Cranial sexual dimorphism in captive adult broad-snouted caiman (*Caiman latirostris*)

Luciano M. Verdade

Laboratório de Ecologia Animal/LPA/ESALQ, Universidade de São Paulo, Caixa Postal 09, Piracicaba, SP
13418-900, Brasil
e-mail: lmv@esalq.usp.br

Living adult crocodilians can be easily sexed by direct examination of the genitalia in the cloaca; however, application of this method in hatchlings and young generally leads to a high rate of misclassification (Chabreck, 1963; Brazaitis, 1969; Allsteadt and Lang, 1995). Although there are some qualitative attempts to describe secondary sexual dimorphism in crocodilians (McIlhenny, 1935; Viosca, 1939; Neill, 1971), few multivariate analyses were done in order to quantify such characteristics. If refined, these methods might be useful to sex smaller individuals. In addition, and possibly more important than that, multivariate methods might possibly be extremely useful to the sex assessment of preserved materials such as skulls and skins.

Using linear discriminant analyses of head measurements and ratios, Webb and Messel (1978) report a proportion of 57.5 to 87% correct sex classification of *Crocodylus porosus* with varying body size (from 40-60 to > 120 cm SVL, respectively). Hall and Portier (1994) compared two methods of multivariate analysis (discriminant analysis and classification tree analysis), using variables located on the complete skull, cranium, and mandible. They report results varying from approximately 36.1% to 97.5% of correct classification of sex for the discriminant and from 0 to 100% for the classification tree analysis (the later is more conservative because of the cross-validation procedure).

"Geometric" morphometrics (as named by Corti, 1993, and Rohlf and Marcus, 1993) led to significant advances on the graphic representation of living organisms' forms. History and implications of these methods are presented in detail by Bookstein (1991, 1993). However, "traditional" multivariate morphometrics (as called by Marcus, 1990) can still be useful for discriminatory studies where the main goal is not the development of fine geometric sketches but the simple separation of statistical groups (Reyment, 1991). A multivariate approach is here presented in order to discriminate gender in broad-snouted caiman (*Caiman latirostris*) based on cranial measurements. For the sake of simplicity, no geometric morphometrics is included.

For the present study eleven head measurements were taken from 18 captive adult broad-snouted caimans (6 males; 12 females). Their body mass varied from 29.2 to 62 kg and their snout-vent length varied from 89 to 115 cm (Appendix 1). Eight measurements are longitudinal in relation to the body (i.e., length-measurements).

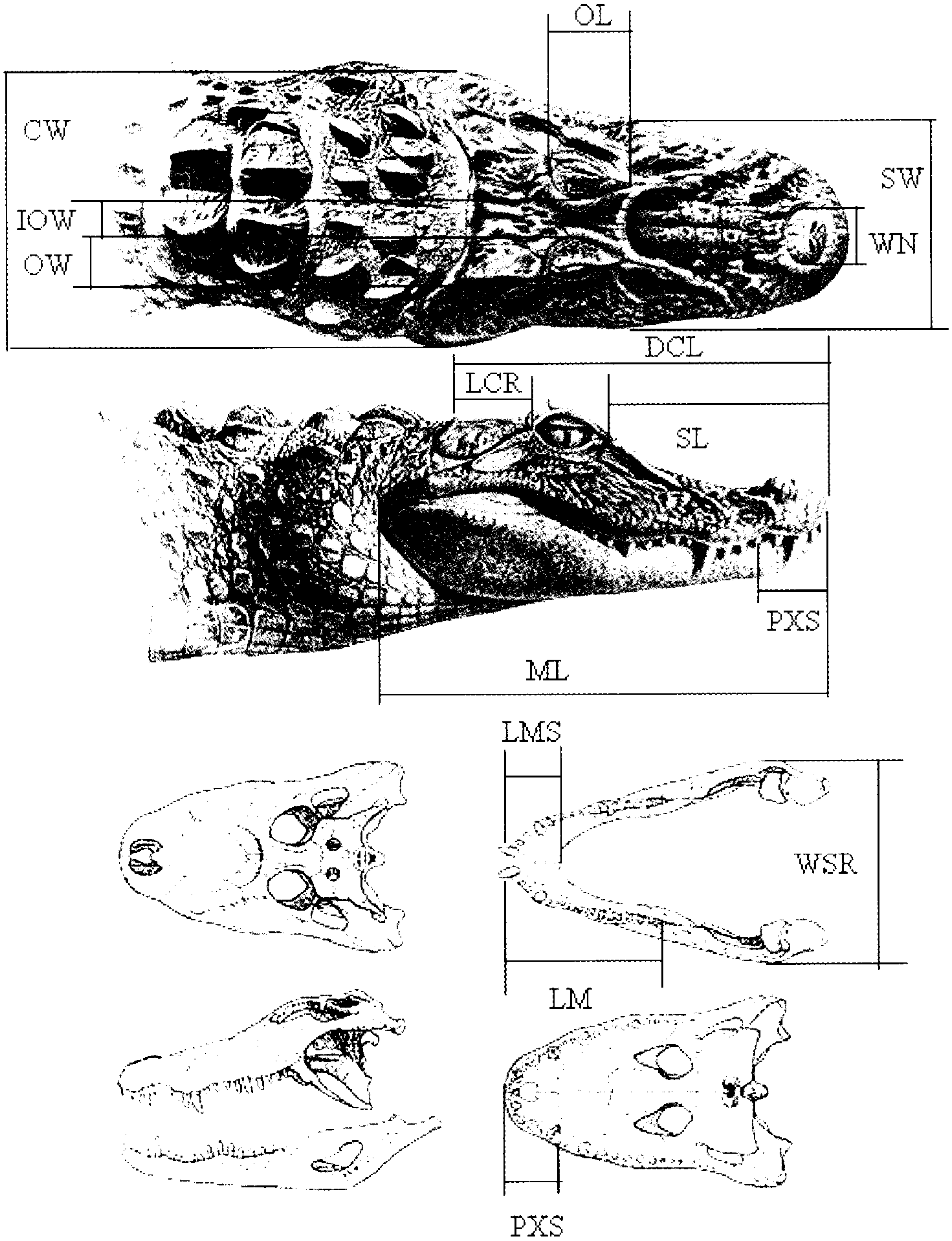

Figure 1. Morphometric variables. Dorsal and lateral view of *Caiman latirostris* head. See below for description of variables. Adapted from Wermuth and Mertens (1961 : 351, Fig. 250, after Natterer, 1840, Ann. nat.-hist. Wien 2: Tab. XXII).

The other six are transversal in relation to the body (i.e., width-measurements). Ten measurements were taken from the upper jaw and cranium, whereas the other four measurements were taken from the lower jaw (fig. 1).

The measurements used in this study are adapted from Iordansky (1973) and Hall and Portier (1994). They are based on linear distances between landmarks placed on the animals head. Hall and Portier used ratios called by them as "relative growth indices". Relative growth represents change of proportions as body size increases. The use of ratios presents several disadvantages. Ratios tend to be relatively inaccurate, not-normally distributed, and discontinuous (Sokal and Rohlf, 1995). For these reasons such ratios are not used in the present study.

Symbol	Explanation	Unit
DCL	Dorsal cranial length: anterior tip of snout to posterior surface of occiptal condyle	mm
CW	Cranial width: distance between the lateral surfaces of the mandibular condyles of the quadrates	mm
SL	Snout length: anterior tip of snout to anterior orbital border, measured diagonally	mm
SW	Basal snout width: width across anterior orbital borders	mm
OL	Maximal orbital length	mm
OW	Maximal orbital width	mm
IOW	Minimal interorbital width	mm
LCR	Length of the postorbital cranial roof: distance from the posterior orbital border to the posterolateral margin of the squamosal	mm
WN	Maximal width of external nares	mm
LMS	Length of the mandibular symphysis	mm
WSR	Surangular width: posterolateral width across surangulars at point of jaw articulation	mm

The animals were located at Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ) University of São Paulo, Piracicaba, State of São Paulo, Brazil (22°42.557′S, 47°38.246′W). The animals were either born at or brought to ESALQ when young, at least six years before the present study. Information about their date of birth and pedigree when available are presented at the Regional Studbook of the species (Verdade and Santiago, 1991; Verdade and Molina, 1993; Verdade and Kassouf-Perina, 1993; Verdade and Sarkis-Gonçalves, in press).

The animals were snared (as described by Chabreck, 1963; Webb and Messel, 1977; Walsh, 1987) and physically restrained during data collection. No chemical immobilization was used. Body measurements were taken with a tape measure (1 mm precision). Head measurements were taken with a steel Summit Vernier caliper (0.02 mm precision, second decimal unconsidered). Body mass was taken with Pesola hanging scales (50 × 0.1 kg). Before final measurements were taken training sessions were carried out and measurements were repeated until variation between replicates were negligible, i.e., less than the desired precision. Animals were sexed through manual probing of the cloaca (Chabreck, 1963; Brazaitis, 1969) and/or visual examination of genital morphology (Allstead and Lang, 1995) with a speculum of appropriate size.

Three following consecutive questions are tested on the present study: a) Is there a significant morphometric variation between males and females?; b) Can the sex of an individual be predicted based on its cranial morphometrics?; and, c) Can the number of measurements be reduced without loosing efficiency? In order to answer these question we used multivariate analysis of variance (MANOVA) (Atchley and Bryant, 1975) followed by linear discriminant analysis with cross validation (LDA), principal components analysis (PCA), and again MANOVA, LDA and PCA for the reduced (best) subset of measurements (Manly, 1994). All statistical analyses were done in Minitab for Windows (Minitab, 2000).

PCA rotates a correlation matrix (i.e., the covariance matrix for the standardized variables) reducing correlation among variables to zero. Its use reduces dimensionality of variation and it is appropriate when differences in variables' scaling could masquerade total variance. PCA helps interpretation of data often revealing relationships among variables that were not previously suspected (Johnson and Wichern, 1992). Size and shape elements can be identified on PCA (Jolicoeur and Mosimann, 1960). The first principal component roughly corresponds to size variation, although in some circumstances there may be some shape variation on it (Hopkins, 1966, Somers, 1986, Marcus, 1990). On the other hand, when there is also a residual shape variation, other principal components may result from the analysis (Dodson, 1975).

Variable selection in this method is based on the fact that the subset model can actually predict future responses with smaller variance than the full model using all predictors (Hocking, 1976). However, caution is necessary

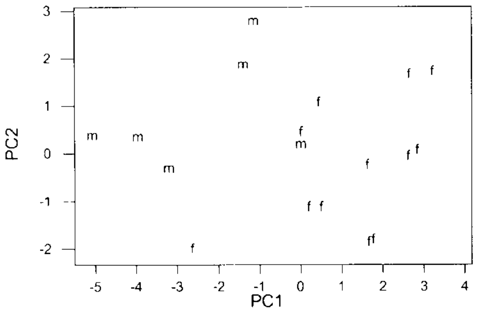

Figure 2. Plots of animals' sex against values for the two principal components (1: males; 2: females) for the whole set of measurements.

when selecting variables because the solution from a purely statistical point of view may not be the best from a substantive perspective (Cody and Smith, 1987). On the other hand, if — as in this case — the study purpose is to distinguish statistical populations, it does not practically matter whether or not the variables make biological sense (Rohlf, 1990).

A significant difference between males and females was found by MANOVA ($P = 0.012$) for the whole set of measurements. The overall proportion of correct classification at the linear discriminant analysis with cross-validation was 72.2%. Males presented the lowest correct classification rate (66.7%) and females the highest (75%).

The first principal component responded for the majority of the variance (55.5%). The coefficients of measurements for the first principal component varied in absolute terms from 0.107 (LCR) to 0.388 (SW). The second principal component responded for 17% of the total variance, with coefficients varying from −0.474 (OW) to 0.589 (IOW). The plots of animals' sex against values for the two principal components for the whole set of measurements are presented in fig. 2.

The following measurements presented a significant relationship with sex: DCL, CW, SL, SW, LMS, and WSR (ANOVA: $P < 0.01$). This subset of measurements slightly improved MANOVA results ($P = 0.002$) and also the overall proportion of correct classification of the linear discriminant analysis with cross-validation (88.9%). Females still presented a higher proportion of correct classification (91.7%) than males (83.3%).

The first principal component for this subset of measurements is responsible for most of the variation (81.9%). The coefficients for the first principal component varied in absolute terms from 0.298 (LMS) to 0.447 (SW). The second principal component responded for 12.1% of the total variance, with coefficients varying from −0.862 (LMS) to 0.466 (SL). The plots of animals' sex against values for the two principal components for the best subset of measurements are presented in fig. 3. Although the measurements used for the present study differ from each other by even one order of magnitude (e.g., IOW and DCL), log-transformation did not improve results (Verdade, unpublished analysis of data). It has been impossible to quantify other factors that possibly can affect cranial morphometrics in captive caimans; however, the animals have been kept in similar pens and have had the

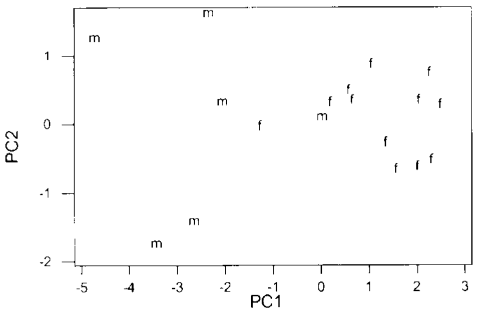

Figure 3. Plots of animals' sex against values for the two principal components (1: males; 2: females) for the best subset of measurements.

same management for years. For this reason, the present study seems to present a consistent cranial sexual dimorphism.

Females presented a higher proportion of correct classification than males at the discriminant analyses possibly because of the female-biased sample. However, it is not easy to improve the number of adult males sampled. Although, the species is relatively common at Brazilian zoos and more recently also at commercial farms the number of animals used at the present study represents a considerable amount of the total captive adult colony in Brazil (Verdade, 2001).

Principal components analyses and their plots are helpful to visualize how much males and females can be clustered apart (fig. 2). Although the first principal component comprises a considerable amount of the total variation (55%), there is a considerable amount of variation on the second principal components (17%) and the others (28%). This suggests that the difference between males and females is not only due to the overall cranial size but also to a considerable difference in cranial shape. However, from the six measurements that were significantly affected by sex there seems to be no distinction between length and width variables: three of each (DCL, SL and LMS are length measurements, whereas CW, SW and WSR are width measurements).

Reducing the number of measurements from eleven to six actually improved the results. MANOVA tests became more significant (P decreased from 0.012 for all measurements to 0.002 for the best subset) and proportion of overall correct classification at linear discriminant analysis with cross-validation increased from 72.2 to 88.9%. This seems to be an effective way to reduce "noise" at the model.

In addition, the amount of variation at the first principal component increased from 55.5% when all variables are considered to 81.9% when only the best subset is taken. This seems to stress that most of the variation between sexes is due to size differences of certain regions of the head, the ones represented by the significant variables (DCL, CW, SL, SW, LMS, and WSR).

Allometric growth shows that there is a consistent sexual dimorphism in captive broad-snouted caiman (Verdade, 2000). Males and females present distinct growth-curves and allometric relations for a considerable number of head and body measurements. However, sexual dimorphism can be detected mostly in the allometric growth of the cranium, not the mandible. This seems to be related with crocodilians' social displays, in which the mandible is usually kept under the water, not seen by conspecifics (Verdade, 2000). It is unlikely that this happens only for captive animals.

However, in order to isolate sexual dimorphism, it is necessary to isolate other sources of morphometric variation, such as age, body size, clutch of origin, environment, nutritional and sanitary status of individuals, and others. Apparently, no study so far took all these factors in consideration. Thus, their results may be including many different things as "sexual dimorphism".

Since it is practically impossible to keep "all other things being equal" in field studies, experimental design using captive animals may help to solve some of these problems. Since sex in crocodilians is temperature determined, and there is a considerable clutch effect (Lang and Andrews, 1994), an experimental design with eggs and hatchlings seems to be the best way to study sexual dimorphism in crocodilians. For such a study, clutches should be equally split into female and male temperatures of incubation. With this procedure, age, size, and clutch effect would be taken off the system. In addition, extra treatments (for instance, involving hormones, as suggested by Guillette et al., 1996a) could be added.

Morphometric studies about sexual dimorphism of crocodilians can be particularly helpful in situations such as the one described by Guillette et al. (1996b), in which chemical pollutants are apparently affecting the primary sexual characteristics of *Alligator mississippiensis* in Lake Apopka, Florida, with deleterious results for the reproduction rate of the whole population. In this case, the analysis of secondary sexual characteristics of individuals may help to identify the occurrence of similar problems in other areas.

The present study shows that there seems to be a consistent statistical distance between caiman males and females concerning cranial form. Geometric morphometric models might help us to visualize those differences by the development of fine graphical representations of it. The following conclusions can be taken from the present study:

a) Although it may be impossible to isolate gender from other sources of variation such as age, environment, and clutch of origin there seems to be a consistent sexual dimorphism in captive adult broad-snouted caiman in terms of their cranial morphometrics;
b) Experimentally designed studies are urged in order to better understand those individual sources of variation not only in captive but mainly in wild animals;
c) Geometric morphometric models might help developing graphical representations of cranial sexual dimorphism in crocodilians.

Acknowledgements. This study is a part of the dissertation presented to the Graduate School of the University of Florida in partial fulfillment of the requirements for the degree of Doctor of Philosophy. This study has been partially supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico — CNPq (Process No. 200153/93-5) and the Fundação de Amparo à Pesquisa do Estado de São Paulo — FAPESP (Process No. 00/01495-3). I am thankful to Prof. F. Wayne King, J. Perran Ross, Lou Guillette, Richard Bodmer, Mel Sunquist, George Tanner, Phil Hall, Bill Magnusson, José Eurico P. Cyrino, and Irineu U. Packer for their comments and suggestions. Fabianna Sarkis, and Rodrigo Zucoloto helped to handle the animals.

References

Allstead, J., Lang, J.W. (1995): Sexual dimorphism in the genital morphology of young American alligators, *Alligator mississippiensis*. Herpetologica **51**(3): 314-325.

Atchley, W.R., Bryant, E.H. [Eds] (1975): Multivariate Statistical Methods: Among Groups Covariation. Strousburg, Dowden, Hutchinson & Hoss.

Bookstein, F.L. (1991): Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge, Cambridge University Press.

Bookstein, F.L. (1993): A brief history of the morphometric synthesis. In: Proceedings of the Michigan Morphometric Workshop, p. 15-40. Rohlf, F.J., Bookstein, F.L., Eds, Ann Arbor, The University of Michigan Museum of Zoology.

Brazaitis, P. (1969): The determination of sex in living crocodilians. British Journal of Herpetology **4**(3): 54-58.

Chabreck, R. (1963): Methods of capturing, marking and sexing alligators. Proc. Ann. Conf. Southeastern Assoc. Game Fish Comm. **17**: 47-50.

Cody, R.P., Smith J.K. (1991): Applied Statistics and the SAS Programming Language. 3rd ed. Englewood Cliffs, Prentice-Hall.

Corti, M. (1993): Geometric morphometric: An extension of the revolution. Trends in Ecology and Evolution **8**: 302-303.

Dodson, P. (1975): Functional and ecological significance of relative growth in *Alligator*. J. Zool., Lond. **175**: 315-355.

Guillette, L.J., Jr., Arnold, S.F., McLachlan, J.A. (1996a): Ecoestrogens and embryos — Is there a scientific basis for concern? Animal Reproduction Science **42**: 13-24.

Guillette, L.J., Jr., Pickford, D.B., Crain, D.A., Rooney, A.A. and Percival, H.F. (1996b): Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. General and Comparative Endocrinology **101**: 32-42.

Hall, P.M., Portier, K.M. (1994): Cranial morphometry of New Guinea crocodiles (*Crocodylus novaeguineae*): ontogenetic variation in relative growth of the skull and an assessment of its utility as a predictor of the sex and size of individuals. Herpetological Monographs **8**: 203-225.

Hocking, R.R. (1976): The analysis and selection of variables in linear regression. Biometrics **32**: 1-49.

Hopkins, J.W. (1966): Some considerations in multivariate allometry. Biometrics **22**: 747-760.

Iordansky, N.N. (1973): The skull of the Crocodilia. In: Biology of the Reptilia. Vol. 4. Morphology D, p. 201-262, Gans, C., Parsons, T.S., Eds, London, Academic Press.

Johnson, R.A., Wichern, D.W. (1992): Applied Multivariate Statistical Analysis. 3rd. ed. Englewood Cliffs, Prentice Hall.

Jolicoeur, P., Mosimann, J.E. (1960): Size and shape variation in the painted turtle: a Principal Components Analysis. Growth **24**: 339-354.

Lang, J.W., Andrews, H.V. (1994): Temperature-dependent sex determination in crocodilians. Journal of Experimental Zoology **270**: 28-44.

Manly, B.F.J. (1994): Multivariate Statistical Methods: a Primer. 2nd. ed. London, Chapman & Hall.

Marcus, L.F. (1990): Traditional morphometrics. In: Proceedings of the Michigan Morphometric Workshop, p. 77-122. Rohlf, F.J., Bookstein, F.L., Eds, Ann Arbor, The University of Michigan Museum of Zoology.

McIlhenny, E.A. (1935). Alligator's Life History. Athens, Society for the Study of Amphibians and Reptiles.

Minitab. (2000): Minitab for Windows Release 13. State College, Minitab, Inc.

Neill, W.T. (1971): The Last of the Ruling Reptiles: Alligators, Crocodiles, and their Kin. New York, Columbia University Press.

Reyment, R.A. (1991): Multidimensional Palaeobiology. Oxford, Pergamon Press.

Rohlf, F.J. (1990): Morphometrics. Annual Review of Ecology and Systematics **21**: 299-316.

Rohlf, F.J., Marcus, L.F. (1993): A revolution in morphometrics. Trends in Ecology and Evolution **8**(4): 129-132.

Sokal, R.R., Rohlf, F.J. (1995): Biometry: the Principles and Practice of Statistics in Biological Research. New York, W.H. Freeman.

Somers, K.M. (1986): Multivariate allometry and removal of size with principal components analysis. Systematic Zoology **35**(3): 359-368.

Verdade, L.M. (2000): Regression equations between body and head measurements in the broad-snouted caiman (*Caiman latirostris*). Revista Brasileira de Biologia **60**(3): 469-482.

Verdade, L.M. (2001): O Programa Experimental de Criação em Cativeiro do Jacaré-de-Papo-Amarelo (*Caiman latirostris*) da ESALQ/USP: Histórico e Perspectivas. In: A Produção Animal na Visão dos Brasileiros, p. 559-564. Mattos, W.R.S., Ed., Piracicaba, Sociedade Brasileira de Zootecnia.

Verdade, L.M., Kassouf-Perina, S. [Eds] (1993): Studbook Regional do Jacaré-de-papo-amarelo (*Caiman latirostris*): 1992/1993. Sorocaba, Sociedade de Zoológicos do Brasil.

Verdade, L.M., Molina, F.B. [Eds] (1993): Studbook Regional do Jacaré-de-papo-amarelo (*Caiman latirostris*): Junho 1991-Junho 1992. Piracicaba, ESALQ.

Verdade, L.M., Santiago, M.E. [Eds] (1991): Studbook Regional do Jacaré-de-papo-amarelo (*Caiman latirostris*). Piracicaba, CIZBAS/ESALQ/USP.

Verdade, L.M., Sarkis-Gonçalves, F. (In press): Studbook Regional do Jacaré-de-papo-amarelo (*Caiman latirostris*): 1993/1997. Piracicaba, ESALQ.

Viosca, P., Jr. (1939): External sexual differences in the alligator, *Alligator mississippiensis*. Herpetologica **1**: 154-155.

Walsh, B. (1987): Crocodile capture methods used in the Northern Territory of Australia. In: Wildlife Management: Crocodiles and Alligators, p. 249-252. Webb, G.J.W., Manolis, S.C., Whitehead, P.J., Eds, Chipping Norton, Surrey Beatty & Sons.

Webb, G.J.W., Messel, H. (1977): Crocodile capture techniques. Journal of Wildlife Management **41**: 572-575.

Webb, G.J.W., Messel, H. (1978): Morphometric analysis of *Crocodylus porosus* from the north coast of Arnhem Land, northern Australia. Australian Journal of Zoology **26**: 1-27.

Wermuth, H., Mertens, R. (1961): Schildkröten, Krokodile, Brückenechsen, Jena, VEB Gustav Fischer Verlag.

Appendix 1. Original dataset.

Tag	Sex	BM	SVL	TTL	DCL	CW	SL	SW	OL	OW	IOW	LCR	WN	LMS	WSR
usp123	f	34.2	89.00	189.0	195.6	148.9	102.7	122.4	42.7	32.7	15.4	45.6	27.6	46.2	137.9
usp124	m	56.4	115.00	187.0	222.0	194.9	117.7	150.2	44.3	35.9	25.4	55.1	31.6	56.0	186.1
usp126	f	57.6	108.00	203.0	215.0	176.9	117.2	136.0	49.1	37.8	20.9	44.4	32.8	46.6	168.6
usp127	f	34.8	99.50	171.5	199.6	167.8	104.7	127.7	44.3	32.0	19.2	47.7	30.6	41.7	154.8
usp128	f	35.0	92.50	158.5	195.7	163.6	99.0	119.7	40.5	29.7	19.9	47.4	28.5	43.8	147.3
usp125	m	45.8	109.00	190.5	214.0	188.7	113.2	147.2	45.2	37.4	23.5	52.1	30.5	52.9	186.9
usp129	f	44.6	104.00	200.0	197.5	174.8	107.4	129.2	44.1	29.8	24.4	41.9	30.3	42.3	162.4
usp130	f	30.8	91.50	174.0	182.4	153.5	93.6	116.5	37.1	25.7	25.3	42.0	27.1	43.3	145.2
usp131	f	30.4	90.50	166.0	176.4	161.7	94.2	120.7	37.0	29.2	27.2	40.6	27.6	43.7	147.3
usp132	f	45.6	102.00	193.5	197.4	171.3	103.5	129.6	44.1	29.3	27.7	44.9	29.6	40.6	159.1
usp113	f	24.2	91.00	167.0	171.3	139.1	90.3	149.9	39.3	34.7	22.2	39.4	25.6	35.4	131.8
usp114	m	31.4	102.00	199.5	210.0	156.9	113.4	128.0	42.8	31.2	23.5	50.7	29.2	44.7	161.6
usp116	f	20.0	81.00	150.0	177.7	133.6	89.5	106.6	33.1	28.4	17.8	41.7	26.0	34.5	121.0
usp117	f	31.0	89.00	173.0	191.3	149.7	104.2	118.7	41.1	28.7	21.1	45.9	27.5	39.0	136.2
usp121	f	27.3	95.00	170.5	184.9	153.3	96.0	114.4	38.4	32.4	*	42.1	26.1	42.6	143.8
usp115	f	44.6	101.50	192.0	195.0	170.1	108.6	121.4	43.7	31.6	20.6	91.7	28.9	39.6	151.6
usp120	m	41.0	101.50	200.0	222.0	186.6	123.1	141.6	39.9	29.5	25.0	56.6	28.5	46.3	169.8
usp119	f	29.2	97.50	189.5	189.7	153.4	103.1	116.6	46.1	32.1	17.9	47.2	27.8	40.9	142.8
usp122	f	33.7	95.00	182.5	180.6	150.1	103.5	114.6	41.1	28.7	22.0	40.3	27.6	40.8	139.7
CL203	m	62.0	115.00	209.0	236.0	215.0	149.1	154.4	48.3	29.6	20.4	54.4	32.7	47.1	185.2
CL1	m	51.0	110.15	210.0	223.0	184.4	129.5	143.1	40.6	26.8	24.9	47.2	27.1	40.5	188.2

Obs: See Figure 1 for explanations on legends and placement of measurements.

Received: September 15, 2001. Accepted: June 10, 2002.