FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-118-R

Endangered and Threatened Species Conservation

Conservation Status of Comanche Springs pupfish (*Cyprinodon elegans*) and Pecos gambusia (*Gambusia nobilis*) in the ciénegas of Balmorhea State Park, Texas

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STATE: ____Texas_____   **GRANT NUMBER:** ___TX E – 118-R____

GRANT TITLE: Conservation Status of Comanche Springs pupfish (*Cyprinodon elegans*) and Pecos gambusia (*Gambusia nobilis*) in the ciénegas of Balmorhea State Park, Texas

REPORTING PERIOD: ____1 September 2009 to 23 May 2013

OBJECTIVE(S):

To (1) assess the current conservation status of the endangered Comanche Springs pupfish and Pecos gambusia in the ciénegas at Balmorhea State Park, Texas, (2) assess the value of restored critical habitats for the conservation of these taxa, and (3) establish ecosystem-based management and conservation strategies for endemic taxa of desert ciénegas; over three seasons.

Segment Objectives:

1. Population estimates
2. Stable isotopes analysis
3. Gut contents.

Significant Deviation: None.

Summary Of Progress: Please see Attachment A (attached).

Location: Reeves County, TX

Cost: ___Costs were not available at time of this report.__

Prepared by: _Craig Farquhar_____   **Date:** _6 September 2013

Approved by: _____ **Date:**___6 September 2013____

               C. Craig Farquhar

ATTACHMENT A

Final Report

USFWS Federal Assistance Grant E-118-1

Conservation Status of Comanche Springs Pupfish (*Cyprinodon elegans*) and Pecos Gambusia (*Gambusia nobilis*) in the ciénegas of Balmorhea State Park, Texas

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Reporting Period: September 1, 2009 to May 28, 2013

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SUMMARY OF OVERALL DELIVERABLES

1. Population Estimates: We estimated populations in three habitats at Balmorhea State Park over a 4 year time period. These habitats included the refuge canal (RC), San Solomon Ciénega (SSC), and Clark Hubbs Ciénega (CHC). The dates sampled per habitat were March 2009 (RC, SSC), July 2009 (RC, SSC), December 2009 (SSC, CHC), April 2010 (SSC, CHC), July 2010 (SSC, CHC), December 2010 (SSC, CHC), March 2011 (SSC, CHC), August 2011(SSC, CHC), January 2012 (SSC, CHC), April 2012 (SSC, CHC), August 2012 (SSC, CHC), December 2012 (SSC, CHC), and April 2013 (SSC, CHC). Findings from population estimates are presented in the results section below

2. Population Age Structure: During each seasonal population estimate we took pictures of all fish and measured each individual in the laboratory using Image-J software. We analyzed size structure and age structure for each fish population from these data. Findings from size structure data are presented in the results section below.

3. Gut contents and Diet Overlap: We preserve ≤10 *Gambusia nobilis*, ≤10 *Cyprinodon elegans*, 20-30 *G. geiseri*, and 20-30 *Astyanax mexicanus* from all habitats during each sample period. We analyzed gut contents for these fishes and calculated diet overlap among taxa using gut content data. The diet overlap results are presented in the results section below.

4. Stable isotopes: In addition to the preserved individuals, we froze ~10 additional individuals of each species from all habitats and sample periods for stable isotope analysis.   Using stable isotope data we examined overall food web structure for SSC and CHC habitats. Stable isotope data are presented in the results section below.

SIGNIFICANT DEVIATIONS

No deviations were made from our original plans.

REPORT
BACKGROUND

Historically, Comanche Springs pupfish (*Cyprinodon elegans*) and Pecos gambusia (*Gambusia nobilis*) inhabited two large desert wetland (ciénega) systems separated by approximately 100 km (Hubbs et al. 1981, Hubbs et al. 1983). One system was fed by the Balmorhea springs complex (Phantom Lake, San Solomon, Giffin, and East Sandia springs), and one by Comanche Springs. These ciénegas and their associated springs provided valuable habitat for these two endemic fishes as well as an entire community of interacting organisms (Hendrickson and Minckley 1984). However, human alteration of the Balmorhea and Comanche spring systems for agricultural irrigation destroyed the associated wetland habitats, and none of these natural systems remain today. This endangered the persistence of Comanche Springs pupfish and Pecos gambusia (Hubbs et al. 1981, Hubbs et al. 1983).

When the original San Solomon ciénega was modified, and for the most part destroyed, the only "aquatic habitat" remaining was in concrete irrigation canals. Although better than no habitat at all, the irrigation canals, at best, provided a tenuous existence for much of the aquatic biota. The Comanche Springs pupfish and Pecos gambusia managed to survive in the irrigation canals, but their numbers were greatly reduced. As a result, these species were elevated to endangered status and conservation efforts were aimed at preventing their extinction (Hubbs et al. 1981, Hubbs et al. 1983).

In an attempt to conserve these endangered species, the Texas Parks and Wildlife Department (TPWD) restored a critical desert wetland habitat by creating the San Solomon Ciénega at Balmorhea State Park in 1996 through a cooperative effort among private, state, and federal entities. This re-creation of a desert wetland habitat within the boundaries of the original, natural ciénega provided critical habitat necessary for survival of desert wetland biota. As a result, the native fish fauna, including Comanche Springs pupfish and Pecos gambusia, have flourished, and this location now provides a natural habitat with the largest known concentration of Comanche Springs pupfish and a viable population of Pecos gambusia. It is believed that the primary benefit of the San Solomon Ciénega to the survival of these endangered fishes is the creation of a "natural" habitat with viable ecosystem-level processes that promote population stability. However, there have been no concerted monitoring efforts since 2001 and there is no information on the ecosystem dynamics of this system.

Based on the success of the San Solomon Ciénega, TPWD is now in the process of creating a second ciénega at Balmorhea State Park by replacing the small, refuge canal with a new 'natural' wetland habitat. This small refuge canal (120 m) was constructed in 1974 at Balmorhea State Park in an effort to improve habitat for the endangered species (Echelle and Hubbs 1978). During a two-year sampling study (Garrett and Price 1993), Comanche Springs pupfish population size in the park refuge canal was estimated to be as low as 968 (May 1990) and as high as 6,480 (September 1990). It is expected that the creation of a second ciénega in this spring system will further promote the successful conservation of Comanche Springs pupfish and Pecos gambusia by doubling the amount of critical habitat and restoring critical ecosystem-level processes that promote population viability.

With the completion of this second ciénega by autumn of 2009 there now exists a great opportunity to document the ecological mechanisms driving the population dynamics of fishes in these desert wetlands. Because successful species conservation not only relies on a thorough understanding of population-dynamics of the species being conserved but also of the ecosystem-level process driving populations, we have begun to implement a 'whole ecosystem' conservation

approach in these ciénega ecosystems. Our goal is to more fully understanding both ecosystem and taxa, so an efficient and effective conservation plan can be established to promote long-term viability of these endangered fishes. With the information gained from this study, we can better manage the existing ciénegas in the San Solomon spring system and have useful insights into the development and functions of the new ciénegas across desert ecosystems.

OVERALL GOALS
Our goal of this multi-year project is to (1) to assess the current conservation status of the endangered Comanche Springs pupfish and Pecos *Gambusia* in the ciénegas at Balmorhea State Park, Texas, (2) to assess the value of restored critical habitats for the conservation of these taxa, and (3) establish ecosystem-based management and conservation strategies for endemic taxa of desert ciénegas.

METHODS
Density & Population Estimates and Size Structure
We estimated population sizes ($\widehat{N}$) and associated variance $V(\widehat{N})$ for all fish species in the old and new ciénegas and refuge canal using the *counts on sample plots method*

$$\widehat{N} = \frac{A}{a}\,\bar{n},$$

where A = total population area (the old ciénega or new ciénega), a = size of the plot, $\bar{n}$ = the average number of animals counted per sample plot, and

$$V(\widehat{N}) = \frac{A^2}{a}\,\frac{V(\widehat{N})}{s}\,\frac{A - s \cdot a}{A},$$

where $V(\widehat{N}) = \sum_{i=1}^{s}\frac{(n_i - \bar{n})^2}{(s-1)}$, n_i = number of animals counted in the ith plot, and s = number of plots used.

To capture fishes for the population estimates, we first blocked off five large sections of each habitat using five 16.7m $\times$ 2m (4.2mm mesh) seines. We repeatedly seined each section using a 7 pass depletion method. We carefully collect all fishes from the net and, immediately following capture, we transferred fishes to insulated coolers containing fresh stream water. We replaced water repeatedly to reduce stress and promote survival of all fishes. We removed individual fishes from the coolers with a dip net and transfer them to a small Plexiglas viewing tank marked with a 1cm grid. While the fishes are in the viewing tank, we identified and counted each species, and we took a digital photograph of the viewing tank (Fig. 1).

Fig. 1. Block off net in San Solomon Ciénega.

Using this digital image and the 1cm grid for reference, we measured each individual using the Image J computer program. From these measurements, we created length frequency histograms and used FiSAT II (2005) software to analyze age cohorts for each population.

Gut content analysis

We collected a random subsample (*N* = 20) of fishes from each of the sample plots described above. This random sample included fish from each size class in proportion to their natural abundance. For example, if 60% of fishes fell in the medium size class, we randomly selected 12 individuals from that group. From this subsample, we froze 10 individuals for stable isotope analysis and preserved 10 individuals in 10% formalin for gut content analysis.

        To evaluate the instantaneous food web structure at the time of sampling, we analyzed gut contents for 10 preserved individuals for each species from both habitats. These data, along with food availability data (collected for stable isotope analysis), will help indicate diet preference by each species in both habitats. To evaluate gut contents, we removed the upper portion of the alimentary tract and spread stomach contents on a 0.8mm gridded Petri dish (Hargrave 2006). We recorded percent area for each food item, which we assumed is proportional to the volume of food in the gut. We then calculated trophic overlap among species using a percent similarity index.

        We used assimilated tissue isotopes to evaluate long-term trophic structure (stable isotope analysis). The 10 frozen individuals for each species were eviscerated and dried at 60C for 5-7 days. Caudal muscle was ground with a mortar and pestle to a fine powder and transferred to 5ml glass vials. Tissue samples were analyzed for the stable isotope ratios C_{13}/C_{12} and N_{15}/N_{14} at the University of Arkansas Stable Isotope Laboratory.

RESULTS

PHYSICAL CHARACTERISTICS OF THE HABITATS

The length of the open habitat (i.e., not inundated by cattail) in the San Solomon Ciénega was 62 m, the average width of this habitat was 7.4m and the average depth was 0.35m. Mud was the dominate substrate type in the San Solomon Ciénega at 77%, followed by *Chara* sp. and *Chlodophora* sp. (13% & 10%, respectively). The length of the Clark Hubbs Ciénega was 52m, the average width is 16.7m and the average depth is 0.79m. The substrate is composed of mud (36.6%) and *Chara* sp. (63.3%). The *Chara* sp. is covered with epithitic algae. The surface area of the Clark Hubbs Ciénega was 80% smaller than the San Solomon Ciénega and the total volume of the new Clark Hubbs Ciénega was about 60% smaller than the total volume of the San Solomon Ciénega. The refuge canal was the smallest of the habitats. The length of the Refuge Canal was 171m, the average width was 3.5m, and the average depth was 0.35m (Fig. 2).

Fig. 2. San Solomon (above) and Clark Hubbs (below) Ciénegas.

FISH DENSITY & POPULATION ESTIMATES

San Solomon Ciénega

We estimated density (no./m^2) and population size (N) of all fishes in San Solomon Ciénega at 13 time periods (March 2009, July 2009, December 2009, April 2010, July 2010, December 2010, March 2011, August 2011, January 2012, April 2012, August 2012, December 2013, April 2013). In Solomon Ciénega, *Cyprinidon elegans* density ranged from 0.3 to 1.6 individuals / m^2, averaging 0.9 ± 0.2 individuals / m^2 across time periods (Table 1). After extrapolating densities to the entire open habitat in the San Solomon Ciénega, population estimates ranged from 209 - 1050 individuals and averaged 560.8 individuals for the sample period (Table 1). There appeared to be temporal trends in the *C. elegans* population over time; however, there the trends appeared independent of season (Fig. 3). Rather, temporal trends were driven by exponential increases in population growth followed by steep declines in density. These fluctuations are possibly a steady state for *C. elegans* populations.

Gambusia nobilis* density ranged from 0.4 to 2.3 individuals / m^2, averaging 1.9 ± 0.7 individuals / m^2 across time periods (Table 1). The population size of *G. nobilis* in the San Solomon Ciénega ranged from 325 to 2023 individuals (Table 1) after extrapolation to the entire open area in this habitat (see above). Population size reflected density, averaging ~901 individuals in this habitat. Temporal variation in population size of *G. nobilis* appeared to be linked to season. Density of *G. nobilis* was greatest in summer. Abundance decreased from summer into winter and to spring. Following spring, populations increased to summer maximums (Fig. 3).

The invasive *G. geiseri* was the most dense species in the San Solomon Ciénega. *G. geiseri* densities in this habitat ranged from 1.9 to 19.2 individuals / m^2 (Table 1). Average *G. geiseri* density in San Solomon Cienega was ~10 individuals / m^2. Population size of *G. geiseri* ranged from 1218 to 12,567 individuals / m^{2}averaging 6673 individuals across sample periods. There appeared temporal patterns of *G. geiseri* abundance in this habitat. Trends of increasing and decreasing populations appeared to reflect a two-year cycle. For example, a year of high density across all seasons was followed by a year of low density across seasons (Fig. 4).

Astyanax mexicanus* was the least dense species in San Solomon Ciénega, with densities that ranged between 0 to 2.5 individuals / m^2. On average, *A. mexicanus* density was estimated at ~0.6 individuals / m^2 (Table 1). The population estimates for *A. mexicanus* in San Solomon Ciénega reflected densities and ranged from 0 to 1500 individuals with an average of 396 individuals in this habitat. There was no pattern in the temporal variation in *A. mexicanus* density (Fig. 4). Abundance was likely driven by stochastic nature of *A. mexicanus* movement into and throughout the ciénega.

The Refuge Canal

Because this locality was drained and filled in October 2009, we estimated fish density and population size of all fishes in the Refuge Canal only at two time periods (March 2009 & July 2009). In general, densities and population estimates of *C. elegans, G. geiseri* and *A. mexicanus* in the Refuge Canal were similar to those estimated in the Old Ciénega (Table 1). For example, *C. elegans* density and population size averaged 1.5 ± 0.6 individuals / m^2 and 861 ± 355 individuals, respectively; *G. geiseri* density and population size averaged ~11 ± 9 individuals / m^2 and 6321 ± 5502 individuals, respectively; and *A. mexicanus* density and population size averaged 0.8 ± 0.8 individuals / m^2 and 488 ± 436 individuals, respectively. *Gambusia nobilis* was about 4 times more dense in the Refuge Canal than in the Old Ciénega (Table 1). *G. nobilis*

density and population size averaged 4.15 ± 1.9 individuals / m^2 and 2456 ± 1125 individuals, respectively.

Clark Hubbs Ciénega
The Clark Hubbs Ciénega was filled and stocked with fish from the Refuge Canal around October 2009.   Therefore, we estimated fish densities and population size for 11 sample periods from this habitat (December 2009, April 2010, July 2010, December 2010, March 2011, August 2011, January 2012, April 2012, August 2012, December 2013, and April 2013). In general, density and population estimates for all species declined from the December 2009 to April 2010 sample periods, but then increased rapidly from the April 2010 to August 2011 samples (Table 1). Populations appeared to reach some level of inter-annual stability (steady state) following August 2011.

     Cyprinidon elegans density and population estimates were about 10 times larger on average to those estimated from San Solomon Ciénega (Table 1). For example, average density was 9 individuals / m^2 and average population size was 8516 individuals in this habitat. There were clear temporal trends in *C. elegans* density in this habitat. *C. elegans* populations peaked during summer of most sample years (Fig. 3). This peak was followed by a steady decline in abundance through spring.   This trend was repeated for all 3 sample years and likely was driven by primary productivity in Clark Hubbs Ciénega.

     G. nobilis density and population estimates in Clark Hubbs Ciénega were about 2 times greater than in San Solomon Ciénega (Table 1). On average, *G. nobilis* density and population estimates were 2 individuals / m^2 and 2062 individuals, respectively. By the second and third years, there were clear temporal trends in *G. nobilis* abundance over time. These trends appeared to be linked to season and likely reflected food availability.   For example, populations were greatest during summer and decreased through spring (Fig. 3).

     Gambusia geiseri density and population estimates in the Clark Hubbs Ciénega were similar to those in the San Solomon Ciénega (Table 1). For example, density ranged from 0.1 to 17.8 individuals / m^2 and averaged 7.8 individuals / m^2. Total population size of *G. geiseri* reflected density with an average of 7375 individuals in this habitat. Temporal trends in *G. geiseri* abundance were likely driven by season in this habitat rather than density dependent population regulation as in San Solomon Ciénega. For example, abundance was greatest in the summer and decreased through spring (Fig. 4). The pattern was repeated across all sample years.

     Astyanax mexicanus density and population estimates ranged from 0 to 4.5 individuals / m^2 (average 1.5 ± 1.1 individuals / m2) and 0 to 3654 individuals (average 924 individuals), respectively. Like *A. mexicanus* populations in San Solomon Ciénega, there was no pattern in the temporal variation in *A. mexicanus* density (Fig. 4). Abundance was likely driven by stochastic nature of *A. mexicanus* movement into and throughout the ciénega.
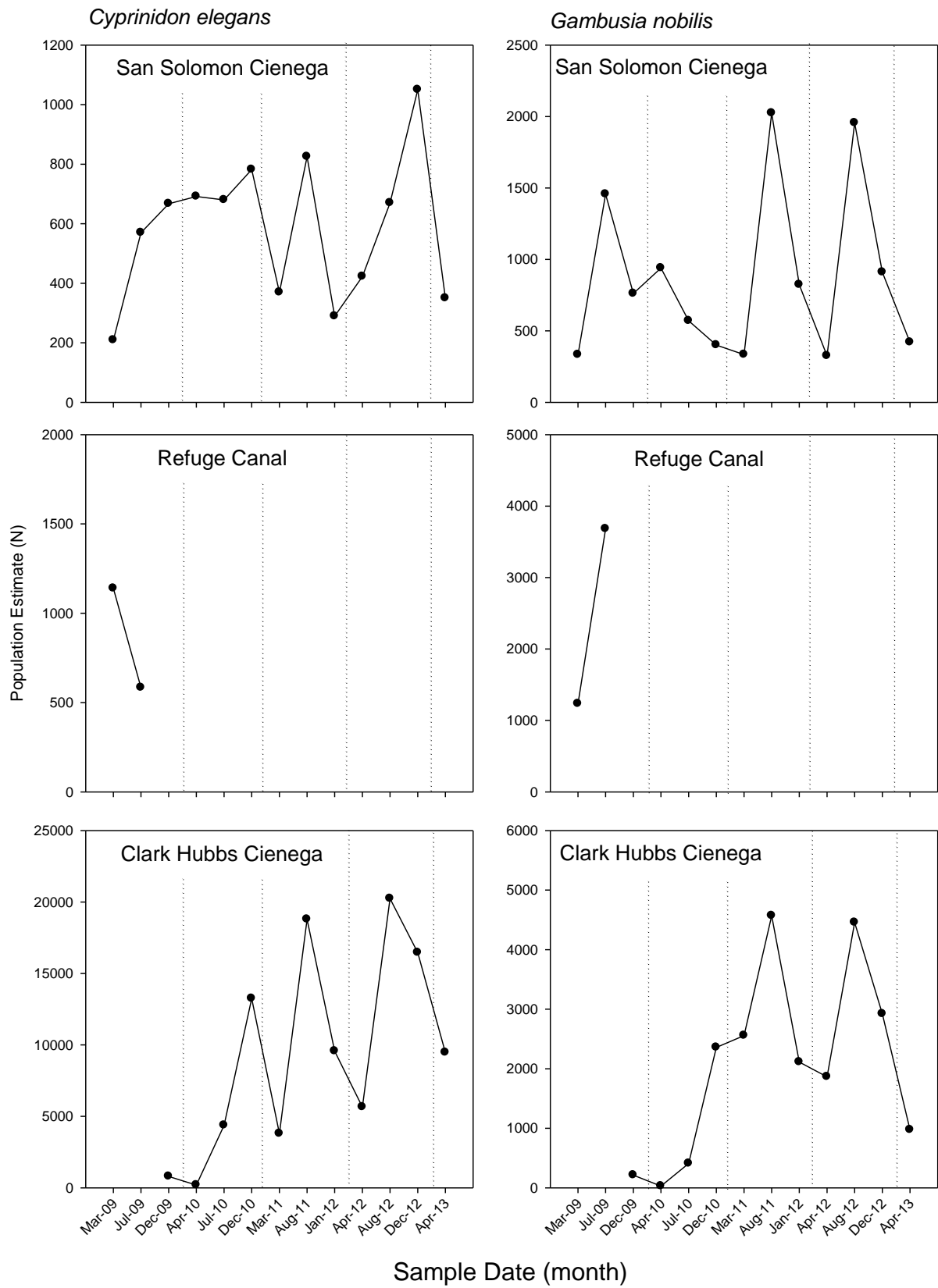
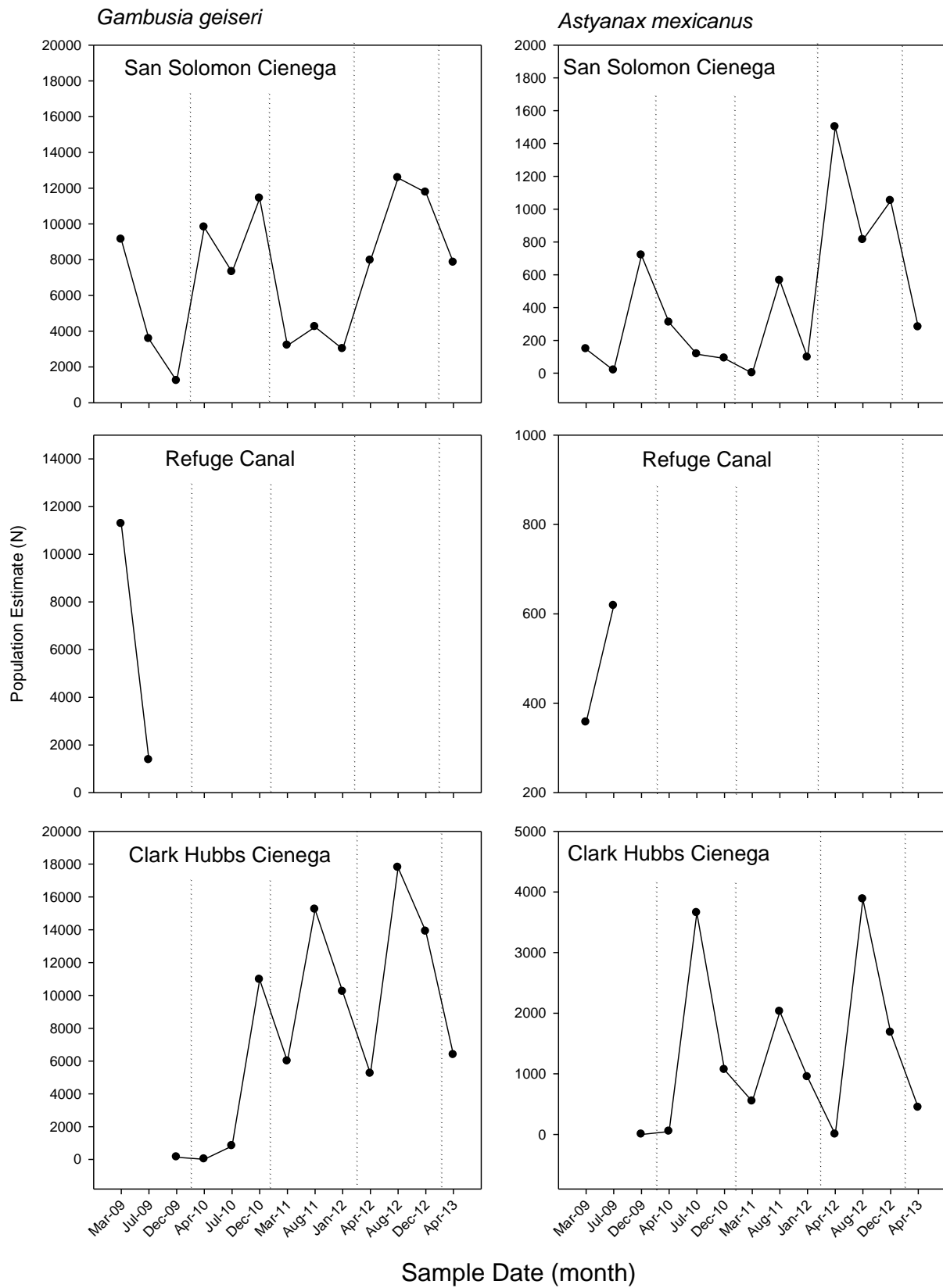Figure 3. Population trends for *C. elegans* and *G. nobilis* in all habitats

Gambusia geiseri

Astyanax mexicanus

Population Estimate (N)

Sample Date (month)

Figure 4. Population trends for *G. geiseri* and *A. mexicanus* in all habitats.

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POPULATION AGE STRUCTURE

We estimated age cohorts for *C. elegans*, *G. nobilis* and *G. geiseri* in both San Solomon and Clark Hubbs Ciénegas, but we did not have enough seasonal data (individuals) to estimate age cohorts for *A. mexicanus*.

Cyprinidon elegans.

Cyprinidon elegans appeared to have 3 age classes in San Solomon Ciénega (age 0, 1, and 2). It appears that age 1 and age 2 individuals reproduce in this habitat. The first reproduction event occurred between winter and spring sample periods. This first age 0 cohort grows about 30mm throughout summer. The second age 0 cohort is born sometime between the spring and summer sample periods. This cohort grows little from summer to winter (~20mm) and likely isn't reproductive until spring or summer when they reach age 1. *Cyprinodon elegans* individuals appear to die between spring and summer when age 2 in this ciénega (Fig. 5).

Age structure of *C. elegans* in Clark Hubbs cienga was similar to that in San Solomon Ciénega with 3 age classes in this habitat (age 0, 1, and 2). It appears that age 1 and age 2 individuals also reproduce in this habitat during two time periods. The first reproduction event occurred between winter and spring sample periods and grows about 30mm throughout summer. The second age 0 cohort is born sometime between the spring and summer sample periods and grows little from summer to winter (~25mm).

Gambusia nobilis

Gambusia nobilis appeared to have 3 age classes in San Solomon Ciénega (age 0, 1, and 2). It appears that only age 1 individuals reproduce in this habitat and that age 2 individuals die sometime between winter and spring when age 2. Age 1 *G. nobilis* reproduce once between spring and summer sample periods. The age 0 cohort grows about 25mm throughout summer (Fig. 6).
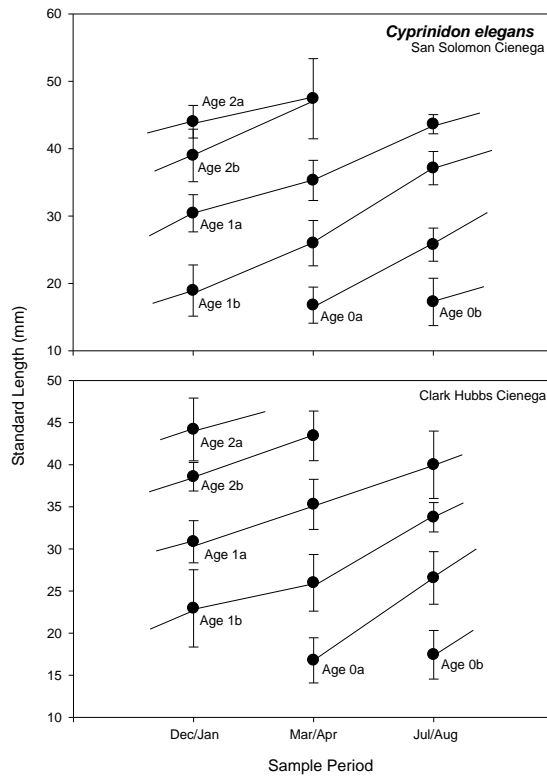

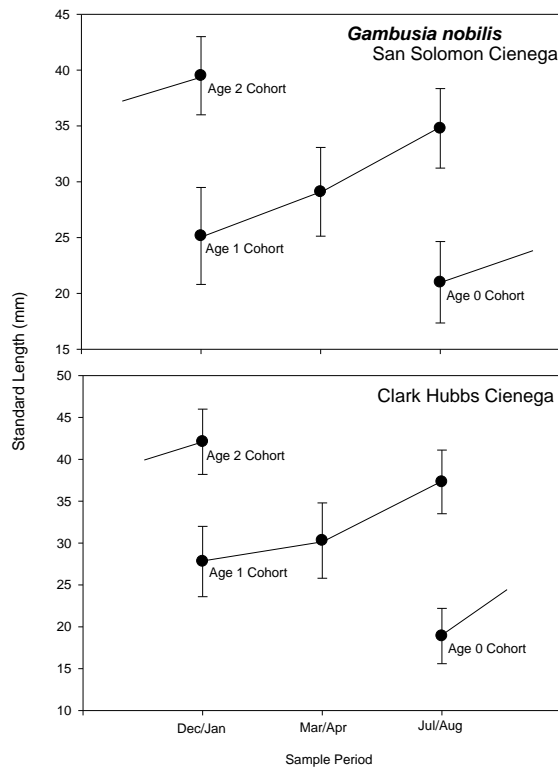Figure 5. Age cohorts for *C. elegans* in two cienegas.

Figure 6. Age cohorts for *G. nobilis* in both cienegas.

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Age structure of *G. nobilis* in Clark Hubbs Ciénega was similar to that in San Solomon Ciénega also with 3 age classes (age 0, 1, and 2). It appears that only age 1 individuals reproduce in this habitat sometime between spring and summer sample periods. The age 0 cohort also grew about 25mm in this habitat from summer to winter.

Gambusia geiseri

Gambusia geiseri appeared to have 2 age classes in San Solomon Ciénega (age 0 and 1). Only age 1 individuals reproduce in this habitat. The first reproduction event occurred between winter and spring sample periods; however, this age cohort reproduces twice per year in this habitat. This first age 0 cohort appears in spring and the second age 0 cohort appears in summer. The first age 0 cohort grows about 30 mm throughout the summer and likely contributes to the first age 0 cohort each year. The second age 0 cohort grows only about 20 mm throughout summer. This second age 0 cohort likely does not reproduce in spring when age 1, but likely contributes to the second age 0 cohort each year. *Gambusia geiseri* individuals appear to die between summer and winter when age 2 in this ciénega (Fig. 7).

Figure 7. Age cohorts for *G. geiseri* in both cienegas.

Age structure of *G. geiseri* in Clark Hubbs cienga was similar to that in San Solomon Ciénega with 2 age classes in this habitat (age 0 and 1). It appears that only age 1 individuals also reproduce in this habitat during two time periods. The first reproduction event occurred between winter and spring sample periods and indivudals of this first age 0 cohort grows about 30mm throughout summer. The second age 0 cohort is born sometime between the spring and summer sample periods and grow little from summer to winter (~20mm). It is likely the first age 0 cohort contributes to the first and second reproduction events the subsequent year, whereas the second age zero cohort likely only contributes to the second reproduction event the subsequent year. *Gambusia geiseri* appear to die between summer and winter when age 1.

FOOD HABITS AND DIET OVERLAP
San Solomon Ciénega – Gut Contents
Algae was the dominant food item (% occurrence) in the guts of *Cyprinodon elegans* for all sample periods. On average, algae comprised about 83% of the stomach contents for this species. Other food items found in *C. elegans* guts included, flocculent material (algae), seeds and some benthic invertebrates. However the percent occurrence of these items rarely exceeded 15% of the gut contents. There appeared to be no temporal trend in the gut contents of *C. elegans* (Fig. 8).
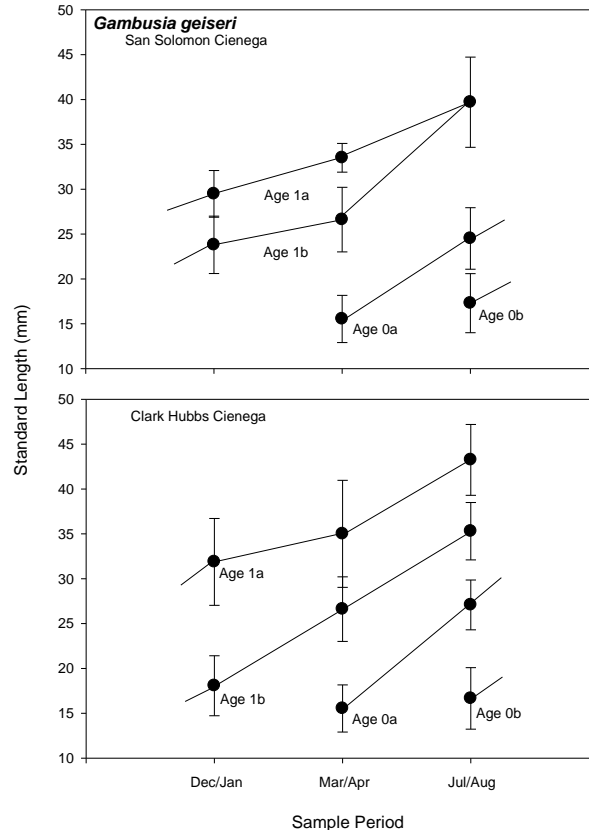
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Algae also was the principal food item consumed by *G. nobilis* across sample periods. On average, algae comprised about 75% of the stomach contents for this species. Although algae was a dominant food item in *G. nobilis* guts, this species also consumed an array of other foods. Benthic invertebrates and terrestrial insects were present in guts of *G. nobilis* during all sample periods. Together, these additional food items comprised about 25% of the gut contents. There appeared to be no temporal trend in the gut contents of *G. nobilis* (Fig. 9).

Gut contents of *G. geiseri* were similar to that of *G. nobilis* with algae comprising the greatest food item (70%) in the guts of this species. *Gambusia geiseri* also consumed benthic invertebrates, terrestrial invertebrates and seeds (~20%). There appeared to be no temporal trend in the gut contents of *G. geiseri* (Fig. 10).

Gut contents of *A. mexicanus* were strikingly different from those of the previous three species. Dominant food items in the guts of this species varied overtime, but generally were either benthic aquatic invertebrates such as chironomids and odonate larvae (40%). This species also consumed terrestrial invertebrates and seeds. Fish were consistently present in the guts of *A. mexicanus* across sample dates. There appeared to be little temporal trend in the gut contents of *A. mexicanus* in this habitat (Fig. 11).
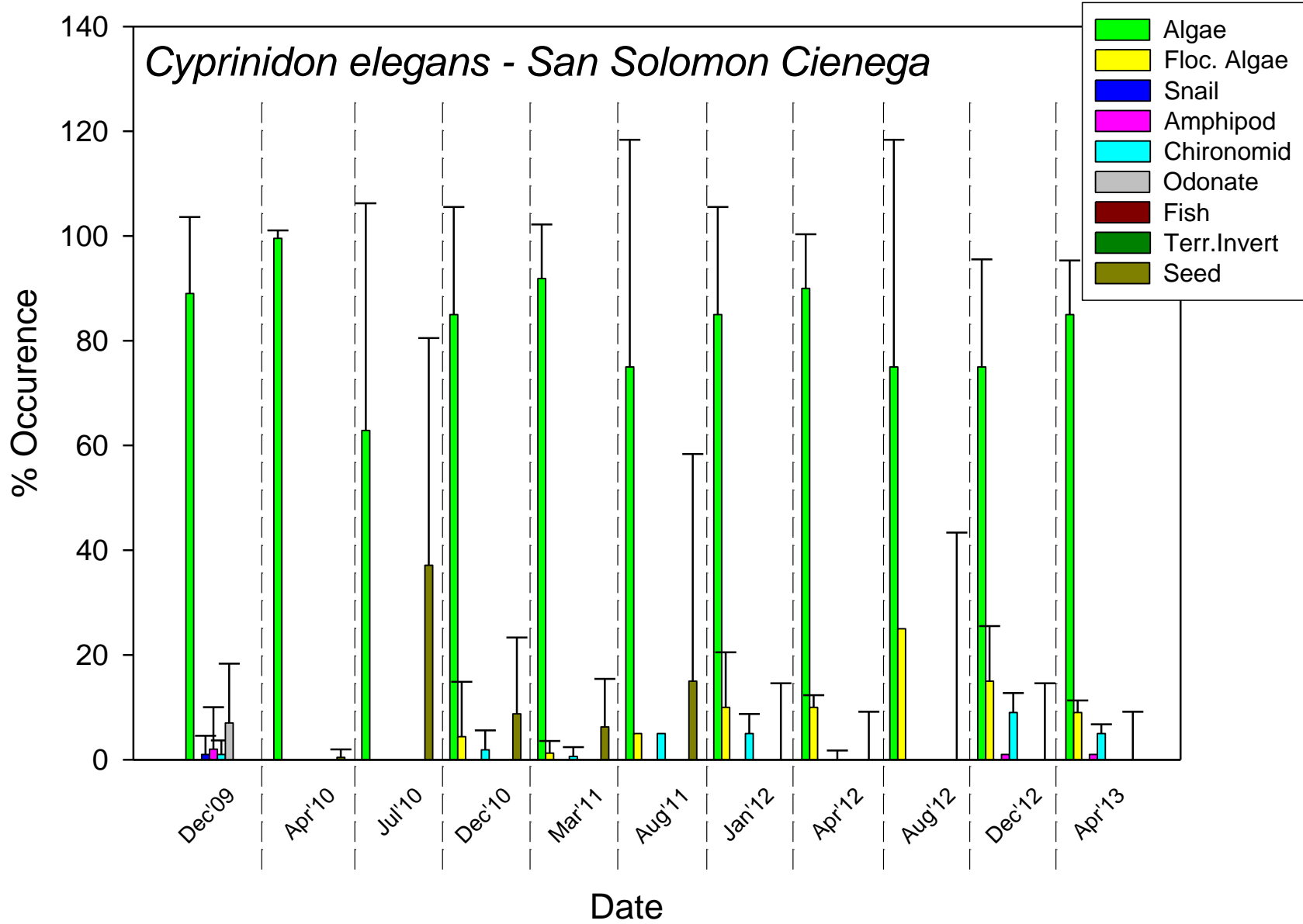
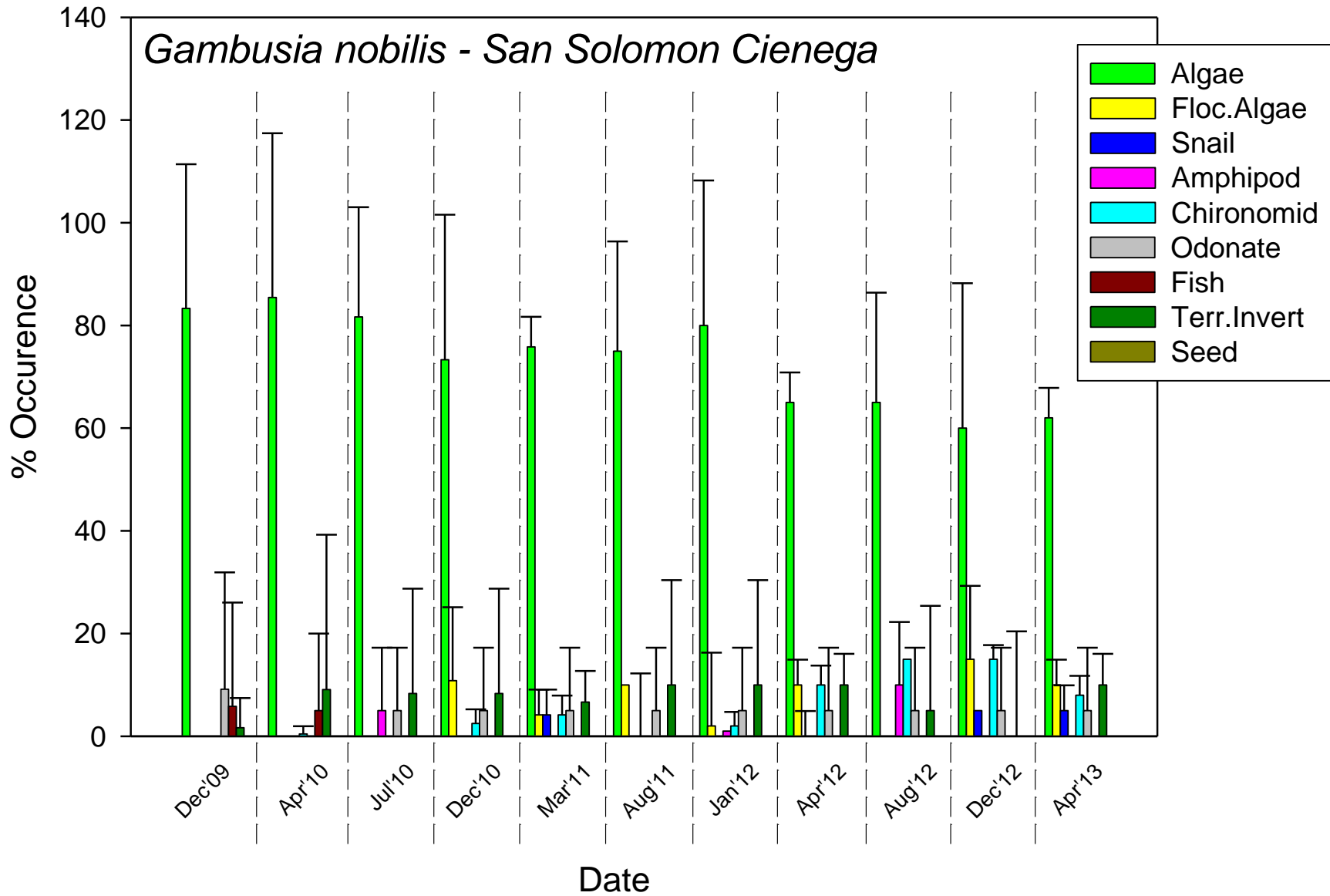Figure 8. Gut contents for *C. elegans* in San Solomon Cienega.

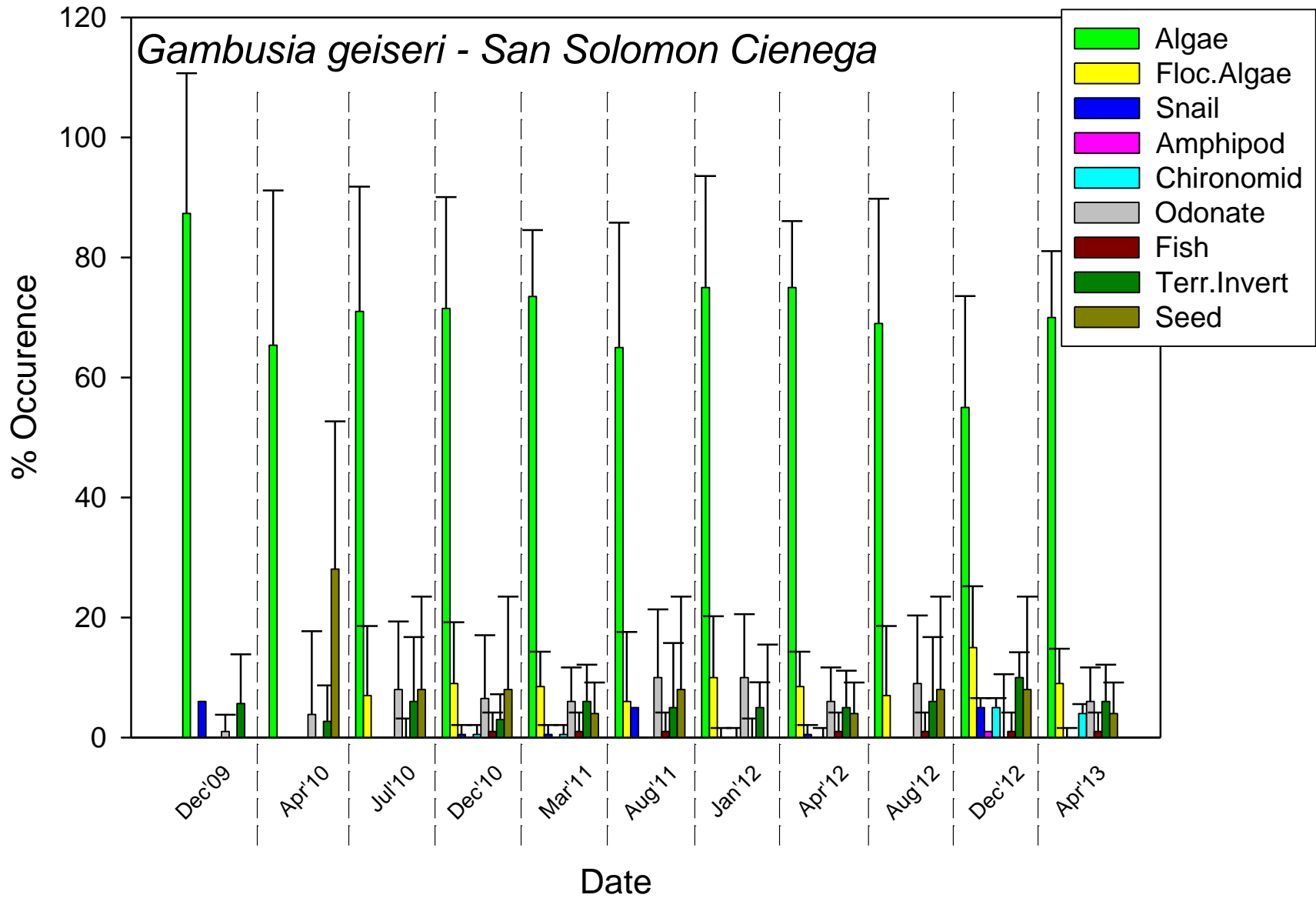Figure 9. Gut contents for *G. nobilis* in San Solomon Cienega.

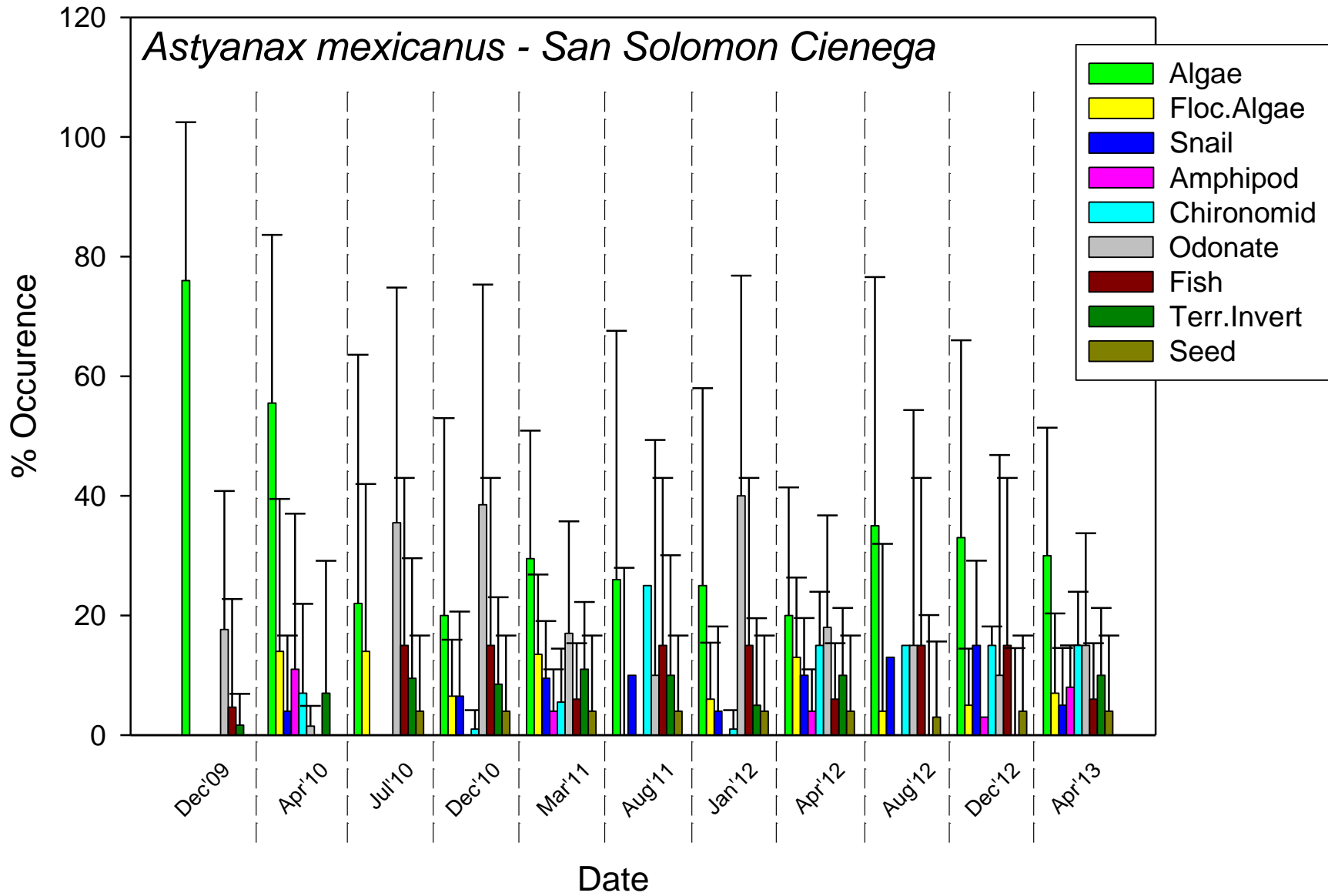Figure 10. Gut contents for *G. geiseri* in San Solomon Cienega.

Figure 11. Gut contents for *A. mexicanus* in San Solomon Cienega.

San Solomon Ciénega – Diet Overlap
Because algae was the dominant food item in the guts of *C. elegans*, *G. nobilis* and *G. geiseri*, diet overlap (% similarity) was high among these taxa for all sample periods. For example, average percent similarity within sample periods in gut contents between *C. elegans* and *G. geiseri* ranged from 75% in Dec. 2009 to 60% in Apr. 2010, averaging about 67% across sample periods. There appeared to be no temporal trend in the diet overlap between *C. elegans* and *G. geiseri*.

Average percent similarity within sample periods in gut contents between C. elegans and *G. nobilis* ranged between 75% in Dec. 2009 to 59% in August 2012. Percent similarity in gut contents between these taxa averaged about 68% across all time periods, and there appeared to be no temporal trend in diet overlap between *C. elegans* and *G. nobilis*.

Gambusia nobilis and *G. geiseri* had highest diet overlap with average values within sample periods ranging from 72% in April 2010 to 88% in April 2012. On average, similarity in gut contents between *G. nobilis* and *G. geiseri* averaged about 80% across all sample periods, and there appeared to be a constant increase in similarity across time (Fig. 12).

Astyanax mexicanus consumed a more diverse array of food items than all three species above. As a result, diet

Figure 12. Diet overlap between species in San Solomon Cienega.

overlap between *A. mexicanus* and *C. elegans*, *G. nobilis*, and *G. geiseri* was far less than presented above. For example, percent similarity in gut contents between *A. mexicanus* and *C. elegans* ranged from 70% in Dec. 2009 to 18% in Apr. 2012. Percent similarity between these two taxa averaged about 35% across all sample periods and appeared to decrease from Dec 2009 to March 2011 where similarity stabilized across sample periods.

Average percent similarity within sample periods in gut contents between *A. mexicanus* and *G. nobilis* ranged from 79% in Dec. 2009 to 21% in Aug. 2012, averaging about 47% across all samples. There appeared to be a decrease in percent similarity of gut contents between these two taxa from Dec. 2009 to Aug. 2012 followed by increasing similarity from Aug. 2012 to Apr. 2013.

Finally, percent similarity in gut contents between *A. mexicanus* and *G. geiseri* were similar to that with *G. nobilis*. Average percent similarity within sample periods for these two
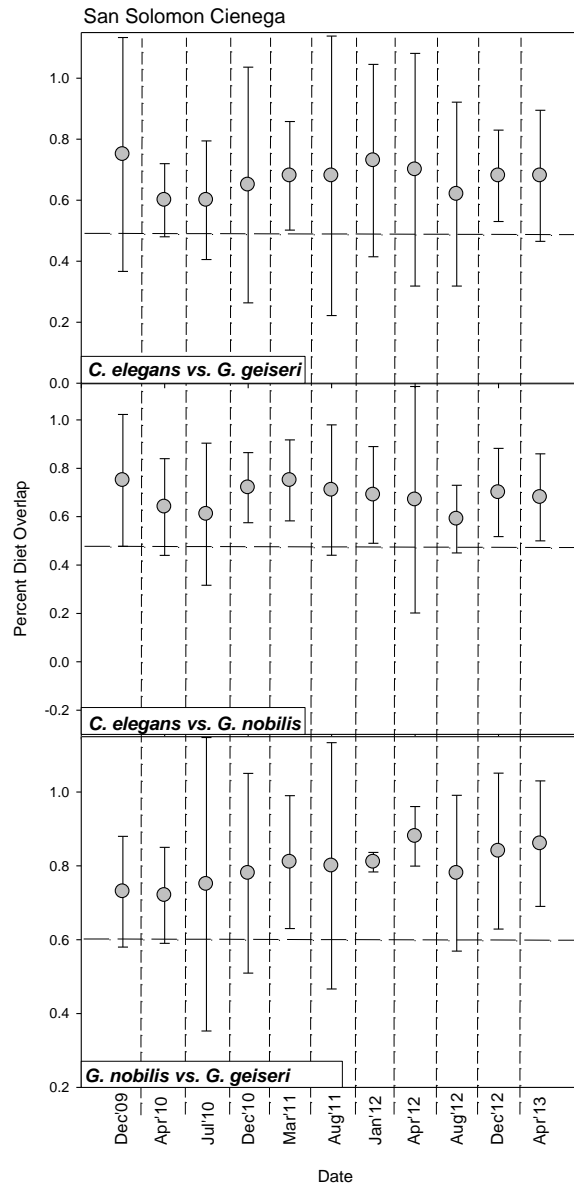
species ranged from 27% on Jan. 2012 to 65% on Dec. 2009, averaging about 40% across all sample periods. There appeared to be a decrease in gut content similarity between these two species from Dec. 2009 to Jul. 2010. After Jul. 2010, percent similarity in gut contents remained similar across sample periods (Fig. 13).

Clark Hubbs Ciénega – Gut Contents

Algae was the dominant food item (% occurrence) in the guts of *Cyprinidon elegans* for all sample periods in Clark Hubbs Ciénega. On average, algae comprised about 68% of the stomach contents for this species. Benthic invertebrates comprised a larger proportion of the gut contents in *C. elegans* guts in this ciénega compared to San Solomon Ciénega, and there appeared to be a slight temporal trend in the gut contents of *C. elegans* in this ciénega. Specifically, the % occurrence of algae in the guts was greatest during spring sample periods and was reduced during summer and fall sample periods (Fig. 14).

Algae comprised only about 45% of the stomach contents for *G. nobilis* in Clark Hubbs Ciénega. Benthic invertebrates and terrestrial insects were abundant in *G. nobilis* guts during all sample periods and comprised a large portion of the diet (~50%). There appeared to be a trend of increasing percent algae in the guts of *G. nobilis* over time in Clark Hubbs Ciénega (Fig. 15).

Figure 13. Diet overlab between species in San Solomon Cienega.

Gut contents of *G. geiseri* were similar to that of *C. elegans* in Clark Hubbs Ciénega with algae comprising the greatest food item (60%) in the guts of this species. *Gambusia geiseri* also consumed benthic invertebrates, terrestrial invertebrates and seeds (~25%). There appeared to be no temporal trend in the gut contents of *G. geiseri* (Fig. 16).

Gut contents of *A. mexicanus* were most carnivorous from the previous three species in Clark Hubbs Ciénega. Although algae was usually the singly most abundant food item, it only averaged 28% of the total gut contents. Other food items such as benthic aquatic invertebrates and terrestrial invertebrates together dominated the guts of *A. mexicanus* (60%). There appeared to be little temporal trend in the gut contents of *A. mexicanus* in this habitat (Fig. 17).
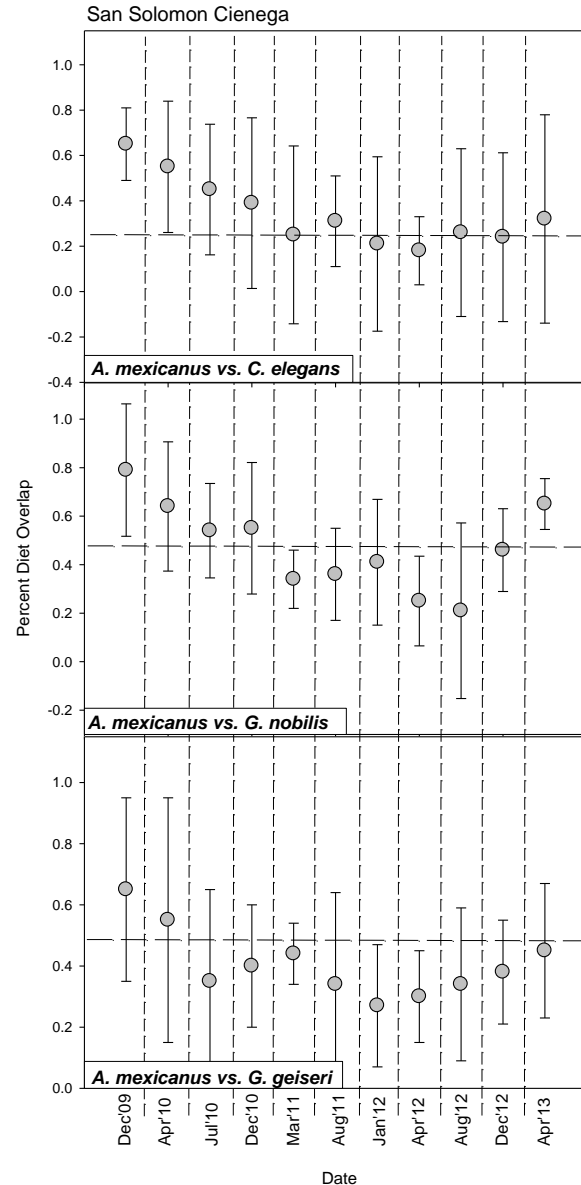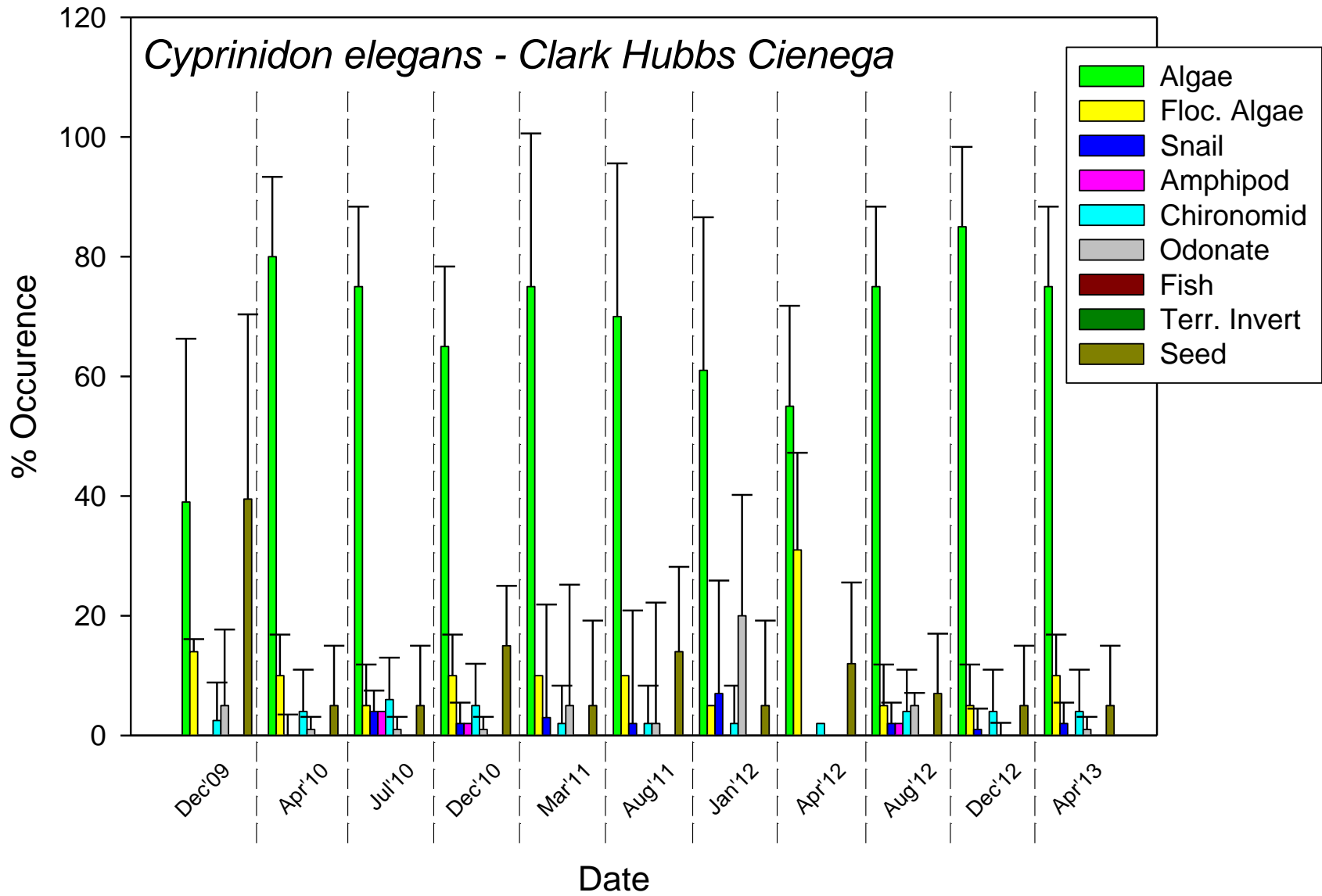
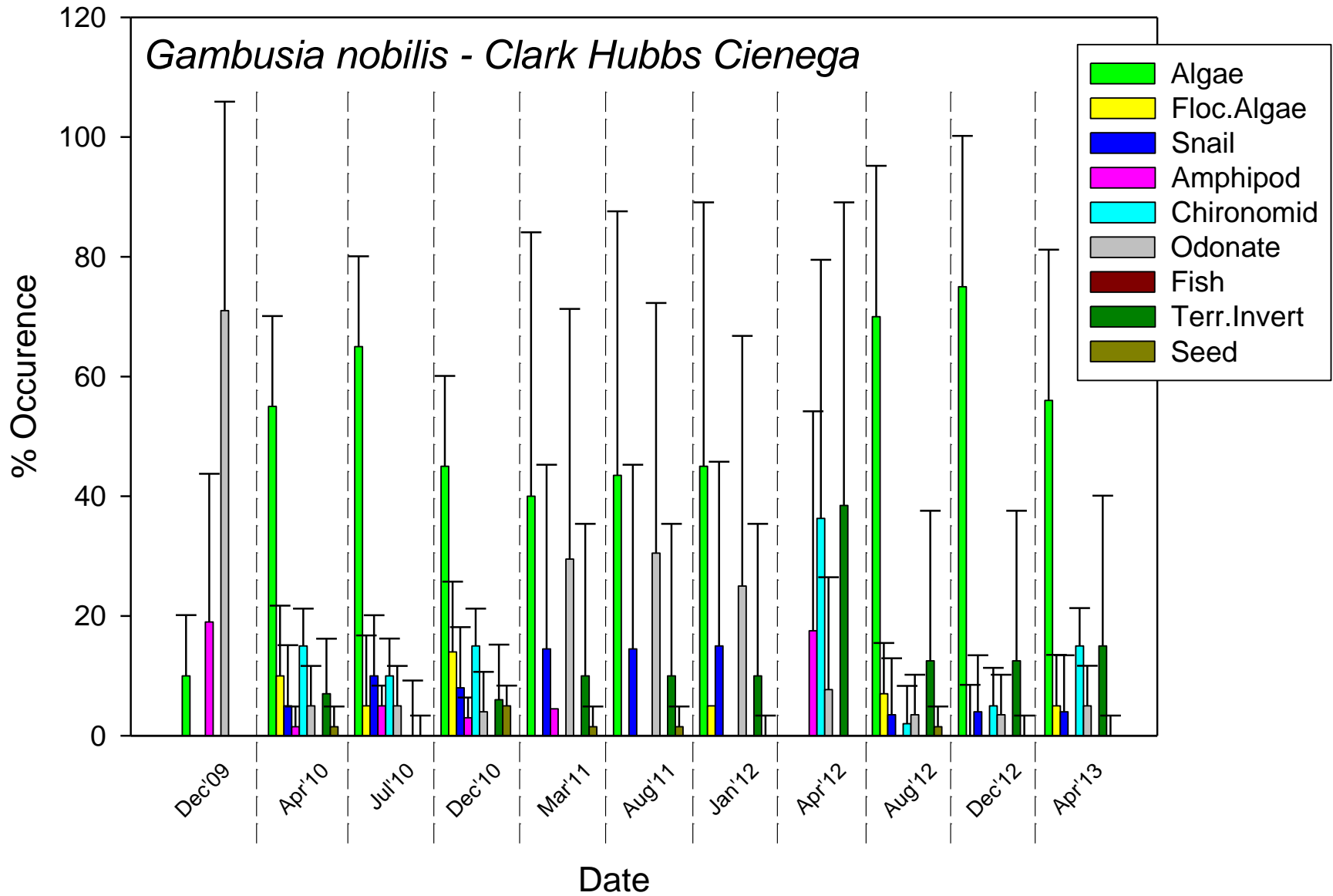Figure 14. Gut contents for *C. elegans* in Clark Hubbs Cienega.

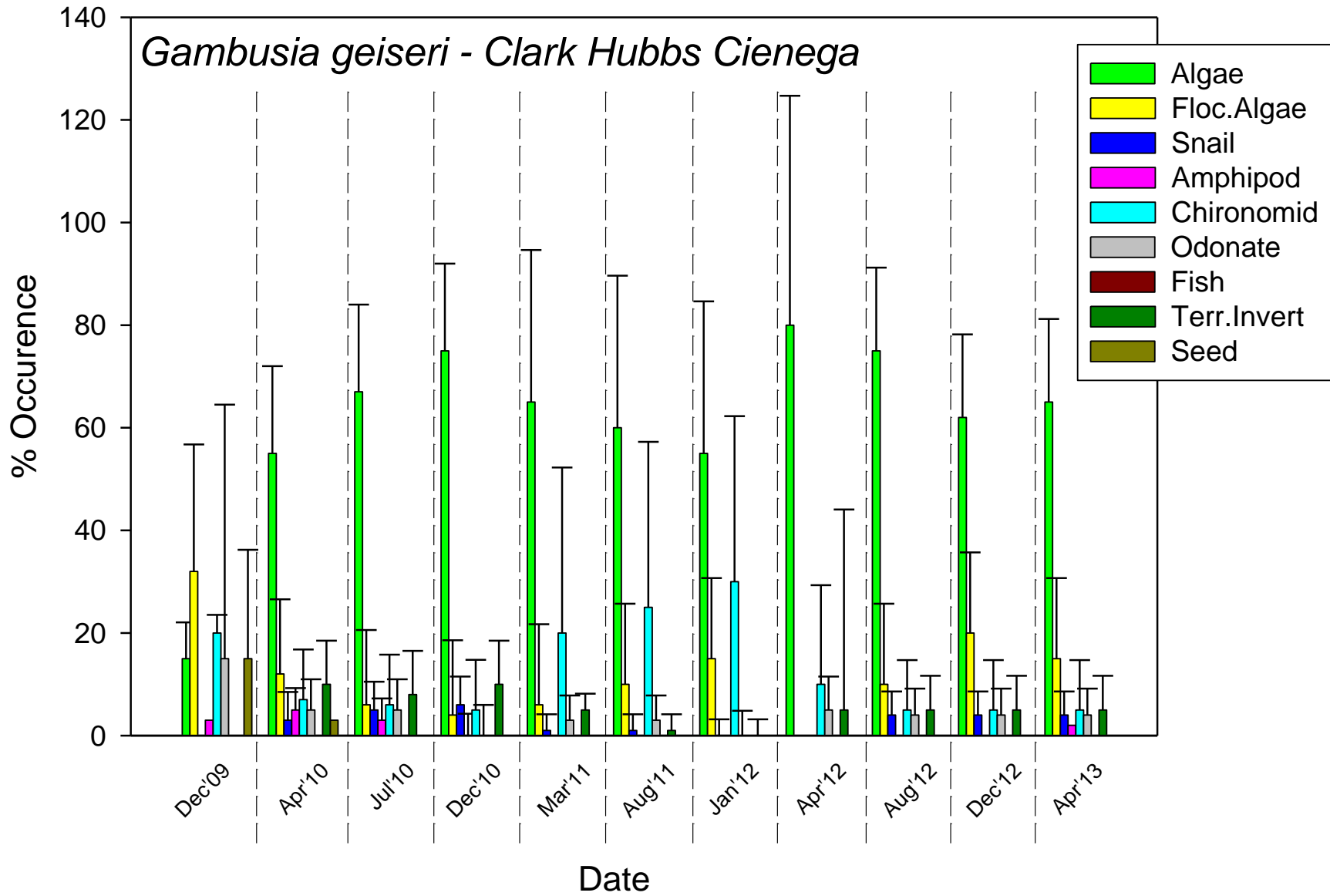Figure 15. Gut contents for *G. nobilis* in Clark Hubbs Cienega.

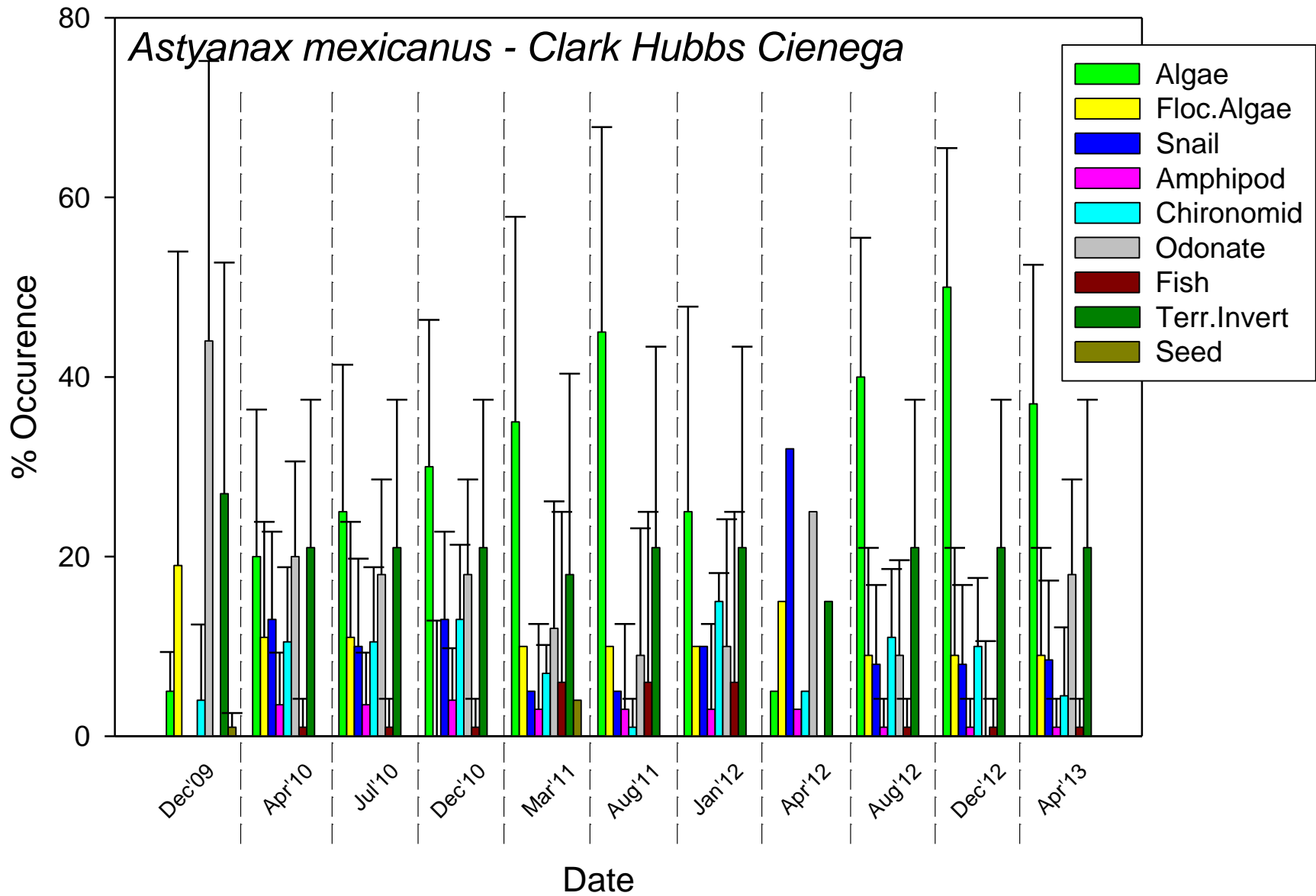Figure 16. Gut contents for *G. geiseri* in Clark Hubbs Cienega.

Figure 17. Gut contents for *A. mexicanus* in Clark Hubbs Cienega.

Clark Hubbs Ciénega – Diet Overlap

Because gut contents were more variable among taxa in Clark Hubbs Ciénega, the percent similarity in diets among species was more variable and somewhat lower than that for San Solomon Ciénega. For example, average percent similarity within sample periods in gut contents between *C. elegans* and *G. geiseri* ranged from 35% in Dec. 2009 to 80% in Aug. 2012, averaging about 65% across sample periods. There appeared to be a temporal increase in the diet overlap between *C. elegans* and *G. geiseri* in this ciénega.

Average percent similarity within sample periods in gut contents between *C. elegans* and *G. nobilis* ranged between 8% in Dec. 2009 to 65% in Dec. 2012. Percent similarity in gut contents between these taxa averaged about 48% across all time periods, and there appeared to be no temporal trend in diet overlap between *C. elegans* and *G. nobilis*.

Gambusia nobilis and *G. geiseri* had average diet overlap values within sample periods ranging from 13% in Dec. 2009 to 76% in Aug. 2012. On average, similarity in gut contents between *G. nobilis* and *G. geiseri* averaged about 62% across all sample periods, and there appeared to be no trend in gut content similarity between species across time (Fig. 18).

As seen in San Solomon Cienga, *A. mexicanus* consumed a more diverse array of food items than the three species above. As a result, diet overlap between *A. mexicanus* and *C. elegans*, *G. nobilis*,

Figure 18. Diet overlap between species in Clark Hubbs Cienega.

and *G. geiseri* was much reduced. For example, percent similarity in gut contents between *A. mexicanus* and *C. elegans* ranged from 15% in Dec. 2009 to 50% in Apr. 2013. Percent similarity between these two taxa averaged about 27% across all sample periods and appeared to increase across sample dates. Average percent similarity within sample periods in gut contents between *A. mexicanus* and *G. nobilis* ranged from 20% in Apr. 2012 to 50% in Dec. 2012, averaging about 34% across all samples. There appeared to be a slight increase in percent similarity of gut contents between these two taxa from Dec. 2009 to Apr. 2013. Finally, percent
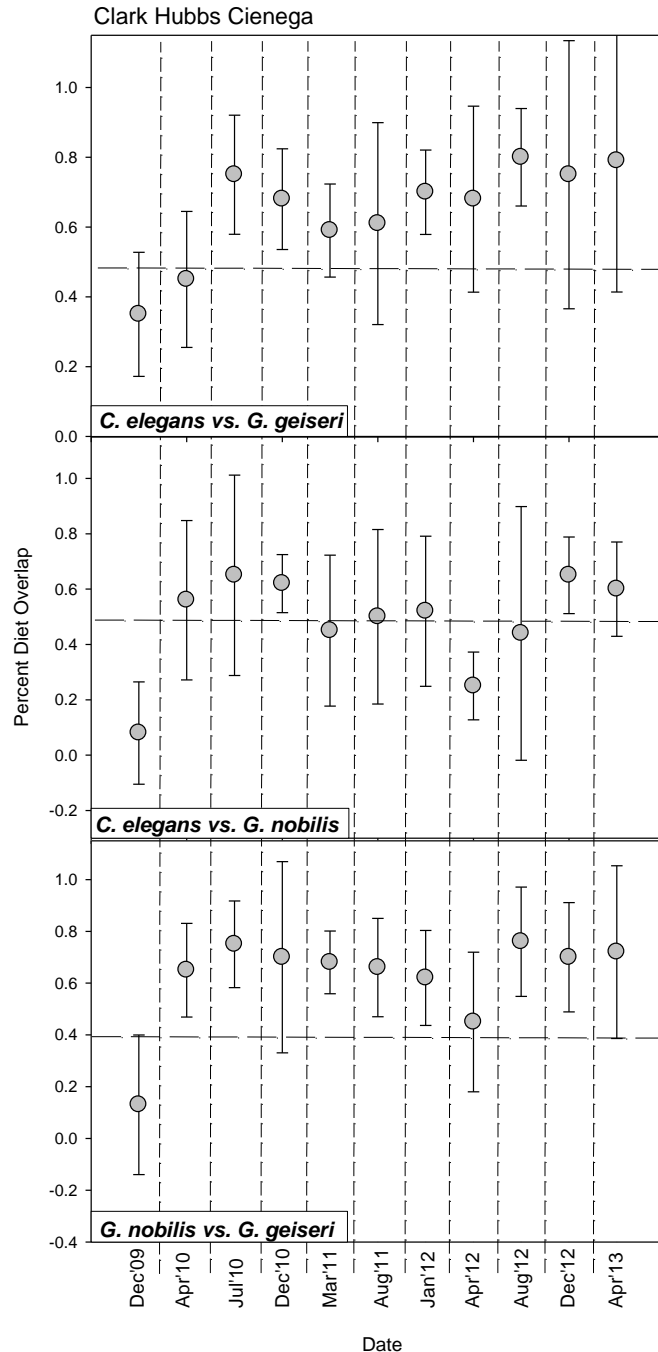
similarity in gut contents between *A. mexicanus* and *G. geiseri* ranged from 15% on Dec. 2009 to 55% on Apr. 203, averaging about 36% across all sample periods. There appeared to be an increase in gut content similarity between these two species across all sample periods (Fig. 19).

FOOD WEB STRUCTURE

San Solomon Ciénega

Based on ratios of the carbon isotopes in all trophic levels above the primary producers, it appears that consumer biomass in the San Solomon Ciénega is driven primarily by carbon from cladophora, terrestrial plants and detritus. It appears that neither terrestrial grasses nor *Chara* sp. provided an important carbon substrate for consumer biomass in this system (Fig. 20; Table 2).

Using ratios of the nitrogen isotopes, we determined that the food web structure in San Solomon Ciénega had three trophic levels – primary producers and detritus, primary consumers and secondary consumers. The primary producer trophic level consisted of algae (cladophora and *Chara* sp.), terrestrial inputs (leaves and grasses), and decomposition plant materials (detritus). The primary consumer trophic level consisted primarily of benthic aquatic invertebrates. This included terrestrial insect larvae such as Odonates, but also aquatic invertebrates such as snails, crayfish, and amphipods. The nitrogen signature for this group of organisms indicated that benthic invertebrates were consuming primarily algae, detritus and some terrestrial plant material. All fish species and a terrestrial vertebrate (Blotched Water Snake – *Nerodia transversa*) comprised the third trophic level – that is the secondary consumers. The nitrogen signature of these taxa indicated that benthic insect larvae and aquatic invertebrates were a dominant contributor to their populations. There was slight trophic separation within fishes. *Gambusia nobilis* occupied the highest position, suggesting that the biomass of this species is driven most by aquatic invertebrates. *Cyprinidon elegans* and *A. mexicanus* occupied the lowest trophic position within the secondary consumers. This suggests that while these taxa are assimilated most biomass from primary consumers, they also are linked somewhat to algae and detritus for biomass. *Gambusia geiseri* fell intermediate to the other fish species (Fig. 20; Table 2).
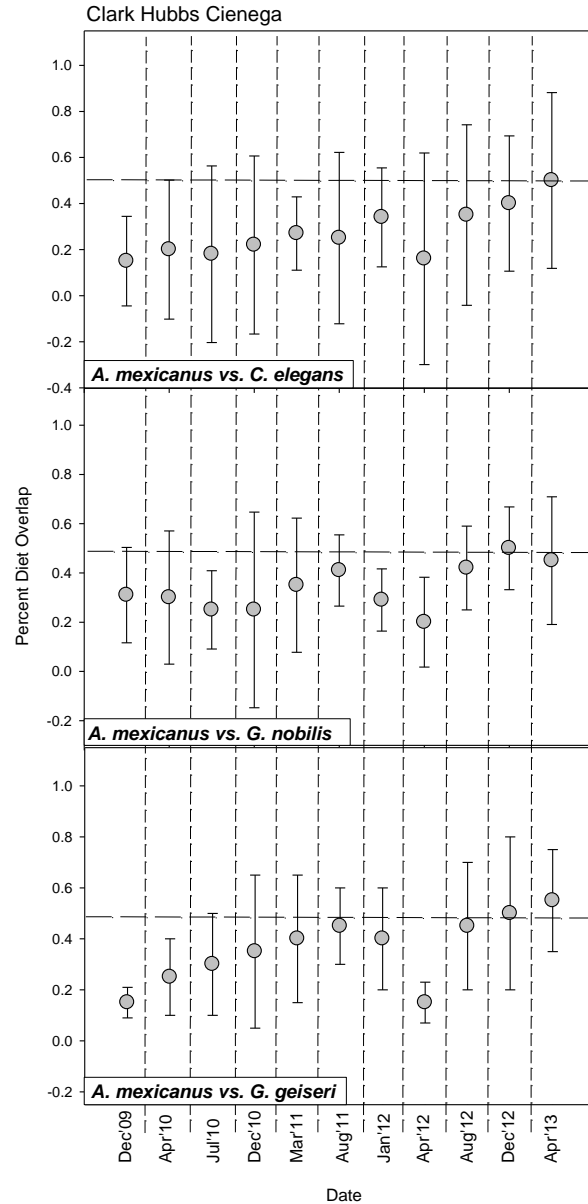

Figure 19. Diet overlap between species in Clark Hubbs Cienga.

Stable isotope data somewhat contract the gut content data. Gut data suggest high diet overlap among most taxa in *San Solomon Ciénega* because of the abundance of algae and flocculent plant material found in their guts. However, these isotope data indicate that most biomass assimilation in the fishes is coming primarily from invertebrates. Therefore, high diet overlap my not be indicative of competitive interactions but rather just inadvertent consumption of algae and detrital material during foraging (Fig. 20; Table 2).
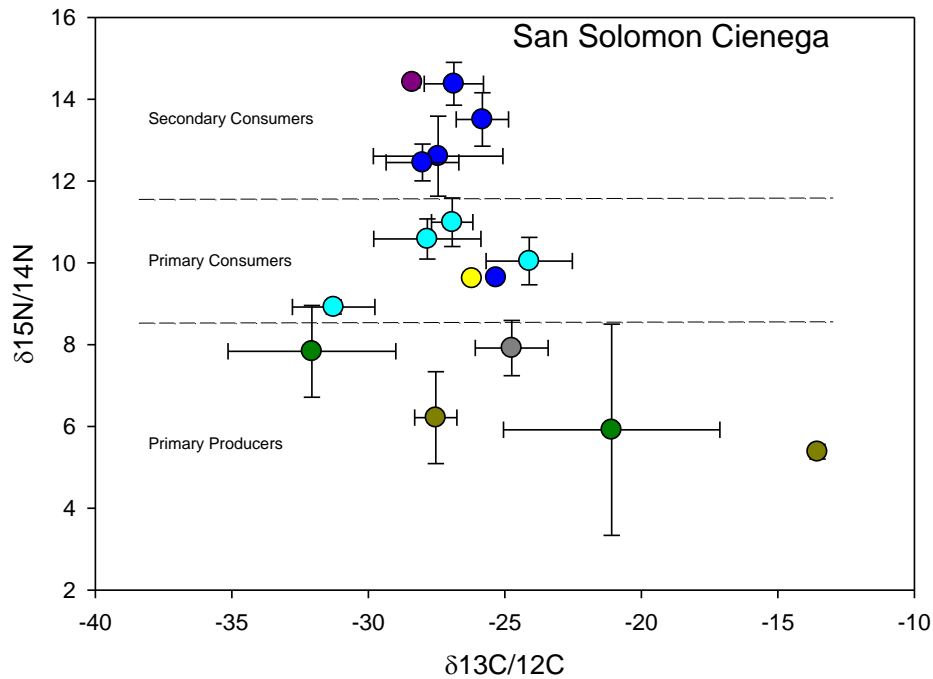

Figure 20. Stable isotope analsysi for San Solomon Cienega see Table 2 for ratio rankings.

Clark Hubbs Ciénega

Food web structure in Clark Hubbs Ciénega was similar to that in San Solomon Ciénega. Based on ratios of the carbon isotopes in all trophic levels above the primary producers, it appears that consumer biomass in the Clark Hubbs Ciénega was driven primarily by carbon from cladophora, terrestrial plant and detritus. It appears that neither terrestrial grasses nor *Chara* sp. provided an important carbon substrate for consumer biomass in this system.

Using ratios of the nitrogen isotopes, we determined that the food web structure in Clark Hubbs Ciénega also had three trophic levels – primary producers and detritus, primary consumers and secondary consumers. The primary producer trophic level consisted of algae (cladophora and *Chara* sp.), terrestrial inputs (leaves and grasses), and decomposition plant materials (detritus). The primary consumer trophic level consisted primarily of benthic aquatic invertebrates. This included terrestrial insect larvae such as Odonates and aquatic invertebrates such as snails, crayfish, and amphipods. The primary consumer trophic level in Clark Hubbs Ciénega also included tadpole larvae and an adult Leopard Frog (*Rana* sp.). The nitrogen signature for this group of organisms indicated that benthic invertebrates were consuming primarily algae, detritus and some terrestrial plant material. All fish comprised the third trophic level –secondary consumers. The nitrogen signature for these taxa was more distinct from the

primary consumers in Clark Hubbs Ciénega. This indicated that benthic insect larvae and aquatic invertebrates were an important contributor to the fish populations in Clark Hubbs Ciénega. There was little trophic separation within fishes in this habitat, but *Gambusia nobilis* still occupied the highest position trophic position in the food web, suggesting that the biomass of this species is driven most by aquatic invertebrates.

Stable isotope data in Clark Hubbs Ciénega also contract the gut content data that suggested high diet overlap among most taxa. However, these isotope data indicate that most biomass assimilation in the fishes is coming primarily from invertebrates.
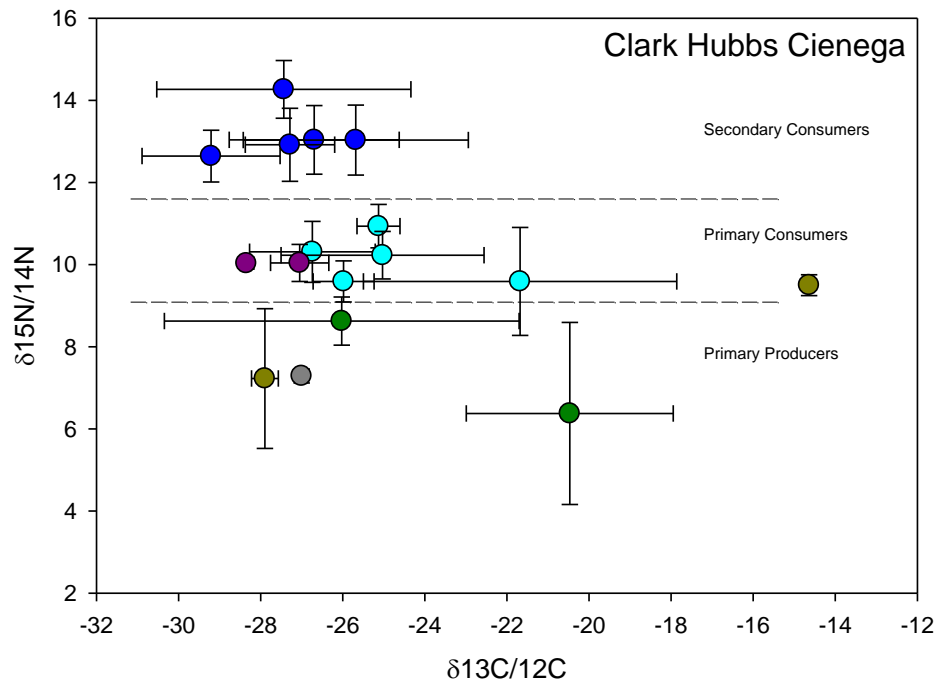

Figure 21. Stable isotope analysis for Clark Hubbs Cienega. See table 2 for isotope rankings.

OVERALL CONCLUSIONS

This research indicates that the populations of the endangered fishes (*C. elegans* and *G. nobilis*) in the Balmorhea State Park ciénegas are currently stable, and, although there is substantial seasonal variation in density and abundance, the populations appear reproductively viable. Food structure and diet overlap data suggest that all fishes with in these ciénegas could be limited by resource availability since they all assimilate biomass from aquatic invertebrates. Therefore, management efforts to enhance population growth should begin with promoting benthic food resources (namely insect larvae) for these taxa. However, the extreme abundance of the invasive *G. geiseri* and its competitive potential with all other taxa, may reduce the benefits any management efforts aimed solely at resources. Therefore, a combined management approach that favors endangered while selecting against the invasive may be the most beneficial management option in this system. I currently have continued research designed to explore additional management options that may selectively favor *C. elegans* and *G. nobilis*.

Table 1.   Seasonal density (no./m^2) and population (N) estimates ($\pm$ 95% CI) for the four most abundant species in three aquatic habitats at Balmorhea State Park – San Solomon Ciénega, Refuge Canal, and Clark Hubbs Ciénega.

SAN SOLOMON CIÉNEGA		*Cyprinidon elegans*	*Gambusia nobilis*	*Gambusia geiseri*	*Astyanax mexicanus*
March 2009	*no./m^2:*	0.3 ± 0.3	0.5 ± 0.1	14.1 ± 14.8	0.2 ± 0.3
	N:	208.9 ± 186.0	332.9 ± 311.0	9126.2 ± 9545.2	147.0 ± 192.5
July 2009	*no./m^2:*	0.9 ± 0.9	2.3 ± 2.7	5.5 ± 7.1	0.03 ± 0.04
	N:	569.5 ± 569.6	1456.2 ± 1751.3	3569.7 ± 4593.7	17.0 ± 23.5
December 2009	*no./m^2:*	1.0 ± 0.9	1.2 ± 2.0	1.9 ± 2.3	1.1 ± 1.7
	N:	667.2 ± 553.8	762.1 ± 1263.1	1218.2 ± 1465.4	718.2 ± 1098.2
April 2010	*no./m^2:*	1.1 ± 0.7	1.5 ± 1.0	15.2 ± 7.9	0.5 ± 0.3
	N:	691.3 ± 451.4	939.2 ± 637.1	9806.8 ± 5104.5	308.8 ± 216.9
July 2010	*no./m^2:*	1.1 ± 0.2	0.9 ± 0.6	11.3 ± 7.1	0.2 ± 0.2
	N:	679.9 ± 160.0	570.9 ± 417.1	7309.4 ± 4580.4	116.2 ± 111.7
December 2010	*no./m^2:*	1.2 ± 0.8	0.6 ± 0.4	17.7 ± 6.5	0.1 ± 2
	N:	781.9 ± 494.8	401.1 ± 278.6	11422.6 ± 4196.9	89.9 ± 138.0
March 2011	*no./m^2:*	0.6 ± 0.4	0.5 ± 0.4	4.5 ± 3.8	0.0 ± 0
	N:	369.6 ± 261.2	333.6 ± 228.5	3194.9 ± 2513.2	0 ± 0.0
August 2011	*no./m^2:*	1.6 ± 0.4	2.2 ± 0.9	5.5 ± 4.8	0.9 ± 0.9
	N:	825.1 ± 261.2	2023.5 ± 500.2	4239.1 ± 2513.2	564.2 ± 564.2
January 2012	*no./m^2:*	0.9 ± 0.2	1.2 ± 0.5	4.5 ± 3.8	0.1 ± 0.1
	N:	289.2 ± 254.2	823.5 ± 250.2	3002.5 ± 2987.5	95.5 ± 95.5
April 2012	*no./m^2:*	0.8 ± 0.3	0.4 ± 0.2	12.5 ± 9.2	2.5 ± 1.1

	N:	422.6 ± 223.2	325.5 ± 222.2	7953.3 ± 4326.2	1500.0 ± 789.2
August 2012	*no./m²:*	1.1 ± 0.5	1.8 ± 0.6	19.2 ± 11.3	1.1 ± 1.1
	N:	669.5 ± 361.2	1955.5 ± 1800.1	12567 ± 7689.2	812.6 ± 812.6
December 2012	*no./m²:*	1.5 ± 0.8	1.3 ± 0.7	16.8 ± 10.0	1.5 ± 1.2
	N:	1050.3 ± 560.1	910.1 ± 490.0	11760.6 ± 7000.1	1050.4 ± 840.3
April 2013	*no./m²:*	0.5 ± 0.4	0.6 ± 0.5	11.2 ± 8.7	0.4 ± 0.4
	N:	350.5 ± 280.8	420.2 ± 350.5	7840.5 ± 6090.5	280.2 ± 280.1

REFUGE CANAL

March 2009	*no./m²:*	1.9 ± 0.5	2.1 ± 1.0	19.1 ± 17.2	0.6 ± 0.6
	N:	1139.5 ± 277.3	1232.9 ± 584.4	11272.8 ± 10148.0	357.3 ± 340.9
July 2009	*no./m²:*	1.0 ± 0.7	6.2 ± 2.8	2.3 ± 1.5	1.0 ± 0.9
	N:	583.7 ± 433.3	3680.3 ± 1666.0	1369.1 ± 857.2	618.0 ± 531.6

CLARK HUBBS

CIÉNEGA

December 2009	*no./m²:*	0.9 ± 0.6	0.2 ± 0.1	0.2 ± 0.2	0.0 ± 0.0
	N:	800.1 ± 537.4	211.9 ± 91.6	133.7 ± 131.3	0.0 ± 0.0
April 2010	*no./m²:*	0.2 ± 0.1	<0.1 ± 0.1	<0.1 ± 0.1	0.1 ± 0.1
	N:	189.0 ± 119.3	28.6 ± 24.4	7.6 ± 7.0	47.7 ± 30.7
July 2010	*no./m²:*	5.0 ± 2.2	0.5 ± 0.3	0.9 ± 1.0	4.2 ± 3.3
	N:	4370.7 ± 1958.9	408.6 ± 280.6	819.1 ± 888.8	3654.6 ± 2838.9
December 2010	*no./m²:*	15.2 ± 13.7	2.7 ± 0.6	12.6 ± 4.1	1.2 ± 0.9
	N:	13248.5 ± 11925.1	2357.8 ± 561.7	10959.8 ± 3593.7	1065.6 ± 819.1

March 2011	*no./m²:*	4.4 ± 2.8	2.9 ± 1.8	6.9 ± 5.1	0.6 ± 0.3
	N:	3791.0 ± 2458.6	2556.8 ± 1606.0	5985.7 ± 4434.9	544.5 ± 324.9
August 2011	*no./m²:*	17.8 ± 12.0	4.2 ± 1.1	15.2 ± 8.2	2.5 ± 2.7
	N:	18789 ± 15621.1	4568.2 ± 2134.5	15234.6 ± 7568.8	2024.4 ± 2987.6
January 2012	*no./m²:*	10.2 ± 7.7	2.3 ± 1.0	10.6 ± 3.3	0.9 ± 0.9
	N:	9568.1 ± 736.5	2111.0 ± 1235.6	10222.2 ± 2935.7	946.2 ± 94.6.2
April 2012	*no./m²:*	6.5 ± 4.5	1.5 ± 0.8	5.5 ± 3.8	0.0 ± 0
	N:	5655.2 ± 4689.2	1865.4 ± 1346.2	5231.8 ± 3335.3	0 ± 0.0
August 2012	*no./m²:*	19.2 ± 15.1	4.1 ± 3.4	17.8 ± 10.0	4.5 ± 3.7
	N:	20235.2 ± 17986.3	4454.8 ± 3956.1	17789.5 ± 11234.2	3879.3 ± 2879.2
December 2012	*no./m²:*	18.6 ± 13.3	3.3 ± 2.4	15.7 ± 13.3	1.9 ± 2.3
	N:	16461.0 ± 1170.5	2920.5 ± 2124.0	13894.5 ± 1177.5	1681.5 ± 2035.5
April 2013	*no./m²:*	10.7 ± 6.3	1.1 ± 1.4	7.2 ± 5.5	0.5 ± 0.5
	N:	946.5 ± 5575.5	973.5 ± 1239.0	6372.0 ± 4867.5	442.5 ± 442.5

Table 2. Isotope ratio rankings across organisms in both ciénegas.

San Solomon Ciénega

Organism	δ13C/12C	Organism	δ15N/14N
Cladophora	-32.07	Snake	14.42
Amphipod	-31.27	G.nobilis	14.38
Snake	-28.39	G.geiseri	13.51
C. elegans	-28.02	A. mexicanus	12.61
Odonate	-27.84	C. elegans	12.45
Terrestrial Plant	-27.53	Crayfish	10.99
A. mexicanus	-27.45	Odonate	10.58
Crayfish	-26.93	InvasiveSnail	10.04
G.nobilis	-26.87	I.lupus	9.64
Cattail	-26.20	Cattail	9.62
G.geiseri	-25.82	Amphipod	8.92
I.lupus	-25.32	Detritus	7.92
Detritus	-24.75	Cladophora	7.84
InvasiveSnail	-24.11	Terrestrial Plant	6.22
Chara	-21.09	Chara	5.92
Grass	-13.55	Grass	5.38

Clark Hubbs Ciénega

Organism	δ13C/12C	Organism	δ15N/14N
A. mexicanus	-29.21	G. nobilis	14.27
Frog	-28.35	G. geiseri	13.04
Terrestrial Plant	-27.90	C. elegans	13.03
G. nobilis	-27.44	Catfish	12.92
I. lupus	-27.29	A. mexicanus	12.64
Tadpole	-27.05	Crayfish	10.94
Detritus	-27.00	Odonate	10.31
Odonate	-26.74	Native Snail	10.23
G. geiseri	-26.70	Tadpole	10.04
Cladophora	-26.02	Frog	10.03
Invasive Snail	-25.98	Backswimmer	9.59
C. elegans	-25.68	Invasive Snail	9.59
Crayfish	-25.13	Grass	9.50
Native Snail	-25.03	Cladophora	8.63
Backswimmer	-21.68	Detritus	7.29
Chara	-20.47	Terrestrial Plant	7.23
Grass	-14.64	Chara	6.38

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