Results:

Drift sampling:

Drift collected during all sampling events was dominated (96-99.9% by weight) by freshwater algae, except following spates or large floods (Figure 7). During the baseflow summer season, algal flux was relatively constant, but it increased after storm events that could detach algae, and entrain loose algal material deposited in deep pools or stranded on emergent rocks and along the shoreline.

The extrapolation of samples collected during these storm events from nets deployed near the shoreline could under-estimate actual drift if material transport were concentrated in deeper faster flows further away from the shore. An analysis of flux across cross-sections during baseflow, however, did not show any such trends (Appendix Figure 2).

The composition of drift varies greatly from one sampling event to another. Drifting green filamentous macroalgae was dominated by *Cladophora* in July, and increasingly made up of other genera of green algae later in the summer and early fall (Figure 9). The drift samples collected in July had high proportions of moderately and heavily epiphytized *Cladophora*. By the end of July, the highly epiphytized rust-colored *Cladophora* made up a much lower proportion of material. This type of algae was subsequently found only late in the season, during storm events (Figure 8). After August 18, 2011, clean or lightly epiphytized *Cladophora* had also disappeared from drift samples, and samples were dominated by genera of filamentous green algae that could resist epiphytization (*Enteromorpha, Zygnema, Hydrodictyon, Spirogyra, Mougeotia,* and *Ulothrix*).

Very little terrestrial material (at most, one or two leaves) was collected over the summer and fall. On November 24 2011, however, during the largest storm event sampled in 2011, terrestrial material made up 61% of the total organic flux (Figure 8). This terrestrial flux was dominated by brown leaves which made up 38% of all the organic material collected. So while aquatic flux makes up the majority of the total organic flux during sampling events throughout the summer, large storm events deliver terrestrial material downstream.

Preference Experiments:

Ansigammarus was the most numerous grazer to recruit to algae deployed in pipettes in the open environment (Fig. 10b). Different amounts of algal material were removed on the fifteen different dates that preference experiments were performed. There was a highly significant effect of algal type on loss, with most losses occurring for moderately-epiphytized *Cladophora* ($\chi 2=26.44$, df=3, $p<<0.001$ Friedman 2-way Analysis of Variance). Weight gains observed for *Ulva* and *Enteromorpha* were probably due to re-hydration, as time was too short for algal growth. Because it was impossible to evaluate losses to consumption versus export, dehydration or rehydration in these open trials, we further evaluated amphipod consumption of various algal types, as well as gains and losses from these non-consumptive processes, in small, enclosed containers.

Consumption:

Algal weights of all types of algae changed significantly during the enclosed feeding experiments (Appendix Table 2, Table 4). All freshwater algae decreased in weight, while most of the marine algae increased in weight (Figure 12), likely due to rehydration. Material that went through the 1 mm mesh screen was primarily fecal matter, and not fragmented or shredded algal strands. Since amphipods had been allowed to clear their guts for 24 hours, it was assumed that these were fecal pellets produced from feeding during the experiment.

On August 1 and 30, in the presence of amphipods, freshwater algae lost significantly more weight than marine algae. In contrast, the difference in freshwater and marine algae was not observed on August 14; however, *Enteromorpha* was less reduced in weight by the presence of amphipods than the other three genera.

Decreases in weight due to amphipod grazing were determined by subtracting the weight losses due to fragmentation and dehydration or rehydration in the absence of the amphipods from average losses measured in their presence (Figure 13).

There was a high amount of variation in the feeding experiments on August 30, 2011, which could have been due to the difference in the number of amphipods (half that used in the earlier experiments).

Discussion:

Rivers discharge organic materials that may affect estuarine and coastal productivity. We found that during summer and fall seasons, coarse particulate organic flux from the Eel River, California was dominated by aquatic primary production from freshwater filamentous green macroalgae. Terrestrial plant detritus did not make up a large proportion of the drift until late November, during the earliest winter storms. Composition of this drift changed seasonally. During mid-summer, drifting algae were predominately *Cladophora spp.*, in particular *Cladophora* that was moderately or heavily epiphytized. Epiphytized *Cladophora* has trophic importance for freshwater grazers, as the carotenoid-laden epiphytic diatoms provide a more nutritious diet (Kupferburg 1997), and can support higher rates of emergence for aquatic insect grazers (Power et al. 2009). As the summer season continued, the *Cladophora* was replaced by other filamentous green macroalgae, *Enteromorpha*, *Zygnema*, *Ulothrix*, and *Spirogyra* that lack nutritionally valuable epiphytic diatoms. Later in the year, during early winter storms, epiphytized *Cladophora* again becomes a significant portion of the algal drift. Our methods could not be used during high flows, and other methods, possibly involving aerial surveillance, bridge-based collection and automated sampling are needed to evaluate quantity and composition of total annual organic drift, particularly that exported during storms.

The flux of freshwater algae into the estuary is likely to be underestimated if algae are quickly consumed by benthic estuarine macrofauna, like copepods or benthic shrimp, amphipods, isopods, and polychaetes. However, these fluxes of riverine algae are of potential trophic and biogeochemical importance to downstream estuarine ecosystems. Estuaries are known to be areas of high productivity due to the delivery of nutrients by rivers and the circulation pattern of water in the estuary itself. Terrestrial detritus, particularly coarse organic debris, can have a long residence time once it reaches the estuary or offshore coastal water. However, riverine algae are a much more labile form of riverine drift-- a flux of “hidden carbon” that be quickly consumed by macroinvertebrates or microbes, but provides a substantial trophic and nutrient subsidy to estuaries and coastal water that would be easy to overlook.

The feeding and preference experiments confirm the quick consumption of riverine algae by a common estuarine consumer in the Eel estuary. In both experiments, more epiphytized *Cladophora* was preferred over cleaner *Cladophora*, and both were preferred over the marine algae. In the short-term preference experiments, small free-swimming crustaceans (mostly *Ansigammarus* amphipods) “preferred” (recruited in larger numbers and more rapidly depleted) moderately epiphytized *Cladophora*, with clean *Cladophora* ranked second, *Enteromorpha* third, and *Ulva* least preferred. The higher nutritional value of epiphytized *Cladophora*, could explain the difference in preference for this type of algae.

In consumption experiments with enclosed algae and amphipods, grazing on algae of different types can be compared while taking into consideration effects of export of small particles during experimental handling and grazer shredding, losses from dehydration, and gains from dehydration or rehydration of the freshwater and marine algae. In control cups without amphipods, freshwater algal dehydration was obvious in feeding experiments. Marine algae, which were collected during low tides, had become dehydrated before use in the experiment, and showed significant rehydration during the feeding experiments. This rehydration explains what looks like growth of algae in cups without amphipods. Paralleling the open environment preference experiment, in closed cup feeding trials, moderately epiphytized *Cladophora* was grazed more than the other types of algae, with clean *Cladophora* second, *Enteromorpha* third, and *Ulva* the least consumed. Unfortunately, heavily epiphytized *Cladophora* was not available for these trials; results from studies on diet preferences by freshwater grazers (Kupferberg 1997, Furey et al. 2011) suggest that these "rusty-red" *Cladophora* growths might have been even more intensively preferred and consumed by amphipods. On August 30, 20, rather than 40, amphipods were used in experimental treatments. This difference in amphipod numbers could have resulted in smaller consumption rates than observed on the other two dates.

With the quick consumption of algae in both the preference and consumption experiments, it is not surprising to see so little freshwater algae drifting in the estuary itself. Our capture of riverine algae just before it entered the estuary, and preference and consumption experiments make it clear that freshwater algae are an important part of the aquatic estuarine food web, and can be consumed quickly by aquatic marine invertebrates. These freshwater subsidies can have indirect effects as well. Estuarine invertebrates feeding on river algae would produce solutes or fecal pellets that can then move to the coastal ocean and influence nearshore phytoplankton primary productivity. Estuarine grazers can feed predators, like fish and birds, which may transport these riverine nutrients to offshore (birds and fish) or upland (birds) habitats, possibly over long distances. Birds can nest and defecate in terrestrial areas, while fish can move both upstream into headwaters, or out into the open ocean.

Algal drift entering the estuary that is not consumed locally could be transported directly to the coastal ocean. The proportion of drift that evades estuarine consumers would depend on grazer abundance and behavior, and on tides, flows, and water circulation. On local scales, diel behavioral patterns, movements and feeding rates of invertebrate consumers will affect how much river drift is consumed in estuaries. Some benthic invertebrates, including some species of isopods and amphipods, prefer to feed in the evening, which might increase riverine algae consumption during the evening hours. During low tide, the influence of the river is highest in the estuary, but low salinity can disturb potential consumers, causing them to hide in the benthic sediments. Thus, we would predict that the most likely time period during which freshwater algae can be an especially important subsidy for estuarine consumers would be during low late summer and autumn flows, after freshwater algal production has peaked, before winter storms, and after the upwelling season.

Over larger scales, tides, river discharge, and water circulation patterns can affect the importance of riverine algae subsidies to consumers. Upwelling is well known on the west coast of continents, and on the northern coast of California the upwelling season occurs from April-September. During this time, nutrients from the deep ocean can increase coastal productivity. High tide can bring nutrients from the open ocean into the estuary, which can diminish the importance of nutritional subsidies from the river (Lalli and Parsons 1999). Storm events scour off attached river algae and remobilize algae deposited in pools or stranded on emergent rocks and shorelines upstream. Floods also move algal drift quickly downstream. Storms and consequent stage elevations also entrained leaf litter that had accumulated in headwater channels and along the banks of larger channels into the drift. Early winter floods deliver this organic material to estuaries. However, large floods can flush riverine material quickly out to sea, allowing little access to this food by most benthic invertebrates. Flood-flushed export would deliver riverine terrestrial and algal detritus to offshore coastal consumers and potentially to eventual burial in the deep sea. Sculley et al. (2009) have documented freshwater diatom frustules in deep marine canyons 10 km west of the present mouth of the Eel River. The abundance of these frustules corresponds closely to magnitudes of river algal blooms documented over 20 years upstream in the South Fork Eel River (Sculley et al. in preparation). Further study on the effects of freshwater algae on both open-ocean and deep sea benthic ecosystems is needed to evaluate potential impacts of riverine algal exports on coastal phytoplankton productivity. Blooms of coastal phytoplankton along coastal waters influenced by the Eel River are large, however, even during periods of weak upwelling or downwelling (NASA Giovanni). The importance of algal export for offshore carbon burial is also of interest, as research on ocean burial of carbon has focused to date on organic matter of terrestrial or marine origin.

Conclusions:

The transport of freshwater biota in rivers has been described in studies on drift and subsidies. The river continuum concept highlights the influences of upstream inputs on downstream habitats (Cummins 1975, Vannote et al. 1980). However, previous studies have focused on drift of invertebrates (Wipfli and Gregovich 2002) or terrestrial coarse organic material derived from terrestrial woody vegetation (Naiman 1982). Blair et al. (2003) have documented large carbon loading into the marine environment from the Eel, but did not distinguish the largely refractory terrestrial carbon (e.g., wood fragments) or ancient (keragen) sources from riverine macroalgae and diatoms. In comparison, the occurrence of freshwater macroalgae or periphyton in riverine drift has received relatively little attention, although aquatic primary producers can dominate drift in productive rivers (Angradi 1991, this study).

This study highlights the dominance of algae in drift throughout the summer and early fall months. We quantify short term rates of flux of freshwater algae into the marine ecosystem and show that freshwater algae is a preferred food source for nearshore marine invertebrates living in the shallow, gravelly intertidal zone at the mouth of the Eel River in northern California. Intertidal marine consumers that live well upstream from upwelling zones, like the small interstitial isopods and amphipods that dominate in the shallow Eel Estuary, may benefit year-round or seasonally from riverine exports, with as yet unknown food web and ecosystem consequences. The timing of drift can be important to downstream consumers. Algal influences would lessen during times of high tidal influence, when marine subsidies would be brought into the estuary; or during times of high discharge, when subsidies would pass quickly over the estuary into the open ocean. Marine *Cladophora* and its epiphytes have been known to be preferred food sources for marine invertebrates, including isopods, amphipods, and gastropods (review in Dodds & Gudder 1992, Jansson 1967, Moore 1975). Freshwater *Cladophora* and its epiphytes in drift could be an important subsidy to marine consumers, especially during the fall, when algal production in the estuary and coastal waters decreases.

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Figure 1. Map of sites that have been sampled with drainage areas.

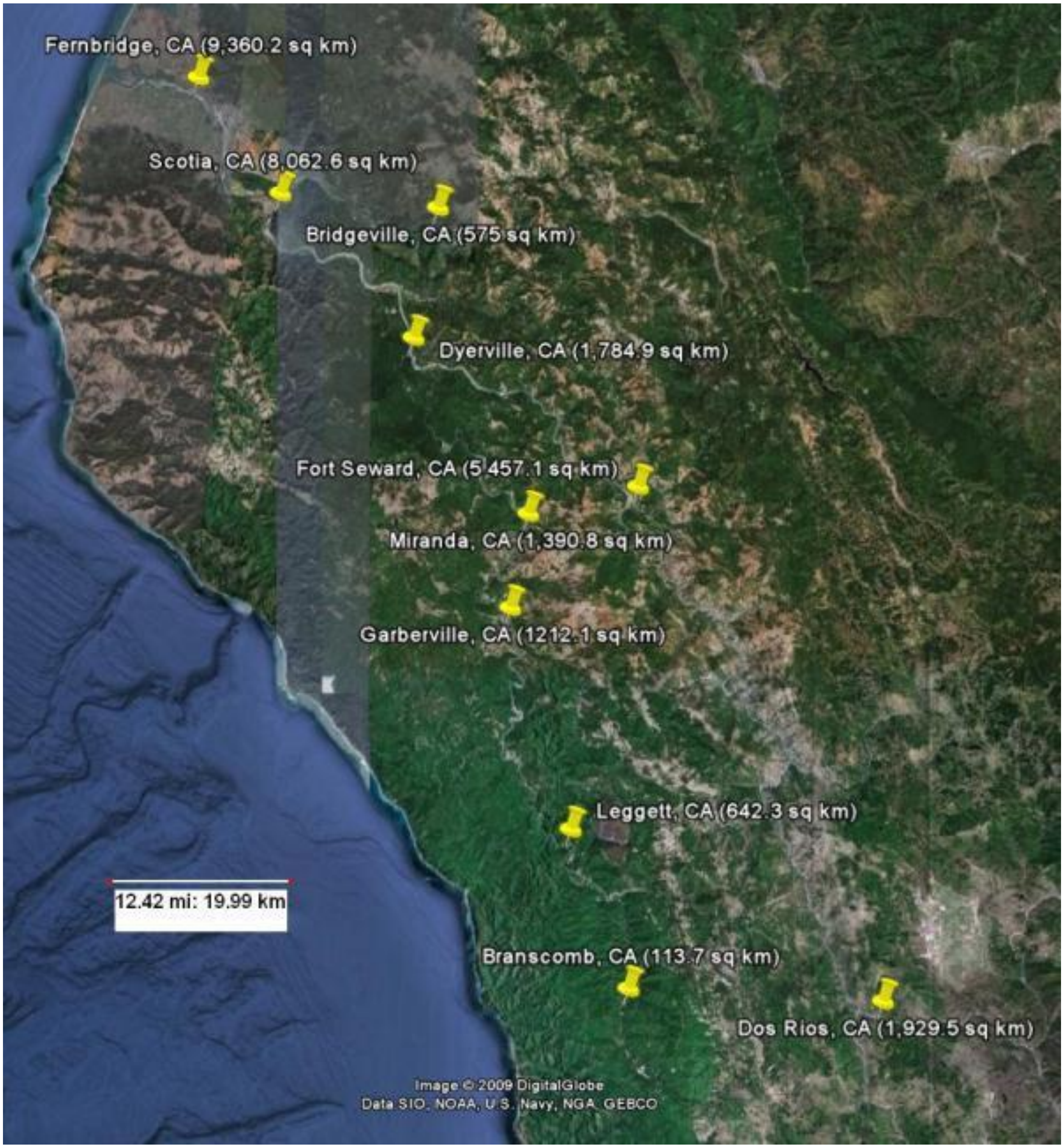


Figure 2: Map of sites in the Eel River Estuary: Crab Park (40º38’36.55” N, 124º18’13.53” W), Cock Robin Boat Dock (40º38’15.85” N, 124º16’57.61” W), South Cock Robin (40º37’11.15” N, 124º16’46.62” W), Fernbridge (40º36’33.20” N, 124º12’0.59” W).

Table 1: Drift net sample descriptions during the summer of 2011, fall of 2011, and early summer 2012.
*Discharge was estimated by assuming that total flow at Fernbridge was the sum of flows gauged by USGS at Scotia and Bridgeville, CA, which are on two different forks of the Eel River.

Date	Cross-section sampled	Sampling period	Discharge sampled (m^3/s)	Total Discharge (m^3/s)	Flux sampled (g/hr)	Total Flux (g/hr)
July 18	Whole	12:30-15:00	23.77	23.77 / 20.64*	14.48	14.48
July 31	1 net every 10 m	15:28-16:47	1.60	13.31*	6.97	57.86
August 15	Whole	11:04-12:38	4.50	4.50 / 7.99*	7.48	7.48
August 31	1 net every 10 m	17:10-18:09	0.83	5.04*	7.24	43.87
September 25	Whole	12:36-14:41	3.52	3.52 / 3.84*	12.42	12.42
October 8	1 m	19:37-19:47	0.50	25.71*	5.39	275.08
November 2	2 m	16:16-16:35	1.07	8.78*	32.49	266.27
November 24	1 m	21:00-21:04	0.55	175.56*	6.82	2188.86

Figure 3: Large seines sampling organic drift at Fernbridge cross-section

Figure 4: Framed drift nets used in sampling.

Figure 5: Algae anchored with disposable pipettes 1.5 cm diameter) into the sediment south of Cock Robin Island, at the beginning of each experiment.

Figure 6: Photos of the closed containers and the experimental set-up floating in the estuary, south of Cock Robin Island.

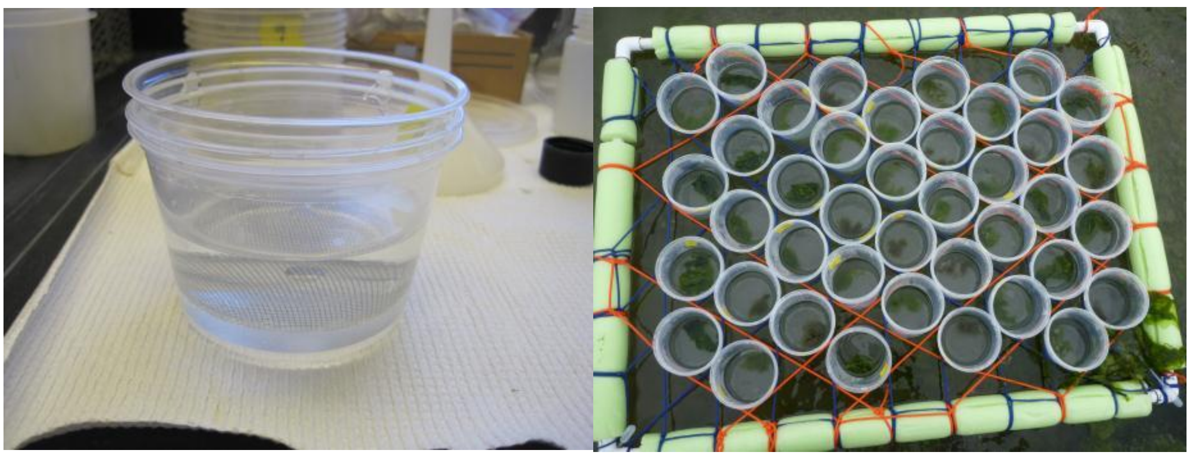


Figure 7: The amount of flux at each sampling date, comparing the amount of aquatic organic material to terrestrial drift. During all sampling events, organic material predominantly originating from the river, while terrestrial material was significant only during the largest storm event sampled (storm events are indicated with blue arrows).

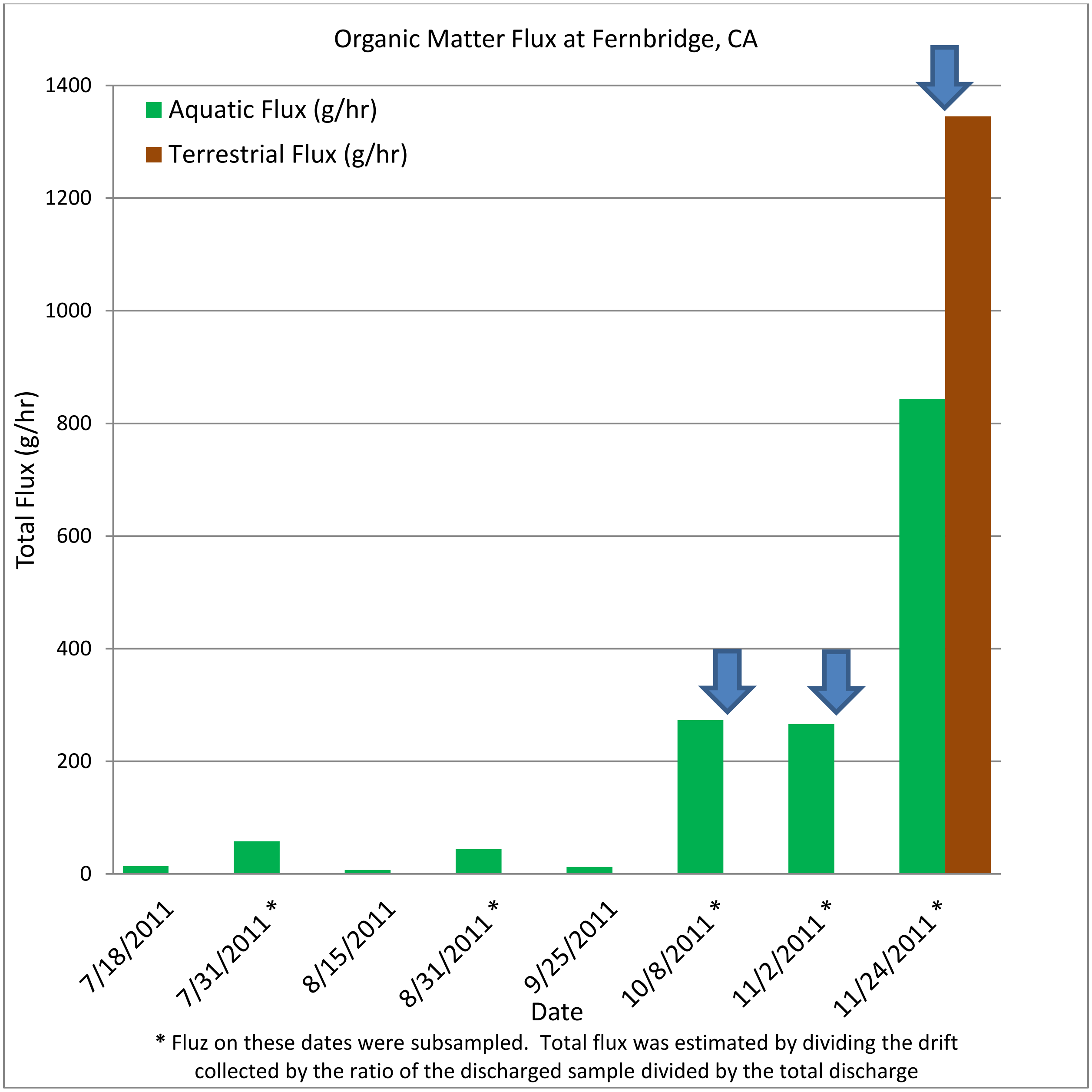


Table 3a and b:

a. Categories used to describe the aquatic drift material collected. Each date is characterized by the percentage of each type of aquatic drift. Macroalgae was characterized as lightly, moderately, or heavily epiphytized.

Date	Macroalgae-light	Macroalgae-moderate	Macroalgae-heavy	Nostoc	Other Aquatic
7/18/2011	25%	47%	27%	0%	0%
7/31/2011 *	73%	26%	1%	0%	0%
8/15/2011	81%	15%	3%	0%	0%
8/31/2011 *	80%	13%	4%	0%	4%
9/25/2011	89%	9%	1%	0%	0%
10/8/2011 *	91%	4%	5%	0%	0%
11/2/2011 *	68%	20%	11%	0%	0%
11/24/2011 *	15%	53%	31%	0%	2%

b. The terrestrial material for each date was characterized and described, with the percentages of each category listed below. Brown and green terrestrial refer to angiosperm leaves that are not from *Alnus* spp.

Date	Brown Alder	Green Alder	Brown Terrestrial	Green Terrestrial	Twig	Other Terrestrial
7/18/2011	0%	0%	15%	18%	10%	58%
7/31/2011 *	0%	0%	0%	0%	0%	0%
8/15/2011	0%	0%	0%	0%	0%	0%
8/31/2011 *	0%	0%	0%	0%	0%	0%
9/25/2011	0%	0%	0%	0%	0%	0%
10/8/2011 *	0%	0%	0%	0%	0%	0%
11/2/2011 *	0%	0%	0%	0%	0%	0%
11/24/2011 *	7%	0%	63%	4%	20%	6%

*indicate flux on these dates were subsampled.

Figure 8: Composition of organic drift from July through November of 2011. Green macroalgae are largely made up of lightly or non-epiphytized *Cladophora*, *Zygnema*, *Mougeotia*, *Spirogyra and Enteromorpha* (see Fig. 9 for proportions of these dominant genera). Yellow and red fractions were largely moderately (mod) and heavily (heavy) epiphytized *Cladophora*.

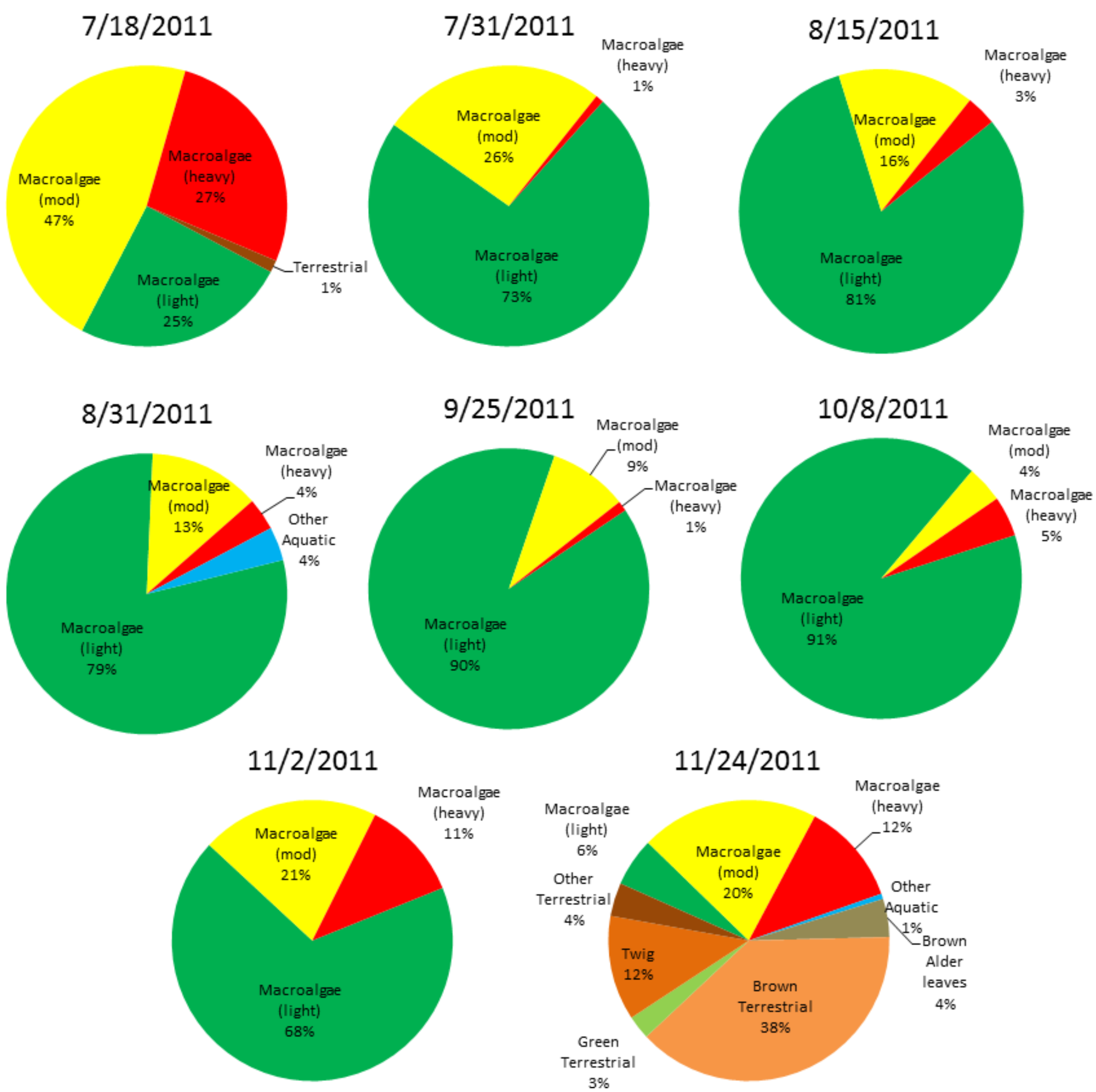


Figure 9: Composition of aquatic component of organic drift.

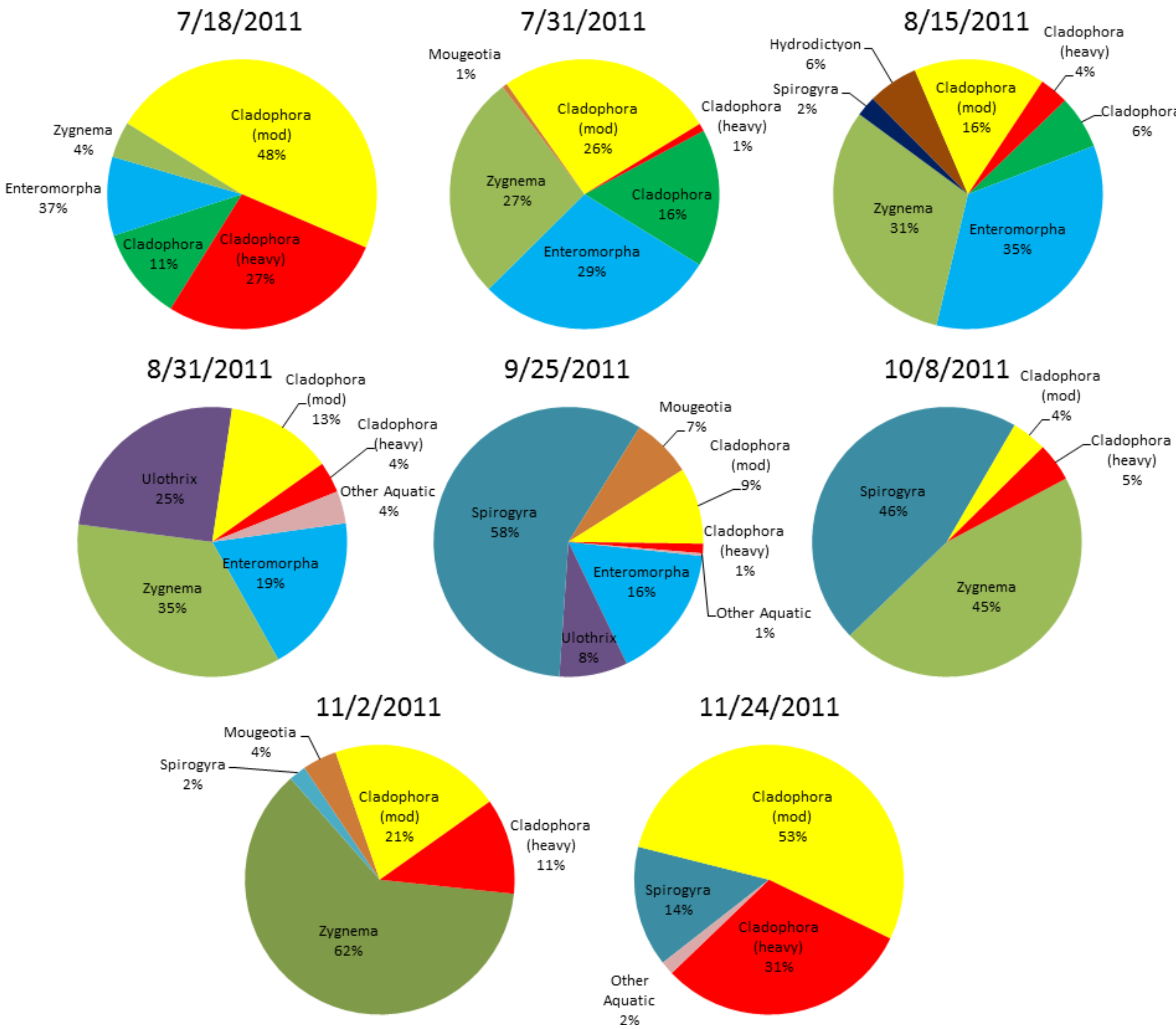


Figure 10. a. Amphipods recruit to algae; notice early recruitment to freshwater algae. b. High amphipod recruitment to moderately epiphytized *Cladophora*.

a.

b.

Figure 11: The average percent weight decrease per hour comparing algal types used during each experiment date. Error bars show standard errors of the mean.

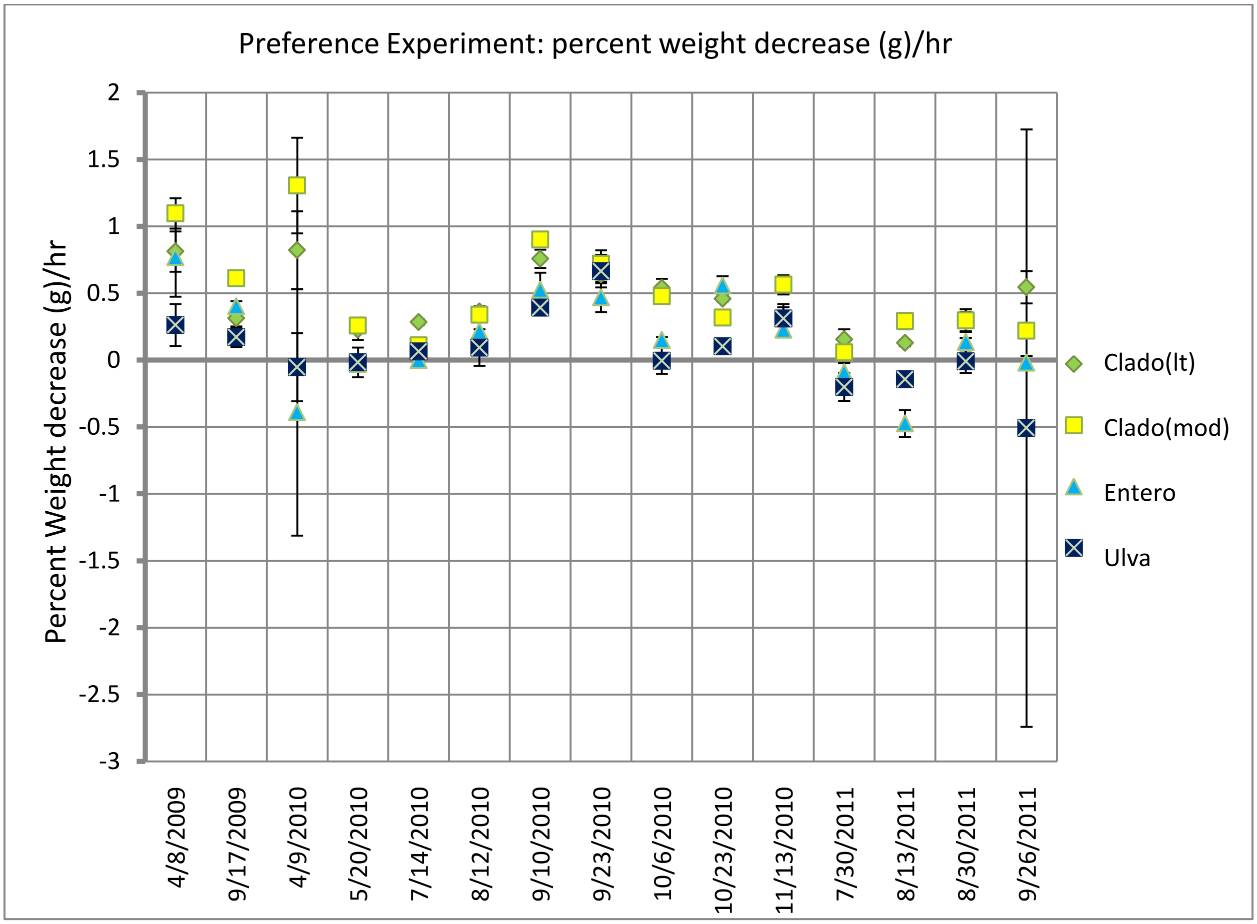


Figure 12: Average weight loss of each algal type per hour (LC =Lightly epiphytized *Cladophora*, MC = Moderately epiphytized *Cladophora*, Ent = *Enteromorpha*, Ulva = *Ulva*). Treatments with amphipods are indicated with a (+) and those without a (-). The number of amphipods stocked in each cup during each experiment is shown in the top righthand corner. Error bars show standard errors of the mean.

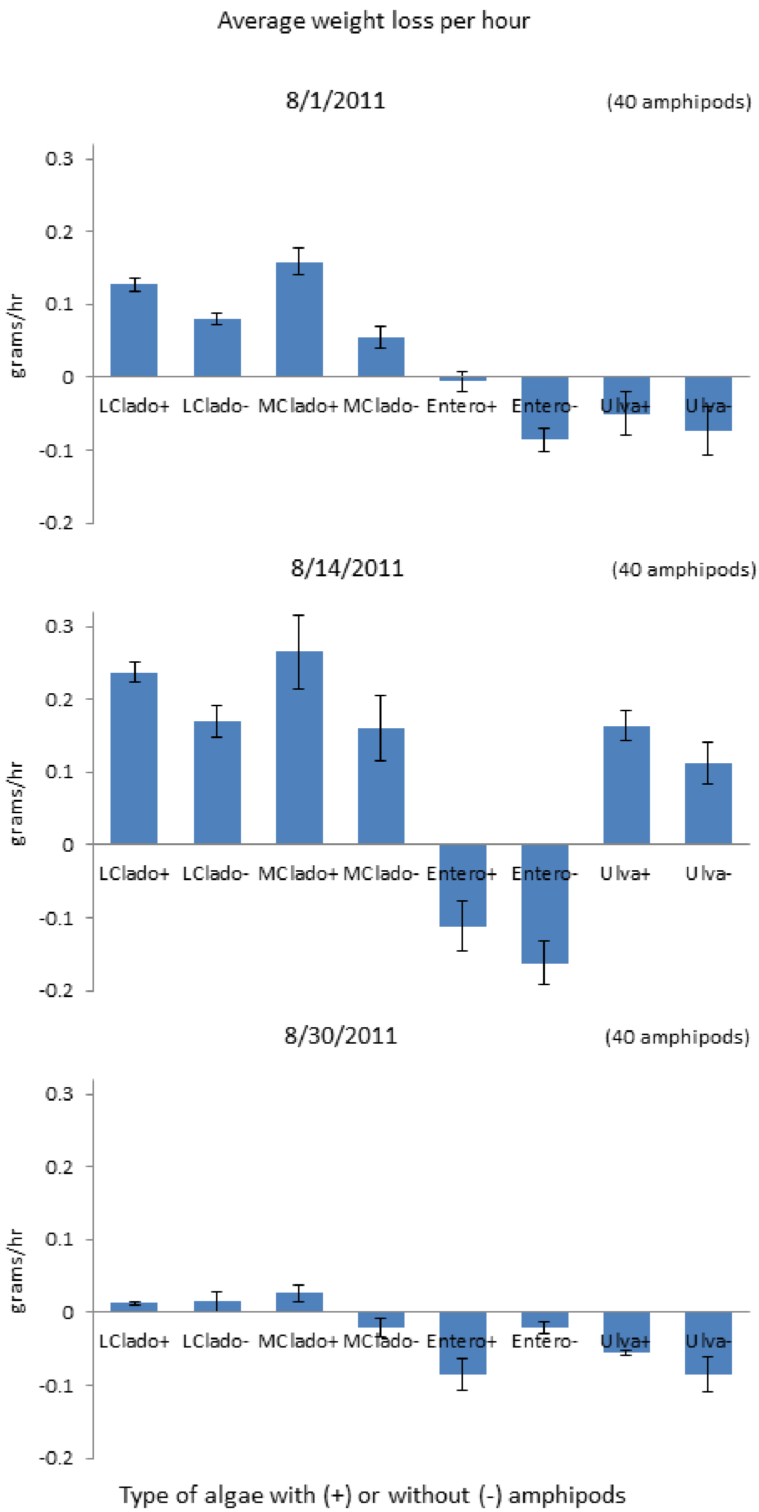
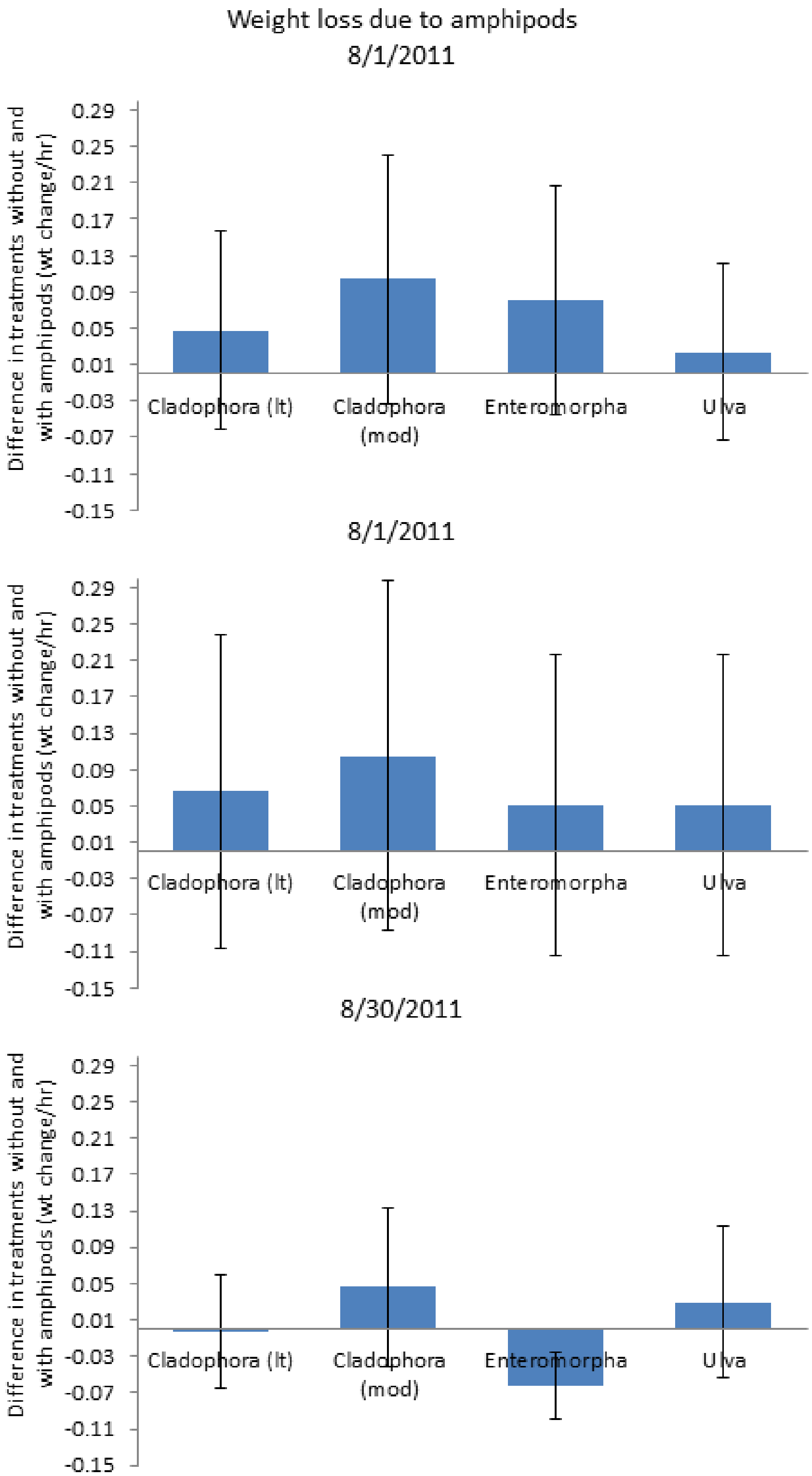


Table 4: Table with ANOVA results for each feeding experiment.

Date	Residual standard error	DF	Multiple R-squared	Adjusted R-squared	F statistic	p-value
8/1/2011	.04435	32	0.8287	0.7912	22.11 on 7 and 32 DF	1.414e-10
8/14/2011	0.07231	32	0.8374	0.8019	23.55 on 7 and 32 DF	6.261e-11
8/30/2011	0.03308	30	0.6752	0.5994	8.908 on 7 and 30 DF	6.591e-06

Figure 13: Effect of amphipods on algal weight change. The weight change per hour due to amphipods was calculated by taking the difference in weight loss with and without amphipods. Error bars show confidence intervals.



Appendix:

Table 1: Table showing the date, the number of nets used, the cross section sampled, the grams of drift collected, the discharge, the amount of time nets were in the water, and the flux values.

Date	Net Number	Ag (g)	Terr (g)	discharge *m^3/s)	total discharge (m^3/s)	time in water (s)	Aq Flux (g/s)	Aq Flux (g/hr)	Total Aq Flux (g/hr)	Terr Flux (g/s)	Terr Flux (g/hr)	Total Terr Flux (g/hr)
7/18/2011	1	19.89	1.04			3720	0.005346774	0.320806452		0.00027957	0.016774194	
7/18/2011	2	14.48	0.56			1740	0.008321839	0.499310345		0.000321839	0.019310345	
7/18/2011	3	18.67	0.94			1200	0.015558333	0.9335		0.000783333	0.047	
7/18/2011	4	86.22	2.02			1860	0.046354839	2.781290323		0.001086022	0.06516129	
7/18/2011	5	26.2	0.14			360	0.072777778	4.366666667		0.000388889	0.023333333	
7/18/2011	6	41.88	1.1			600	0.0698	4.188		0.001833333	0.11	
7/18/2011	7	8.51	0.03			900	0.009455556	0.567333333		3.33333E-05	0.002	
7/18/2011	thalweg	1.33	0.33			660	0.002015152	0.120909091		0.0005	0.03	
7/18/2011	8	2.88	0.13			780	0.003692308	0.221538462		0.000166667	0.01	
7/18/2011	9	1.41	0.18			600	0.00235	0.141		0.0003	0.018	
7/18/2011	TOTAL			23.7744			0.235672578	14.14035467	14.14035467	0.005692986	0.341579162	0.341579162
7/31/2011	1	20.21				600	0.033683333	2.021		0	0	

7/31/2011	2	15.634				600	0.026056667	1.5634		0	0	
7/31/2011	3	6.515				900	0.007238889	0.434333333		0	0	
7/31/2011	4	21.497				900	0.023885556	1.43313333		0	0	
7/31/2011	5	3.584				900	0.003982222	0.238933333		0	0	
7/31/2011	6	16.482				900	0.018313333	1.0988		0	0	
7/31/2011	7	2.641				900	0.002934444	0.176066667		0	0	
7/31/2011	TOTAL			1.6023	13.30892		0.116094444	6.965666667	57.85776722	0	0	0
8/15/2011	1	13.15	0.027			900	0.014611111	0.876666667		3E-05	0.0018	
8/15/2011	2	41.43	0.01			600	0.06905	4.143		1.66667E-05	0.001	
8/15/2011	3	15.15	0.06			1260	0.01202381	0.721428571		4.7619E-05	0.002857143	
8/15/2011	4	6.86	0.07			840	0.008166667	0.49		8.33333E-05	0.005	
8/15/2011	5	9.84	0.6			600	0.0164	0.984		0.001	0.06	
8/15/2011	7	0.29	0.71			300	0.000966667	0.058		0.002366667	0.142	
8/15/2011	TOTAL				4.49599		0.121218254	7.273095238	7.273095238	0.003544286	0.212657143	0.212657143
8/31/2011	1	25.77				600	0.04295	2.577		0	0	
8/31/2011	2	2.24				600	0.003733	0.224		0	0	

							333					
8/31/2011	3	3.7				50	0.074	4.44		0	0	
8/31/2011	TOTAL			0.832	5.040399		0.120683333	7.241	43.86722255	0	0	0
9/25/2011	1	5.7	0.154			600	0.0095	0.57		0.000256667	0.0154	
9/25/2011	2	31.57	0.096			600	0.052616667	3.157		0.00016	0.0096	
9/25/2011	3	3.71				340	0.010911765	0.654705882		0	0	
9/25/2011	4	2.73				563	0.004849023	0.290941385		0	0	
9/25/2011	5	25.75	0.036			630	0.040873016	2.452380952		5.71429E-05	0.003428571	
9/25/2011	7	19.2	0.033			330	0.058181818	3.490909091		1E-04	0.006	
9/25/2011	8	12.4	0.094			540	0.022962963	1.377777778		0.000174074	0.010444444	
9/25/2011	9	3.7	0.053			605	0.006115702	0.366942149		8.76033E-05	0.005256198	
9/25/2011	10	0.098	0.004			610	0.000160656	0.009639344		6.55738E-06	0.000393443	
9/25/2011	TOTAL				3.52105		0.20617161	12.37029658	12.37029658	0.000842044	0.050522657	0.050522657
10/8/2011	1	80.38	0.502			900	0.089311111	5.358666667		0.000557778	0.033466667	
10/8/2011	TOTAL			0.504	25.7117		0.089311111	5.358666667	273.3738685	0.000557778	0.033466667	1.707311296
11/2/2011	1	55.32	0.001			120	0.461	27.66		8.33333E	0.0005	

										-06		
11/2/2011	2	24.16	0.002			300	0.080533333	4.832		6.66667E-06	0.0004	
11/2/2011	TOTAL			1.0712	8.778223		0.541533333	32.492	266.2640233	1.5E-05	0.0009	0.007375281
11/24/2011	1	10.524	16.78			240	0.04385	2.631		0.069916667	4.195	
11/24/2011	TOTAL			0.5475	175.5645		0.04385	2.631	843.6715973	0.069916667	4.195	1345.192836

Table 2: Table with experiment start time, duration, treatments, number of individuals per cup, and average weights before and after the experiment. Standard deviations and standard errors of the mean are listed in the last four columns.

date	start time	average duration (h)	amphipods/cup	with+ or without- amphipod	average weight before (g)	average weight after (g)	StDev before	SEM before	StDev after	SEM after
8/1/2011	10:15	3	40	GC+	1.03	0.65	0.05	0.02	0.06	0.03
8/1/2011	10:15	3	40	GC-	1.04	0.80	0.04	0.02	0.08	0.03
8/1/2011	10:15	3	40	YC+	1.01	0.53	0.04	0.02	0.13	0.06
8/1/2011	10:15	3	40	YC-	1.01	0.85	0.04	0.02	0.07	0.03
8/1/2011	10:15	3	40	Ent+	0.99	1.01	0.05	0.02	0.08	0.04
8/1/2011	10:15	3	40	Ent-	0.98	1.24	0.02	0.01	0.10	0.05
8/1/2011	10:15	3	40	Ulva+	1.00	1.14	0.04	0.02	0.17	0.08
8/1/2011	10:15	3	40	Ulva-	1.01	1.23	0.05	0.02	0.22	0.10
8/14/2011	9:05	2.19	40	GC+	0.96	0.46	0.13	0.06	0.10	0.05
8/14/2011	9:05	2.19	40	GC-	0.93	0.57	0.09	0.04	0.10	0.04
8/14/2011	9:05	2.19	40	YC+	1.12	0.62	0.17	0.07	0.09	0.04
8/14/2011	9:05	2.19	40	YC-	1.10	0.79	0.17	0.07	0.05	0.02
8/14/2011	9:05	2.19	40	Ent+	1.06	1.28	0.10	0.04	0.11	0.05
8/14/2011	9:05	2.19	40	Ent-	1.06	1.46	0.10	0.05	0.18	0.08
8/14/2011	9:05	2.19	40	Ulva+	1.11	0.76	0.14	0.06	0.14	0.06
8/14/2011	9:05	2.19	40	Ulva-	1.22	0.99	0.16	0.07	0.05	0.02
8/30/2011	10:17	4	20	GC+	0.97	0.92	0.16	0.07	0.17	0.08
8/30/2011	10:17	4	20	GC-	1.00	0.94	0.18	0.08	0.27	0.12
8/30/2011	10:17	4	20	YC+	0.86	0.76	0.08	0.04	0.13	0.06
8/30/2011	10:17	4	20	YC-	0.97	1.05	0.15	0.07	0.20	0.09
8/30/2011	10:17	4	20	Ent+	1.00	1.34	0.05	0.02	0.20	0.09
8/30/2011	10:17	4	20	Ent-	1.10	1.18	0.06	0.03	0.54	0.24
8/30/2011	10:17	4	20	Ulva+	0.95	1.14	0.13	0.06	0.54	0.24
8/30/2011	10:17	4	20	Ulva-	0.86	1.20	0.17	0.08	0.36	0.16

Figure 1: Weights of algae before and after the feeding experiments.

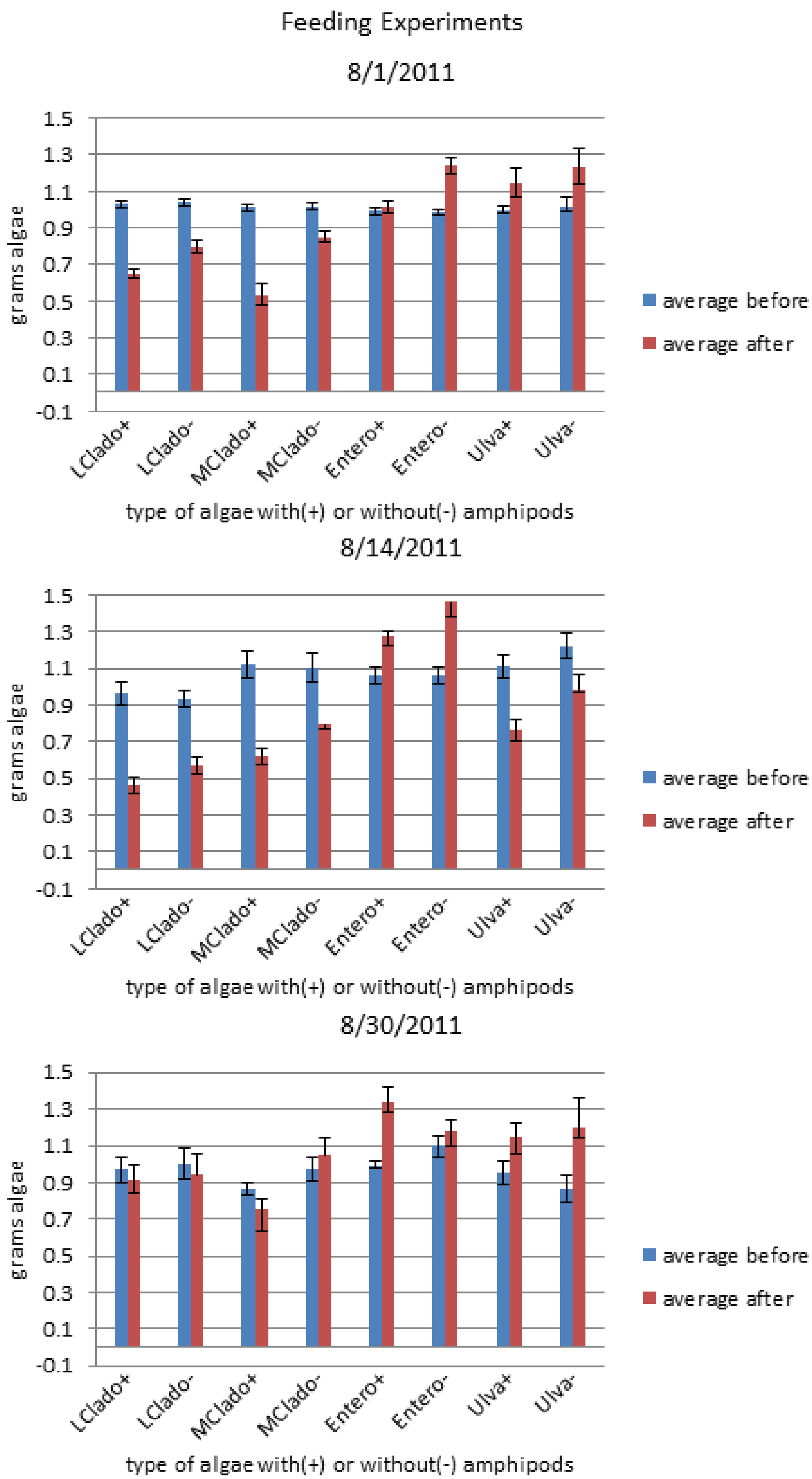


Figure 2: Flux of organic material through each net on dates when the whole cross section was sampled.