Section 6 (Texas Traditional) Report Review

Form emailed to FWS S6 coordinator (mm/dd/yyyy): 2/5/2016

TPWD signature date on report: 10/26/2015

Project Title: Habitat expansion, genetic characterization, and population assessments of the highly endangered Leon Springs pupfish, *Cyprinodon bovinus*.

Final or Interim Report? Final

Grant #: TX E-150-R

Reviewer Station: Austin ESFO

Lead station concurs with the following comments: NA (reviewer from lead station)

Interim Report (check one):

☐ Acceptable (no comments)

☐ Needs revision prior to final report (see comments below)

☐ Incomplete (see comments below)

Final Report (check one):

☒ Acceptable (no comments)

☐ Needs revision (see comments below)

☐ Incomplete (see comments below)

Comments:

FINAL PERFORMANCE REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-159-R

(F13AP00688)

Endangered and Threatened Species Conservation

Developing a predictive habitat model for the Comanche Springs pupfish (*Cyprinoden elegans*) to be used in species recovery.

Prepared by:

Dr. Chad Hargrave

TEXAS PARKS & WILDLIFE

Carter Smith
Executive Director

Clayton Wolf
Director, Wildlife

17 October 2017

FINAL REPORT

STATE: ____Texas_______________ GRANT NUMBER: ___TX E-159-R-1__

GRANT TITLE: Developing a predictive habitat model for the Comanche Springs pupfish (*Cyprinoden elegans*) to be used in species recovery.

REPORTING PERIOD: ____1 September 2013 to 30 September 2017

OBJECTIVE(S). To quantify effects of substrate type and vertical structure on population growth rate and size of *C. elegans*, and develop a quantitative habitat-model that accurately predicts *C. elegans* population growth rate and size based on substrate composition and areal coverage of vertical structure within a ciénega.

Segment Objectives:

Task 1: Manipulative experiments
The manipulative experiments will test the importance of two potentially critical habitats on population growth and size of the *C. elegans* in a controlled, statistically robust framework.

Task 2: Monitor natural fish populations and local habitat
Support the growing dataset and extend our population tracking by an additional 3 years. To provide essential data for conservation of the endangered fishes in the Balmorhea State Park and will extend monitoring to other local habitats supporting *C. elegans.*

Task 3: Quantitative habitat model for *C. elegans*
All tasks above will result in a simple, quantitative habitat model for predicting *C. elegans* density and population size within a ciénega.

Significant Deviations:

None.

Summary Of Progress:

Please see Attachment A.

Location: Balmorhea State Park, Phantom Cave, East Sandia Ciénega near Balmorhea & Toyahvale, Reeves County, Texas USA

Cost: ___Costs were not available at time of this report, they will be available upon completion of the Final Report and conclusion of the project.__

Prepared by: _Craig Farquhar_____________ Date: _17 October 2017

Approved by: ______________________________ Date:_____17 October 2017_
C. Craig Farquhar

ATTACHMENT A

Final Report

USFWS Federal Assistance Grant TX E-159-R

DEVELOPING A PREDICTIVE HABITAT MODEL FOR THE COMANCHE SPRINGS PUPFISH (*CYPRINODON ELEGANS*) TO BE USED IN SPECIES RECOVERY

Prepared by: Chad W. Hargrave (PI)
Department of Biological Sciences
Box 2116
Sam Houston State University
Huntsville, TX 77341
cwhargravae@shsu.edu

Date: October 13, 2017

CO-PIs David Riskind
Natural Resources
Texas Parks and Wildlife Department
4200 Smith School Road
Austin, TX 78744
David.riskind@tpwd.state.tx.us
512-912-7120

Gary Garrett
Texas Museum of Natural History
University of Texas, Austin
GaryGarrett@utexas.edu
830-866-3356

ABSTRACT

We monitored natural populations of four fish species (targeting *Cyprinodon elegans*) and local habitat parameters in five natural desert wetlands (ciénegas) in west Texas. We found that populations of all 4 taxa varied over space and time. *Cyprinidon elegans* densities and abundances were somewhat correlated with local availability of gravel substrate and submerged vegetation that grew vertically in the water column. We hypothesized that these patterns of abundance were driven by the increase in basal resources (algae and invertebrates) consumed by *C. elegans*. To test this hypothesis, we conducted a controlled mesocosm experiment where we manipulated the degree of gravel comprising the substrates and the areal coverage of vertical vegetative structure. We monitored population density and total abundance of *C. elegans* in response to these habitat manipulations. We found that increased gravel and vertical vegetative structure increased basal resources and increased the density and abundance of *C. elegans* in a controlled environment. This supported our hypothesis based on field observations. We used the results for the manipulative experiment to parameterize a population growth model and compared the density and abundance predictions from that model to field observations. The comparison of predicted versus actual *C. elegans* densities revealed that our predictive population model was significantly over predicting natural *C. elegans* populations in the field. We suggest that other variables that affect *C. elegans* densities and abundance, e.g., competition with other fishes and invertebrates, resulted discrepancy between the predictive model and actual field estimates. Although our model did not accurately predict *C. elegans* in the field, our controlled experiment and resulting predictive model demonstrate that increased gravel substrate and vertical vegetative structure can positively affect *C. elegans*. Therefore, we suggest that habitat management for this species should include both management strategies that increase course substrates and promote vegetation that grows vertically in the water column.

INTRODUCTION

The endangered Comanche Springs Pupfish (*Cyprinodon elegans*) continues to face global-extinction for two reasons. First, most naturally flowing springs in West Texas that could support viable populations of *C. elegans* have failed or are failing because of continued mining for groundwater throughout the desert southwest (Brune 1981). Second, local wetland habitats supported by natural spring flows have been altered by humans for irrigation purposes (Brune 1981). As a result, a once extensive network of flowing wetland habitats throughout the native range of *C. elegans* is now completely absent or has been modified into channelized irrigation canals (Garret et al. 2002). The extent of groundwater withdraw has restricted the distribution of *C. elegans* to four localized areas (Hubbs et al. 1991) and local habitat alteration through channelization likely has prevented *C. elegans* population densities from reaching their maximum potential (Hubbs 2001). As a result, habitat loss is one of the 3 major threats to the persistence *C. elegans* (U.S. Fish and Wildlife Service 1981).

In general, habitat requirements have been based on anecdotal observations from the field. These reports indicate that *C. elegans* is most abundant in stenothermal spring outflows and in channelized areas with low current velocity (Garret et al. 2002). The species is omnivorous foraging often in the benthos (Winemiller and Anderson 1997). It spawns in both stagnant and flowing water, but structure for male territories is necessary for reproduction (Leiser and Itzkowitz 2003). Based on these habitat associations, San Solomon Spring in Balmorhea State Park could be the most important localized spring habitats for the continued persistence of *C. elegans* throughout its range (Hubbs 2001).

San Solomon Spring continues to have stenothermal freshwater flows (14,000,000 to 20,000,000 gallons/day), and, although the entire natural wetland habitat associated with this this spring was once drained and converted into irrigation canals, two reconstructed wetland habitats (San Solomon Ciénega and Clark Hubbs Ciénega) were built in 1995 and 2009, respectively, by Texas Parks and Wildlife Department (TPWD). These wetlands provide stenothermal areas of low water velocity that may provide critical habitat for the endangered fishes within this system (*C. elegans* and *Gambusia nobilis* – Pecos Gambusia).

The Hargrave Lab at Sam Houston State University has a large scale monitoring study in San Solomon and Clark Hubbs ciénegas in Balmorhea State Park as well as in other ciénegas around the state park (Phantom Cave Ciénega, Giffin Spring Ciénega, and East Sandia Ciénega). The goals of these monitoring studies were to (1) establish a monitoring program and quantify population size of the endangered *C. elegans* and *Gambusia nobilis* in all Ciénegas, and (2) identify abiotic and biotic properties that correlate with large populations of both endangered *C. elegans* and *G. nobilis*. We have gained some important insights into the population dynamics of both species from this work. Specifically for *C. elegans*, we have found that vertical structure (i.e., *Chara* sp.) and high benthic primary productivity positively correlate with *C. elegans* population size.

We believe there are three possible explanations for the observation described above. First, *C. elegans* forages primarily on benthic algae (Winemiller and Anderson 1997); therefore, ciénegas with high primary productivity can support larger *C. elegans* populations. Second, rocky substrates and vertical structure provide attachment sites for benthic algae, supporting primary productivity; therefore, habitats with these characteristics have greater primary production and large *C. elegans* populations. Finally, rocky substrates and vertical structure can provide breeding territories for male *C. elegans* (Leiser and Itzkowitz 2002); therefore, habitats with these characteristics can have greater reproduction rates and larger populations.

The monitoring program established by the Hargrave Lab has provided a solid base-line for targeting hypothetical habitat types necessary for *C. elegans* population viability. However, no true quantitative studies have examined the direct effects of specific types of habitat on *C. elegans* populations. Because monitoring and enhancing critical habitats are two major goals in the *C. elegans* Recovery Plan (U.S. Fish and Wildlife Service 1981), there is great need for an in-depth study examining the link between *C. elegans* and ciénega habitats. To meet the goals established in *C. elegans* recovery plan, it is essential to first conclusively identifying critical habitat for *C. elegans*. It also is essential to quantify the extent of this habitat across the current range of *C. elegans* and establish a habitat monitoring program. Finally, it is necessary that a predictive habitat model be developed for *C. elegans*, so that an effective habitat management plan can be created that best meets the needs of this endangered species.

Herein, we tested the direct effects vegetation and substrate composition on the reproductive success of *C. elegans*. We used the experimental results to produce a predictive model to be used in habitat management decision for *C. elegans* reproduction. Finally, , we continued the monitoring program of natural *C. elegans* populations in the field, and used these data to field verify the results of our predictive model.

OBJECTIVES

To quantify effects of substrate type and vertical structure on population growth rate and size of *C. elegans*, and develop a quantitative habitat-model that accurately predicts *C. elegans* population growth rate and size based on substrate composition and areal coverage of vertical structure within a ciénega.

METHODS

<u>Natural Population and Habitat Monitoring</u>

Fish Populations – We continued to monitor fish populations (all species including *C. elegans*) on during winter and summer from the San Solomon and Clark Hubbs ciénegas throughout this study period. We also monitored populations in E. Sandia, Phantom Cave Ciénegas, and Giffin Spring ciénega for both seasons. We estimated fish density, and population sizes ($\hat{N}$) and associated variance $V(\hat{N})$ for all fish species in the ciénegas using the *counts on sample plots method*

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

where A = total population area (the San Solomon Ciénega or Clark Hubbs Ciénega), a = size of the plot, $\bar{n}$ = the average number of animals counted per sample plot, and

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

where n_i = number of animals counted in the i^{th} plot, and s = number of plots used.

To estimate fish densities, we will first blocked off five large sections of each habitat using five 16.7m × 2m (4.2mm mesh) seines. We seined within each blocked off section with a smaller 15' 1/8'' mesh seined using a 3 to 5 pass depletion. We then slowly pull each seine from the shoreline, and carefully collected all fishes from the net. Immediately following capture, fishes were transferred to insulated coolers containing fresh stream water. Fish were held in large cattle tanks during processing. The water in the cattle tanks was replaced repeatedly and fish densities were kept low to reduce stress and promote survival. We removed individual fish from the cattle tanks with a dip net and transfer them to aquaria for viewing. While the fishes are in the viewing tank, we identified and counted each species.

Local habitat – We quantified local habitat characteristics (namely substrate composition and vertical structure coverage) within all ciénegas on a seasonal basis (when populations were estimated). We established ten evenly spaced transects perpendicular to the length of the ciénega. Each transect spaned the width of the ciénega. We measured the length of the transect (i.e., ciénega width) and at ten evenly spaced points along each transect will record the following data. We measured depth of the ciénega, we measured the height of any vertical structure, we estimated the % areal coverage of the vertical structure within a 1×1m grid, and we estimated the substrate composition (% substrate type – silt, sand, gravel, & cobble). Delineation data from all ciénegas was georeferenced and local habitat maps were created for each ciénega.

Manipulative Experiments

Experimental Design – This experiment had two main treatments: Substrate and Vertical Structure. The substrate treatment had 3 treatment levels, and the vertical structure treatment will had 4 treatment levels. All treatment levels were completely crossed, in a full factorial ANOVA design. We replicated all treatment levels 4 times, totaling 48 independent experimental mesocosms in this experiment (Table 1).

The levels within substrate treatment represented different degrees of gravel comprising the substrate. The substrate treatment included 100% sand 0% gravel, 50% sand 50% gravel, and 100% gravel. To create the substrate treatments, all mesocosm were first filled with sand substrate that covered the entire bottom of the mesocosm to a depth of ~10 cm. Then, gravel was added to the mesocosms at the rates per assigned to across mesocosm units .

The levels within vertical structure treatment represented the different amounts of vertical structure observed in the field at a local scale of $1m^2$. Thus, we quantified vertical structure treatments as the percent areal coverage. These levels of vertical structure reflected the following 0%, 25%, 50%, 75% areal coverages. Vertical structure was created using frayed Polypropylene rope attached to an anchoring substrate (e.g., pvc pipe). The length (height of vertical structure) was be 15cm or ½ of the water depth in the experimental mesocosm. Vertical structures treatments were randomly assigned within each substrate treatment.

Experimental mesocosms – Experimental mesocosms were designed to mimic abiotic and biotic conditions of the natural wetlands in the San Solomon Spring complex. All conditions except for the experimental treatments variables (above) were constant across experimental units, providing a powerful test of the objectives of this study. Each experimental mesocosms consisted of a 110 gallon plastic cattle tank with a benthic surface area of $\sim 2.0m^2$, and a depth of about 75cm. Substrate covered the bottom of the mesocosm at a depth of 10cm, and water was maintained at a depth of about 30cm from the substrate. Water was circulated through each tub using a water delivery pipe and a standing drain pipe. Water was refrigerated using a water chiller at a collection point, and was distributed among all units using submersible pumps and PVC piping. Water circulation was adjusted to an appropriate rate to maintain a constant water temperature around 25±3C (natural temperature in San Solomon Ciénega). All mesocosms were housed within a greenhouse facility at the Sam Houston State University Center for Biological Field Studies (CBFS), which maintained constant external environmental conditions.

Similar facilities have been used to successfully house and rear many spring species including *G. geiseri* and *G. nobilis* (per Edith Marsh-Matthews at University of Oklahoma and Raelynn Deaton at Saint Edwards University). Water chemistry (alkalinity, conductivity and salinity) was adjusted to field conditions using dissolved ions. The experimental mesocosms were seeded with a natural periphyton and invertebrate slurry collected from ciénegas at Balmorhea State Park in November 2014. The periphyton, microbe and invertebrate communities were allowed to establish in the mesocosms for 6 months before beginning the experiment with *C. elegans*.

Primary Production and Benthic Invertebrates- We estimated primary production by measuring the 24-h change in oxygen from each mesocosm. Primary production was estimated monthly and averaged across all sample periods. Using a YSI-85 meter, we took oxygen measurements from every mesocosm on an hourly basis for a period of 24h. The change in oxygen during this time period was used as a surrogate for net primary production from each mesocosm.

In addition to the primary production measurements, we estimated benthic invertebrate densities monthly by collecting a single core sample from each mesocosm at monthly intervals. Invertebrates were collected collecting a small core (0.1 m^2) from the substrate. The core was through a 500µm sieve and invertebrates preserved in 70% ethanol. The invertebrates were returned to the lab and counted under a dissecting microscope.

Fish – We collected fishes from Clark Hubbs Ciénega at Balmorhea State Park in January 2015 and transported the fishes in insulated coolers back to CBFS. We will stocked 2 females and 2 males in each experimental mesocosm. Fish were maintained in the mesocosms from June 2015 – August 2017. The mesocosms were checked daily and reproductive behaviors were monitored weekly for the duration of the study. We collected and counted all offspring on a monthly weekly basis via visual observation. At the end of the experiment, all fish were removed, counted and measured. Abundance and population growth was calculated from these measurements.

<u>Quantitative Habitat Model</u>

Model development – We used the following derivation to the logistic growth model to create a habitat-dependent population model that predicts *C. elegans* population size for a habitat based on ciénega substrate composition and percent vertical structure

$$dN_c/dt = r_{\alpha sv}\, N_c(1-(N_c/K_{\alpha sv}) + error$$

where $r_{\alpha sv}$ is the habitat-specific per capita rate of increase of *C. elegans* across differing substrates and vertical structure treatments, N_c is population size of *C. elegans*, $K_{\alpha sv}$ is the habitat specific carrying capacity of *C. elegans* across differing subtrates and vertical structure treatments.

We used data from the manipulative experiments to parameterize the above model. For example, the average population growth of *C. elegans* across substrate and vertical structure treatments was used to estimate $r_{\alpha sv}$ in the population model. The final population sizes of *C. elegans* across substrate and vertical structure treatments was used to estimate $K_{\alpha sv}$ in the population growth model.

Model Testing & Calibration – We tested the theoretical model by applying field data for αs and αv and comparing predicted N to actual population estimates from the field.

RESULTS

<u>Natural Population and Habitat Monitoring</u>

Fish populations – Fish densities varied across season and across localities (Table 2). Gambusia geiseri, an invasive to the system, densities ranged from ~1 to ~28 fish/m^2 and was the most abundant species across all localities and across all seasons. *Gambusia nobilis* densities ranged from 0.1 to ~5.5 fish/m^2, and, on average, was the least abundant species in the system. *Cyprinidon elegans* densities ranged from 0 to 15.6 fish/m^2, and was the third most abundant fish in the system. Finally, *Astyanax mexicanus* densities ranged from 0 to 9.1 fish/m^2 and, on average, was the second most abundant fish in the system.

Local habitat – Most local habitat parameters varied more over space than time (Table 3). In general, temperature was constant across localities, averaging ~24C for all localities and sample periods. Substrates were dominated by fine particulates (sand and silt) in Clark Hubbs, San Solomon, East Sandia and Giffin Spring ciénegas. Substrate in Phantom Cave Spring Ciénega was dominated by cobble/gravel. Vegetation cover varied more temporally than the other abiotic variables. In general, vegetation had the greatest cover in summer months than in winter. The type of vegetative cover differed among ciénegas. For example, *Chara* sp. (a macroalgae) was most dominant in Clark Hubbs and Phantom Cave ciénegas. Giffin Spring Ciénega had both a mix of *Chara* sp. and submerged macrophytes. Filamentous algae (*Chlodophora* sp.) dominated San Solomon Ciénega. East Sandia Ciénega had very little benthic vegetative cover.

<u>Manipulative Experiments</u>

Primary Production and Benthic Invertebrates – Whole system primary production increased on average by about 1.5-times with the percent vegetative cover and proportion of gravel comprising the substrate. This pattern was present throughout the course of the experiment (Fig. 1). Benthic invertebrates were about 1.8 times greater in treatments with greater areal coverage of vertical structure and percent gravel substrate at the onset of the experiment. However, this difference in benthic invertebrates across treatments was absent when the study ended (Fig. 2).

Fish – Fish reproduced successfully in all mesocosms. There was an average intrinsic rate of increase (r) of 0.04 ± 0.004 fish/day across all mesocosms, but reproduction rates differed significantly among treatments (Table 4; Fig. 3). For example, *r* increased by about 65% with increasing proportion of gravel in the substrate (Fig. 4). The effect of vertical structure on *r* was non-linear, increasing by about 153% from the 0% to 50% increasing areal coverage of vertical structure and decreasing at the highest (75%) areal coverage of vertical structure (Fig. 5). Although there was no interaction between substrate composition and areal coverage of vertical structure (P = 0.079), the intermediate levels of vertical structure (i.e., 25% & 50%) had the greatest effect on *r* in the 100% gravel treatments (Fig. 5).

Quantitative Habitat Model

Model Parameterization – We parameterized the quantitative model using multiple regression to predict $r_{\alpha sv}$ and $K_{\alpha sv}$. The best-fit models for both $r_{\alpha sv}$ (eq. 1) and $K_{\alpha sv}$ (eq. 2) were explained by a combined quadratic-linear function:

$$\text{eq. 1.}\ \ r_{\alpha sv} = [(-0.0000275V^2)+(0.0023V)] + 0.0002968S + 0.0118$$

$$\text{eq. 2.}\ \ K_{\alpha sv} = [(-0.0198V^2)+(1.657V)] + 0.2138S + 8.5$$

where V^2 ($P < 0.0001$) is the square of the percent cover of vertical structure, V is percent cover of vertical structure ($P < 0.0001$), and S ($P < 0.0036$) is percent gravel comprising the substrate.

Based on the above equations the overall population growth-rate model for *C. elegans* based on our manipulative experiment is below (eq. 3):

$$\text{eq. 3.}\ \ dN_c/dt = [[(-0.0000275V^2)+(0.0023V)] + 0.0002968S + 0.0118]\ N_c\,[1 - (N_c / [(-0.0198V^2)+(1.657V)] + 0.2138S + 8.5)] \pm 0.22$$

Using the above model, we calculated the predicted number of individuals C. elegans for each treatment in the manipulative experiment, and then compared this predicted value to the average number of C. elegans recovered from each of these treatments at the end of the experiment. This relationship was slightly greater than a 1:1 (r=1.02) highly correlated ($P<0.0001$; Fig. 6).

Model Calibration & Testing – Using the above models and the field estimates of substrate composition and percent cover of vertical structure, we predicted the maximum population size for *C. elegans* in each ciénega for all each sample period. In this comparison, our model over predicted the maximum population size of *C. elegans* in all localities and all sample dates (Table 5). Specifically, our model over estimated C. elegans populations by as much as 58,752 individuals and as little as 2,021 individuals (Average (17,640 individuals).

DISCUSSION

Our field observations, manipulative experiment, and mathematical model demonstrate that vertical vegetative structure and gravel substrate may benefit *C. elegans* populations in their natural habitat.

Cyprinodon elegans were most dense in ciénegas with the greatest amount of vertical vegetative structure and in habitats with courser substrates (e.g., Clark Hubbs Ciénega and

Phantom Spring Ciénega). Moreover, *C. elegans* were most dense during seasons when growth of vegetative structure was also greatest. Densities and populations were comparatively smaller in systems where vegetative structure was rare/absent (East Sandia), or where substrates were dominated by clay/silt/sand (San Solomon Ciénega). We hypothesize that vertical structure and gravel substrates provide increased surface area for primary production and benthic invertebrates – important resources for *C. elegans* populations.

Our controlled experiment supports these hypotheses. Mesocosms with greater vegetative structure and gravel substrates had greater rates of primary production and greater benthic invertebrate densities at the onset of the experimental – i.e., at the introduction of fishes. These rates of primary production remained throughout the duration of the experiment, but the densities of invertebrates were reduced over time. We suggest that the fishes consumed the benthic invertebrates alleviating any positive affect on this ecosystem parameter at the end of the experiment. Thus, we believe the invertebrate biomass moved into the fish trophic group by this time. This hypothesis is supported by the greater density of fish supported in the high vegetation and gravel treatments.

The results from the manipulative experiment support the findings from the field study. Specifically, fish density and total number of individuals recovered from the mesocosm increased with the proportion of gravel in the substrate and increased with the areal coverage of vertical vegetative structure. As discussed above, the basal resources that support *C. elegans* production also increased with these local habitat changes, suggesting that the experimental manipulations benefited *C. elegans* from the bottom up.

The results of the manipulative experiment were used to parameterize a general population growth model. This model was then used to predict the maximum potential densities and population size in natural habitats based on the substrate and vegetative structure in those systems. Although our predictive model was able to accurately predict the densities observed in the mesocosm experiments (indicating the model was calculating correctly), the predicted populations densities and population size for the natural ciénegas were well above observed values. Thus, our predictive model was relatively inaccurate by over predicting the abundance that we found in our monitoring surveys. This suggests that there were potentially other factors (in addition to substrate and vertical structure) that affected *C. elegans* populations in the natural system. Most notable of these is the likely effect of competitors on *C. elegans* populations in the natural systems. For example, at least three other fish species co-occur with *C. elegans* in these natural habitats and there exists a number of snail species, a crayfish species and other invertebrates. All of these coexisting taxa likely rely on algae and/or benthic invertebrates as a food resource. Thus, the availability of these basal resources for *C. elegans* were likely artificially elevated in our controlled mesocosm experiment resulting in a model that over-predicted actual densities and population sizes. Further research may include other community level interactions with fishes, snails and crayfish on the *C. elegans*.

Regardless of the inaccuracy of our predictive models, our data clearly show that elevated vertical vegetative structure and greater proportion of gravel in the sediments will benefit *C. elegans* either directly or indirectly. Therefore, we suggest that any habitat management for *C. elegans* in their native habitats should include addition of course substrates and promotion of vertical vegetative growth.

LITERATURE CITED

Brune, G. 1981. Springs of Texas, Vo. 1. Branch-Smith, Inc., Fort Worth. 566 pp.

Garret, G. P., C. Hubbs, and R. J. Edwards. 2002. Threatened fishes of the world: *Cyprinodon elegans* Baird and Girard, 1853 (Cyprinodontidae). Environmental Biology of Fishes 65:288.

Hubbs, C. 2001. Environmental correlates to the abundance of spring adapted versus stream adapted fishes. Texas Journal of Science 53:299-326

Hubbs, C., R. J. Edwards, and G. P. Garret. 1991. An annotated checklist of the freshwater fishes of Texas, with keys to identification of species. Texas Journal of Science, Supplement 43:1-56.

Leiser, J. K., and M. Itzkowitz. 2002. The relative costs and benefits of territorial defense and the two condional male mating tactics in the Comanche Springs poupfish (*Cypinodon elegans*). Acta Ethol 5:65-72.

Leiser, J. K., and M. Itzkowitz. 2003. The breeding system of an endangered pupfish (*Cyprinodon elegans*). Western North American Naturalist 63:118-121.

U.S. Fish and Wildlife Service. 1981. Recovery plan for the Comanche Springs pupfish. U.S. Fish and Wildlife Service, Albuquerque, New Mexico

Winemiller, K. O., and A. A. Anderson. 1997. Response of endangered desert fish populations to a constructed refuge. Restoration Ecology 5:204-213.

Table. 1. Experimental Design showing 2 main treatments, levels within each treatment and the number of replicates per treatment-level.

		Vertical Structure (% areal cover)			
		0	25%	50%	75%
Substrate	0	n=4	n=4	n=4	n=4
Treatment	50%	n=4	n=4	n=4	n=4
(% gravel)	100%	n=4	n=4	n=4	n=4

Table 2. Fish density ± 1 SE for four fish species collected from the 5 localities within the Balmorhea Spring complex during From August 2014 through November 2015.

	Fish density individuals m^{-2}			
Summer 2014	*Gambusia geiseri*	*Gambusia nobilis*	*Cyprinidon elegans*	*Astyanax mexicanus*
Clark Hubbs Cienega	7.0 ± 1.1	2.9 ± 0.5	9.6 ± 4.3	5.9 ± 4.3
San Solomon Cienega	20.8 ± 15.4	1.1 ± 0.8	0.3 ± 0.13	0
East Sandia Spring	17.8 ± 19.2	0.7 ± 1.0	0.27 ± 0.2	3.5 ± 3.3
Phantom Cave Spring	0.7 ± 0.5	5.3 ± 2.5	2.5 ± 1.4	3.3 ± 1.2
Giffin Spring	20.2 ± 16.8	2.4 ± 1.5	0	1.1 ± 0.9
Winter 2014				
Clark Hubbs Cienega	2.0 ± 2.1	1.1 ± 1.5	4.2 ± 2.0	4.5 ± 4.5
San Solomon Cienega	8.7 ± 5.5	0.8 ± 1.8	3.3 ± 1.1	3.7 ± 3.0
East Sandia Spring	21.9 ± 15.3	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 2.0
Phantom Cave Spring	2.2 ± 1.1	3.2 ± 1.1	3.0 ± 2.5	1.1 ± 0.5
Giffin Spring	15.5 ± 12.7	0.2 ± 1.0	0	2.1 ± 1.9
Summer 2015				
Clark Hubbs Cienega	10.0 ± 9.1	5.5 ± 4.3	15.6 ± 6.6	6.6 ± 5.1
San Solomon Cienega	27.8 ± 19.5	3.2 ± 2.2	5.5 ± 3.3	9.1 ± 7.9
East Sandia Spring	16.2 ± 11.4	0.5 ± 1.0	0.0 ± 0.0	1.3 ± 1.3
Phantom Cave Spring	1.1 ± 0.4	7.7 ± 5.3	3.3 ± 2.2	1.2 ± 1.0
Giffin Spring	24.3 ± 11.1	1.4 ± 1.1	0.0 ± 0.0	3.5 ± 4.0
Winter 2015				
Clark Hubbs Cienega	1.5 ± 2.1	0.5 ± 1.5	5.1 ± 4.2	1.2 ± 2.0
San Solomon Cienega	11.1 ± 6.4	0.8 ± 1.0	2.2 ± 1.8	0.4 ± 1.2
East Sandia Spring	19.9 ± 13.3	0	0	1.9 ± 1.9
Phantom Cave Spring	3.4 ± 2.9	2.8 ± 2.0	3.6 ± 4.1	1.5 ± 0.9
Giffin Spring	12.1 ± 10.0	0.1 ± 1.0	0	3.3 ± 1.2

Table 3. Average habitat parameters (±1SD) estimated base on point measurements along 5 transects from all localities within the Balmorhea spring complex during August 2014 and June 2016.

	Clark Hubbs Ciénega	**San Solomon Ciénega**	**East Sandia Spring**	**Phantom Cave Spring**	**Giffin Spring**
Summer 2014					
Temperature (C)	25.3 ± 1.2	26.1 ± 1.7	24.1 ± 0.2	24.0±0.1	26.7 ± 3.2
Area (m2)	375	495	250	58	700
Substrate Cover					
% Cobble	1 ± 1	10 ± 10	0 ± 0	90 ± 2	0 ± 0
% Gravel	1 ± 1	3 ± 2	0 ± 0	10 ± 5	10 ± 7
% Sand	5 ± 3	15 ± 15	0 ± 0	0 ± 0	35 ± 15
% Silt	94 ± 15	72 ± 30	100 ± 0	0 ± 0	55 ± 22
Vegetation Cover					
% Fil. Algae	15 ± 20	50 ± 34	0 ± 0	10 ± 3	7 ± 6
% Chara	66 ± 32	1 ± 1	0 ± 0	55 ± 12	15 ± 25
% Macrophyte	0 ±0	0 ± 0	5 ± 5	0 ± 0	30 ± 12
Winter 2014					
Temperature (C)	23.3 ± 3.2	22.1 ± 5.7	24.0 ± 0.2	24.0±0.4	22.7 ± 5.3
Area (m2)	375	495	250	58	700
Substrate Cover					
% Cobble	2 ± 1	15 ± 5	0 ± 0	95 ± 2	0 ± 0
% Gravel	1 ± 1	5 ± 5	0 ± 0	5 ± 1	15 ± 5
% Sand	4 ± 2	10 ± 15	0 ± 0	0 ± 0	20 ± 5
% Silt	94 ± 15	70 ± 15	100 ± 0	0 ± 0	65 ± 20

Vegetation Cover					
% Fil. Algae	0 ± 0	10 ± 20	0 ± 0	5 ± 1	1 ± 1
% Chara	45 ± 10	0 ± 0	0 ± 0	25 ± 10	10 ± 5
% Macrophyte	0 ±0	0 ± 0	0 ± 0	0 ± 0	10 ± 10
Summer 2015					
Temperature (C)	24.3 ± 2.2	24.1 ± 3.7	24.1 ± 1.2	24.0±0.1	25.2 ± 5.2
Area (m2)	375	495	250	58	700
Substrate Cover					
% Cobble	1 ± 1	11 ± 10	0 ± 0	95 ± 2	0 ± 0
% Gravel	1 ± 1	5 ± 2	0 ± 0	3 ± 2	5 ± 2
% Sand	3 ± 1	12 ± 8	0 ± 0	3 ± 1	45 ± 10
% Silt	95 ± 5	72 ± 45	100 ± 0	0 ± 0	50 ± 25
Vegetation Cover					
% Fil. Algae	65 ± 10	75 ± 25	0 ± 0	25 ± 5	15 ± 5
% Chara	75 ± 15	0 ± 0	0 ± 0	80 ± 30	30 ± 20
% Macrophyte	0 ±0	0 ± 0	10 ± 10	0 ± 0	45 ± 15
Winter 2015					
Temperature (C)	23.1 ± 1.9	22.5 ± 4.1	24.2 ± 0.1	24.0±0.1	23.1 ± 6.7
Area (m2)	375	495	250	58	700
Substrate Cover					
% Cobble	2 ± 1	5 ± 5	0 ± 0	80 ± 2	0 ± 0
% Gravel	0	5 ± 5	0 ± 0	5 ± 1	20 ± 15
% Sand	4 ± 2	10 ± 15	0 ± 0	0 ± 0	35 ± 10
% Silt	94 ± 15	75 ± 25	100 ± 0	15 ± 10	45 ± 15

Vegetation Cover					
% Fil. Algae	5 ± 5	25 ± 150	0 ± 0	15 ± 5	5 ± 5
% Chara	15 ± 10	5 ± 5	0 ± 0	40 ± 5	15 ± 10
% Macrophyte	0 ±0	0 ± 0	0 ± 0	0 ± 0	15 ± 20
Summer 2016					
Temperature (C)	24.0 ± 1.2	23.8 ± 4.4	24.5 ± 3.3	24.0±0.1	25.7 ± 7.3
Area (m2)	375	495	250	58	700
Substrate Cover					
% Cobble	1 ± 1	5 ± 5	0 ± 0	80 ± 2	0 ± 0
% Gravel	1 ± 1	5 ± 5	0 ± 0	5 ± 5	0
% Sand	3 ± 1	5 ± 5	0 ± 0	0	45 ± 15
% Silt	95 ± 5	85 ± 25	100 ± 0	15 ± 5	55 ± 30
Vegetation Cover					
% Fil. Algae	35 ± 15	85 ± 35	0 ± 0	20 ± 10	10 ± 10
% Chara	90 ± 25	20 ± 15	0 ± 0	95 ± 15	15 ± 10
% Macrophyte	0 ±0	0 ± 0	20 ± 25	0 ± 0	35 ± 20

Table 4. Statistical ANOVA table showing overall model and main-treatment affects.

Source of Variation	df	F-Value	P-Value
Overall Model	5	5.44	<0.0001
Substrate	2	6.48	0.0035
Vertical Structure	3	9.34	<0.0001

Table 5. Comparison of predicted maximum population size, observed population size and the difference between the two for each sample locality and sample period.

	Date	Predicted Max-N	Observed N	Difference
Clark Hubbs Cienega				
	Summer 2014	19,415	3600	15,815
	Winter 2014	29,671	1575	28,096
	Summer 2015	11,308	5850	11,308
	Winter 2015	20,983	1912	20,983
San Solomon Ciénega				
	Summer 2014	13,121	112	13,121
	Winter 2014	12,648	1237	12,648
	Summer 2015	11,801	2062	11,807
	Winter 2015	17,798	825	17,798
East Sandia Ciénega				
	Summer 2014	7,920	101	7,920
	Winter 2014	4,250	0	4,250
	Summer 2015	11,095	0	11,095
	Winter 2015	4,250	0	4,250
Phantom Cave Spring				
	Summer 2014	6,515	937	6,515
	Winter 2014	6,575	1125	6,574
	Summer 2015	3,259	1237	3,259
	Winter 2015	6,690	1350	6,690
Giffin Spring Ciénega				
	Summer 2014	57,481	0	57,481
	Winter 2014	49,178	0	49,177
	Summer 2015	22,006	0	22,006
	Winter 2015	58,752	0	58,752

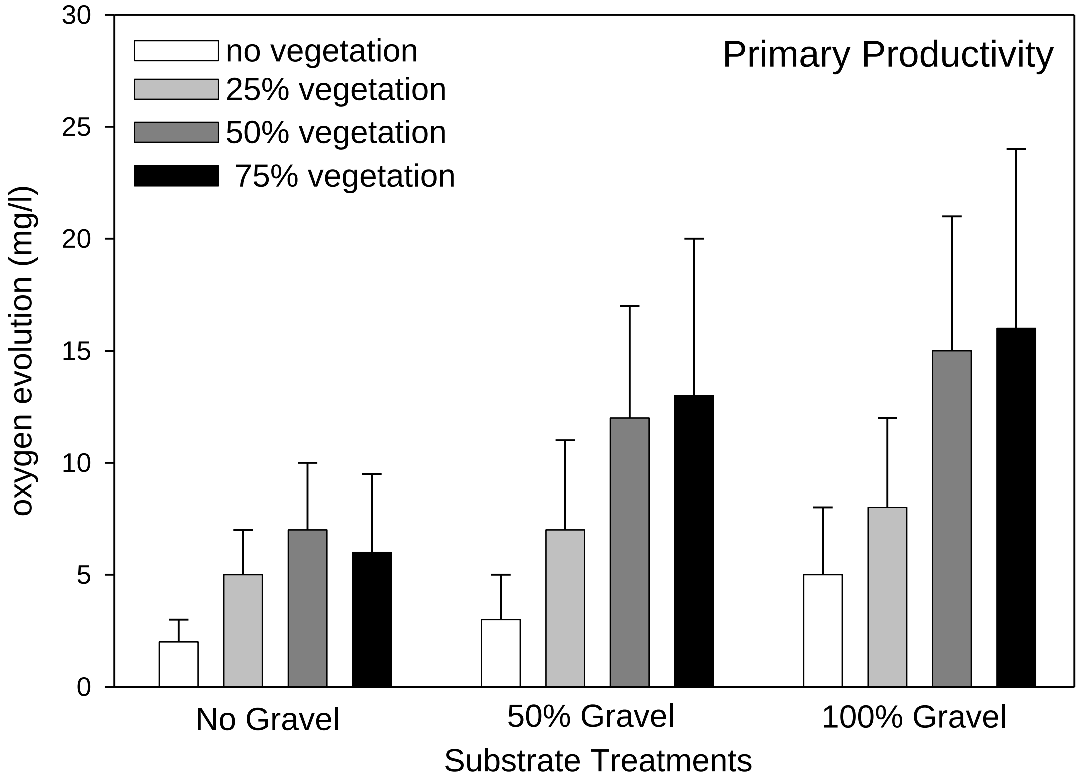

Fig. 1. Effects of substrate composition and areal coverage of vegetative vertical structure on whole-system primary production.

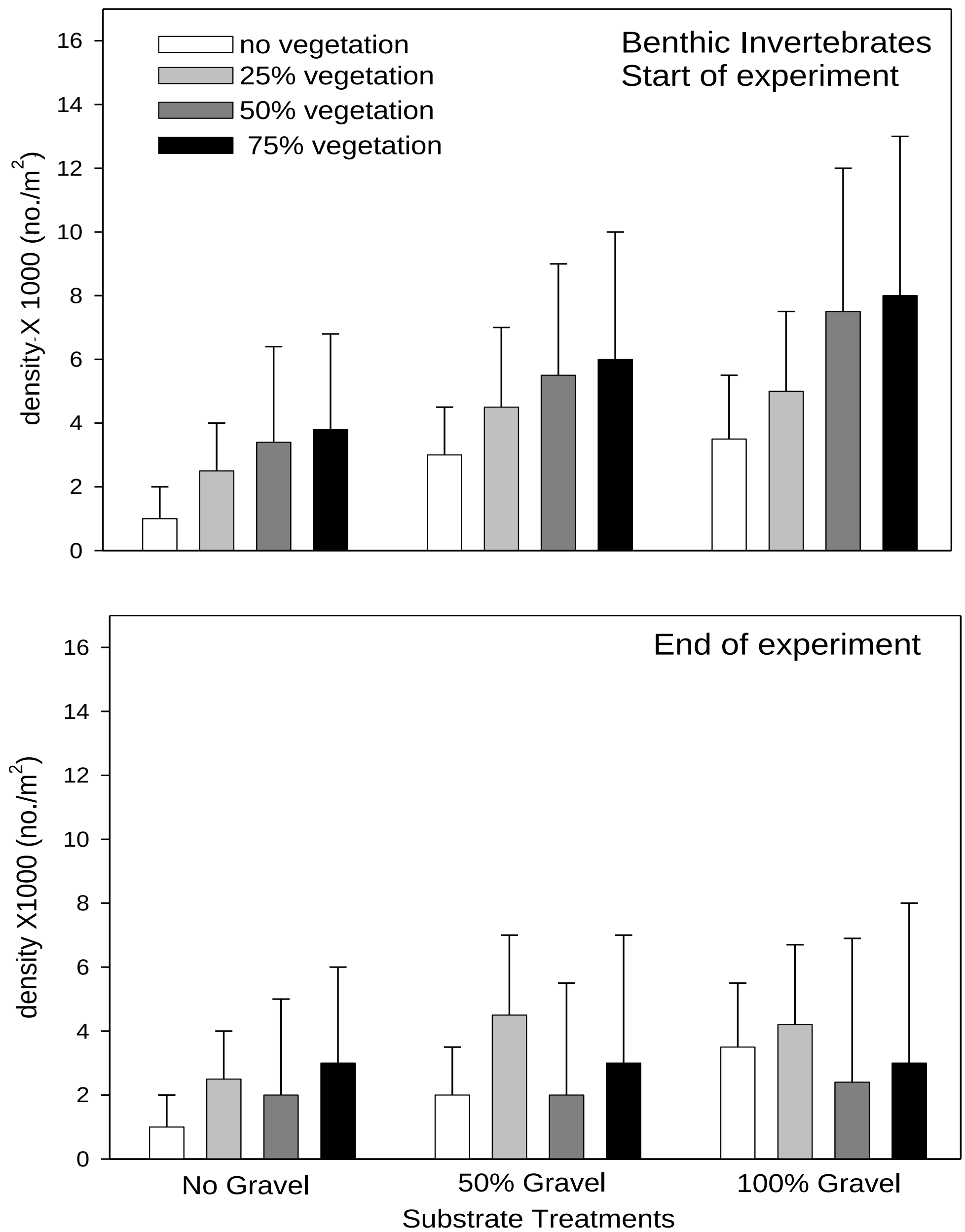

Fig. 2. Effects of substrate composition and areal coverage of vegetative vertical structure on benthic invertebrate density at onset and end of experiment.

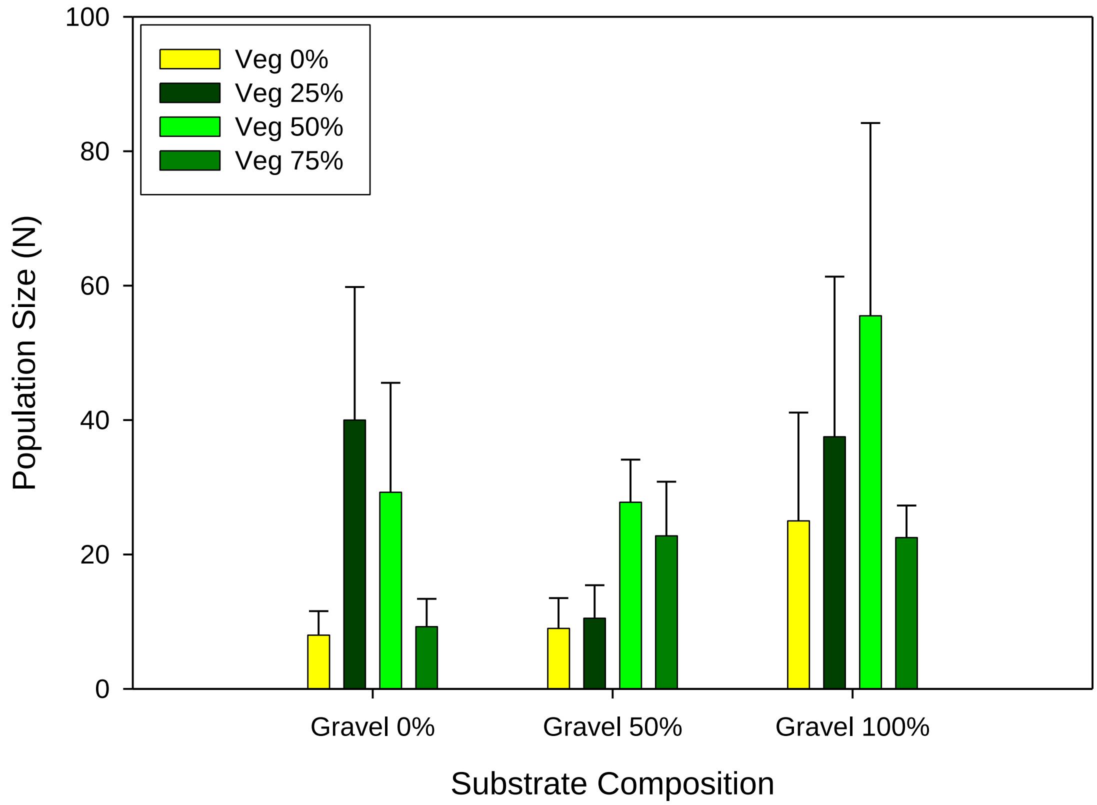

Fig. 3. Effects of substrate composition and vertical structure on population size from experimental mesocosms.

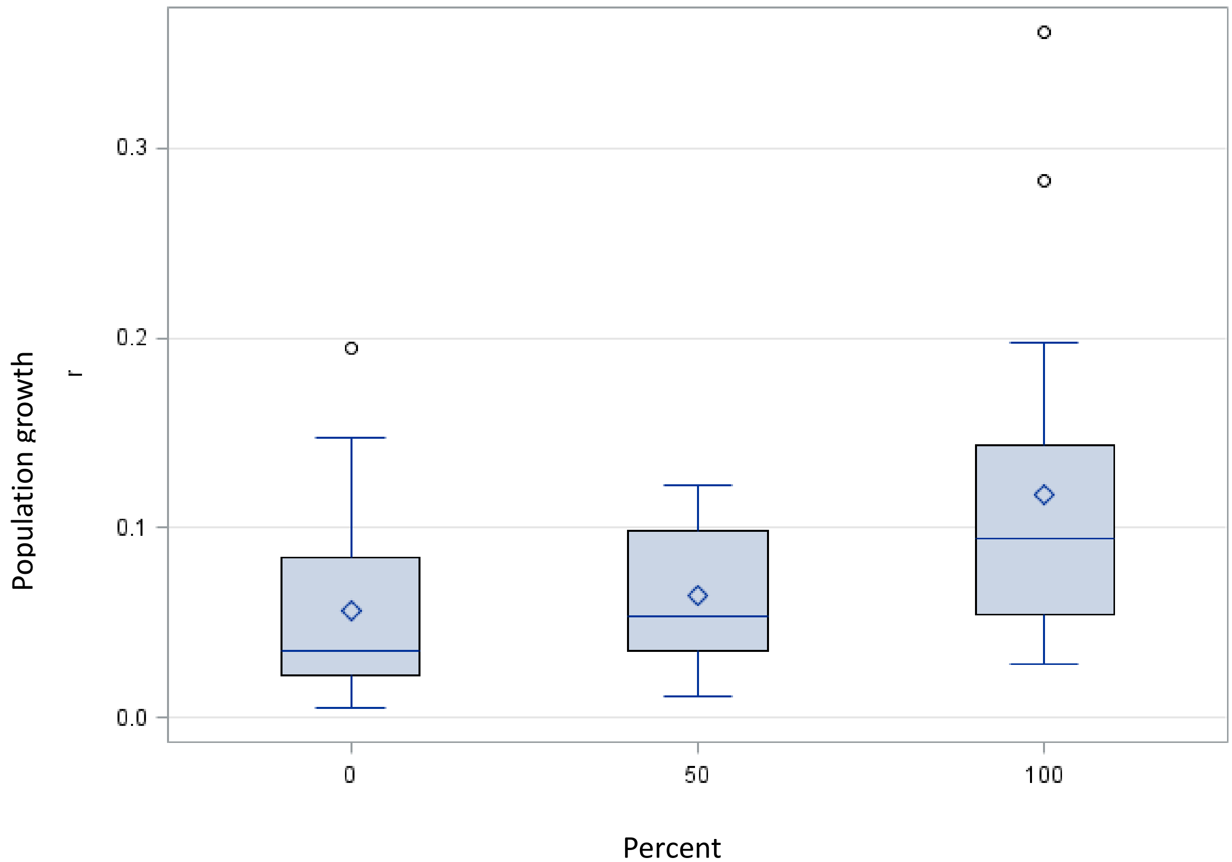

Fig. 4. Independent effect of substrate composition on population growth

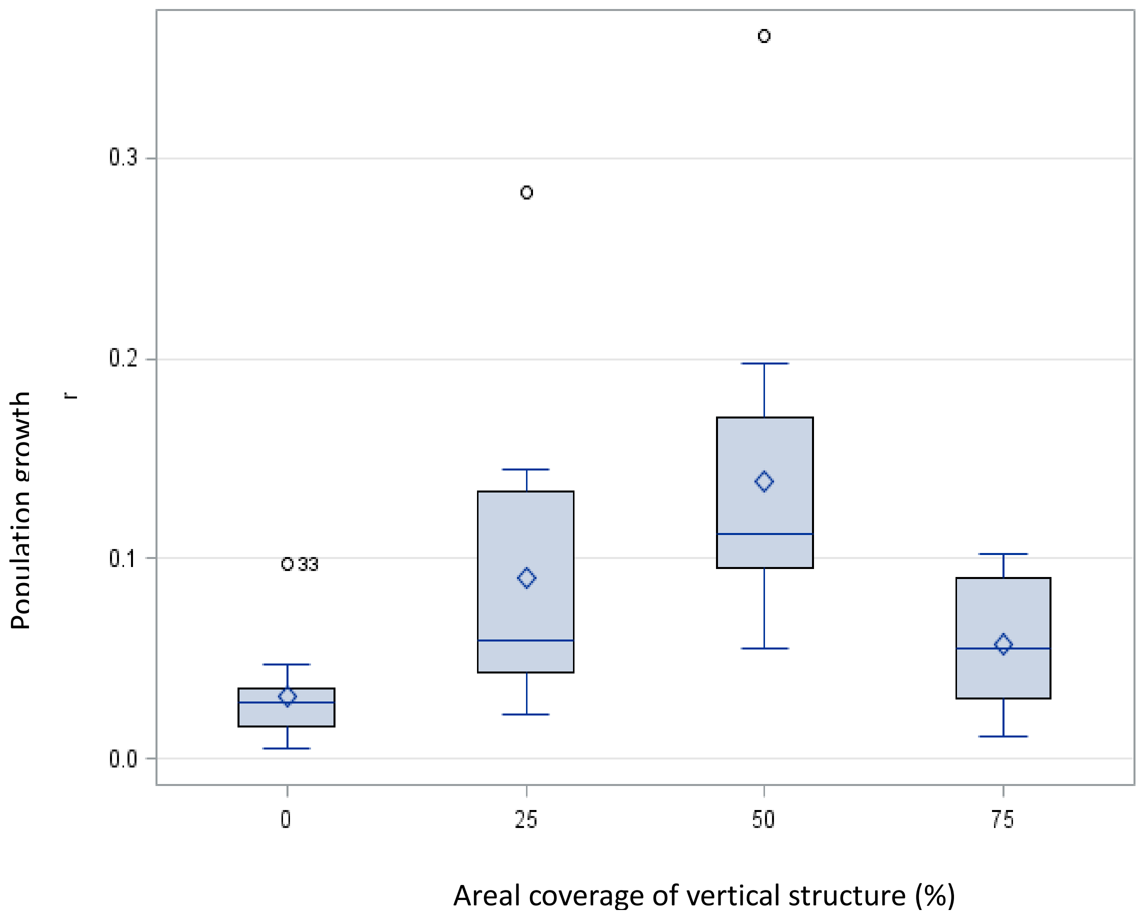

Fig. 5. Independent effect of areal coverage of vegetative vertical structure on population growth rates.

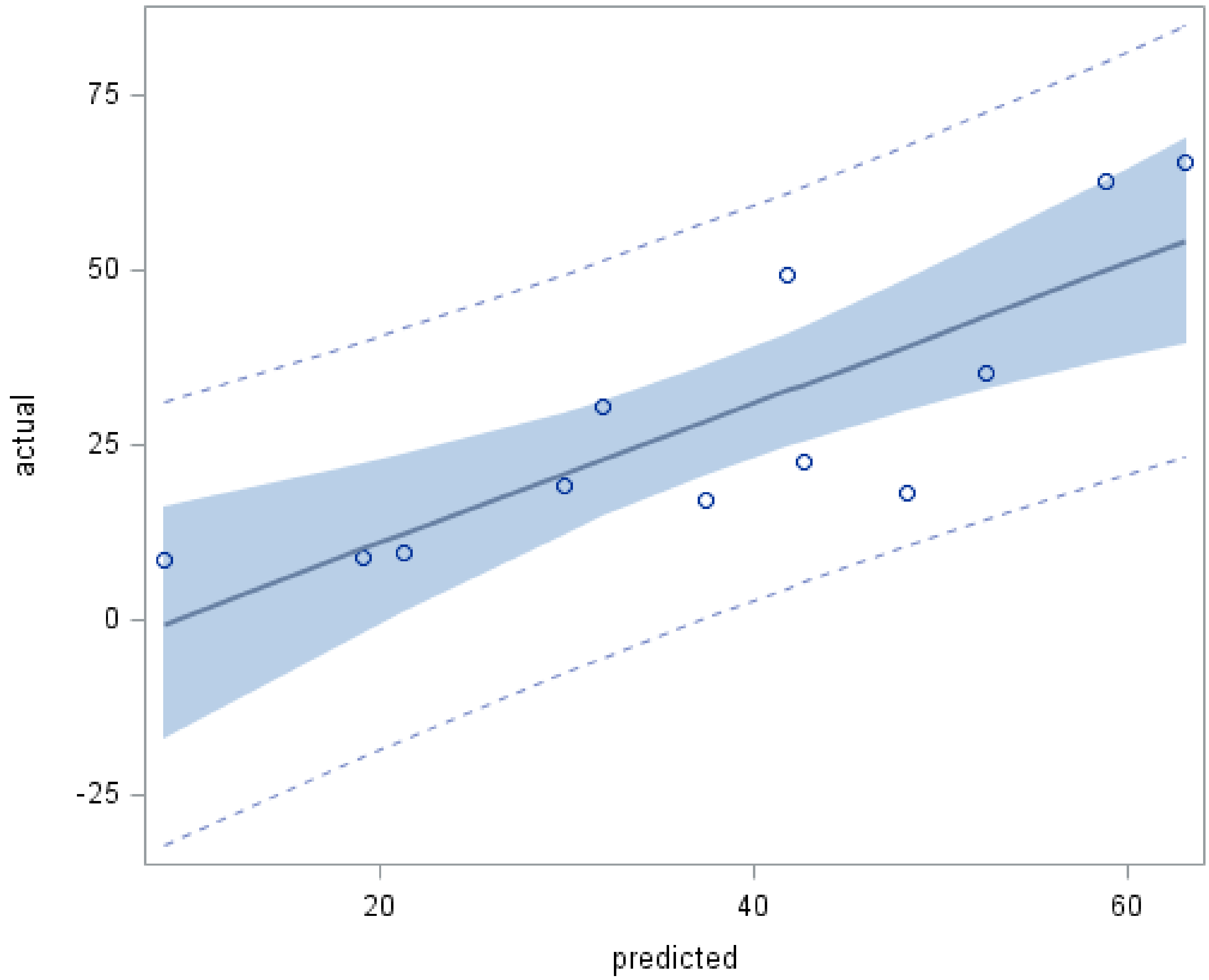

Fig. 6. Relationship between predicted and actual number of *C. elegans* recovered from each treatment.