

Morphometric Model for Sex Assessment in Hatchling Olive Ridley Sea Turtles

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ABSTRACT. – A non-lethal method for sex assessment in olive ridley sea turtle hatchlings, *Lepidochelys olivacea*, was investigated. Four nests were incubated in the laboratory under controlled conditions, with a part of each clutch at a female-producing temperature (32°C) and the remainder at a male-producing temperature (28°C). The phenotypic variability in hatchlings obtained was characterized. We recorded 51 variables, 21 meristic and 30 morphometric, in our search for specific characteristics of each sex. With multivariate methods, a continuously variable discriminant function of 30 morphometric characters was found that gave a definite sex assessment. Because we were searching for simpler methods to use in sea turtle conservation programs, we reduced the number of variables to 9 morphometric characters, and achieved correct estimation of sex with 95% confidence. Whether this methodology is also effective for natural nests remains to be investigated.

KEY WORDS. – Reptilia; Testudines; Cheloniidae; *Lepidochelys olivacea*; sea turtles; conservation; sexing method; temperature-dependent sex determination; discriminant function; morphometrics; Mexico

Sea turtles are a natural resource of great socioeconomic importance. Because of their high value and demand in national and international markets, in Mexico there has been massive capture of adults offshore and intensive egg poaching on beaches used for turtle nesting.

In Mexico, there have been *in situ* programs for sea turtle conservation since 1966 (Márquez et al., 1976), and the banning of taking of all sea turtles since 1990 (Anonymous, 1990). The conservation strategy used for the protection of sea turtles is based on the protection of gravid females in nesting areas and translocation of eggs from natural nests to hatchery beaches or styrofoam boxes.

Limpus and Miller (1980), Mrosovsky and Yntema (1980), and Standora and Spotila (1985) have suggested that nest translocation to a place with different temperatures from those under natural conditions affects the population sex ratio, because sea turtles like many other reptiles, have temperature-dependent sex determination (TSD).

This mechanism of sex determination has been found by laboratory and field studies in at least 16 turtle genera (Janzen and Paukstis, 1991), including the olive ridley, *Lepidochelys olivacea* (Morreale et al., 1982; Dimond and Mohanty-Hejmadi, 1983; McCoy et al., 1983; Merchant-Larios et al., 1989).

McCoy et al. (1983) and Silva et al. (1986) determined that olive ridley eggs incubated at temperatures of 28°C produced 100% male hatchlings. When incubated at 30°C, the result was about 50% males and 50% females, and at 32°C, 100% female hatchlings were produced. However, hatchlings do not appear to show external sexual dimorphism. In reptiles, hatchling sexual dimorphism has only been found in crocodiles, *Alligator mississippiensis*, for which incubation temperatures affect pigmentation and size (Deeming and Ferguson, 1989), and in softshell turtles, *Apalone spinifera spinifera*, which develop sex-specific shell markings (Graham and Cobb, 1998).

In conservation strategies for sea turtles (Limpus, 1993), it is important to develop techniques for sex assessment in hatchlings to determine the sex ratio produced using various conservation practices. Current methods for hatchling sex assessment are either lethal (Yntema and Mrosovsky, 1979; Van der Heiden et al., 1985) or highly sophisticated (Crain et al., 1994), and therefore their application is limited.

We determined phenotypic variability of known-sex laboratory-incubated olive ridley hatchlings in order to discover meristic and morphometric characteristics to make the assessment of sexual dimorphism possible and to develop a non-lethal method to estimate sex ratios. Hopefully such non-lethal assessment might also become applicable in conservation programs under natural conditions.

METHODS AND MATERIALS

Our work was based on the hypothesis that there are different characteristics, meristic and morphometric, between female and male olive ridley hatchlings. Incubation under controlled laboratory conditions was done at female and male temperatures to have a representative sample of hatchlings with known sex.

Four nests of olive ridley turtles were collected on 15 August 1990 at El Verde Camacho, Sinaloa, Mexico, and incubated at the Centro Regional de Investigaciones Pesqueras (CRIP) in Mazatlán. Forty eggs were selected at random from each nest, incubating 20 eggs at female-producing temperatures (32°C) and 20 at male-producing temperatures (28°C), using electric incubators in styrofoam boxes with vermiculite and recording the incubation temperatures daily. In this way, 160 eggs were incubated, with 80 at each of the two temperatures.

In all the hatchlings obtained (females and males), 51 variables were recorded; 21 meristic (scute and claw number)

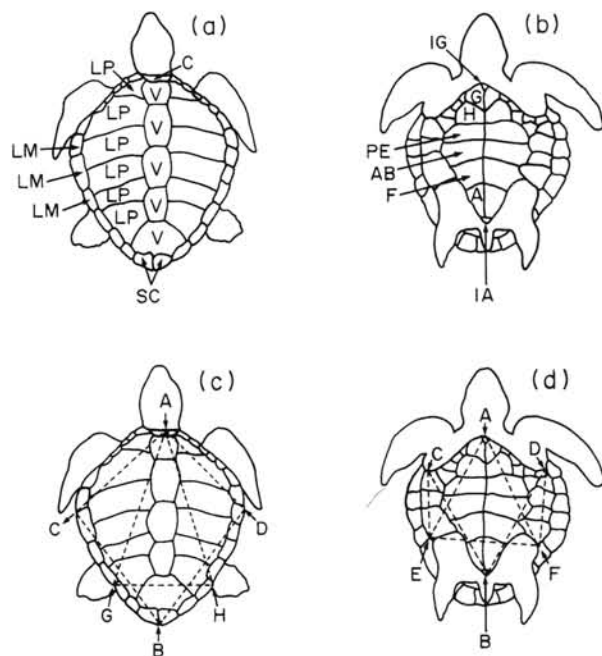


Figure 1. Some meristic and morphometric variables recorded in olive ridley hatchlings: **a)** scute numbers for carapace; cervical (C), vertebral (V), left pleural (LP), left marginal (LM), supracaudal (SC); **b)** plastron; intergular (IG), gular (G), humeral (H), pectoral (PE), abdominal (AB), femoral (F), anal (A), infraanal (IA); **c)** body shape measurements for carapace; point A to C (AC), A to D (AD), A to G (AG), A to H (AH), B to C (BC), B to D (BD), G to H (GH); **d)** body shape measurements for plastron: point A to E (PAE), A to F (PAF), B to C (PBC), B to D (PBD), C to E (PCE), D to F (PDF).

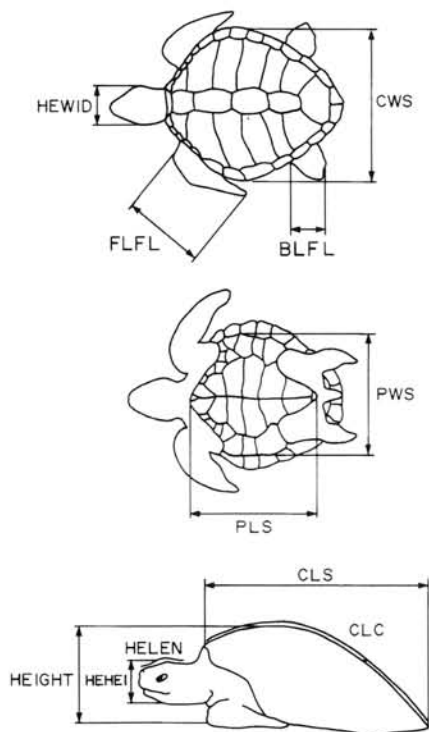


Figure 2. Some morphometric variables recorded in olive ridley hatchlings: carapace length straight (CLS), carapace length curved (CLC), carapace width straight (CWS), plastron length straight (PLS), plastron width straight (PWS), head length (HELEN), head height (HEHEI), body height (HEIGHT), front left flipper length straight (FLFL), back left flipper length straight (BLFL) (see Table 1 for the rest).

and 30 morphometric (body measurements). The latter were made by using a millimeter caliper, a metric tape, and a scale (Figs. 1 and 2; Table 1).

The meristic variables we recorded were scute and claw numbers. We recorded scute number for the carapace: cervicals (C), vertebrals (V), left pleurals (LP), right pleurals (RP), left marginals (LM), right marginals (RM), and supracaudals (SC). For the plastron we recorded left inframarginals (LI), right inframarginals (RI), intergulars (IG), gulars (G), humerals (H), pectorals (PE), abdominals (AB), femorals (F), anals (A), and infraanals (IA). We recorded the front left claw number (FLCN) and right (FRCN), and for the hind limbs, left claw number (BLCN) and right (BRCN).

The morphometric variables recorded were: carapace length straight (CLS), carapace length curved (CLC), carapace width straight (CWS), carapace width curved (CWC), plastron length straight (PLS), plastron length curved (PLC), plastron width straight (PWS), plastron width curved (PWC), head length (HELEN), head width (HEWID), head height (HEHEI), front left flipper length straight (FLFL) and right (FRFL), back left flipper length straight (BLFL) and right (BRFL), body height (HEIGHT), and wet weight (WEIGHT).

Additionally, body shape measurements were recorded (Humphries et al., 1981; Strauss and Bookstein, 1982; Bookstein et al., 1985). These measurements, shown in Fig. 1(c, d) are carapace; point A to C (AC), A to G (AG), A to H (AH), B to C (BC), B to D (BD), and G to H (GH), and plastron; point A to E (PAE), A to F (PAF), B to C (PBC), B to D (PBD), C to E (PCE), and D to F (PDF). The body reference points were from Frazier (1983). To verify the sex

Table 1. Meristic and morphometric variables recorded in olive ridley hatchlings; all length measurements are in cm, weight in g.

Meristic Variables

| | |
|--------------------------|--|
| Cervical (C) | Humeral (H) |
| Vertebral (V) | Pectoral (PE) |
| Left pleural (LP) | Abdominal (AB) |
| Right pleural (RP) | Femoral (F) |
| Left marginal (LM) | Anal (A) |
| Right marginal (RM) | Infraanal (IA) |
| Supracaudal (SC) | Front left flipper claw number (FLFN) |
| Left inframarginal (LI) | Front right flipper claw number (FRFN) |
| Right inframarginal (RI) | Back left flipper claw number (BLFN) |
| Intergular (IG) | Back right flipper claw number (BRFN) |
| Gular (G) | |

Morphometric Variables

| | |
|--|---------------------------------|
| Carapace length straight (CLS) | Body height (HEIGHT) |
| Carapace length curved (CLC) | Wet weight (WEIGHT) |
| Carapace width straight (CWS) | Points A to C on carapace (AC) |
| Carapace width curved (CWC) | Points A to D on carapace (AD) |
| Plastron length straight (PLS) | Points A to G on carapace (AG) |
| Plastron length curved (PLC) | Points A to H on carapace (AH) |
| Plastron width straight (PWS) | Points B to C on carapace (BC) |
| Plastron width curved (PWC) | Points B to D on carapace (BD) |
| Head length (HELEN) | Points G to H on carapace (GH) |
| Head width (HEWID) | Points A to E on plastron (PAE) |
| Head height (HEHEI) | Points A to F on plastron (PAF) |
| Front left flipper length straight (FLFL) | Points B to C on plastron (PBC) |
| Front right flipper length straight (FRFL) | Points B to D on plastron (PBD) |
| Back left flipper length straight (BLFL) | Points C to E on plastron (PCE) |
| Back right flipper length straight (BRFL) | Points D to F on plastron (PDF) |

Table 2. Results of olive ridley egg incubations; temperatures in °C.

| | Female Nests | | | | Male Nests | | | |
|----------------------------|--------------|------|------|------|------------|------|------|------|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Mean temperature | 32.7 | 32.7 | 32.4 | 32.4 | 28.7 | 28.7 | 28.3 | 28.3 |
| Std. dev. temperature | 0.5 | 0.5 | 0.2 | 0.2 | 0.7 | 0.7 | 0.2 | 0.2 |
| Maximum temperature | 33.8 | 33.8 | 33.6 | 33.6 | 30.1 | 30.1 | 29.0 | 29.0 |
| Minimum temperature | 32.1 | 32.1 | 31.9 | 31.9 | 28.7 | 28.7 | 27.5 | 27.5 |
| Eggs hatching (<i>n</i>) | 19 | 19 | 16 | 0 | 19 | 20 | 19 | 0 |
| Hatching % | 95 | 95 | 80 | 0 | 95 | 100 | 95 | 0 |
| Incubation days | 42 | 42 | 42 | - | 52 | 51 | 51 | - |

of each hatchling, the cleared-gonads glycerin technique (Van Der Heiden et al., 1985) was used.

From a univariate point of view, descriptive statistics were obtained and the frequency distributions of each variable, meristic and morphometric, was analyzed with the two-sample Kolmogorov-Smirnov test between females and males (Zar, 1996).

From multivariate methods, in morphometric variables, a discriminant analysis with the objective of group definition was made (Morrison, 1976). As a first step, a correlation matrix per sex and a covariance matrix were obtained. A discriminant function and a graphical solution with all 30 variables considered were obtained. Afterward, a "discarded variables analysis" was done using correlation coefficients. The statistical analysis was made using STATGRAPHICS for MS-DOS.

RESULTS

The variation in the observed clutch temperatures was within the permissible limits for the production of high percentages of individuals of single sex (McCoy et al., 1983; Silva et al., 1986). The mean temperature was 32.7°C for female nests 1–2 and 32.4°C for female nests 3–4, and 28.7°C and 28.3°C for male nests 1–2 and 3–4. Hatching percentage was between 80 and 100%, excepting nest 4, which was an unfertilized nest in which no hatchlings were found (Table 2).

We obtained 112 hatchlings, 54 in the "female" incubator (32°C) and 58 in the "male" incubator (28°C). All the hatchlings showed histologic evidence of the expected sex as a function of incubation temperature.

Once the meristic and morphometric measurements were made, a 39 x 112 matrix was obtained, eliminating from the

analysis 12 variables that did not demonstrate variation: supracaudal (SC), left inframarginal (LI), gular (G), humeral (H), pectoral (P), abdominal (A), femoral (F), and anal (A) scutes; front left claw number (FLCN) and right (FRCN), and back left claw number (BLCN) and right (BRCN).

Meristic variables showed similar trends in observed frequencies for all hatchlings of each sex, with an important proportion of overlapping between them (Table 3). Although some characters approached significance (e.g., intergular scutes) the high error values and small sample sizes precluded using these characters for sex assessment.

The univariate analysis of the 30 morphometric measurements showed that some were not useful for sexual assessment (Table 4), even though the Kolmogorov-Smirnov test showed significant differences between distributions for the total hatchlings in 13 variables. However, plots of each variable showing minimum, maximum, and standard deviation values demonstrated a substantial range overlap between sexes in all cases (Fig. 3).

By contrast, multivariate methods, like discriminant analysis with all 30 morphometric variables, showed a clear separation between the sexes, with positive function values corresponding to females and negative function values to males (Fig. 4).

Sex assessment was definite if we considered all 30 variables, but because our goal was also to develop a practical method for sex assessment for sea turtle conservation programs, taking 30 measurements on a large number of hatchlings might not be a practical protocol.

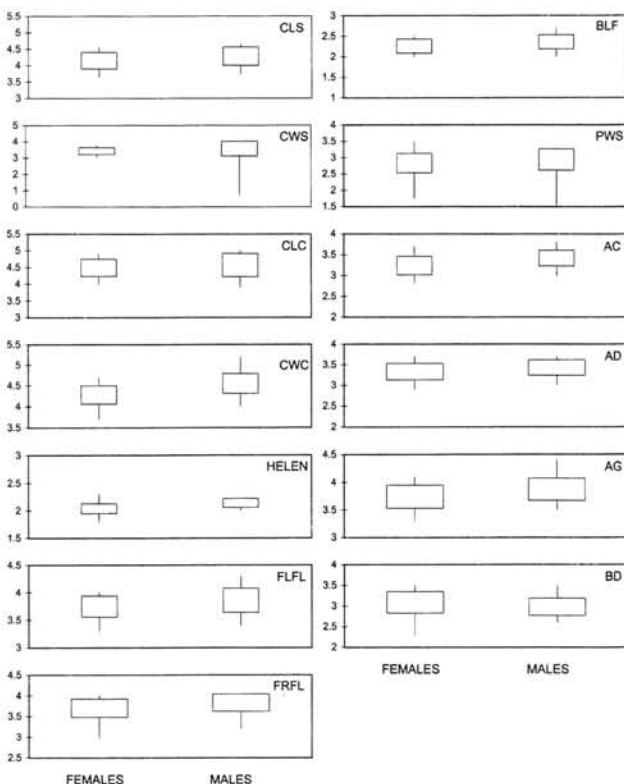
For this reason, using correlation coefficients between each pair of variables, 21 variables were discarded and only 9 independent variables considered in an adjusted model. These variables were selected because of the

Table 3. Descriptive statistics from carapace and plastron scutes in female and male olive ridley hatchlings, with Kolmogorov-Smirnov test for differences between sexes; * = same value for each phenotype; * = significant difference (D) for Kolmogorov-Smirnov test ($p < 0.05$)

| Scute | Females | | | Males | | | Significance between sexes | | | |
|------------------|---------|------|--------|-------|------|--------|----------------------------|---|---|---|
| | Range | Mode | % Mode | Range | Mode | % Mode | Nests | | | |
| | | | | | | | Total | 1 | 2 | 3 |
| Cervical | 1-3 | 1 | 75.9 | 1-2 | 1 | 96.5 | * | * | | |
| Vertebral | 5-7 | 6 | 35.1 | 5-8 | 5 | 43.1 | | | | |
| Left Pleural | 5-8 | 6 | 53.7 | 5-8 | 6 | 56.8 | * | | | |
| Right Pleural | 5-8 | 6 | 51.8 | 5-8 | 6 | 51.7 | | | | |
| Left Marginal | 12-13 | 12 | 94.4 | 12-13 | 12 | 89.6 | | | | |
| Right Marginal | 12-13 | 12 | 94.4 | 12-13 | 12 | 96.5 | | | | |
| Right Inframarg. | 3-4 | 4 | 98.1 | 4 | 4 | 100.0 | | | | |
| Intergular | 0-2 | 1-2* | 35.1 | 0-2 | 0 | 82.7 | * | * | * | * |
| Infraanal | 0-2 | 1 | 88.8 | 0-3 | 1 | 55.1 | * | * | * | |

Table 4. Descriptive statistics for female ($n = 54$) and male ($n = 58$) olive ridley hatchlings (all length measurements are in cm, weight in g) with Kolmogorov-Smirnov test (D) for differences between sexes (* = significant differences, $p < 0.05$).

| | Females | | | | Males | | | | Significance between sexes | | | |
|--|---------|------|------|------|-------|------|------|------|----------------------------|--------|--------|--------|
| | Mean | SD | Min | Max | Mean | SD | Min | Max | Total | Nest 1 | Nest 2 | Nest 3 |
| Carapace length straight (CLS) | 4.15 | 0.25 | 3.64 | 4.56 | 4.29 | 0.28 | 3.73 | 4.65 | * | | * | * |
| Carapace width straight (CWS) | 3.44 | 0.22 | 3.05 | 3.74 | 3.57 | 0.45 | 0.73 | 3.92 | * | * | * | * |
| Carapace length curved (CLC) | 4.50 | 0.26 | 4.00 | 4.90 | 4.58 | 0.35 | 3.90 | 5.00 | * | | * | * |
| Carapace width curved (CWC) | 4.29 | 0.23 | 3.70 | 4.70 | 4.55 | 0.24 | 4.00 | 5.20 | * | * | * | * |
| Head width (HEWID) | 1.50 | 0.09 | 1.32 | 1.65 | 1.53 | 0.11 | 1.32 | 1.65 | | | | |
| Head height (HEHEI) | 1.28 | 0.10 | 1.14 | 1.82 | 1.32 | 0.07 | 1.14 | 1.56 | | | | |
| Head length (HELEN) | 2.04 | 0.10 | 1.80 | 2.30 | 2.14 | 0.09 | 2.00 | 2.20 | * | * | * | * |
| Front left flipper length straight (FLFL) | 3.76 | 0.20 | 3.30 | 4.00 | 3.87 | 0.22 | 3.40 | 4.30 | * | | * | * |
| Front right flipper length straight (FRFL) | 3.70 | 0.22 | 3.00 | 4.00 | 3.83 | 0.21 | 3.20 | 4.00 | * | | * | * |
| Back left flipper length straight (BLFL) | 2.26 | 0.18 | 2.00 | 2.50 | 2.37 | 0.18 | 2.00 | 2.70 | * | | | |
| Back right flipper length straight (BRFL) | 2.22 | 0.18 | 2.00 | 2.60 | 2.32 | 0.19 | 2.00 | 2.60 | | | | |
| Plastron length straight (PLS) | 3.35 | 0.28 | 2.64 | 3.73 | 3.42 | 0.31 | 2.91 | 3.83 | | | | |
| Plastron width straight (PWS) | 2.83 | 0.04 | 3.50 | 1.75 | 2.94 | 0.33 | 1.55 | 3.24 | * | | * | * |
| Plastron length curved (PLC) | 3.68 | 0.30 | 3.00 | 4.20 | 3.68 | 0.30 | 3.00 | 4.20 | | | | |
| Plastron width curved (PWC) | 3.43 | 0.24 | 2.90 | 3.90 | 3.42 | 0.30 | 2.90 | 4.60 | | | | |
| Body height (HEIGHT) | 1.85 | 0.11 | 1.64 | 2.06 | 1.87 | 0.20 | 1.06 | 2.50 | | | | |
| Points A to C on carapace (AC) | 3.25 | 0.22 | 2.80 | 3.70 | 3.42 | 0.19 | 3.00 | 3.80 | * | * | * | * |
| Points A to D on carapace (AD) | 3.34 | 0.20 | 2.90 | 3.70 | 3.44 | 0.20 | 3.00 | 3.70 | * | | | |
| Points A to G on carapace (AG) | 3.74 | 0.21 | 3.30 | 4.10 | 3.88 | 0.21 | 3.50 | 4.40 | * | | * | * |
| Points A to H on carapace (AH) | 3.79 | 0.21 | 3.40 | 4.20 | 3.85 | 0.21 | 3.30 | 4.20 | | | * | * |
| Points B to C on carapace (BC) | 3.06 | 0.29 | 2.30 | 3.60 | 2.94 | 0.22 | 2.50 | 3.50 | | * | * | * |
| Points B to D on carapace (BD) | 3.09 | 0.26 | 2.30 | 3.50 | 2.99 | 0.22 | 2.60 | 3.50 | * | | * | * |
| Points G to H on carapace (GH) | 2.69 | 0.23 | 2.20 | 3.50 | 2.73 | 0.36 | 1.70 | 3.70 | | | | |
| Points A to E on plastron (PAE) | 3.06 | 0.26 | 2.20 | 3.50 | 3.08 | 0.26 | 2.50 | 3.80 | | | | |
| Points A to F on plastron (PAF) | 3.18 | 0.27 | 2.60 | 3.50 | 3.16 | 0.21 | 2.60 | 3.60 | | | | |
| Points B to C on plastron (PBC) | 2.98 | 0.22 | 2.50 | 3.40 | 2.96 | 0.23 | 2.50 | 3.40 | | | | |
| Points B to D on plastron (PBD) | 2.99 | 0.24 | 2.30 | 3.60 | 3.00 | 0.21 | 2.60 | 3.50 | | | | |
| Points C to E on plastron (PCE) | 1.52 | 0.08 | 1.40 | 1.70 | 1.56 | 0.08 | 1.40 | 1.70 | | | | * |
| Points D to F on plastron (PDF) | 1.52 | 0.08 | 1.40 | 1.80 | 1.58 | 0.09 | 1.40 | 1.80 | | | | * |
| Wet weight (WEIGHT) | 16.0 | 2.89 | 10.9 | 20.6 | 16.2 | 2.97 | 9.70 | 19.8 | | | | |

**Figure 3.** Plot for several morphometric measurements (length in cm) in female and male olive ridley hatchlings (abbreviations from Table 4).

high correlation among them. These were the more common measurements; carapace length curved (CLC), carapace width straight (CWS), carapace width curved (CWC), head length (HELEN), front left flipper length straight (FLFL), plastron length straight (PLS), wet weight (WEIGHT), point B to C (BC) on the carapace, and point D to F (PDF) on the plastron.

With these variables, the discriminant function was recalculated and showed a good level of definition between the sexes. Of 112 hatchlings used, the discriminant function classified only 6 incorrectly. This adjusted sampler method was thus effective in 95% of the cases (Fig. 5).

We now have a discriminant function (Fd) for 9 morphometric measurements, from which sexual assessment was possible in 95% of olive ridley hatchlings incubated under controlled conditions, with a positive value indicating females, and a negative value for males:

$$\begin{aligned}
 Fd = & ((1.63 * CLC) + (-5.9 * CWS) + (-4.95 * CWC) \\
 & + (-3.54 * HELEN) + (2.75 * FLFL) + (3.56 * PLS) \\
 & + (0.29 * WEIGHT) + (3.20 * BC) + (-3.94 * PDF)) \\
 & + 32.99
 \end{aligned}$$

DISCUSSION

Modification of the sex ratio of sea turtles and the implications for conservation programs have been widely discussed, because variance in the sex ratio released could

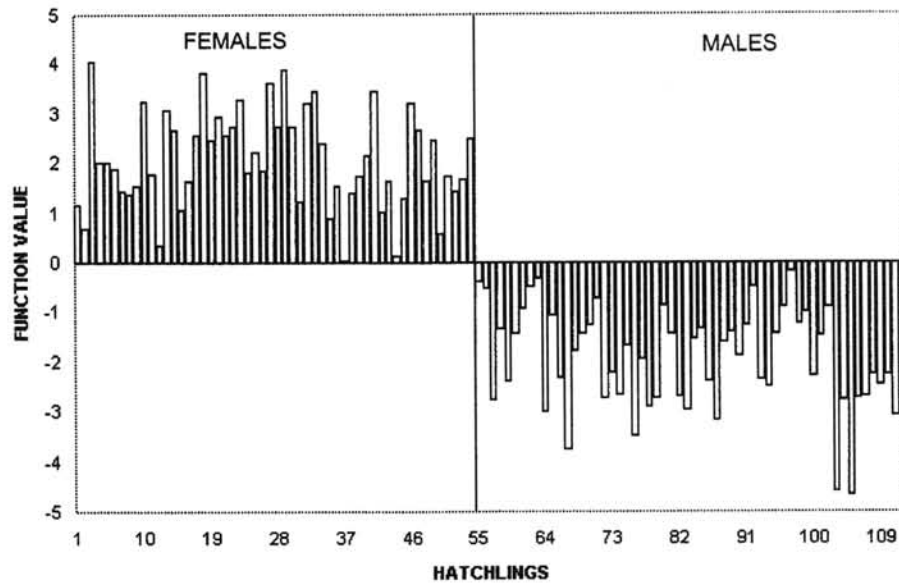


Figure 4. Discriminant function value for each female and male hatchling based on all 30 morphometric measurements.

cause important changes in the population structure, with long term consequences (Vogt, 1994; Lovich, 1996). Because it is not common practice to know the sex ratio of hatchlings released to the sea in sea turtle protection programs, it is hard to make an educated guess about population changes and conservation program goals. Through multivariate analysis of morphologic characteristics, such as this method for sexual assessment, we can make a theoretical consideration of these aspects and, if necessary, suggest some methodological changes in conservation programs.

Moreover, estimation of sex ratio is an important factor for population size estimation in the wild. Theoretically, a bias in the sex ratio could reduce the effective population size and therefore genetic variability and loss of reproductive potential.

On the other hand, some authors have suggested that the sex ratio in wildlife hatchlings tends to favor females (Godinez and Silva, 1994). Vogt (1994) recommended that turtle nests be thermally manipulated in conservation programs to pro-

duce a larger number of females, because a male has the possibility of mating with several females (Peare et al., 1994), and therefore the overall population reproductive potential could be greater.

Results from this work were obtained in hatchlings incubated under controlled conditions and stable temperatures, using those for which we know only one sex is produced. This method needs to be investigated under field conditions with diurnal temperature oscillations as well as at pivotal temperatures (at which a 1:1 proportion of sexes is produced within each clutch).

We need to take a representative sample of hatchlings from natural hatcheries, record the measurements, make inferences about the sex of the turtle with the use of the discriminant function, and then compare the results with histological methods of sexual assessment.

Because sexual dimorphism has also been reported in other hatchling reptiles, (i.e., in *Alligator mississippiensis* and

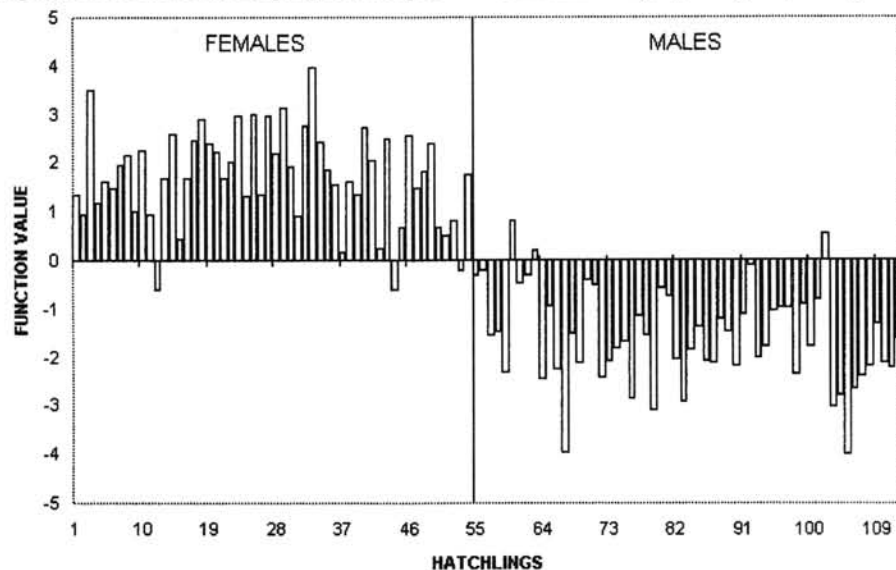


Figure 5. Discriminant function value for each female and male hatchling based on only 9 morphometric measurements.

Apalone spinifera), we suggest the possibility of also detecting morphometric sexual dimorphism in other hatchling sea turtle species.

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