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MORPHOLOGIC MEASUREMENTS FROM SPECIMENS AND THEIR X-RAYS: TEST OF A METHOD FOR THE STUDY OF ALLOMETRY AND PHENOTYPIC PLASTICITY IN FISHES

by

AXEL MEYER

(Museum of Vertebrate Zoology and Department of Zoology, University of California, Berkeley, California 94720, U.S.A.)

SUMMARY

I present a method of repeated X-raying of individual fish as a means to study ontogenetic changes in morphology. This method also allows the direct testing of environmental influences on development of genetically similar groups of animals. The methodology is validated by statistical comparison between measurements taken from specimens and their X-rays.

INTRODUCTION

Interest in the study of development of morphology has surged recently. Particularly questions of growth and allometry and the relation between ontogeny and phylogeny (GOULD, 1977; DULLEMEIJER, 1980; WILHELM, 1984) have been addressed. Likewise, investigations determining the influence of the environment on morphology during the individual's ontogeny (phenotypic plasticity) particularly in cichlids fishes have recently been published (WITTE, 1984; HOOGERHOUD, 1986, MEYER, 1987; WITTE & WITTE-MAAS, 1987).

New insights into these questions may be gained by a combination of this approach with recent trends in the quantification of shape (*i.e.* STRAUS, 1984; BOOKSTEIN *et al.*, 1985; STRAUSS & FUIMAN, 1985). The utility of these methods of quantification of shape is large.

In most studies of growth patterns, individuals are typically sampled only once as preserved specimens. The allometric coefficients typically gathered by these transverse growth studies therefore are static allometric coefficients. Thus, these data, may not strictly represent patterns of growth (COCK, 1966; GOULD, 1966). It would be desirable to avoid this pitfall by following the ontogenetic trajectories (MEYER, 1987) of individuals, quantifying their morphology as they grow. These longitudinal growth studies would yield true dynamic allometric coefficients.

In my studies on the ontogeny of morphology and the importance of environmental influences on the development of morphology in Neotropical cichlid fishes (MEYER, 1987, MS) I raised juvenile cichlids quantifying their morphology throughout much of their ontogeny (beginning at sizes smaller than 20 mm SL). Treatment groups of fish were fed on different diets for varying lengths during their ontogeny and a radiographic record of the morphology of the fish was obtained repeatedly.

This paper tests the reliability of measurements from X-rays, in order to validate the method of repeatedly X-raying for the study of morphology during ontogeny.

MATERIALS AND TECHNIQUES

Fish were lightly anaesthetized with phenoxyethanol (SEHDEV *et al.*, 1963). A Picker Industrial Mini Shot X-ray machine with a fixed current of 10mA was used to X-ray the fish. The fish were approximately 80 cm away from the X-ray tube and laid on their side directly onto Kodak X-ray film. This arrangement made distortion and magnification effects very unlikely. An X-ray exposure for 30-35 sec with a voltage of 35 KV gave adequate results for small fishes. This dosis of soft X-rays has probably much less than 0.1 r exposure, which is the dose of a normal chest X-ray (HICKS & D'AMATO, 1968); however, without proper dosimetry the absolute dose of X-rays cannot be determined.

These X-rays became instantaneous records of the fish's skeletal morphology. I am not the first to use radiographs for morphological studies of fishes (i.e. BAREL *et al.*, 1977; BOOKSTEIN *et al.*, 1985).

To allow comparisons between measurements from (two-dimensional) X-rays and (three-dimensional) specimens, I took the same measurements from a group of 55 specimens of the Neotropical cichlid fish *Cichlasoma managuense*. Specimens ranged in size from 20.1 mm to 59.2 mm. The measurements included standard length (SL), head length (HL), lower jaw length (LJL), snout length (SnL), eye length (EyL), cheek depth (ChD), and snout acuteness (SnA). These measurements (fig. 1) were measured as defined by BAREL *et al.* (1977). I used digital calipers (Fowler Co.) to measure these distances in the specimens and their X-rays. However, the use of a digitizing tablet will probably speed up the measurements of X-rays without a loss of acuracy.

These measurements and their ratios are important in the determination of the potential mode of feeding employed by cichlid fishes (BAREL, 1983; WILHELM, 1984; WITTE, 1984; MEYER, 1987, MS).

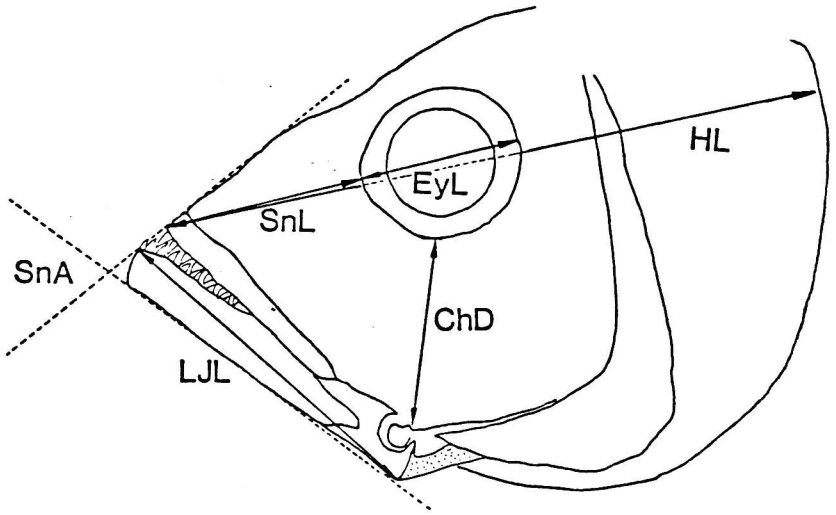

Fig. 1. Measurements taken as defined by BAREL *et al.* (1977) (Adapted from BAREL *et al.*, 1977, fig. 12).

RESULTS

Table I summarizes the results of t-tests conducted to test for differences between the two methods of measuring morphology. Only one measurement, SnA, showed significant differences.

If one conducts multiple statistical comparisons between groups one needs to adjust the type I error rate. A type I error rate of $p = 0.05$ means that in one out of twenty comparisons one significant difference is expected by chance alone. The simplest solution to this problem is the Bonferroni procedure; to maintain an overall significance level of

TABLE I

Means and standard deviations of all log-transformed measurements from specimens and their X-rays (N = 55). SnA was not log-transformed.

Measurement	*Specimens*		*X-rays*			
	Mean	*SD*	*Mean*	*SD*	*t-value*	*p-value*
SL	1.46	0.123	1.47	0.125	0.57	0.572
HL	1.08	0.126	1.06	0.120	0.84	0.404
LJL	0.79	0.136	0.78	0.142	0.23	0.817
SnL	0.50	0.141	0.52	0.158	0.67	0.501
EyL	0.60	0.080	0.60	0.88	0.23	0.815
ChD	0.28	0.177	0.34	0.172	1.84	0.064
SnA	68.19	4.25	64.03	5.39	4.60	0.000

$p = 0.05$ one divides the type I error rate by the number of comparisons. This adjusted significance level will be approximately equal the original type I error rate (SOKAL & Rohlf, 1981). I conducted seven comparisons of means in table I and therefore the adjusted type I error rate is (0.05/7) about 0.007.

Steeper angles in snout acuteness were measured from specimens than from X-rays. The difference in SnA was significant. This points to a problem in taking measurements from X-rays. The fishes mouths were not closed entirely in some specimens. For most measurements this does not create a problem because the lengths of bones or the count of meristic characters is not effected by this, however, the measurement of angles may be impaired. Similarly, the measurement of SnA from specimens is equally difficult if the jaws are not closed. In the cases of fish with their mouths open I estimated the angle of the snout acuteness with closed jaws. This procedure may account for the differences observed in both measurement techniques.

Table II shows the results of slope-comparisons between the two ways of measuring. If one uses the head measurements from the specimens plotted against SL as a standard slope then a parallel slope with a higher or lower Y-intercept in the X-ray measurements means that the measurements are higher or lower than the standard way of measuring irrespective of the size of the fish. A steeper slope in X-ray measurements than from specimens in a positive correlation with SL means that the measurements are (1) lower in small sized fish and/or (2) higher in larger sized fish. The opposite is the case in a less steep slope than the standard slope.

With a adjusted significance level none of the slopes are significantly different (table II). The measurement techniques do not differ in their results and the X-ray method is validated by this analytic comparison.

TABLE II

Comparison of slopes regressed on SL; p-values stem from tests for heterogenity of slopes. The statistical tests were performed on log-transformed data (except SnA). The type I error rate was adjusted for multiple comparisons (see Results) at about $p = 0.008$, yielding an overall significance level of $p = 0.05$. Hence, none of the slopes are significantly different.

Measurement	*Y-intercept*		*Slope*		*p-value*
	Specimens	*X-rays*	*Specimens*	*X-rays*	
HL	-.491	-.417	1.03	1.00	0.021
LJL	-.813	-.872	1.00	1.12	0.468
SnL	-1.12	-1.19	1.11	1.16	0.490
Eyl	-.330	-.388	0.64	0.67	0.292
ChD	-1.74	-1.61	1.39	1.33	0.410
SnA	103.4	100.1	-24.2	-24.6	0.948

DISCUSSION

X-rays are usefull for small living or delicate fish utilized, for example, in studies on the ontogeny of morphology because the fish are not harmed and particular individuals can be sampled repeatedly during ontogeny. Ontogenetic allometric coefficients can be calculated more accurately, avoiding the problem of measuring an individual only once.

To the best of my knowledge, nothing is known about the potentially adverse effects of low dose X-rays on the allometric growth of fishes (HICKS & D'AMATO, 1968; JACOBSON, 1968). One reviewer pointed out that fish may be more susceptable to X-rays than land animals. My studies (MEYER, 1987) let me to believe that this procedure does not adversely influence ontogenetic development. However, any potentially adverse effects of X-rays and anaesthesia can easily be accounted for with proper controls. It is possible to test for the effect of the anaeasthesia by repeatedly X-raying a group of fish without aneasthesia and compare their morphology after a predetermined time or number of X-rays with a group of (genetically similar organisms like siblings) fish that had been anaesthetized during the X-ray procedure. Similarly the effect of the X-rays could be tested by following the same procedure as above but to only anaesthetize the fish without X-raying them.

Some potential problems with the method remain. Young fish may not be fully ossified and therefore give incomplete radiographs. The measurement of morphologic characters from radiographs of small fishes (less than 15 mm SL) creates technical problems. However, it is also difficult to measure living fish of that size without harming them. Cichlid fishes used for this study seem ideal subjects because most of them are compressed fishes. My method may not be applicable to ontogenetic studies of more robust or even depressed fishes. If during ontogeny the head width changes in some positive allometric way, which is faster than allometric growth in other dimensions, potentially a distortion of measurements could arise through the change of the angle of some measurements with the plane of the X-ray film. In the species tested this did not seem to be a problem.

Longitudinal studies on the development of morphology which directly test the environmental influence on morphology are important, and warrant increased attention to and refinements of this method.

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