**Appendix 2. Table of MANOVA and linear model results for each analysis**

TABLE B1 An evaluation of the trend in invertebrate numbers in Rio Hondo *spring-vents* from 2014-2021, accounting for a likely season effect and sample effects. Sample location (individual spring-vents) was included in the model as a random effect and the model took into account a trap size change in 2015. Seasons were summer and winter. Sample locations were vent, mid-run, and end-run.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Noel’s Amphipods: Comparison of linear and quadratic models evaluating trend in Noel’s amphipod (Noels) through time. A poisson distribution was assumed (poisson(log)). All competing models included sample location as a random effect and an offset for trap size [offset(log(trap size))] | | | | | | | | |
| Model | | | | | df | AIC*c* | | ΔAIC*c* |
| Noels~I((Yr-2014)/10)+I(((Yr-2014)^2)/10)+I(((Yr-2014)^3)/10)+Season+Sample | | | | | 8 | 1050.0 | | 0.0 |
| Noels~I((Yr-2014)/10)+I(((Yr-2014)^2)/10)+Season+Sample | | | | | 7 | 1234.2 | | 184.21 |
| Noels~I((Yr-2014)/10)+Season+Sample | | | | | 6 | 1305.1 | | 255.14 |
|  | | | | | | | | |
| *Output of preferred model* | | | | | | | | |
| Noels~I((Year-2014)/10)+I(((Year-2014)^2)/10)+I(((Year-2014)^3)/10)+Season+Sample +offset(Trap size)+(1|Sample) | | | | | | | | |
| Fixed effects | Estimate | Std. Error | *z* value | Pr(>|z|) | | |  | |
| (Intercept) | -3.4397 | 0.2475 | -13.899 | <0.001 | | |  | |
| I((Year - 2014)/10) | 30.8315 | 2.4473 | 12.598 | <0.001 | | |  | |
| I(((Year - 2014)^2)/10) | -111.8085 | 8.9084 | -12.551 | <0.001 | | |  | |
| I(((Year - 2014)^3)/10) | 101.2784 | 8.5692 | 11.819 | <0.001 | | |  | |
| SeasonWinter | -2.2861 | 0.1823 | -12.539 | <0.001 | | |  | |
| SampleMid-run | -0.3088 | 0.1129 | -2.734 | <0.01 | | |  | |
| SampleEnd-Run | -1.4092 | 0.1657 | -8.503 | <0.001 | | |  | |
| Random effect = Sample Location: Variance 0.1308 | | | | | | | | |
|  | | | | | | | | |
| Springsnails: Comparison of linear and quadratic models evaluating trend in springsnails (SSnails) through time. A poisson distribution was assumed (poisson(log)). All competing models included sample location as a random effect and an offset for trap size [offset(log(trap size))] | | | | | | | | |
| Model | | | | | df | AIC*c* | | ΔAIC*c* |
| SSnails~I(Year-2014)+I((Year-2014)^2)+Sample | | | | | 6 | 2032.0 | | 0.0 |
| SSnails~I(Year-2014)+I((Year-2014)^2)+Season+Sample | | | | | 7 | 2033.3 | | 1.29 |
| SSnails~scale(Year)+I(scale(Year)^2)+I(scale(Year)^3)+Sample | | | | | 7 | 2034.0 | | 1.97 |
|  | | | | | | | | |
| *Output of preferred model* | | | | | | | | |
| SSnails~I(Year-2014)+I((Year-2014)^2)+ Sample+offset(Trap size)+(1|Sample) | | | | | | | | |
| Fixed effects | Estimate | Std. Error | *z* value | Pr(>|z|) | | |  | |
| (Intercept) | -49.86Aa72 | 3.1661 | -15.75 | <0.001 | | |  | |
| I(Year - 2014) | 144.0756 | 9.7140 | 14.83 | <0.001 | | |  | |
| I((Year - 2014)^2) | -111.6790 | 7.6428 | -14.61 | <0.001 | | |  | |
| SampleMid-run | 2.1681 | 0.1435 | 15.11 | <0.001 | | |  | |
| SampleEnd-run | 2.9901 | 0.1393 | 21.46 | <0.001 | | |  | |
| Random effect = Sample Location: Variance 4.011 | | | | | | | | |
|  | | | | | | | | |

TABLE B2. An evaluation of the trend in springsnail numbers in the Rio Hondo *midstream* area from 2014-2021, accounting for a likely season effect and sample effects. Sample location (or sample site) was included in the model as a random effect and the model took into account a trap size change in 2015. Seasons were summer and winter. Sample locations were bank, quarter, and center.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Springsnails: Comparison of linear and quadratic models evaluating trend in springsnails (SSnails) through time. All models included an offset for trapsize [offset(log(trap size))] and sample location as a random effect. A poisson distribution was also assumed for all models (poisson(log)) | | | | | | | | |
| Model | | | | | df | AIC*c* | | ΔAIC*c* |
| SSnails~I(Year-2015)+I((Year-2015)^2)+Season+Sample | | | | | 7 | 1405.8 | | 0.0 |
| SSnails~I(Year-2015)+Season+Sample | | | | | 6 | 1429.1 | | 23.34 |
|  | | | | | | | | |
| *Output of preferred model* | | | | | | | | |
| SSnails~I(Year-2015)+I((Year-2015)^2)+Season+Sample+offset(log(Trap size))+(1|Sample) | | | | | | | | |
| Fixed effects | Estimate | Std. Error | *z* value | Pr(>|z|) | | |  | |
| (Intercept) | -7.94108 | 1.25237 | -6.340 | <0.001 | | |  | |
| I(Year - 2015) | -0.52071 | 0.33901 | -1.536 | 0.125 | | |  | |
| I((Year - 2015)^2) | 0.28368 | 0.03597 | 7.887 | <0.001 | | |  | |
| SeasonWinter | -3.12696 | 0.14019 | -22.305 | <0.001 | | |  | |
| SampleQuarter | 1.68105 | 0.09105 | 18.462 | <0.001 | | |  | |
| SampleCenter | 0.92040 | 0.09887 | 9.309 | <0.001 | | |  | |
| Random effect = Sample Location: Variance 4.184 | | | | | | | | |
|  | | | | | | | | |

TABLE B3. Comparison of springsnail abundance across spring-systems for 2020 and 2021. Spring-systems include Bitter Lake, Rio Hondo (both spring-vents and midstream), Sago Springs, and Snail Unit. A poisson distribution was assumed (poisson(log)). Purpose of analysis was to see if springsnail abundance in Rio Hondo spring-system were comparable to those in the three reference spring-systems. Sample location was included in model as a random effect. Summer and winter data were analyzed separately.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Summer**  Springsnails ~ Spring-system + (1 | Sample Location) + (1 | Year) | | | | | |
| Fixed effects: |  |  |  |  |  |
|  | Estimate | Std. Error | *z* value | Pr(>|z|) |  |
| (Intercept) | -2.4263 | 0.5493 | -4.417 | <0.001 |  |
| Spring-systemRH Midstream | 1.5566 | 1.1291 | 1.378 | 0.16886 |  |
| Spring-systemRH Spring-vents | 1.1341 | 0.9938 | 1.141 | 0.25338 |  |
| Spring-systemSago Springs | 3.0084 | 1.0416 | 2.888 | <0.01 |  |
| Spring-systemSnail Unit | 1.2755 | 1.0450 | 1.221 | 0.22225 |  |
| Random effect = Sample Location: Variance 4.75052 | | | | |  |
| Random effect = Year: Variance 0.01797 | | | | |  |
|  | | | | | |
| **Winter**  Springsnails ~ Spring-system + (1 | Sample Location) + (1 | Year) | | | | | |
| Fixed effects: |  |  |  |  |  |
|  | Estimate | Std. Error | *z* value | Pr(>|z|) |  |
| (Intercept) | -3.4929 | 0.5075 | -6.883 | <0.001 |  |
| Spring-systemRH Midstream | -0.7991 | 1.0658 | -0.750 | 0.4534 |  |
| Spring-systemRH Spring-vents | 1.7494 | 0.8717 | 2.007 | <0.05 |  |
| Spring-systemSago Springs | 3.9105 | 0.9083 | 4.305 | <0.001 |  |
| Spring-systemSnail Unit | 1.0793 | 0.9191 | 1.174 | 0.2403 |  |
| Random effect = Sample Location: Variance 3.55064 | | | | |  |
| Random effect = Year: Variance 0.05051 | | | | |  |
|  | | | | | |

TABLE B4. Evaluation of Rio Hondo *midstream* water quality variables through time, accounting for a likely season effect and allowing for a non-linear trend/response. Response variables are water temperature (C°), dissolved oxygen (mg/L), pH, salinity (part per thousand), and water depth (cm).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Y <- cbind(Water Temperature, Dissolved Oxygen, Salinity, pH, Water Depth)  modelManova < manova(Y~I(Year)+I((Year)^2)+Season) | | | | | | | | | | |
|  | Df | | Wilks’ Λ | F (approx) | | | num df | | den df | Pr(>F) |
| Year | 1 | | 0.50576 | 8.7950 | | | 5 | | 45 | <0.001 |
| Year^2 | 1 | | 0.66080 | 4.6198 | | | 5 | | 45 | <0.01 |
| Season | 1 | | 0.40366 | 13.2961 | | | 5 | | 45 | <0.001 |
| Residuals | 49 | |  |  | | |  | |  |  |
|  | | | | | | | | | | |
| *Subsequent linear models for each water variable* | | | | | | | | | | |
| Water Temperature (C°): Linear preferred | | | | | | | | | | |
| Coefficients: | Estimate | | Std. Error | | t value | Pr(>|t|) | |  | | |
| (Intercept) | 177.74830 | | 339.26546 | | 0.524 | 0.602 | |  | | |
| Year | -0.07765 | | 0.16813 | | -0.462 | 0.646 | |  | | |
| SeasonWinter | -5.17586 | | 0.71383 | | -7.251 | <0.001 | |  | | |
|  | | | | | | | | | | |
| Dissolved Oxygen (mg/L): Quadratic preferred | | | | | | | | | | |
| Coefficients: | Estimate | Std. Error | | | t value | Pr(>|t|) | |  | | |
| (Intercept) | -2216000 | 759500 | | | -2.918 | <0.01 | |  | | |
| Year | 2198 | 752.8 | | | 2.920 | <0.01 | |  | | |
| Year^2 | -0.5449 | 0.1865 | | | -2.922 | <0.01 | |  | | |
| SeasonWinter | -2.122 | 1.272 | | | -1.668 | 0.1011 | |  | | |
|  | | | | | | | | | | |
| pH: Linear preferred | | | | | | | | | | |
| Coefficients: | Estimate | Std. Error | | | t value | Pr(>|t|) | |  | | |
| (Intercept) | 5.358255 | 31.959332 | | | 0.168 | 0.867 | |  | | |
| Year | 0.001004 | 0.015838 | | | 0.063 | 0.950 | |  | | |
| SeasonWinter | 0.005862 | 0.067243 | | | 0.087 | 0.931 | |  | | |
|  | | | | | | | | | | |
| Salinity (ppt): Quadratic preferred | | | | | | | | | | |
| Coefficients: | Estimate | Std. Error | | | t value | Pr(>|t|) | |  | | |
| (Intercept) | 975100 | 239400 | | | 4.072 | <0.001 | |  | | |
| Year | -966.6 | 237.3 | | | -4.073 | <0.001 | |  | | |
| Year^2 | 0.2396 | 0.0588 | | | 4.074 | <0.001 | |  | | |
| SeasonWinter | -0.94 | 0.3998 | | | -2.351 | <0.05 | |  | | |
|  | | | | | | | | | | |
| Water Depth (cm): Linear preferred. Water depth data were log transformed | | | | | | | | | | |
| Coefficients: | Estimate | Std. Error | | | t value | Pr(>|t|) | |  | | |
| (Intercept) | -217.2212 | 63.25115 | | | -3.434 | <0.01 | |  | | |
| Year | 0.10895 | 0.03135 | | | 3.476 | <0.01 | |  | | |
| SeasonWinter | -0.33335 | 0.13308 | | | -2.505 | <0.05 | |  | | |
|  | | | | | | | | | | |

TABLE B5. Evaluation of Rio Hondo *spring-vent* water quality variables through time, accounting for a likely season effect and allowing for either a linear or non-linear trend/response. Response variables are water temperature (C°), dissolved oxygen (mg/L), pH, salinity (parts per thousand), water depth (cm) and pH.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Y <- cbind(Water Temperature, Dissolved Oxygen, Salinity, pH, Water Depth)  modelManova < manova(Y~I(Year)+I((Year)^2)+Season) | | | | | | |
|  | Df | Wilks’ Λ | F (approx) | num df | den df | Pr(>F) |
| Year | 1 | 0.55696 | 12.0910 | 5 | 76 | <0.001 |
| Year^2 | 1 | 0.60603 | 9.8811 | 5 | 76 | <0.001 |
| Season | 1 | 0.69838 | 6.5645 | 5 | 76 | <0.001 |
| Residuals | 80 |  |  |  |  |  |
|  | | | | | | |
| *Subsequent linear models for each water variable* | | | | | | |
| Water Temperature (C°): Quadratic preferred | | | | | | |
| Coefficients: | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 389000 | -134700 | 2.887 | <0.01 |  | |
| Year | -385.8 | -133.5 | -2.889 | <0.01 |  | |
| Year^2 | 0.09566 | -0.03309 | 2.891 | <0.01 |  | |
| SeasonWinter | -0.6428 | -0.2855 | -2.252 | <0.05 |  | |
|  | | | | | | |
| Dissolved Oxygen (mg/L): Quadratic preferred | | | | | | |
| Coefficients: | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 1613000 | 459200 | -3.513 | <0.001 |  | |
| Year | 1599 | 455.2 | 3.513 | <0.001 |  | |
| Year^2 | -0.3963 | 0.1128 | -3.514 | <0.001 |  | |
| SeasonWinter | 0.5076 | 0.9731 | 0.522 | 0.603276 |  | |
|  | | | | | | |
| pH: Quadratic preferred | | | | | | |
| Coefficients: | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | -87840 | 25530 | -3.440 | <0.001 |  | |
| Year | 87.04 | 25.31 | 3.439 | <0.001 |  | |
| Year^2 | -0.02156 | 0.006272 | -3.437 | <0.001 |  | |
| SeasonWinter | 0.03322 | 0.05411 | 0.614 | 0.540826 |  | |
|  | | | | | | |
| Salinity (ppt): Quadratic preferred | | | | | | |
| Coefficients: | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 876700 | 167600 | 5.231 | <0.001 |  | |
| Year | -869.2 | 166.1 | -5.232 | <0.001 |  | |
| Year^2 | 0.2154 | 0.04116 | 5.23 | <0.001 |  | |
| SeasonWinter | 0.2154 | 0.3482 | 0.619 | 0.538 |  | |
|  | | | | | | |
| Water Depth (cm): Linear preferred. Water depth data were log transformed | | | | | | |
| Coefficients: | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | -419.9826 | 118.4199 | -3.547 | <0.001 |  | |
| Year | 0.2090 | 0.0587 | 3.560 | <0.001 |  | |
| SeasonWinter | -0.9927 | 0.2714 | -3.657 | <0.001 |  | |
|  | | | | | | |

TABLE B6. Relationship of *summer* water parameters to *spring-systems*, including Rio Hondo and three reference systems. Purpose of analyses were to compare parameters in Rio Hondo (both midstream and streamvents) to those in Bitter Creek, Sago Springs and Snail Unit. Response variables were water temperature (C°), dissolved oxygen (mg/L), pH, salinity (parts per thousand), and water depth (cm).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Y <- cbind(Water Temperature, Salinity, pH, Water Depth, Dissolved Oxygen)  manova(Y ~ Spring System) | | | | | | |
| Variable | Df | Wilks’ Λ | F (approx) | num df | den df | Pr(>F) |
| Season | 4 | 0.1028 | 12.305 | 20 | 249.7 | <0.001 |
| Residuals | 79 |  |  |  |  |  |
|  | | | | | | |
| *Subsequent linear models for each water parameter* | | | | | | |
| Water Temperature (C°) | | | | | | |
| Coefficients | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 29.0694 | 0.5811 | 50.023 | <0.001 |  | |
| SiteRH Midstream | -7.7794 | 1.2464 | -6.242 | <0.001 |  | |
| SiteRH Spring-vents | -10.5837 | 1.0982 | -9.637 | <0.001 |  | |
| SiteSago Springs | -8.8861 | 1.1622 | -7.646 | <0.001 |  | |
| SiteSnail Unit | -8.0194 | 1.1622 | -6.900 | <0.001 |  | |
|  | | | | | | |
| Dissolved Oxygen (mg/L) | | | | | | |
| Coefficients | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 6.4639 | 0.3485 | 18.549 | <0.001 |  | |
| SiteRH Midstream | -0.5339 | 0.7474 | 0.714 | 0.477 |  | |
| SiteRH Spring-vents | -4.2139 | 0.6586 | -6.399 | <0.001 |  | |
| SiteSago Springs | -0.5306 | 0.6969 | -0.761 | 0.449 |  | |
| SiteSnail Unit | -3.3222 | 0.6969 | -4.767 | <0.001 |  | |
|  | | | | | | |
| pH | | | | | | |
| Coefficients | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 7.6614 | 0.0608 | 126.005 | <0.001 |  | |
| SiteRH Midstream | -0.2694 | 0.1304 | -2.066 | <0.05 |  | |
| SiteRH Spring-vents | -0.5264 | 0.1149 | -4.581 | <0.001 |  | |
| SiteSago Springs | -0.2972 | 0.1216 | -2.444 | <0.05 |  | |
| SiteSnail Unit | -0.2081 | 0.1216 | -1.711 | 0.0910 |  | |
|  | | | | | | |
| Salinity (ppt) | | | | | | |
| Coefficients | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 7.6203 | 0.2920 | 26.098 | <0.001 |  | |
| SiteRH Midstream | -0.5483 | 0.6262 | -0.875 | 0.383958 |  | |
| SiteRH Spring-vents | -2.6517 | 0.5518 | -4.805 | <0.001 |  | |
| SiteSago Springs | -2.3836 | 0.5840 | -4.082 | <0.001 |  | |
| SiteSnail Unit | -4.4186 | 0.5840 | -7.566 | <0.001 |  | |
|  | | | | | | |
| Water Depth (cm) | | | | | | |
| Coefficients | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 13.6028 | 1.5628 | 8.704 | <0.001 |  | |
| SiteRH Midstream | 11.0372 | 3.3519 | 3.293 | <0.01 |  | |
| SiteRH Spring-vents | -0.1242 | 2.9535 | -0.042 | 0.96656 |  | |
| SiteSago Springs | -0.6028 | 3.1257 | -0.193 | 0.84757 |  | |
| SiteSnail Unit | -5.8111 | 3.1257 | -1.859 | 0.06673 |  | |
|  | | | | | | |

TABLE B7. Relationship of *winter* water parameters to spring-systems, including Rio Hondo and three reference systems. Purpose of analyses were to compare parameters in Rio Hondo (both midstream and streamvents) to those in Bitter Creek, Sago Springs and Snail Unit. Response variables were water temperature (C°), dissolved oxygen (mg/L), pH, salinity (parts per thousand), and water depth (cm).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Y <- cbind(Water Temperature, Dissolved Oxygen, Salinity, pH, Water Depth)  manova(Y~Spring-system) | | | | | | |
| Variable | Df | Wilks’ Λ | F (approx) | num df | den df | Pr(>F) |
| Season | 4 | 0.0325 | 22.596 | 20 | 249.7 | <0.001 |
| Residuals | 79 |  |  |  |  |  |
|  | | | | | | |
| *Subsequent linear models for each water parameter* | | | | | | |
| Water Temperature (C°) | | | | | | |
| Coefficients | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 8.7639 | 0.3689 | 23.759 | <0.001 |  | |
| SiteRH Midstream | 6.7861 | 0.7911 | 8.578 | <0.001 |  | |
| SiteRH Spring-vents | 8.6361 | 0.6971 | 12.389 | <0.001 |  | |
| SiteSago Springs | 8.0361 | 0.7377 | 10.893 | <0.001 |  | |
| SiteSnail Unit | 7.1028 | 0.7377 | 9.628 | <0.001 |  | |
|  | | | | | | |
| Dissolved Oxygen (mg/L | | | | | | |
| Coefficients | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 8.6889 | 0.6035 | 14.398 | <0.001 |  | |
| SiteRH Midstream | -1.8289 | 1.2943 | -1.413 | 0.161583 |  | |
| SiteRH Spring-vents | -1.3960 | 1.1405 | -1.224 | 0.224561 |  | |
| SiteSago Springs | -1.4222 | 1.2070 | -1.178 | 0.242197 |  | |
| SiteSnail Unit | -4.7556 | 1.2070 | -3.940 | <0.001 |  | |
|  | | | | | | |
| pH | | | | | | |
| Coefficients | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 7.65694 | 0.03771 | 203.022 | <0.001 |  | |
| SiteRH Midstream | -0.25294 | 0.08089 | -3.127 | <0.01 |  | |
| SiteRH Spring-vents | -0.36409 | 0.07127 | -5.108 | <0.001 |  | |
| SiteSago Springs | -0.26861 | 0.07543 | -3.561 | <0.001 |  | |
| SiteSnail Unit | -0.28278 | 0.07543 | -3.749 | <0.001 |  | |
|  | | | | | | |
| Salinity (ppt) | | | | | | |
| Coefficients | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 5.1936 | 0.1118 | 46.453 | <0.001 |  | |
| SiteRH Midstream | 1.0704 | 0.2398 | 4.464 | <0.001 |  | |
| SiteRH Spring-vents | 0.9821 | 0.2113 | 4.648 | <0.001 |  | |
| SiteSago Springs | -0.1561 | 0.2236 | -0.698 | 0.487 |  | |
| SiteSnail Unit | -2.0403 | 0.2236 | -9.124 | <0.001 |  | |
|  | | | | | | |
| Water Depth (cm) | | | | | | |
| Coefficients | Estimate | Std. Error | t value | Pr(>|t|) |  | |
| (Intercept) | 9.8167 | 0.8590 | 11.428 | <0.001 |  | |
| SiteRH Midstream | 3.8833 | 1.8423 | 2.108 | <0.05 |  | |
| SiteRH Spring-vents | -6.5310 | 1.6233 | -4.023 | <0.001 |  | |
| SiteSago Springs | -0.4417 | 1.7180 | -0.257 | 0.797779 |  | |
| SiteSnail Unit | -1.1917 | 1.7180 | -0.694 | 0.489935 |  | |
|  | | | | | | |