

SMALL, FAT MALE MOORHENS *GALLINULA CHLOROPUS* ARE HEALTHIER

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SUMMARY.—*Small, fat male moorhens Gallinula chloropus are healthier.*

Aims: To find out whether the fatter and smaller moorhen males (which are preferred by females as mates) are also healthier.

Location: Southwestern Andalusia (southern Spain).

Methods: Analysing the variation in time along the autumn and winter of the extent of the fat reserves of adult male moorhens. In the period preceding pair formation (February 2003), the relationship was obtained between body size (represented by tarsus length) and an effective index of fat reserves (body mass x tarsus length⁻³) with tarsi fluctuating asymmetry, haematocrit, leukocyte index, heterophil/lymphocyte ratio and albumin/globulins ratio.

Results: The extent of the fat reserves decreases during the autumn and winter. The heterophil/lymphocyte ratio was found to be significantly and positively related to body size, and negatively so to the fat index. In addition, the fat index was found to be significantly and negatively related to tarsi fluctuating asymmetry.

Conclusions: The findings that preferred fatter males are more symmetrical, and that these fatter males, as well as those of smaller size, are healthier with respect to the heterophil/lymphocyte ratio point to the election of male mates, not only for the benefits derived from maintaining incubation, but also of good health.

Key words: body condition, *Gallinula chloropus*, mate choice, Moorhen.

RESUMEN.—*Machos de Gallineta Común Gallinula chloropus con más grasa y menor tamaño corporal son más sanos.*

Objetivos: Determinar si los machos de Gallineta Común con mayores depósitos grasos y de menor tamaño corporal (preferidos por las hembras para formar pareja) son más sanos.

Localización: Sudoeste de Andalucía (España).

Métodos: Se analizó la variación temporal durante el otoño y el invierno del nivel de los depósitos grasos subcutáneos de machos adultos de Gallineta. En el periodo previo a la formación de parejas (febrero de 2003) se obtuvo la relación entre el tamaño corporal (longitud del tarso) y un índice efectivo de grasa subcutánea (masa corporal x tarso⁻³) con la asimetría fluctuante en longitud del tarso, hematocrito, índice leucocitario, relación heterófilos/linfocitos, y relación albúmina/globulinas en plasma.

Resultados: El nivel de los depósitos grasos disminuye durante el otoño e invierno. La relación heterófilos/linfocitos resultó estar significativa y positivamente relacionada con el tamaño corporal, y negativamente con el índice de reserva grasa. Además, el índice de grasa se relaciona significativa y negativamente con la asimetría fluctuante en la longitud de los tarsos.

Conclusiones: El que los machos con mayores reservas grasas, preferidos por las hembras como pareja, sean más simétricos, y que estos machos más gruesos, así como los de menor tamaño corporal, sean más

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sanos con respecto a la relación heterófilos/linfocitos sugiere que la elección no se basa únicamente en la mayor constancia en la incubación por parte de los machos de mayor calidad, sino también en su buena salud.

Palabras clave: condición física, elección de pareja, Gallineta, *Gallinula chloropus*.

INTRODUCTION

The Moorhen *Gallinula chloropus* is a socially monogamous (sometimes polyandrous) rallid with partial sex-role reversal, females being more aggressive than males in the winter flocks, and more active in courtship and pair formation, thus acting according to sociobiological theory (Trivers, 1972), since the males perform most of the incubation (Ripley, 1977; Petrie, 1983a, 1983b, 1988; Siegfried & Frost, 1975; Del Hoyo *et al.*, 1996).

Petrie (1983a) showed that female moorhens compete for exclusive access to males with large fat reserves which tended to be of small body size. According to Petrie, females would actually benefit from their choice of good male incubators, since, due to the high energetic cost of incubation, fat males are able to sit on the eggs for longer periods, while possibly because of energetic constraints on large body size, such high quality males are also smaller.

High fat reserves have been observed to accompany a good health state in migrating birds (Merilä & Svensson, 1995; Svensson & Merilä, 1996). On the other hand, and particularly for male moorhens, the energetic requirements of the costly incubation, and the higher number of clutches per season in charge of the fatter males (Petrie, 1983a), suggest that fatter birds may also be in better health conditions. To analyze this hypothesis, the potential relationships of body size and extent of fat deposits of male moorhens with several indices of condition (namely fluctuating asymmetry, haematocrit, leucocytes count, heterophil/lymphocyte ratio, and albumin/globulin ratio) were explored in the period just preceding pair formation.

MATERIAL AND METHODS

The study was carried out at La Lantejuela (SW Spain, 37°21' N, 5°13' W). Moorhens in the area form flocks during autumn and winter, and pair off by the months of March and April. Values of the extent of the fat reserves were obtained for 93 adult males caught from the beginning of September 2002 to the end of February 2003. In addition, values of several indices of adult male body condition were obtained before the start of the breeding season (during the month of February) (of 60, 30 and 25 subjects, respectively, for tarsi fluctuating asymmetry, haematocrit and counts of leukocytes and blood parasites, and plasma proteins estimates). All subjects were caught in the early morning, at the shores of fresh water pools.

The subjects were weighed (portable electronic balance of 1 g accuracy), and both tarsi were measured with digital callipers (± 0.01 mm accuracy). In the same way as Petrie (1983a), tarsus length was used as an index of body size, and (body mass \times tarsus length⁻³) (which was found by Petrie 1983a to indicate the weight of representative fat pads) was used as an index of subcutaneous fat reserves. Fluctuating asymmetry (FA) of length of tarsi was computed as length difference ($|\text{Right-Left}|$), since it did not change with length (Palmer, 1994).

Blood samples (about 1.5 ml) were taken from the leg vein. From each sample two capillaries were used to obtain haematocrit values, and two blood smears for leukocyte and blood parasites counts. Two drops of blood were used for sex determination, and the rest was used for plasma proteins assays.

Capillaries were centrifuged for 8 min at 11500 rpm in a portable centrifuge (Bayer M

TABLE 1

Values ($X \pm SD$) of the variables of body condition of male free-living moorhens during the month of February 2003 at La Lantejuela (Spain).

[Valores ($X \pm DT$) de las variables de condición física de machos de Gallineta viviendo en libertad durante el mes de febrero de 2003 en La Lantejuela (España)]

| | $X \pm SD$ | n |
|--|-------------------|----|
| Body size (tarsus length, mm) [Tamaño corporal (longitud del tarso, mm)] | 51.33 ± 1.30 | 60 |
| Fat index (body mass x tarsus length ⁻³) [Índice de grasa subcutánea (peso x long. del tarso ⁻³)] | 2.42 ± 0.32 | 59 |
| Tarsi FA (length difference, mm) [FA de tarsos (diferencia en longitud, mm)] | 0.40 ± 0.21 | 60 |
| Blood parasites [Parásitos sanguíneos] | 0 | 30 |
| Haematocrit [Hematocrito] | 0.44 ± 0.04 | 30 |
| Leukocyte count per field in blood film [Número de leucocitos por campo en frotis] | 0.65 ± 0.41 | 30 |
| Leukocyte index (leukocyte count x PCV) [Índice leucocitario (total de leucocitos x PCV)] | 27.96 ± 18.08 | 30 |
| H/L ratio (heterophils / lymphocytes) [Relación H/L (heterófilos / linfocitos)] | 1.36 ± 1.32 | 30 |
| Alb/Glo ratio (albumin / immunoglobulins) [Relación Alb/Glo (albúmina / inmunoglobulinas)] | 1.75 ± 0.35 | 25 |

1101), and the haematocrit was expressed as a volume of the part of the capillary occupied by blood cells/ total blood volume in the capillary. Blood smears were fixed in absolute methanol for 10 min and then stained following the May Grünwald-Giemsa technique. Leucocytes were counted on 100 fields under oil, using magnification of X 100. Erythrocytes and leucocytes were also counted in 10 of those 100 fields, and their average numbers per field were positively correlated ($r = 0.438$, $n = 30$, $P = 0.015$). A leucocyte index was obtained by multiplying each subject's leucocyte count by its observed PCV (Campbell, 1995, modified), and the ratio of the counts of heterophils to lymphocytes (H/L ratio) was also used as a parameter of health (Gross & Siegel, 1983). Although blood

parasites were looked for in 100 fields, using magnification of X 100, none was found in the samples.

Two drops of blood preserved in absolute ethanol were used for sex determination. The technique used was the polymerase chain reaction (PCR) amplification of CHD1 genes with primers P2 and P8, following Griffiths *et al.* (1998). Females were identified by the amplification of two PCR products of 380 and 340 base pairs, corresponding to CHD1-Z and CHD1-W genes, respectively, while males yielded only the longer fragment.

Plasma samples for protein electrophoresis were obtained by centrifuging blood for 5 min at 4000 rpm (vials contained gel aided the separation of plasma from clots) and then stored at 4°C. Data to obtain the relative abundance

TABLE 2

Simple linear correlations of body size and fat index (body mass x tarsus length⁻³) with the variables of body condition of male free-living moorhens during the month of February 2003 at La Lantejuela (Spain). [*Correlaciones simples del tamaño corporal y del índice de grasa subcutánea (masa corporal x tarso⁻³) con las variables de condición física de machos de Gallineta viviendo en libertad durante el mes de febrero de 2003 en La Lantejuela (España)*]

| | Body size [Tamaño corporal] | | | Fat index [Índice de grasa subcutánea] | | |
|--|--------------------------------|--------------------|------|---|---------------------|------|
| | r | P | (n) | r | P | (n) |
| Tarsi FA [FA de tarsos] | -0.110 | 0.40 | (60) | -0.355 | 0.006* | (59) |
| Haematocrit [Hematocrito] | -0.011 | 0.96 ^{ns} | (30) | -0.064 | 0.74 ^{ns} | (30) |
| Leukocyte index [Índice leucocitario] | -0.061 | 0.75 ^{ns} | (30) | -0.001 | 0.99 ^{ns} | (30) |
| H/L ratio [Relación H/L] | 0.468 | 0.009* | (30) | -0.542 | 0.002* | (30) |
| Alb/Glo ratio [Relación Alb/Glo] | 0.306 | 0.14 ^{ns} | (25) | 0.030 | 0.886 ^{ns} | (25) |

^{ns} : not significant, * : Significant at $P < 0.05$ after a sequential Bonferroni correction for multiple comparisons.
[^{ns} : no significativo, * : Significativo al nivel $P < 0.05$ tras realizar la corrección secuencial de Bonferroni para comparaciones múltiples]

of albumin and of immunoglobulins (alpha-, beta-, and gamma-globulin) (Alb/Glo ratio, i.e., the ratio between areas in the densitometric profile) were obtained with the instrument Paragon CZE 2000 by capillarity-zone electrophoresis.

In order to assess repeatability, two samples were obtained for all variables of random choice subjects. Since values for all variables did not deviate from a normal distribution ($P > 0.20$, Kolmogorov-Smirnov test), simple linear correlation tests were used. Comparisons were tested by the Bonferroni correction for multiple comparisons (Sokal & Rohlf, 1995). The repeatability of all recorded variables (tarsus length, tarsi FA, body weight x tarsus length³, haematocrit, leukocyte index, and H/L and Alb/Glo ratios) was high (coefficient of intraclass correlation: $0.810 < r_1 < 0.984$, $13 < df < 65$, $P < 0.001$).

RESULTS

The relationship of the fat index with the date of observation of each male when caught for the first time (number of days from September 1) was found to be negatively significant ($r = -0.281$, $n = 93$, $P = 0.006$). In other words, with the passage of time during autumn and winter, males progressively lose their fat reserves.

Considering now the relationships of body condition in the period previous to pair formation (values are in Table 1), as expected, the two dependent variables (body size and fat index) were highly related to each other ($r = -0.340$, $n = 59$, $P = 0.005$), since body size is represented by tarsus length, and the fat index is (body mass x tarsus length⁻³).

The result of the correlations of body size and fat reserves with the parameters of body

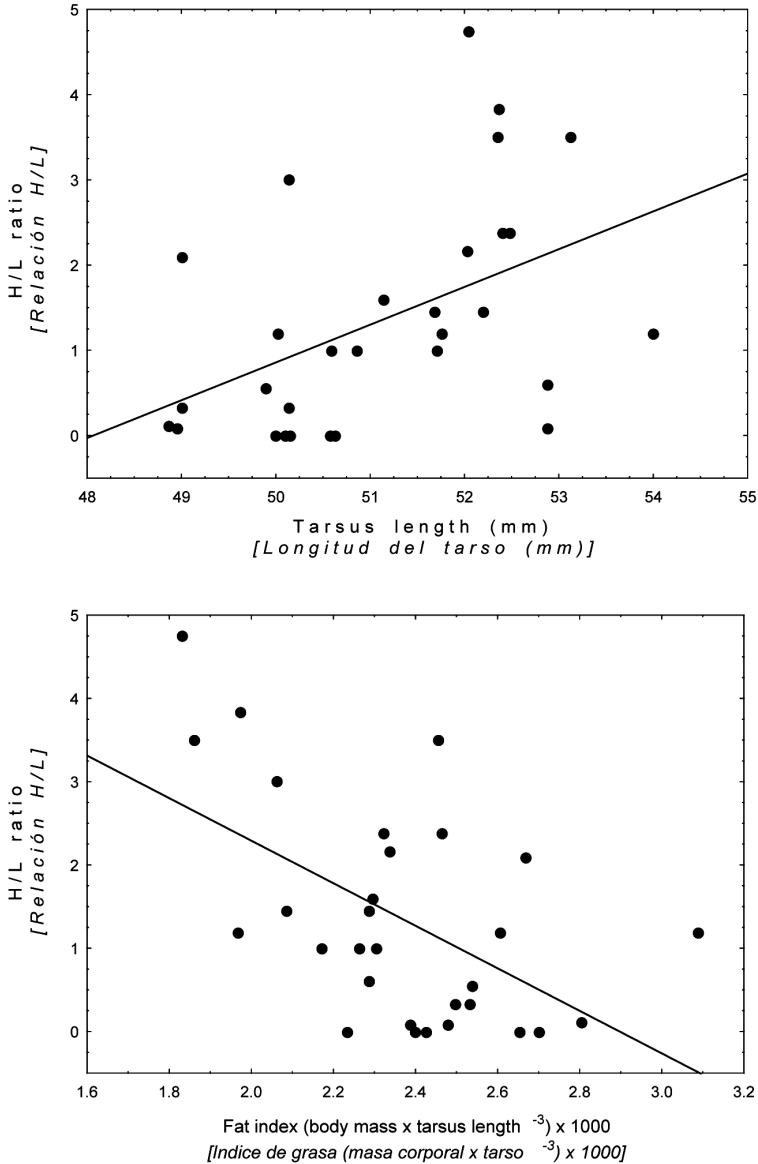


FIG. 1.—Relationship of the heterophil/lymphocyte (H/L) ratio with body size (above) and with the extent of their fat reserves (below) of male free-living moorhens during February of 2003 at La Lantejuela (Spain).

[Relación de la razón heterófilos/linfocitos (H/L) con el tamaño corporal (arriba) y con el índice de grasa subcutánea (abajo) de machos de *Gallineta* viviendo en libertad durante el mes de febrero de 2003 en La Lantejuela (España).]

condition, corrected according to the Bonferroni test for multiple comparisons, yielded significant results for the comparisons of the H/L ratio with both the body size and fat index (Table 2 and Fig. 1). The H/L ratio was shown to be positively related to body size (smaller males are healthier in this respect), and negatively so with their fat index (fatter males are also healthier). In addition, the fat index was found to be negatively related to tarsi FA (i.e., fatter males are more symmetrical).

DISCUSSION

In the same way as reported by Acquarone *et al.* (1998) for moorhens (sex undetermined) wintering in North Italy, the male moorhen population studied here decreases its fat reserves through the autumn and winter. Therefore, it will be those males skilful enough to resist that fat-losing trend, and reaching the month of February, prior to pair formation in March and April, with sufficient subcutaneous reserves that will probably be qualified as mates and perhaps chosen as such by the females. Efficient feeding would probably renew the fat deposits mobilized by the winter low temperatures (Acquarone *et al.*, 1998)

Concerning the relationships with body condition, the H/L ratio is widely used as an index of stress, increasing in response to infectious disease and other stressors, and resulting from a decrease in the lymphocyte number and a simultaneous increase in the heterophil number (Gross & Siegel, 1983; Maxwell 1993; Hůrak *et al.*, 1998). Although an elevated number of heterophils can be interpreted as enhancing resistance to bacterial infection, tissue damage due to inflammatory processes may take place (Fulton *et al.*, 1996).

Consequently, when opting for fatter and smaller males (and, therefore, with lower H/L ratio), females would not only be choosing high quality male incubators, but also healthier mates

less prone to suffering from infectious diseases, starvation or psychological disorders.

Because high H/L ratio is indicative of a disease process, it may be that moorhen males showing signs of infectious diseases are unable to feed efficiently enough to fill up their fat reserves. Alternatively, restricted feeding (and, consequently, followed by a descent of fat reserves) may produce high H/L ratio. In connection with this, Maxwell *et al.* (1990) reported a slight H/L increase in restricted-fed broilers.

Thus, when female moorhens are choosing fatter and more symmetrical males, they are probably securing access to healthier mates, since FA appears to be a good indicator of environmental and genetic stress (Leary & Allendorf, 1989; Parsons, 1992; Swaddle & Witter 1994; Møller *et al.*, 1995; Yang *et al.* 1997).

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