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Trophic Ecology of Two Sympatric Frogs with Contrasting Morphology and Habitat Use in a Subtropical Wetland

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ABSTRACT: Frog diets are influenced by multiple factors, including morphological constraints, habitat use, and seasonal variation in environmental conditions and food availability. This study combined stomach content analysis (SCA), stable isotope analysis (SIA), and estimates of prey availability to investigate the influence of body size and microhabitat use on seasonal variation of the trophic ecology of two sympatric hylids (*Pseudis minuta* and *Scinax squalirostris*). We evaluated two hypotheses: (1) the species with larger body and mouth sizes or broader use of microhabitats will have greater diet breadth, and (2) regardless of differences in morphological traits and microhabitat use, diet breadth of both species will be greater during the warmer of two periods. *Pseudis minuta* exhibited larger body size and mouth width and revealed broader use of microhabitats (mostly within and near major water bodies), whereas *S. squalirostris* had smaller body size and mouth gape and was found exclusively within or near phytotelmata (plant-held water bodies). SCA revealed that *P. minuta* had a more diverse diet than *S. squalirostris*. Only *P. minuta* showed temporal dietary differences, but these findings did not corroborate our prediction of greater diet diversity during the warmer and drier period when prey densities were higher. The two species had distinct carbon and nitrogen stable isotope ratios, indicating assimilation of different resources, except during the colder wetter season when their isotope spaces overlapped partially. We concluded that the two hylids did not use the same food resources on account of their differences in morphology and microhabitat use, and environmental seasonality did not influence their feeding strategies.

Key words: Anuran; Isotopic space; Prey availability; *Pseudis*; *Scinax*; Stable isotope analysis; Stomach content analysis

GIVEN their diverse life histories, feeding strategies, and microhabitats, frogs are useful for studying trophic ecology in wetlands (Hocking and Babbitt 2014). Frogs play different ecological roles throughout their ontogeny, with aquatic tadpoles consuming benthic algae and detritus, with potential to have top-down effects on aquatic primary production (Ranvestel et al. 2004). Most adult frogs use both aquatic and terrestrial habitats and consume invertebrates, and therefore might influence the flow of matter and energy between aquatic and terrestrial compartments of wetland food webs (Huckembeck et al. 2014). Wetlands tend to have high productivity and rapid nutrient cycling that support essential ecosystems services and high biodiversity, including herpetofauna (Zedler 2000). At subtropical and tropical latitudes, wetlands are influenced by rainfall and, to a lesser degree, temperature (Simioni et al. 2017), which directly affect environmental conditions, productivity, and community dynamics. During periods of low rainfall, the area of water bodies and flooded zones is reduced and aquatic organism density increases, which might increase predator–prey encounter rates and foraging success of aquatic consumers (Maltchik et al. 2007).

In addition to the influence of these extrinsic environmental factors, intrinsic factors, such as body size, morphology, and behavior, affect frog feeding (Lima and Magnusson 1998; Maneyro and da Rosa 2004). Morphological constraints on diet can influence microhabitat use and competitive interactions. For example, the presence of well-developed interdigital membranes on the hind feet and eyes positioned dorsolaterally are common features of

adult anurans that forage in flooded areas (Huckembeck et al. 2012). Prior studies inferred high dietary similarity between frogs coexisting in the same habitat (Moser et al. 2017), but few studies have evaluated frog dietary overlap in relation to food availability (e.g., Toft 1980; Huckembeck et al. 2014). Even fewer studies have investigated how seasonal changes in frog trophic ecology are associated with morphology and microhabitat use (but see Gutiérrez-Cárdenas et al. 2016; Moser et al. 2017; Ordoñez-Ifarraguerri et al. 2017).

We investigated the trophic ecology of two hylids sympatric in a subtropical wetland to assess relationships of seasonal environmental variation and morphology with dietary diversity and interspecific overlap. Hylidae is the most diverse anuran family (~982 species) with the widest geographic distribution (distributed on all continents, except the poles; Frost 2017). Most tree frogs are arboreal, but some species are aquatic, semiaquatic, or fossorial (Macale et al. 2008). In the southern Neotropical region, *Pseudis minuta* and *Scinax squalirostris* often inhabit wetlands in sympatry, but with contrasting microhabitat use (Huckembeck et al. 2012). *Pseudis minuta* forages at the water–land interface, whereas *S. squalirostris* is more often associated with shrubs and phytotelmata in plants (Huckembeck et al. 2012; Kittel and Solé 2015). Both species are trophic generalists with diets dominated by insects and spiders (Huckembeck et al. 2014; Kittel and Solé 2015). These species differ morphologically (e.g., body size, mouth width) and anatomically (e.g., *P. minuta* has interdigital membranes that should enhance swimming; Macale et al. 2008; Kittel and Solé 2015).

We studied trophic ecology using stomach content analysis (SCA), stable isotope analysis (SIA), and field estimates of prey availability. During recent decades, SIA

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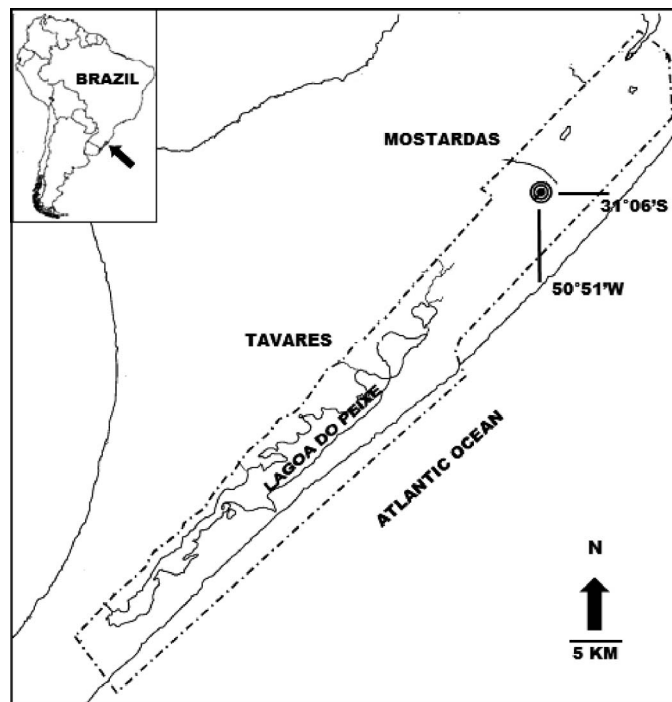


FIG. 1.—Location of the studied wetland (enclosed circle) in Lagoa do Peixe National Park (area enclosed by dotted line) within the extent of the coastal plain habitat of southern Brazil (indicated by black arrow within inset).

has become an important tool for investigating trophic ecology (Layman et al. 2012; Phillips et al. 2014), including studies of anurans (Araújo et al. 2007; Trakimas et al. 2011; Carvalho-Rocha et al. 2017). Ratios of heavier and lighter isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) are often used to estimate the proportional assimilation of food resources into consumer tissues as well as the consumer's vertical trophic position (Peterson and Fry 1987). Stable isotope data have been used to make inferences about trophic ecology, including niche width (Bearhop et al. 2004; Jackson et al. 2011; Cloyd and Eason 2017) and interspecific niche overlap (Swanson et al. 2015). SIA has become more effective when combined with SCA, which allows a more precise and detailed description of a consumer's diet (Winemiller et al. 2011; Condini et al. 2015).

Using two sympatric hylids (*P. minuta* and *S. squalirostris*), we investigated the following questions: (1) are there differences in diet composition and food assimilation between coexisting frog species with contrasting morphology and microhabitat use; and (2) do dietary and assimilation patterns in each species change with seasons? We hypothesized that the species with the larger body and mouth sizes and broader use of microhabitats would have a more diverse diet, and that both species would undergo seasonal shifts in diet and food assimilation. More specifically, we hypothesized that, regardless of interspecific differences in morphology and microhabitat use, diets would be more diverse during the warmer and drier period when prey are more available and frog metabolic rates are higher. Aside from adding to the knowledge about the trophic ecology of Neotropical frogs that are relatively understudied, our

findings yielded insights about potential mechanisms that facilitate amphibian coexistence.

MATERIALS AND METHODS

Study Area

The study was conducted in a wetland (31.0651°S, 50.5121°W; datum WGS84) in the northern portion of the Lagoa do Peixe National Park, an area of ~1.63 ha (Fig. 1). The wetland contains permanent and intermittent water bodies with a maximum depth of 50 cm. Predominant terrestrial vegetation was grasses of Family Poaceae, diverse shrubs, and herbaceous plants (*Eryngium* spp.) that commonly contain phytotelmata. The dominant aquatic macrophytes were *Salvinia herzogii*, *Azolla filiculoides*, *Eichhornia crassipes*, and *Cabomba* sp. The regional climate is classified as subtropical humid. Precipitation, evapotranspiration, and temperature data were obtained from Brazil's National Institute of Meteorology (Inmet 2010). We defined a cold/wet and a warm/dry period on the basis of the mean monthly temperature, rainfall, evapotranspiration, and water surplus (net balance rainfall and evapotranspiration; Fig. 2). The cold/wet period during our study period occurred from April through September, when water temperature ranged from 14.1°C to 19.8°C. The warm/dry period occurred from October through March, when air temperature ranged from 18.3°C to 23.7°C.

Fieldwork and Data Collection

Fieldwork was conducted monthly (1 d/mo) between April 2008 and May 2009 to sample frogs (*P. minuta* and *Scinax squalirostris*) and representative species of invertebrates and primary producers. Specimens of *P. minuta* and *S. squalirostris* were collected by hand and euthanized in an ice bath before being transported to the laboratory for examination and processing. Frogs were collected at dusk, when these anurans were most active, and each monthly survey involved 2.5 h of searching. Aquatic macroinvertebrates and macrophytes (*Salvinia herzogii* and *Eichhornia crassipes*) were sampled using a drop sampler, which was a bottomless plastic bucket covering an area of 0.045 m². Each month, 18 samples were collected (three samples in three stands per macrophyte species). After collection, aquatic vegetation samples were washed in tap water over a 500-mm mesh sieve that retained associated macroinvertebrates. In addition to aquatic macrophytes, samples of periphyton, particulate organic matter (POM), and leaves from terrestrial plants were collected for estimation of isotopic composition of basal resources. Suspended samples of POM were obtained by filtering water through a precombusted (450°C, 4 h) Whatman glass fiber filter (porosity = 1.2 μm) with the aid of a manual vacuum pump. Periphyton was collected by carefully scraping a thin upper layer of flocculent or consolidated sediment from substrates. Samples of terrestrial vegetation (*Kyllinga vaginata* and *Sporobolus virginicus*) were collected by clipping with scissors, and invertebrates associated with terrestrial vegetation (ants, hemipterans, spiders) were sampled using pitfall traps consisting of 500-mL cans buried in the soil and containing 100 mL of water ($n = 10/\text{mo}$). A light trap also was used to collect winged insects (e.g., beetles, mosquitoes, moths). The pitfall traps and light traps were haphazardly distributed in

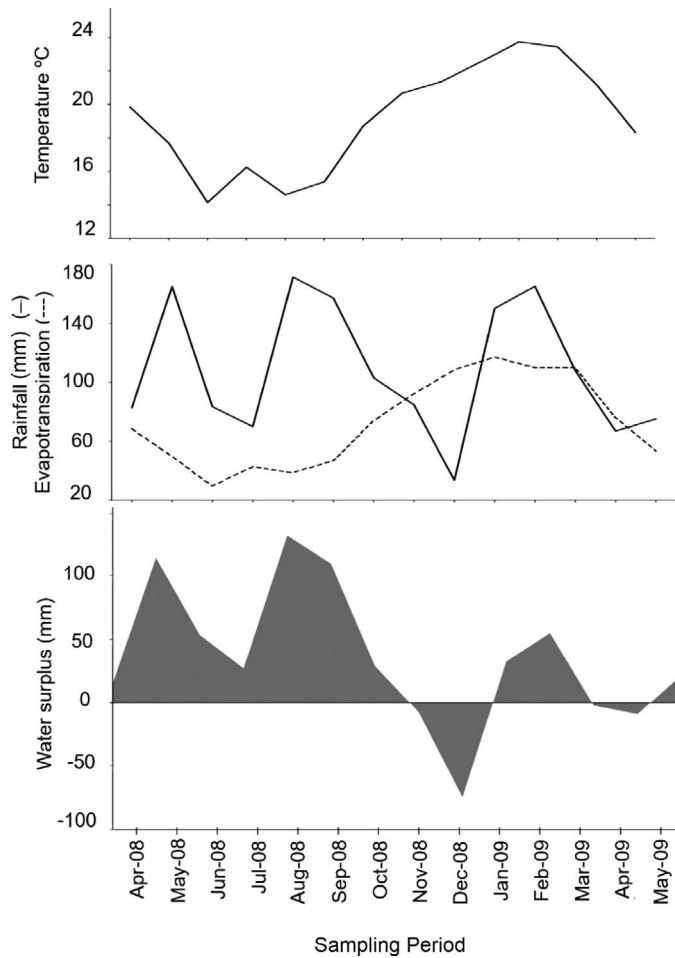


FIG. 2.—Monthly variation of temperature (°C), rainfall (mm³), and water surplus (mm) during the study period (April 2008–May 2009) at Lagoa do Peixe National Park, Brazil.

the grassland near the edge of the main water body. All samples (primary producers, invertebrates, and anurans) were stored on ice until transported to the lab where they were kept frozen until processing for SIA and SCA.

Snout–vent length (SVL, ± 1 mm) and mandibular width (MW, ± 0.1 mm) were measured for each frog specimen, and stomachs were removed through an incision in the abdomen. Food items recovered from stomachs were preserved in 70% ethanol and later identified at the lowest feasible taxonomic level given available identification keys and degree of digestion. Partially digested prey (e.g., fragments of appendages, exoskeleton, or muscle tissue) were classified as animal remains. Following Huckembeck et al. (2014), we estimated the numerical abundance of each prey category in each stomach and its area when spread in a Petri dish (with thickness ~ 1 mm and eliminating empty spaces) with the bottom marked in a grid with 1-mm² squares.

Each frog specimen was dissected to obtain samples of muscle tissue (< 5 g) from the posterior limb. Because of their small size (< 10 mm), invertebrates could not be dissected to obtain sufficient samples of pure muscle tissue; therefore, invertebrates were processed whole for SIA. Macrophyte leaf samples were rinsed with distilled water

and placed in a sterile Petri dish, then dried in an oven at 60°C (for 48 h) before being ground into a fine powder with a mortar and pestle, and placed in Eppendorf tubes for storage. Powdered material was weighed (1 to 3 mg), pressed into ultrapure tin capsules (Costech Analytical Technologies), and sent to the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, for analysis of carbon and nitrogen stable isotope ratios. We compared our samples against the carbon and nitrogen standards of marine sedimentary limestone and atmospheric nitrogen, respectively (Peterson and Fry 1987). On the basis of the SD of internal standard replicate samples, analytical precision was 0.14‰ and 0.13‰ for carbon and nitrogen stable isotope ratios, respectively.

Analysis of Microhabitat Use and Morphology

To evaluate habitat use by the two frog species, we followed the characterization of microhabitats performed by Huckembeck et al. (2012) that included the following parameters: vegetation spatial coverage (% grasses, shrubs, phytotelmata plants, and aquatic plants); average height of the vegetation (cm); average water depth (cm); substrate type (dry, wet, flooded, or underwater); and average distance from a water body (cm). Relative frequencies of occurrence of both species in each microhabitat were evaluated with chi-square tests (Zar 1994). Differences in SVL and MW between species, and warmer vs. colder periods, were assessed by the Mann–Whitney *U*-test. Data were examined for normality (Kolmogorov–Smirnov tests), homoscedasticity (*F*-tests), and independence (autocorrelations of residuals; Hammer 2017).

Analysis of Diet and Prey Availability

Food items encountered during SCA were quantified by the prey-specific index of relative importance (%PSIRI) adapted from Brown et al. (2012), according to the formula:

$$\%PSIRI = \%FO(\%NP_i + \%AP_i)/2,$$

where %FO is the relative frequency of occurrence of item *i* based on all stomachs; %NP_{*i*} is the relative abundance of prey *i* based on its numerical abundance; and %AP_{*i*} is the relative abundance of prey *i* based on its estimated area. Relative abundance values were calculated as the number or area of item *i* divided by the number of stomachs containing item *i*.

Frog diet diversity was calculated by Shannon's index, $H = -\sum p_i(\log p_i)$, where p_i is the proportion (by area) of each prey item found in the diet. Potential differences in *H* values within species, between seasons, and between species were evaluated by the diversity *t*-test (Hammer 2017). We also calculated interspecific dietary overlap on the basis of Pianka's index:

$$O_{jk} = O_{kj} = \left(\sum [p_{ij} \times p_{ik}] \right) / \sqrt{\left(\sum [p_{ij}^2 \times p_{ik}^2] \right)},$$

where p_{ij} and p_{ik} are the proportions (by area) of food item *i* consumed by species *j* and *k*, respectively. Differences in prey availability (based on prey abundance and diversity) were evaluated by the student's *t*-test and diversity *t*-test (Hammer 2017).

Analysis of Stable Isotope Ratios

To verify the assumption that the isotopic composition of consumer muscle tissue was derived from food assimilated during periods when consumers and resources were collected together (Phillips et al. 2014), we only analyzed isotopic composition of frogs sampled during the final 3 mo of both cold/wet and warm/dry periods. To evaluate patterns of isotopic variation within and between species and periods, we constructed biplots of $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ for frogs, representative prey, and dominant aquatic and terrestrial basal sources. The statistical significance of differences between average values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was evaluated with the Mann–Whitney U -test. The relative importance of material assimilated by frogs from various prey and basal food sources and relative vertical trophic positions of the two frog species in the food web were indicated by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. Following Phillips et al. (2014), food sources with similar isotopic values were pooled together to achieve better resolution in the isotopic mixing models used to estimate assimilation of material from sources. Therefore, when the variability (SD) around the average $\delta^{13}\text{C}$ values of a prey group (e.g., Aranea) was high ($\text{SD} > 2.85$), the group was divided using a nonhierarchical cluster-analysis K -means (Hammer 2017). We attributed this variability to the diversity of species that compose each group. This procedure resulted in most prey taxa being divided into two groups, one relatively depleted (D) and one enriched (E) in ^{13}C (e.g., Araneae-D vs. Araneae-E; Table 1). We used Mann–Whitney U -tests to assess the statistical significance of differences in prey isotopic values between periods. On the basis of isotopic similarity, basal production sources aggregated into two groups: aquatic producers (aquatic and emergent macrophytes, POM, and periphyton) and terrestrial producers (plants with C_4 photosynthesis). Fully terrestrial C_3 plants were uncommon in the riparian zone of the wetland and, therefore, were not included in the sampling.

Relative contributions of food sources to the biomass of the two anuran species, expressed as 95% credibility intervals, were estimated using a Bayesian isotopic mixing model as implemented in the Stable Isotope Analysis in R program (SIAR; Parnell et al. 2010). On the basis of the $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ biplots in which we visualized the spatial trends in the isotopic composition of frogs and their stomach contents, we considered the following resources for the mixing model: Araneae depleted, Odonata, Coleoptera depleted, and Coleoptera enriched for *P. minuta*; Araneae depleted, Araneae enriched, and Coleoptera enriched for *Scinax squalirostris*. Mixing models were fit using the Markov chain Monte Carlo method, which generates simulations of the resource contribution to the anurans. These simulations were generated using a Dirichlet prior distribution (Parnell et al. 2010). Each model was run on the basis of 500,000 iterations, discarding the first 50,000 because they are considered noninformative for guiding simulations. We used the mean values (± 1 SD) for trophic discrimination factor as determined for postmetamorphic anurans ($\delta^{13}\text{C}$: $1.13 \pm 0.50\text{‰}$; $\delta^{15}\text{N}$: $2.56 \pm 0.50\text{‰}$; Cloyd et al. 2015; Appendix).

The isotopic space (i.e., areas occupied in C-N isotopic space; Newsome et al. 2007) of each frog species was plotted as a standardized ellipse area corrected for small samples

(SEAc) using the program Stable Isotope Bayesian Ellipses in R (SIBER; Jackson et al. 2011). SEAc is unaffected by bias associated with sample size, allowing comparison among groups with distinct sample sizes (Jackson et al. 2011). Overlap between isotopic spaces was calculated between periods and species and reported as a percentage of each SEAc (asymmetrical overlap; Albernaz et al. 2016).

RESULTS

Morphological Traits and Microhabitat Use

Forty-three *P. minuta* specimens (20 from the warm/dry and 23 from the cold/wet period) and 21 *S. squalirostris* specimens (11 from the warm/dry and 10 from the cold/wet period) were collected in the wetland. The values for body size and mouth width were both greater in *P. minuta* ($\text{SVL} = 29.6 \pm 4.9$ mm, $\text{MW} = 10.35 \pm 1.60$ mm) than in *S. squalirostris* ($\text{SVL} = 22.2 \pm 2.1$ mm, $\text{MW} = 6.40 \pm 0.90$ mm; SVL : $U = 36$, $z = -5.33$, $P < 0.001$; MW : $U = 7.5$, $z = -3.79$, $P < 0.001$). Values for SVL and MW in *P. minuta* were similar between periods (SVL : $U = 177.50$, $z = -0.83$, $P > 0.40$; MW : $U = 42.50$, $z = -0.32$, $P > 0.74$). The mean SVL of *S. squalirostris* was greater during the warm/dry period (23.0 ± 0.7 mm) than the cold/wet period (20.2 ± 3.1 mm; $U = 72$, $z = -2.44$, $P < 0.01$).

We observed interspecific differences in microhabitat use. Most specimens of *S. squalirostris* were captured on or near plants containing phytotelmata (13 occurrences vs. 3.5 expected at random; $\chi^2 = 25.78$, $\text{df} = 4$, $P < 0.0001$), with only one specimen captured from aquatic macrophytes (1 occurrence vs. 3.5 expected; $\chi^2 = 1.78$, $\text{df} = 4$, $P < 0.0001$). In contrast, all *P. minuta* were captured from aquatic microhabitats, including floating macrophytes and wetted margins of the main water body, and none was observed on plants bearing phytotelmata.

SCA and Prey Availability

Regardless of the study period, the diversity of diet differed between the two frog species (cold/wet: $t = 18.98$, $\text{df} = 119.61$, $P < 0.001$; warm/dry: $t = 13.05$, $\text{df} = 58.03$, $P < 0.001$; Table 1), with *P. minuta* having greater diet diversity (cold/wet: $H = 2.08$; warm/dry: $H = 1.89$) than *S. squalirostris* (cold/wet: $H = 0.26$; warm/dry: $H = 0.34$). Interspecific dietary overlap was low in both periods (cold/wet: $O_{ij} = 0.12$; warm/dry: $O_{ij} = 0.17$).

For both frog species, diet diversity did not differ between study periods (*P. minuta*: $t = -1.59$, $\text{df} = 87.79$, $P > 0.11$; *S. squalirostris*: $t = -0.31$, $\text{df} = 21.19$, $P > 0.75$). Proportional consumption of prey categories varied between periods for *P. minuta*: Araneae (%PSIRI = 14.96), Coleoptera (%PSIRI = 9.65), and Hymenoptera (%PSIRI = 8.14) were predominant in the diet during the cold/wet season, whereas Odonata (%PSIRI = 17.19), Hemiptera (%PSIRI = 16.20), and Coleoptera (%PSIRI = 10.88) were more important in the diet during the warm/dry period. In contrast, diet composition of *S. squalirostris* revealed relatively little temporal variation. Hemiptera was the more important prey in the diet of *S. squalirostris* during both seasons (%PSIRI, cold/wet = 22.36; warm/dry = 18.80; Table 1).

Prey abundance in the wetland was greater during the warm/dry period ($t = -1.92$, $P < 0.05$), but prey diversity

TABLE 1.—Relative importance of food items, as measured by frequency of occurrence (FO), numerical percentage (%NP), and area percentage (%AP), and prey-specific index of relative importance (%PSIRI) in diets of *Pseudis minuta* and *Scinax squalirostris* during cold and warm periods at the wetland of Lagoa do Peixe National Park. Bold font indicates highest %PSIRI values for each period.

Food items	<i>Pseudis minuta</i>							
	Cold/wet (n = 23)				Warm/dry (n = 20)			
	FO	%NP	%AP	%PSIRI	FO	%NP	%AP	%PSIRI
Araneae	0.30	8.38	8.24	14.96	0.15	6.94	6.21	5.40
Cladocera					0.05	6.94	0.53	1.02
Coleoptera	0.22	6.84	8.16	9.65	0.20	8.67	11.17	10.88
Diptera	0.09	12.21	6.69	4.86	0.10	10.40	4.70	4.14
Gastropoda	0.04	4.89	0.69	0.72				
Hemiptera	0.13	4.89	3.07	3.07	0.45	10.79	2.34	16.20
Hydracarina	0.09	4.89	0.17	1.30	0.10	6.94	0.18	1.95
Hymenoptera	0.17	12.21	3.60	8.14	0.20	6.94	2.44	5.14
Isopoda	0.04	4.89	0.52	0.70	0.10	13.87	4.70	5.09
Lepidoptera	0.04	4.89	0.35	0.67				
Odonata	0.04	4.89	4.34	1.19	0.20	6.94	24.43	17.19
Orthoptera	0.04	4.89	26.06	3.98	0.05	6.94	26.60	4.60
Ostracoda	0.13	4.89	0.17	1.95				
Trichoptera	0.04	4.89	8.69	1.75				
Others (unidentified)								
Animal remains	0.74	6.61	11.69	40.02	0.45	7.71	4.89	15.53
Plant remains	0.13	4.89	8.86	5.30	0.25	6.94	11.81	12.85
Eggs	0.04	4.89	8.69	1.75				
Food items	<i>Scinax squalirostris</i>							
	Cold/wet (n = 10)				Warm/dry (n = 11)			
	FO	%NP	%AP	%PSIRI	FO	%NP	%AP	%PSIRI
Diptera	0.25	8.21	0.48	2.54				
Hemiptera	0.17	24.63	71.93	18.80	0.20	19.05	71.93	22.36
Hydracarina	0.17	12.31	0.72	2.54	0.20	19.05	2.88	5.39
Hymenoptera	0.17	12.31	0.72	2.54	0.40	28.57	2.16	15.10
Isopoda	0.92	2.24	0.39	2.82				
Others (unidentified)								
Animal remains	0.92	40.30	25.76	70.76	0.80	33.33	24.81	57.16

was higher during the cold/wet period (cold/wet, $H = 2.79$; warm/dry, $H = 2.59$; $t = 4.87$, $P < 0.0001$; Fig. 3).

Isotopic Variability and Food Assimilation

Relative positions of the two frog species and their prey in the $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ biplot indicated interspecific differences in assimilation of carbon and nitrogen from prey and basal sources (Fig. 4). Mean $\delta^{13}\text{C}$ values of *P. minuta* ranged from -26.64 to -22.28‰ during the cold/wet period, and from -25.08 to -21.79‰ during the warm/dry period (Fig. 4A). These values were similar between periods ($U = 66$, $z = -0.68$, $P > 0.49$; Table 2), and largely reflected isotopic values of important prey that varied between seasons (Fig. 4A). Mean $\delta^{15}\text{N}$ values of *P. minuta* differed between study periods ($U = 36$, $z = -2.21$, $P < 0.02$; Fig. 4A), and ranged from 5.66 to 7.48‰ during the cold/wet period and 4.25 to 7.13‰ during the warm/dry period.

Mean $\delta^{13}\text{C}$ values of *S. squalirostris* differed between periods ($U = 9$, $z = -2.32$, $P < 0.01$; Table 2), with values ranging from -23.53 to -19.35‰ during the cold/wet period and from -20.84 to -17.28‰ during the warm/dry period (Fig. 4B). Mean $\delta^{15}\text{N}$ values of *S. squalirostris* differed between periods ($U = 13$, $z = -2.09$, $P < 0.03$; Table 2), with values ranging from 6.16 to 7.51‰ during the cold/wet period and from 3.08 to 7.24‰ during the warm/dry period (Fig. 4B).

Carbon isotope ratios of aquatic producers during both periods (cold/wet = $-27.26 \pm 2.49\text{‰}$; warm/dry = $-29.68 \pm 1.91\text{‰}$) were lower than those of terrestrial producers (cold/

wet = $-13.91 \pm 2.40\text{‰}$; warm/dry = $-10.52 \pm 2.40\text{‰}$; Fig. 4). Average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were similar between periods for any insect prey group (Table 2).

Proportional assimilation of food resources by frogs was estimated using isotopic mixing models. *Pseudis minuta* had a similar pattern of prey assimilation during the two periods (Fig. 5). Odonata-E was estimated to be the prey assimilated in greatest proportions by *P. minuta* during the cold/wet period (95% Bayesian credibility interval = 12–61%), followed by Coleoptera-E (30–59%) and Araneae-D (0–42%). This species assimilated prey in similar proportions during the warm/dry period. In contrast, *S. squalirostris*

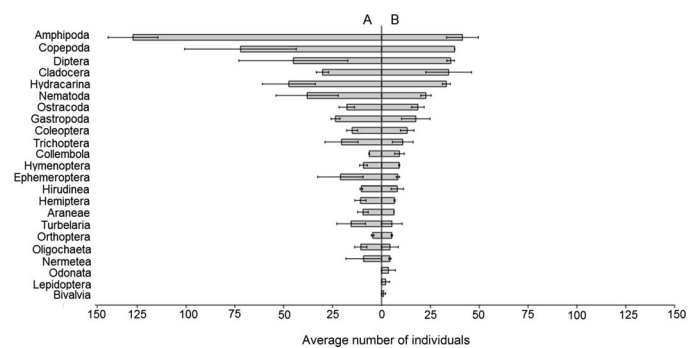


FIG. 3.—Mean (± 1 SD) number of individual invertebrate taxa obtained from surveys conducted during cold/wet (A) and warm/dry (B) periods at the wetland in Lagoa do Peixe National Park, Brazil. Data were square-root transformed.

TABLE 2.—Number of samples (n), mean (± 1 SD) values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and P -values from Mann–Whitney U -tests comparing mean isotopic values of frogs and their prey in the wetland of Lagoa do Peixe National Park.

Group	$\delta^{13}\text{C}$					$\delta^{15}\text{N}$				
	Cold/wet		Warm/dry		<i>P</i> -values	Cold/wet		Warm/dry		<i>P</i> -values
	<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD		<i>n</i>	Mean $\pm$ SD	<i>n</i>	Mean $\pm$ SD	
Amphibians										
<i>Pseudis minuta</i>	8	−23.74 $\pm$ 1.31	20	−23.22 $\pm$ 0.96	0.49	8	6.46 $\pm$ 0.72	20	5.67 $\pm$ 0.79	0.03
<i>Scinax squaleirostris</i>	10	−21.64 $\pm$ 1.74	7	−19.25 $\pm$ 1.15	0.02	10	6.79 $\pm$ 0.49	7	5.58 $\pm$ 1.36	0.04
Prey										
Araneae D	4	−27.39 $\pm$ 1.44	3	−27.55 $\pm$ 1.98	0.86	4	5.83 $\pm$ 0.84	3	4.7 $\pm$ 0.53	0.15
Araneae E	5	−17.46 $\pm$ 2.79	11	−19.46 $\pm$ 1.77	0.26	5	7.36 $\pm$ 2.03	11	6.28 $\pm$ 1.2	0.28
Coleoptera D	2	−29.87 $\pm$ 2.05	6	−25.55 $\pm$ 2.43	0.15	2	1.95 $\pm$ 2.48	6	5.59 $\pm$ 2.48	0.39
Coleoptera E	3	−21.13 $\pm$ 2.54	3	−15.42 $\pm$ 2.42		3	4.97 $\pm$ 1.53	3	7.40 $\pm$ 1.34	
Hemiptera D	4	−27.49 $\pm$ 0.8	2	−27.76 $\pm$ 1.14	0.15	4	3.85 $\pm$ 1.16	2	3.30 $\pm$ 1.51	0.39
Hemiptera E	5	−21.64 $\pm$ 0.88	2	−22.00 $\pm$ 1.22		5	4.61 $\pm$ 0.26	2	4.40 $\pm$ 0.42	
Odonata D	3	−28.39 $\pm$ 1.49	5	−23.54 $\pm$ 2.02	0.15	3	4.50 $\pm$ 0.05	5	4.21 $\pm$ 1.46	0.39
Odonata E		−17.52 $\pm$ 2.21								
Orthoptera D	2	−14.50 $\pm$ 1.53	6	−	0.15	2	5.34 $\pm$ 1.15	6	5.03 $\pm$ 1.75	0.39
Orthoptera E	6	—								
Producers										
Aquatic	13	27.26 $\pm$ 2.49	5	−29.68 $\pm$ 1.91	0.09	13	0.97 $\pm$ 0.83	5	−0.55 $\pm$ 1.64	0.1
Terrestrial	2	−13.91 $\pm$ 2.4	2	−10.52 $\pm$ 2.4		2	0.03 $\pm$ 0.23	2	−0.30 $\pm$ 0.23	

assimilated prey in different proportions during the two periods. During the cold/wet period, Coleoptera-E was assimilated in greatest proportions (37–86%), followed by Araneae-D (11–47%) and Araneae-E (0–22%). Coleoptera-E, Araneae-D, and Araneae-E made similar contributions to *S. squaleirostris* biomass during the warm/dry period (9–58%, 10–56%, and 1–57%, respectively; Fig. 5).

Both frog species showed shifts in the relative position and size of their isotopic spaces between survey periods (Fig. 6). *Pseudis minuta* had a larger isotopic space during the cold/wet period ($\text{SEAc} = 3.41$ for cold/wet, 2.45 for warm/dry), and ellipses of the two periods overlapped in isotopic space (Fig. 6). In contrast, the isotopic space occupied by *S. squaleirostris* was greater during the warm/dry period ($\text{SEAc} = 5.88$ for warm/dry, 2.45 for cold/wet). The two periods had little overlap within isotopic space, with separation mostly

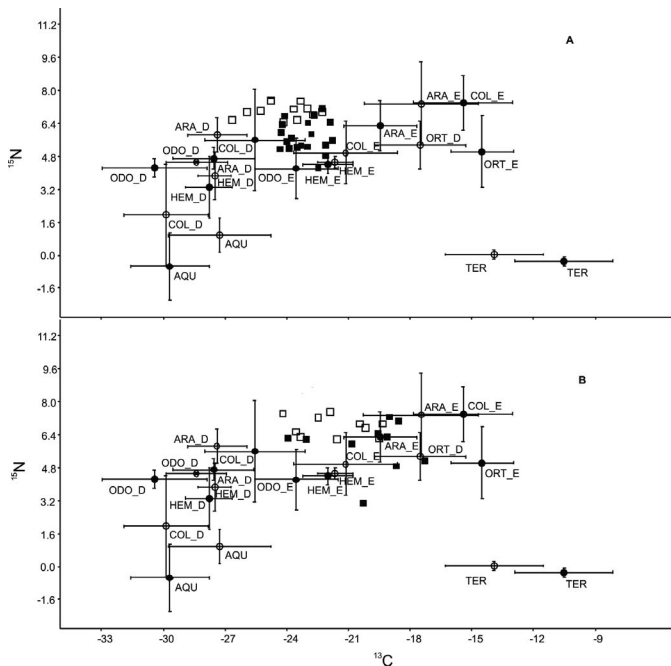


FIG. 4.—Mean values (± 1 SD) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in sympatric frogs (squares: *Pseudis minuta* [A] and *Scinax squaleirostris* [B]) and their potential prey (circles) during the cold period (open symbols) and the warm period (filled symbols) at the wetland in Lagoa do Peixe National Park, Brazil. ARA-D = Araneae-depleted, ARA-E = Araneae-enriched, COL-D = Coleoptera-depleted, COL-E = Coleoptera-enriched, HEM-D = Hemiptera-depleted, HEM-E = Hemiptera-enriched, ODO-D = Odonata-depleted, ODO-E = Odonata-enriched, ORT-D = Orthoptera-depleted, ORT-E = Orthoptera-enriched, AQU = aquatic producers, and TER = terrestrial producers.

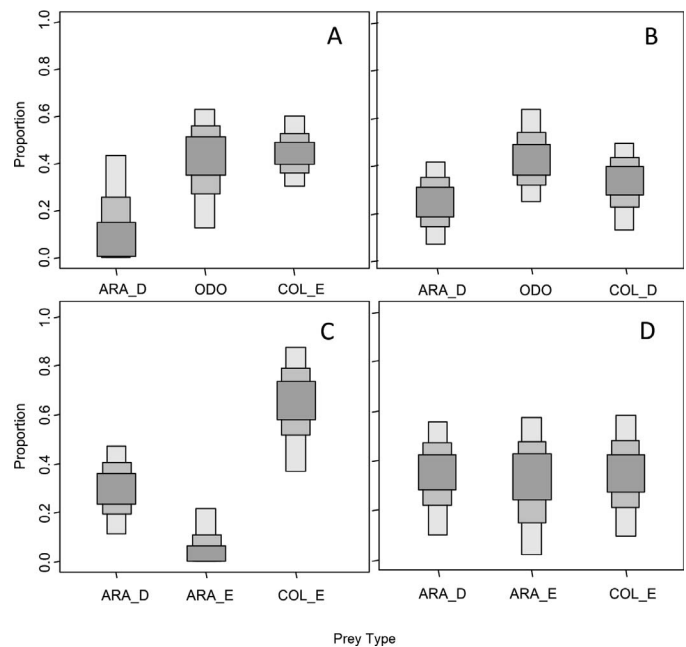


FIG. 5.—Relative contribution of potential prey to biomass of *Pseudis minuta* (A = cold/wet period; B = warm/dry period) and *Scinax squaleirostris* (C = cold/wet period; D = warm/dry period) at the wetland in Lagoa do Peixe National Park, Brazil. Bayesian credible intervals of the feasible contributions of each prey category: 50% (dark gray), 75% (medium gray), and 95% (pale gray). ARA-D = Araneae-depleted, ARA-E = Araneae-enriched, COL-D = Coleoptera-depleted, COL-E = Coleoptera-enriched, ODO-D = Odonata-depleted.

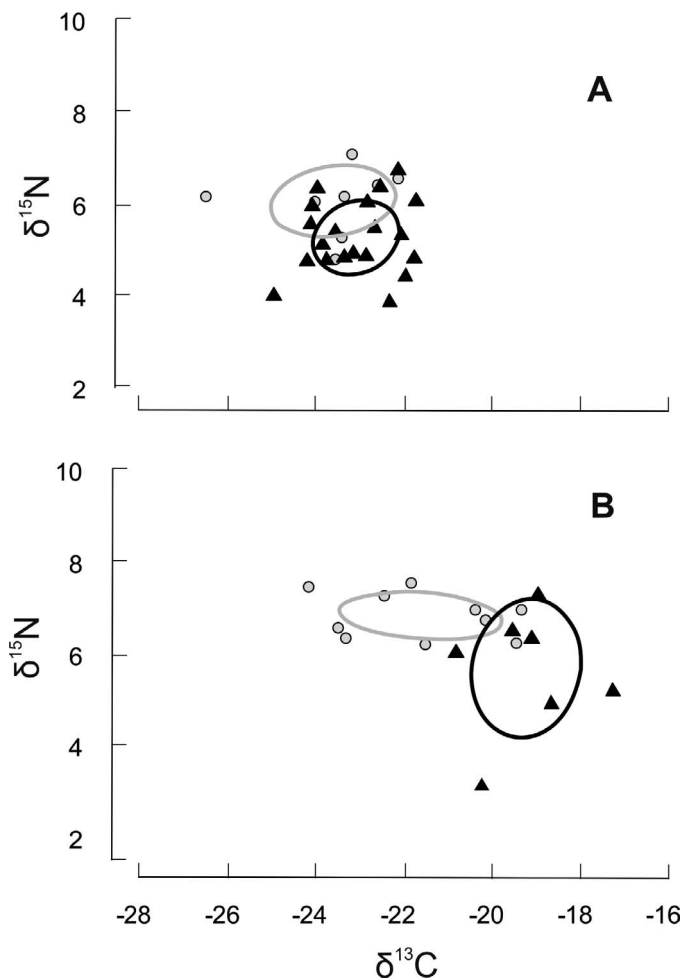


FIG. 6.—Isotopic niche space of *Pseudis minuta* (A) and *Scinax squalirostris* (B) during the cold/wet period (gray circles) and the warm/dry period (black triangles) at the wetland in Lagoa do Peixe National Park, Brazil.

along the $\delta^{13}\text{C}$ axis, with the cold/wet-period having lower values (Fig. 6). Overlap between isotopic ellipses of the two periods was much lower for *S. squalirostris* (0.008) than for *P. minuta* (0.86). This finding indicated that the resources assimilated by *P. minuta* had similar isotopic composition during both periods, whereas the resources assimilated by *S. squalirostris* varied between periods (Fig. 6). There was interspecific overlap in isotopic spaces of the two anurans during only the cold/wet period (asymmetrical overlap = 17.64% for *P. minuta*, 20.68% for *S. squalirostris*).

DISCUSSION

Our findings support the hypothesis that among sympatric frog species, those that have a larger body and mouth gape and that use a greater array of microhabitats should have a broader trophic niche. Prior studies have suggested that anurans representing several families exhibit a correlation between body size and diet composition, with larger individuals consuming larger prey and having greater volumes of stomach contents (Kittel and Solé 2015). Our findings are consistent with patterns observed for hylid frogs

inhabiting a permanent pond in southern Brazil (Miranda et al. 2006). *Pseudis minuta* is larger, occurred in a wider range of microhabitats, and, as predicted, had a more diverse diet than *S. squalirostris*. *Pseudis minuta* often was captured from aquatic microhabitats, including floating macrophytes, but sometimes could be found in terrestrial microhabitats of riparian areas (Huckembeck et al. 2012). In our study system, *P. minuta* was not associated with herbaceous plants bearing phytotelmata. *Scinax squalirostris* had a more restricted distribution and was usually captured from plants growing along the wetland margin, either within, or in proximity to, phytotelmata. Similar to our findings, an investigation of hylids inhabiting wetlands in Colombia found interspecific differences in microhabitat use and diet that were associated with variation in body size (Muñoz-Guerrero et al. 2007). Other studies have concluded high niche overlap among frogs sharing the same habitat in tropical and subtropical forests (Toft 1980; Wu et al. 2005). In addition to being larger, *Pseudis* has traits that are adaptive in aquatic habitats, such as interdigital membranes on the hind feet and the absence of digital pads that are possessed by most hylids (Huckembeck et al. 2014). *Scinax* lacks interdigital membranes on the hind feet and possesses digital pads typical of tree frogs.

Contrary to our prediction, the isotopic space occupied by the smaller species, *S. squalirostris*, was larger than that occupied by the larger species. Large isotopic variation among prey inhabiting phytotelmata could explain this pattern. Phytotelmata in subtropical wetlands harbor diverse assemblages of terrestrial and semiaquatic invertebrates (Campos 2010). Given that invertebrates can exhibit substantial variation in their isotopic values over small spatial scales in systems with high environmental heterogeneity (e.g., Willson et al. 2010), we suggest that large levels of isotopic variation might exist among invertebrates from phytotelmata.

Results from SCA did not provide strong evidence corroborating our hypothesis that diets of both frogs would be more diverse during the warm/dry period. We also disregard the influence of body size on the diet between the periods. *Scinax squalirostris* was greater in the warm/dry period, but we did not consider this minor difference (<3 mm) biologically significant. Diets of both species were fairly consistent during the two survey periods. The relative importance of only a few prey categories varied seasonally within the diet of *P. minuta*, and Hemiptera were dominant in the diet of *S. squalirostris* during both periods. In contrast, the diet of a hylid (*Boana pulchella*) inhabiting ponds in temperate southern Uruguay varied among microhabitats and seasons (Maneyro and da Rosa 2004). Our dietary results should be interpreted with caution, because sample sizes for SCA were small, and some of the material recovered from stomachs was in an advanced state of digestion, especially during the cold/wet period.

Stable isotope analysis did not show differences in proportions of prey assimilated by *P. minuta* during the two periods. In contrast, *S. squalirostris* revealed between-period differences in the proportional assimilation of prey categories. The isotope space occupied by *S. squalirostris* during the warm/dry period was more than double the size of that occupied during the cold/wet period, with higher values of $\delta^{13}\text{C}$ during the warmer period. We speculate that this

difference in carbon isotopic ratios might reflect assimilation of material from herbivorous insects that feed on plants using the C_4 photosynthetic pathway (e.g., terrestrial grasses) that typically are enriched in ^{13}C . During the warm/dry period, these insects probably become more available to frogs as wetted marginal areas shrink. In Panamanian highland streams, frogs showed a variation in their isotopic values, indicating that sources of prey vary in riparian areas (Whiles et al. 2006). To evaluate our hypothesis, we suggest that future studies at this site examine the isotopic variation of more invertebrate taxa, as well as C_4 grasses.

Our prey availability survey revealed higher prey densities during the warm/dry period. Seasonal variation in invertebrate communities has been reported in other studies of subtropical wetlands, with invertebrate densities increasing and species richness decreasing as water levels recede (Moraes et al. 2014). Both adult and larval communities of anurans in tropical latitudes have been found to respond to changes in resource availability (Toft 1980; Whiles et al. 2006; Altig et al. 2007). However, the two hylids in the present study did not exhibit seasonal dietary variation. Both hylids have been described as opportunistic, generalist predators (Huckembeck et al. 2014; Kittel and Solé 2015). Changes in diets of both frog species seemed to be driven largely by changes in the relative proportions of prey types in their respective microhabitats. Given small sample sizes, however, this inference is tentative.

Isotopic spaces occupied by *P. minuta* and *S. squalirostris* overlapped broadly during the cold/wet period when higher rainfall produced a hydrologic pulse that connected aquatic habitats in the wetland (Garcia et al. 2017). Hydrologic connectivity promotes entry of terrestrial invertebrates, vegetation, and riparian detritus into aquatic habitats (Rezende and Mazzoni 2005). During floodplain inundation, new growth of floating aquatic vegetation and emergent riparian plants support spiders, ants, and other terrestrial invertebrates (Campos 2010). Terrestrial arthropods are probably more vulnerable to predation by hylid frogs under these conditions, resulting in stronger linkages between terrestrial and aquatic food-web compartments. Moreover, higher dispersal of invertebrate prey between phytotelmata housed in emergent plants and floating aquatic vegetation (Zilli and Marchese 2011) could have contributed to higher overlap between isotopic spaces of the two frog species during the cold/wet period.

Aquatic ecosystems in subtropical and tropical latitudes are strongly influenced by unimodal or bimodal annual rainfall (Winemiller 1990; Bunn and Arthington 2002), and amphibian ecology should show corresponding temporal responses (Babbitt 2005; Maltchik et al. 2008). Our study revealed an appreciable influence of seasonality on the trophic ecology in only one of the two hylid species. Both of these anurans are trophic generalists that feed opportunistically on insects, but only *P. minuta* underwent a major seasonal diet shift. Microhabitat use and morphological constraints appear to be important factors that influence the trophic ecology of these species. Nonetheless, both species link aquatic and terrestrial compartments of the wetland food web, and therefore can serve as sensitive indicators of environmental impacts to the system.

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APPENDIX.—Percent contributions of prey taxa (Bayesian credible interval of 95%) to *Pseudis minuta* and *Scinax squalirostris* muscle tissue during cold and warm periods in the wetland of Lagoa do Peixe National Park based on calculations using different values for the trophic discrimination factor: (1) Cloyed et al. (2015), (2) Post (2002), and (3) Vanderklift and Ponsard (2003). ARA-D = Araneae depleted, ARA-E = Araneae enriched, COL-D = Coleoptera depleted, COL-E = Coleoptera enriched, ODO-D = Odonata depleted, ODO-E = Odonata enriched, PSE = *Pseudis minuta*, SCI = *Scinax squalirostris*, TDF = trophic discrimination factor.

TDF	Cold/wet					
	PSE			SCI		
	1	2	3	1	2	3
ARA-D	0–42	0–45	0–40	11–47	3–43	4–40
ARA-E				0–22	0–35	0–28
COL-E	30–59	35–65	35–67	37–86	26–94	37–92
ODO-D	12–61	3–53	7–56			
ODO-E						
TDF	Warm/dry					
	PSE			SCI		
	1	2	3	1	2	3
ARA-D	7–42	13–49	1–37	10–56	6–52	6–52
ARA-E				1–57	3–61	2–59
COL-D	13–49	4–38	13–51			
COL-E				9–58	9–58	12–62
ODO-E	25–63	30–64	28–68			