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Population distribution and variability of mucosal-sheath mass of Calastoma cinnabarium in the Cloud Forest of Monteverde, Costa Rica

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ABSTRACT

Comprising an entire kingdom to themselves, fungi have been poorly studied relative to their diversity and importance across ecosystems. *Calastoma cinnabarium* is one of these widely distributed but understudied mushrooms found throughout the Americas. The purpose of this study was to examine its elevational distribution at Monteverde, Costa Rica and adaptive value of its distinctive mucosal-sheath to investigate its possible role in moisture regulation. Population censuses were conducted along an elevational gradient through two life zones in the Monteverde Cloud Forest in parallel with a study determining mucosal-sheath mass to cap size diameter. Additionally, the adaptive value of mucosal-sheathing was examined through the removal and subsequent 16-day exposure to high elevation environmental conditions. Population size was not significantly correlated with elevation (R^2 = 0.077, p-value = 0.315, n = 15). Mucosal-sheath mass to cap diameter ratio varied significantly between elevations (F-value = 4.89, p-value = 0.001, df = 4, n = 128) and supported the prediction that it serves to prevent desiccation at lower elevations. The mucosal sheath was found to confer a fitness advantage to *C. cinnabarium* by helping it cope with abiotic factors such as moisture and temperature, but not for biotic conditions, such as fungivory (λ^2 = 6.15, p-value = 0.046, df = 2, n = 40). Results from this experiment reinforce the notion of high fungal environmental specificity and shows how this constraint drives specialized morphology.

RESUMEN

Comprendiendo un reino en si mismos, los hongos han sido pobremente estudiados en relación a su diversidad e importancia a lo largo de los ecosistemas. *Calastoma cinnabarium* es uno de estos hongos ampliamente distribuidos y muy poco estudiados que se encuentra en todo el continente de América. El propósito de este estudio fue examinar la distribución altitudinal y el valor adaptativo de la capa mucosa de *C. cinnabarium* con el fin de investigar su possible papel en la regulación de humedad. Se condujeron censos de poblaciones a lo largo de un gradiente altitudinal a lo largo de dos zonas de vida en el bosque nuboso de Monteverde junto con otro estudio para determinar la masa de la capa mucosa con respecto al diámetro del tamaño de la copita. En forma adicional, se examino el valor adaptativo de la capa de mucosa por medio de la remocion de la misma y con una subsecuente exposicion de dieciseis dias a condiciones ambientales tipicas de elevaciones altas. El tamaño de la poblacion no estuvo significativamente correlacionado con la elevacion (R^2 = 0.077, valor de p = 0.315, n = 15). La tasa de la masa de la capa mucosa con respecto al diametro de la copita vario significativamente entre las elevaciones (valor de F = 4.89, valor de p = 0.001, df = 4, n = 128) y apoya la predicción de que esta sirve para prevenir desecación a elevaciones bajas. Se encontro que la capa mucosa confiere una ventaja adaptativa a *C. cinnabarium* ayudándole a resistir los factores abióticos tales como temperatura y humedad, pero no a resistir los factores bióticos,tales como funguivorismo (λ^2 = 6.15, valor de p = 0.046, df = 2, n = 40). Los resultados

de este experimento reenforzan la noción de una alta especificidad ambiental de los hongos y muestra cómo esta restricción conlleva a una morfología especializada.

INTRODUCTION

Conservation efforts have tried to preserve tropical ecosystems threatened by development and deforestation because of their rich biodiversity. However, these efforts have failed to acknowledge one crucial component of these rich ecosystems. Unique enough to comprise their own Kingdom, fungi are understudied relative to their diversity and function throughout biological communities. This distinct group of eukaryotic organisms is estimated to have over 1.5 million species and is found in virtually every ecological niche (Alexopoulos et al. 1996). The role of fungal communities within forest ecosystems is essential to the recycling of organic nutrients. By breaking down complex organic compounds, fungi replenish vital resources to nutrient-depleted tropical soils (Arora 1979). It is essential to study fungal ecology and their interaction within tropical systems to assist in conservation and preservation efforts aimed at maintaining such rich biodiversity.

Belonging to the division Basidiomycota, *C. cinnabarium* is a distinct fungus found throughout the Americas. Like most members of the fungi Kingdom, little is known about the natural history of the *C. cinnabarium.* It is grouped within the Order Tulostomatales, otherwise known as the "stalked puffballs," referring to the shape of their fruiting body (Knopf 1981). It is unique among close relatives by having a bright red fruiting body covered with a thick mucosal-sheathing and an affinity for humid climates (Arora 1979). Little is known about the specific function or adaptive significance of *C. cinnabarium's* mucosal-sheath.

Home to five life zones, the Monteverde Cloud Forest provides a unique physical environment with an array of altitudinally-compressed life zones. Straddling the Continental Divide in the Cordillera de Tilarán, this area can be characterized by its steep elevational gradients that influence the region's precipitation, temperature, and trade winds (Hartshorn 1983). High levels of rainfall and constant temperatures make the area an optimal habitat for fungal growth. With a highly documented variation of precipitation associated with elevation and season, the area provides a wide range of abiotic conditions for a diversity of fungal life (Clark et al. 2000).

The direct contact that fungi have with their environment makes them vulnerable to climatic fluctuations in humidity and temperature (McCracken 1995). Requiring particular ranges of abiotic factors for growth and development such as temperature, precipitation, carbon sources, and pH, fungi are dispersed throughout a wide range of microhabitats. For successful reproduction and germination, each species requires particular humidity levels and temperature (Arora 1979). It has been a challenge to determine the sizeable influence for each abiotic factor on each species because of their high environmental specificity alongside wide habitat distributions (Alexopoulos et al. 1996).

In the tropical life zones of Monteverde, as moisture and temperature levels fluctuate between regions, fungal development and community diversity are directly affected (Wales 1998). Furthermore, high levels of beta-diversity have been determined across life zones of the Monteverde Cloud Forest, indicating that distinct habitats support different communities of fungi (Ciocca 2000). These studies indicate high fungal environment specificity within tropical life zones, which are defined by precipitation and temperature. Our study explores the distribution and function of the mucosal-sheath of *C. cinnabarium* within two life zones of the Monteverde Cloud Forest around the Estación Biológica Monteverde (EBM). We hypothesize that the distribution of *C. cinnabarium* will vary with elevation, which is a proxy for temperature and precipitation within the Monteverde Cloud Forest. We predict that *C. cinnabarium* population sizes will be larger at higher elevations, where favorable precipitation and temperatures conditions exist.

Additionally, previous studies of *C. cinnabarium* in the Monteverde area have attempted to examine the functional significance of it's mucosal-sheathing (Veysey and Brown 2000). Limited by time and a small sample size, they found no association between individuals with removed mucosal-sheathing and fungivory or water logging. With a more detailed and long-term study of *C. cinnabarium's* mucosal-sheathing, our study will investigate the possible adaptive function as a water regulating mechanism affecting the fitness of individuals within our study sites. Therefore, if mucosal-sheaths are part of a water regulating mechanism to prevent desiccation, we will see thicker mucosal-sheaths at lower elevations where there is less precipitation and, conversely, if sheaths prevent water logging, we will see thicker sheaths at higher elevations. Furthermore, removal of the mucosal-sheathing will reduce the fitness of *C. cinnabarium*, making it vulnerable to either fluctuating moisture levels or fungivory or both. If the mucosal sheath provides an increase in fitness, we predict that individuals with their sheaths removed will suffer higher levels of mortality compared to control individuals with their sheaths intact.

METHODS

Three related studies were conducted within and around the property boundaries of the EBM in July and August of 2006. The areas surveyed occurred on the Pacific slope and included Tropical Montane Moist and Wet Forest, as classified by the Holdridge Life Zone System.

A population survey of *C. cinnabarium* was conducted within a 1550–1850 m elevational gradient along the Senderos Principal and Mirador and along a nearby ridgeline transect. These trails spanned the same elevational gradient, however, the ridgeline which runs north-west to south-east along the continental divide is consistently exposed to trade winds. In contrast the Senderos Principal and Mirador run perpendicular to the continental divide and are partially blocked from trade winds. Fruiting bodies within one meter to either side of the trail were counted in the survey. Approximately equal sampling efforts were put forth along each transect with time and energy. Linear regression analysis was used to examine possible correlations between population size and elevation.

Fruiting bodies along both transects were collected for further study. Approximately twenty to forty fruiting bodies were randomly selected to represent each of five fifty-meter elevational categories between 1600 and 1850 m. No *C. cinnabarium* were found below 1600 m. Fruiting bodies were rinsed and massed before and after the complete removal of the mucosal sheath, and the diameter of the spore case was measured to the nearest millimeter. A mucosal mass to spore case diameter ratio was calculated for each body and an average value was determined for each elevational category. Utilizing a Kolmogorov-Smirnov test to analyze the distribution of data (Rohlf and Sokal 1969), a one-way ANOVA test was then used to determine whether significant differences in sheath mass to diameter ratio existed between means. Furthermore, a Tukey's post-hoc pair-wise contrast was completed to compare pairs of means to detect significant differences between each elevation.

To examine the functional significance of the mucosal sheath, a gregarious cluster of *C. cinnabarium* at 1800 m was chosen. The population, located in a light gap on a steep trail embankment, was divided into two experimental groups (n = 40). To one group, the mucosal sheath was mostly removed using a razor. The other group served as a control. Care was taken to ensure that each group contained a similar demographic distribution. After sixteen days of exposure and frequent monitoring, mortality in each group was accessed and recorded. Data were analyzed using contingency tests to detect differential survivability between the groups.

RESULTS

Population Distribution

A total of 394 individual fruiting bodies from 15 different elevations were observed on the Sendero Principal, Mirador and ridgeline covering an elevational range of 1645 to 1840 m (Fig. 1). No fruiting bodies were observed from the start of our elevational transect at 1550 m until 1645 m. The population sizes ranged from gregarious groups of up to 60 individuals (80% of populations) to isolated patches of three or less individuals (20% of populations). Populations were present on an array of substrates with varying slopes along the trail edges. The population size of *C. cinnabarium* showed a positive trend with increasing elevation, but no significant correlation was determined between the two factors (Fig. 2; $R^2 = 0.077$, p-value = 0.315 n = 15).

Mucosal-Sheath Mass to Cap Diameter Analysis

A total of 128 individual samples were collected from five different elevational transects for mucosal-sheath mass analysis. Using a mucosal-sheath mass to cap diameter ratio for each individual within elevation zones, elevation averages were calculated (Fig. 3). The lowest elevational transect contained the largest mucosal-sheath mass to cap diameter (4.52 g/cm) while the highest elevational transect contained the smallest (2.73 g/cm). Each elevational group's mucosal-sheath mass to cap diameter was normally distributed about its mean (Kolmogorov-Smirnov, all p-value < .01). Further analysis showed that the elevational groups' mean mucosal-sheath masses to cap diameter ratios were significantly different from each other (One-way ANOVA, F-value = 4.89, p-value = 0.001, df = 4, n = 128). Table 1 displays the relationship between pairs of elevational zones means, with pairs of significantly different means highlighted by plus signs (Tukey's post-hoc pair-wise contrast, p-value < 0.05, n = 128). The pairs of elevational groups that had significantly different mucosal-sheath mass to cap diameter ratios were

1600-1650 m and 1650-1700 m, 1600-1650 m and 1700-1750 m, and 1600-1650 m and 1800-1850 m.

Mucosal-Sheath Removal

After a 16-day treatment on our control and experimental groups of 20 fruiting bodies at an elevation of 1800 m on the Senderos Principal and Mirador, mortality associated with fungivory and water regulation was witnessed (Fig 4 & 5). Of the 20 control fruiting bodies, three individuals were found dead, 12 were found alive, five were unaccounted for in the study site, and three were at the stage of spore dispersal. Of the 20 experimental individuals, seven were found dead, 14 were found alive, and one was at the stage of spore dispersal. It was also observed that after mucosal-sheath removal, smaller individuals within the experimental group appeared to regenerate a minimal mucosal barrier.

Initial statistical analysis of known survivorship and presumed mortality showed that mortality was independent of the absence of a mucosal-sheath (Fig. 4; Contingency analysis: $\lambda^2 = 0.440$, p-value = 0.507, df = 1, n = 40). These assumptions lumped the presumed dead (missing individuals) with the known dead individuals. By only utilizing two groupings, presumably dead or alive, an observed number of unknown individuals outcomes were associated as presumably dead. Further statistical analysis isolated these individuals into a third category to differentiate the number of known dead and the number of unknown fate. Mortality in this grouping was found to be significantly dependent on the absence of a mucosal-sheath (Fig 5; Contingency analysis: $\lambda^2 = 6.15$, p-value = 0.046, df = 2, n = 40).

DISCUSSION

Fungi are known to have high environmental specificity in their geographic distribution (Arora 1979). Therefore, it was hypothesized that the distribution of *C. cinnabarium* would be correlated with abiotic factors such as moisture and temperature, both of which are tightly linked to elevation. In this study, population size was not significantly correlated with elevation (Fig. 2) above 1645 m. However, there was a positive trend between population size and elevation, suggesting that elevation may account for some, but not the all the distribution pattern observed. Below 1645 m no individuals were observed, which reinforces the notion of high fungal environmental specificity (McCracken 1995), and shows that *C. cinnabarium* prefers higher elevations in Tropical Montane Moist and Wet Forest. It is possible that a stronger correlation between population and elevation does actually exist. Our methodology was biased toward lower elevations sites due to the fact that we passed by them twice on each transect where as higher elevation sites were only viewed once, resulting in a more thorough censusing of lower elevations. Thus the true trendline describing population versus elevation may have a steeper, possibly more significant slope. Future censusing studies should consider the systematic bias present in this methodology.

Our study sought to answer the question of whether mucosal sheaths provide a fitness advantage to *C. cinnabarium* by acting as a water-regulating mechanism. Initial statistical analysis did not support this hypothesis. Mortality was found to be independent of the presence or absence of mucosal sheath (Fig. 4). However, when we considered the unexplained disappearance of 25% of the individuals from our control site, we saw that mortality was significantly higher for individuals without mucosal sheaths (Fig. 5). These results suggest that the mucosal sheath confers a fitness advantage. The advantage is probably not anti-fungivory because the deaths appeared to be due to rot. This study would benefit by repetition due to the large fraction of unexplained disappearances in the control group.

We hypothesized that the mucosal sheath regulates the aqueous environment of the developing spores, by either preventing desiccation in dryer habitats, or preventing water logging in moister habitats. The results from this experiment suggest that the mucus has a more crucial role in preventing desiccation in drier environments. *Calastoma cinnabarium* populations occurring between 1600 and 1650 m were found to produce significantly more mucus relative to cap diameter than populations between 1800-1850 m, 1650-1700 m and 1700-1750 m (Fig. 3). The significant variation in mucus sheath production between elevations implies that quantity of mucus production is an environmentally-induced trait of *C. cinnabarium*. That the fungus produces more mucus at lower elevations suggests that individuals rely more heavily on the protective qualities of the mucus at lower elevations where the dryness may lead to membrane damage and desiccation (McCracken 1995). Interestingly, we observed that after scraping, some individuals appeared to regenerate their sheath. It appears that the physiological mechanisms regulating mucus production are continuously open to environmental signals and are quite complex. The energy invested in producing and maintaining this elaborate structure suggests how valuable it may be for successful reproduction.

Previous studies in the Monteverde area showed that mucous-sheathed fungi have greater abundance at high elevations (Veysey and Brown 2000). Due to this observation, researchers suggest that the mucus sheathing protects fungi against water logging. Our study of *C. cinnabarium* reveals a similar positive trend between abundance and elevation. We hypothesize that mucus-sheathed fungi may compete better at higher elevations due to water regulation mechanisms conferred in the mucosal sheath. In addition, based on the significantly greater thickness of the mucosal sheaths at lower elevations and the environmental inducibility of the mucosal sheath, we hypothesize that this physiological mechanism allows mucus-sheathed fungi to extend their range into lower elevations.

The presence of a mucosal-sheath is thought to be a plesiomorphic trait, suggested by fact that it is found in seven orders of fungi in both the Ascomycete and Basidiomycete divisions (Veysey and Brown 2000). There are no obvious similarities in the habitats occupied by fungi with sheaths, showing there is an overarching fitness advantage conferred in the sheath that has been selected for on many different occasions under varying environmental conditions. Further studies could investigate whether the inducibility of the mucosal sheath is present in these other orders. A possible explanation for why mucosal sheathing has evolved independently so many times is that it creates a favorable microclimate for reproduction, thus ameliorating the climatic variability in their habitats.

To understand whether precipitation, temperature, or some other abiotic factor more strongly influence the range and growth pattern of *C. cinnabarium* than others, further studies should sample different life zones with contrasting abiotic parameters in order to isolate variables. Another factor to consider when examining the distribution of a fungus is that mycorrhizal interactions are extremely common, occurring in over 80% of plant species (Johnson and Raven 1996). It is unknown whether *C. cinnabarium* participates in such an interaction.

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FIGURES AND TABLES

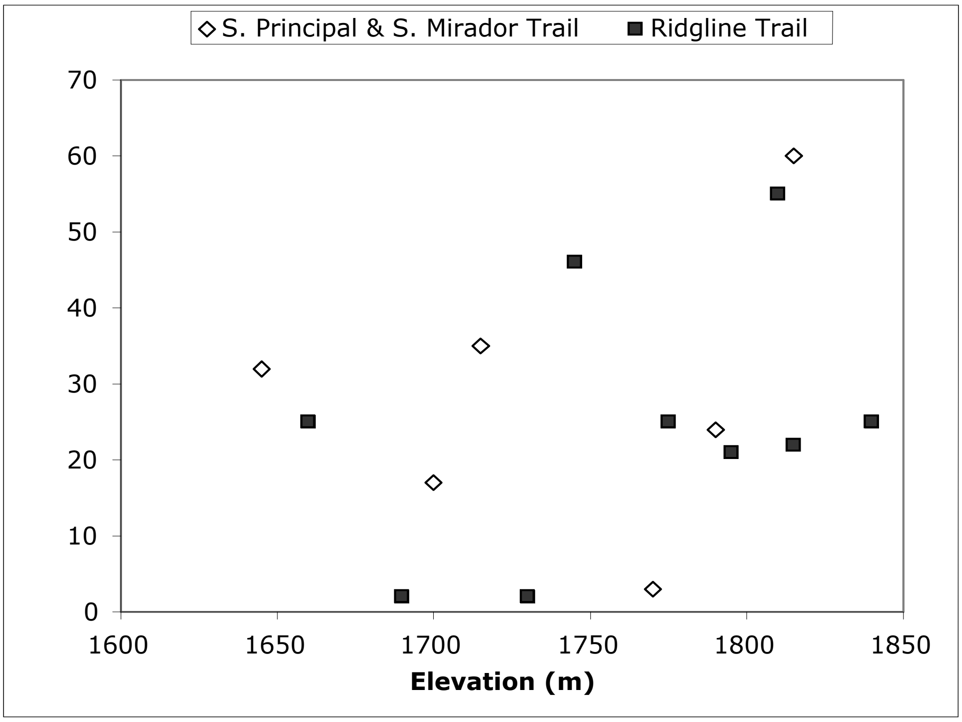

FIGURE 1. The distribution of *C. cinnabarium* along an elevational gradient on both the S. Principal/Mirador (diamonds) and ridgeline (squares) trails around the EBM.

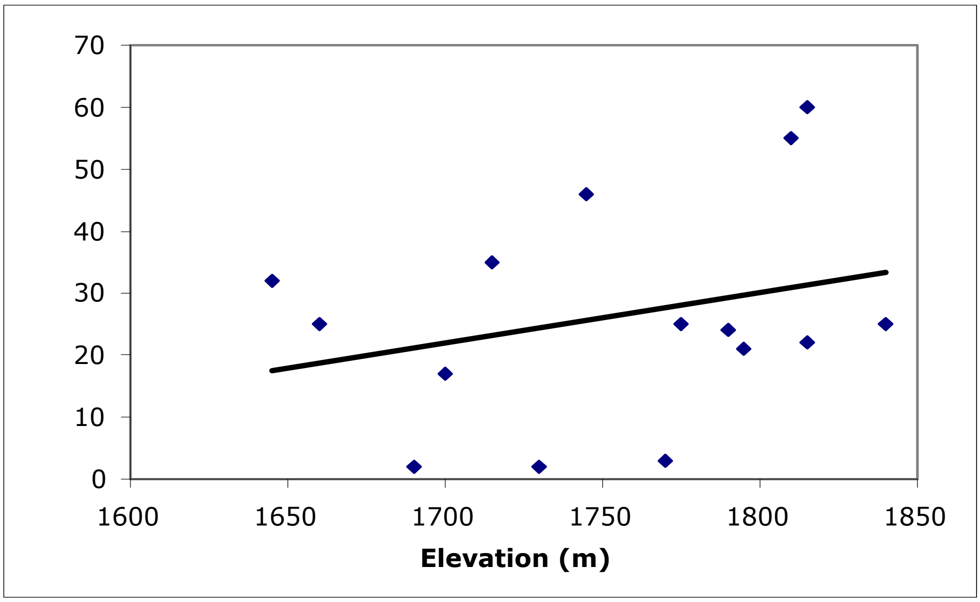

FIGURE 2. The positive trend between *Calastoma cinnabarium* population size and elevation (m) between 1550-1850 meters around the EBM. There was no significant correlation between elevation and population size for the observed groups ($R^2 = 0.077$, p-value = 0.315 n = 15).

FIGURE 3. Shows the average mucosal-sheath mass to cap diameter ratio (g/cm) for each elevational transect within the Life Zones around the EBM. A significant difference in mucosal-sheath mass to cap diameter ratio between three of the elevational zones existed (refer to Table 1 & 2) (One-Way ANOVA: F-value = 4.89, p-value = 0.001, df = 4, n = 128).

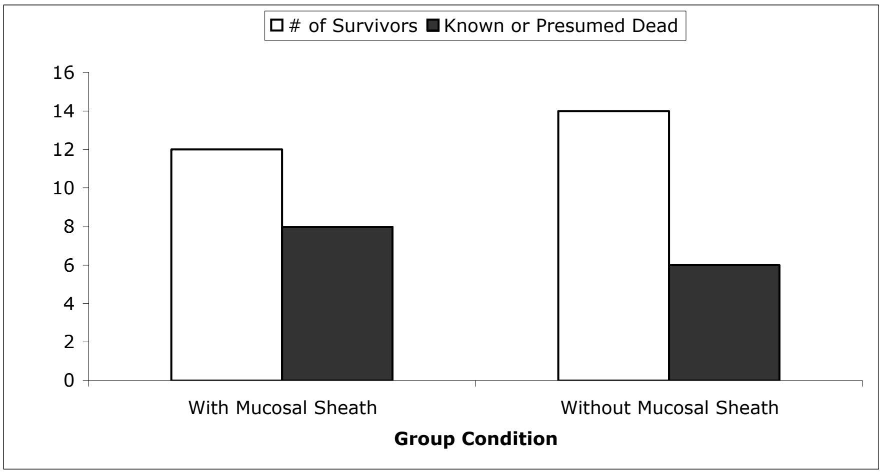

FIGURE 4. Shows the mortality and survivorship between the control and experimental groups in the mucosal-sheath removal test site at 1800 meters. The frequency of mortality was independent of the presence or absence of a mucosal-sheath (Contingency Analysis: λ^2 = 0.440, p-value = 0.507, df = 1,n = 40).

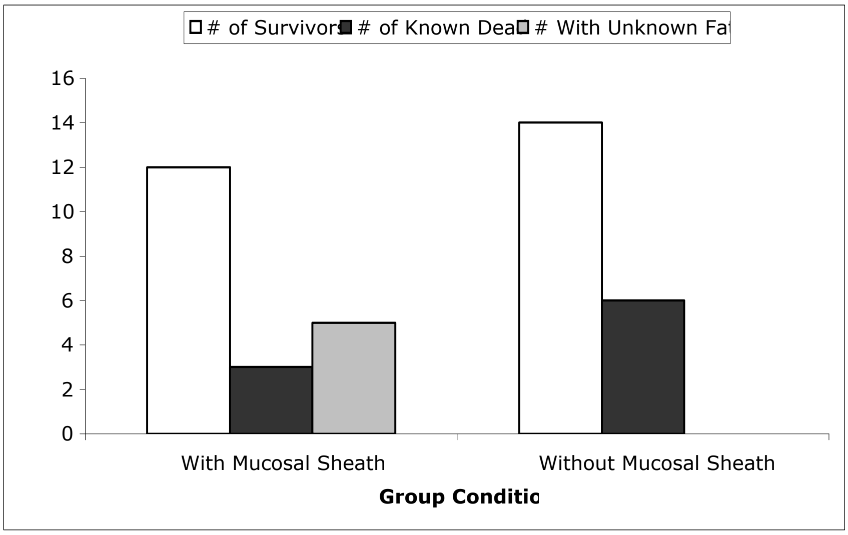

FIGURE 5. Shows the mortality, survivorship, and isolated unknown outcome condition between the control and experimental groups in the mucosal-sheath removal test site at 1800 meters. The frequency of mortality was dependent on the presence or absence of a mucosal-sheath (Contingency Analysis: $\lambda^2 = 6.154$, p-value = 0.0461, df = 2, n = 40).

TABLE 1. Shows the significant results between average mucosal-sheath mass to cap diameter ratio (g/cm) for each elevational transect. Positive values show pairs of means that are significantly different (Tukey's Post-Hoc Pairwise Contrast: p-value = 0.05, n = 128).

Elevation	1600-1650	1650-1700	1700-1750	1750-1800	1800-1850
1600-1650	-	+	+	-	+
1650-1700		-	-	-	-
1700-1750			-	-	-
1750-1800				-	-
1800-1850					-