INTRODUCTION

Given its correlation to a number of other life history parameters including reproduction and survival, growth remains one of the most widely studied life history traits of fishes (Martin 1949, Beverton and Holt 1957, Beverton 1992, Hotos and Katselis 2011). Length–weight relation (LWR), Fulton’s condition factor (K), and the relative condition factor (Kn) are some of the most widely used morphometric indices to assess fish condition (Froese 2006). Differences in the parameters of LWRs and other condition indices represent spatial variations (Sparre and Venema 1998) and mirror the influence of abiotic factors and food availability on fish growth (Mommsen 1998).

In natural populations, it seldom is possible to delineate the influences of extrinsic (environmental) and intrinsic or genetic (age, sex) factors on fish growth. Besides, younger and older life history stages are ill represented in the commercial fish catches, which provide the bulk of samples from natural populations. Alternatively, laboratory studies offer a suitable alternative by offering sufficient control over the environmental variables and also by providing access to all life history stages. Nonetheless, laboratory growth experiments are not always an easy option (Magnussen 2007).

As an attempt to overcome the inherent problems associated with acquiring the age-related growth indices and with understanding the effects of age on such indices, an annual fish—the redtail notho, Notobranchius guentheri (Actinopterygii: Cyprinodontiformes: Notobranchiidae) was used as a model for aging. The redtail notho was chosen for the following reasons:

• Being a laboratory model (Gerhard 2007), it gives a possibility to exercise control over the environmental variables and thus discern the effect of genetic variables (age and sex) on the growth indices;
• Having a median lifespan of 12 months (Markofsky and Perlmutter 1972) it gives a possibility to obtain information on the growth indices for all the life history stages in a relatively short period; and

MORPHOMETRIC INDICES IN AN ANNUAL FISH—THE REDTAIL NOTHO, NOTOBRANCHIUS GUENTHERI (ACTINOPTERYGII: CYPRINODONTIFORMES: NOTOBRANCHIIDAE): INFLUENCE OF AGE AND GENDER

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Pawar R., Zhang S., Liu C. 2012. Morphometric indices in an annual fish—the redtail notho, Notobranchius guentheri (Pfeffer, 1893), were obtained by hatching the diapause eggs of the same parental lineage and reared over their entire lifespan. Length–weight measurements were recorded from 3–12 months and various indices (length–weight relation, Fulton’s condition factor, and relative condition factor) were computed and compared statistically.

RESULTS. Mean lengths, weights, length–weight relations (LWRs), Fulton’s condition factor (K) and relative condition factor (Kn) varied significantly leading to differential indices based on age and sex. Age influenced all indices positively, which is indicative of the indeterminate growth typical of fishes.

CONCLUSION. Age was not seen to suppress the growth indices in the annual fish, which is suggestive of a healthy and delayed senescence in the annual fish N. guentheri. Whether other short- and long-lived finfish follow the same pattern needs further investigation.

Keywords: Annual fish; aging, growth, morphometric indices, condition indices, indeterminate growth
• Being a model for vertebrate aging studies (Genade et al. 2005), the redtail notho provides new insights on understanding the influence of intrinsic factors, especially of aging, on the growth dynamics in fishes as a whole by covering the entire lifespan effectively.

Male *N. guentheri* have been assessed for their cross-sectional growth and body composition, age at sexual maturity in relation to longevity, and for growth differences in subgroups of varying longevities (Markofsky and Perlmutter 1972, 1973, Markofsky 1976). The presently reported study was thus conducted to elucidate the effects of age and sex on the growth dynamics of the annual fish *N. guentheri* and interpret them in the broader contexts of the evolution of aging and senescence. The study also assumes great relevance since condition indices covering the entire lifespan are lacking for the species with or without context to age and gender.

**MATERIAL AND METHODS**

**Fish and husbandry.** Laboratory-hatched embryos of *N. guentheri* were reared in 1-L beakers (at 15 embryos per L) for two months followed by rearing in 40-L glass aquaria (at one fish per L of water) till the end of the experiment. Larvae were fed freshly hatched artemia nauplii twice a day; live tubifex worms were fed ad libitum from the third month onward in two rations per day. Water parameters were maintained in strict accordance with the requirements for the species, which prefers soft waters with near neutral pH. Mean water temperature and pH during the entire culture duration was maintained at 27 ± 1°C and 7.1 ± 0.1, respectively. Experimental protocols involving the use of live fish were in accordance with the Ocean University of China’s guidelines.

**Data collection.** LWR estimates for *N. guentheri* were recorded separately for males and females for 3–12 month-old *N. guentheri* population obtained from the same parents. Prior to each sampling, fish were not fed for 24 h to ensure uniform condition and were anesthetized using MS 222 (Sigma, St. Louis, USA) at 120 mg · L⁻¹. Individual fish were measured for their total length (TL) [cm] to the nearest 0.1 cm and body weight (BW) [g] to the nearest 0.001 g. Lengths were measured by placing the fish on a graph paper; weights were determined using an analytical balance. Fish were briefly placed on a soft moist cloth to remove excess body moisture before weighing them individually.

**Data recording.** Length–weight data were recorded separately under three categories based on sex namely, both or combined sexes (B; n = 92), males (M; n = 29), and females (F; n = 63). Since the average lifespan of *N. guentheri* stocks raised and maintained in the laboratory was ~12 months (Pawar, unpublished*), the length–weight data were further segregated into three periods of four months each namely 1, 2, and 3 representing the first, second and the third four-month intervals of lifespan respectively. This resulted in a total of 12 categories or subsets: (B, M, F); (B1, B2, B3); (M1, M2, M3) and (F1, F2, F3). The same nomenclature has been adopted for all analyses and discussions in the presently reported study. However, in order to fulfil the requirements of ANOVA associated with the estimation of b-value of the LWRs, the length–weight data were segregated into two halves (1 and 2) spanning the first and second six months of the lifespan for each category instead of three four-monthly groups as mentioned earlier. Thus, b-values were derived for nine subsets in all (B, M, F); (B1, B2); (M1, M2) and (F1, F2).

**Models used.** Age- and sex-based length–weight data were analyzed using different models for obtaining of following growth indices. Parameters a and b were obtained by logarithmic transformation of the length–weight relation (Ricker 1975) bearing the form

\[ W = aL^b \]  

where, \( W \) = body weight [g], \( L \) = total length [cm], \( a = \) constant, and \( b = \) regression coefficient. Fulton’s condition factor, \( K \) (Fulton 1904) was estimated from the length–weight data using the equation

\[ K = 100WL^{-3} \]

where, \( W \) = fish weight [g], \( L \) = total length [cm]. Multiplying \( WL^{-3} \) by 100 brings the value of \( K \) to unity (Froese 2006). Relative condition factor, \( K_n \) (Le Cren 1951) was estimated by the equation \( K_n = WW_E^{-1} \), where \( W_E \) = the length-specific expected mass predicted from the LWR. In the presently reported study, the common (and not the sub-set specific) LWR derived for the *N. guentheri* population as a whole was employed in deriving the length-specific expected mass (\( W_E \)).

**Statistical analyses.** Ordinary least square regression of log-transformed weight on length was performed. Significance of the LWR regressions were assessed by analysis of variance (ANOVA) in testing the hypothesis \( H_0: \beta = 0 \) against \( H_a: \beta \neq 0 \) (Zar 1996). Student’s t-test (Zar 1996) was used to:

- test whether \( b \) were significantly different from 3, the isometric growth (\( H_0: \beta = 3 \) against \( H_a: \beta \neq 3; \alpha = 0.05 \));
- compare the slopes of the LWRs for differences between sexes and/or ages; and
- compare the mean lengths, weights, \( K \) and \( K_n \) between different sub-subs.

Shapiro–Wilk normality test was used to test the normality of distribution of \( b \) (\( \alpha = 0.05 \)). Outliers for parameters \( a \) and \( b \) were detected by determining the 95% confidence limits (CL) for mean \( a \) and \( b \).

**RESULTS**

**Distribution of length and weight in *N. guentheri*.** The total length of *N. guentheri* ranged from 1.5 to 5.5 cm; mean = 2.9 cm (standard error of the mean SE = 0.8) and the body weight from 0.044 to 2.036 g; mean = 0.371 g (SE = 0.314). Mean lengths, weights, and their ranges for the 12 subsets are given in Table 1.

Among the sexes, females (F) had the lowest-, combined sexes (B) the intermediate-, and males (M) the highest mean length–weight values thus following the pattern (F < B < M). Within categories, the trend was for the younger groups (F1, B1, M1) to have lower mean length–weight values as

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compared to the median age group (F2, B2, M2), which had intermediate values, followed by the older subsets of the population (F3, B3, M3). Thus, the overall length–weight distribution trend for *N. guentheri* in the presently reported study could be summarized by the formula:

\[(F1 < F2 < F3) < (B1 < B2 < B3) < (M1 < M2 < M3)\]  

(3)

In statistical terms, the observed trend for the distribution of length across age and gender was:

\[(F1 <<< F2 <<< F3) ~ (B1 <<< B2 <<< B3) << (M1 <<< M2 <<< M3)\]  

(4)

where, ( <) = \(P < 0.05\), (<<) = \(P < 0.01\), (<<<) = \(P < 0.001\), and (~) = \(P > 0.05\).

Mean weights closely followed the distributional trends in length with but one difference. For length, F did not differ significantly from B (F ~ B) whereas, in terms of weight F differed significantly from B (F < B).

Statistically, distribution of weight in *N. guentheri* followed the trend:

\[(F1 <<< F2 <<< F3) < (B1 <<< B2 <<< B3) << (M1 <<< M2 <<< M3)\]  

(5)

Formulae (4) and (5) are depicted graphically in Figs. 1a and 1b, respectively.

**Length–weight relation.** Regression parameters of LWR, namely \(a\) and \(b\) (and their CL95%), the coefficient of determination (\(r^2\)), and associated growth patterns for *N. guentheri* subsets are given in Table 2. All LWRs were highly significant (\(P < 0.001\)). Except for F1 and F2, remaining \(b\)-values were found to lie within the valid range of 2.5–3.5 as proposed by Carlander (1969), with values of \(b < 2.5\) and \(> 3.5\) often reflecting a narrow size range (Carlander 1977). Considering the overall small-sized stature of *N. guentheri* and particularly its females (F) and their sub-classes (F1 and F2) within the whole population, \(b\)-values < 2.5 seem more to be an exception rather than an aberration in the proposed range. In addition, significant differences in the \(b\)-values between the subset groups were evident from formula (6)

\[(F1 << F2 ~ F3) ~ (B1 ~ B2 ~ B3) <<< (M1 ~ M2 ~ M3)\]  

(6)

Formula (6) is depicted in Fig. 2.

**Fulton’s and relative condition factor.** Fulton’s (\(K\)) and the relative condition factor (\(Kn\)) were estimated for all subsets of *N. guentheri* (Table 3). \(K\) values ranged from 0.70 to 1.62 (mean = 1.2109); and \(Kn\) values from 0.61 to 1.44 (mean = 0.9844). Mean \(K\) was about 1.23 times mean \(Kn\). Both \(K\) and \(Kn\) were highly correlated (\(r = 0.89\)) and had significant difference between their means (\(P < 0.05\)).

Between the three major categories, F had the lowest, B the median, and M the highest \(K\) and \(Kn\) values. Overall, \(K\) and \(Kn\) distribution thus followed the pattern (F < B < M).

Within each category, the trend was for the younger groups (F1, B1, M1) to have lower mean \(K\) and \(Kn\) values; the median age groups (F2, B2, M2) to have intermediate values; and the older subsets (F3, B3, M3) to have the highest condition. In statistical terms, the distribution of \(K\) and \(Kn\) can be expressed respectively as follows:

\[(F1 ~ F2 ~ F3) ~ (B1 ~ B2 ~ B3) <<< (M1 ~ M2 ~ M3)\]  

(7)

\[(F1 <<< F2 ~ F3) <<< (B1 <<< B2 <<< B3) <<< (M1 <<< M2 <<< M3)\]  

(8)

Formulae (7) and (8) are represented graphically in Fig. 3a and 3b respectively.

**DISCUSSION**

In the presently reported study the length–weight data covered the young and old fish alike. Age- and sex-based subsets of *N. guentheri* were found to have differential LWRs; all LWRs conformed to most statistical standards and were highly significant (\(P < 0.001\)).

Except for F1 and F2, remaining \(b\)-values were found to lie within the valid range of 2.5–3.5 as proposed by Carlander (1969), with values of \(b < 2.5\) and \(> 3.5\) often reflecting a narrow size range (Carlander 1977). Considering the overall small-sized stature of *N. guentheri* and particularly its females (F) and their sub-classes (F1 and F2) within the whole population, \(b\)-values < 2.5 seem more to be an exception rather than an aberration in the proposed range.

LWR parameters are known to be influenced by water quality or food availability via fish growth (Mommsen...
Temperature and/or starvation influence the parameter $b$ so much that it is possible to produce different growth stanzas experimentally through marked temperature changes or by feed restrictions alone (Martin 1949). In the presently reported study, diurnal and inter-annual variations of abiotic- (temperature, pH, dissolved oxygen) and biotic (food availability) components were effectively taken care of. Thus, the observed differences in the $b$ values and of the condition factors for *N. guentheri* could therefore be ascribed to the endogenous (genetic) influences in the form of age and sex on the growth. This is in accordance to the differential LWRs obtained for perch for different life stages, sexes, and stages of gonad development (Le Cren 1951). The existence of differential LWRs in *N. guentheri* validates these findings and justifies the need for examining and determining separate LWRs taking into account the sex and/or age (growth stanzas) of the population.

All the female- (F, F1, F2) and the common subsets (B, B1) displayed negative allometric growth ($b < 3$); all the male subsets (M, M1, M2) and B2 followed an isometric pattern ($b = 3$) (Table 2). Thus, age had a conspicuous positive influence on the growth pattern of *N. guentheri* as the older half of the population (B2) showed highly significant ($P < 0.001$) $b$-values than B1. The mean $b$ of nine LWRs representing different growth stanzas (ages and sizes) showed negatively allometric growth pattern ($b < 3$; mean $b = 2.711$) which, appropriately (Froese 2006) describes the growth type for *N. guentheri* as a whole.

Condition factors yield values close to unity and values $> 1$ indicating better condition than an average individual.

| Subset | $n$ | $a$  | $b$  | SE ($b$) | $r^2$ | $a$ (CL$_{95\%}$) | $b$ (CL$_{95\%}$) | Growth pattern |
|--------|-----|------|------|----------|-------|-----------------|-----------------|----------------|
| B      | 92  | 0.0167 | 2.711 | 0.075 | 0.94 | 0.0143–0.0196 | 2.562–2.860 | N              |
| B1     | 66  | 0.0191 | 2.517 | 0.100 | 0.91 | 0.0158–0.0231 | 2.317–2.717 | N              |
| B2     | 26  | 0.0150 | 2.870 | 0.197 | 0.90 | 0.0088–0.0256 | 2.464–3.276 | I              |
| M      | 29  | 0.0121 | 3.057 | 0.092 | 0.98 | 0.0096–0.0152 | 2.869–3.245 | I              |
| M1     | 17  | 0.0135 | 2.929 | 0.112 | 0.98 | 0.0103–0.0176 | 2.690–3.168 | I              |
| M2     | 12  | 0.0124 | 3.062 | 0.274 | 0.93 | 0.0054–0.0285 | 2.451–3.672 | I              |
| F      | 63  | 0.0192 | 2.516 | 0.097 | 0.92 | 0.0159–0.0233 | 2.322–2.711 | N              |
| F1     | 49  | 0.0219 | 2.309 | 0.126 | 0.88 | 0.0176–0.0274 | 2.055–2.563 | N              |
| F2     | 14  | 0.0248 | 2.425 | 0.211 | 0.92 | 0.0138–0.0446 | 1.966–2.885 | N              |

SE = standard error of the mean; F = females, B = both sexes, M = males; Suffixes 1, 2, and 3 represent up to 4-, up to 8-, and up to 12- month periods of age, respectively within each category; The growth pattern is based on t-test for $H_0$: $\beta = 3$ against $H_A$: $\beta \neq 3$; N = negative allometric growth ($b \neq 3; P < 0.05$); I = isometric growth ($b = 3; P > 0.05$); CL: confidence limit.
vidual with the same length and vice versa (Knaepkens et al. 2002). Based on the mean Fulton’s condition factor (K) value of 1.21, *N. guentheri* population as a whole appeared to conform to good health condition. Besides, all subsets also possessed K values > 1. The mean relative condition (*Kₙ*) value of 0.98 was marginally < 1 (*P > 0.05*). However, all the older subsets: B3, M3, and F3 had values > 1 indicating an improvement in health status with age. For sexes combined, *Kₙ* increased consistently with age (formula 8).

Fulton’s condition factor, tended to overestimate condition since K is based on the assumption of isometric growth (*b* = 3) whereas, the average *N. guentheri* population, in this study, exhibited negative allometric growth (*b* < 3). Relative condition factor (*Kₙ*) thus seems more sensitive and appropriate in predicting the condition of the redtail notha as it better accounts for the deviations of an individual from the average weight for given length (Le Cren 1951) and is therefore recommended for exploring relative condition of individuals within a sample (Froese 2006).

The morphometric indices (*L, W, b, K, Kₙ*) investigated in the presently reported study displayed conspicuous age- and gender- based trends. Age appeared to influence the indices positively. In particular, within a subset the older subsets (B3, M3, and F3) possessed higher indices for all the parameters tested as compared to the younger subsets. The findings indicate that aging does not retard growth or the health condition in the annual fish *N. guentheri* and thus validate the phenomenon of indeterminate growth as evidenced in most ectotherms. Secondly, gender or sex of the specimens also influenced all the morphometric indices greatly. Specifically, all the female subsets had the lowest values for all the indices as compared to their male counterparts with the combined subset (B) taking the median position. Thirdly, the Fulton’s condition factor (K) cannot provide a good estimate of the condition differences between the different growth stanzas since the growth pattern of the species investigated does not conform to the isometric type. Therefore, the relative condition factor appears to be a reliable index of assessing fish condition spanning all life history stages.

From an evolutionary perspective, the results ratify the phenomenon of delayed senescence arising out of indeterminate growth as common to fishes. Although the data presented herein covered the majority lifespan of *N. guentheri*, no signs, if any, of aging or senescence were evident from the obtained health indices at any stage for any age group for the combined population or the individual sexes. On the contrary, the condition indices improved with age, which is in complete agreement with Reznick et al. (2002) that the older age classes contribute greater to fitness in fishes and, according to Hamilton (1966) and Abrams (1993), such conditions favour and support delayed senescence.

In summarizing, the intrinsic factors—age and sex—were found to influence the growth indices pronouncedly in *N. guentheri*. The aging process proper did not seem to influence somatic growth in *N. guentheri* negatively since fish of the older age groups possessed better condition as compared to the younger groups. Sex-wise, the males displayed higher indices on all counts when compared to their female counterparts. Collectively, the results are indicative of negligible senescence (Keller and Murtha 2004).

### Table 3

| Subset | *n* | *K* | *Kₙ* |
|--------|-----|-----|------|
| B      | 92  | 1.21| 0.98 |
| B1     | 29  | 1.21| 0.89 |
| B2     | 63  | 1.20| 0.98 |
| B3     | 35  | 1.26| 1.12 |
| M      | 35  | 1.30| 1.10 |
| M1     | 22  | 1.26| 0.99 |
| M2     | 10  | 1.27| 1.09 |
| M3     | 10  | 1.45| 1.31 |
| F      | 9   | 1.17| 0.92 |
| F1     | 25  | 1.16| 0.82 |
| F2     | 25  | 1.18| 0.93 |
| F3     | 13  | 1.16| 1.01 |

SE = standard error of the mean; *K* = Fulton’s condition factor, *Kₙ* = relative condition factor, F = females, B = both sexes, M = males; Suffixes 1, 2, and 3 represent up to 4-, up to 8-, and up to 12- month periods of age, respectively within each category.

![Fig. 3](image-url)
in the annual fish *N. guentheri* in terms of the morphometric growth indices. Condition indices describing a majority of the lifespan of a finfish in the form of a statistical equation have been proposed for the first time.

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