# Interpopulational variation in sexual dimorphism, reproductive output, and parasitism of *Liophis miliaris* (Colubridae) in the Atlantic forest of Brazil

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**Abstract.** Sexual maturity, sexual dimorphism, reproductive output, and parasitism of the colubrid snake *Liophis miliaris* were compared among populations inhabiting four regions of Brazil: (1) northern coastal Atlantic forest, (2) southern coastal Atlantic forest, (3) northern inland Atlantic forest (4) southern inland Atlantic forest. Females delayed maturity and attained larger body sizes than males in all regions. Males and females from northern Atlantic forest were smaller and attained maturity with a smaller body size than males of other regions. The sexual size dimorphism index ranged from 0.19 to 0.23 and was lowest in the northern Atlantic forest. There was no sexual dimorphism in head length in any of the populations studied. Clutch size was similar in all populations and increased with maternal body size. The reproductive frequency was lower in the northern coastal Atlantic forest and in contrast to the other regions, tended to increase with female body size. The nematoda *Ophidiascaris* sp. and cystacanths of *Oligacanthorynchus spira* (Acanthocephala) occurred equally in both sexes. Fewer snakes from the northern coastal Atlantic forest were infested by parasites compared to the other regions and parasitism apparently did not influence reproduction.

## Introduction

Sexual maturity, sexual dimorphism, and reproductive output are important aspects of reproduction. The age at which sexual maturity occurs in snakes is difficult to estimate (cf. Shine, 1978; Quinn, 1979; Marques, 1996) but can be easily assessed by the snout-vent length (SVL). Size at sexual maturity is positively correlated with mean body sizes such that large snakes usually attain maturity with larger body sizes (cf. Shine, 1994). Snake body size and sexual dimorphism differ greatly among species (cf. Shine, 1994) and populations (Shine and Fitzgerald, 1995). These variables can be influenced by abiotic factors, food availability, reproductive strategy, microhabitat use, species diversity and/or population dynamics (Begon et al., 1990; Shine and Fitzgerald, 1995).

Reproductive output is the proportion of energy stored in the body that is allocated to reproduction in a given time (Begon et al., 1990). Female reproductive output can be estimated by the fecundity (number of eggs, size of eggs and neonates), relative clutch mass (RCM, the rate of clutch mass to maternal mass) and reproductive frequency (Begon et al., 1990). Clutch size is usually correlated with female body size (Darwin, 1874; Seigel and Ford, 1987) and, in some snakes decreases with increasing egg size (Pizzatto and Marques, 2002). Data on the RCM frequently reveal patterns of reproductive investment.

Despite phylogenetic constraints, the RCM is also related to body shape (Shine, 1992) and habitat, and is lower in aquatic than in terrestrial snakes (Shine, 1988). Female snakes commonly reproduce annually or biennially (Fitch, 1970; Seigel and Ford, 1987). Moreover, the reproductive frequency of individuals can be irregular (cf. Capizzi et al., 1996) and dependent on phenotype (such as color, see Capula and Luiselli, 1994), age and/or size (Blem, 1982; Shine, 1986; Madsen and Shine, 1996; Shine et al., 1998a).

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*Liophis miliaris* is a common highly polymorphic (Dixon, 1989) colubrid snake with a wide distribution in South America (Dixon, 1983). Although there is some ecological data for this species, nothing is known about interpopulational variation, although some studies have reported significant variation in fecundity, related to phenotype (see also Capula and Luiselli, 1994 for another case study, i.e. that relative to the European adder, *Vipera berus*). The aim of this study was to provide data on the sexual maturity, sexual dimorphism, reproductive output, and parasitism of *Liophis miliaris* in four populations in Brazil.

## Material and methods

The water snake *Liophis miliaris* occurs mainly in the Atlantic forest, which includes rainforest in eastern Brazil and semi-deciduous forest in southeastern Brazil (cf. Dixon, 1983; fig. 1). Four populations of *Liophis miliaris* from forest areas were studied for the present study:

1. Northern Coastal Atlantic Forest (NCAF): this population occurs in southern Bahia state, between 13°48′N, 18°04′S, 30°08′E and 40°43′W. This area is being increasingly occupied by cacao plantations that grow well in rainforest. The climate is aseasonal, with high temperatures and high rainfall throughout the year (fig. 2).

2. Southern coastal Atlantic forest (SCAF): located in the states of São Paulo and Paraná, between 23°26′N, 25°52′S, 45°04′E and 48°50′W, on the eastern slopes of the Serra do Mar. This region is covered by rainforest. The climate in this area is seasonal, with a
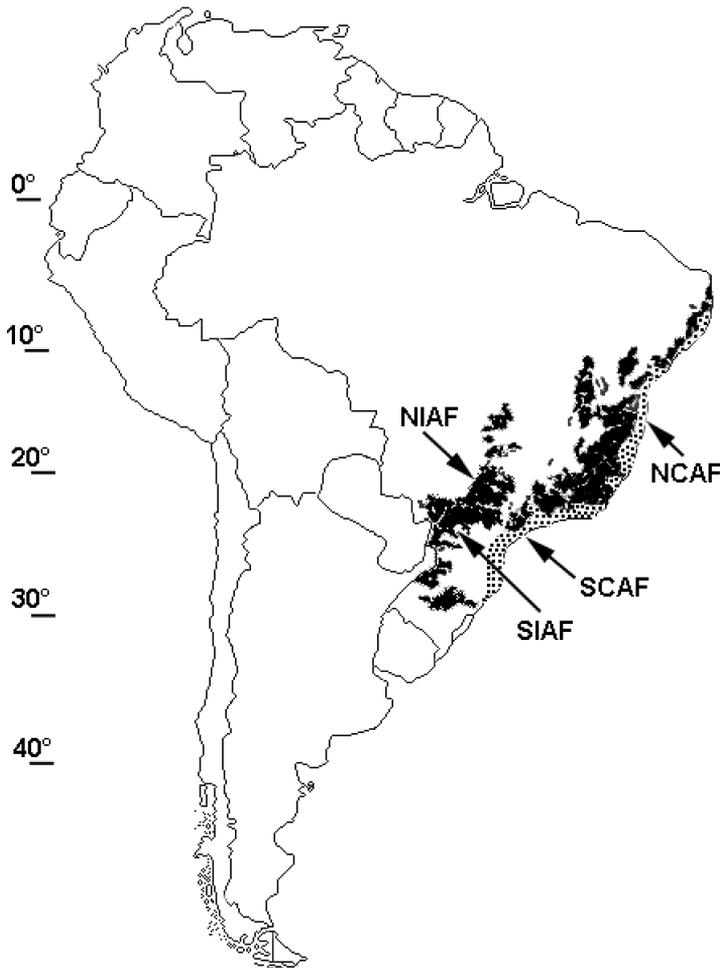


**Figure 1.** Distribution of the *Liophis miliaris* populations studied. NCAF = northern coastal Atlantic forest, SCAF = southern coastal Atlantic forest, NIAF = northern inland Atlantic forest, SIAF = southern inland Atlantic forest. Dark areas = inland Atlantic forest domain, dotted areas = coastal Atlantic forest domain.
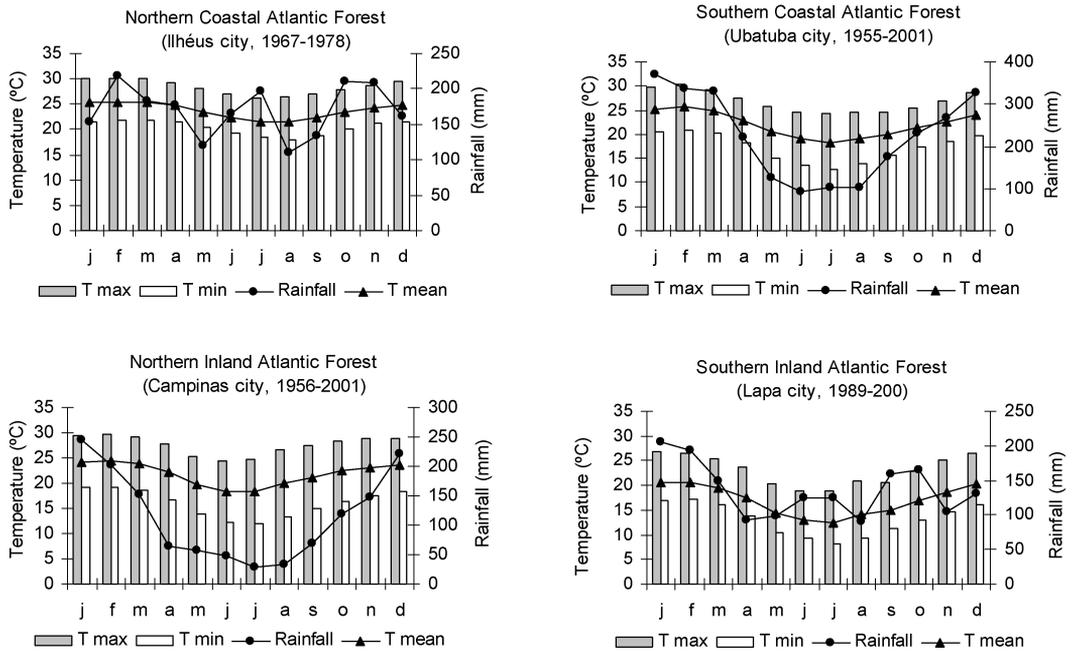
**Figure 2.** Climatic patterns in the areas where specimens of *Liophis miliaris* were collected.

hot, rainy spring and summer (September to March) and warm, dry autumn and winter (April to August) (fig. 2).

3. Northern inland Atlantic forest (NIAF): located in São Paulo state, between 20°12′N, 24°41′S, 45°06′E and 51°06′W, on the western slopes of the Serra do Mar. This area is covered mainly by semi-deciduous seasonal forest. The climatic variation is similar to SCAF, but the fluctuations are more marked (fig. 2).

4. Southern inland Atlantic forest (SIAF): located in Paraná state, between 22°51′N, 27°17′S, 45°18′E and 54°35′W. This area is covered by semi-deciduous seasonal forest and *Araucaria* forest. The spring and summer (September to March) are hot but the temperatures are lower than in the other areas. The autumn and winter (April to August) are cold and rains are abundant throughout the year; there is no dry season (fig. 2).

According to the most recent taxonomic review of *Liophis* species, *L. miliaris* shows extensive morphological variation (mainly in color patterns) and is represented by seven subspecies (Dixon, 1989). Based on this classification, the NCAF population was considered to be *L. m. merremii* whereas the other populations studied (SCAF, NIAF, SIAF) were classified as *L. m. orinus*. In addition, individuals from SCAF and inland Atlantic forest sites (NIAF, SIAF) differ in color pattern: adults from SCAF have dorsal scales with yellow centers (more obvious in live snakes and not recorded by Dixon 1983, 1989) whereas snakes from NIAF and SIAF are dark green olive to brown (see colors plates in Marques et al., 2004). Adults from NCAF have a brown dorsum [see Dixon (1983, 1989) for a more detailed

description]. Since the populations from SCAF and NIAF-SIAF may belong to distinct taxa (see Gans, 1964 and Marques et al., 2001), the populations studied here may belong to three subspecies (and not just two, as proposed by Dixon, 1983, 1989). Despite these unresolved taxonomic problems, for practical reasons we have followed the current classification by Dixon (1983, 1989).

A total of 289 preserved specimens of *Liophis miliaris* was examined from NCAF (127 adult females, 140 juveniles and 22 adult males); 249 (84 adult females, 106 juveniles and 59 adult males) from SCAF; 167 (49 adult females, 80 juveniles and 38 adult males) from NIAF and 201 (84 adult females, 64 juveniles, and 53 adult males) from SIAF. The specimens belong to the collections of the Museu de Zoologia da Universidade de Santa Cruz (MZUESC), Comissão Executiva do Plano da Lavoura Cacaueira (CE-PLAC), Museu de Historia Natural da Universidade Estadual de Campinas (ZUEC), Instituto Butantan (IB), and Museu de História Natural do Capão da Imbuia (MHNCI). Additionally, we also examined nine live gravid females received at the reception room of the Instituto Butantan.

Each specimen was sexed and measured for snout-vent length (SVL, to the nearest 1 mm using a plastic ruler), head length (HL) and head width (HW, both with a vernier caliper to the nearest 0.1 mm). After dissection, the following information was recorded: (1) reproductive condition – mature or immature (females were considered mature when the diameter of the ovarian follicles was >10 mm or if they had oviductal eggs; males were considered mature if the testes were large and turgid or if the deferent ducts were opaque and convoluted, indicating the presence of sperm – see Shine 1977a, b), (2) number of eggs and well-developed

**Table 1.** Snout-vent length and sexual size dimorphism in adult males and females of *Liophis miliaris*.

| Population | Mean female SVL ± SD (mm) | Min-max SVL (mm) | n | Mean male SVL ± SD (mm) | min-max SVL (mm) | n | SSD |
|---|---|---|---|---|---|---|---|
| NCAF | 555.8 ± 101.0 | 400-1015 | 127 | 465.4 ± 78.4 | 355-650 | 23 | 0.19 |
| SCAF | 713.3 ± 111.7 | 534-985 | 83 | 580.6 ± 84.8 | 410-745 | 59 | 0.23 |
| NIAF | 676.2 ± 118.5 | 505-1005 | 49 | 539.1 ± 94.4 | 417-905 | 37 | 0.25 |
| SIAF | 670.9 ± 115.1 | 507-876 | 80 | 532.3 ± 85.1 | 380-744 | 104 | 0.26 |

NCAF = Northern Coastal Atlantic Forest, SCAF = Southern Coastal Atlantic Forest, NIAF = Northern Inland Atlantic Forest, SIAF = Southern Inland Atlantic Forest. SSD = sexual size dimorphism index. SD = standard deviation.

follicles (>20 mm in NCAF and >25 mm in the other populations in which snakes were larger), (3) length, width and thickness of eggs (measured to the nearest 0.1 mm with a vernier caliper), (4) length, width and thickness of testes (measured to the nearest 0.1 mm with a vernier caliper), and (5) level of parasitism by nematodes in the stomach and by cystacanths of Acanthocephala in the peritoneum. The level of parasitism was graded as: L1 – 1-15 parasites, L2 – 16-30 parasites and L3 – >30 parasites.

The eggs and testicular volumes were estimated using the ellipsoid volume formula $V = 4/3abc$ where $a =$ half of the length, $b =$ half of the width and $c =$ half of the thickness (see Pleguezuelos and Feriche, 1999). The testicular volume reflects the degree of spermatogenic activity (Volsøe, 1944; Shine 1977b). Clutch size was estimated from the number of eggs and vitellogenic follicles (>15 mm) in preserved specimens and from clutches laid by captive snakes. Reproductive frequency was estimated based on the ratio between the total number of females and the number of reproductive females (cf. Shine et al., 1998a).

Live gravid females (detected by palpation) were maintained in captivity. After egg laying, the number of eggs and clutch mass (to the nearest 0.1 g) were recorded. The length, width and thickness of the eggs were measured to the nearest 0.5 mm. Females were measured and weighed after egg-laying. All of these measurements were made soon after egg-laying in order to calculate the RCM (relative clutch mass = clutch mass/female mass after egg-laying – Shine, 1980). The eggs were incubated in moist vermiculite at room temperature (19.3-31.9°C). The (SVL) and body mass of the neonates were recorded.

Differences in SVL between females and males and among populations (areas) were compared using factorial ANOVA with SVL as dependent variable and sex and area as factors (Zar, 1999). The sexual size dimorphism index was calculated as (mean SVL of the largest sex/mean SVL of the smallest sex) −1 (cf. Shine, 1994). Since head length and width are related to SVL, we used the residuals of these variables and the Mann-Whitney test to compare the sexes. Fecundity was compared among the populations by the Kruskal-Wallis test using the residual number of eggs relative to the female SVL (Zar, 1999). Non-parametric tests were used when the assumptions of parametric statistics were not met. The exact Fisher test was used to compare the number of reproductive and non-reproductive females infected by nematodes and Acanthocephala (Zar, 1999). In this comparison, only two infection levels (L1 and L2 + L3) were considered.

## Results

### Sexual maturity and sexual dimorphism

Females attained sexual maturity with a larger body size than males in all the populations studied. In NCAF, both sexes attained sexual maturity with a smaller body size than in the other areas (table 1).

Adult females were larger than adult males in all populations (table 1) and the mean SVL of both sexes differed among individuals from different populations (table 1). The sexual size dimorphism index was similar in all populations (table 1). The SVL of snakes was highly but separately dependent on sex and on the area where the snakes occurred. However iteration between these two factors was not significant (factorial ANOVA – sex: $F = 163.65$, df $= 1$, $P < 0.00001$; area: $F = 30.47$, df $= 3$, $P < 0.00001$; iteration: $F = 1.15$, df $= 3$, $P = 0.328$), indicating that there was no geographic variation in sexual size dimorphism. The relative head length and width were similar between males and females in all areas (Mann-Whitney test; head length – NCAF: Zadj $= 0.014$, $P = 0.988$; SCAF: Zadj $= 0.169$, $P = 0.866$; NIAF: Zadj $= -0.167$, $P = 0.865$; SIAF $= 0.744$, $P = 0.467$; head width – NCAF: Zadj $= -0.901$, $P = 0.367$; SCAF: Zadj $= -1.464$, $P = 0.143$; NIAF: Zadj $= -0.297$, $P = 0.766$; SIAF $= -0.762$, $P = 0.446$).

**Table 2.** Clutch size, egg size and reproductive frequency in *Liophis miliaris*.

| Population | Mean clutch size (± SD, range) | Mean egg volume (± SD in mm, range) | Reproductive females in the reproductive season (%) | Reproductive females all though the year (%) |
|---|---|---|---|---|
| NCAF | 9.0 ± 3.4 (5-17, $n = 20$) | 1520.0 ± 557.3 (503.0-2693.3, $n = 53$ eggs, 5 clutches)[a] | 32.8 | 32.8 |
| SCAF | 10.2 ± 2.8 (5-17, $n = 19$) | 1782.2 ± 701.9 (1051.7-3805.0, $n = 41$ eggs, 5 clutches)[a] | 70.0 | 52.5 |
| NIAF | 10.9 ± 6.5 (4-30, $n = 14$) | 1812.5 ± 532.9 (971.2-3263.5, $n = 63$ eggs, 4 clutches)[a] | 70.2 | 58.3 |
| SIAF | 10.0 ± 4.4 (1-18, $n = 28$) | 2926.3 ± 1090.9 (1682.0-2828.2, $n = 51$ eggs, 7 clutches)[b] | 77.0 | 53.6 |

Superscripts letters indicate the result of the post-hoc Kruskal-Wallis test.

## Reproductive output

Clutch size was similar in all populations, both in absolute number ($H = 1.885$, df $= 3$, $P = 0.597$; table 2) and relative to SVL (fig. 3, $H = 1.43$, df $= 3$, $P = 0.6983$). The mean egg volume differed among populations, with eggs from females of SIAF being the largest compared to all the other regions ($H = 66.09$, df $= 3$, $P < 0.0001$; table 2). Neonates from NCAF were smaller than those from other areas (table 3). The RCM could be only estimated in NIAF and was $0.26 \pm 0.05$ ($n = 3$ clutches).

The reproductive frequency was lower in NCAF than in the other areas throughout the year ($\chi^2 = 14.977$, df $= 3$, $P = 0.0018$; table 2). When the reproductive frequency in NCAF was compared with that of the other areas, and considering it only in the reproductive period (it is seasonal in SCAF, NIAF and SIAF – see Pizzatto, 2003), the frequency was still lower in snakes from NCAF ($\chi^2 = 50.227$, df $= 3$, $P < 0.0001$; table 2). Moreover, in snakes from NCAF, the reproductive frequency was lower in smaller adult females than in larger ones, while in other populations it was high in all size classes (fig. 4).

Clutch size, but not egg volume, was correlated with female SVL (table 4). In captive snakes from NIAF, clutch size decreased with increasing mean egg volume (table 4).
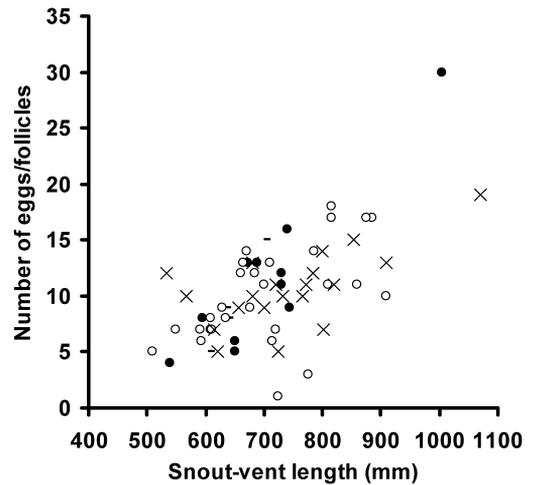


**Figure 3.** Fecundity in *Liophis miliaris*: clutch size versus SVL. –: northern coastal Atlantic forest ($R^2 = 0.506$, $P = 0.0004$, $n = 20$), ×: southern coastal Atlantic forest ($R^2 = 0.387$, $P = 0.0034$, $n = 20$), •: northern inland Atlantic forest ($R^2 = 0.923$, $P = 0.0006$, $n = 7$), ○: southern inland Atlantic forest ($R^2 = 0.387$, $P = 0.0034$, $n = 20$).

## Parasitism

Two parasites were identified: adults of *Ophidiascaris* sp. (Nematoda) in the stomach and cystacanths of *Oligatanthorynchus spira* (Acanthocephala) in the peritoneum.

The prevalence of both parasites was lowest in snakes from NCAF and highest in snakes from SCAF (table 5). The level of infestation did not differ between males and females from any population (table 5). Female reproductive status was independent of the level of infection

**Table 3.** Neonatal size in *Liophis miliaris*.

| Population | Smallest newborn in collection (SVL, mm) | Mean SVL from hatchling in captivity ($\pm$ SD in mm, range) | Mean SVL from hatchling in field ($\pm$ SD in mm, range) |
|---|---|---|---|
| NCAF | 113 | – | – |
| SCAF | 169 | $127.3 \pm 7.0$ (118-130, $n = 8$) | $172.4 \pm 6.6$ (162-178, $n = 5$) |
| NIAF | 170 | $184.3 \pm 4.5$ (173-190, $n = 17$) | $175 \pm 4.6$ (172-182, $n = 5$) |
| SIAF | 150 | – | – |

Mean snout-vent length (SVL) for hatchlings (both in captivity and field) refers to individuals of the same clutch.

**Table 4.** Fecundity parameters (mean egg volume in mm$^3$ and clutch size relative to female SVL) in *Liophis miliaris*.

| | NCAF | SCAF | NIAF (collection) | NIAF (captivity) | SIAF |
|---|---|---|---|---|---|
| n° eggs/follicles × female SVL | $R^2 = 0.506$, $P = 0.0004, n = 20$ | $R^2 = 0.160$, $P = 0.089, n = 19$ | $R^2 = 0.923$, $P = 0.0006, n = 7$ | $R^2 = 0.463$, $P = 0.137, n = 6$ | $R^2 = 0.542$, $P = 0.0030, n = 28$ |
| n° eggs × mean eggs volume | $R^2 = 0.174$, $P = 0.484, n = 5$ | $R^2 = 0.608$, $P = 0.120, n = 5$ | $R^2 = 0.171$, $P = 0.586, n = 4$ | $R^2 = 0.897$, $P = 0.053, n = 4$ | $R^2 = 0.094$, $P = 0.554, n = 6$ |
| Female SVL × mean eggs volume | $R^2 = 0.041$, $P = 0.744, n = 5$ | $R^2 = 0.217$, $P = 0.428, n = 5$ | $R^2 = 0.207$, $P = 0.545, n = 4$ | $R^2 = 0.621$, $P = 0.211, n = 4$ | $R^2 = 0.272$, $P = 0.288, n = 6$ |

**Table 5.** Prevalence of infection of *Liophis miliaris* by *Ophidiascaris* sp. (Nematoda) and cystacanths of *Oligacanthorynchus spira* (Acanthocephala) in.

| Population | *Ophidiascaris* sp. | | | | *Oligacanthorynchus spira* | | | |
|---|---|---|---|---|---|---|---|---|
| | infected snakes (%) | infected females (%) | infected males (%) | $\chi^2$ between males and females | infected snakes (%) | infected females (%) | infected males (%) | $\chi^2$ between males and females |
| NCAF | 2.0 (3/149)* | 1.3 (3/127) | 0 (0/22) | – | 0.67 (1/149) | 0.79 (1/127) | 0 (0/22) | – |
| SCAF | 41.9 (60/143)* | 48.8 (41/84) | 32.2 (19/59) | $\chi^2 = 3.27$, $P = 0.07$ | 9.1 (13/143) | 10.7 (9/84) | 6.8 (4/59) | – |
| NIAF | 19.5 (17/87) | 12.2 (6/49) | 28.9 (11/38) | $\chi^2 = 2.8$, $P = 0.009$ | 3.4 (3/87) | 2.0 (1/49) | 5.3 (2/38) | – |
| SIAF | 16.0 (22/137) | 16.7 (14/84) | 15.1 (8/53) | $\chi^2 = 0.06$, $P = 0.996$ | 3.6 (5/137) | 4.8 (4/84) | 1.9 (1/53) | – |

Numbers in parenthesis are infected snakes/total of snakes. *Significative values for the Chi-squared test between infected snakes from the four populations: $\chi^2 = 73.4$, df = 1, $P < 0.0001$.

**Table 6.** Relationship between the prevalence of infection by *Ophidiascaris* sp. (Nematoda) and cystacanths of *Oligacanthorynchus spira* (Acanthocephala) and the reproductive state of females of *Liophis miliaris*.

| Population | *Ophidiascaris* sp. | | | | | *Oligacanthorynchus spira* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Reproductive females | | Non-reproductive females | | Fisher exact test | Reproductive females | | Non-reproductive females | | Fisher exact test |
| | L1 | L2-3 | L1 | L2-3 | | L1 | L2-3 | L1 | L2-3 | |
| NCAF | 1 (100%) | 0 (0%) | 1 (100%) | 0 (0%) | – | 0 (0%) | 0 (0%) | 1 (100%) | 0 (0%) | – |
| SCAF | 10 (43.5%) | 13 (56.5%) | 7 (38.9%) | 11 (61.1%) | $P = 1$ | 5 (71.4%) | 2 (28.6%) | 2 (66.7%) | 1 (33.3%) | $P = 0.99$ |
| NIAF | 5 (71.4%) | 2 (28.6%) | 2 (66.7%) | 1 (33.3%) | $P = 1$ | 1 (100%) | 0 (0%) | 0 (0%) | (0%) | – |
| SIAF | 8 (66.7%) | 4 (33.3%) | 1 (50%) | 1 (50%) | $P = 1$ | 1 (100%) | (0%) | 1 (100%) | 0 (0%) | – |

L1 – from 1 to 15 parasites, L2 – from 16 to 30 parasites and L3 – more than 30 parasites.
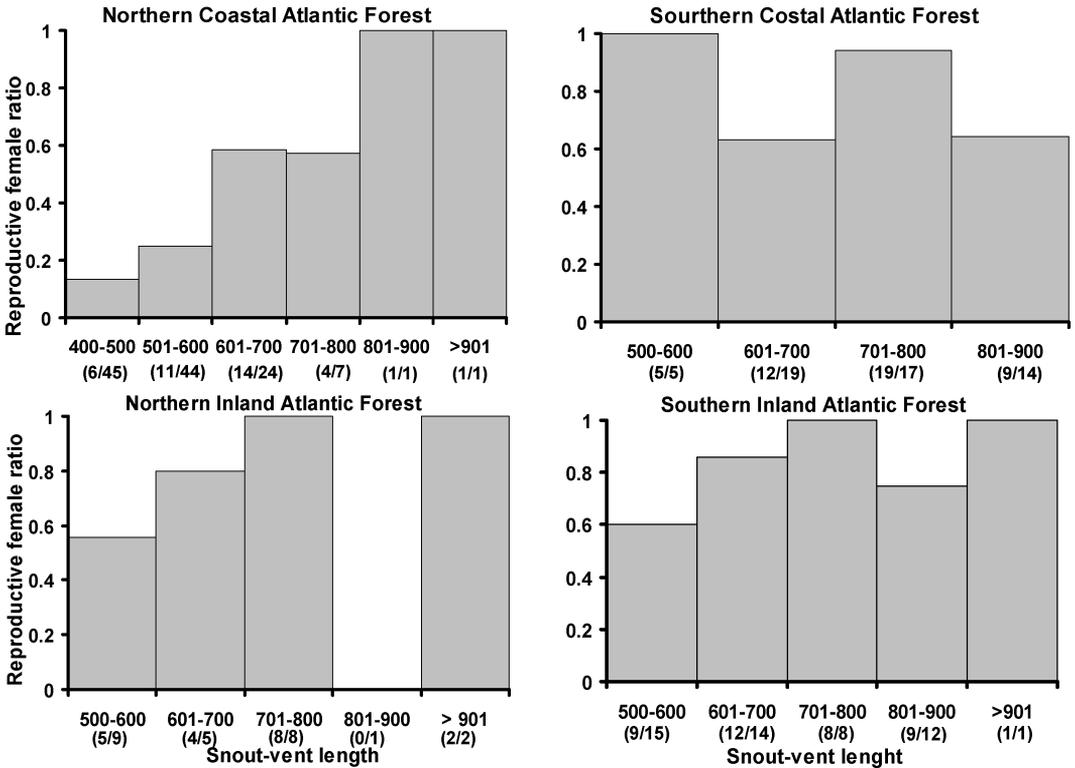
**Figure 4.** Ratio of reproductive females of *Liophis miliaris* in different body size classes. The numbers in parentheses indicate the numbers of reproductive females/total number of females.

in any population (table 6). In SIAF, the number of eggs did not differ between females infected ($9.9 \pm 5.68$, $n = 10$) and not infected ($10.3 \pm 5.50$, $n = 25$) by nematodes (intersample difference: $t = -0.202$, $P = 0.841$). In addition, the number of eggs was not related to SVL in females infected ($R^2 = 0.375$, $P = 0.0796$, $n = 9$) or not infected ($R^2 = 0.202$, $P = 0.224$, $n = 9$) by nematodes in SIAF. In contrast, in NIAF, the number of eggs and well-developed follicles ($>25$ mm) was positively correlated with SVL in not infected ($R^2 = 0.425$, $P = 0.003$, $n = 18$) and infected ($R^2 = 0.875$, $P = 0.026$, $n = 7$) females. The residual testicular volume was not correlated to the level of parasitism (L1, L2, and L3) by nematodes (NAF: $R^2 = 0.018$, $P = 0.458$; $IAF_{SP}$: $R^2 = 0.00001$, $P = 0.994$; $IAF_{PR}$: $R^2 = 0.016$, $P = 0.454$) or cystacanths (NAF: $R^2 = 0.070$, $P = 0.136$).

## Discussion

### Sexual dimorphism and reproductive output

Adult male and female body sizes differed significantly among all *L. miliaris* populations, with snakes from NCAF being the smallest. Body size may differ either because of local genetic modification or because of a direct phenotypic effect of food availability on the growth rates (see Madsen and Shine, 1993). Ecological characteristics such as species diversity, interspecific competition and niche dimensions vary geographically and may affect food availability in distinct populations.

There are no dimorphism in head size in *Liophis miliaris*, which agrees with the findings for most colubrid species studied so far (Shine, 1991). Head size dimorphism may be associated with inter-sexual dietary divergence (Houston and Shine, 1993; Shine, 1986; Shetty and Shine, 2002), but there are no dietary differences be-

tween the sexes of *L. miliaris* (Pizzatto, 2003). However, some snake species may show head size dimorphism without any divergence in diet (Luiselli et al., 2002). In such cases, authors attributed the dimorphism to a "ghost" of past competition or, more probably, to sexual selection (Luiselli et al., 2002).

The sexual size dimorphism index for *L. miliaris* ranged from 0.19 to 0.23, and colubrid snakes with this index are not expected to exhibit male combat behavior (*cf.* Shine, 1994). Indeed, male combat has not been reported in Xenodontini (Schuett et al., 2001; Shine 1994), which suggests that this behavior does not occur in this snake lineage. The absence of male combat and the small size of *L. miliaris* males may reflect phylogenetic conservatism.

Clutch size increased with SVL in *L. miliaris*, as in many snakes (Shine, 1994). Although body sizes differed among populations, fecundity (in terms of clutch size) was similar. This finding suggests that even the small snakes from NCAF invest heavily in producing large clutches. Although relative clutch mass and reproductive frequency are parameters of reproductive output, as important as clutch size, they are difficult to estimate and unavailable for most species (cf. Seigel and Ford, 1987). The reproductive frequency of females from NCAF was lower than in the other areas, but the causes of this remains unknown.

The reproductive frequency of small females (<600 mm SVL) from NCAF was lower when compared to the other size classes, as already reported for another colubrid (*Coronella austriaca*) and one viperid (*Vipera berus*) (Capula and Luiselli, 1994; Capizzi et al., 1996; Reading, 2004). This pattern suggests that juvenile females from NCAF may invest more in growth rather than in reproduction. Initiating reproduction with larger body sizes allows for the production of more eggs. Furthermore, since females in NCAF can reproduce continuously (Pizzatto, 2003), this initial delay in reproduction may not represent a particular disadvantage. In contrast, females from the other areas reproduce only seasonally (Pizzatto, 2003) and a relatively short delay in becoming involved in reproduction may result in the failure to reproduce in that year. This would force the snakes to postpone reproduction until the next reproductive season.

Despite the influence of senescence on reproductive frequency, i.e., large snakes have a lower reproductive frequency (Shine et al., 1998a), species that grow fast can be large but young (cf. Madsen and Shine, 1996; Shine et al., 1998a). However, estimates of reproductive frequency based on preserved specimens in collections must to be interpreted with caution because pregnant females and larger non-pregnant females have a greater probability of being collected than non-gravid or smaller females (Shine, 1979, 1980a; Seigel et al., 1987).

*Parasitism and fecundity*

Parasitism is a poorly understood aspect of snake ecology and our current knowledge does not allow us to infer the extent of its influence on natural populations (Shine et al., 1998b). The incidence of parasitism may be related to the snakes' feeding behavior and immunological resistance (Shine et al., 1998b). As shown here, the incidence of parasitism was similar in males and females of all populations and did not adversely affect the reproductive capacity of the snakes, in contrast to some lizards infested by blood parasites (Schall, 1983). However, it is possible that the parasites may have influenced other life history parameters (such as growth) or may have affected reproduction on a very small scale that was not perceptible here.

Anurans, lizards and snakes are accidental hosts of *O. spira* cystacanths (Travassos, 1917; Yamaguti, 1963; Schmidt and Roberts, 1996; Pizzatto and Madi, 2002), with insects being the intermediate hosts and birds the definitive hosts (Travassos, 1917; Schmidt and Roberts, 1996). Hence, snakes generally do not show any symptoms of illness when infested by this parasite (Schmidt and Roberts, 1996). Adult *Ophidiascaris* spp. is common parasites in snakes

(Sprent, 1955; Ash and Beaver, 1963; Araujo and Machado, 1980; Hamir, 1986; Sprent, 1988; McAllister et al., 1993; Goldberg and Bursey, 1999; Hering-Hagenbeck and Broomker, 2000) and anurans (Bursey et al., 2001). We suggest that snakes are the definitive hosts of these nematodes and that the parasites are acquired from ingested prey, mainly anurans in the case of *L. miliaris*. Snakes from NCAF were less susceptible to infeciton by *Ophidiascaris* sp. and *O. spira*. Since the diet is similar in the four populations studied here (Pizzatto, 2003), the cause of this low susceptibility remains unclear.

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