Rev. Biol. Trop., 30(2): 113-120, 1982

Embryological stages of the marine turtle *Lepidochelys olivacea* (Eschscholtz)

Fernando Crastz

Escuela de Biología, Universidad de Costa Rica. Present Address: Círculo Herpetológico de Panamá. Apdo. 10762. Estafeta Universitaria. Panamá.

(Received for publication March 5, 1982)

Abstract: Embryos of the Pacific Ridley Turtle, *Lepidochelys olivacea*, obtained from eggs from the Pacific Coast of Costa Rica and Panama, developing on a beach and others incubated in the laboratory respectively, were examined and evaluated in terms of development by means of an Index of Morphological Development (IMD); selected morphological characters and measurements were used to diagnose 31 different developmental stages from gastrula to neonate.

Investigators (Carr and Hirth, 1961; Schulz, 1975; Mrosovsky and Yntema, 1980) who have dealt with embryological development of marine turtles have often been obliged to improvise classification of developmental stages based on time or to use stages described for other groups of animals. This report consists of a descriptive catalogue of 31 stages of embryological development of the Pacific Ridley, *Lepidochelys olivacea* (Eschscholtz), which hopefully will prove useful to other investigators and perhaps applicable to other species of marine turtles as well.

MATERIAL AND METHODS

Embryos of *L. olivacea* were obtained at Playa Nancite (10° 47' N, 85° 50' W), Costa Rica between November 6 and December 2, 1979; and freshly laid eggs taken on Isla Cañas (7° 25' N; 80° 20' W), Panamá on 16 December, 1979, 21 July and 6 December 1980.

Eggs from Nancite were collected randomly and from marked nests on the beach and examined *in situ* while those from Isla Cañas were transported to the laboratory within a maximum of 23 hours after laid, incubated in beach sand to which distilled water was occasionally added, as recommended by Bustard and Greenham (1968), and maintained at an average temperature of 30 C throughout the development.

Eggs were sampled daily in both places, and if turgid, puctured with a fine needle to release pressure before opening them. Observations on spontaneous motility of embryos were made during the 10 minutes immediately following opening of the egg. Measurements were made with vernier calipers to 0.005 mm accuracy before preservation of the embryos in 10% formalin.

In total, 210 eggs examined were considered normal and used in the following analysis. These embryos were examined for morphological information with the aid of a stereoscope (0.7 - 4.2X).

Ninety-three distinct characters were observed for each embryo and scored as inapplicable (zero) or on an ascending integer scale from one to a maximum of nine according to the number of progressive states discernible for the characteristic. Of these, 70 were selected for mathematical analysis. A score for each character was tabulated and an Index of Morphological Development (IMD) was computed for each embryo with the following formula:

$$IMD = (\Sigma\ SoSm^{-1}) T^{-1}$$

where So = score obtained, Sm = maximum score possible for the respective characteristic and T = total number of characteristics applicable for the respective embryo. Scores for each

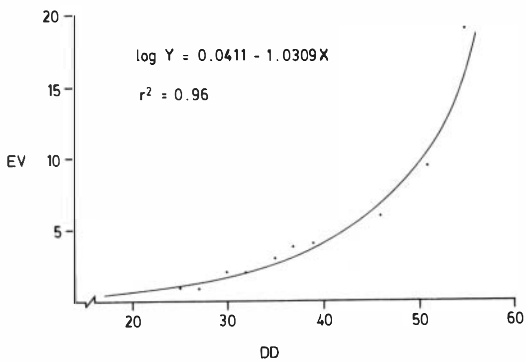


Fig. 1. Parabola of Embryonic Volume (EV) in cc with Development Days (DD) of 12 incubated embryos.

characteristic of each embryo were reordered according to their respective ascending IMD values. Those characteristics whose ascending scores displayed few incongruities were considered for the descriptions that follow.

The significance of the simple linear regression of days of development upon IMD and selected measurements upon IMD was examined. The logarithmic Y formula corresponding to the parabolic curve of the embryonic volume obtained by water displacement, against days of development was calculated.

The figures in the catalogue were drawn with the aid of a stereoscope equipped with a camera lucida. Each embryo was placed in the best position to illustrate its diagnostic characteristics.

RESULTS

The artificial incubation temperature (30 C) was within the range of temperatures in nests at different development levels found at Nancite (27.0 - 34.5 C) and 10 neonates hatched in a span of 55 or 56 days.

The correlation of IMD with days of development is 0.97 for the embryos before the middle of development and 0.84 for older embryos. The lower correlation in older embryos reflects the fact that during later stages, morphological characteristics other than size do not change significantly.

On the basis of the ordered IMD's, morphological characteristics were selected to diagnose stages from gastrulation to near full term development. Of 31 measurements taken, distance from axilla to groin, width of carapace and diameter of vitelline sac were selected for diagnosing these terminal stages of development.

The correlation of embryonic volume against development days describes a parabola (Fig. 1) with a rapid increment beginning about the middle of the incubation period.

CATALOGUE OF EMBRYOLOGICAL STAGES OF *L. olivacea*

The diagnostic characters presented for each of the following stages are given in order of usefulness, together with Days of Development (DD) and Index of Morphological Development (IMD).

Stage 1 (Fig. 2): Late gastrula with oval area opaca enclosing the area pellucida wherein the rounded germinative disk lies to one side; primitive plate very narrow and closely joined to the area opaca; blastopore arched. DD = 0, IMD = 0.00.

Stage 2 (Fig. 3): Post-gastrula with primitive plate extending laterally between area opaca and area pellucida, bordering the oval germinative disk; cephalic process extended ventrally; anterior neuropore evident; appearance of head (amniotic) fold. DD= 2, IMD = 0.00.

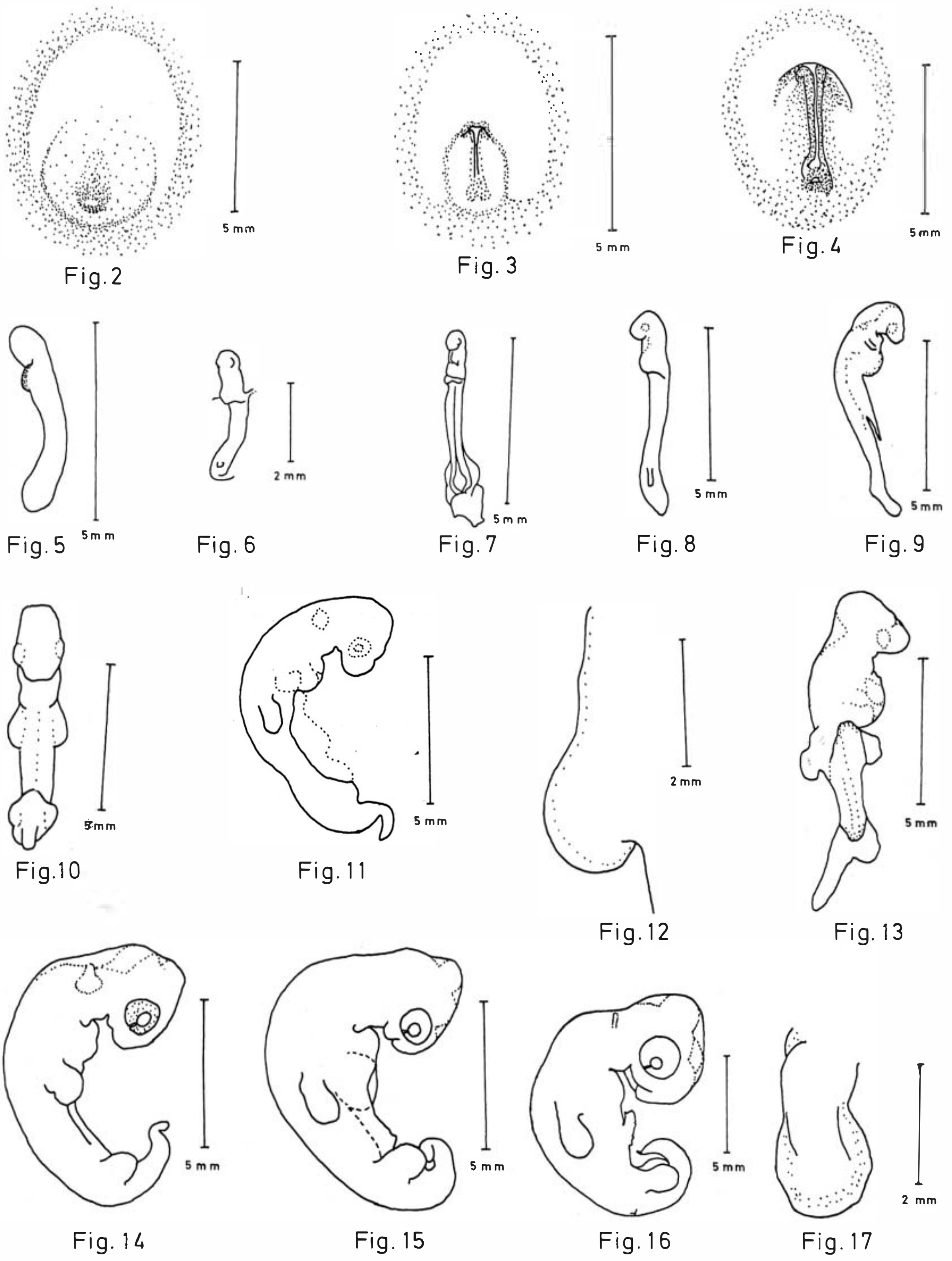
Stage 3 (Fig. 4): Neural canal still open; cephalization evident with amniotic fold beginning to extend over anterior extremity. DD = 3, IMD = 0.00.

Stage 4 (Fig. 5): Head formed, but facial characteristics lacking; erythrocytes concentrated; circulatory pulsations in living embryo permitting recognition of the extended tubular heart; amniotic fold partially covering heart. DD = 4, IMD = 0.00.

Stage 5 (Fig. 6): Optic cup and otic invagination discernible; hindgut conspicuous. DD = 5, IMD = 0.11.

Stage 6 (Fig. 7): Anterior cephalic projection present but face still unformed. DD = 5, IMD = 0.11.

Stage 7 (Fig. 8): Foregut evident; tail bud present. DD = 6, IMD = 0.12.



Figs. 2-17. Sketches of development of *L. olivacea* from laying of egg to stage 13, when identifiable as reptile embryo.

Stage 8 (Fig. 9): Telencephalon, diencephalon and pineal body evident; tail present. DD = 7, IMD = 0.15.

Stage 9 (Fig. 10, 11): Both pairs of limb buds evident; maxillary. process not yet reaching level of optic capsules. DD = 9, IMD = 0.21.

Stage 10 (Fig. 12, 13): Limb buds without invaginations, rounded; abdominal opening not more than 4/10 total length of embryo. DD = 10, IMD = 0.22.

Stage 11 (Fig. 14): Maxillary process reaching choroid fissure; pigment evident in iris. DD = 12, IMD = 0.26.

Stage 12 (Fig. 15): Maxillary process reaching beyond choroid fissure; limbs paddle shaped; elbow formed, and one interdigital groove present on forelimb; genital prominence evident between hindlimbs. DD = 12, IMD = 0.26.

Stage 13 (Fig. 16, 17): Maxillary process reaching anterior border of eye; iris uniformly pigmented; pupil distinct; forelimb with two interdigital grooves. DD = 15, IMD = 0.34.

Stage 14 (Fig. 18): Lateral prominences behind the axillae representing the primordial carapace; maxillary process reaching beyond anterior border of eye; general opacity of embryo; otic capsule no longer evident. DD = 17, IMD = 0.37.

Stage 15 (Fig. 19, 20, 21): Posterior border of primordial carapace evident as lumbar projections in vertebral region; third interdigital groove present in forelimb; second interdigital groove present in hindlimb. DD = 20, IMD = 0.45.

Stage 16 (Fig. 22, 23): Five phalanges on each limb; carapace present but its anterior border not yet defined; ribs evident; mandibular process reaching to below choroid fissure. DD = 21, IMD = 0.46.

Stage 17 (Fig. 24): Mandibular process reaches anterior border of eye; head more opaque, only the mesencephalon and diencephalon being evident; endolymphatic duct no longer evident. DD = 21, IMD = 0.50.

Stage 18 (Fig. 25, 26, 27): Appearance of rostral caruncle as a white point on the tip of the snout; free claw (proximal) on anterior border of foreflipper. DD = 22, IMD = 0.51.

Stage 19 (Fig. 28): Presence of pigment on the neural plates; proximal free claw on anterior border of hindflipper; vertebral and lateral longitudinal crests evident. DD = 23, IMD = 0.54.

Stage 20 (Fig. 29): Pigmentation evident in eyelids, neck, limbs and marginal plates. DD = 24, IMD = 0.56.

Stage 21 (Fig. 30): Two claws present on each flipper. DD = 26, IMD = 0.61.

Stage 22 (Fig. 31): Jaws developed to the occlusion point; proximal claw of each flipper pigmented. DD = 27, IMD = 0.65.

Stage 23 (Fig. 32): Crest present on plastron; scales and plates evident on head, eyelids and limbs. DD = 28, IMD = 0.72.

Stage 24 (Fig. 33): Caruncle thickened and widened, covering the tip of the snout; all free claws pigmented; scales evident on throat and skin of pectoral region. DD = 29, IMD = 0.76.

Stage 25 (Fig. 34): Appearance of the triangular unpigmented mentonian plate of the lower jaw; sutures between neural and costal plates darker than the plates; pigment present on throat and plastron; scales on pelvic region and tail. DD = 30, IMD = 0.81.

Stage 26 (Fig. 35): Forelimbs completely covered by scales and plates; mentonian plate pigmented; ventro-posterior portion of thighs and inframarginal plates slightly pigmented. DD = 33, IMD = 0.82.

Stage 27 (Fig. 36): Definite pigmentation of ventro-posterior portion of thighs; slight pigmentation of intermandibular region posterior to the mentonian plate. DD = 36, IMD = 0.84.

Stage 28: Plastron pigmented, retaining a light oval zone between its crests; width of carapace between 17.0 and 19.5 mm. DD = 40, IMD = 0.86.

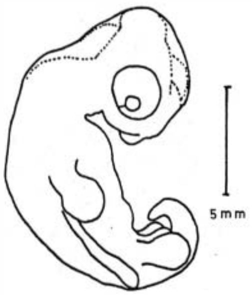


Fig. 18

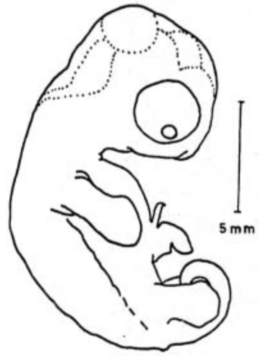


Fig. 19

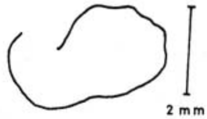


Fig. 20

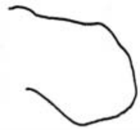


Fig. 21

Fig. 22

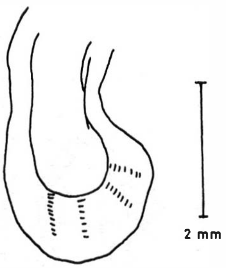


Fig. 23

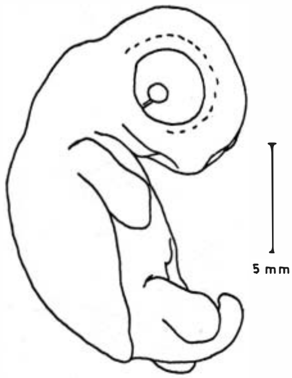


Fig. 24

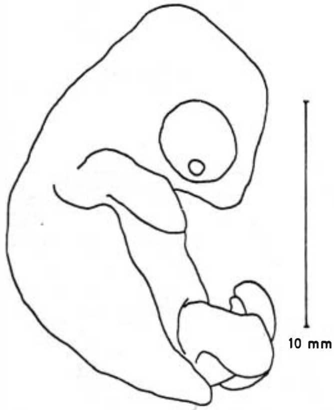


Fig. 25

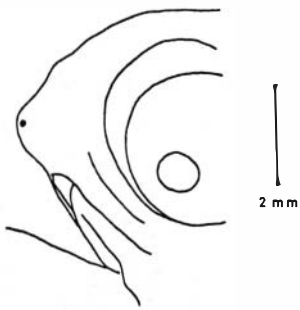


Fig. 26

Fig. 27

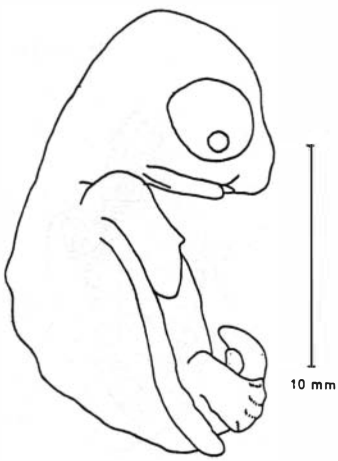


Fig. 28

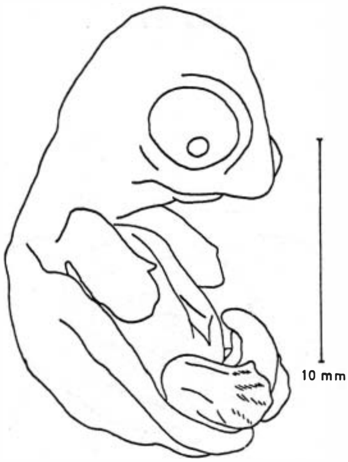


Fig. 29

Figs. 18-29. Sketches of development of *L. olivacea* from stage 14 when identifiable as turtle embryo, to stage 20.

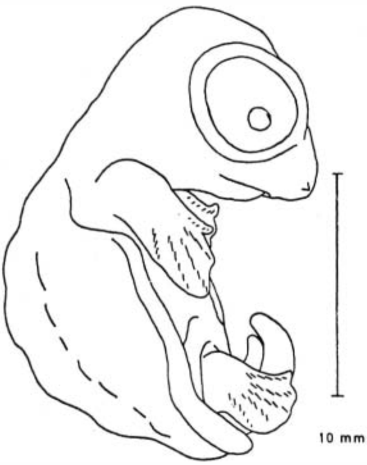


Fig. 30

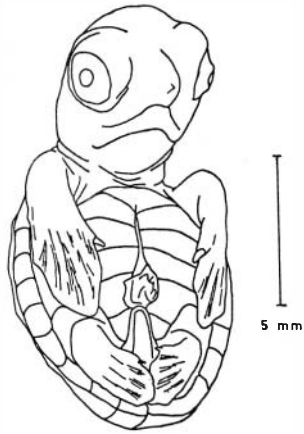


Fig. 31

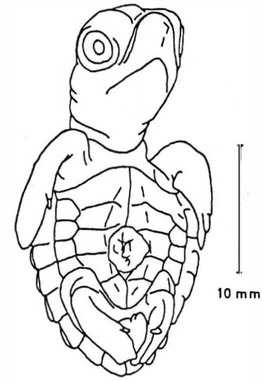


Fig. 32

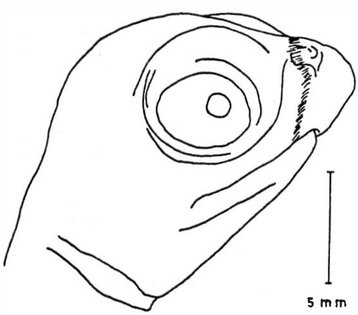


Fig. 33

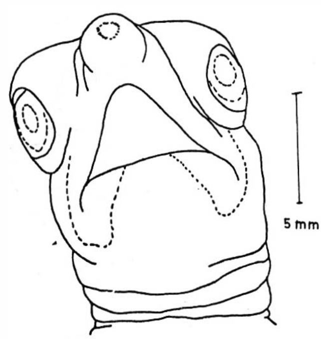


Fig. 34

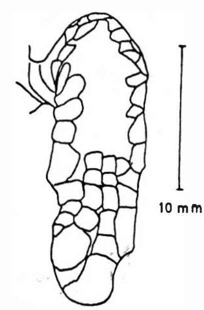


Fig. 35

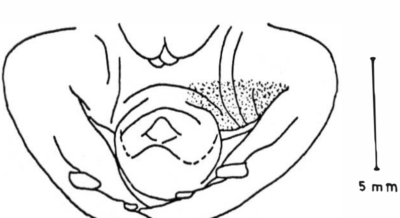


Fig. 36

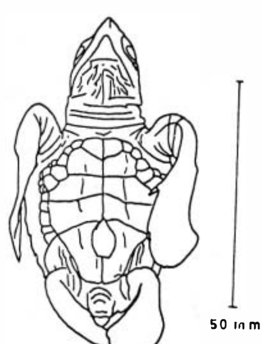


Fig. 37

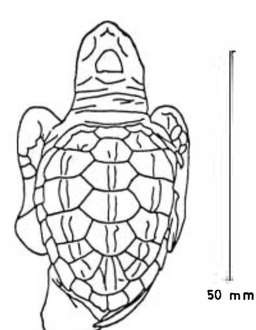


Fig. 38

Figs. 30-38. Sketches of development of *L. olivacea* from stage 21 to stage 31, hatchling.

Stage 29 Pigmentation resembling that of neonate; width of carapace between 26.0 and 28.4 mm. DD = 45, IMD = **0.87**.

Stage 30: Diameter of vitelline sac between 10.0 and 19.2 mm. DD = 50, IMD = 0.88.

Stage 31 (Fig. 37, 38): Hatchling; diameter of vitelline sac between 6.1 and 2.5 mm; alternatively, distance from axilla to groin between 26.1 and 28.9 mm. DD = 55, IMD = 0.89.

DISCUSSION

This catalogue is designed for the identification of 31 stages of development of *L. olivacea* and utilizes morphogenesis and measurements as diagnostic parameters since physiological and behavioral parameters were impossible to evaluate without disturbing the embryo to an unquantifiable extent.

The temperature and duration of the laboratory incubation were comparable to those reported by Schulz (1975), Márquez *et al.* (1976) and Acuña (1980); the high correlation (0.98) of days of development with IMD for the 71 embryos for which days of development was known supports the use of the IMD as a valid measure and an effective tool for selecting reliable characteristics and delimiting stages.

As shown in Fig. 1, embryonic volume increases slowly during early development until morphogenesis is essentially completed, after which growth rate increases rapidly.

Deraniyagala (1939) described embryos of *L. olivacea* at five different levels of development, which correspond to stage 18, 22, 26+, 27+ and 30+ of this catalogue. Between the ages reported by him and those given here, differences in general are no greater than two or three days.

Agassiz (1857) illustrated eight of the stages of his system for "Thalassochelys Caouana" = *Caretta caretta* which resemble in many aspects the development observed in *L. olivacea*, but with significantly longer periods between successive stages.

Variations in developmental rates in certain oviparous reptiles have been attributed to ecological differences in the nests (Packard *et al.*, 1977) caused by seasonal changes occurring during the reproductive period or to local conditions of distinct biogeographical zones, whereas embryonic death at different stages of development has been considered in relation with salinity, season, microorganisms, etc. for *L. olivacea* (Acuña, 1980).

ACKNOWLEDGEMENTS

I thank Douglas C. Robinson for his patience and interest, Francisca de Sousa provided logistic facilities for the laboratory work; A. Stanley Rand made many useful sugestions; Servicio de Parques Nacionales de Costa Rica granted permission to obtain samples at Playa Nancite; Departamento de Recursos Naturales Renovables de Panamá helped in obtaining eggs at Isla Cañas, and the Smithsonian Tropical Research Institute provided numerous facilities during the course of this work. This study was partially aided by USFWS grant, 14-16-0002-80-228 to S. Cornelius and D. Robinson. To these and for the help of many friends mentioned in the thesis on which this work was based, my sincere thanks.

RESUMEN

Embriones de la tortuga marina, *Lepidochelys olivacea,* unos obtenidos de huevos desarrollándose en la playa (Playa Nancite, Costa Rica) y otros incubados en el laboratorio (Isla Cañas, Panamá), fueron examinados y evaluados en términos de desarrollo mediante un Indice de Desarrollo Morfológico (IDM); características morfológicas y medidas corporales seleccionadas de los embriones examinados, se usaron para diagnosticar 31 diferentes etapas, desde la gástrula hasta el neonato.

LITERATURE CITED

Acuña, R.A. 1980. Aspectos de la fase terrestre de la Tortuga Lora *Lepidochelys olivacea.* Tesis de Maestría. Universidad de Costa Rica. 115 p.

Agassiz, L. 1857. Embryology of the turtle. Contr. Nat. Hist. U. S., II vol. Boston. p. 451-643.

Bustard, H.R., & P.M. Greenham, 1968. Physical and chemical factors affecting hatching in the green sea turtle, *Chelonia mydas* (L). Ecology, 49: 269-276.

Carr, A., & H. Hirth. 1961. Social facilitation in green turtle siblings. Anim. Behav., 9: 68-70.

Deraniyagala, P.E.P. 1939. Tetrapod reptiles of Ceylon. Vol. I. Testudines and Crocodilians. Ceylon J. Sci., Colombo Mus. Publ. 412 p.

Márquez, M., R.A. Villanueva, & C. Peñaflores. 1976. Sinopsis de datos biológicos sobre la Tortuga Golfina, *Lepidochelys olivacea* (Eschscholtz, 1829). INP/S2. México, D.F. 63 p.

Mrosovsky, N. & C.L. Yntema. 1980. Temperature dependence of sexual differentiation in sea turtles: implications for conservation practices. Biol. Conserv., 18: 271-280.

Packard, G.C., G.R. Tracy, & J. Roth. 1977. The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class Reptilia. Biol. Rev., 52: 71-105.

Schulz, J.P. 1975. Sea turtle nesting in Surinam. Nederlandsche Commissie voor Internationale Natuurbescherming. Mededelingen No.23. 143 p.