



## Scientific Note

### Substrate color selection by tadpoles of *Physalaemus gracilis* (Boulenger, 1883) (Anura, Leiuperidae)

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**Abstract.** In the present study, we performed laboratorial experiments to evaluate the substrate selection in tadpoles of *Physalaemus gracilis*. Our findings reveals the ability of tadpoles to actively select specific portions of the microhabitat based on substrate color, using predominantly darker ones.

**Key words:** anuran, behavior, microhabitat selection

**Resumo.** Seleção quanto à cor do substrato em girinos de *Physalaemus gracilis* (Boulenger, 1883). Neste estudo, realizamos experimentos para avaliar a seleção de substrato em girinos de *Physalaemus gracilis*. Nossos resultados revelam a capacidade dos girinos selecionar ativamente porções específicas do micro-habitat com base na cor do substrato, utilizando predominantemente as de coloração mais escuras.

**Palavras chave:** anuros, comportamento, seleção de micro-habitat

The selection of a habitat by individuals of a species is one of the central themes in ecology (Huey 1991). In general, the main factors determining the presence of organisms in a given portion of the habitat are food availability, tolerance to local physico-chemical conditions and predation intensity (Connell 1975, Rittschof *et al.* 1995, Pereira *et al.* 2009). These trade-offs in habitat selection have implications, including the structuring of ecological communities (e.g. rodents: Kotler 1984, birds: Suhonen *et al.* 1994, primates: Cowlshaw 1996). Habitat selection can be examined from different perspectives, from a broad geographic range to a scale compatible with individuals, usually referred

as microhabitat selection. At this scale the microclimate and other physical characteristics of the substrate may influence habitat use (Turra & Denandai 2002, Bezerra *et al.* 2006, Townsend *et al.* 2006). However, the ability of species to discriminate specific portions of the habitat is still poorly known in most vertebrate groups. This process may involve environmental cues, such as population density, pheromones, terrain characteristics, microclimate, or type of substrate. In some benthic fish, habitat selection may also be influenced by substrate color (Scott 1982, Iwata & Kikuchi 1997). Like benthic fish, larvae of many amphibians spend long periods of time at the bottom

of water bodies. Therefore tadpoles may exhibit some mechanism to evaluate the substrate. If present, this ability could enhance foraging and thermal exchanges with the habitat, and reduce predation. Since tadpole predation may be a key element to determine the spatial and temporal distribution of anuran communities in the environment (Wilbur 1980, Hero *et al.* 1998, Gunzburger & Travis 2004), species with a broad distribution and/or relatively abundant in some areas might exhibit efficient mechanisms to select microhabitats. The frog *Physalaemus gracilis* occurs throughout southern Brazil, Uruguay, Paraguay and Argentina (Frost 2011), with tadpoles developing at the bottom of temporary puddles, lakes and lagoons (Loebmann 2005, Lingnau & Di-Bernardo 2006), making it a suitable species to examine a possible ability to select different microhabitats based on substrate characteristics, since substrate color may be a basic indication for the use of this habitat by the specie.

Tadpoles were obtained from four clutches (Figs. 1a-1b) collected in temporary ponds in September 2010 in a swampy area in the municipality of Rio Grande (32°08'52.14"S – 52°11'57.68"O), Rio Grande do Sul State, southernmost region of Brazil. The clutches were transferred to the laboratory and individually maintained in plastic trays (38 x 22 x 8 cm, Fig. 1c) filled with water from the collecting site, with natural lighting and ambient temperature. Tadpoles were raised under these conditions until they reached the stage 26 (Gosner 1960). Tadpoles were then divided in groups and transferred to an experimental circular tank (70 cm diameter x 30 cm height and capacity for 250 L) with the bottom divided in three equal sections lined with synthetic material (EVA) in either white, gray or black (Fig. 1d). For each experiment, the tank was filled to a depth of 4 cm high with water from the collecting site and a group of 50 tadpoles from the same clutch was added. The number of tadpoles used in each experiment was aimed at maximizing the tadpole/tank area ratio and minimizing the risks of intoxication by excreted. Groups with fewer individuals were avoided so that individual variations in behavior would not bias the results. In preliminary tests, larger groups had higher mortality rates. The same lighting and temperature conditions were maintained for all trays during experiments. After acclimating for 12 h, the animals were maintained for six additional hours in the tank for the experiments and four photographs were taken automatically at predefined times: 8 h 00min, 10 h 00min, 12 h 00min, 14 h 00min. These times were

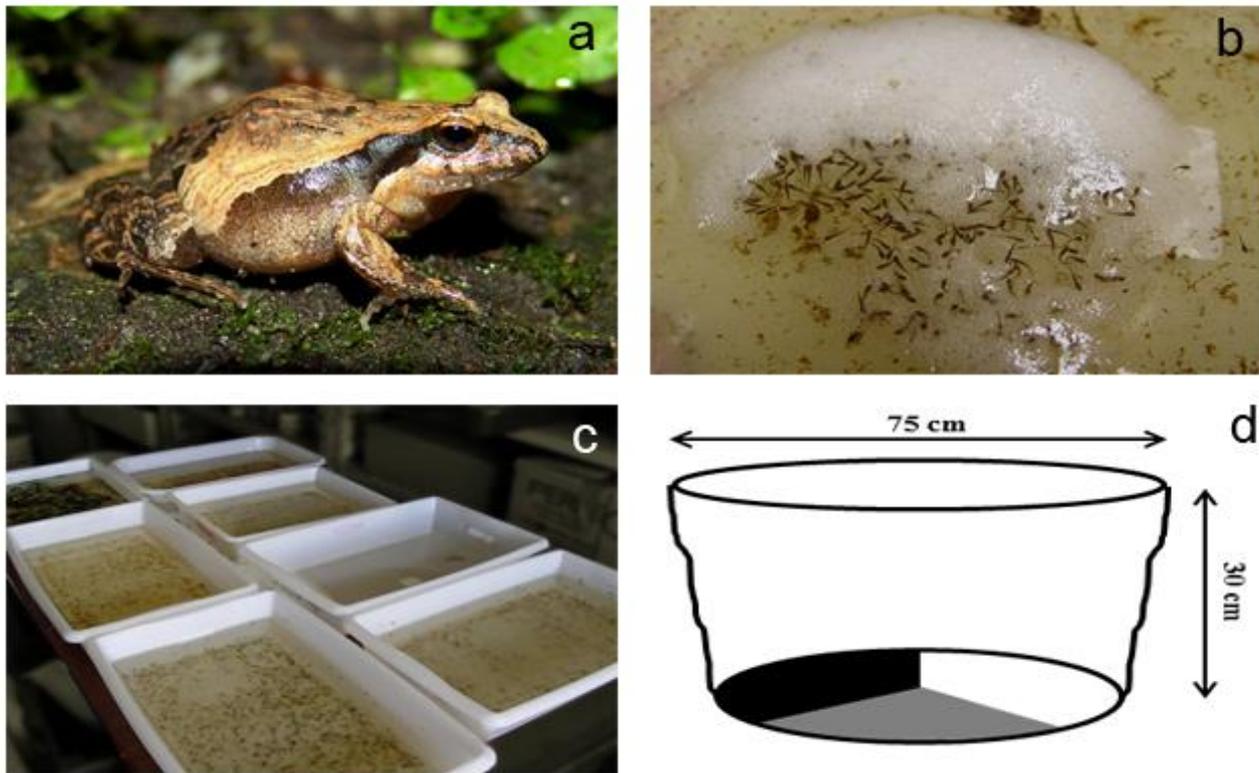
defined to limit the observation of the selection of microhabitats for resting during the day when the visual search for different substrate colors would be more efficient. To obtain the photographs, a camera was attached at a distance of approximately 2 m above the tank and the photos were taken automatically. The number of individuals on each substrate was counted in each image. The tadpoles on two substrate colors were counted as on the color where most of the body (more than 50%) was resting. At the end of the assay, tadpoles were removed, the tank was cleaned and sanitized with ethanol (GL 70), the water was changed, and the test was repeated with a new group of 50 tadpoles. Ten animals of each group were euthanized to confirm the identification of the species with the aid of the identification key proposed by Machado & Maltchik (2007). To evaluate variations in substrate use, we compared the number of tadpoles on each substrate color. Each animal observed in each image was considered a record. The comparisons among the number of records for each substrate color were carried out with the Kruskal-Wallis analysis of variance (Zar 1999). This test was also used to examine whether the number of records for each substrate color varied among the four times sampled (8 h 00min, 10 h 00min, 12 h 00min, 14 h 00min). For all analysis, the significance level was set at  $p < 0.05$  (Zar 1999).

The number of tadpoles on each substrate color varied significantly ( $U = 2$ ;  $p < 0.001$ ;  $n = 48$ ). More tadpoles were observed on the black substrate (623 records or 77.87% of records), followed by the gray substrate (117 / 14.63%), while fewer tadpoles were observed on the white substrate (60 / 7.5%, Table I). Our results suggest that tadpoles of *P. gracilis* can distinguish different portions of the microhabitat based on substrate color and tend to rest on darker substrates. One possible advantage of this behavior is that darker-colored substrates make detection or recognition of tadpoles more difficult by visually-oriented predators (Stevens & Merilaita 2009). Several studies have shown that tadpole coloration is correlated to an antipredator mechanism when a darker color seems to be associated with camouflage (Duellman & Trueb 1994, Wells 2007, Nomura *et al.* 2010). Also, cryptic coloration seems to be the first line of defense of tadpoles of *P. gracilis* against visual predators (Hödl & Amézquita 2001, Toledo 2007).

Substrate selection might also reflect different options of thermoregulation, since darker substrates have peculiarities regarding heat absorption (Schmidt-Nielsen 2002). In this case, the

selection of darker substrates would provide warmer water, thus favoring metamorphosis (Blouin & Brown 2000, Álvarez & Nicieza 2002). Because of the subtemperate climate in the study area, the populations of *P. gracilis* are subjected to a

relatively cold climate for tropical standards (Maluf 2000). Speeding the metamorphosis process could increase the survival of the species, especially in temporary water bodies and short hydroperiods (Wilbur & Collins 1973).



**Figure 1.** General aspect of the assay. (a) Adult of *Physalaemus gracilis* (Photo by Daniel Loebmann), (b) detail of foam nest of *P. gracilis*, (c) tray for development of tadpoles and (d) a diagram of the tank and substrate pattern used for the experiments.

**Table I.** Total number of records, mean of records per sample and percentage of tadpoles of *Physalaemus gracilis* observed on each substrate color.

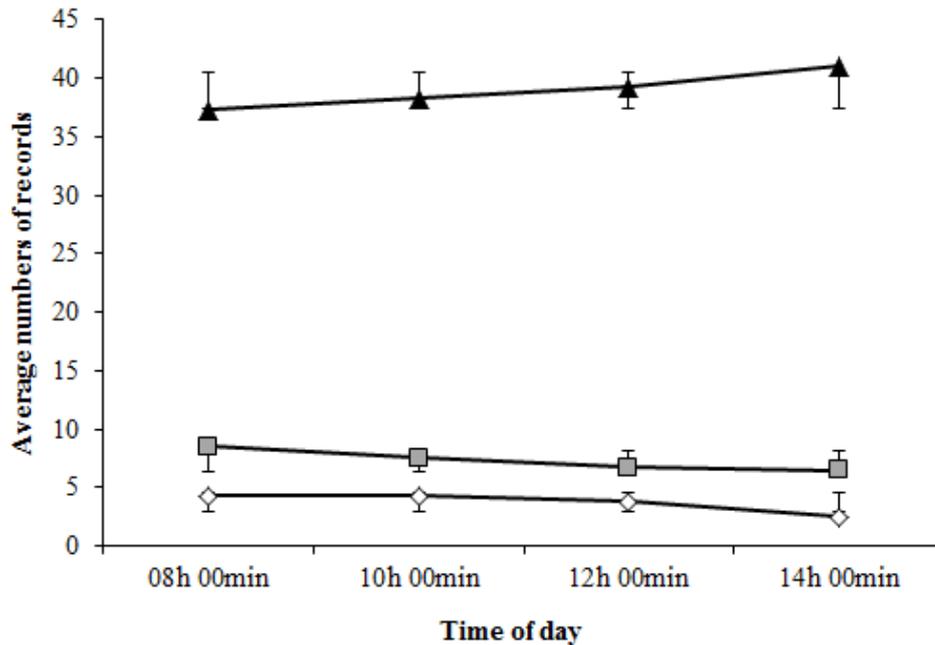
Substrate color	Number of records	Mean $\pm$ SD	Percentage of records
Black	623	38.93 $\pm$ 8.05	77.87
Gray	117	7.31 $\pm$ 5.25	14.63
White	60	3.68 $\pm$ 3.26	7.5
Total	800	-	100

The average number of tadpoles registered on each color of substrate did not vary significantly throughout the day for the white (8 h: 4.24  $\pm$  3.20; 10 h: 4.25  $\pm$  4.92; 12 h: 3.75  $\pm$  3.59; 14 h: 2.50  $\pm$  1.73;  $H_{[3;16]} = 0.3778$ ,  $p = 0.9448$ ), gray (8 h: 8.50  $\pm$  6.10; 10 h: 7.50  $\pm$  4.79; 12 h: 6.75  $\pm$  5.67; 14 h: 6.50

$\pm$  6.45;  $H_{[3;16]} = 0.7188$ ,  $p = 0.8688$ ) and black substrates (8 h: 37.25  $\pm$  9.28; 10 h: 38.25  $\pm$  9.60; 12 h: 39.50  $\pm$  8.54; 14 h: 41  $\pm$  8.16;  $H_{[3;16]} = 1.0182$ ;  $p = 0.7969$ , Fig. 2). This suggests that after selecting a substrate color, tadpoles were likely to remain on it, avoiding moving to other colors, probably reducing

the exposure to predators (Sih 1987, Ludwig & Rowe 1990, McIntyre *et al.* 2004). Despite the predominance of temporary ponds in the area, their connection to permanent bodies of water during rains allows the access of fishes, important tadpole predators (Wilbur 1987, Wellborn *et al.* 1996). Thus, the reproductive success of this species in this

region (Loebmann 2005) might be associated, not only to the ability to exploit resources that require microhabitat selection during early developmental stages, but also an efficient mechanism to avoid predation resulted of active selection of specific portions of the microhabitat based on substrate color.



**Figure 2.** Average number of records of tadpoles of *Physalaemus gracilis* on each substrate color (Δ = Black; □ = Gray; ◇ = White) at different times during the assay with corresponding standard deviations.

The ability of tadpoles of *P. gracilis* to detect different microhabitats based on color suggests the importance of visual evaluation in habitat selection and use. This ability might be more advantageous in predominantly open areas, such as grasslands (e.g. Pampa) and savannas (e.g. Cerrado) than in forests. The higher incidence of light in water bodies in open habitats might favor the use of visual cues (especially substrate color) in the microhabitat selection by tadpoles.

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#### References

- Álvarez, D. & Nicieza, A. G. 2002. Effects of temperature and food quality on anuran larval growth and metamorphosis. **Functional Ecology**, 16: 640-648.
- Bezerra, L. E. A., Dias, C. B., Santana, G. X. & Matthews-Cascon, H. 2006. Spatial distribution of fiddler crabs (genus *Uca*) in a tropical mangrove of northeast Brazil. **Scientia Marina**, 70(4): 759-766.
- Blouin, M. S. & Brown, S. T. 2000. Effects of temperature-induced variation in anuran larval growth rate on head width and leg length at metamorphosis. **Oecologia**, 125: 358-361.
- Connell, J. H. 1975. Some mechanisms producing structure in natural communities: a model and evidence from field experiments. Pp.460-490. *In*: Cody, M. L. & Diamond, J. M. (Eds).

- Ecology and evolution of communities.** Belknap Press, Cambridge, USA, 545 p.
- Cowlshaw, G. 1996. Trade-offs between foraging and predation risk determine habitat use in a desert baboon population. **Animal Behaviour**, 53(4): 667-686.
- Duellman, W. E. & Trueb, L. 1994. **Biology of amphibians.** John Hopkins University Press, Baltimore and London, 670 p.
- Frost, D. R. 2011 (Ed). **ASW - Amphibian Species of the World 5.5**, accessible at <http://research.amnh.org/vz/herpetology/amphibia/> (accessed 21/02/2012).
- Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. **Herpetologica**, 16: 183-90.
- Gunzburger, M. S. & Travis, J. 2004. Evaluating predation pressure on green treefrog larvae across a habitat gradient. **Oecologia**, 140: 422-429.
- Hero, J. M., Gascon, C. & Magnusson, W. E. 1998. Direct and indirect effects of predation on tadpole community structure in the Amazon rainforest. **Australian Journal of Ecology**, 23: 474-482.
- Hödl, W. & Amézquita, A. 2001. Visual signaling in anuran amphibians. Pp.121-141. *In*: Ryan, M. J. (Ed.). **Anuran communication.** Smithsonian Institution Press, Washington, 252 p.
- Huey, R. B. 1991. Physiological consequences of habitat selection. **American Naturalist**, 137: 91-115.
- Iwata, N. & Kikuchi, K. 1997. Effects of sand substrate and light in hypermelanosis of the blind side in cultured Japanese flounder *Paralichthys olivaceus*. **Environmental Biology of Fishes**, 52: 291-297.
- Kotler, B. P. 1984. Risk of predation and structure of desert rodent communities. **Ecology**, 65: 689-701.
- Lingnau, R. & Di-Bernardo, M. 2006. Predation on foam nests of two leptodactylid frogs by *Solenopsis* sp. (Hymenoptera, Formicidae) and *Liophis miliaris* (Serpentes, Colubridae). **Biociências**, 14(2): 223-224.
- Loebmann, D. 2005. **Guia Ilustrado: Os anfíbios da região costeira do extremo sul do Brasil.** Coleção manuais de campo USEB, Pelotas, 76 p.
- Ludwig, D. & Rowe, L. 1990. Life history strategies for energy gain and predator avoidance under time constraints. **American Naturalist**, 135: 686-707.
- Machado, I. F. & Maltchik, L. 2007. Check-list da diversidade de anuros no Rio Grande do Sul (Brasil) e proposta de classificação para as formas larvais. **Neotropical Biology and Conservation**, 2(2): 101-116.
- Maluf, J. R. T. 2000. Nova classificação climática do estado do Rio Grande do Sul. **Revista Brasileira de Agrometeorologia**, 8(1): 141-150.
- Mcintyre, P. B., Baldwin, S. & Flecker, A. S. 2004. Effects of behavioral and morphological plasticity on risk of predation in a Neotropical tadpole. **Oecologia**, 141: 130-138.
- Nomura, F., Prado, V. H. M., Silva, F. R., Borges, R. E., Dias, N. Y. N. & Rossa-Feres, D. C. 2011. Are you experienced? Predator type and predator experience trade-offs in relation to tadpole mortality rates. **Journal of Zoology**, 284(2): 144-150.
- Pereira, P. H. C., Junior, J. Z. & Jacobucci, G. B. 2009. Ocupação de conchas e utilização de microambientes por caranguejos ermitões (Decapoda, Anomura) na Praia da Fortaleza, Ubatuba, São Paulo. **Biotemas**, 22(2): 65-75.
- Rittschof, D., Sarrica, J. & Rubenstein, D. 1995. Shell dynamics and microhabitat selection by striped legged hermit crabs, *Clibanarius vittatus* (Bosc). **Journal of Experimental Marine Biology and Ecology**, 192: 157-172.
- Schmidt-Nielsen, K. 2002. **Fisiologia animal – adaptação e meio ambiente.** Livraria Santos, Editora Com. Imp. Ltda, São Paulo, 600 p.
- Scott, J. S. 1982. Selection of bottom type by groundfishes of the Scotian Shelf. **Canadian Journal of Fisheries and Aquatic Sciences**, 39(7): 943-947.
- Sih, A. 1987. Predators and prey lifestyles: an evolutionary and ecological overview. Pp. 203-224. *In*: Kerfoot, W. C. & Sih, A. (Eds). **Predation: direct and indirect impacts on aquatic communities.** University Press of New England, Hanover, 386 p.
- Stevens, M. & Merilaita, S. 2009. Animal camouflage: current issues and new perspectives. **Philosophical Transactions of the Royal Society B**, 364: 423-427.
- Suhonen, J., Norrdahl, K. & Korpima, K. I. E. 1994. Avian predation risk modifies breeding bird community on a farmland area. **Ecology**, 75: 1626-1634.
- Toledo, L. F. 2007. Predação e defesa em anuros: revisão, descrição e evolução. **PhD. Thesis.** Universidade Estadual Paulista “Julio de Mesquita Filho”, Campus de Rio Claro, São Paulo, 237 p.

- Townsend, C. R., Begon, M. & Harper, J. L. 2006. **Fundamentos em ecologia**. Artmed, Porto Alegre, 592 p.
- Turra, A. & Denadai, M. R. 2002. Substrate use and selection in sympatric intertidal hermit crab species. **Brazilian Journal of Biology**, 62(1): 107-112.
- Wellborn, G. A., Skelly, D. K. & Werner, E. E. 1996. Mechanisms creating community structure across a freshwater habitat gradient. **Annual Review of Ecology, Evolution, and Systematics**, 27: 337-363.
- Wells, K. D. 2007. **The ecology and behavior of amphibians**. The University of Chicago Press, Chicago and London, 1148 p.
- Wilbur, H. M. & Collins, J. P. 1973. Ecological aspects of amphibian metamorphosis. **Science**, 182: 1305-1314.
- Wilbur, H. M. 1980. Complex life cycles. **Annual Review of Ecology, Evolution, and Systematics**, 11: 67-93.
- Wilbur, H. M. 1987. Regulation of structure in complex systems: experimental temporary pond communities. **Ecology**, 68: 1437-1452.
- Zar, J. H. 1999. **Biostatistical Analysis**. Prentice-Hall, New Jersey, 664 p.

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