Diet and foraging ecology of an assemblage of Western Pond Turtles (*Actinemys marmorata*) living in two Northern California urban streams

by

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ABSTRACT

Diet and foraging ecology studies of freshwater turtles are a way to determine the habitats they use. Over a 4-year period, we studied an aggregation of Western Pond Turtles (Actinemys marmorata) living in 2 urban streams in Northern California that carried urban stormwater runoff. By developing and using the gastric lavage procedure to retrieve stomach contents in the field, we found their diet was 31% less taxonomically diverse than that of turtles living in wildland streams. Further, >56% of their diet was the non-native Red Swamp Crayfish (Procambarus clarkii). In addition, these urban dwelling turtles foraged on the streamside terrestrial landscape and consumed previously unreported prey taxa. Moreover, by observing turtles forage and then collecting their stomach contents we found they incidentally ingested sand, soil, vegetation, and other nearby habitat of their prey. Our results show that habitat loss from stream maintenance program (SMP) activity to prevent urban flooding reduced their dietary taxonomic diversity by 69%. In addition, after SMP activity removed crayfish habitat the turtles abandoned foraging in one of the streams. Our results provide convincing evidence that *A. marmorata* is a carnivore rather than a dietary generalist.

Acknowledgement

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Introduction

Habitat loss linked to the urbanization of the world's landscape is a major reason that many freshwater turtles are in jeopardy worldwide (Gibbon and Buhlmann 2000; Buhlmann and Georges 2010). This pressure is likely to continue since urban human populations are expected to grow by 15 % by 2050, at which time over 65 % of the world's population will be living in urban centers (United Nations, Department of Economic and Social Affairs 2014). In the United States, especially along the West Coast, the estimated urban population will reach over 85 % (United Nations, Department of Economic and Social Affairs 2014; Hawley and Vietz 2016). Since the West Coast is the home range of the Western Pond Turtle (*Actinemys, a.k.a. Clemmys marmorata*) many of these turtles living in urban waterways that carry urban stormwater runoff will likely be affected by urban growth (Bury and Germano 2008; Radeloff and Hammer 2005). As urban centers grow, the additional demands to prevent urban flooding are likely to produce further stream maintenance program (SMP) modifications that may affect *A. marmorata* (Morse and Huryn 2003; Radeloff and Hammer 2005; Hawley and Vietz 2016). For instance, benthic macroinvertebrates (BMI) are the primary food resource of *A. marmorata* in wildland streams (Bury 1986). Since these taxa frequently have lower diversity and biomass in urban streams (Morse and Huryn 2003; Booth and Roy 2016). One of our research goals was to examine the diet and foraging ecology of an assemblage of *A. marmorata* living in 2 urban streams and compare our results to those of an earlier study done by R. Bruce Bury in wildland streams. Another goal was to examine if additional SMP modifications to accommodate urban growth altered the diet and foraging ecology of these turtles.

Based on published information on the diet and foraging behavior of *A. marmorata* in wildland streams we expected the diet of urban turtles would have significantly lower dietary taxonomic diversity. This is because some BMI that *A. marmorata* consume in wildland streams (e.g., caddisflies; *Trichoptera*, mayflies; *Ephemeroptera*) do not thrive in urban streams (Alberti and Booth 2007; Urban and Skelly 2006; Bury 1986). Furthermore, *A. marmorata* were reported to also consume filamentous algae and vegetation (Bury 1986). Since BMI prey of all kinds are often scarce in urban streams (Alberti and Booth 2007) we anticipated that algae and vegetation might be a more significant portion of the diet of these turtles.

Materials and Methods

Study Sites — The study sites were located downstream of the city of Santa Rosa, the largest city in Sonoma County, California. The climate of the region is a Mediterranean one, meaning that there is a somewhat distinct rainy and dry season, with the rainy season ending on or about May 15. The assemblages of *A. marmorata* we studied primarily foraged along two 280m reaches of two streams that were within 1.1 km of one another. One site, SRC2 (38° 26.733'N, 122° 46.734'W), is a reach of Santa Rosa Creek and is the major stormwater runoff channel for the city of Santa Rosa. The other site, ARC1 (38° 26.751'N, 122° 48.187'W), is a reach of Abramson Creek, a seasonal or ephemeral waterway that carries stormwater that drains from the nearby residential and agricultural landscape. The geomorphology of both streams is similar; both have unnaturally straight channels flanked on either side by steep flood banks with a 1:1 gradient that are 5.0 m high (SCWA 2009). Atop each flood bank is a narrow riparian zone 10 m wide bordered by chain-link fencing (SCWA 2009). A single lane asphalt or gravel service road bisects the riparian zone along both sides of SRC2 and ARC1. The overall appearance of SRC2 was more spacious because the channel is wider (10 m) than that of ACR1 (3.7 m) (SCWA 2009).

Turtle Capturing — For three years, from 2010 to 2012, we trapped *A. marmorata* using an equal effort protocol at both sites starting May 15th and ending August 15th (D. J. Brown & Mali, 2011). We used custom-made hoop net traps that we adapted for use in these modified streams with rip-rap banks and streambeds to prevent the turtles from drowning. For an equal number of 4-day periods we trapped for turtles at each site throughout each field season. On the first day, we set out traps baited with a punctured can of sardines packed in oil. Subsequently, each morning for the next 3-days we checked each trap for captured turtles, and removed them. We transported captured turtles to a temporary field station where we marked the turtles and recorded their weight and carapace length (Cagle 1939; Bury 1986).

Stomach Content Sample Collection —To collect stomach content samples we developed the gastric lavage procedure (GLP). We developed GLP because none of the current published stomach flushing methods met the current Institutional Animal Care and Use Committee (IACUC) standards for using animals in research established by the California State University System. After testing GLP in a clinical setting, we found it to be a safe (100%) and effective (>95%) method for collecting stomach content samples from *A. marmorata*. GLP features the use of Ketamine (15 mg/kg) and Dexmedetomidine (0.2 mg/kg) as an anesthetic that relaxes the alimentary canal, allowing for safe intubation. This along with the use of an adjustable-flow peristaltic pump and medical grade tubing permitted effective stomach content removal. After retrieving the stomach contents, we administered Atipamezole (1mg/kg) to reverse the effects of Dexmedetomidine._We used GLP 71 times in the field and laboratory on any *A. marmorata* that was ≥ 100mm in carapace length, not a gravid female, and considered free of injury and disease (Kaplan 1958).

Stomach Content Sample Analysis —We first sorted through each stomach content sample to identify and count the number of individuals of each taxonomic group using standard methodologies (Huber 1998; Merritt and Cummins 1996; Barbour and Gerritsen 1999). We then macro-photographed each taxonomic subset in the stomach content sample using a digital camera and archived the images. Afterwards, we used the images to reexamine each sample and confirm our taxonomic identifications. At the end of each field season, we combined all the samples for each taxonomic group to measure the volume (V_i). We made these measurements using a standard water displacement method for turtle diet studies (Bjorndal and Bolten 1997; Souza and Abe 2000).

Data Analysis — To determine the percentage of the diet for each taxonomic group we first calculated their index of relative importance (IRI) value and then calculated its percentage of the overall diet (Hyslop 1980; Bjorndal and Bolten 1997). The IRI for each taxon was calculated using the formula: $IRI_i = (\%N_i + \%V_i) + (\%F_i)$ where N_i is the number individuals of a specific taxonomic group, V_i the net volume for each taxon in all the samples, and F_i the frequency number of samples where each taxon occurs. To calculate the $\%N_i$ we used the formula: $\%N_i = \frac{N_i}{\Sigma N_i}$, to calculate the $\%V_i$ we used the formula: $\%V = \frac{V_i}{\Sigma V_i}$, and to calculate the $\%F_i$ value we used the formula: $\%F = \frac{F_i}{\Sigma F_i}$

(Hyslop 1980). To calculate the percentage of the diet for each taxon we used the equation: $\% D = \frac{IRI_i}{\Sigma IRI}$ where %D is the percentage of the diet for each taxonomic group, IRI_i is the taxon's IRI, and $\sum IRI$ is the sum of all taxa IRI values (Bjorndal and Bolten 1997).

Field Observations — Each year we epoxied Advanced Telemetry Systems (ATS) submersible transmitters to the anterior vertebral scutes of 4 turtles we captured at each site. One day per week, we located each of them using an ATS R410 Scanning Receiver outfitted with a 3-element folding Yagi antenna. Subsequently, we observed their foraging behavior and feeding style. At the outset of each 15-day trapping period we recorded the surface water temperature (STS) using standard methods (Barbour and Gerritsen 1999).

Crayfish Abundance Survey — From June 15 – June 30, 2013 to assess the abundance of *Procambarus clarkii* (Red Swamp or Louisiana Crayfish) at each site we set Frabill conical traps baited with chicken wings. Each morning we checked traps and re-baited them. We marked any captured crayfish by notching the telson to ensure we did not recount them. Afterwards, we filled out an individual numbered data sheet for each crayfish where we recorded the cephalothorax length (CL). Using a digital camera outfitted with a macro lens, we then photographed each crayfish ventrally along with the datasheet. Later, we used these digital images to determine the sex of each crayfish (Oluoch, 1990; McClain & Romaire, 2012). To determine the age/class of each individual we used a conventional CL measurement protocol (Leavitt & Haley, 1989; Mkoji et al., 1999; Gutierrez-Yurrita & Montes, 1999) . Specifically, any crayfish with a

CL of ≤ 30mm was considered to be juvenile and any individual with a CL of ≥ 31mm was considered an adult.

Results

Diet Composition — We collected 54 stomach content samples from 17 female and 34 male turtles (Fig. 1). The non-native crayfish *P. clarkii* occurred in 37 of the 54 stomach content samples we collected and comprised 56.74% of the total diet in 2010, reached 83.54% in 2011, and declined in 2012 to 57.75% (Table 1). At the outset of our research in 2010, 8 other taxa were in the diet. These include the larvae of midges (*Cecidomyiidae;* 13.68%) found in galls on the terrestrial landscape. Taxa found in mats of filamentous algae include scuds (*Amphipoda*; 8.47%), segmented worms (*Oligochaeta*; 4.68%), snails (*Gastropoda*; 1.62%), and water striders (*Hemiptera*; 5.37%). Free swimming taxa found in the littoral zone included dragonfly nymphs (*Odonata*; 3.66%) and a native fish, the 3-spined Stickleback (*Gasterosteus aculeatus;* 5.57%). We logged terrestrial and aerial arthropod taxa that accidentally end up on the water's surface (*e.g*., beetles, caterpillars, adult dragonflies, spiders) as carrion (0.83%). In the succeeding 2 years, the number of taxa in the diet declined from 9 taxa to 4 taxa. In 2011 when SMP activity restricted the turtles to either stream, the diet was largely crayfish (83.54%), followed by dragonfly nymphs (11.03%), three-spined sticklebacks (1.40%), and carrion (4.03%). During 2012, when turtles were once again able to forage between the streams, the percentage of crayfish in the diet was 57.75%, nearly the same as it was in 2010. Of the remaining taxa in the diet, dragonfly nymphs rose to 28.69% and three-spined sticklebacks to 12.48%, while carrion dropped off to 1.08%.

Foraging Observations — Based on our radio telemetry results, we found that all the turtles appeared to have home range fidelity and in 2010 remained at or near the site of capture. In 2011, after July 1 and until the end of the field season, each time we located a specific turtle it was at the same cluster of aquatic vegetation. Moreover, we did not observe any *A. marmorata* basking after that date. In 2012, the *A. marmorata* captured and released at SRC2 remained there during May but by the end of June they had moved downstream and out of tracking range. The turtles captured and released at ARC1 remained at the site the entire field season. We observed *A. marmorata* actively foraging, capturing and consuming prey 11 times. Afterwards, we captured them and collected stomach content samples using GLP. In 2010, we observed 3 of the turtles foraging on the streamside terrestrial landscape. Each turtle appeared to deliberately be searching through the vegetation. When the turtle encountered a specific plant, it would consume a portion. We captured each of them ≤ 12 hours later, collected their stomach contents, and found that each gall they ate contained from 2 – 5 *Cecidomyiidae* or midge larvae (Fig. 3b). In addition, we observed 2 turtles foraging in algal mats and occasionally consume a portion of the mat. When we captured ≤12 hours later, each turtle had a bolus of algae and soft-bodied BMI (i.e., *Amphipoda*, *Oligochaeta*) in the stomach content sample. All other *A. marmorata* that we observed doing this that were captured ≥ 24 hours later had a bolus of algae its stomach and no organisms. On 5 occasions, we observed *A. marmorata* capture and swallow crayfish resting on banks of soil, sand, or gravel. After capturing them, we found the substrate material along with crayfish in the stomach contents (Fig. 3d). We also observed one *A. marmorata* capture and consume a crayfish entangled in filamentous algae, and then collected its stomach contents 72 hours later. The stomach contents included a bolus of filamentous algae and semi-transparent pieces of the exoskeleton of the crayfish (Fig. 3a). In addition, we observed some *A. marmorata* scavenge and eat terrestrial and aerial arthropods that fell onto the water's surface (Fig. 3c). For instance, we observed some of the turtles ingest a floating butterfly chrysalis and 2 caterpillars (*Lepidoptera*), 2 adult dragonflies (*Odonata*), and a spider (*Araneae*).

SMP Modifications — Stream maintenance work in 2011 and 2012 followed the work plan outlined in the SMP that included vegetation management and channel maintenance (SCWA 2009). Vegetation management included the mechanical and chemical removal of bankside and instream vegetation beginning June 1 each year. Channel maintenance work included sediment removal and additional streambed channelization (Fig. 4). Beginning in 2011, a 2.2 km section of Santa Rosa Creek between both study sites underwent sediment removal and channel modification by earthmoving equipment (SCWA 2009). Because of this work, both study sites were isolated from one another and turtles were unable to move freely between the sites. An artificial pipe channel allowed water to flow in Santa Rosa Creek throughout this period. Consequently, the surface water temperature (STS) never rose above 19˚C in Santa Rosa Creek and a wetland did not form in late summer, as had been the case in 2010 and earlier years (Fig. 3). Subsequently, in 2012 and 2013, water flowed year around in Santa Rosa Creek and the STS never exceeded 19˚C. In contrast, in Abramson Creek, the waterflow and STS remained the same from 2010 - 2013. As a result, the wetland that once formed each year during the summer only persisted in Abramson Creek where the STS rose to 22˚C in July and remained that temperature until August 15. In addition, bankside vegetation along this section of the stream was largely absent (Fig. 4).

Crayfish Abundance Survey — The overall results of the *P. clarkii* abundance survey in 2013 is that ACR1 had 4.8 times as many crayfish as did SRC2, with a ratio of 72:15. In the case of juvenile crayfish, the ratio between ACR1 and SRC2 was 42:1 and for adults was 2:1.

Discussion

Urban streams that carry stormwater runoff suffer from lower biodiversity and habitat loss from SMP activity (Booth and Roy 2016; Roy and Walsh 2016). Our results confirm that the diversity of taxa in the diet of urban *A. marmorata* was lower than that of turtles living in wildland streams. Specifically, a single taxon of non-native crayfish was the mainstay of the diet of these urban dwelling turtles. In addition, we found that the *A. marmorata* in our study were carnivores rather than an opportunistic dietary generalist or omnivore, as was previously reported. Consequently, when SMP activities removed prey habitat the diversity of taxa in the diet was smaller. Lastly, when SMP modifications in one stream removed crayfish habitat and they became scarce, the turtles moved to a nearby creek or downstream.

Diet — As we anticipated, the overall diet of the *A. marmorata* we studied was less diverse than the turtles studied earlier in wildland streams (Bury 1986). Of the stomach contents we examined, 37 of 54 samples contained the non-native Red Swamp Crayfish (*P. clarkii*). Specifically, this taxon of crayfish was >56% of the diet, whereas an equivalent value in turtles living in wildland stream was comprised of 4 different taxa, all of which were native taxa. These results are not surprising since *P. clarkii* are ubiquitous in urban and agricultural waterways worldwide (Cruz and Rebelo 2007; Scalici and

Gherardi 2007). The presence of a non-native such as *P. clarkii* is a conservation concern (Barbaresi, Tricarico, and Gherardi 2004; Cruz and Rebelo 2007). Nevertheless, the presence of this taxon in urban streams where the diversity and amount of native species of BMI is low made them an important alternative food resource for the turtles we studied. Consequently, although the presence of such a non-native species is a conservation concern, they can make it possible for freshwater turtles to persist in these modified ecosystems.

Carnivory — Despite earlier reports that suggested that *A. marmorata* are dietary generalists, our research results provide persuasive evidence that they are opportunistic carnivores; especially when turtle-feeding kinematics is taken in to consideration. When turtles bite and consume the food they seek, they also consume any nearby items in the habitat because of their feeding kinematics (Van Damme and Aerts 1997; Summers and Darouian 1998). Also, once the food and other items are in their mouth they have no buccopharyngeal features in the mouth that would enable them to separate food from incidentally ingested items (Winokur 1988). For instance, after observing *A. marmorata* capture crayfish resting on the substrate, we found soil, sand, gravel, detritus, and bits of plastic in the stomach content samples. In addition, when crayfish were resting on vegetation or in filamentous algae, we found these items in their stomachs. Similarly, when *A. marmorata* consumed insect larvae found in terrestrial plant galls we found them in their stomachs.

A basic precept of optimal foraging theory is that animals will not expend energy unnecessarily or risk foraging failure unless food resources are scarce (Charnov 1976; Layman and Quattrochi 2007; Ford 1983). Moreover, when prey is scarce, predators will relocate to habitats that better support the presence of prey (Thompson and Stelle 2014; Thygesen and Sommer 2016; Fields and Simpson 2003). Our findings regarding the carnivory of *A. marmorata* began with the SMP activity downstream of SRC2 in Santa Rosa Creek in 2011. This work lowered the STS so that conditions were no longer ideal for *P. clarkii* reproduction. During 2012, our trapping results and observations indicate that the turtles abandoned foraging at SRC2 by July. We caught no turtles at SRC2 after June and those turtles that previously foraged at SRC2 were now foraging at ARC1. Using radio telemetry, we tracked some turtles from SRC2 swimming downstream. In 2013, we surveyed both sites for vegetation, algae, and crayfish abundance. Our results show that *P. clarkii* adults were scarce and juveniles largely absent while vegetation and algae were abundant at SRC2. Conversely, *P. clarkii* of all age/classes were abundant at ARC1, as was vegetation and algae. Consequently, a carnivore whose primary prey was *P. clarkii* would abandon SRC2 when they became scarce. In contrast, omnivores would likely remain in place and alter their dietary focus to vegetation.

Habitat Loss — Urban wildlife habitat loss is a major conservation concern (Jeltsch and Moloney 2011; Fahrig 2007; Fischer and Lindenmayer 2007). The decline in the biodiversity of taxa in the diet of the *A. marmorata* we studied between 2010 and 2011 is an indication of urban habitat loss linked to SMP activity. Specifically, the taxonomic diversity in the diet dropped from 9 to 4 when terrestrial vegetation management and stream modification removed habitat. For instance, terrestrial vegetation management removed habitat for one taxon, midge larvae (*Cecidomyiidae*) found in streamside plant galls. Similarly, stream modifications removed algal mat habitats of 4 taxa, scuds

(*Amphipoda*), water boatman (*Hemiptera*), snails (*Gastropoda*), and worms (*Oligochaeta*).

Conservation Implications — Studies such as ours that add to the available information on the life history of freshwater turtles can be applied when wildlife agencies examine land management activity proposals such as SMP's (Rosenberg and Gervais 2009). An example such information is that *A. marmorata* is a carnivore, relies on a non-native crayfish as its primary food resource, and forages on the streamside landscape. Likewise, understanding the ways that conventional SMP activity produces habitat loss which can negatively affect freshwater turtles adds to the growing body of information that supports the incorporating of wildlife conservation into stormwater management policy (Grimm & Morgan, 2000; National Research Council Report, 2009; Walsh, Booth, & Roy, 2016).

Appendix A

Table 1. Dietary taxonomic diversity and percentage of the diet of *A. marmorata* in 2 urban streams from 2010 – 2012. Data showing the number of stomach content samples collected and examined each year (N), and the percentage of the diet for each taxonomic group (% Diet).

	2010 (N = 36)	2011 (N = 12)	2012 (N = 24)
Taxonomic Group	% Diet	% Diet	% Diet
Procambarus clarkii	56.74	83.54	57.75
Odonata (L)	03.66	11.03	28.69
Gasterosteus aculeatus	01.61	01.40	12.48
Carrion	00.12	04.03	01.08
Cecidomyiidae (L)	17.73	00.00	00.00
Hemiptera (A)	05.37	00.00	00.00
Amphipoda	08.47	00.00	00.00
Oligochaeta	04.68	00.00	00.00
Gastropoda	01.62	00.00	00.00

Appendix B — Figures

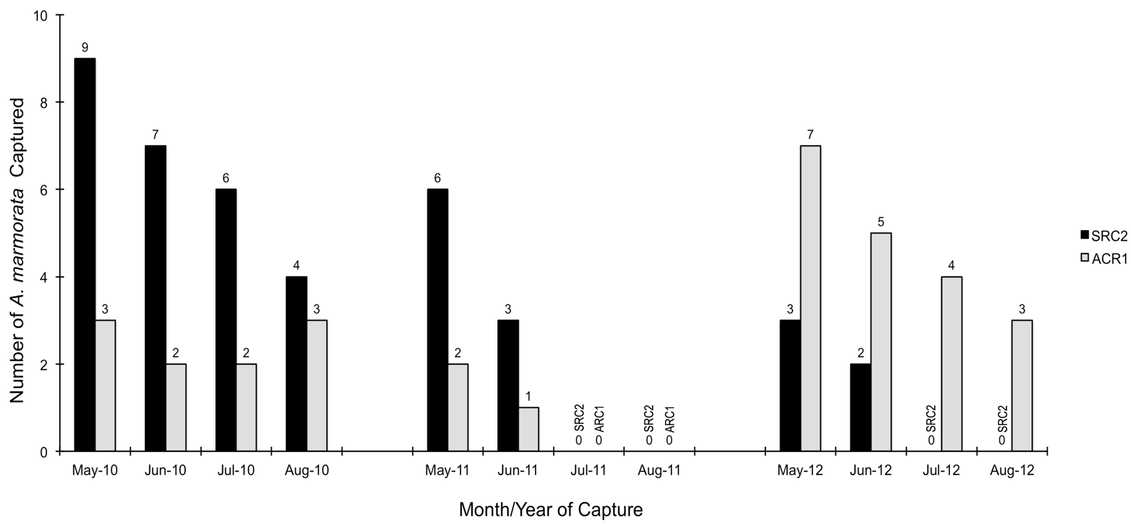

Figure 1. Turtle captures by month and year. Black bar is the number of turtles captured at site SRC2 and the gray bar is the number of turtles captured at ARC1.

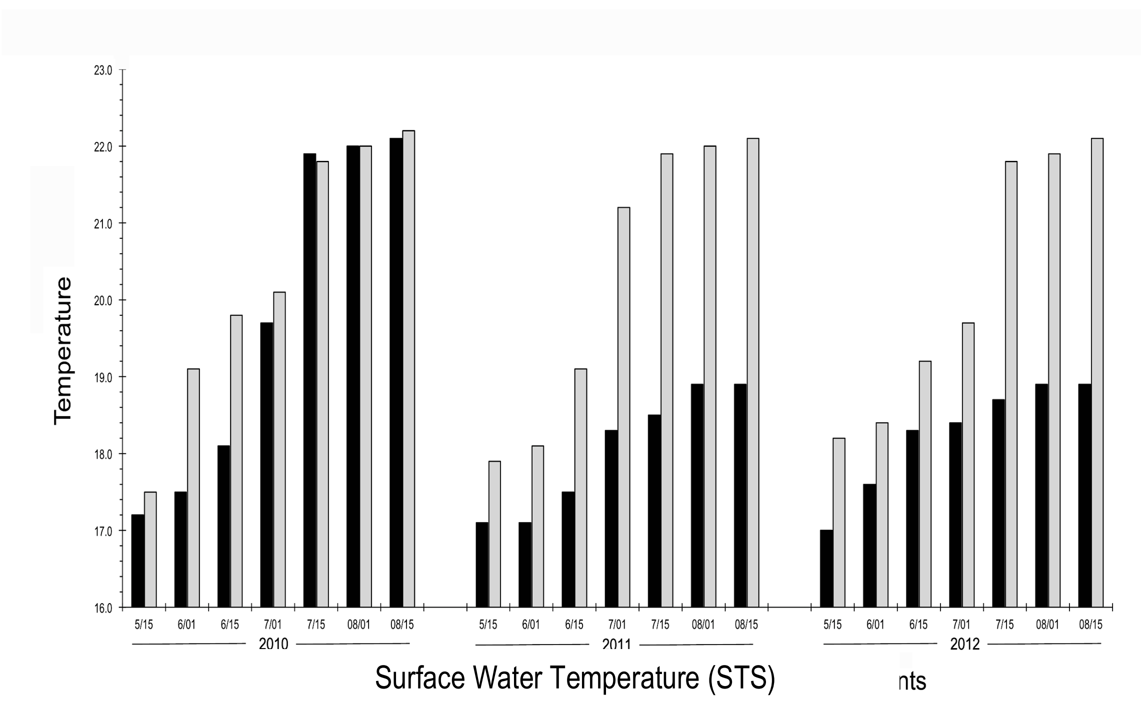

Figure 2. Surface water temperature (STS) measurements taken at 15-day intervals during the field seasons 2010 – 2012. The black bar represents site SRC2 in Santa Rosa Creek and the gray bar site ARC1 in Abramson Creek.

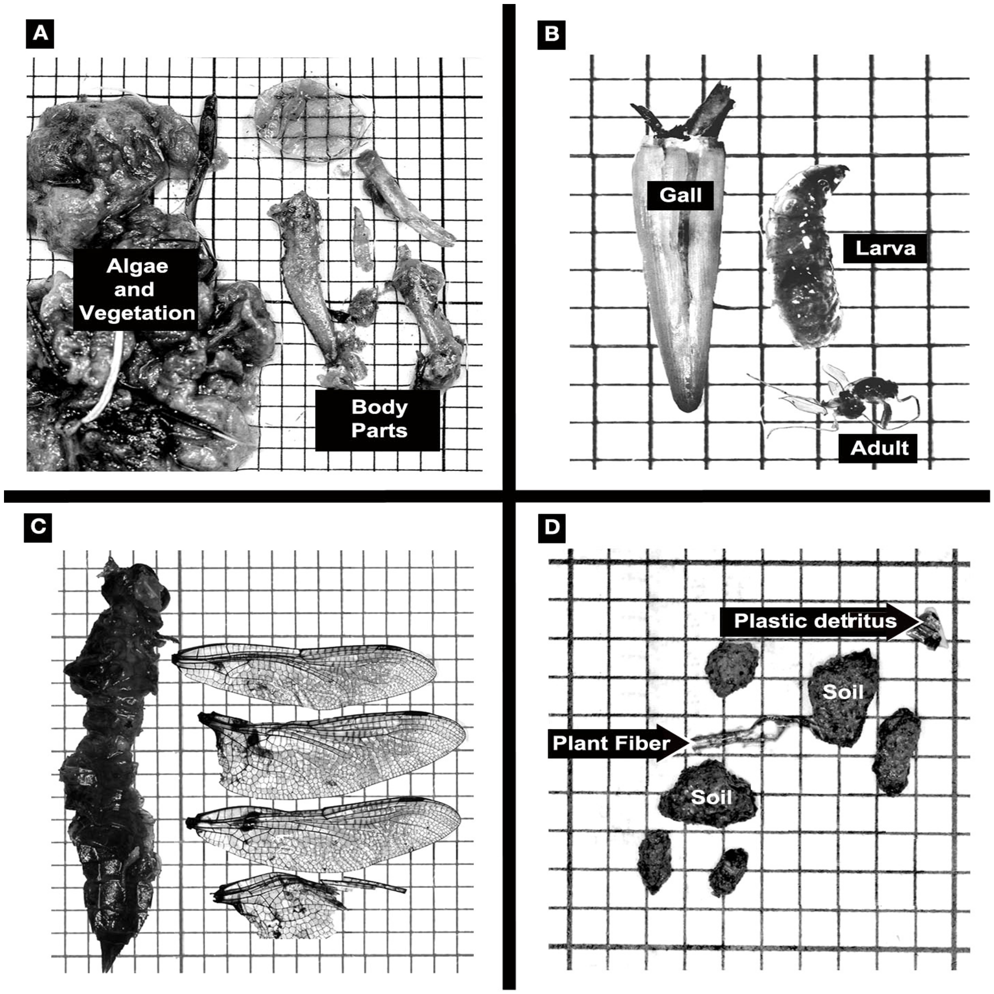

Figure 3. Stomach content samples collected from *A. marmorata*. Example A is from a turtle captured >24 hours after it was seen capturing and consuming a crayfish. Example B is from a turtle that consumed a *Cecidomyiidae* gall, larvae, and adult while foraging in bankside vegetation. Example C is an adult dragonfly that was on the water surface and consumed by a turtle. Example D is inorganic material collected from a turtle that captured a crayfish while it was foraging along a stream bank.

Figure 4. Results of SMP work in Santa Rosa Creek. These 2 infrared photographs are of the same section of a section but from a different viewpoint of the 1.1km reach of Santa Rosa Creek downstream of both study sites that underwent modification in 2011. Image A was taken at the start of SMP work in June 2011 and image B was taken in December 2011 after the SMP work was completed.

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