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An Application of Automated Feature Extraction and Geometric Morphometrics: Temperature-related Changes in Body Form of *Cyprinus carpio* Juveniles

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ABSTRACT

Automated image acquisition and geometric morphometric analysis are applied to the study of morphological ecophenotypism in the fry of carp, Cyprinus carpio, *of the same progeny reared in aquaria varying the water temperature. The image analysis procedure described here provides an* in vivo *method of detection and the extraction of simple features: in this sense it can be considered a powerful tool to obtain a preliminary insight of the occurring shape changes and the acquisition of large amounts of data without sacrifying fishes. On the other hand, the recently established geometric morphometrics offers a detailed description of shape differences and growth trends in the external morphologies by analysing the geometric properties of the form. Thus, the approach here described could be applied to a wide range of fisheries topics.*

INTRODUCTION

The morphometric approach is becoming a powerful technique for a variety of biological problems in fishery biology. In fact, multivariate morphometrics, first applied in systematic and autoecological studies of fish (Bookstein *et al.*, 1985; Strauss, 1985; Meyer, 1987; Meyer, 1993) as multivariate statistics of distance measures, has been recently applied in aquaculture and fishery biology. Problems such as identification of fish larvae (Froese, 1989; Edwards & Morse, 1995), estimating stock composition in mixed stock fisheries (Fournier *et al.*, 1984), wild stock characterization (Claytor and MacCrimmon, 1988; Corti *et al.*, 1988), discrimination among growth patterns of reared populations (Loy *et al.*, 1995) and monitoring of the larval quality on the basis of the external morphological traits (Boglione *et al.*, 1993; Marino *et al.*, 1993), all could take advantage of the integration of morphometric tools and automated systems in defining standard procedures.

Recent advances in the field of image acquisition and analysis and of morphometric techniques could provide an excellent tool for quickly acquiring and accurately analysing biological features, enhancing the quality and increasing the quantity of morphological data. The measuring of several shape features used in morphometric analysis, such as areas, perimeters, profiles and landmarks, is usually a time-consuming operation. Conversely, the same features are easily detected and acquired and partially analysed with suitable machine vision techniques. Many systems are available, ranging from inexpensive PC systems (see Becerra *et al.*, 1993 for a review) to more sophisticated hardware, such as the Quantimet 970 of the Cambridge Instruments. The recently established geometric morphometrics treats biological forms as geometric objects (Bookstein, 1991; Rohlf and Marcus, 1993), maintaining all their properties in space. In this way it is possible to reconstruct and compare forms and eventually extrapolate morphological tendencies according to particular rearing conditions.

In this paper some preliminary results are reported on automated image acquisition and geometric morphometric analysis. These results take their origin from a research programme aimed at the study of morphological ecophenotypism in the carp, *Cyprinus carpio*. The great plasticity of the carp and the temperature as a rearing condition easily kept under strict control, offered a valuable tool to design and check a standard procedure. The possibility to use the Quantimet 970 for automated feature acquisition and to apply geometric morphometrics is verified.

MATERIALS AND METHODS

The progeny of a male and a female of mirror carp was obtained by induced reproduction (Woynarovich and Horváth, 1981). Fertilized eggs were incubated in aquaria at different temperatures, i.e. 20, 25 and 30°C, and larvae at hatching reared at the same temperatures, with all other rearing conditions (density, feeding, etc.) held constant. Larvae hatched after 40 hr from spawning at 30°C, after 45 hr at 25°C, and between 80 and 200 hr at 20°C. After 3 months 130 individuals were sampled, approximately 40 individuals for each experimental condition (Table 1). Specimens were anaesthetised with MS222 (40 mg/litre) and directly posed under the macro-viewer of the computerized image analysis system Quantimet 970 (Cambridge Instruments), equipped for the image acquisition with the high resolution scanner Chalnicon (Fig. 1).

TABLE 1

Means and standard deviations of total length (mm) and weight (g) of *Cyprinus carpio* juveniles reared at different temperatures

Temperature (°C)	*TL ± SD (mm)*	*W ± SD (g)*
20	39·33 ± 6·77	1·22 ± 0·79
25	60·31 ± 9·38	3·51 ± 1·64
30	51·36 ± 8·74	2·38 ± 1·44

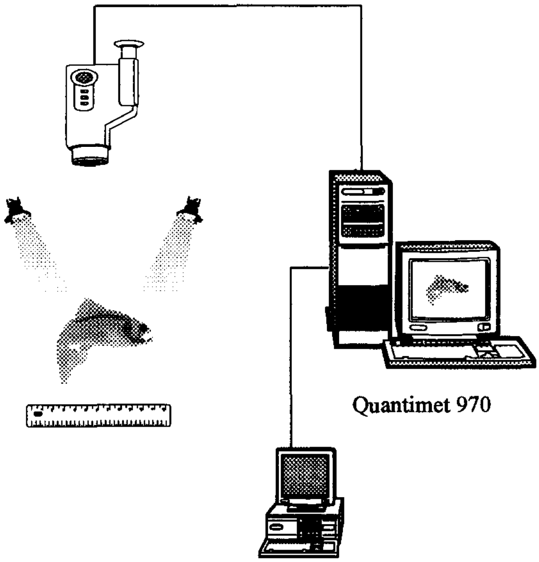

Fig. 1. Automated feature acquisition: devices.

The following features were automatically recorded: the *coefficient of roundness* (RO), defined as $P2/4\pi A$, where P is the perimeter and A the area and utilized in other studies such as Canini *et al.* (1992); *total area* (TA) and *partial area* (PA), that portion of the area between the caudal insertion of the dorsal fin and the tip of the mouth. Partial area was already considered a highly variable character among carp populations (Schäperclaus, 1962). Both areas are surfaces projected onto the two-dimensional sagittal plane. Characters were automatically extracted using the software Quips vol. 7·1.

Geometric morphometrics is based on the detection of *homologous landmarks*, in the form of x, y and, eventually, z coordinates, which can be located unambiguously from specimen to specimen. In the present study 15 landmarks were collected (Fig. 2) using the pointing device of the Quantimet 970. Different methods have been developed for the study of shape change as superimposition of individual landmark configurations according to different fitting criteria (see Rohlf and Marcus, 1993 for a review). Here we want to show the results of the 'thin-plate spline' interpolating function (Bookstein, 1991). Landmarks configurations are first properly translated, scaled and rotated with a least square superposition (Generalised Least Square superposition, option GLS of the software GRF-nD; Slice, 1993). The residuals after superposition are then fitted by an interpolating function (the 'Thin-plate Spline' of Bookstein, 1989; Bookstein, 1991). The differences among individuals are then interpreted as variation of the coefficients of the interpolating function and are summarized by the 'Weight matrix'. The weight matrix contains then the projections of the deviation of landmarks locations of each specimen from a consensus configuration onto the

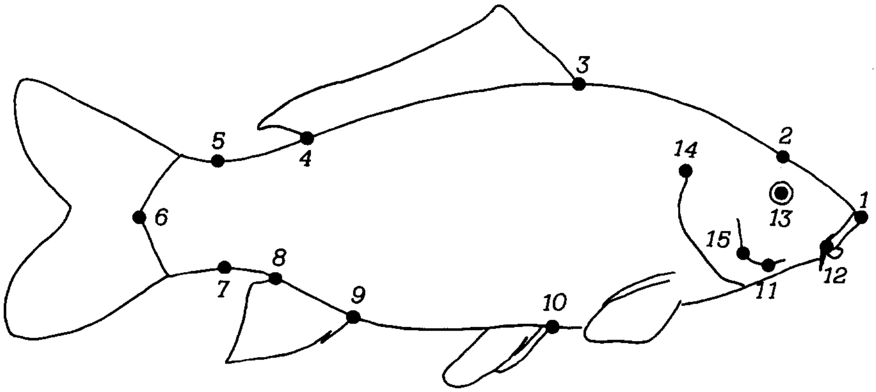

Fig. 2. The 15 landmarks collected on the carp, *Cyprinus carpio*.

interpolating function. The statistical, geometrical and biological rigour of this procedure is described by Bookstein (1995). One advantage of this new technique is that it allows to reconstruct geometrically and visualize size-free shape changes, representing them as deformation of a grid, taking into account the covariation of landmarks displacements. Moreover, the weight matrix can be analyzed with any traditional multivariate technique since it bares all the informations contained in the original data set. Size differences are previously partialled out and can be analyzed independently.

The weight matrix was computed with the software TPSRW (Rohlf, 1993a) and was then regressed on the scores of each individual on canonical axes 1 and 2, using the software TPSREGR (Rohlf, 1993b). Shape changes relative to extreme values of canonical variate scores were then visualized as splines. Examples of application of geometric morphometrics in the study of fish forms are provided by Loy *et al.* (1995) and by Walker (1993).

Both roundness and ratio log(PA)/log(TA) were compared with analysis of variance and studentized Tukey–Kramer intervals were computed using SAS (procedures ANOVA and GLM) to detect significant differences among samples.

As an estimate of size we used centroid size (CS), i.e. the square root of the sum of all the squared distances between the x and y coordinates and the specimen centroid.

Centroid size was calculated with a SAS-IML program written by L. F. Marcus (Appendix of Reyment, 1991).

RESULTS

Mean centroid size values and studentized intervals are shown in Fig. 3. Centroid size was significantly highest at 25°C. Differences were not significant between 20°C and 30°C.

Differences in roundness and in the log(PA)/log(TA) ratio are presented in Fig. 4. Roundness was maximum at 20°C and minimum at 30°C, although differences were not significant (Fig. 4a). The ratio log(PA)/log(TA) was highest at 25°C, while it showed similar values at the other temperatures (Fig. 4b).

Both characters may represent a preliminary key to discriminate among rearing conditions: in particular young carps at 25°C were recognizable by their relatively wider surface of the partial area.

Splines (shape changes relative to the general consensus) related to extreme values of canonical variates 1 and 2 are shown besides and

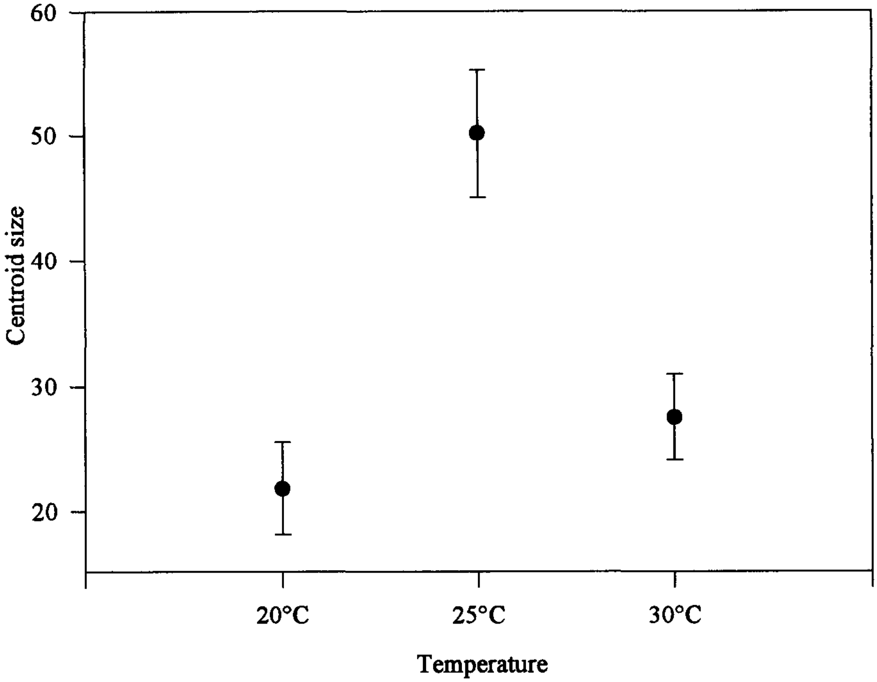

Fig. 3. Means and Tukey–Kramer confidence intervals of centroid size values relative to temperatures. Bars do not overlap where means are not significantly different ($\alpha = 0{\cdot}05$).

below the scatter plot of individuals scores on canonical axes 1 and 2 (Fig. 5). Mahalanobis distances between each pair of samples were all significantly different ($P < 0{\cdot}0001$). The group reared at 20°C was well discriminated along axis 1, which expresses major shape differences (83% of total variance). The splines describe the tendency of the warmer water samples to acquire a deep bodied profile if compared with the 20°C sample. The residual variance summarized by the second canonical axis represents shape differences between the group reared at 25°C and the other two. Again, the splines relative to these residual among-group differences showed the tendency of the 25°C sample to acquire a deeper bodied shape.

In the light of such considerations, the differences described by ratio log(PA)/log(TA) (Fig. 4b) are congruent with the statistics of canonical variate analysis and the splines associated with them.

DISCUSSION

Besides the differences in growth rate (Table 1, Fig. 3) at the three

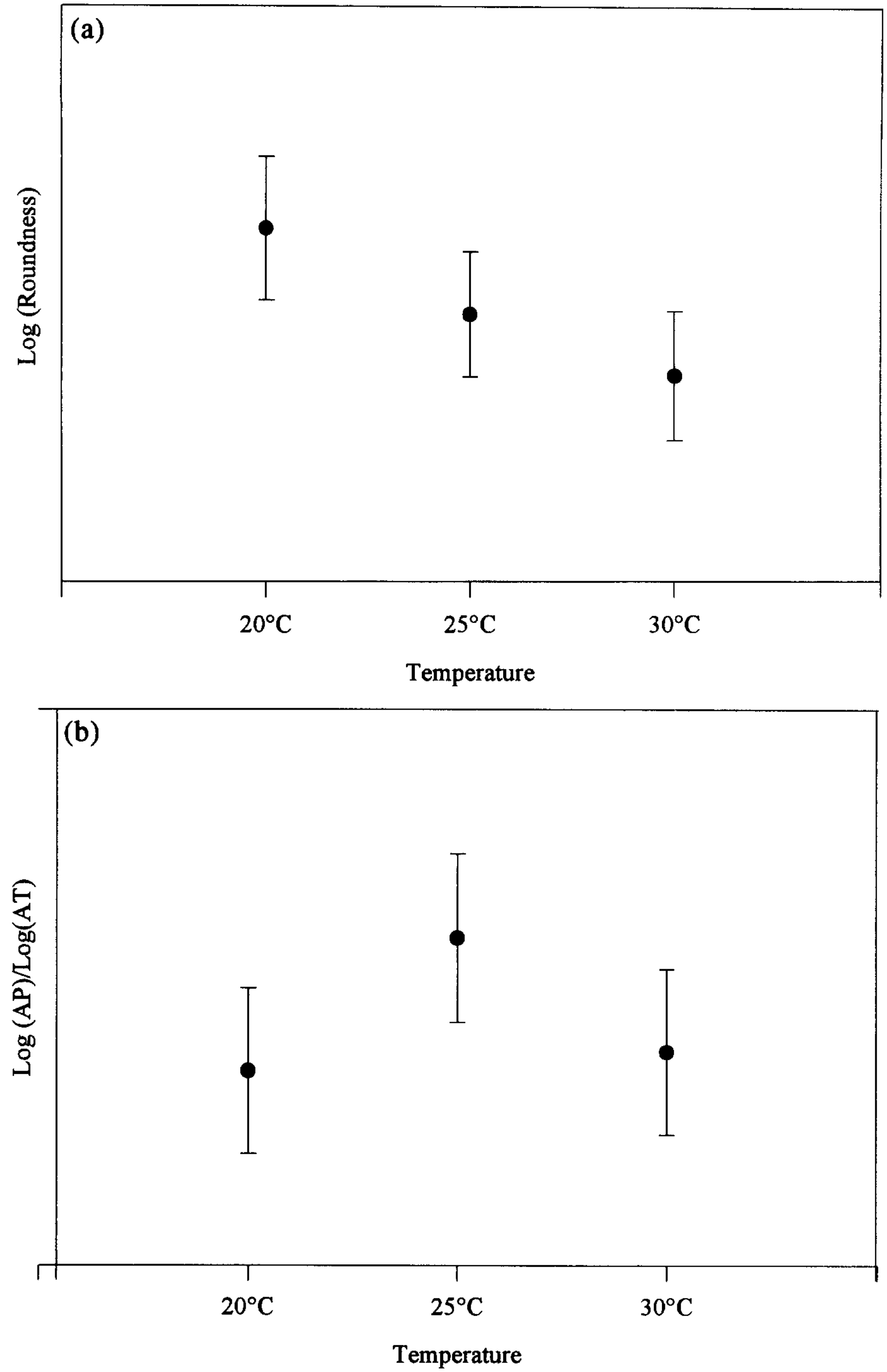

Fig. 4. (a) Means and Tukey–Kramer confidence intervals of the logarithm of the roundness coefficient relative to temperatures. (b) The same as above for the ratio log(PA)/log(TA). Bars do not overlap where means are not significantly different ($\alpha = 0{\cdot}05$).

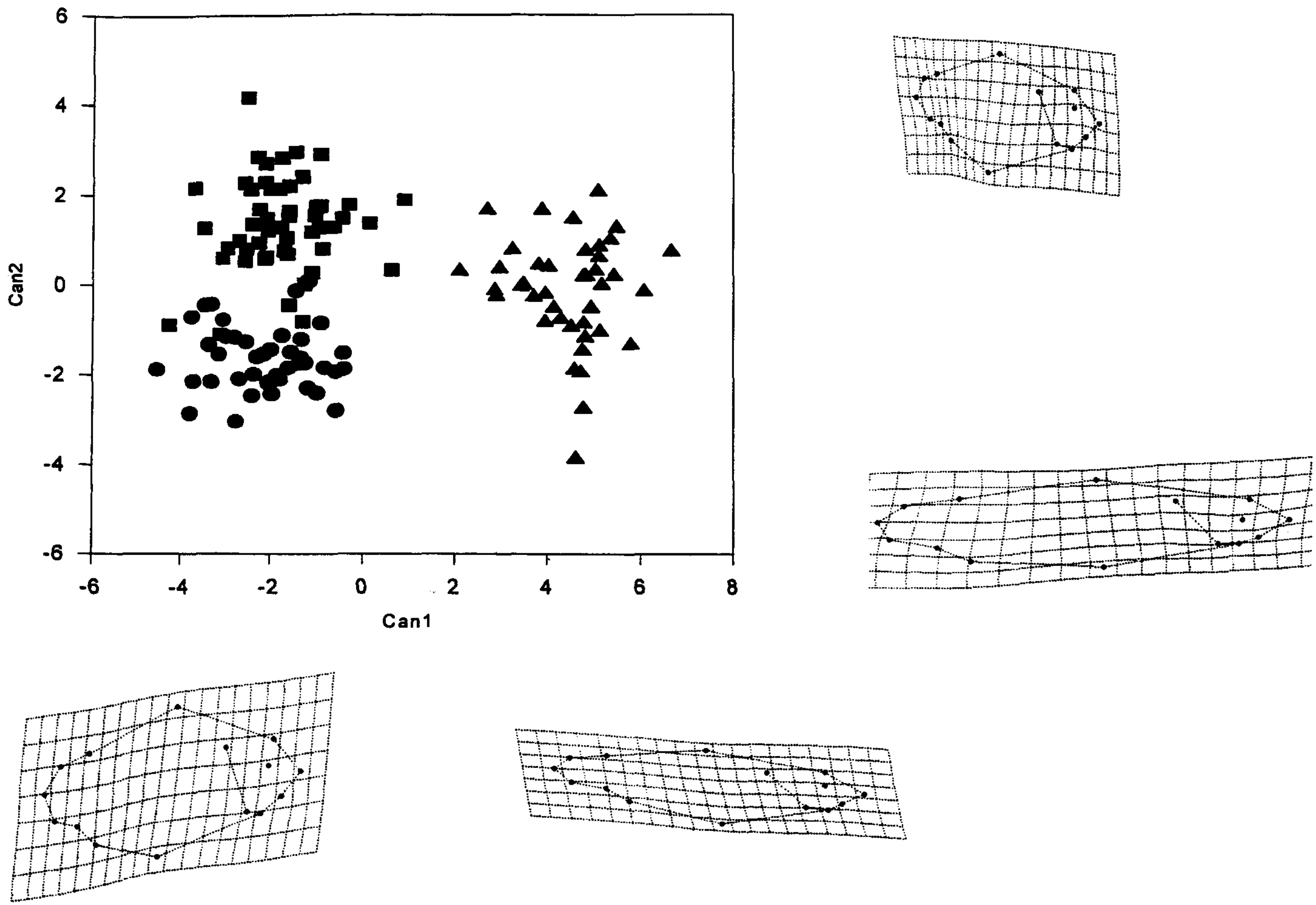

Fig. 5. Canonical variate scores for each trial and splines relative to extreme values of canonical axes 1 and 2. Circles, 30°C; squares, 25°C; triangles, 20°C.

temperatures, 3-month old carp juveniles showed marked differences in shape. The 25°C specimen tended to acquire a deep bodied profile, young carps reared at 20°C showed a more fusiform profile, while the 30°C sample showed intermediate characteristics. Those results reflect probably the great plasticity of the body shape of the common carp in response to environmental factors, as reported by Schäperclaus (1958), Stegman (1966) and Suzuki and Yamaguchi (1980). Morphological plasticity is here confirmed by the fact that all juveniles shared a common genetic origin, as they are progeny of the same parents, and has been characterized both quantitatively and qualitatively in relation to temperature.

Multivariate morphometrics has already been proved to be a convenient tool both for stock identification and monitoring in aquaculture. Corti *et al.* (1988) applied multivariate morphometrics in characterizing different morphae of common carp. Landmark-based geometric morphometrics offer the advantage of allowing to consider at a glance major trends in the external morphology (Loy *et al.*, 1995). Simple features such as the ratio log(PA)/log(TA) roughly summarize the results obtained with landmark-based morphometrics. Roundness gives information which can be integrated with the results of geometric morphometrics. By comparing RO with the ratio log(PA)/log(TA) one may suggest that the relatively smaller PA of the 20°C sample is compensated by a more rounded profile of the ventral part of the body (Figs 4a, 4b). Roundness is also related to the geometric morphometrics results: it can be noted that results based on RO (20°C sample more similar to 25°C sample than to the 30°C sample) summarize the ordering of the specimen along the canonical axis 1 (Fig. 5).

The image analysis procedure here described provides an *in vivo* method of detection and the extraction of simple features (RO, PA and TA): in this sense it can be considered a powerful tool for obtaining a preliminary insight of the occurring shape changes. In this respect, dealing with anaesthetized fish (in accordance with EEC Directive 86/609) rather than with preserved specimen guarantees unbiased results, as MS222 allows complete muscle relaxation (Sylvester, 1975), in addition to having the chance to follow the same fish during growth. The latter aspect is of particular interest when studying species for whom it may be difficult to obtain specimens, such as rare species, biomanipulated specimens or cases of complicate captive breeding.

In conclusion, this approach has a number of significant advantages in the morphometric analysis of fish shape. As a consequence, it is possible to state that this approach can be applied to a wide range of aquaculture topics, where automated feature acquisition combined with simple feature extraction might represent a radical change in the quality of results. In fact, this method offers the possibility of obtaining observations quickly for large samples, thus reducing the inaccuracy of the measurements and enabling one to acquire large amounts of data without sacrifying fish.

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