Appendix B TYC Reintroduction Methods and Analysis

1.0 Seed collection

Seeds for the 2003 pilot project were collected in September, 2001 at 9 high priority and core restoration sites: Blackwood North and South (combined for planting), Cascade, Edgewood, Lighthouse, Tallac Creek, Taylor Creek, Tahoe Meadows, and Upper Truckee East (UTE) (Table 1).

Table 1. Seed sources of container-grown TYC and year of outplanting.

Seed Source/Year Planted	2003	2004	2005	2006
Blackwood	X	X		
Cascade	X	X		
Taylor Creek	X	X	X	X
Tallac Creek	X	X		
Lighthouse	X	X		
Upper Truckee East	X	X	X	X
Regan Al Tahoe		X		
Tahoe Meadows	X	X		
McKinney Creek				X
Edgewood	X		X	

Seeds for the 2004 pilot project replication and experimental reintroduction were collected in September 2002 at the same sites, except that Regan Al Tahoe was substituted for Edgewood. Seeds for the 2005 experimental reintroduction and restoration plantings were collected in September 2003 at three sites: Edgewood, Taylor Creek, and UTE. Seeds for the 2006 experimental reintroduction and restoration plantings were collected in September 2005 at the Taylor Creek, McKinney Creek and UTE source populations. Each year, seed lots were cleaned and hand-sorted into two equal lots and stored in manila envelopes at room temperature and humidity. Seeds were delivered to two nurseries in the fall of the collection year.

2.0 Greenhouse propagation

Two nurseries propagated TYC: the Nevada Division of Forestry (NDF) facility at an elevation of 5,000 ft. in Washoe Valley, NV; and privately-owned Sierra Valley Farms at an elevation of 5,000 ft. in Beckwourth, CA, 25 miles north of Truckee. The nurseries were directed to utilize all seed lots and both followed the same propagation protocol of top sowing-seed in plastic supercells with standard greenhouse soil-less potting mix. A light layer of Lake Tahoe beach sand was sprinkled on the surface to cover the seeds.

In 2003 and 2004, TYC seed were fall-sown in the greenhouse for outplanting the following summer. For the 2006 outplanting, seed were sown in January. Prior to planting, all plants were sorted according to seed source and then assigned a vigor code (Low (L), Medium (M), or High (H)). The vigor code was a subjective measure of apparent plant health that partially reflected variability from different planting dates, but also the uneven effects of neglect during cultivation. In 2003 and 2004, all three levels were recorded. In 2006, only Low or High vigor was used.

3.0 Site selection

Site selection was based on ecological characteristics, patterns of recreational use, and the availability of the agency landowner to install fencing and make in-kind contributions of personnel for outplanting and monitoring.

In 2003, the pilot project was installed in different microhabitats at four sites: Avalanche/Eagle Creek in Emerald Bay (California State Parks (CSP)), Taylor Creek at Baldwin Beach (U.S. Forest Service (USFS)), Zephyr Cove (USFS), and Sand Harbor (Nevada Division of State Parks (NDSP)) (Table 2). Descriptions of these "pilot" sites may be found in the 2003 pilot project report (Pavlik and Stanton 2004).

In 2004, the pilot project design was repeated at Taylor Creek and Sand Harbor by installing the new cohort in and among the 2003 plots, effectively doubling the size of the outplanting at each site. Avalanche and Zephyr Cove were not re-planted, but two new sites were selected that were large enough to accommodate the installation of replicated plots within a microhabitat: UTE (California Tahoe Conservancy (CTC)) and Nevada Beach (USFS). Descriptions of these sites may be found in Pavlik and Stanton 2005.

In 2005, the experimental design was repeated at UTE and Nevada Beach and three additional sites were outplanted: Ebright Beach and Pope Beach (USFS), both on the south shore, and Hidden Beach (NDSP), located in the northeast corner of the lake. Replicated plots within a microhabitat were not possible in the limited space available at these sites. Descriptions of these sites may be found in Pavlik and Stanton 2006.

In 2006, the experimental designs of 2005 were repeated at the USFS sites and UTE. Hidden Beach was not planted because storms in January 2006 destroyed the fence and covered the plot with beach wrack and trash. The high lake level also significantly reduced the amount of available planting space, so the site was abandoned and its fence removed in late summer of 2006. Two additional sites were outplanted during 2006: D.L. Bliss State Park (CSP) on the west shore, and Tallac Creek at Baldwin Beach (USFS). Both plantings occurred within the existing enclosures. Descriptions of these sites may be found in Pavlik and Stanton 2007.

Table 2. Location and numbers of TYC installed at 11 sites from 2003 to 2006.

Site Name	CS2015 Rank	2003	2004	2005	2006
Avalanche	Medium	300			
Zephyr Cove	Low	286			
Taylor Creek	Core	541	546		150
Sand Harbor	Ephemeral	172	281		
Upper Truckee East	Core		1,425	650	250
Nevada Beach	Core		582	534	200
Ebright	Unranked			418	100
Pope Beach	Low			250	150
Hidden Beach	Ephemeral			180	
D.L. Bliss	Ephemeral				100
Tallac Creek	Core				225
Total		**3,302**	**2,814**	**2,032**	**1,175**

4.0 Outplanting design

In all years, plant installations consisted of outplanting container-grown TYC in "transect" configurations perpendicular to the shore, extending from the waterline into different, upslope microhabitats. Transects were placed 3.28 ft. (1 m) apart and plants within a single transect were outplanted in rows at 1.6 ft. (0.5 m) intervals. Individuals were marked with wooden stakes. For plots outplanted with individuals from different seed sources, the stakes were color coded. Within a plot, a stratified random planting scheme was employed to distribute TYC from different seed sources and low vigor plants as evenly as possible. After planting, plants were hand-watered for three days.

In 2004, replication of the 2003 pilot design at Taylor Creek and Sand Harbor and the installation of 45 two year-old TYC at UTE in two microhabitats was meant to test the ideas of age-structured outplanting and "founder–cost averaging". In experimental re-introductions, individual plants have been referred to as founders (Pavlik 1996). The age-structure of a rare plant population may be important for the maintenance of high levels of reproductive output (seeds and clones). Building an optimized age-structure in reintroduced populations can be accomplished by planting multiple age classes (e.g. one year-olds, two year-olds, etc.) in a single year or by promoting survival of founders across years. Members of different classes often differ in size and, therefore, in resources available for reproduction. Presumably, older and larger founders would produce more seeds or clones than younger, smaller founders, and could boost the overall production of new plants in a given year. "Founder-cost averaging" is the successive outplanting of founders of any age class in different years. In this way the risk of outplanting all founders in an unfavorable year (e.g. drought, high lake level) is reduced. This minimizes stochastic effects and is analogous to "dollar-cost averaging" in financial investment. Instead of maximizing monetary return, this ecological restoration technique could be used to maximize "return" (survival and reproductive output) on the investment of founders among all outplanting years.

5.0 Microhabitats and lake levels

The level of Lake Tahoe fluctuated across a full spectrum during the four years of experimental reintroductions. Lake Tahoe was low in 2003 and 2004, increased by more than one foot in 2005, and rose to capacity (6,229.1 ft. LTD) in 2006 (Table 3).

TYC microhabitats that occur on the shores of Lake Tahoe may be defined by geomorphology, elevation, and other environmental factors and may include moist shoreline, berm, low beach, high beach, dune trough, scrub, and meadow (Table 4).

A laser level was used to determine the elevation of each experimental plot and microhabitat type using the known level of Lake Tahoe on that day (from the USGS) as a reference point.

Table 3. The level of Lake Tahoe during TYC plantings in 2003 through 2006.

	Elevation (ft. LTD)			
	2003	2004	2005	2006
Lake elevation at planting	6,224.3	6,224.3	6,225.1	6,228.9
Peak lake elevation	6,224.9	6,224.3	6,225.6	6,229.1
September lake elevation	6224.2	6223.3	6,225.1	6228.1

Table 4. Microhabitat descriptions and associated lake levels in 2003 through 2006.

Microhabitat Description	Elevation (ft LTD)			
	2003	2004	2005	2006
Moist shoreline Found in plots adjacent to the lake with saturated soil and wave inundation.	6,224.3 - 6,225.7	6,224.3 - 6,225.7	6,225.5- 6,226.5	6,229.1 to 6,230
Berm Formed by sediment deposition, generally at creek mouths	NA	6,225.30	NA	NA
Low beach Found between the moist shoreline and high beach.	6,225.8- 6,227.9	6,225.8- 6,227.9	6,226.6- 6,227.9	
Dune trough Formed by water accumulation in back barrier beach.	6,224.6-6,226	6,224.6-6,226	NA	NA
High beach Characterized by dry soil, lack of wave action or inundation, very sparse vegetation.	6,228-6,230.6	6,228-6,230.6	6,228-6,230.6	6,229.1 - 6,231.3
Meadow Characterized by stabilized vegetation of sedges and rushes in the back barrier beach.	>6,230	>6,230	NA	6,228.3 -6,230
Scrub Characterized by stabilized vegetation of shrubs in the back barrier beach.	NA	NA	NA	6,229.5- 6,231.3

The assumption behind the microhabitat description is that the water table is at the level of Lake Tahoe and, therefore, the height of a plot above the lake is equivalent to the depth to the water table. In the design of the experimental reintroduction, plants were installed in rows running parallel to the lake beginning 0.5 m to 1m from the water's edge. The moist shoreline microhabitat included plants installed adjacent to Lake Tahoe, generally in rows 1-5, at an elevation that depended on lake level the day of planting. The moist shoreline was characterized by saturated soil conditions, wave impacts and some level of inundation and disturbance from waves. Low beach encompassed the range in elevation on the beach from 6,225.8 – 6,228 ft. between the moist shoreline and high beach and could contain competing vegetation. High beach occurred above 6,228 ft. and has drier surface conditions, a lack of vegetation, and is almost never inundated and provides a refuge in times of high water. The dune trough occurred only at Taylor on the margins of a persistent inland lagoon supporting water lilies (*Nuphar* sp.) and other aquatic vegetation. Plants were installed in the moist sand on either side of the lagoon and these plots developed had high levels of competing vegetation.

Meadow habitat included stabilized perennial vegetation of rushes and sedges (*Juncus* sp., *Carex* sp.) in the barrier beach, while scrub habitat was drier and included shrubs like yellow rabbitbrush (*Chrysothamnus viscidiflorus* (Hook.) Nutt.). The berm microhabitat only formed at UTE where the Upper Truckee River deposits benches of sand adjacent to the shoreline that were elevated by one or two feet above the lake level but were generally protected from wave impact and inundation.

6.0 Biological monitoring

In all years, plants were evaluated at 2 weeks and 4 weeks after planting and thereafter on a monthly basis through October. Site factors that were tracked included plot, habitat, and elevation (Table 5). Initial plant characters included seed source, phenology, and vigor. Plant size (length x width) was measured in September at peak reproduction.

An assessment of reproductive output found that the mean number of seeds per silique at three sites varied from 22 to 30 (Pavlik *et al.* 2002). Seed output per plant was calculated by multiplying the number of fruits/plant by the mean number of seeds/fruit for each of three sites. This product was regressed against canopy area measured on 30 plants at each site and fit to a line. The slopes of the regression lines of all three sites were similar and could be described by a single line ($y=3.609x -109.542$; $r^2=0.81$) where y is the number of seeds per individual and x is canopy area in square centimeters (see Figure 4 in Pavlik *et al.* 2002).

Vegetative reproductive output was estimated from counts of plantlets (vegetative ramets) that appeared within up to 25 cm centimeters of the original plant. In some cases the plantlets connection to the parent were confirmed by digging around to uncover the lateral underground roots, but it was not practical to do this for every plant.

Table 5. Monitoring variables for TYC planting from 2003 to 2006 including site factors, plant characters, and plant response in September.

Site Factors
• Plot: 1-12, containing from 50 to over 200 plants • Habitat: moist shoreline, berm, dune trough, low beach, high beach, meadow, scrub • Elevation: above the lake, ranging from -0.2 to 5.8 feet
Plant characters
• Seed Source: 1-8 tested in 03 and 04, only 2 tested in 2006 • Initial Vigor: L, M, H (Low, Medium, High) in 2003 only L/H in 04 and 06 • Initial Phenology**:** V, F, S (Vegetative, Flower/Fruit, Senescent)
Plant Responses in September
• Status: L, D (live dead) • Phenology: V, F (Vegetative, Flower/Fruit) • Canopy size**:** in cm^2 • Seeds per plant (determined from canopy size) • Number of plantlets (vegetative reproduction)

7.0 Precision seeding experiment

A precision seeding experiment was installed at Upper Truckee East at the time of the outplanting in June 2004. Plywood sowing frames (1.25 x 1.25 m) with 100 hole grids (10 x 10) were used as guides for precisely locating seeds in four microhabitats; low beach, high beach, berm, and moist shoreline. A frame (one replicate) was placed on the ground and a one foot piece of rebar was inserted in each of three small holes (two at the top and one at the bottom) on each frame. The rebar was then driven into the ground and left in place so the frame could be removed and returned to the exact same location for subsequent monitoring. Next, a small number (3–10 seeds) of clean TYC seeds were placed on the beach sand surface in each sowing hole and lightly covered with sand taken from just outside the frame. Three frames were sown in each microhabitat for a total of 300 sown holes per microhabitat. To avoid any displacement of sown seeds, and to test for more natural patterns of germination, plots were not watered. Plots were monitored one month after sowing.

8.0 Outplanting timing

Seed collection and plant propagation

The greenhouse propagation of TYC was conducted at the NDF nursery in Washoe, Nevada. For the 2008 cohort, seed was collected in September 2007 at five sites: McKinney Creek, Blackwood Creek, Taylor Creek, Upper Truckee East, and Nevada Beach. Seed collected in September 2004 from UTE was used for the 2009 cohort.

In both years, seed was sown in January in supercell containers with greenhouse potting mix and covered with a thin layer of vermiculite to hold the seed in place. Seedlings were watered regularly with a light fertilizer solution. The planting racks held 98 containers each and were periodically thinned to space plants and give them more light and opportunity for root growth. In both years the first batch of plants was removed from the greenhouse for planting in May 2009 or June 2008 and thereafter at four week intervals into September for each planting event.

Site selection and planting design

In 2008, four sites were planted; private property on the north side of Blackwood Creek, CTC land at UTE, private property at Edgewood Golf Course, and California State Parks land just north of General Creek at Sugar Pine Point SP. Descriptions of these sites are in Stanton and Pavlik (2009). In 2009, four sites were planted: USFS beaches at Zephyr Cove, Pope Beach, and Ebright Beach and CTC land at UTE. Descriptions of these sites are in Stanton and Pavlik (2010).

A standard planting protocol was utilized in both years. Container-grown plants were spaced one half meter apart in a regular grid design and marked with wooden stakes color-coded by month. The planting treatments were randomly distributed throughout the grid. In both years, planting began the third week of June and continued every four weeks through September. In 2008, the lake declined from 6,225.5 ft–6,224 ft LTD during the season and in 2009, the lake declined from 6224.6 ft–6223.5 ft LTD. After each planting, newly planted individuals were hand-watered for 3 days. Plant status and phenology was monitored at every planting time, concluding in October. The canopy of surviving plants was also measured in October.

9.0 Data analysis

TYC plant performance was evaluated in the following ways:

- Percent survivorship (# live/ # planted)*100
- Survivorship to reproduction (# FR/# planted)*100
- Canopy size (cm^2)
- Seeds/plant (derived from canopy size)

- Number of plantlets (vegetative reproduction)

The factors that may affect TYC performance include:

- Site
- Seed source
- Initial vigor/ initial phenology
- Microhabitat
- Microhabitat elevation
- Year of planting (persistence through time)

9.1 Logistic regression analysis of TYC survival and survival to reproduction

Logistic regression was employed using the statistical software R version 3.1.1 (R Core Team 2014) to examine the factors impacting TYC survival and survival to reproduction. Logistic regression is a form of multivariate regression designed for modeling binary outcomes (e.g. survival versus death). Each cohort was modeled separately to examine the impact of each predictor variable for each year on both survival, and survival to reproduction. Survival and reproductive status was determined in September of planting year. Predictor variables considered during the model selection process included: site, elevation, habitat, initial vigor, initial phenology, and seed source. For the 2003 and 2004 cohorts neither seed source, nor initial phenology significantly improved model fit when predicting survival (as determined via comparison of AIC scores), therefore these variables were not included in the final regression. This concurs with preliminary data exploration that showed no substantive impact of either variable on TYC performance. It should be noted that initial phenology was not collected for all seedlings in the 2004 and 2006 cohorts. However, since initial phenology may be correlated with initial vigor (i.e. plants that had already flowered in the greenhouse and were senescing may have been graded as lower vigor), only initial vigor was considered during the model selection process for the 2004 and 2006 cohorts. In 2006, plants were graded as low or high only. Habitat type was not included in the model selection process for either cohort, as this factor is largely determined by elevation. Therefore, due to collinearity, it would be inappropriate to include both variables in the model. Elevation was chosen over habitat type because it is a continuous variable rather than a subjective descriptor. Final models were examined for multicollinearity via variance inflation factor calculation and model fit statistics computed.

The area under the receiver operator characteristic curve (AUC) is a commonly used gauge of logistic regression predictive power. The AUC is representative of the proportion of times the model predicts a true positive versus a false positive. In other words, the AUC is a measure of the predictive ability of the model, with a higher AUC implying better model performance.

Nagelkerke's R^2 was also computed as a measure of model fit. This statistic compares the final model with a model fitted with means from each predictor variable to examine how much better the fitted model is than the null expectation. The output statistic represents this ratio, therefore a higher number signifies a better model. This statistic should not be interpreted as one would the R^2 output from ordinary least squares linear regression.

9.2 Analysis of factors influencing Tahoe yellow cress canopy size

The impact of site, planting elevation above the lake, seed source, and initial vigor and phenology were all examined for their impact on canopy size of TYC, as measured in September of the planting year. Analysis was performed on data from surviving plants via multiple linear regressions. Each cohort from 2003, 2004, and 2006 was analyzed separately.

The model selection process was as follows. For each cohort, a saturated model was first constructed that included all predictor variables mentioned above. Following examination of the saturated model, variables were eliminated in a stepwise fashion to determine the best model for predicting canopy size. Model comparison was performed using the Akaike information criterion (AIC). The response variable for each cohort was examined, and transformed as necessary (see below). Residuals of final models were examined, and showed only slight evidence of heteroscedasticity, and departure from normality as evidenced by examination of QQ plots. Multiple regression is robust to such modest violations of assumptions (see Lorenzen and Anderson 1993, and van Belle 2002). Outlier analysis was done using Cook's distance, and outliers removed from analyses (typically outliers were plants with canopy sizes in excess of 700 cm^2).

The response was square-root transformed for the 2003 cohort, and log_{10} +1 transformed for the 2004 and 2006 cohorts. In 2003 and 2006, initial phenology was not collected for all plants, therefore initial phenology was not considered in the model selection process for these cohorts. Because response variables were transformed differently, beta coefficient values should not be compared between the 2003 cohort and the other cohorts. Rather, the relative performance of predictor variables within a cohort, should be used to examine the overall impact of that variable across years.

9.3 Multiple comparison analysis of canopy size and seed output

The impact of elevation on canopy size was examined using ANOVA with a Tukey's honest significant differences post-hoc test for each cohort and for each site. For some of the subsets of data analyzed, the assumption of normality required of ANOVA was mildly violated as evidenced by examination of QQ plots. Several data transformations were explored (including square root, and log10 transformations) and model fit was slightly improved, however the benefit of transformation was marginal. Given that ANOVA is robust to such mild violations of normality, and that data transformation reduces the interpretability of results, no data transformation was used. Repeating analysis of several subsets of the data using a Kruskal-Wallis approach confirmed the results of the ANOVA.

10.0 Key Results and Discussion

10.1 Logistic regression analysis of TYC survival, reproduction, and growth

Site, elevation, and initial vigor were included in the top models as significant predictors of TYC survival and survival to reproduction for each of the cohorts installed in 2003, 2004, and 2006. Seed source and initial phenology did not improve model fit and were not included in the final regressions.

For the 2003 and 2004 cohorts, elevation had a negative effect on survival (Table 6). The coefficients resulting from the regression can be exponentiated to return odds ratios describing the impact of a one-unit change in the predictor variable on the dependent variable. For instance, in Table 8 for the 2003 cohort the exponentiated coefficient for elevation is 0.91. This means that for every one unit increase in elevation the odds of survival are multiplied by 0.91 (so survival decreases with increasing elevation). The interpretation of these odds ratios is similar for other factors, though the change in odds ratios is relative to the reference condition. Taylor Creek was selected as a reference site because it was planted in both years, but the choice does not have any effect on the model itself, only the relative values. For example, the coefficient describing the impact of being planted at Avalanche versus Taylor Creek in 2003 is 1.34 (Table 6). Exponentiating this coefficient gives 3.81 and means that the odds of survival at Avalanche are 3.81 times the odds of survival at Taylor Creek. If Sand Harbor had been chosen as the reference site, the beta coeffcients would have been negative for all sites because survival was lowest. Exponentiated coefficients greater than one mean the odds of survival increase for that group as compared with the reference group, while coefficients less than one mean the odds of survival decrease for that group as compared with the reference group. It is important to remember that these coefficients are calculated under the assumption that all other model terms are held constant. In other words, these coefficients are accurate after accounting for variation in survival predicted by other model terms.

Table 6. Summary of logistic regression analysis of TYC survival for 2003 and 2004. Planting cohorts were analyzed separately to examine the effect of each variable for a given year. β refers to the beta coefficient of the model. SE β refers to the standard error for that coefficient. e^{β} is the exponentiated beta coefficient (for interpretation see main text)

Predictor variable	2003 Cohort			2004 Cohort		
	β	SE β	e^{β}	β	SE β	e^{β}
2003 Cohort - Site: Taylor Creek	***Reference category***					
Avalanche	1.34***	0.19	3.81	-	-	-
Zephyr Cove	0.04	0.16	1.05	-	-	-
Sand Harbor	-1.01***	0.17	0.36	-1.17***	0.16	0.31
Upper Truckee (UTE)	-	-	-	0.36**	0.14	1.43
Nevada Beach	-	-	-	0.74***	0.15	2.10
Elevation	-0.09*	0.04	0.91	-0.38***	0.03	0.68
Initial vigor: High	***Reference category***					
Medium	-0.46***	0.13	0.63	-0.24	0.22	0.79
Low	-1.55***	0.19	0.21	-0.43***	0.11	0.65
Intercept	0.98	0.15		1.9***	0.12	
AUC		0.73			0.783	
Nagelkerke R^2		0.21			0.17	
*denotes significance at $p < 0.05$; ** denotes significance at $p < 0.01$; *** denotes significance at $p < 0.001$						

Table 7. Summary of logistic regression analysis of TYC survival for 2006. β refers to the beta coefficient of the model. SE β refers to the standard error for that coefficient. e^{β} is the exponentiated beta coefficient, and is the odds ratio with respect to the reference condition of each factor.

Predictor variable	β	SE β	e^{β}
Site: Upper Truckee East (UTE)	***Reference category***		
DL Bliss	-3.41***	0.48	0.03
Ebright	-3.51***	0.35	0.03
Nevada Beach	-0.30	0.42	0.74
Pope Beach	-1.62***	0.24	0.20
Tallac	-0.75**	0.25	0.62
Taylor Creek	-1.09***	0.25	0.34
Elevation	-1.37***	0.18	0.27
Initial vigor: High	***Reference category***		
Low	-1.26***	0.18	0.29
Seed Source: UTE	***Reference category***		
Taylor Creek	-0.57***	0.16	0.57
Intercept	3.46***	0.29	
AUC		0.84	
Nagelkerke R^2		0.543	
*denotes significance at $p < 0.05$; ** denotes significance at $p < 0.01$; *** denotes significance at $p < 0.001$			

For the 2006 cohort, site, elevation, initial vigor, and seed source were all significant (Table 7). Survival was significantly higher at UTE than any other site and elevation had a much stronger negative effect on survival than was observed in 2003 or 2004. The effect of plant quality was similar to the 2003 cohort, although plants were not graded as medium. The significant effect of seed source may be due to the uneven distribution of seed lots among sites.

Site elevation and initial vigor were also included in the top models for the multiple linear regression evaluating survival to reproduction in the 2003 and 2004 cohort (Table 8). Seed source was not included as a predictor variable in the 2006 cohort (Table 9). The model fit was poor in both cohorts.

Table 8. Summary of logistic regression analysis of TYC survival to reproduction for 2003 and 2004. Cohorts of seedlings were analyzed separately to examine the effect of each variable for a given year. β refers to the beta coefficient of the model. SE β refers to the standard error for that coefficient. e^{β} is the exponentiated beta coefficient (for interpretation see main text)

Predictor variable	2003 Cohort			2004 Cohort		
	β	SE β	e^{β}	β	SE β	e^{β}
2003 Cohort - Site: Taylor Creek	***Reference category***					
Avalanche	0.68***	0.16	1.97	-	-	-
Zephyr Cove	0.03	0.16	1.03	-	-	-
Sand Harbor	-0.88***	0.19	0.41	-1.99***	0.22	0.14
Upper Truckee (UTE)	-	-	-	0.35	0.18	1.42
Nevada Beach	-	-	-	0.59***	0.18	1.81
Elevation	-0.09*	0.05	0.92	-0.66***	0.03	0.52
Initial vigor: High	***Reference category***					
Medium	-0.28**	0.12	0.76	0.35	0.28	1.42
Low	-0.97***	0.20	0.38	-0.31***	0.12	0.73
Intercept	0.03	0.15		1.68***	0.15	
AUC		0.66			0.83	
Nagelkerke R^2		0.11			0.43	
*denotes significance at $p < 0.05$; ** denotes significance at $p < 0.01$; *** denotes significance at $p < 0.001$						

Table 9 Summary of logistic regression analysis of TYC survival to reproduction for 2006. β refers to the beta coefficient of the model. SE β refers to the standard error for that coefficient. e^{β} is the exponentiated beta coefficient, and is the odds ratio with respect to the reference condition of each factor.

Predictor variable	β	SE β	e^{β}
Site: Taylor Creek	***Reference category***		
DL Bliss	-0.44	0.90	0.64
Ebright	-2.26*	1.08	0.10
Nevada Beach	0.26	032	1.30
Pope Beach	-0.31	0.35	0.73
Tallac	1.03**	0.34	2.79
Upper Truckee East (UTE)	0.03	0.28	1.04
Elevation	0.09	0.20	1.10
Initial phenotype: High	***Reference category***		
Low	0.40	0.28	1.49
Intercept	-0.42***	0.37	
AUC		0.62	
Nagelkerke R^2		0.09	
*denotes significance at $p < 0.05$; ** denotes significance at $p < 0.01$; *** denotes significance at $p < 0.001$			

Model selection for data pertaining to the 2003 cohort showed that site, elevation, and initial vigor were included in the top model predicting canopy size of TYC (Table 10). For the 2004 cohort, site, elevation, seed source, and initial phenology were included in the top model (Table 11). However, seed source was not significant. For the 2006 cohort, site, elevation, and seed source were included in the top model (Table 12). For every cohort, canopy size was smaller for plants installed higher above lake level. However, for all models multiple R^2 was low, indicating that canopy size is poorly predicted by any of the factors analyzed. Light, water, and nutrient levels all affect plant growth and none of these variables were directly measured. It is not surprising that the factor of site did not adequately integrate the factors that support plant growth because canopy size was extremely variable across the elevational gradient within a site (see Tables 16-18 below) and was generally very low when averaged for the entire site (see Tables 13–15 below).

Table 10. Multiple regression analysis of factors impacting TYC canopy size in 2003. Only data from survivors were included in analysis. The response for this cohort was square-root transformed. β refers to the beta coefficient of the model. SE β refers to the standard error for that coefficient.

Predictor variable	β	SE β
Site: Taylor Creek	***Reference level***	
Avalanche	-2.00***	0.45
Zephyr Cove	-0.31	0.53
Elevation	-0.63***	0.19
Initial vigor: High	***Reference level***	
Medium	-1.14**	0.41
Low	-1.02	0.76
Intercept	11.49***	0.44
Multiple R^2	0.05	
*denotes significance at $p < 0.05$; ** denotes significance at $p < 0.01$; *** denotes significance at $p < 0.001$		

Table 11. Multiple regression analysis of factors impacting canopy size of TYC in 2004. Only data from survivors were included in analysis. The response for this cohort was log10 +1 transformed. β refers to the regression coefficient. SE refers to the standard error of that coefficient.

Predictor variable	β	SE β
Site: Taylor Creek	Reference level	
Nevada Beach	0.40***	0.06
Sand Harbor	0.05	0.05
Upper Truckee (UTE)	0.13*	0.05
Elevation	-0.12***	0.01
Seed Source: Lighthouse	Reference level	
Regan Al Tahoe	0.01	0.06
Taylor	-0.1	0.06
Cascade	0.08	0.06
Tahoe Meadows	0.1	0.08
Tallac	0.13	0.08
Blackwood	-0.1	0.08
UTE	-0.25***	0.07
Initial phenology: Flowering	Reference level	
Senescent	0.03	0.04
Vegetative	-0.18	0.04
Intercept	1.63***	0.05
Multiple R2	0.27	

Table 12. Multiple regression analysis of factors impacting canopy size of TYC in 2006. Only data from survivors were included in analysis. The response for this cohort was log10 +1 transformed. β refers to the regression coefficient. SE refers to the standard error of that coefficient.

Predictor variable	β	SE β
Site: Taylor Creek	Reference level	
Nevada Beach	-0.26**	0.09
Ebright	-0.60***	0.18
Upper Truckee (UTE)	-0.26**	0.08
Pope	-0.25**	0.1
Tallac	0.15	0.1
Elevation	-0.09	0.06
Seed Source: UTE	Reference level	
Taylor Creek	-0.15**	0.05
Intercept	1.63***	0.11
Multiple R2	0.14	

10.2 Multiple comparison analysis of canopy size and seed output

10.2.1 The effect of site on TYC survival, reproduction, and growth

The multivariate analysis revealed that site had significant impact on survival, reproduction, and plant growth. To further explore the impact of site on canopy size and/ or seed output, ANOVA with a Tukey's honest significant differences post-hoc test was utilized for each cohort and for each site. Reproductive success varied from 22 to 59% among the four sites in the 2003 cohort and canopy size was lower at Avalanche than Taylor Creek or Zephyr Cove (Table 13).

Table 13. The number of container-grown TYC planted, survival to reproductive success, canopy size, and seed output in September of 2003. Within in a column, canopy size and seeds/ plant followed by different letters are significantly different (ANOVA, F=12.1 p<.0001, Tukey HSD). Canopy size was not measured at Sand Harbor.

2003 Cohort	Year 1	September		% survival to reproduction	canopy (cm2)		
Site	# planted	# Live	# Fruiting		Fruit	Veg	seeds/ plant
Avalanche	297	254	176	59	107a	22	352a
Sand Harbor	221	78	49	22			
Taylor Creek	541	320	230	43	175b	24	574b
Zephyr Cove	286	168	115	40	167b	15	555b

A similar pattern was observed in the 2004 cohort (Table 14), but reproduction and growth were reduced under the high lake stand in the 2006 cohort (Table 15). The number of seeds/ plant is not presented for these cohorts because the metric is directly derived from canopy size.

Table 14. The number of container-grown TYC planted, survival to reproductive success, and canopy size in September of 2004. Within in a column, canopy size followed by different letters are significantly different (ANOVA, F=49.2 p<.0001, Tukey HSD).

2004 Cohort	Year 1	September		% survival to reproduction	canopy (cm2)
Site	# planted	# Live	# Fruiting		Fruit
Nevada Beach	578	436	311	54	106a
Sand Harbor	281	122	41	15	35b
Taylor Creek	540	389	259	48	36b
Upper Truckee East	986	726	472	48	49b

Table 15. The number of container-grown TYC planted, survival to reproductive success, and canopy size in September of 2006. Within in a column, canopy size followed by different letters are significantly different (ANOVA, F=19.2 p<.0001, Tukey HSD).

2006 Cohort	Year 1	September		% survival to reproduction	
Site	# planted	# Live	# Fruiting		canopy (cm2)
DL Bliss	100	6	2	2	
Ebright	100	14	1	1	
NV	198	154	72	36	37bc
Pope	150	79	28	19	13c
Tallac	225	178	119	53	86a
Taylor	150	71	31	21	50b
UTE	250	207	89	36	26bc

10.2.2 The effect of elevation on TYC survival, reproduction, and growth

The multivariate analysis also revealed that planting elevation had a significant negative impact on survival, reproduction, and plant growth. To further explore the impact of elevation on canopy size, ANOVA with a Tukey's honest significant differences post-hoc test was utilized for each cohort and for each site. Canopy size was not measured at Sand Harbor in 2003. Inundation was the likely cause of reduced growth at the lowest elevation at Avalanche and Sand Harbor in the 2003 cohort, but the TYC that survived in that microhabitat at Taylor grew significantly bigger than plants at any other elevation (Table 16). Competing vegetation was a likely factor in the small canopy size observed at Taylor creek in the 1.1 and 1.7 m elevation plots located around a back beach lagoon. Plants installed in stabilized vegetation in the meadow at 5.6 m at Taylor did not survive at all.

Table 16. The planting elevation (m) and canopy size (cm2) of surviving TYC in September of 2003. Within in a column, for each site, canopy size followed by different letters are significantly different (ANOVA with Tukey HSD).

Site	F ratio p value	elevation (m)	canopy (cm2)
Avalanche	F=37.8	0.5	41a
	P<0.0001	1.3	130b
		3	71c
Taylor Creek	F=18.96	0.3	395a
	P<0.0001	0.8	127bc
		1.1	85b
		1.7	63b
		2.4	122bc
		4.3	171c
		5.6	No live
Zephyr Cove	F=49.6	0.4	16a
	P<0.001	1.4	196b
		3.4	28a

In the 2004 cohort, inundation was again the likely cause of small canopy size at the lowest elevation at UTE (Table 17). At Nevada Beach, plants that were installed on both banks of Burke Creek had similar growth regardless of elevation and canopy size was only reduced in plants installed at the highest elevation away from the bank. Lake level was low in both 2003 and 2004, but rose to capacity in 2006. The experimental reintroductions failed completely at two sites in the 2006 cohort and canopy sizes were small at three of the five sites where TYC survived (Table 18).

Table 17. The planting elevation (m) and canopy size (cm2) of surviving TYC in September of 2004. Within in a column, for each site, canopy size followed by different letters are significantly different (ANOVA with Tukey HSD).

Site	F ratio p value	elevation (m)	N Live	canopy (cm2)
NV	F=18.5	0.8	132	132a
NV	P<0.001	1.7	95	126a
NV		3.3	134	117a
NV		5.8	75	17b
SH	F=12.98	1	41	69a
SH	P<0.001	2.4	63	25b
SH		4	18	8b
TY	F=8.5	0.3	40	74a
TY	P<0.001	0.8	138	38b
TY		1.7	113	34b
TY		2.4	45	14b
TY		4.3	53	24b
TY		5.6	233	no live
UTE	F=49.7	0.3	235	22a
UTE	P<0.0001	1	18	108b
UTE		1.7	77	19a
UTE		3.3	163	24a
UTE		5	1	17a

Table 18. The planting elevation (m) and canopy size (cm2) of surviving TYC in September of 2006. Within in a column, for each site, canopy size followed by different letters are significantly different (ANOVA with F ratio and p value for each site, Tukey HSD).

Site	F ratio p value	elevation (m)	N Live	canopy (cm2)
DL Bliss		1.8	6	
Ebright		0.5	14	
Ebright		2.1	0	
NV	F=5.8	0.4	72	54a
NV	P<0.001	0.8	23	15b
NV		1.1	52	29ab
NV		1.8	7	0
Pope	F=1.6	0.3	15	5a
Pope	P=.19	1.2	64	17a
Tallac	F=16.1	-0.2	63	45a
Tallac	P<0.0001	0.7	93	143b
Tallac		1.6	22	31a
Taylor	F=8.5	1.1	42	134a
Taylor	P<0.005	1.7	29	8b
UTE		1.1	207	26

10.2.3 Persistence through time

Once container-grown TYC plants established, they tended to persist through time. Patterns of survival and reproduction observed across sites in the first year in 2003 cohort persisted into subsequent years (Table 19). This was also observed within sites in 2003 cohort at different planting elevations in the first year following the reintroduction (Table 20).

Table 19. The number of container-grown TYC planted, survival to reproductive success, and number alive in September in years 1-3 (2003 to 2005).

2003 Cohort		Year 1		Year 2		Year 3	
Site	# planted	% survival to reproduction	N	% survival to reproduction	N	% survival to reproduction	N
Avalanche	297	59	176	67	198	46	137
Sand Harbor	221	22	49	30	66	19	46
Taylor Creek	541	43	230	51	225	17	65
Zephyr Cove	286	40	115	40	114	37	107

Table 20. The planting elevation (m) and canopy size (cm2) of surviving TYC from the 2003 cohort in September, 2004. Within in a column, for each site, canopy size followed by different letters are significantly different (ANOVA with F ratio and p value for each site, Tukey HSD).

Site	F ratio p value	elevation (m)	N live	canopy (cm2)
AV	F=36.2	0.5	175	22a
AV	P<0.0001	1.3	94	208b
AV		3	57	105b
SH	F=6.4	0.5	67	
SH	P<0.01	1.9	62	202a
SH		3.5	16	78b
Taylor	F=25.8	0.8	168	65c
Taylor	P<0.0001	1.1	66	76c
Taylor		1.7	28	157b
Taylor		2.4	25	244a
Taylor		4.3	33	47c
Zephyr	F=33.3	0.5	9	107a
Zephyr	P<0.001	1.3	91	133a
Zephyr		3.4	69	24b

Variation in total estimated seed production among sites in the 2003 cohort partly reflects differences in the number of plants installed (see Table 2). Many more TYC were installed at Taylor Creek than the other sites. In the year after planting, seed production increased at Avalanche but declined at Taylor Creek and Zephyr Cove where more inundation occurred during winter storms (Table 21). Lake levels were similarly low in 2003 and 2004, but at transition levels in 2005

Table 21. Total estimated seed production in the 2003 cohort in September in year 1, year 2 (2004), and year 3 (2005). Seed production was estimated from canopy size and canopy was not measured at Sand Harbor in 2003 or 2005 or at Avalanche in 2005.

2003 Cohort	Year 1	Year 2	Year 3
Lake level in September	6,224.2	6,223.3	6,228.1
Site	**Total seed production**		
Avalanche	51,334	73,827	NA
Sand Harbor	NA	38,960	NA
Taylor Creek	122,158	64,176	22,873
Zephyr Cove	56,993	36,467	62,253
Total seed per year	**230,484**	**213,430**	**85,126**

While site and elevation are important to establishment, lake level is one of the most important determinants of TYC persistence through time. Every single plant that was installed in the experimental re-introductions between 2003 and 2005 was inundated by the high lake levels in 2006. The pattern of total seed production observed among the cohort reflects this inundation (Table 22). Total estimated seed production for the four experimental cohorts exceeded 1.5 million seeds, but the fate of these seeds is unknown. 2005 has been excluded from all other analyses because of poor container-grown plant quality, but seed were produced in the first year.

Table 22. Total estimated seed production in the 2003 -2006 cohorts in September in years 1- 3. Seed production was estimated from canopy size and canopy was not measured at Sand Harbor in 2003 or 2005 or at Avalanche in 2005.

		Estimated number of seeds produced			
Cohort	**Number planted**	**Year 1**	**Year 2**	**Year 3**	**Total**
2003	1,423	220,097	193,166	84,607	**497,870**
2004	2,814	291,071	409,472	0	**700,543**
2005	2,032	95,394	0	NA	**95,394**
2006	1,175	96,700	143,429	NA	**241,304**
					1,535,111

10.3 Precision seeding

One month after sowing, no seedlings were present in any of the low beach or high beach plots, two seedlings were present in one berm plot, and nineteen seedlings in one moist shoreline plot. However, small amounts of shifting of the beach sand surface (perhaps by wind or water) made it difficult to know for certain if seedlings were actually the products of sown seed. Only one of the seedlings was directly under the planting frame hole in the berm plot and some seedlings in the moist shoreline plot may have come from natural recruitment. Inspection around the area of the frames found new seedlings emerging beyond the edges of each plot. Even if all the seedlings were attributed to sown seed, the maximum of 24 seedlings emerging from a total sowing of 1,200 frame holes (each hole received more than one seed) would constitute very low germination and recruitment (2%). These results indicate that sowing TYC seed on the soil surface is an ineffective method for enhancing or creating TYC populations.

10.4 Age-structured reintroduction

In June 2004, 50 container-grown TYC held in the greenhouse since 2003 were installed at UTE at three elevations to test the ideas of age-structured outplanting and "founder–cost averaging". In experimental re-introductions, individual plants have been referred to as founders (Pavlik 1996). The age-structure of a rare plant population may be important for the maintenance of high levels of reproductive output (seeds and clones) (Pavlik 1996). Building an optimized age-structure in reintroduced populations can be accomplished by planting multiple age classes (e.g. one year-olds, two year-olds, etc.) in a single year or by promoting survival of founders across years. Members of different classes often differ in size and, therefore, in resources available for reproduction. Presumably, older and larger founders would produce more seeds or clones than younger, smaller founders, and could boost the overall production of new plants in a given year. "Founder-cost averaging" is the successive outplanting of founders of any age class in different years. In this way the risk of outplanting all founders in an unfavorable year (*e.g.* drought, high lake level) is reduced. This minimizes stochastic effects and is analogous to "dollar-cost averaging" in financial investment. Instead of maximizing monetary return, this ecological restoration technique could be used to maximize "return" (survival and reproductive output) on the investment of founders among all outplanting years.

Survival to reproduction in September of the two year old TYC varied by elevation, with highest survival at 1m in the berm habitat (Table 23). The mean survivorship to reproduction of the one year old TYC planted at the same time was higher in the berm plots (1m) (90%), but similar at 5m (14%). At 3.3 m it was only 6%, but competing vegetation was a factor at that elevation. These limited results indicate that holding container-grown TYC in the greenhouse does not appear to confer any advantage in the survival to reproduction of two year old TYC.

Table 23. Survival to reproduction in September, 2004 of container-grown TYC held in the greenhouse for two years planted at UTE in June.

Elevation (m)	# planted	# Live	# Fruiting	% survival to reproduction
1	10	7	6	60
3.3	20	3	2	10
5	20	12	3	15
Total	50	22	11	22

10.5 The effect of planting time on TYC survival, reproduction, and growth

In the 2008 cohort, planting time had a significant effect on canopy size at three of the four sites (Table 24) and on seed output (mean number of seeds produced per plant) at all sites (Table 25). Planting early in June or July appeared to confer an advantage in reproduction for TYC at all sites except Edgewood, where reproduction failed completely in the first year. Plants installed in June or July at UTE grew to be five times larger than plants installed in August. No seed output per plant was measureable in TYC planted in September. Despite planting time, reproduction was very poor except for UTE; only 12 (6%) plants fruited at Blackwood and only 24 (13%) at Sugar Pine SP. In contrast, 113 plants (57%) reproduced at UTE.

Table 24. The mean canopy size (cm^2) and B) seed output of surviving TYC in September, 2008. Within in a row different letters are significantly different (ANOVA with F ratio and p value for each site, Tukey HSD).

YEAR 1	F ratio	Canopy size (cm^2)			
Site	p value	June	July	August	September
Blackwood	F=2.01 p=0.116	32a	25a	23a	17a
Edgewood	F=8.92 p<.0002	36a	16b	9bc	2d
Sugar Pine SP	F=48.81 p<.0001	167a	63b	30c	16c
UTE	F=30.94 p<.0001	252a	240a	28b	5b

Table 25. The mean seed output of surviving TYC in September, 2008. Within in a row different letters are significantly different (ANOVA with F ratio and p value for each site, Tukey HSD).

Year 1		seeds/plant					
Site	F ratio p value	June	N	July	N	August	N
Blackwood	F=0.85 p=0.377	170a	7	101a	5	0	0
Sugar Pine SP	F=4.72 p<.018	600a	14	214b	8	178b	4
UTE	F=7.94 p<.0007	823a	38	882a	39	103b	11

In the second year after planting, a greater number of plants in the 2008 cohort became reproductive at all sites for each planting month and the effect of planting month was similar or more pronounced (Table 26). Planting in June or July resulted in greater seeds/plant than in August or September. AT UTE, TYC planted in July produced an average of 67% less seed per plant than those planted in June. Seed output in the August planting was only 15% of the June planting, and the September planting again failed to produce seed in the second year.

Table 26. The mean seed output of fruiting TYC planted in 2008 in September, 2009. Within in a row different letters are significantly different (ANOVA with F ratio and p value for each site, Tukey HSD).

Year 2		seeds/plant							
Site	F ratio p value	June	N	July	N	August	N	September	N
Blackwood	F=9.76 p=0.0007	786a	10	553a	13	0	0	189b	6
Sugar Pine SP	F=11.35 p<.0001	934a	19	672ab	28	246c	25	219c	6
UTE	F=43.2 p<.0001	3160a	41	2185b	44	472c	28	16c	28

Unfortunately, the results observed in 2008 were not duplicated in the 2009 cohort because reproduction failed at all sites but UTE. However, the effect of planting time was again highly significant at that site. TYC planted in June grew more than three times larger than those planted in July in the first year and the pattern was maintained into the second year (Table 27).

Table 27. The mean seed output of fruiting TYC planted in 2009 in September in 2009 and 2010. Within in a row different letters are significantly different (ANOVA with F ratio and p value for each site, Tukey HSD).

Upper Truckee East		seeds/plant							
Year	**F ratio p value**	**June**	**N**	**July**	**N**	**August**	**N**	**September**	**N**
YR 1	F=21.7 p=0.0001	1990a	43	600b	27	139b	15	0	0
YR 2	F=34.3 p=0.0001	3045a	48	1190b	40	673b	38	274b	17

11.0 References

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