Follicular hierarchy evaluation of pateros ducks (*Anas platyrhynchos domestica*) from semi free-range farms of Zamboanga del Sur and Misamis Occidental, Mindanao, Philippines

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Abstract. The egg-type pateros ducks, *Anas platyrhynchos domestica*, in the Philippines are raised in the traditional semi free-range management system which allows farmers to keep the ducks in flocks as extra activities in between their crop farming routines. This study assessed the follicular development in ducks grown from farms in semi free-range system and evaluated the different conditions of the gonads using the follicular ranks and the frequency of occurrence of follicular atresia. Nineteen ducks from Zamboanga del Sur (9 aged 6-12 months; 10 aged 13-24 months) and 20 ducks from Misamis Occidental (10 each for 6-12 and 13-24 months age group) were dissected and evaluated (n=39). Results showed that the gonadosomatic index (GSI) of 6-12 month-old ducks from Zamboanga del Sur (Group 1) was 0.367% while GSI of the 6-12 month-old ducks from Misamis Occidental (Group 2) was 0.323%. Lower GSI of 0.298% was observed for Group III (13-24 month-old ducks from Zamboanga del Sur) and Group IV with 0.222% (13-24 month-old ducks from Misamis Occidental). Results also revealed atretic, unresponsive, and undeveloped follicles in almost all of the ducks sampled which appear to be affected by the type of feeding management by the farmers.

Key Words: Endocrine disruptor, feeding, follicles, gonads, gonadosomatic index, management.

Introduction. The Philippine duck industry is dominated by *balut* (partially hatched embryos) production and by smallholder production that accounts for more than 75 percent of total duck output (Dagaas & Chang 2004). The demand for duck products in the Philippines continually rises indicating that the duck industry can be a lucrative enterprise. The pateros ducks, *Anas platyrhynchos domestica*, are raised under two farm management systems – complete confinement and semi free-range system (Lambio et al 1988). In the latter, ducks are allowed to range freely the whole day usually in rice fields and are then herded back to their shelter at night time or during severe weather. Semi free-range management system allows the ducks to have varying food sources as compared to the commercial management system wherein the food they eat depends on the farmer's choice. However, semi free-range management system exposes the ducks to a number of ecological factors which could alter their egg-laying capacity. Data over a 10-year period (2003-2013) showed that there is stagnation, if not contraction, in the duck inventory of five out of six regions of Mindanao (Philippine Statistics Authority 2014).

The low egg productivity must be taken into account in order to help the farmers overcome decline in marketing as well as low-quality breeding stocks. One of the ways duck egg production fitness can be assessed is by checking on its follicular hierarchy and occurrences of follicular atresia on the ranks of rapidly growing follicles. Birds have unique characteristic in their ovaries wherein follicles from all stages of development exist at the same time once they reach egg-laying stage (Johnson & Woods 2007). Examination of the preovulatory follicles (POF) may provide a viable method to estimate breeding probability in free-range mallard populations (Lindstrom 2005). POF belonging to a clutch is a good tool for researchers to assess the egg-laying health of ducks and thus draw conclusions for use, enforcement, and policy development by concerned individuals.

The main goal of the study was to determine the state of the gonads of randomly sampled ducks in two provinces in the Philippines. Documenting the growing follicles of duck layers in varying stages of growth and development can give us a physiological knowledge on the state of duck gonads in semi free-range farms of Mindanao.

Material and Method. Thirty-nine randomly selected 6–12 months (early laying, EL) and 13–20 months old (late laying, LL) pateros ducks were sampled from semi free-range farms located in Zamboanga del Sur (ZDS) and Misamis Occidental (MSO) (Figure 1). The birds were divided into four groups based on location and age. Group I consisted of nine EL ducks from Zamboanga del Sur (EL-ZDS); Group II with 10 EL ducks from Misamis Occidental (EL-MSO); Group III with 10 LL ducks from Pagadian City (LL-ZDS); and Group IV with 10 LL ducks from Misamis Occidental (LL-MSO). Body weight of each individual was recorded prior to dissection. After dissection, the gonads were carefully isolated and imaged, weighed, and then stored in a freezer (-4^{0}C) for future use. Mature follicles of follicular hierarchies were measured using ImageJ v1.46r.

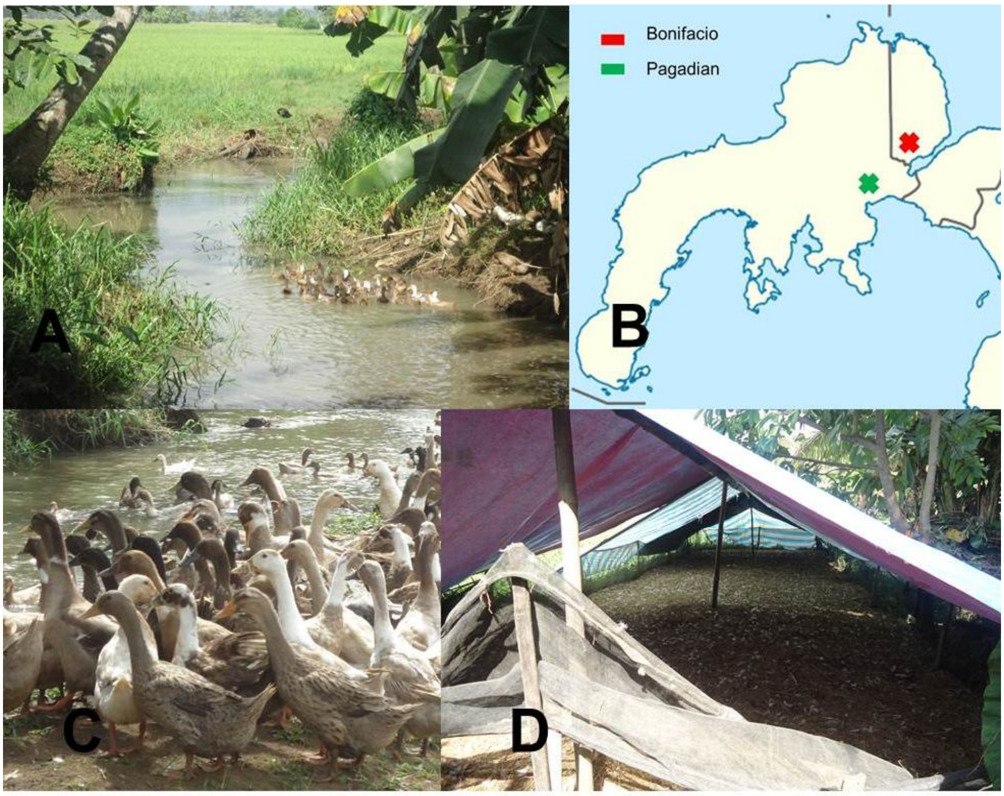

Figure 1. (A) Ducks freely ranging in the streams of a farm in Misamis Occidental. (B) Location of the sampling sites marked “x”. (C) A herd of *Anas platyrhynchos domestica* from Misamis Occidental and the (D) shelter at night. (Photos A, C, D are original photos; B, from www.mindanaomaps.com with modifications).

Results and Discussion. The mean live weights (LW) of groups I at 1,304 g and II at 1,326 g were more or less similar (Table 1) while ducks belonging to Group III were heavier compared to Group IV with mean live weights of 1,472 g and 1,387 g, respectively (Table 2). The mean gonadal weights (GW) and mean gonadosomatic index (GSI) between groups I and II were of values close to each other but varied greatly between Groups III and IV. The GWs of ducks with preovulatory follicles are indicated in red; the ones colored black signify absence of preovulatory follicles.

Table 1
Live weights (LW), gonadal weights (GW), and gonadosomatic index (GSI) of duck layers from Groups I and II (6-12 months)

Sample	*LW (g)*		*GW (g)*		*GSI (%)*	
	I	*II*	*I*	*II*	*I*	*II*
1	1460	1350	1.6	3.5	0.11	0.26
2	1250	1320	3.5	4.7	0.28	0.356
3	1320	1420	1.7	3.3	0.129	0.232
4	1240	1320	2.7	3.4	0.218	0.258
5	1150	1380	11.5*	1.8*	1	0.13
6	1340	1420	6.9*	12.9	0.515	0.908
7	1600	1240	2.1	3.2	0.131	0.258
8	1000	1100	1.8	3.5	0.18	0.318
9	-	1280	-	2.7	-	0.211
10	1380	1430	10.2	4.2	0.739	0.294
Mean	1304	1326	4.667	4.32	0.367	0.323

* - presence of preovulatory follicles in the gonads.

Table 2
Live weights (LW), Gonadal weights (GW), and Gonadosomatic index (GSI) of duck layers from Groups III and IV (13-24 months)

Sample	*LW (g)*		*GW (g)*		*GSI (%)*	
	III	*IV*	*III*	*IV*	*III*	*IV*
1	1780	1340	5.1*	2.6	0.287	0.194
2	1440	1400	3.4	2.8	0.236	0.2
3	1470	1420	6.4	1.6	0.435	0.113
4	1540	1470	3.7*	1.6	0.24	0.109
5	1240	1370	5.3	5.9*	0.427	0.431
6	1340	1170	3.4	1.4	0.253	0.12
7	1320	1260	2.8	1.2	0.212	0.0952
8	1310	1500	1.6	5.2*	0.122	0.347
9	1620	1480	-	4.2	-	0.284
10	1660	1460	7.8*	4.9	0.47	0.336
Mean	1472	1387	4.388889	3.14	0.298	0.22292

* - presence of preovulatory follicles in the gonads.

A comparison on the LWs and GWs in each group showed two opposite trends. Group I data give a negative correlation between LW and GW (Figure 2). Group II data however showed that increasing LW is related with rapidly increased GW. Group III ducks showed the linearity between the LW and GW relationship while Group IV duck samples showed almost similar pattern as Group II with more confined increasing GW within a very small range of LWs. Generally, all duck samples showed that increasing LW was related with increased GW as shown in both semi-ranged farms except for Group I consisting of 6-12 month old ducks from Pagadian City.

Although the graph above shows a good relationship between the LW and GW, the actual condition of the gonads tells otherwise. Of the nine ducks in Group I, only two individuals (samples 5 and 6) had preovulatory follicles (follicles >9 mm), all the rest have follicles at an early stage (Table 3). The inconsistent gonadal weight to live weight relationship in Group I is another thing that needs revisiting. This observed trend in the mature ovary of ducks in a flock would be quite alarming.

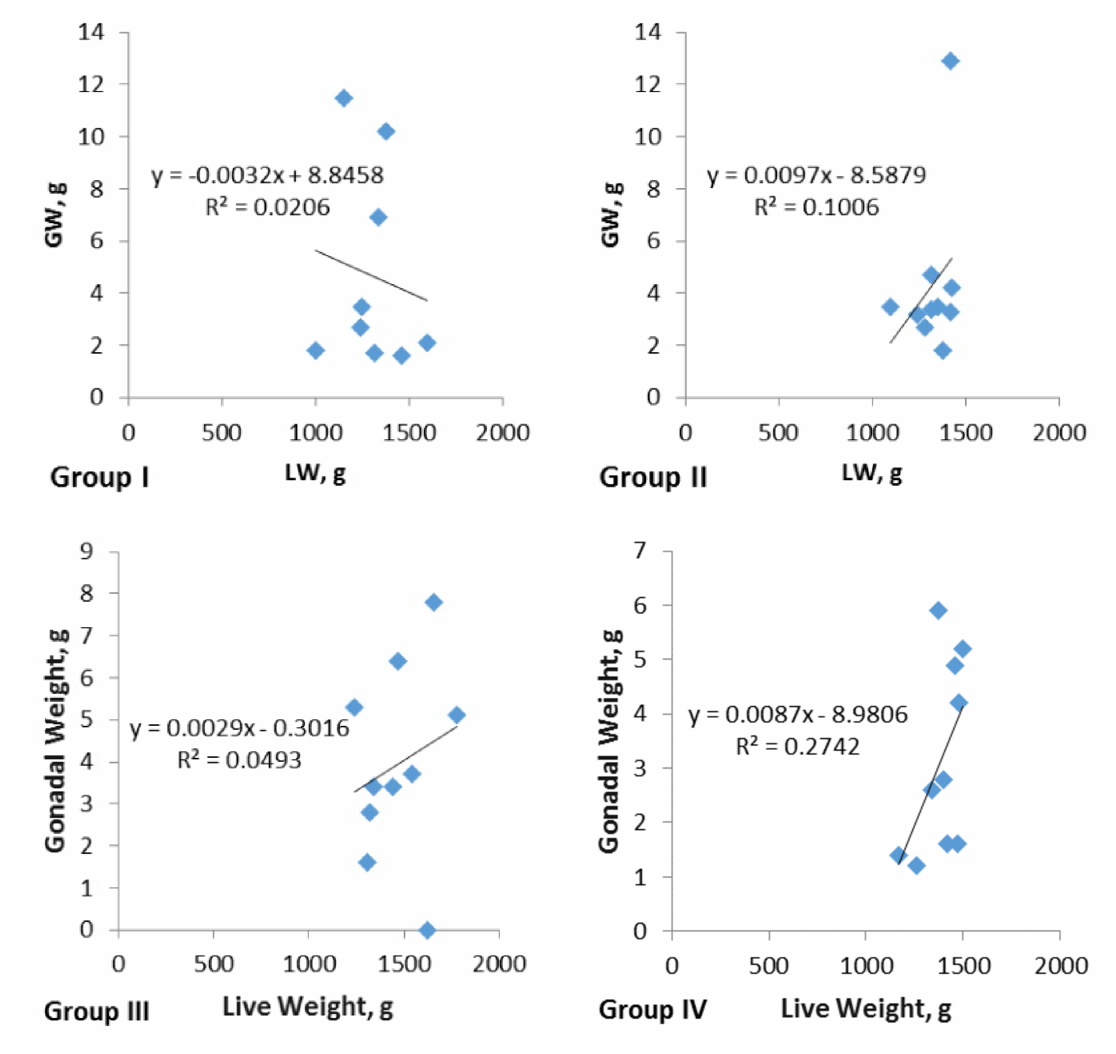

Figure 2. Correlation coefficient and regression equation between gonadal weight (GW) and live weight (LW) of ducks in all groups.

Table 3

Area of preovulatory follicles (mm^2) from duck layers and the number of preovulatory follicles each produced (>9mm)

	F1	*F2*	*F3*	*F4*	*F5*	*F6*	*Mean*
Group I-5	189.572*	130.138*	133.826	137.717*	-	-	147.813
Group I-6	103.842	-	-	-	-	-	103.842
Group II-5	1,243.214	335.138*	347.039*	178.586*	238.573	169.658*	418.701

* - atretic large follicle.

The advanced preovulatory follicles (F1 and F2) of sample 5 were atretic with only the F3 appearing as viable (Figure 3). Already on its second clutch, sample 6 of Group I only had one preovulatory follicle in which succeeding prehierarchal follicles are discolored and have an irregular shape.

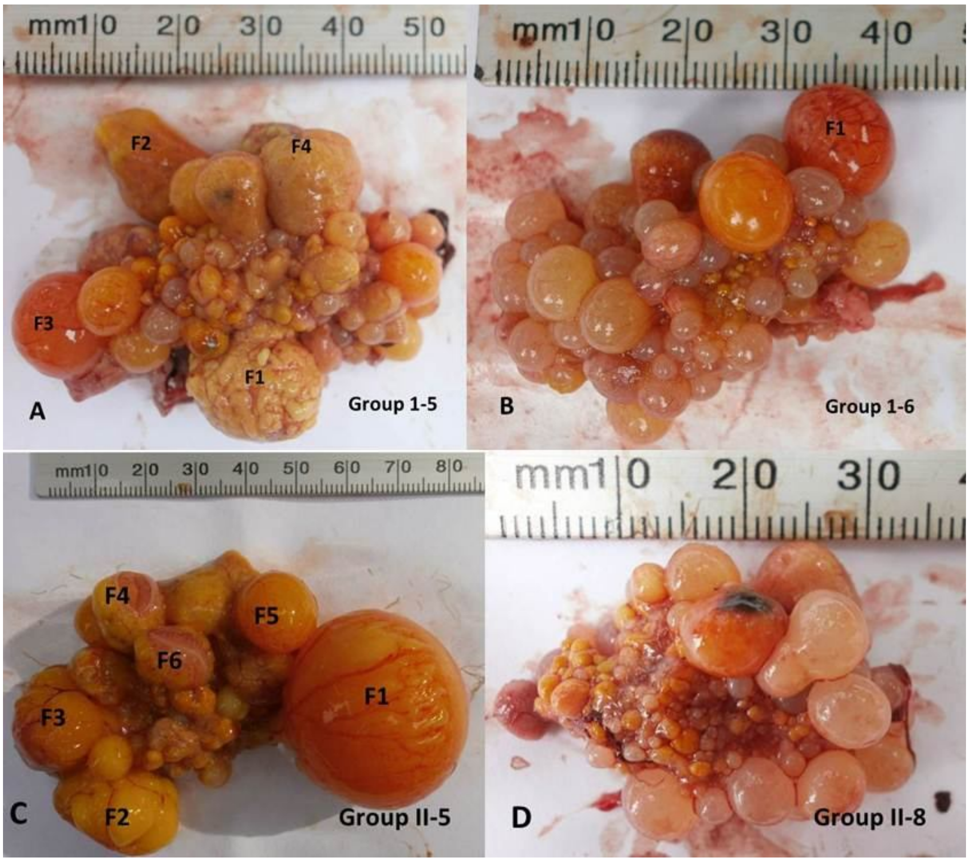

Figure 3. Image compilation of ducks with follicular hierarchy. (A) Group I sample 5 with four preovulatory follicles wherein three are atretic. (B) Group I sample 6 with one preovulatory follicle and a discolored prehierarchal follicle. (C) Group II sample 5 with six preovulatory follicles, note the irregular yolk deposition from in F2-F4 and F6. (D) An ovary with prehierarchal follicles with the largest having an abnormal blood deposition on its surface (original).

Ducks from Group II only had one sample, sample 5, with follicular hierarchy wherein only two are viable preovulatory follicles, F1 and F5. All succeeding follicles were all atretic, with irregular coloration and shapes due to abnormal yolk deposition. Sizes of the preovulatory follicles in Group I were rather small with mean area of 147.813 mm^2 and 103.842 mm^2 compared to the sizes of follicles in Group II which had a mean area of 418.701 mm^2.

Figure 4 shows the follicular hierarchy of Group III and Group IV duck samples with distinct preovulatory follicles. Among the 20 sampled individuals, only a few preovulatory follicles were noted while most have inhibited, unresponsive, and atretic conditions. The noticeable delay in the egg laying of the ducks can be attributed to the presence of atretic follicles.

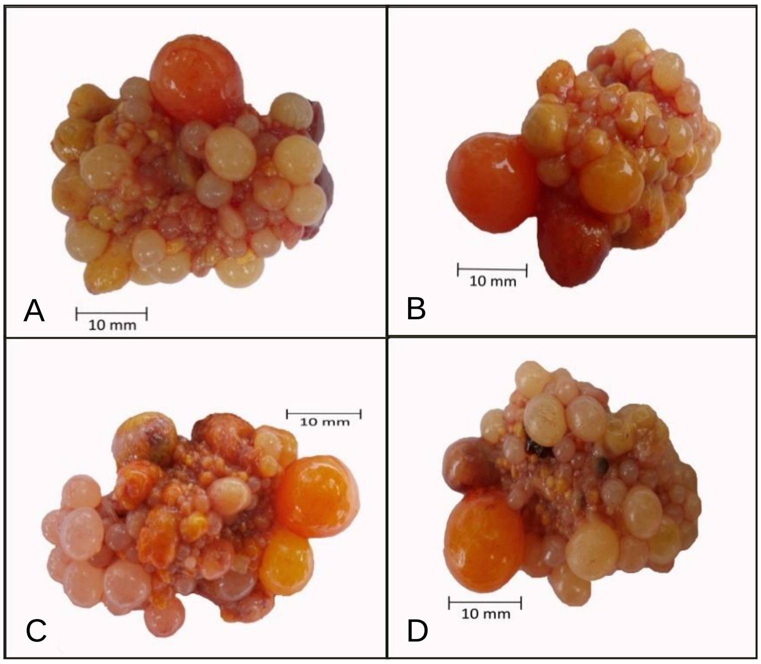

Figure 4. Image compilation of duck gonads (13-24 months old) with follicular hierarchy. (A.) Group III sample 1 with single preovulatory follicle wherein most surrounding follicles are undeveloped and unresponsive. (B.) Group III sample 4 with two preovulatory follicles and irregular yolk deposition. (C.) Group IV sample 5 with five pre-hierarchal follicles wherein 3 are atretic. (D.) Group IV sample 8 gonad with two preovulatory follicles wherein one is unresponsive (original).

Table 4 presents the sizes of the follicles in Group III and IV duck samples as well as the number of preovulatory follicles each one produced. Group III has small sizes of follicles with a mean area of 95.656 mm^2, 124.939 mm^2 and 146.5647 mm^2 compared to Group IV with a mean area of 210.8916 mm^2 and 120.039 mm^2.

Table 4

Area (mm^2) and number of preovulatory follicles of duck layers (13-20 months)

Sample	*F1*	*F2*	*F3*	*F4*	*F5*	*Mean*
Group III - 1	95.656	-	-	-	-	95.656
Group III - 4	95.663	58.552	-	-	-	124.939
Group III – 10	88.996	46.781	32.363	-	-	146.5647
Group IV - 5	83.347	56.829	43.335	22.931	22.248	210.8916
Group IV - 8	100.929	38.22	-	-	-	120.039

Data showed that from Groups III and IV, only five (three and two, respectively) were seemingly reproductively capable with the other seven having delayed maturity since no mature follicles were present despite their age. Follicles in these samples were all in PGC, primary, or prehierarchal stage and most have abnormal atresia. The apparent delay in the reproductive maturity of the ducks and the presence of abnormal atresia can be partially attributed to management system of the farm and its feed administration.

A study of Arora & Samples (2011) reported a linear relationship between body weight and the number of large yellow follicles in heavier birds compared to lighter birds at the onset of sexual maturity. Data collected exhibit the role of body weight as a means of assessing reproductive and physiological traits since body weight is the most important factor in determining the onset of egg production (Ocak et al 2004). This is partially supported with the data gathered since duck samples with distinct follicles were also the ones exhibiting an increased weight. Previous studies have shown that larger follicles yield oocytes with increased rates of development, fertilization, and embryo development than those yielded by smaller follicles (Thomas et al 2000). Large follicles may indicate the beginning of preovulatory luteinization which has a potential role in ovulation (Rice et al 1996). However, most of the ducks with good LW and GW had low follicle quality, even late growing follicles have intervening atresia.

The primordial follicles of all ducks, except for one, were already exhibiting yolk deposition. More advanced follicles in the ovary have no yolks while those in earlier stages were already exhibiting yolk deposition. In addition, all viable preovulatory follicles found had irregular blood vessel formation on its vitelline membrane and all samples with preovulatory follicles are coupled with severely atretic follicles. Data indicate that a large number of individuals have delayed maturity since there were no mature follicles observed in the gonads despite their age along with the fact that they are supposed to be in the peak of laying eggs. Regression of follicle size often makes it difficult to distinguish between POFs and atretic follicles at later stages of envelopment (Lindstrom 2005). Atresia can occur when large yolk-filled follicles or pre-ovulatory follicles are reabsorbed prior to ovulation. Involution atresia occurs when the oocyte and yolk are enveloped into the ovary and the follicle becomes discolored and irregularly shaped. During bursting atresia, ovarian follicles rupture and their yolk contents are released into the body cavity (Johnson 2000).

It was gathered through key informant interviews that the farmers transport the ducks almost every three months to places where there are rice harvests for ducks to feed and help in the consumption of snails which are considered pests on rice. Transportation to long distances causes stress in ducks which is a probable cause for the atresia. This can be verified by the accounts of the farmers that egg production significantly decreases after long distance transport of the ducks.

The noticeable delay in the reproductive maturity of the ducks can be attributed to the presence of these atretic follicles. A study conducted by Urata et al (2006) suggested that atretic follicles present lower estrogen values which reduce their antioxidant mechanisms.

The ducks sampled in this study were ranged in rice fields in order to control snail populations. These rice fields, however, are regularly sprayed with pesticides to increase rice production. Studies have shown that reproduction may be at risk from chemical discharge into the environment particularly those which exist throughout the food chain where ducks were involved. Feeds laced with pesticide and cadmium cause abnormalities which include atretic follicles, deformed follicles, and fibrosis in the ovary of female *A. platyrhynchos domestica* (Anggraeni 2010). Likewise, exposure of 7-11 month old mallard ducks to organophosphate pesticide reduces body weight, egg production, and egg quality (Gile & Meyers 1986). This can explain the presence of abnormally occurring atresia in the ducks from both sampling sites since the ranging ducks drink the water and feed on rice fields which are sprayed with pesticides. In a separate study of Renema et al (1999), it was found that hens fed ad libitum had a sharp increase in follicular atresia, the reason for this is still unknown (Robinson & Renema 2003). From this we can say that the abnormal atresia in the ducks sampled is contributed upon by the feeding style and the quality of feeds being obtained by the ducks. Although the ducks are used to control snail population in rice fields, they appear to be unknowing victims of the adverse effects of pesticides.

In a study on *Coturnix japonica* layers (Arora & Samples 2011), body weight was found to have a distinct bearing on the weight of reproductive and physiological traits. One disadvantage of the feeding method the farmers use is that each duck does not have a regular intake of food as food availability in rice fields is limited since ducks can only graze on fields that have been freshly harvested and the food the ducks eat are not being regulated. Feed restriction results in loss of body weight which ultimately causes alterations in body composition and reduction of ovarian follicles (Arora & Samples 2011).

This paper found that the semi free-range duck management system that some Mindanao farmers employ can have adverse effects on the reproductive physiology of ducks as evidenced by the apparent delayed reproductive maturity of the supposed duck layers and the presence of abnormal atresia in their ovaries. The system in itself does not cause the physiological problems of the ducks, instead, it is the quality of food and water the ducks are getting from the rice fields.

Conclusions. All the ducks sampled although reaching the required live weights to be considered to belong to laying age, showed problematic follicular development which includes irregular yolk deposition, abnormal atresia, and delayed gonadal maturity. It appears that these problems can be attributed to the diet of ducks sourced from pesticide-laced rice fields. This study acknowledges the significant implication of farming system to the reproductive performance of sexually mature ducks.

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