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Evaluation of external anchor tags for estimation of growth and survival in Tilapia (*Oreochromis shiranus chilwae* Trewavas).

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ABSTRACT

The effects of tagging with Floy FD-68B T-bar anchor tags on estimates of growth in Tilapia (*Oreochromis shiranus chilwae*) were investigated in a pond and in a field experiment. In the pond experiment, mean length increments of tagged and marked fish were compared. In the field experiment growth of tagged and "untouched" individual wild fish were compared by measuring scale circuli spacing (Circ.), which is correlated to instantaneous growth rate. Length increments of tagged and untagged/marked fish were not significantly different in either experiment . In the pond experiment, the total mortality rate in the small tagged fish was significantly higher than in the marked fish. The recoveries of tagged fish in the pond experiment increased with fish size. Recoveries exceeded 80% at lengths over 13 cm TL. The ratios of tagged to marked recoveries were 1.02 and 0.74 for large and small fish respectively.

The study shows that tagging of Tilapia with Floy anchor tags does not in general alter the growth rates of the fish.

INTRODUCTION

Growth and mortality rates are essential inputs in many models of fish populations. In temperate waters the required information can be deduced from analyses of annual growth rings, formed on hard parts such as scales and otoliths as an effect of seasonal variation in growth, combined with the size structure of the population. Further, in temperate waters spawning usually occurs once a year in a short and well-defined period, which facilitates the recognition of year classes or cohorts by size alone.

In the tropics, seasonality is less pronounced and reproduction may take place over extended periods, sometimes spanning most of the year. Seasonal growth checks on hard parts are often absent or weakly developed, and year classes may be difficult to distinguish by size. An alternative technique developed for tropical fish uses daily growth rings in otoliths (PANELLA, 1971; PANELLA, 1973; GJØSÆTER *et al., 1984),* which is particularly suited for short-lived species and juveniles. However, this method is time consuming and difficult to apply on routine basis (SPARRE and VENEMA, 1992). Furthermore, collection of otoliths means that the fish have to be sacrificed. An alternative approach is measurement of marginal scale circuli spacing, which is correlated to instantaneous growth rate (DOYLE, *et al.*, 1987; TALBOT and DOYLE, 1990). However, the extrapolation of scale circuli-spacing into growth increments requires extensive calibration experiments to correct for factors such as size, age, sex, strain and seasonality of growth.

Tag-recapture investigations are difficult and costly to carry out, but provided that the assumptions hold, give direct estimates of growth and survival as well as other population parameters (JONES, 1977). In addition, tag-recapture is often the only practical way of calibrating direct methods for estimations of growth in wild populations. Growth may be generally influenced by marking and tagging, and size-specific effects can cause further variation in growth and mortality estimates (e.g., RICKER, 1949; VAN SOMEREN and WHITEHEAD, 1961; CARLINE and BRYNILDSON, 1972; ISAKSSON and BERGMAN 1978; McFARLANE and BEAMISH, 1990; McALLISTER, *et al.*, 1992).

The study consists of a pond experiment and field experiment. Pond experiments are advantageous because of the ease with which experimental conditions can be controlled. However, certain aspects of natural waters may be markedly different from the pond environment. For example, levels of predation and competition are normally higher in natural waters, which may affect tag-recapture data.

Another aspect is that cultivated fish usually are less sensitive to handling than their wild counterparts. The field experiments were designed to estimate the effect of tagging on growth in *Oreochromis shiranus chilwae* Trewavas in a small un-managed reservoir by employing the relation between spacing of marginal circuli of scales and recent growth rate (DOYLE *et al.*, 1987). The experimental design allowed comparison of growth between tagged and "untouched" fish, *i.e.*, essentially wild fish that have not been handled previously. The objective was to corroborate tag-recapture estimates of growth in *O.s. chilwae* and to get an indication of tag losses and tag-induced mortality.

MATERIALS AND METHODS

Pond experiment

A total of 594 *O. s. chilwae* of two sizes, large (mean ± S.D.; 15.4 ± 2.1 cm TL and 60.7 ± 22.7 g wwt), and small (10.4 ± 1.2 cm TL and 19.2 ± 6.8 g wwt), were randomly assigned to three treatments as shown in Table 1. All fish were marked by staining with alcian blue, dissolved in water using Pan Jet injector (Wright Hea, Kingsway West, Dundee DD2 3QD, Scotland). It was assumed that this had negligible effect on growth and survival, and the treatment was labelled "unmarked" fish.

Table 1—Distribution of marks identifying treatments applied to *O.s.chilwae* in each of the ponds

	FISH SIZE	
Treatment	*Large*	*Small*
Tagged	1. Floy tag 2. Left fin clip 3. Pan Jet, front left	1. Floy tag 2. Right fin clip 3. Pan Jet, front right
Marked	1. Left fin clip 2. Pan Jet, anal fin left	1. Right fin clip 2. Pan Jet, anal fin right
Unmarked	1. Pan Jet, front left	1. Pan Jet, front right

Two thirds of the fish were marked by clipping of the pelvic fin ("marked fish") and half of these (one third of all the fish) were tagged with blue Floy FD-68B T-bar anchor tags (Floy Tag, 4616 Union Bay Place, N.E Seatle, WA 98105, USA) ("tagged" fish). The tags were attached, using a tagging gun, through the dorsal musculature between the fourth and fifth dorsal fin spine so that the anchor locked behind the pterygiophore and neural spines, and with the tag projecting rearward. The fish were randomly distributed into three 200 m^2 earthen ponds at the national Aquaculture Centre (NAC), Domasi, Malawi, on 25th March 1993. Each pond received 33 fish from each size and treatment making a total of 198 fish per pond (9,900 fish. ha^{-1}). No anaesthetics were employed, and only fish in the good physical condition were used.

The ponds were added 280 $kg.ha^{-1}.week^{-1}$ chicken manure, 82.5 $kg.ha^{-1}.week^{-1}$ maize husks, and 12.5 $kg.ha^{-1}.week^{-1}$ diammonium phosphate. If necessary, agricultural lime was applied to keep the alkalinity above 20 $mg.l^{-1}$ equivalent $CaCO_3$. The oxygen concentration and water temperature at 5.00 a.m. varied between 0.7 and 6.3 $mg.l^{-1}$, and 16.5 and 25.1^{o}C, respectively. The ponds at NAC are guarded day and night against bird predation, etc.

After 81 days the fish were harvested and enumerated by the same personnel that tagged and marked them, and therefore it was unlikely that distinct marks were overlooked. The growth (ΔTL) was calculated as the difference between mean final and mean initial length, divided by the time in days. The rate of mortality, Z, was calculated as: $Z = -\ln(N_1/N_0)/T$, where N_0 and N_1 are numbers of fish stocked and harvested, respectively, and T is the time in years, i.e., T = 81/365 = 0.222. Thus, tag and mark losses were included in the calculation of Z. Growth (ΔTL) and mortality (Z) in the pond experiment were analyzed separately for small and large fish by a 2-way ANOVA with treatment (tagged vs. marked) as fixed and pond as random effects.

Field Experiment

The Chisombezi Dam is a small reservoir (2.5 ha) located in the southern Region of Malawi. *O.s. chilwae* were captured from Chisombezi on the 13th March 1995 using a 100 m long beach seine with one inch stretched mesh, and placed in cages until tagging started. A total of 300 fish (118 males and 182 females) in the size range 9.5 - 19.1 cm TL were tagged using the same tags and procedure as in the pond experiment. After 41 days, on the 25th April 1995, 35 fish were measured and sexed by external morphology. Scales were removed from recaptured fish and untagged fish of similar size. The scales were taken from the second row above the lateral line and four scales back from the operculum on both sides of the body (TALBOT & DOYLE, 1990). Sampled scales were preserved in 10% buffered formalin. Subsequently, the scales were mounted on microscopic slides and the distance from the first to the fourth circular ridge, or the width of the outer three intercirculus spaces, was measured on the five radii per scale. The ten measurements obtained from each fish were averaged before analysis (DOYLE *et. al.,* 1987; TALBOT and DOYLE, 1990). The measurement of the first three intercirculus spaces (*Circ.*) represents 1 - 3 weeks of growth (DOYLE *et al.*, 1987). Besides the scales collected in Chisombezi Dam, *Circ.* was also measured on scales from a sub-sample of the tagged fish in the pond experiment (n = 33). The growth of the recaptured tagged fish (TL_i) was calculated for each individual as the difference between final and initial length, divided by the time in days.

Length is correlated with both growth and *Circ.*, and to test differences between treatments it is desirable to correct for variation in length (DOYLE *et al.,* 1987). As the initial length was only known for the tagged fish, the final length (= length at sampling) was used. The correlation between initial and final length among the tagged fish in the field experiment was high (R = 0.98). Variation in growth between the sexes in Tilapia has been well described (e.g., PAULY *et al.*, 1988). Therefore, the data from the field study was analyzed by regressing *Circ.* on treatment (0 = untagged; 1 = tagged), ln (length at sampling) and sex (1 = males; -1 = females). In addition, using pooled data from tagged fish in both experiments, Circ. was regressed on growth, ln (length at sampling) and sex. A few of the tagged fish in the pond experiment did not grow during the experimental period; these cases were not used. The growth of the untagged fish and tagged fish in the field experiment was then calculated individually by inverse prediction, i.e., the multiple linear regression equation was arranged so that growth became the predicted variable (ZAR, 1984).

RESULTS

Pond Experiment

The total recoveries were on average 92.3% and 83.2% for large and small fish, respectively (Table 2). In one case among the large fish and in two cases among the small fish were the number of recoveries of unmarked fish higher than the number stocked.

Table 2—Number of *O.s.chilwae* recovered after 81 days rearing in fish ponds

		POND		
Size	*Treatment*	*A*	*B*	*C*
Large	Tagged	31	29	30
	Marked	27	32	30
	unmarked	30	36	29
Small	Tagged	20	19	19
	Marked	25	26	28
	unmarked	33	35	42

This was most likely due to tags that had been lost and/or fin clipped fish were overlooked. The Pan Jet stain spots were distinct in all but two cases. Within size the tagged and unmarked fish were identical with respect to the Pan Jet stain spots, whereas the marked fish had unique Pan Jet spots (Table 1). Therefore, tagged fish probably contributed more to the counts of unmarked fish than the marked fish, and therefore only the effects of tagging vs. marking (fin clipping) are considered here.

The ratios of tagged to marked recoveries were on average 1.02 and 0.74 for large and small fish, respectively (Table 2). Figure 1 shows the proportion of recoveries among tagged fish plotted against midlength class at tagging. A function of form y = 1- exp (a- (x-b)) was fitted to the data. The intercept with the data x-axis, b, was fixed at 7.5 TL (the smallest tagged fish measured 7.8 cm TL) and fitted with least squares as the loss function. The function fitted was: *Proportion recovered* = 1-exp (-0.308(TL - 7.5)). The recovery fish measuring 13 cm TL or more exceeded 80%.

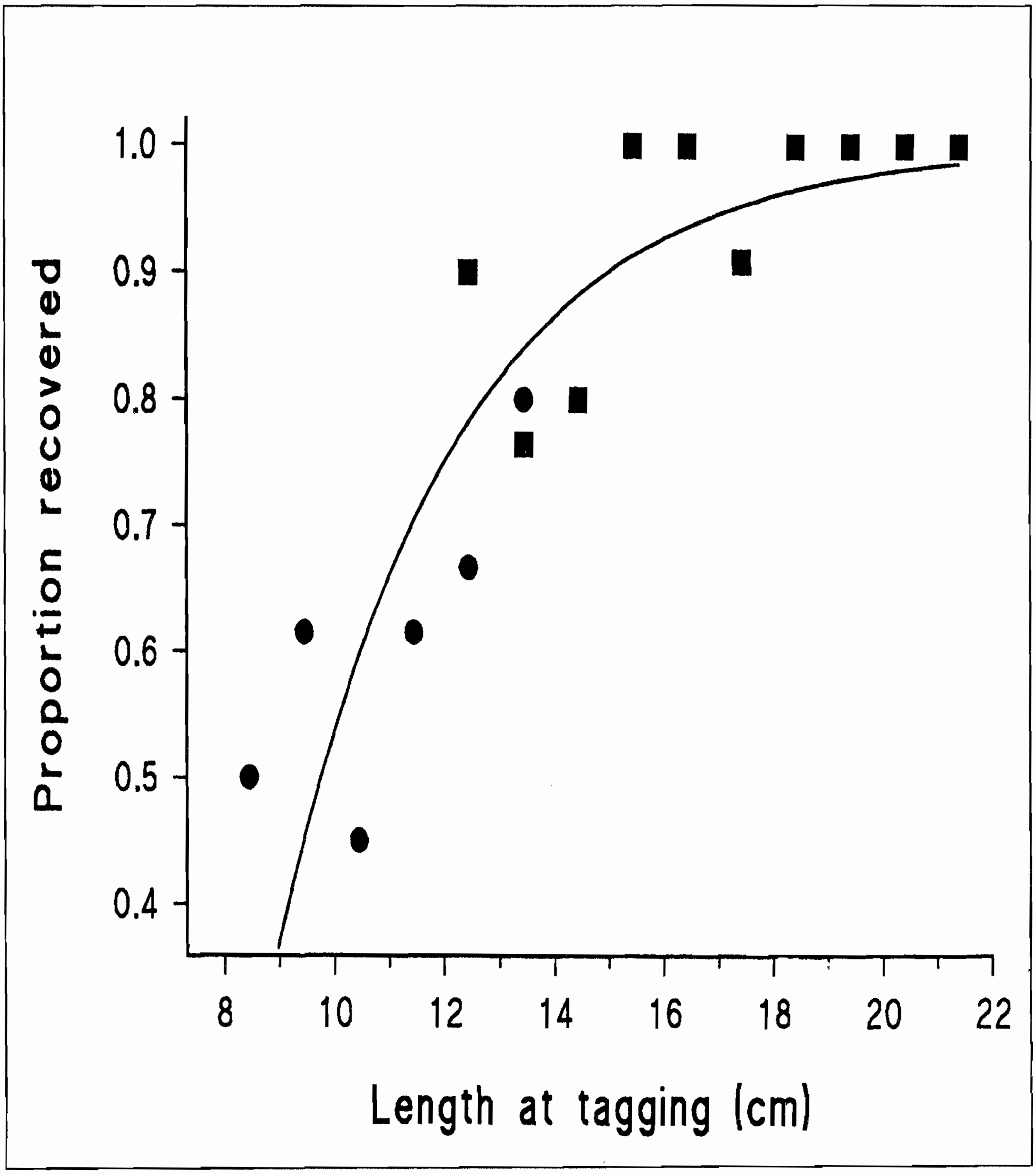

Fig. 1. Proportion of tagged *O. s. chilwae* recovered after 81 days rearing in fish ponds plotted against midlength-class at tagging. The fitted funtion is: Proportion recovered = 1-exp(-0.308(Tl-7.5), where TL is total length(R^2 = 0.74. large fish; small fish.

This experiment showed no significant differences in growth between the tagged and marked treatments (Table 3). The average growth of the small untagged fish was slightly, but not significantly (p=0.305), higher than the tagged fish. The power (1-B) of this test at a = 0.05 was low at about 0.11 (SEARCY - BERNAL, 1994). The mortality rate, Z, was significantly higher in small tagged than in small marked fish (Table 3).

Table 3. Mean growth (_TL; mm.day^{-1}), and mortality rate (Z; year includes tag and mark losses), in two sizes of tagged and marked *O. s. chilwae* reared in three ponds over a time period of 81 days. Significance of the difference between treatment means were tested by 2-way ANOVA with treatment as fixed and pond as random effect. The figures in parenthesis are standard errors of the mean.

Size	Treatment Means				
	Variable	*Tagged*	*Marked*	*F(1,2)*	*p - level*
Small					
	_TL	0.273 (0.065)	0.304 (0.045)	1.870	0.305
	Z	2.41 (0.077)	1.02 (0.150)	41.92	0.023
Large					
	_TL	0.119 (0.030)	0.119 (0.045)	0.000	0.999
	Z	0.432 (0.087)	0.491 (0.223)	0.037	0.865

Field Experiment

The length at tagging of the recaptured fish did not differ significantly from the length of the tagged fish that were not recaptured (Mann-Whitney U-test). The lengths of the recaptured and untagged fish from which scale samples were collected are given in Table 4. The tagged fish grew on average 14.4 mm (range 9 -25 mm) during 41 days that elapsed between tagging and recapture.

Table 4. Break down of results from the field experiment. TL is the mean of total lengths at sampling. Circ. is the mean of individual measurements of the width of the outer scale intercircular spaces. $\_TL_i$ is the mean of the individual length increments of *O. s. chilwae* over 41 days. $\_TL_{i.est}$ is the mean of the predicted individual length increments using reverse prediction and the linear model of Table 5 Figures in parenthesis are standard deviations. For further explanation see text.

	n	*TL (cm)*	*Circ. (µm)*	$\_TL_i$ *(mm.day^{-1})*	$\_TL_{i.est}$ *(mm.day^{-1})*
Untagged	37	16.40 (1.78)	111.5 (6.1)		0.419 (0.061)
Males	16	17.39 (1.86)	113.4 (6.4)		0.457 (0.057)
Females	21	15.64 (1.31)	110.0 (5.5)		0.390 (0.047)
Tagged	35	15.75 (1.74)	107.9 (8.4)	0.351 (0.075)	0.390 (0.07)
Males	10	17.23 (92.09)	111.9 (7.4)	0.371 (0.057)	0.447 (0.040)
Females	25	15.16 (1.17)	106.3 (8.3)	0.343 (0.081)	0.367 (0.067)

Figure 2 shows the relation between length at sampling and *Circ.* categorized by sex and treatment (tagged/no tag.). Simple linear regressions were fitted for each sex and treatment.

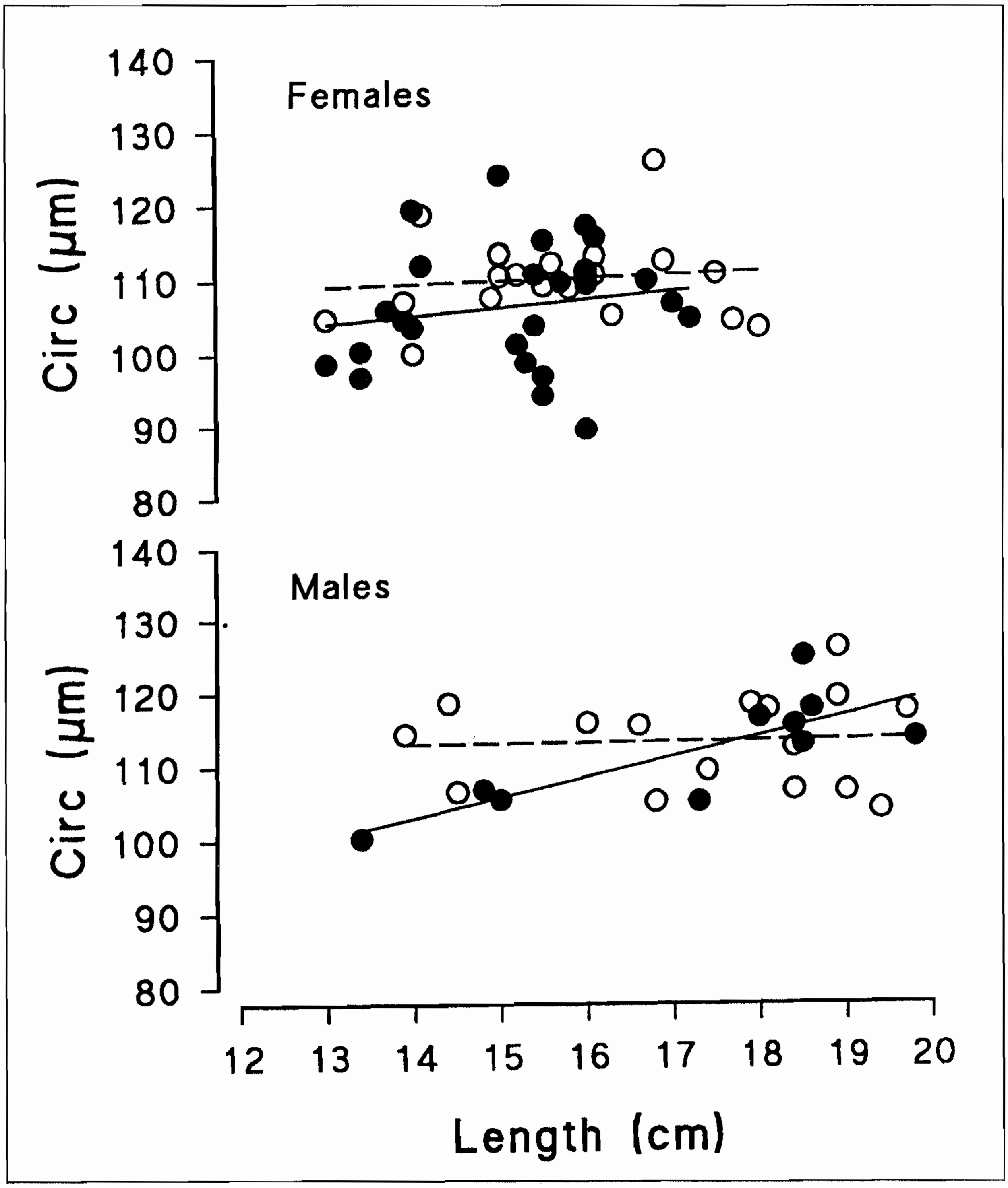

Fig. 2. Spacing of marginal circuli of scales (Circ.) of tagged, and untagged, male and female *O.s.chilwae.* Lines are simple linear regressions:, ----untagged, —tagged.

Multiple linear regressions of *Circ.* on ln (length at sampling), growth and sex, using pooled data from tagged fish in both experiments were highly significant (p<<0.001). Significant effects were detected from all the three independent variables but with growth as the main contributor (Table 5). Regressing *Circ.* from the field experiment on treatment (tagged/no tag.), ln (length at sampling) and sex yielded a significant regression (p <0.01), but the explained variance was small (Table 6). Neither of the independent variables contributed significantly on their own, although p< 0.10 for ln (length at sampling). Inverse prediction of the growth of tagged and untagged fish, using the linear model of Table 5, yielded the growth estimates presented in Table 4. Residual analysis did not reveal any serious violations of multiple linear regression assumptions.

Table 5.—Results for Circ regressed on growth (mm.day^{-1}), ln (length at sampling) (cm) and sex (1=male; - 1=female). Pooled data of tagged O. s. chilwae from pond and field experiment. ANOVA for multiple linear regression. F(3.64)=41. 08; p<<0.001, R=0.811, adjusted R=0.624.

N=68	Beta	Standard error of Beta	b	Standard error of b	t(64)	p-level
Intercept			-23.81	34.15	-0.0697	0.488
Growth	0.8062	0.0740	124.20	11.40	10.90	<<0.001
ln(Length)	0.1765	0.0740	29.63	12.42	2.386	0.020
Sex	-0.1849	0.0748	-3.965	1.605	-2.470	0.016

Table 6.—Results for Circ regressed on treatment (0=no tag; 1=tagged), ln(length at sampling (cm) and sex (1=male; -1=female). Data from the field experiment. ANOVA for multiple regression: F(3.68)=5.08, p<0.01, R=0.428, adjusted R=0.147.

N=72	Beta	Standard error of Beta	b	Standard error of b	t(68)	p-level
Intercept			64.61	24.40	2.648	-0.010
Treatment	-01708	0.1118	-2.527	1.654	-1.528	0.131
ln(Length)	0.2488	0.1286	16.84	8.709	1.934	0.057
Sex	0.1625	0.1279	1.251	0.9849	1.270	0.208

DISCUSSION

Three types of practical errors connected with tag-recapture, termed Type A, B and C errors, that may affect estimates of fishing mortality, survival rate, and population sizes have been identified (RICKER, 1976, JONES, 1977). Briefly, type A errors are associated with mortality at the time of tagging or incomplete reporting of tags, and influence the intercept in regression analysis. Type B errors occur if the mortality rates of the tagged fish, following release, is higher than for untagged fish, or if there is a continuous loss of tags throughout the experiment. This affects the slope in regression analysis. Finally, type C errors result if the initial recoveries are biased due to, *e.g.* incomplete mixing of the tagged fish within the population.

In the pond experiment, recoveries among the tagged fish diminished with decreasing size (Figure 1 and Tables 2 and 3). The type A and B error components could have been separated by addition of sampling occasions between stocking and harvesting. However, the collection of accurate data on survival would have required that the ponds were drained at each sampling occasion, and this would probably have affected growth rates, and would possibly also have induced tag losses and mortalities. Therefore, as the effect of the tags on growth was prime interest, it was decided against additional sampling. The high Z among the small tagged fish in the pond experiment may thus have been due to a high rate of tag loss rather than mortality. Nonetheless, Figure 1 shows that tagging of small Tilapia is likely to lead to biased estimates.

Clipped pelvic fins in *Oreochromis spirulus niger* (GÜNTHER) regenerated in as little as one month (VAN SOMEREN and WHITEHEAD, 1959), although the branching of the fin rays was altered and recognizable by trained personnel for six months or more. Similar regeneration times have been observed in *O.s. chilwae* (N.S. MATTSON , unpublished observation). In the present study, no separate records were kept of the number of fish with regenerated fins, and the identification of the marked fish relied mainly on the Pan Jet stain spots. In general, marking by fin clipping is not considered to affect the growth and survival of fish, but effects have been associated with marking of juveniles (RICKER, 1947, NICOLA and CORDONE, 1973) and spawning fish (LAIRD and STOTT, 1978).

CARLINE and BRYNILDSON (1972) detected retarded growth in brook trout (*Salvelinus fontinalis*) (Mitchill) tagged with Floy FD-67 anchor tags. However, the effect on growth was only present during the first three months following tagging. The survival of tagged and untagged trout was similar. VAN SOMEREN and WHITEHEAD (1959) described a method of tagging *O.s. niger* using numbered plastic strips attached with silver wire through the dorsal musculature. They reported that tag losses in ponds never exceeded 8%, and observed high growth rates in fish over 6 cm TL. However, in a later paper (VAN SOMEREN and WHITEHEAD, 1961) they reported reduced recovery and growth of small male Tilapia, measuring 7.5 cm TL at tagging, compared to fish that were 13 cm TL at tagging. After 9 months rearing in ponds the recoveries were 40% and 87% of the small and large fish, respectively.

This study shows that tagging of tilapia measuring 13-14 cm TL or more probably does not affect growth rates compared with marked or "untouched" fish, even during the first few months. However, the slope of the effect of tagging on *Circ* was negative (Table 6 and Figure 2). In the pond experiment the small untagged fish grew slightly, but not significantly, more than the tagged fish (Table 3). The power of the test was, however, low, and the probability of detecting a difference in mean growth was consequently small.

Considered in combination with the findings of VAN SOMEREN and WHITEHEAD (1961), these observations suggest that a small difference in growth may be present between the smaller sized fish, i.e. less than 13-14 cm TL. To assess the magnitude of such an effect, it may be assumed that the differences between the predicted mean length increments in the field experiment (Table 4) accurately describe the effect of tagging. Under this assumption, the effect of tagging in terms of mean length increments would be 0.010 and 0.023 $mm.day^{-1}$ for males and females, respectively. In the pond experiment, the difference in mean growth between small tagged and marked fish was 0.031 mm.day^{-1} (Table 3). With the growth rates recorded in these experiments this translates into a retardation in length increment of between 2 and 10%. In both experiments this corresponds to a difference in growth of less than one mm per month. This is well within the error of length measurements, and unless the period between release and recapture exceeds half a year or so, the influence of tagging with Floy anchor tags on length increments should be negligible in most experimental situations, even for small fish.

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