Effect of Protein level in Broodstock Diet on Egg size of *Oreochromis shiranus*

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Abstract
A study to investigate the effect of varying protein levels in broodstock diet (25% and 30% crude protein (CP)) on egg size of *Oreochromis shiranus* was conducted at Bunda College Fish Farm, Lilongwe, Malawi. The study was carried out in 6 concrete tanks of $3m^2$ (1.5*2.0*1m) each. Each tank was filled with water up to 0.75m depth and stocked at a density of 5 female fish/m^2. Fish were examined weekly and mature eggs from ripe females were sampled for 108 days after the onset of the experiment. Fish fed with higher protein levels (30%) had higher egg wet and dry weights than fish fed with 25% protein level. The results suggest that broodstock diet has a significant effect on the reproductive strategy in *O. shiranus* since in general, larger embryos hatch from larger eggs.

Introduction

Nutritional status of broodstock is of great importance in aquaculture as it has been shown that the nutritional status of broodstock affects the quality of eggs. Egg quality determines the quality of fish larvae, hatchability and survival of larvae (De Silva & Anderson 1995; Silverstein & Hershberger, 1992). For example, Springate *et al.* (1985) showed that rainbow trout, *Oncorhynchus mykiss*, broodstock fed on an inferior diet produced smaller eggs than those fed on normal diet. Therefore, quality of broodstock diet can affect the reproductive strategy in fish, since the egg size is known to affect the embryo size (e.g. Wallace & Aasjord, 1984) and can also affect subsequent life history stages of fish, e.g. age and size at maturation (Balon, 1985; Silverstein & Hershberger, 1992).

The species used in the present study, *Oreochromis shiranus,* is a mouthbrooding tilapia known as "makumba" in Malawi. Two subspecies have been described in Malawi, *O.s.shiranus*, mainly found in the Upper Shire River, and *O.s.chilwae*, found in Lake Chilwa and Lake Chiuta. Both species have been used in aquaculture and for stocking for man-made waterbodies in Malawi. The species breed easily in ponds to the extent that overcrowding, early maturation and stunted growth become a problem (Balarin, 1983; Maluwa, 1990). Early maturation and stunted growth represent a shift in life history strategy of the fish and could be affected by quality and/or quantity of a broodstock diet(Noakes & Balon, 1982; Springate *et al.*, 1985). As the effect of broodstock diet on egg size has never been studied in *O.shiranus,* the present study was conducted to determine the effect of different protein levels in broodstock diet on egg weight of *O. shiranus*.

Materials and methods

The experiment was conducted at Bunda College Fish Farm (BCFF), Lilongwe, Malawi in six $3m^2$ (1.5*2*1m) concrete tanks. The experiment had two treatments, broodstock diet of 25%CP and 30%CP, replicated three times in a completely randomized design (CRD). Diets were formulated from locally available material that were purchased from Bunda College and Lilongwe. The ingredients included soybean meal, maize bran, fish meal, vitamin and mineral premixes and wheat flour as a binder in proportions shown in Table 1.

Female broodstock of *O. shiranus* obtained from BCFF were stocked in $3m^2$ concrete tanks at 5 fish/m^2. The average weight of fish at stocking was 9.5g and the experiment was run until all fish were ripe, for 108 days.

Fish were fed pelleted feed twice per day (9:00 am and 2:00 p.m.) at 4% body weight per day, which was reduced to 3% body weight at day 65 to the end of the experiment.

Eggs were sampled weekly. Each fish was squeezed gently on the abdomen in order to force out ripe eggs. The fish that had ripe running eggs was then dissected to obtain only ripe eggs that were collected and counted. Fish that had ripe eggs was weighed to the nearest 0.01g and its total length measured to the nearest 0.1cm. From the collected bunch of ripe eggs, 20 eggs were randomly sampled from each fish and individually weighed to the nearest 0.001g. The eggs were then

Table 1: Relative weight of the feed ingredients in the two different diets used in the experiment

Ingredient	Low CP (25%)	High CP (30%)
Fish meal	9.88%	4.5%
Soybean	16.9%	17.96%
Maize bran	70.2%	74.54%
Vitamin premix	0.5%	0.5%
Mineral premix	0.5%	0.5%
Binder	2.0%	2.0%

dried for 72 hours (3 days) at 60°C and the dried eggs were weighed.

Analyses of variance (ANOVA) and covariance (ANCOVA) were used to test differences between treatment means, at 0.05 level of significance (Sokal & Rohlf, 1981) using SYSTAT 7.0-computer package.

Results and Discussion

Egg weight was not statistically different between replicates within treatments allowing us to pool the data among the replicates in further testing of the results. In both high and low protein diets there was a significant positive correlation between the weight of the fish and the wet weight of the eggs produced (r^2=0.59; P<0.001) (Figure 1). The same relationship was found between egg dry weight and female weight (r^2=0.66; P<0.001) (Figure 2). Fish fed on high CP (30%) diet had significantly higher mean weight (23,2g) than those fed on low CP (25%) diet (17,9g) (t=-4.66; P<0.001).

The condition (K) factor was not different between the fish in the two treatments. The average weight of eggs from fish fed 30% protein diet was 8.0 mg and 3.4 mg wet and dry weight, respectively while fish fed with 25% protein diet had an average wet and dry weight of 5.0 mg and 2.3 mg, respectively. However, since there was a positive correlation between female weight and egg weight and a difference in female weight between the treatments, egg weights could not be compared directly, female weight effects were removed. Fish fed on high CP diet produced significantly heavier eggs independent of their body weight compared to fish fed on low protein diets (ANCOVA using female weight as a covariate: $F_{[1,65]}$=112,4; P<0.001)

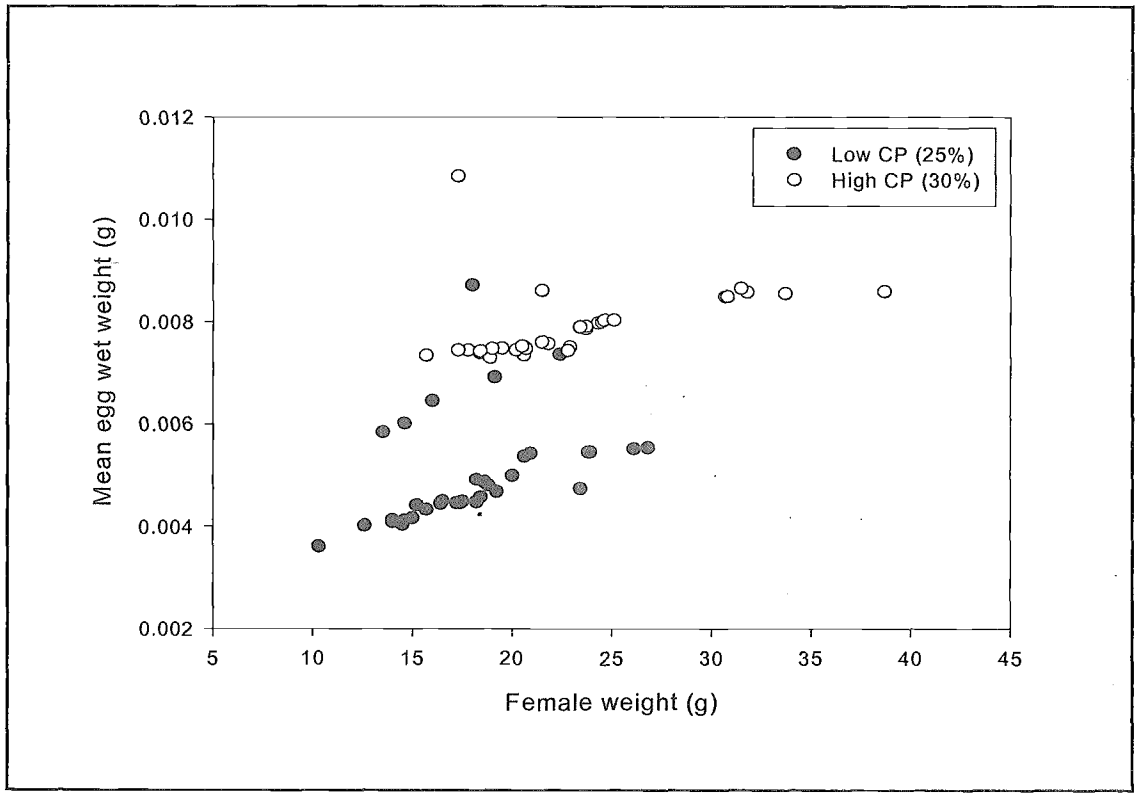

Figure 1. The relationship between female weight and ripe egg wet weight in *Oreochromis shiranus* fed on two different diets.

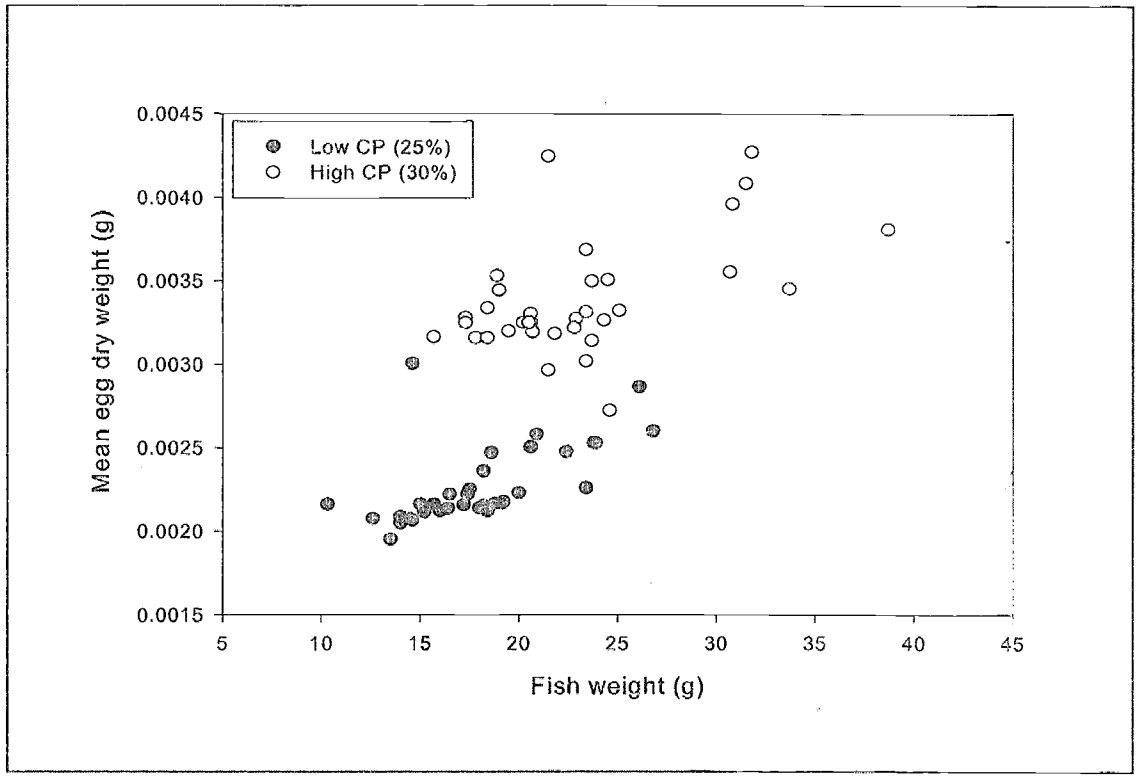

Figure 2. The relationship between female weight and ripe egg dry weight in *Oreochromis shiranus* fed on two different diets

(Figure 1). Also, egg dry weight was higher in fish fed on high CP diet compared to low CP diet, independent of female weight (ANCOVA using female weight as a covariate: $F_{[1,65]}=179.8$; $P<0.001$) (Figure 2).

In the present study it is shown that protein level in broodstock diet and female size affect the egg size in *O.shiranus*. These findings suggest that the reproductive strategy may be affected by broodstock diet since larger embryos hatch from larger eggs (Wallace & Aasjord, 1984) and that survival rate of large embryos is higher than small ones (Bagenal, 1969; Rana, 1985; B De Silva, 1995). Fry production of *O. shiranus* might be enhanced by improving broodstock diet. Further studies are needed to determine the optimal protein level in the broodstock diet.

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