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Spawning frequency, fecundity, egg size and ovarian histology in groups of *Tilapia zillii* maintained upon two distinct food ration sizes from first-feeding to sexual maturity

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Abstract – Duplicate groups of first-feeding (4–5-d-old) *Tilapia zillii* (Gervais) were maintained on two feeding regimes (high and low ration size) for a total period of 550 d (~17 months) in environmentally-controlled aquaria. Differences in mean individual fish weight were evident between fish fed the different ration sizes after just 3 months, though these differences were not statistically significant until day 180. Thereafter, fish maintained upon the higher ration were significantly larger than fish fed a reduced ration. Mortality in high ration fish over the course of the experiment was ~60 % compared to ~88 % in low ration fish. Mortality in both treatment groups was most pronounced during the first three months (particularly during the first 30 d) but was much reduced thereafter. Spawning trials undertaken from day 450 onwards indicated that there was no significant difference between the two ration groups in terms of spawning periodicity over a discrete time period of 60 d. Mean total fecundity was significantly higher in fish on high rations though mean egg diameter and gonadosomatic index remained unchanged. Adjustment of spawning data to a common maternal fish size using one-factor ANCOVA failed to detect significant differences between the two ration levels in terms of either mean total fecundity or mean egg diameter. Stereological analysis of plastic-embedded ovarian tissue revealed that the ovaries of low ration fish possessed significantly more stage 2 and 3 (early and late perinucleolar) oocytes and significantly less stage 6/7 (late-vitellogenic/maturing) oocytes than the ovaries of fish fed the higher ration. No significant differences were detected between the two rations in terms of ovarian atresia. It is suggested that under prolonged food restriction, female *T. zillii* sacrifice somatic growth such that reproductive investment can be maintained. © Ifremer/Elsevier, Paris

Food ration size / ovarian histology / fecundity / egg size / *Tilapia zillii*

Résumé – Fréquence des pontes, fécondité, taille des œufs et histologie de l'ovaire, chez deux groupes de *Tilapia zillii* nourris, de la 1re alimentation au stade de maturité sexuelle, avec deux rations alimentaires distinctes. Deux groupes de *Tilapia zillii* (Gervais) au stade de 1re alimentation (âgés de 4–5 j) sont maintenus à deux régimes alimentaires différents (ration faible et forte) durant 550 j (~17 mois) en milieu contrôlé. Des différences entre les poids moyens individuels des deux groupes sont évidentes après juste trois mois d'élevage, bien qu'il n'y ait pas de différence statistiquement significative jusqu'au 180e jour. Par la suite, les poissons nourris de fortes rations alimentaires étaient de plus grande taille que ceux nourris avec des rations faibles. Sur toute la durée de l'expérience, la mortalité est plus faible (~60 %) chez les poissons nourris de fortes rations comparée à celle des autres poissons (~88 %). La mortalité est plus importante durant les trois premiers mois (les 30 premiers jours en particulier) quel que soit le traitement. Les essais de reproduction effectués à partir du 450e jour montrent qu'il n'y a pas de différence dans la périodicité des pontes sur une période de 60 j entre les deux groupes. La fécondité totale moyenne est significativement plus élevée chez les poissons nourris de fortes rations, bien que le diamètre des œufs et l'indice gonado-somatique restent inchangés. En termes de fécondité moyenne totale ou de diamètre des œufs, aucune différence n'a été observée (ANCOVA à un facteur) si les données de ponte sont ajustées à une femelle de même taille afin de comparer les deux groupes. L'analyse stéréologique du tissu ovarien révèle que les ovaires des poissons nourris de faibles rations possèdent significativement davantage d'ovocytes de stades 2 et 3 (stades périnucléaires) et moins de stades ovocytaires 6/7 (stades vitellogéniques tardifs/de maturation) que les poissons nourris de fortes rations alimentaires. Aucune différence n'a été notée en terme d'atrésie ovarienne entre les deux rations. Il est suggéré qu'avec une restriction alimentaire prolongée, les femelles de *T. zillii* sacrifient la croissance somatique de façon à maintenir l'investissement nécessaire à la reproduction. © Ifremer/Elsevier, Paris

Ration alimentaire / histologie de l'ovaire / fécondité / taille des œufs / *Tilapia zillii*

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1. INTRODUCTION

Supplies of high quality fry can become a major limiting factor in semi-intensive tilapia culture due to their inherently low fecundity [33]. As a result, there is increasing emphasis on reducing maintenance costs (particularly feed costs) by the adoption of appropriate husbandry practices. Potential techniques include restriction of food ration, the development of practical diets incorporating plant ingredients and the evaluation and use of alternative protein sources other than fishmeal which, over recent years, has suffered from uncertain supply and rising costs. The potential influence of such practices, however, particularly in terms of reproductive performance is not clearly understood.

Previous studies on the effects of food ration size manipulation on reproductive function in fish have mainly focused upon temperate species, particularly salmonids [6, 37, 39, 43, 44], three-spined stickleback *Gasterosteus aculeatus* (L.) [17, 56, 57] and European sea bass *Dicentrarchus labrax* (L.) [7–9]. Studies of tropical species are less common and are largely restricted to the convict cichlid *Cichlasoma nigrofasciatum* (Günther) [47, 48] or mouth-brooding species of tilapia [18, 19, 34, 38, 45, 46]. Despite the continued increase in distribution and development of commercial tilapia culture to over 100 countries, available data concerning the effect of dietary quality and quantity on reproductive physiology remain sparse and are exclusive to mouth-brooding genera. Moreover, most previous studies conducted with tilapia were short-term and were undertaken in outdoor tanks or ponds. Under these circumstances, dietary influences are difficult to quantify and separate from other environmental variables.

Reductions in food ration size have been shown, in various teleosts, to delay maturation and spawning [32], reduce fecundity [39, 47] and reduce egg size [43, 47]. Reduced food supply also retards or inhibits gonadal development in salmonids [3, 39], guppy *Poecilia reticulata* (Peters) [20] and roach *Rutilus rutilus* (L.) [26]. Decreased food abundance also significantly increased egg mortality amongst the smallest eggs spawned by brook trout *Salvelinus fontinalis* (Mitchill) [24]. Model simulations indicated that maternal fitness was a curvilinear function of egg size; food supply influenced both the height and shape of the function.

Little is known on how manipulation of ration size may affect the reproductive physiology of tilapia broodstock though Toguyeni et al. [45] report that food ration size did not significantly influence plasma sex steroid levels. Results of an earlier study involving *Oreochromis mossambicus* (Peters) by Mironova [34] suggest that reproduction was apparently stimulated by food limitation which is of immense interest considering the current need to reduce maintenance costs of tilapia broodstock. Whilst food restriction resulted in growth limitation and reduction of fecundity, there were increases in spawning frequency, the total number of eggs produced over a discrete period and the proportion of energy allocated to egg production [34].

The present study aimed to investigate the effect of two contrasting food ration sizes upon spawning performance, fecundity, egg size and ovarian histology in stocks of *T. zillii* held under closely-controlled environmental conditions. *T. zillii* is cultured extensively throughout Africa and the Middle-East, is used as a means of bio-control in the irrigation canals of southern California [28] and is one of the most adaptive tilapias in terms of temperature, salinity and diet [5]. Rationing commenced at first-feeding (when fish were just 4–5-d-old) and continued for approximately 17 months, thereby providing batches of sexually-mature fish that had been subject to controlled feeding regimes throughout on-growing.

2. MATERIALS AND METHODS

2.1. Aquarium conditions and fish maintenance

Fish were maintained in gravity-fed recirculating aquaria incorporating fibreglass or glass tanks linked to settling tanks, faecal traps and filtration units appropriate to system size and capacity. Four separate types of aquaria were used in the present study, each forming discrete recirculating units:

– (1) Fry system: incorporating several covered plastic holding tanks (42 × 26 × 30 cm). One communal tank per replicate of each treatment group. Rate of water inflow was ~1 $L \cdot h^{-1}$.

– (2) On-growing system: incorporating several covered 'bucket' type plastic tanks (50 × 38 × 41 cm). One communal tank per replicate of each treatment group. Rate of water inflow was 190 $L \cdot h^{-1}$.

– (3) Holding aquaria: incorporating several covered fibreglass holding tanks (114 × 114 × 42 cm). One communal tank per replicate of each treatment group. Rate of water inflow was 400 $L \cdot h^{-1}$.

– (4) Experimental aquaria: consisting of eight 122.0 × 47.0 × 47.5-cm glass tanks. Water inflow rate was 327 $L \cdot h^{-1}$. Each glass tank contained two vertical dividers constructed from translucent perspex (0.5 cm thick), thus creating three separately partitioned 'holding spaces' within each glass tank into which three sexually-mature female broodstock could be introduced and maintained individually.

Previous experiments with *T. zillii* have demonstrated that this species is very sensitive to the degree of confinement within a culture system [12]. Sexually-mature fish held collectively in large holding aquaria such as those used in the present experiment (system 3 above) are inhibited from spawning due to a mechanism involving the suppression of sex steroid levels. This inhibition is removed once fish are transferred into aquaria that are partitioned such that each fish is held in its' own individual space [12]. In order to

investigate reproductive function in the present study, it was therefore necessary to transfer rationed fish, once sexually-mature, to individually-partitioned aquaria so that spawning could begin. Once transferred to individually-partitioned aquaria, females were deprived of all manner of male conspecific contact; female tilapia spawn regularly even when deprived of conspecific stimulation [10–12, 40, 41].

All aquaria experienced a constant daily photoperiod of 12L:12D (controlled by a 24-h timer) and a water temperature of 27 ± 1 °C (controlled by a thermostatically controlled water heater). System filters and settling tanks were cleaned every two weeks concommitant with a 10–20 % water change.

2.2. Effect of food rationing on growth

A clutch of first-feeding fry (4–5-d-old) were randomly divided into four groups (each containing approximately 190 fry): groups 1 and 2 (duplicate high ration) and groups 3 and 4 (duplicate low ration). Each group was placed into pre-cleaned 'fry system' (system 1) tanks. Outflow pipes were protected with fine mesh to avoid inadvertant loss of fry. Fry were allowed to acclimatise for 24 h prior to any further interference.

High ration was initially set at 40 % body weight per day (bw·d^{-1}) and low ration at 10 % bw·d^{-1} (*table I*). These ration levels were similar to those used by Macintosh and De Silva [30] in a similar study of early growth and survival in mouth-brooding tilapia. First-feeding tilapia fry are normally fed daily ration sizes of 30–40 % bw·d^{-1}. The rations designated as being 'high' in the present study (initially 40 % bw·d^{-1}), therefore, represent a 'normal' ration size whilst the low ration (initially 10 % bw·d^{-1}) represents a far more restrictive ration. The most commonly reported daily ration sizes for broodstock tilapia lie between 1 [38] and 5 % bw·d^{-1} [27]. Recent data, however, suggest that broodstock should be fed 2–3 % bw·d^{-1}, the ration being provided over at least three feeds [31].

Table I. Allocation of ration sizes throughout the experiment.

Age (d)	Ration allocation (% bw·d^{-1})	
	High ration (groups 1 & 2)	Low ration (groups 3 & 4)
0–60	40	10
60–80	30	10
80–100	10	5
100–130	8	4
130–160	5	2
160–190	3	1
190–270	2	1
270–450	1.5	0.8
450–550	0.8	0.4

Two groups of ten fry were sacrificed and weighed to the nearest 0.0001 g. These data were then used to calculate the average initial wet weight of fry for each treatment tank. Daily rations, initally provided by grinding commercial pelleted trout feed ('Fry 02 Granules'-B.P./Ewos, UK) to a suitable size (initially 250–500 µm), were given to treatment tanks three times daily. Faeces and excess food were siphoned from each tank twice daily. Treatment tanks were also cleaned daily to prevent build-up of algae. Mortalities were removed as soon as discovered and rations altered accordingly. Rations were reduced nine times over the course of fry development (see *table I*), with alterations taking place when increasing amounts of excess (uneaten) food became apparent in treatment tanks. On these occasions, fish in the high ration tanks were fed to satiation three times during a 24-h period. The amount of food provided at each of these feedings was totalled to give the appropriate daily amount to be supplied to high ration fish. Low ration groups were fed no more than 50 % of the ration given to the high ration groups. Particle and pellet size were gradually increased as fish grew in weight. Ration sizes were ultimately reduced to 0.8 and 0.4 % bw·d^{-1} in high and low ration groups respectively (from day 450 onwards).

Every ten days, three groups of at least ten fry from each group were re-weighed and the daily ration altered accordingly. To minimise damage/stress to young fry, batches of ten fry were weighed in a pre-weighed beaker of water. Once fish in each group had grown to a weight of approximately 5 g, individual fish were anaesthetised and weighed to the nearest 0.01 g. Due to the fragility of young fry, length was not measured. Every 30 d, the true number of fish remaining in each treatment tank was ascertained by netting and counting manually. This was to avoid relying upon the presence of carcasses as an indicator of mortality, since cannibalism may lead to over-estimation of population size. From day 410 onwards, fish were weighed every 20 d. Once deemed large enough, fry were transferred into a second recirculating aquarium (on-growing system, system 2) and finally into a third system (holding aquaria, system 3). Rationing was maintained for a total period of 550 d (~17 months).

Due to an unfortunate mechanical fault with one of the aquarium systems, all of the fish in group 2 (one of the high ration duplicates) were killed on day 170. Prior to this point however, there had been no significant difference ($P > 0.05$, ANOVA) in terms of mean fish weight between the two duplicate tanks for each treatment. Furthermore, the extent of tank-tank variation between the two duplicate groups of each treatment prior to the loss of group 2 (i.e. days 0–170) was tested by using a blocked ANOVA; variation was negligible. Subsequent experiments involving spawning performance and ovarian histology were therefore undertaken with the three remaining groups.

2.3. Effect of food rationing on spawning performance, fecundity and egg size

Commencing on day 450 of rationing (i.e. at approximately 15 months of age), six females from each of the remaining rationed groups (i.e. one group of high ration fish and two duplicate groups of low ration fish) were randomly removed from their respective holding aquaria (system 3) and placed into experimental glass aquaria (system 4) such that each female was held individually (three individually-held fish per partitioned glass tank). This allowed females to commence spawning. Females were stocked into individually-partitioned aquaria in such a way that each individual was held in a partition adjacent to females maintained upon the same ration size. Fish were sexed by comparison of the external appearance of the genitalia. Partitions between 'holding spaces' were fitted tightly such that food provided to one holding space could not inadvertently pass into the next. Immediately prior to transfer, fish were anaesthetised in a 1:20 000 (v/v) solution of 2-phenoxyethanol (Sigma Chemicals, UK), weighed and total length determined. Fish were also individually tagged in the peritoneal cavity with P.I.T. tags (Labtrac Systems, UK) as described in Coward et al. [12].

Broodfish were maintained in individual aquaria for a total period of 60 d. Ration manipulation was maintained as detailed earlier with high ration fish allocated 0.8 % bw·d^{-1} and low ration fish 0.4 % bw·d^{-1} (*table I*). Fish were reweighed every 20 d and the daily ration adjusted accordingly.

Each 'holding space' was assessed daily (at approximately 3-h intervals during daylight hours) for evidence of spawning (clutches of eggs adhered to tank base or sides). Spawning periodicity was assessed for each treatment group using the index 'mean days elapsed per spawn' [10]. This figure represents the mean number of days taken by an individual fish within the treatment group to undergo one complete spawning cycle and accounts for both spawning and non-spawning fish within an experimental group. This index was calculated by dividing the total length of observation period in individual aquaria (i.e. 60 d) by the cumulative number of spawnings observed in a specific treatment group.

Post-spawning females were anaesthetised, weighed and measured. Total fecundity, defined by Rana [35] as the number of eggs in a freshly spawned clutch, was determined directly by tally counter. Long and short axes of a subsample of 50 eggs from each spawn were measured using a microscope containing a calibrated eyepiece graticule. Mean egg diameter was calculated for each spawn as follows: mean egg diameter (mm) = (length of long axis + length of short axis)/2.

2.4. Effect of food rationing on ovarian histology

Beginning on day 510 of rationing, five females were removed from each of the three remaining treatment groups (one high ration and two duplicate low ration groups). Since the spawning cycles of tilapia tend to be very asynchronous when compared amongst a batch of individuals, it was important to compare ovarian histology amongst rationed fish at similar points within the reproductive cycle. This was achieved by removing females from holding aquaria (system 3, where as a result of confinement, *T. zillii* fail to spawn) and not from individually-partitioned aquaria where spawning cycles (and therefore stage of ovarian development) could have been very asynchronous [12]. Fish were anaesthetised, weighed, and measured. Fish were then sacrificed by anaesthetic overdose followed by spinal transection. Ovaries and liver were removed from each fish; gonadosomatic index (GSI) and hepatosomatic index (HSI) were determined as follows:

$$\text{GSI}\ (\%) = \text{gonad weight}/(\text{body weight} - \text{gonad weight}) \times 100$$

$$\text{HSI}\ (\%) = \text{liver weight}/\text{body weight} \times 100$$

Five-mm transverse sections of the right ovary were fixed in Bouin's fluid and embedded in 'Historesin' as described in Coward and Bromage [11]. In brief, tissue was dehydrated with a series of alcohol solutions and infiltrated at 4 °C with 'Historesin' (hydroyethylmethacrylate) according to the manufacturer's instructions (Reichert-Jung, Gmbh, Germany). Tissue samples were then embedded in 'Historesin' medium, mounted on wooden blocks, sectioned at 3 µm and stained in haematoxylin and eosin. Oocytes that that had been sectioned directly through the nucleus were classified into established developmental stages according to Coward and Bromage [11]. Volume fractions (the relative area/volume of each oocyte developmental stage in the tissue of interest) were determined using stereological methods [11, 12, 55].

2.5. Statistical analysis

Data were tested for normality and logarithmically transformed if necessary. Volume fraction, GSI and HSI data were arc sine transformed prior to analysis. Fish weight and mortality data were compared between the two rations using one-way and two-way ANOVA. Blocked ANOVA (with tank duplicate as the 'block' factor) was undertaken from days 0–170 (i.e. where all four groups were present) to investigate the extent of tank-tank variation between duplicates of each treatment. Mean total fecundity and mean egg diameter were initially compared between groups using one-way ANOVA. Such analysis, however, fails to account for differences in fish size between treatment groups; previous studies of *T. zillii* have demonstrated that fecundity is significantly related to both fish length and weight [10]. One factor analysis of covariance (ANCOVA) was thus used to adjust data to a common maternal size and therefore provide a more rigorous comparison. ANCOVA was performed upon $\log_{10}$ transformed data as described by Snedecor and

Cochran [42] and using the SUPERANOVA (version 1.11) statistical package (Abacus Concepts, USA). Data used in ANCOVA were also tested (by F-tests) for homogeneity of residual variance and slope. Ovarian histology was compared amongst the different groups using one-way ANOVA.

3. RESULTS

3.1. Effect of food rationing on growth and fry survival

3.1.1. Fish growth

Throughout the experiment, mean fish weight was not significantly different ($P > 0.05$) between the two duplicate tanks at either of the two ration levels. For this reason, growth data for each ration level were pooled from the two respective duplicate tanks. Due to an untimely mechanical fault with one of the aquarium tanks, one of the treatment groups maintained on high ration (group 2) was lost on day 170. Prior to this point however, there had been no significant difference ($P > 0.05$) in terms of mean fish weight between the two duplicate tanks being fed a high ration. Blocked ANOVA undertaken from days 0–170 demonstrated that tank-tank variation between the duplicates of the two treatments was minimal ($P > 0.05$). This suggests that the chances of confounding tank-tank variation with treatment variation (owing to the loss of one treatment replicate) were very small.

Fish fed the higher ration size grew from 0.0022 ± 0.0002 (on day 0) to 190.4 ± 13.4 g (by day 550) whilst low ration fish grew from 0.00023 ± 0.0002 to 48.8 ± 6.8 g (*figure 1*a). Differences in mean fish weight were evident from day 80 onwards (*figure 1*b) though these differences were not significant until day 180. Thereafter, high ration fish were significantly larger (either $P < 0.05$, $P < 0.01$ or $P < 0.001$, see *figure 1*a) than those fed the lower ration.

3.1.2. Fry survival

Fry mortality rate was significantly lower ($P < 0.05$) in fish maintained upon the high ration size (approximately 60 %) than fish fed the reduced ration (approximately 88 %). Mortality in both treatment groups was most pronounced during the first 90 d of the experiment, particularly during the first 30 d but was much reduced thereafter. Mortality during the first 30 d was clearly higher (more than double, $P < 0.05$) in fish fed the low ration. During this period, observed mortality (recovery of carcasses from experimental tanks) closely matched actual mortality (calculated from counts of remaining live fish) (*figure 2*). Thereafter, actual mortality (in both treatment groups) consistently exceeded observed mortality. Actual mortality was approximately 29–45 and 41–44 % higher than observed mortality in fish fed high ration and low ration, respectively. There were no mortalities in any treatment group after day 290.

3.1.3. Ration allocation

At the start of the experiment, treatment groups were allocated daily ration sizes of 40 (high) and 10 (low) % bw·d^{-1}. These ration sizes were altered as fry devel-

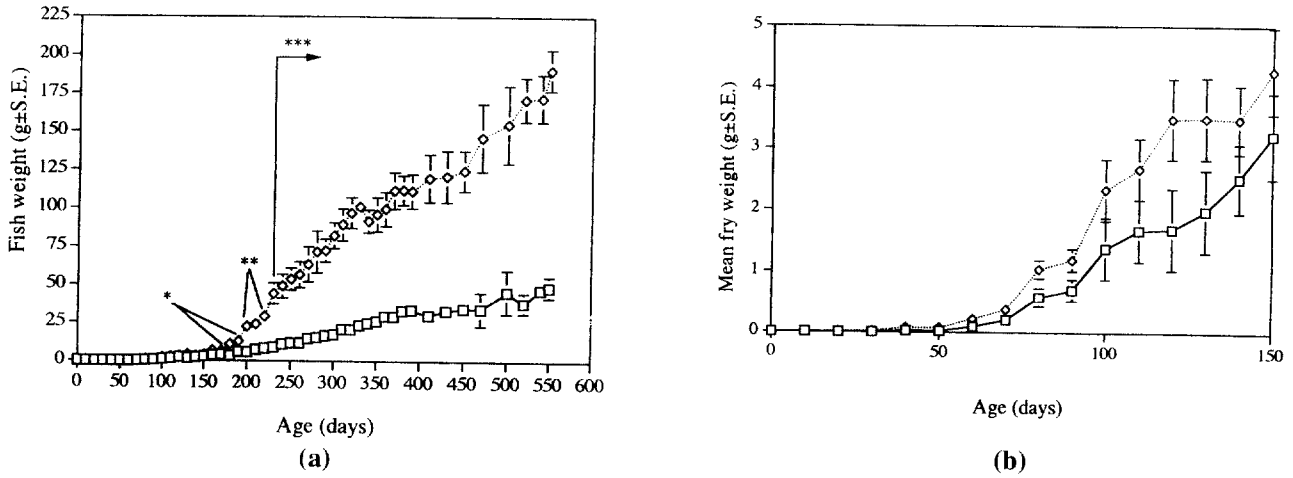

Figure 1. (a) Growth of *T. zillii* from day 0 (first-feeding) to day 550 maintained on either high ration (groups 1 and 2, ◊) or low ration (groups 3 and 4, ❑). Weights are given as mean ± SE (pooled from duplicate groups). Where no SE is visible, it lies within the confines of the symbol. Differences between high and low rationed groups are significant from day 180 onwards (*, $P < 0.05$, days 180–190; **, $P < 0.01$, days 200–230; ***, $P < 0.001$, days 240+). Between days 0 and 150, fish were held in plastic fry tanks (system 1, see Materials and Methods). Fish were transferred into system 2 on day 150 and finally transferred into holding aquaria on day 170 (see Materials and Methods for explanation of each aquaria system). (b) Growth of *T. zillii* from day 0 (first-feeding) to day 150 maintained on either high ration (groups 1 and 2, ◊) or low ration (groups 3 and 4, ❑). Weights are given as mean ± SE (pooled from duplicate groups). Where no SE is visible, it lies within the confines of the symbol. Differences between the two rations are not significant ($P \geq 0.05$)

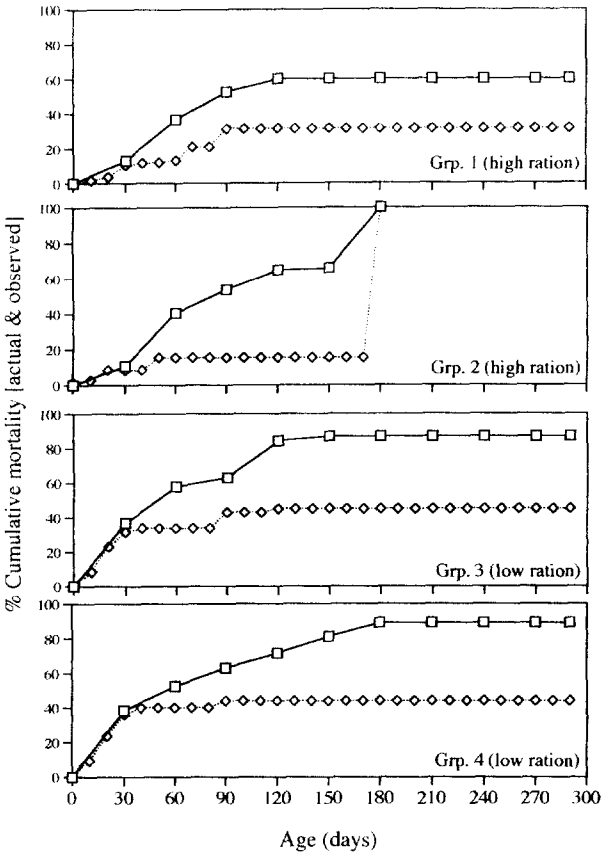

Figure 2. Percentage cumulative mortality (expressed as 'actual' [❑] and 'observed' [◊] mortality) in 4 groups of *T. zillii* fry maintained on either high (groups 1 and 2) or low (groups 3 and 4) ration sizes. 'Observed' figures represent physical mortalities removed from tanks during the courses of the experiment. 'Actual' data represent the true mortality found in each treatment group and was determined by counting the number of live fish remaining in each tank at 30-d intervals. There were no mortalities after day 290.

oped and by the end of the experiment (day 550) were set at 0.8 (high) and 0.4 (low) % bw·d^{-1} (see *table I*).

3.2. Effect of food rationing on spawning performance, fecundity and egg size

There was no significant difference between the two low ration duplicates (groups 3 and 4) over the 60-d experimental period ($P > 0.05$) in terms of either mean weight or length. On this basis, groups 3 and 4 were pooled and compared to the single remaining high ration group (group 1) in the following analyses.

3.2.1. Fish size

Mean weight and mean length were both significantly higher ($P < 0.001$ and $P < 0.01$, respectively) in fish fed the higher ration size.

3.2.2. Spawning performance, fecundity and egg size

There were no spawnings in any of the aquaria utilised in the present study except for the individually-partitioned aquaria; transfer of fish from holding aquaria (system 3) to individually-partitioned aquaria (system 4) allowed fish to begin spawning. First spawns in the high ration and low ration groups were observed 8.7 ± 4.2 and 7.1 ± 1.8 d after transfer, respectively (*table II*). There was little difference between the two rations in terms of spawning periodicity; the mean number of days elapsed per spawn was very similar between the two ration sizes (15 and 13.5 d for high and low ration, respectively) (*table II*). Mean total fecundity was significantly higher ($P < 0.01$) in high ration fish than low ration fish (*table II*). Mean egg diameter did not differ significantly ($P > 0.05$). Further analysis by one-factor ANCOVA found no significant difference ($P > 0.05$) between the two ration levels in terms of total fecundity or egg diameter (*table III*). It should be noted, however, that only in the case of total fecundity were all criteria for ANCOVA fully satisfied (i.e. homogeneity of both residual variances and slope).

3.3. Effect of food rationing on ovarian histology

No significant differences ($P > 0.05$) were found between the two duplicate low ration groups (groups 3

Table II. Spawning characteristics of female *T. zillii* maintained on two distinct food ration sizes (high and low). Fish were held in individually-partitioned glass aquaria for a period of 60 d.

Spawning characteristic	High ration Group 1	Low ration Groups 3 & 4 (data pooled)
Number of fish observed	6	12
Total number of spawns	4	8
Number of fish spawning once	2	4
Number of fish spawning twice	1	2
Mean days elapsed spawn^{-1}	15	13.5
Mean weight of spawning fish (g ± SE)	89.3 ± 13.9	28.7 ± 3.2 ***
Mean length of spawning fish (mm ± SE)	162.0 ± 7.7	117.5 ± 3.7 **
Mean total fecundity (± SE)	2 608.2 ± 338.2	1 190.6 ± 94.6 **
Mean egg diameter (mm ± SE)	1.44 ± 0.03	1.53 ± 0.03

Significant differences (one-way ANOVA) between the two groups are indicated by annotation with ** ($P < 0.01$) or *** ($P < 0.001$) otherwise, differences are not significant.

Table III. Analysis of covariance (ANCOVA) of $\log_{10}$ post-spawned fish weight and (a) $\log_{10}$ total fecundity and (b) $\log_{10}$ mean egg diameter (mm^3) for *T. zillii* maintained upon high (group 1) and low (groups 3 and 4 pooled) food ration sizes. NS = not significant.

Test	Homogeneity of residual variances		Homogeneity of slopes		Intercept (elevation)	
	F	Result	*F*	Result	*F*	Result
(a)	1.83	Homogeneous ($P \geq 0.05$)	0.53	Homogeneous ($P \geq 0.05$)	0.06	NS ($P \geq 0.05$)
(b)	7.56	Homogeneous ($P \geq 0.05$)	320.2	Heterogeneous ($P < 0.05$)	1.68	NS ($P \geq 0.05$)

and 4) in terms of weight, length, HSI or GSI. These data were therefore pooled and compared to the single remaining high ration group (group 1) in the following analyses.

3.3.1. Fish size, GSI and HSI

Mean weight and length were both significantly higher ($P < 0.001$ and $P < 0.01$, respectively) in fish fed the higher ration size (*figure 3*a, b). No significant differences ($P > 0.05$) in GSI were found between the two groups although mean HSI was significantly larger ($P < 0.01$) in high ration fish (*figure 3*c, d).

3.3.2. Stereological analysis of ovarian histology

No significant differences ($P > 0.05$) were found between the two duplicate low ration groups (groups 3 and 4) in terms of the mean volume fraction (VF) of any oocyte developmental stage. For this reason, VF data from these two groups are pooled and compared to the remaining high ration group (group 1) in the following analyses (*figure 4*).

There was no significant difference between the two groups of rationed fish in their ovarian volume fractions of stage 4 (cortical alveolar) oocytes or stage 5 (early vitellogenic) oocytes. However, low ration fish exhibited a significantly higher ($P < 0.05$) VF of stage 2 and 3 oocytes (early and late perinucleolar stage, respectively) and a significantly lower ($P < 0.05$) VF of stage 6/7 oocytes (late vitellogenic/maturing) oocytes. No significant difference ($P > 0.05$) was detected between high and low ration groups in terms of ovarian atresia.

4. DISCUSSION

This paper forms the first detailed attempt to investigate the effect of food ration size on the reproductive physiology and endocrinology of substrate-spawning tilapia. Unlike most previous studies involving mouthbrooding tilapia, the present investigation took place under controlled laboratory conditions where water temperature, photoperiod, flow rate and water quality were strictly controlled. Furthermore, earlier studies have also failed to utilise appropriate statistical techniques such as ANCOVA to adjust reproductive data for differences in fish size.

Groups of first-feeding *T. zillii* (fish approximately 4–5-d-old) were allocated either a high ration size or a

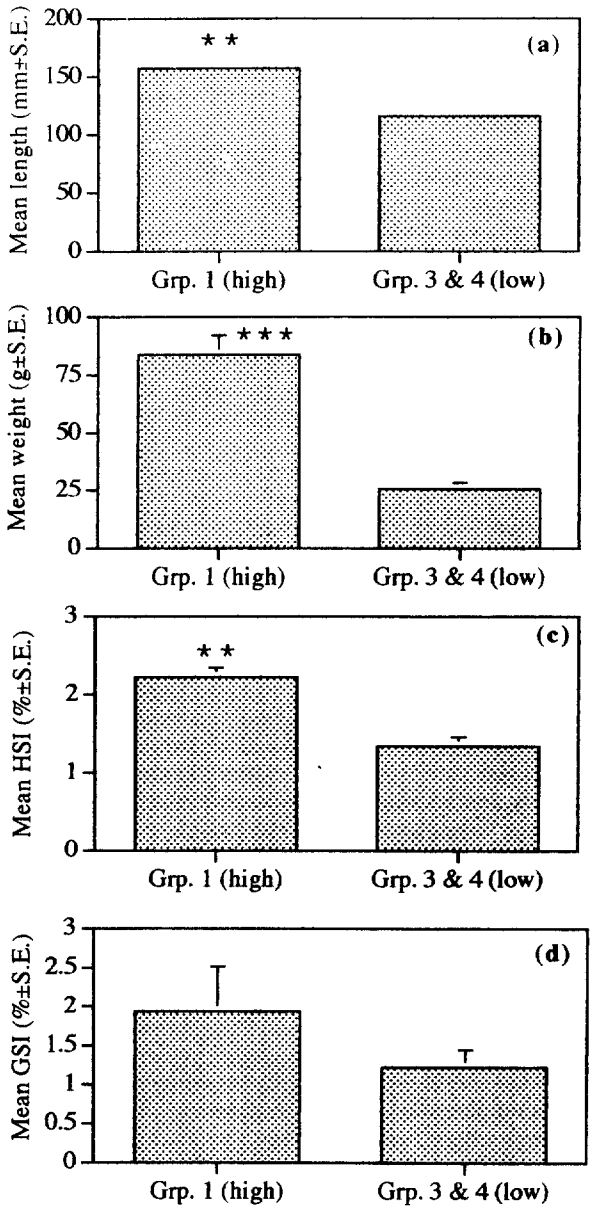

Figure 3. Comparison of (a) mean length, (b) mean weight, (c) mean HSI and (d) mean GSI between two groups of *T. zillii*. Group 1 was fed a high ration and groups 3 and 4 were fed a low ration (here, low ration data are pooled). Significant differences between groups are indicated by ** ($P < 0.01$) or *** ($P < 0.001$).

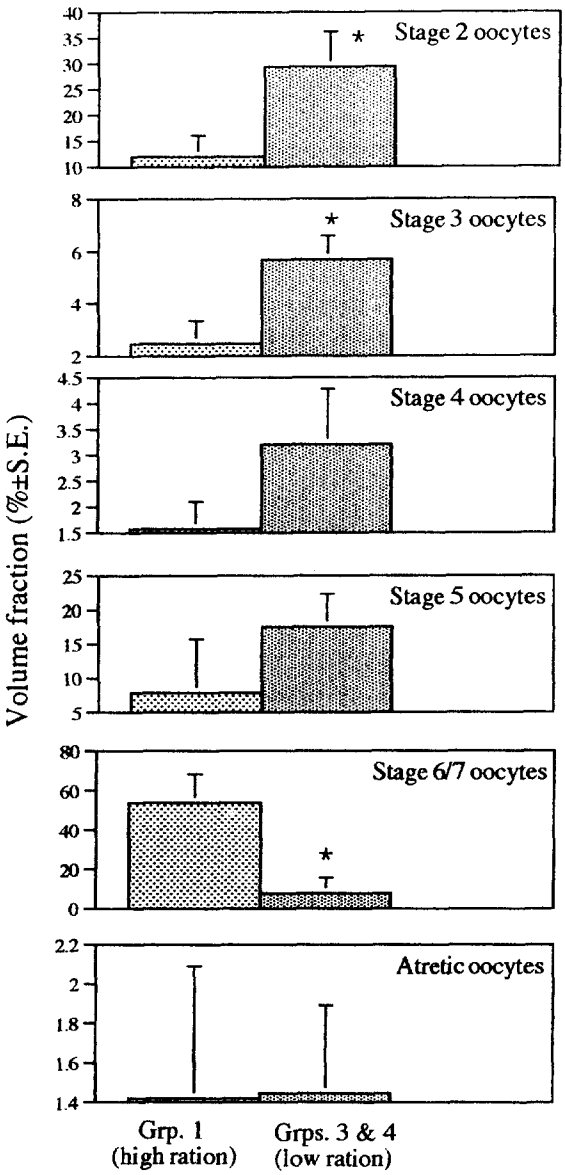

Figure 4. Mean volume fraction (% ± SE) of oocyte developmental stages in two groups of *T. zillii*; group 1 (high ration, ▒) and groups 3 and 4 (low ration, data pooled, ▒). Statistical differences are indicated by * ($P < 0.05$).

restricted ration size and were maintained upon the allocated rations for the following 17 months. The commencement of rationing at first-feeding was planned so as to coincide with the process of sexual differentiation. Onset of sexual differentiation (movement of primordial germ cells from the mesoderm and endoderm to the presumptive gonadal sites to form an ovarian cavity or ovocoel) in female tilapia appears to depend upon species, rearing temperature and rearing density. Sexual differentiation in *O. mossambicus* reared at 20 °C, for example, occurs by 20-d post-hatching [2]. In *T. zillii* held at 30 °C, an ovarian cavity forms by 20-d post-hatching [59]. Since rationing was undertaken in the present study from first-feeding (i.e. 4–5 d after hatching) onwards, it seems likely that most, if not all, of the process of sexual differentiation would have occurred during ration manipulation.

Over the course of the first 60–70 d of the experiment, there was little difference between the two ration sizes (initially 40 and 10 % bw·d^{-1} for high and low ration sizes, respectively) in terms of mean individual fish weight. Fish maintained on the higher ration were clearly larger between days 80 and 160; though these differences were not significant until day 180. Thereafter, fish fed the higher ration were significantly larger and, by the end of the experiment, high ration females were three and a half times larger than those fed the reduced ration. That there was no significant difference in terms of individual fish weight between the high and low ration groups until day 180 suggests that the low ration employed during this period (10 % bw·d^{-1}) did not have a restrictive effect on fish growth compared to fish fed a ration size four times as large. In other words, the provision of an excess of food did not appear to significantly enhance fish growth until 180 d into the experiment. This finding, however, may have been partially influenced by maternal effects.

There were, however, clear differences between the two food ration sizes in terms of fry mortality in the early stages of the experiment, suggesting that the lower food ration size was indeed having a detrimental effect. Over the first 290 d of the experiment, mortality rates were much higher in fish fed the low ration (at ~88 %) than those fed the higher ration (~60 %). Mortality was particularly high during the first 30 d of the experiment. Even at this early stage, mortality rates in the low ration groups were more than twice that observed in the high ration groups. High early mortality probably reflects the stressful transfer of such young fry from incubators to experimental set-ups. This most likely would have been exacerbated in the group experiencing restricted food supply. Macintosh and De Silva [30] found a similar relationship between fry survival and food ration size. In their study of *O. mossambicus* and *O. niloticus* × *O. aureus* hybrids, Macintosh and De Silva [30] found that although cannibalism occurred in all experimental groups (and accounted for 10–35 % of fry mortality), the incidence of cannibalism was much reduced as food ration size was increased. There was little difference in the incidence of cannibalism when comparing the two ration sizes used in the present experiment; cannibalism accounted for 29–45 and 41–44 % of total mortality in high and low ration groups, respectively. Cannibalism amongst tilapia of the same age is a common problem associated with tilapia production, largely due to the appearance of 'shooters' that are much larger than the rest of the stock [31].

It should also be noted that differences in mortality between the two food rations employed here inevitably

resulted in differences in population density in the two treatments. In salmonids, dominance status is inversely related to a chronic state of stress [15] and it has been demonstrated that increasing population/stocking densities lead to reduced growth [16]. In tilapias, the converse is true. It is thought that high population density prevents the establishment of stable territories; as a result, tilapia dominance ranks change much more often [29]. Fish would therefore tend to avoid confrontation and although brief bouts of aggression would tend to occur more often, its effect upon individual fish and on population growth would be much reduced [23]. The improved growth rate of fish fed the higher ration after day 180 of the present study may therefore represent a combination of both improved nutrition and increased population density within the treatment tanks.

Spawning trials demonstrated that spawning periodicity (mean number of days taken for an individual fish to complete one spawning cycle, i.e. mean days elapsed per spawn) was remarkably consistent across the rationed groups despite very significant differences in fish size. Previous studies on the three-spined stickleback [57] and the convict cichlid [47] have also observed a positive relationship between the number of spawns and ration size, though the underlying physiological mechanism remains unclear. In *O. mossambicus* however, ration restriction resulted in increased spawning frequency [34].

Studies involving other species of fish have generally found that food restriction results in a decline in fecundity (for example, rainbow trout [6, 43], brown trout [3], guppy [20], winter flounder *Pseudopleuronectes americanus* (Walbaum) [49], stickleback [56], convict cichlid [47, 48] and *O. mossambicus* [34]). The present study also observed significantly reduced total fecundity at low ration levels. This was attributable, however, to size differences between high and low ration fish; significant relationships exist between fecundity and fish size in *T. zillii* [10]. After adjustment to a common body size using ANCOVA, no significant difference was found between high or low ration fish in terms of either fecundity or egg diameter. This is especially interesting since rationing occurred throughout the period of sexual differentiation.

Neither ANOVA nor ANCOVA found significant differences between the two rationed groups of fish in terms of egg diameter. Although some previous studies have found changes in egg size attributable to ration ([6, 39, 43], all in the rainbow trout), several report that food rationing has no effect upon egg size [4, 17, 20, 26, 56]. Moreover, Fletcher and Wootton [17] found no relationship between egg protein or lipid content (nor DNA or RNA content) and food ration in the three-spined stickleback. Similarly, ration size was found to have no effect on rainbow trout eggs in terms of total fat, protein or amino acid profiles [43]. Conservation of egg composition has also been found in several marine species [25]. Such results suggest, as detailed in Fletcher and Wootton [17], that female fish appear to ensure consistent egg composition (as diet quality and body reserves allow). Studies in the vivaparous guppy, however, demonstrated that food restriction resulted in fewer but larger offspring; females receiving a reduced ration possessed lower fat reserves of their own but incorporated increased fat reserves into each embryo [36].

Sacrifice of fish at the age of ~16 months revealed that GSI did not differ significantly between high or low ration groups. HSI of high ration fish was significantly larger than that of low ration fish, perhaps reflecting greater loading on the liver in terms of protein and fat regulation at high ration levels, increased VTG production or increased glycogen or fat storage. Positive relationships between liver size and food ration were also found by Tyler and Dunn [49] in the winter flounder and by Allen and Wootton [1] in the three-spined stickleback. The livers of sticklebacks maintained on low ration exhibited high rates of weight loss in the pre-spawning period as ovary and carcass weight increased and decreased, respectively. At high rations, post-spawning sticklebacks exhibited high rates of weight gain in their livers as ovary and carcass weight decreased and increased, respectively. Allen and Wootton [1] suggested that the observed autonomy of the stickleback ovary to food supply, particularly outside of the spawning season, may depend upon the buffering action of the liver.

Large differences in ovarian histology were evident between rationed groups in the present study. Low ration fish possessed ovaries containing predominantly pre-vitellogenic and early vitellogenic oocytes whilst high ration fish contained predominantly late-vitellogenic/maturing oocytes. These findings concur with those of Tyler and Dunn [49] in the winter flounder and Townshend and Wootton [47] in the convict cichlid, i.e. that the proportion of vitellogenic oocytes present in the ovary was related to food supply. Reductions in ration were suggested to have such an effect by reducing the recruitment of vitellogenic oocytes. It is possible that the low ration size utilised in the present study altered the usual pattern of ovarian development such that there was a delay in the appearance of vitellogenic oocytes in low ration fish. Similar results have been observed in the European sea bass [8]. There was no significant difference between the two ration sizes in terms of ovarian atresia. This was contrary to the findings of Springate et al. [43] in the rainbow trout where atresia was found to be far more common in fish maintained upon a restricted ration. Atresia has been suggested as a mechanism that could reduce fecundity during food restriction [3, 6, 47, 50, 51, 58]. Although there were significant differences between the two rations levels in terms of ovarian histology, there were no differences in terms of spawning rate. This may suggest that there were differences in the rhythm of ovarian development in fish from differing ration sizes. For example, although the ovaries of low ration fish exhibited significantly fewer vitellogenic oocytes, it is

possible that the process of vitellogenesis in these fish occurred much quicker, once oocytes had been recruited, than in the fish fed high rations. Indeed, previous studies of *T. zillii* have demonstrated that the spawning cycle tends to be much shorter in smaller individuals [10]. The length of the spawning cycle is also known to be much reduced in tilapia that are stunted; prolific breeding by stunted tilapia can rapidly cause over-population within a discrete environment [31].

There are several mechanisms in which food supply could affect egg production in a multiple-spawning fish such as *T. zillii*. Typical mechanisms include the size of females at maturity, the number of spawnings per female and the number of eggs produced by each female. A reduction in egg production may reflect the result of one or more of these mechanisms. A model describing typical effects of such mechanisms is given by Wootton [56]. Further descriptions of the effect of food restriction upon various fish life-history traits can be found in the literature [21, 24, 36, 37, 44, 52–54]. Further investigations of spawning rate, fecundity and egg size in tilapia, in relation to environmental and genotypic factors, are given in Duponchelle et al. [14] and Desprez and Mélard [13], respectively.

In summary, despite very large (and significant) differences in fish size, ANCOVA found no difference in total fecundity or egg diameter in groups of *T. zillii* fed upon two ration sizes (high and low) from first-feeding. That no differences were found in terms of GSI in the sexually-mature ovary suggests that despite large differences in supplied ration throughout sexual differentiation and on-growing, investment in reproductive function remained remarkably consistent. Low ration fish appeared to sacrifice growth to maintain reproductive capacity. This has also been reported in the haddock *Melanogrammus aeglefinus* (L.) [22] and the European sea bass [8]. Under conditions of restricted food supply, an animal can invest its limited income into either somatic maintenance and growth or channel energy into egg production at the expense of body weight [57]. Under food restriction, the winter flounder maintains body weight at the expense of egg production [49]; the proportion of fish with vitellogenic ovaries was reduced at low ration due to increased rates of resorption and a reduction in the proportion of oocytes beginning vitellogenesis. In the present study, since (i) low ration females were significantly smaller than high ration females, (ii) GSI did not differ according to ration size and (iii) ANCOVA found no differences in terms of total fecundity, egg diameter or total egg volume, it is suggested that female *T. zillii* adopt an approach to food restriction different to that of the winter flounder: somatic growth is sacrificed such that reproductive investment can be maintained.

In conclusion, during a prolonged period of food restriction, female *T. zillii* appeared to sacrifice body weight/growth so as to maintain reproductive investment. This implies that if long-term food restriction was used as a practical means of reducing the maintenance costs of this and closely-related species, a given broodstock would only exhibit a predictable modification in those reproductive factors that are closely related to body-size (e.g. total fecundity and spawning periodicity); there should be no change in egg size nor gonado-somatic index.

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