# Microhabitat use of the Strawberry dart-poison frog (*Oophaga pumilio*) in relation to individual body condition

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**ABSTRACT**

The Strawberry poison dart frog, *Oophaga pumilio* is abundant throughout its range, but like in all amphibians a population decline has been detected in the last years (IUCN, 2010). In order to manage and conserve a species in a more targeted manner, it is important to assess the relation of habitat together with fitness, measured as a body condition (Babbit et al. ,2010). This study aims to reveal the relation between microhabitat characteristics and individual body condition of *O. pumilio*, and the body condition of males and females within the *O. pumilio* population of a Neotropical lowland rainforest in Costa Rica. Data collection took place in October and November 2014. Transects were set out in a garden area, a Neotropical lowland rainforest area and a forest path within this area. Microhabitat and morphological measurements were taken for the encountered individuals of *O. pumilio*. The measured variables found to be significantly associated with individual fitness measured as body condition index were the presence of calling sites, rainfall, substrate and gender. Furthermore, a significant relationship between sex and substrate type was found.

**INTRODUCTION**

Territorial behaviour in animals develops when the benefits of defending an area outweighs the costs in fitness (Carpenter and McMillen, 1976). Fitness as a central concept of biology and ecology is determined by the survival and reproductive schedule of an individual (McGraw *et al*., 1996). In all vertebrates, differences in fitness often correspond to differences in phenotypic quality, suggesting that larger individuals have greater fitness (Gaillard *et al*., 2000). Additionally in anurans, time and size at metamorphosis is largely related to adult fitness (Semlitsch, 1988).

Fitness can be gained through access to limited resources such as food, shelter and preferred calling or breeding sites (Pröhl, 2005). Even in a rich habitat such as a tropical rainforest where food, shelter and breeding sites are abundant (Pröhl, 2005), territory choice can influence the individual fitness. The spatial and temporal distribution of limited resources can have major influence on fitness (Wells, 1977).

Males and females are known to use different microhabitats due to gender specific requirements (Townsend, 1989, Resetarits and Wilbur, 1991, Pröhl, 2002).

Male anurans have two crucial reproductive activities, calling and nesting (Pröhl, 2002). Breeding and calling represent a trade-off between fitness via hatching success and fitness via mating success (Townsend, 1989). Territories which include an elevated calling site and a well-protected nesting site can represent a limited resource in the forest ecosystem (Townsend, 1989).

For females on the other hand, a high abundance of tadpole rearing sites represents the limiting resource (Resetaris and Wilbur, 1991 and Pröhl, 2002).

The choice of a territory, and therefore the choice for a certain microhabitat, influences the life history of an individual and therefore its general fitness (Pröhl, 2002). According to Murphy (2003), the selection of appropriate microhabitats is an important component of anuran fitness.

Individual fitness has been defined as a body condition measurement including snout-vent length (L) and weight as measurements. This body condition measurement has been used in different ecological and ethological studies to calculate an index for individual fitness (Jacob *et al.*, 1996 and Dziminski *et al.* 2009).

In order to link microhabitat use and therefore use of resources of anurans in a Neotropical rainforest to individual fitness, it has been chosen to study the strawberry dart-poison frog

(*Oophaga pumilio*). This species belongs to the family of the *Dendrobatidae*, the dart-poison frogs (Amphibiaweb, 2014). *O. pumilio* is a leaf-litter frog that can be found on the forest floor and on low branches or bush vegetation in Central America (Savage, 2002; Whitefield and Pierce, 2005; Whitefield et al., 2007). The species is abundant throughout its range, but like in nearly all amphibian populations, a decline in numbers has been detected in recent years (IUCN, 2010). Males exhibit strong territorial behaviour (Pröhl, 2005 and Staudt *et al*., 2010,). The core area of their territory which extends over 1-4m² (Staudt *et al*., 2010 and Rudh et al., 2012) is defended through calling and to a lesser extent through physical combat (Pröhl, 1997, Pröhl and Hödl, 1999). Due to its abundance and this strong territorial behaviour – an indication of microhabitat preference - this species has been chosen for this study.

According to Narins and Capranica (1976), calling sites of male frogs are mostly elevated and relatively free of surrounding cover which enables better sound propagation. Nesting sites however, can be found in well-enclosed habitats to shelter the eggs from predation and weather (Townsend, 1989). Males of the strawberry dart-poison frog are known to prefer territories with elevated places such as dead wood, branches or tree buttresses that can be used as calling sites (Whitefield and Pierce, 2005 and Staudt *et al.*, 2010). Furthermore, habitats with a high female abundance have been defined by Pröhl (2002) as a reproductive resource for males. Females show a preference for habitats with a high abundance of oviposition sites, tadpole rearing sites (bromeliad plants, banana plants and *Heliconia sp*.) and high mate abundance (Resetaris and Wilbur, 1991 and Pröhl, 2002). According to Donnelly (1989) and Pröhl (2002) tadpole-rearing sites are the main limiting resource for female Strawberry dart-poison frogs and Pröhl (2002) assumed that they are the determining factor for their territory size.

This study focused on the questions what is the relationship between microhabitat characteristics and individual body condition of *O. pumilio* , and what is the body condition of males and females on different substrates, within the *O. pumilio* population of a Neotropical lowland rainforest in Costa Rica.

## MATERIAL AND METHODS

### STUDY AREA

The study area is located in a tropical lowland forest in the Costa Rican Caribbean close to Caño Palma Biological Station (N 103536.1/W 833139.4). It is owned by the Canadian Organization for Tropical Education and Rainforest Conservation (COTERC), whose land covers an area of 40 ha of Atlantic lowland tropical wet rainforest. It is situated in the Barra del Colorado Wildlife Refuge and belongs to municipality of Límon, Costa Rica, 8km north the small town Tortuguero and 200m from the Caribbean coast. The daily temperatures of 23°C to 32°C (mean: 26°C) and a humidity range from 60 to 95% make it a very wet rainforest (Lewis et al, 2010).

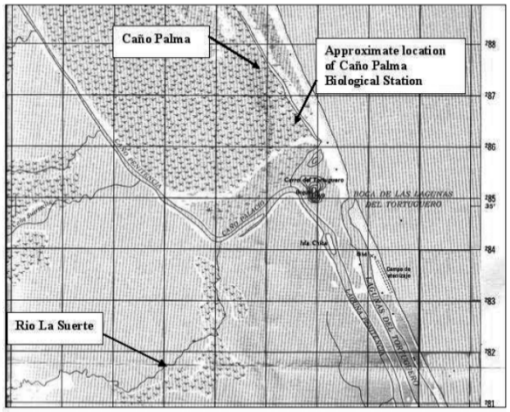


Figure 1: Location of Caño Palma and surrounding, Lewis , 2010

### SPECIES

*O. pumilio* is a small (17-24 mm) frog with a bright coloration which includes a mostly bright red to orange back, some small black spots, black to dark blue hind legs and a red belly (University of Michigan, 2014). It has a mean weight of 0,93g (Pröhl, 1999). Like all poison dart frogs, their skin contains an alkaloid-based toxin (Saporito, 2007) which they assimilate through their food intake (Staudt *et al.,* 2010). *O. pumilio* is a leaf-litter species which is mostly found on the forest floor and on low branches or bush vegetation (Savage, 2002, Whitefield and Pierce, 2005, Whitefield *et al*., 2007). Eggs are laid under dead leaves and kept moist by the male. After the tadpoles hatch, the female carries them one by one on her back to small water bodies in bromeliad plants or other similar vegetation, such as banana plants, that function as tadpole-rearing sites, where each tadpole has its own small water pool (Pröhl, 2002).

DATA SAMPLING & COLLECTION

Data collection took place over eight weeks in October and November 2014. 149 Transects of twenty-five meters length were set up in the secondary forest close to the Biological Station in advance. Eighty-two of the transects started perpendicular on the forest path, leading into the vegetation. The 1640 meters of forest path were then divided into another sixty-seven transects. To get the most diverse set of microhabitats, additional samples were taken in the garden area of the research station at Caño Palma.

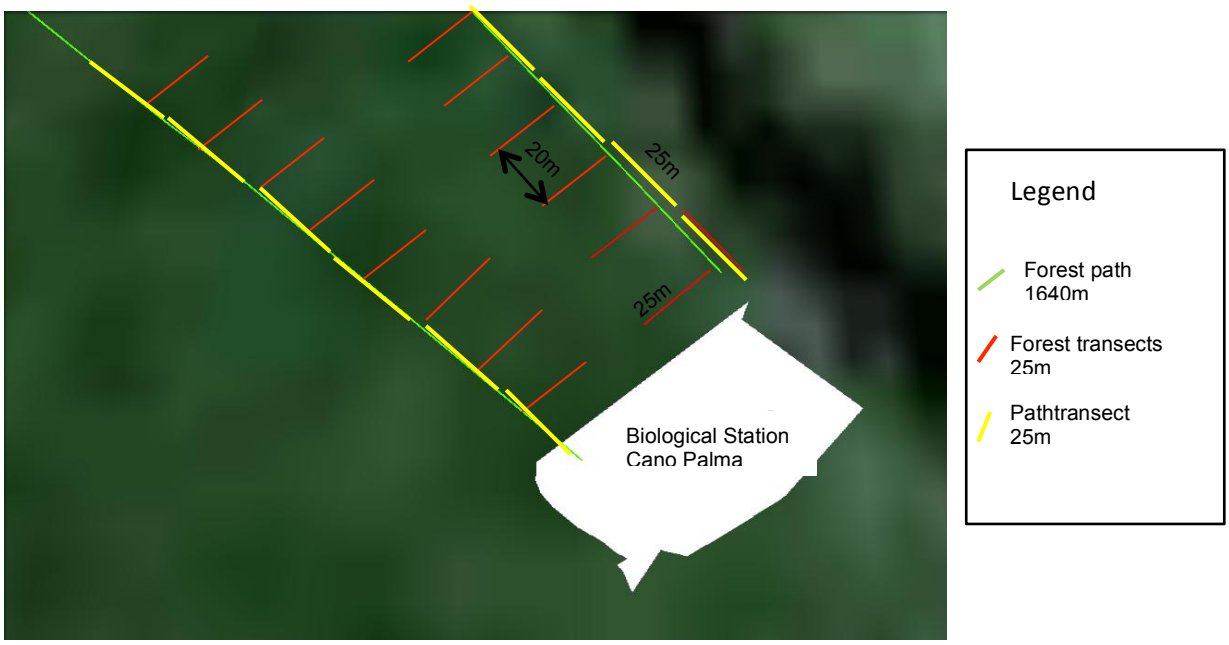


Figure 2: Setup of transetcs in primary rainforest of Caño Palma, Google Earth, Cappello, 2015

Two surveyors walked the transects in a slow pace, looking for *O.pumilio* around two meters to the left and the right. Data were recorded on every individual encountered on the transect and within a distance of at least 5 meters from the last encountered frog. The exact location of the individual was marked and a circular plot with a radius of 50cm was set up around the frog´s location. One-hundred-and-forty-nine transects were surveyed in total. Sampling took place inside and outside of daily peak activity times (07:00 a.m. – 12:00 a.m. (Pröhl, 2002)) to cover all possible circumstances.

MICROHABITAT CLASSIFICATION

Differences in microhabitats for frogs depend mostly on substrate type, height above ground, humidity and light (Townsend, 1989 and Whitefield & Pierce, 2008).
Within this study twelve microhabitat variables and abiotic factors were observed for every individual encountered.

**Table 1: Microhabitat variables:**

| Variable | Explanation |
|---|---|
| Substrate | Type of substrate the frog was encountered on (eg. Leaves attached to tree/bush (green/brown), leaf litter, rocks, branches, bare soil, dead wood, palm) |
| Elevation | Height above ground in cm |
| Humidity | Percentage of air humidity, measured directly above the location of the frog |
| Temperature | Temperature in °C measured right above the location of the frog |
| Oviposition site | Presence of possible oviposition site within the plot |
| Tadpole rearing site | Presence of possible tadpole rearing site within the plot |
| Calling site | Presence of possible calling site within the plot |
| Exposure to sun | Whether the frog has been found in the sun or not |
| Dry/wet ground | Estimated percentage of dry/wet ground within the plot |
| Sun/shadow | Estimated percentage of sun/shadow within the plot |
| Rain | Amount of rain over the day (mm) |
| Weather | Weather during data collection (eg. sunny, cloudy, rainy) |

MORPHOLOGICAL MEASUREMENTS

For this study, individual fitness was defined as a body condition measurement using snout-vent length and weight as measurements. The following formula was used to calculate a body condition index for every individual:

$$BCI = M\,[L_0/L]^R$$

Where M is the mass, L is the snout-vent length for a given individual, $L_0$ is the arithmetic mean of the L's for the whole population and R (the scaling component) is equivalent to the regression value (R value) of M on L for the whole population (Michaels *et al.*, 2014).
This measure of body condition has been found to accurately represent actual body condition and energy reserves in amphibians (Jacob *et al.,* 1996, Dziminski *et al.*, 2009, McCracken and Stebbings, 2012 and Michaels *et al*., 2014)
For each individual the individual fitness is thus assessed by measuring the body condition in SVL and weight.

GENDER

According to Pröhl (1999) and Richards-Zawacki (2010) sexes can be distinguished by throat coloration which is red in females and black or grey in males. During data collection all individuals with a dark throat and calling individuals were defined as males.

DATA ANALYSIS

The Fitness Index was calculated within the program Excel. A Univariate General Linear Model was performed with the program IBM SPSS Statistics 20. Within the model, the fitness Index was used as dependent variable. Covariates and Factors with a significance higher than 0.5 were not included in the final model. Furthermore a Chi-Square test was used to test the association between sex and substrate.

**RESULTS**

The variables found to be significantly associated with individual fitness measured as body condition index were presence of a calling site (d=10.287; Sig=0,001), the substrate the individual was found on (d=2.157; Sig=0,031), rainfall (d=9.511; Sig=0,002) and the gender of the individual (d=39.029; Sig=0,00). Within the general linear model used to analyze the relationships between the variables, the named significant variables explain 21 percent of the differences found in individual fitness. Furthermore, a significant relationship (X(1)=20,75, p= 0,08) between sex and substrate type was found. Figure 3 shows the relationship between substrate type and mean body condition index of males and females. Males show a strong peak of fitness on the substrate `leaf` (mean 0.8), whereas for females the fitness index shows the highest values for the substrate types palm (mean 0,76) and bare soil (mean 0,75). Even though males show a stronger peak in fitness on one substrate type, the overall mean for fitness is higher in females (mean body condition 0,72 ±0,09) than in males (mean Body condition 0,67 ± 0,08).

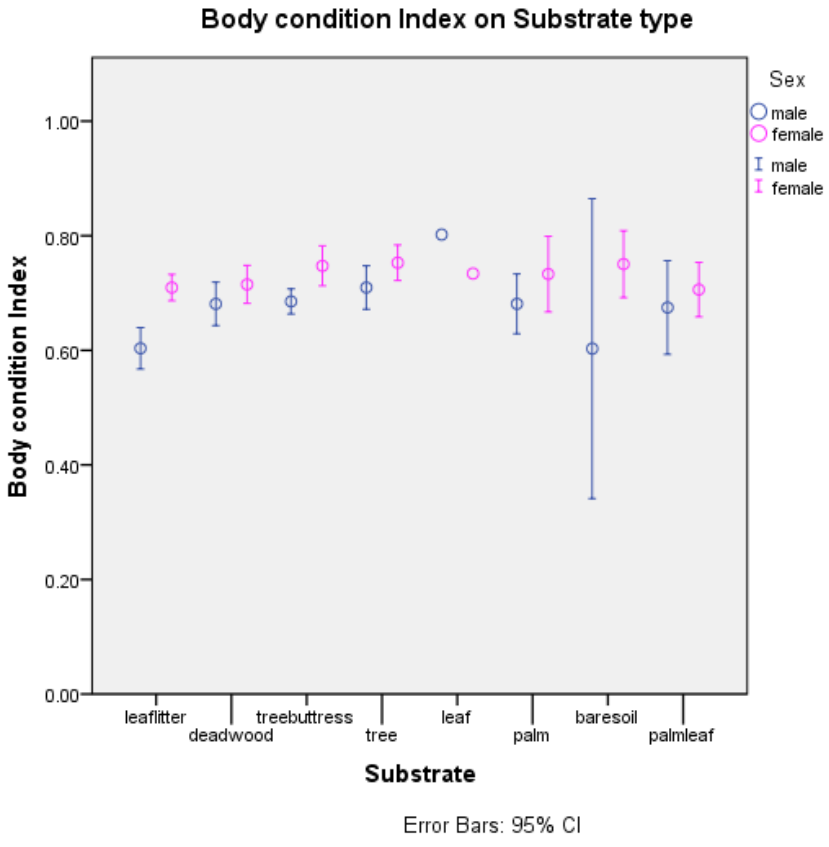


**Figure 3: This figure shows the relationship between type of substrate and mean fitness of males and females.**

**DISCUSSION:**

This study focused on the questions what is the relationship between microhabitat characteristics and individual body condition of *O. pumilio* , and what is the body condition of males and females on different substrates, within the *O. pumilio* population of a Neotropical lowland rainforest in Costa Rica.

Since *O. pumilio* is a species with a resource-based mating system, this study focused mainly on microhabitat traits that are related to reproduction because those are expected to be the limiting factors for individual fitness. The general linear model representing the relationships between the variables found that the presence of calling sites, rainfall, substrate and gender have a significant influence on the body condition of *O. pumilio*.

The presence of a calling site within the territory of *O. pumilio* represents a crucial element for every male. This variable is highly related to the resource based mating system of the studied species and the results of this study underline the fact that fitter individuals have a calling site and can therefore attract more mates. Just as the presence of a calling site is a microhabitat trait connected to the mating system of this species so is substrate use. According to Nowakowsky *et al*. (2013), *O. pumilio* uses different amounts of energy for movements over different substrates. This findings give a possible explanation to the significant correlation between substrate use and body condition that has been found in this study. Furthermore, the gender specific differences concerning substrate use and body condition can be explained with the resource based mating system of O. pumilio. Females move over larger distances, looking for males while the males protect their territory more aggressively and spend time and energy calling for mates (Pröhl and Hödl, 1999, 2002).

Schulte and Lötters (2013) state that rainfall variability and humidity in general are important factors for reproductive success in many anurans, especially because Dendrobatidae depend critically on water since the small pools that function as tadpole rearing sites are exceptionally sensitive to drought. In many anuran species a sexual size dimorphism towards the females has been found (Zhang and Lu, 2013). Even though *O. pumilio* is mostly described as a species with no significant sexual size dimorphism (University of Michigan, 2014), the significant influence of gender on body condition that has been found, indicates than females are often heavier and larger than males.

Suitable tadpole rearing sites have been defined as a major limiting resource for *O. pumilio* (Pröhl and Hödl, 1999, 2002). The results of this study however; did not reveal a significant relationship between the presence of suitable tadpole rearing sites and individual fitness. Pröhl and Berke (2001) did not find that the presence of tadpole rearing sites had influence on the spatial distribution of *O. pumilio*, they also did not find that territorial males defend tadpole rearing sites. Furthermore, Pröhl (2002) states that *O. pumilio* do not only use bromeliad and banana plants as tadpole rearing sites as other studies state (Donnelly, 1989) but they also use water pools in tree buttresses, and other water bodies that are available. Females climbing palm trees, with and without tadpoles on their back, were observed frequently by the researchers during the period of data collection which suggests an additional use of palms as tadpole rearing sites. As previously mentioned, Pröhl and Hödl (1999, 2002)state that tadpole rearing sites represent one of the major limiting resources for *O. pumilio.* Due to the wet climate (mean daily rainfall 9,25mm and mean daily humidity 82% during the data collection period) of the study area however, it can be expected that small water bodies, representing potential tadpole rearing sites, are not a limiting factor in this environment.

The results of this study indicate that body condition is linked to microhabitat use. Nevertheless, more intensive research is needed to reveal the strength of this relationship and to investigate its variations in different habitats and populations.

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