How does seasonality affect the fitness of cane toads (*Bufo marinus*) in the Wet Tropics of Northeast Australia?

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Abstract

Understanding the viability and overall success of cane toad (*Bufo marinus*) populations is critical to exploring their impact on ecosystems and to develop management strategies. Our study looks to investigate how seasonality affects cane toad fitness. While past studies have mainly focused on the effect of lung worms (*Rhabdias pseudophaerocephala*) on toad fitness, we identified several factors that may indicate cane toad viability and tested their variability throughout the year. We collected toads at three sites across the dry, winter, and wet season in Northeast Queensland, Australia. Our results supported our hypothesis that cane toad fitness is at its lowest during the dry season due to unfavorable abiotic factors and poor foraging opportunities. During the wet season, when conditions are most favorable, percent body fat and average stomach content weight were at their highest. We found no significant difference between nematode infections across seasons. Future work should investigate demographic processes and how seasonality affects population dynamics throughout the year.

Introduction

Recognizing the importance of invasive species as an integral part of ecosystems helps guide critical decisions in the future conservation of our natural world. However, invaders often change their overall ecology and life history patterns when occupying a new habitat, which makes understanding their impact difficult.

Cane toads (*Bufo marinus*) were first introduced into the Wet Tropics of Australia in 1935 as a pest control.[1] Since their introduction, they have spread across the Wet Tropics and negatively affected ecosystem processes.[1] Past research has fallen under two main categories: investigating the behavioral ecology of cane toads and investigating the role of the lung worm (*Rhabdias pseudophaerocephala*) in cane toad viability. Seebacher and Alford revealed that the wet season and moist soils are extremely important for cane toad movement and therefore an integral part of their life history strategy.[2,3] Other studies have shown that toad behaviors are altered across age strata due to cannibalistic habits.[4] The lung worm has shown to be the main negative force on the population and acts as the primary bio-control of the cane toad in the Wet Tropics.[1,3,5] Initial research worked mainly to identify the toad's role as host and identify which parasites inhabited its body.[6] However, more current research has explored how lung worms negatively affect their fitness.[1,3,5]

Little work has looked into the role of seasonality on their overall fitness. Exploring the variability in cane toad fitness across multiple seasons is essential to understanding the overall impact of the toad in the Wet Tropics of Australia. We predict to find cane toad fitness lowest in the dry season due to food resource availability and abiotic conditions. The dry season poses unfavorably low temperatures and a dry climate that we predict will lower the overall success of cane toads.

Methods

We surveyed three field sites between 1730 to 2100 hours on September 19, 2011, and September 20, 2011. Groups were designated and sent out to collect cane toads at Crawford Plantation (-17.268, +145.64), Bonodios Plantation (-17.250, +145.534), and Centre for Rainforest Studies (-17.2045, +145.678), located near Yungaburra, Queensland. A total of 98 individuals were collected during the season. Toads were caught roaming individually and in groups found in holes. GPS data was collected on a Garmin 12. At each hole, readings of diameter, depth, temperature, and humidity were recorded using a Kestrel and metric measuring tape. Cane toads were then brought back to the Centre for Rainforest Studies Warrawee Field Station and euthanized using 3% chloral hydrate solution within two hours of capture.[7] Toads were analyzed in the lab at the Centre, where each toad was weighed using a Pesola spring scale before and after removal of all organs. Additionally, using a caliper each toad was measured from snout to vent in order to make future inferences about the age structure of the toads. After each toad was dissected, measurements focused on three main internal aspects. First, the total fat bodies were removed from each toad and weighed. Fat bodies are found inside cane toads and have the charactersitic resemblance of white oval-like sacs located near the digestive system. Second, the stomachs were weighed before and after the internal contents were removed in order to assess the mass of the contents. Finally, the lungs were weighed and then analyzed under a Prism Optical dissecting scope for presence of lung worms. The scale used in all instances was an Ohause Adventurer.

Additional data was collected in the winter and wet seasons by previous School for Field Studies students.[8-10] Data were used from these years to investigate seasonality in ecological patterns of cane toads. A total of 199 toads had been collected during the wet season and a total of 99 toads had been collected during the winter season.

Data were split into three main seasons of study. First, the dry season was designated as data collected between the beginning of September and the end of December, as past studies have done.[2] Second, we designated a winter transition season, which was data collected during the months of June and July. Finally, the wet season was designated between the beginning of January and the end of April.[2] We used an ANOVA to test variables in the dry, winter, and wet seasons. Our study focused on four indicator variables of fitness: average percent body fat, average number of nematodes per gram of lung, average total mass, and average stomach content mass. In cases where an overall significant difference was found, additional Tukey's Pairwise tests were run to identify specific variation between seasons.

Results

ANOVA tests and Tukey's Pairwise tests were run to investigate the effects of seasonality on cane toad fitness.

When comparing the average percent body fat between seasons, an overall significance was found (ANOVA, $F_{2,383}=18.13$, $p<0.001$)(Figure 1). Using Tukey's Pairwise test we found that the dry season was significantly different from both the winter and wet seasons (Tukey's, $p<0.001$,

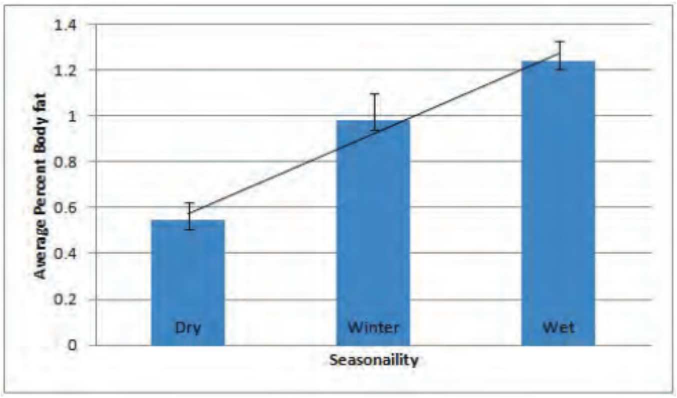

Figure 1: Average percent body fat between the three seasons.(ANOVA, $F_{2,383}$=18.13, p<0.001). Significance between dry and winter (Tukeys, p<0.001). Significance between dry and wet (Tukeys, p<0.008194). No significance between wet and winter seasons (Tukeys, p=0.175).

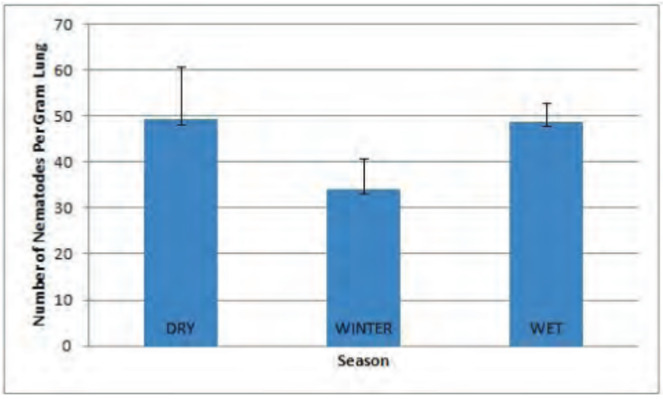

Figure 2: Average number of nematodes per gram of lung mass between the three seasons.(ANOVA, $F_{2,385}$=1.794, p=0.1693). No significance between the seasons.

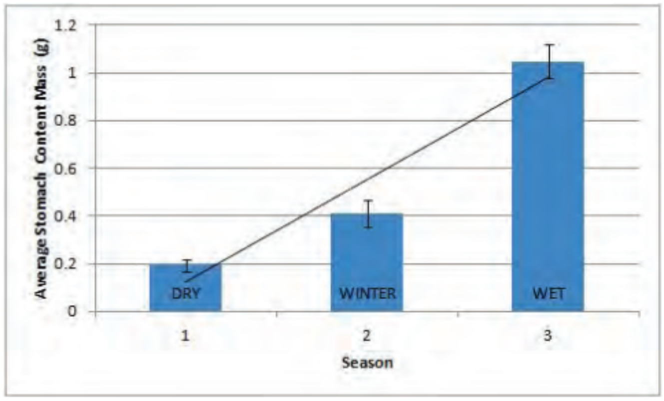

Figure 3: Average stomach content mass between the three seasons. (ANOVA, $F_{2,347}$=64.15, p<0.001). Significance between wet and both dry and winter (Tukeys, p<0.001). No significance between dry and winter (Tukeys, p=0.1677).

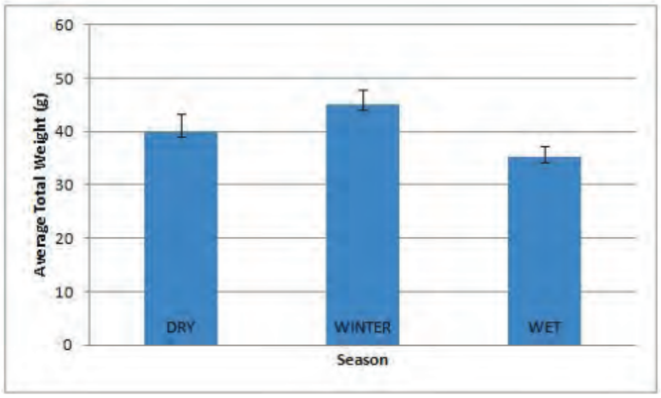

Figure 4: Average total body weight between the three seasons. (ANOVA, $F_{2,383}$=4.166, p=0.01).Wet and winter significance (Tukey's, p=0.0295). No significance between dry and both winter and wet (Tukey's, p=0.3801; p=0.443)

p<0.008194). There was no significant difference between the wet and winter seasons (p=0.175).

No significant difference was found when comparing the average number of nematodes per gram of lung mass between the three seasons (ANOVA, $F_{2,385}$=1.794, p=0.1693)(Figure 2).

Analysis showed an overall significant difference when comparing average stomach content mass between the three seasons. (ANOVA, $F_{2,347}$=64.15, p<0.001)(Figure 3). Further analysis showed the greatest variation between the wet season, and both the dry and winter seasons (Tukey's, p<0.001; p<0.001). However, analysis between the dry and winter seasons revealed no significance (Tukey's, p=0.1677).

Analysis showed a significant difference when comparing average total body weight across all three seasons (ANOVA, $F_{2,383}$=4.166, p=0.01)(Figure 4). However, further tests only showed one significant difference between the wet and winter seasons (Tukey's, p=0.0295). Additional analysis between the dry and winter seasons (Tukey's, p=0.3801) and the dry and wet seasons (Tukey's, p=0.443) showed no significance.

Discussion

Our study investigated the role of seasonality on overall cane toad fitness. Due to lack of food resources and overall colder temperatures and drier climate, we predicted to find cane toad viability at its lowest in the dry season. Additionally, because past studies have mainly based cane toad fitness on the presence of nematode lung worms, we also wanted to investigate seasonality affects on lung worm concentrations.

First, our results revealed that percent body fat was at its lowest in the dry season (Figure 1). Moreover, analysis of variance showed that the greatest difference in percent body fat was between the wet and dry seasons (p<0.008194). We contribute this finding to the likelihood of higher frequency of foraging opportunities in the wet season than in the dry. This supports our belief that toad fitness may be at its lowest in the dry season as low percent body fat is an indicator for poor viability.

Second, our results found no significant difference between the concentrations of lung worms across all three seasons (Figure 2). This result is interesting as past studies have attributed cane toad viability mainly to nematode concentrations.[1,5]

Thirdly, analysis of stomach content found significance between the wet season with both the dry and winter seasons (p-value<0.001). We found stomach contents to be significantly higher in the wet season (Figure 3). Because cane toads are general opportunistic feeders, often stomach content could not be identified. But in some of the cases when it could be identified, it generally consisted of ants, beetles, and on occasion other frogs and toads. We attribute this finding to the availability of food in the wet season compared to other seasons. This supports our hypothesis that cane toad fitness is lowest in the dry and highest in the wet season under ideal abiotic conditions and foraging opportunities.

Finally, our investigation of total weight revealed that the most variance was between the wet season and the winter (p-value=0.0295). We found the average total weight to be highest in the winter and lowest in the wet season (Figure 4). This result is somewhat surprising as we predicted lowest cane toad fitness in the dry season and believed that total cane toad weight is a predictor variable of overall fitness. One possible explanation could be that overall metabolic processes are somewhat slow and that high foraging rates in the wet season (Figure 1 and 2) are not transformed into total body weight until the dry and winter. This claim is also supported by our findings of lowest total body weight in the wet season (Figure 4). Low total body weight in the wet could be attributed to the use of stored resources throughout the winter and dry seasons.

Our study results coincide with previous finding in a study conducted on desert toads (*Scaphiopus couchii*) in Arizona, USA.[11] These results revealed that fat bodies and reproductive organs were at their peak in the most profitable foraging season.[11] Furthermore, the study showed that toads were most susceptible to parasite infection when fat bodies were at their lowest, and therefore, overall toad fitness was at its lowest in the winter. Although our study found no particular increase in nematode presence, we believe that seasonality has an overall effect on toad fitness. Additionally, previous studies found that night activity increases with rising temperatures.[12] Assuming that more activity is related to more foraging opportunities and an overall increase in fitness, this coincides with our hypothesis that overall fitness may be affected by abiotic factors. The data supported the hypothesis that cane toad fitness is at its lowest when foraging opportunities are low and abiotic factors are non-favorable. However, our study was limited by variability in sample sizes across seasons as certain seasons have been better studied than others. Ongoing collections, especially in the dry season, are essential to fully understanding the role of seasonality. Future work should investigate demographics in cane toads across seasons to investigate predation, survival, and mortality rates in cane toad populations. This research will be an integral part of fully understanding the effect of seasonality on cane toads.

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