Vol. 152: 61–71, 2022
https://doi.org/10.3354/dao03705

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published online November 17

Leucism: the prevalent congenital malformation in the olive ridley sea turtle of northwestern Mexico

Ingmar Sosa-Cornejo[1,2], Rodolfo Martín-del-Campo[3], Juan A. González-Flores[4], Zuleika B. González-Camacho[2], Blanca A. Cabrera-Cuellar[5], Alessandra Bielli[6], Jorge E. Valdes-Flores[1], Vicente Olimón-Andalón[7,*]

[1]Doctorado en Ciencias Biológicas, Facultad de Biología, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa 80040, Mexico
[2]Programa de Tortugas Marinas de la Facultad de Biología, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa 80040, Mexico
[3]Department of Oral Health Sciences, Faculty of Dentistry, Life Sciences Institute, University of British Columbia, Vancouver, BC V6T 1Z3, Canada
[4]División de Ciencias Naturales y Exactas, Departamento de Biología, Universidad de Guanajuato, Guanajuato 36050, Mexico
[5]Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango 35010, Mexico
[6]Pro Delphinus, Lima 15074, Peru
[7]Laboratorio de Inmunogenética, Facultad de Biología, Universidad Autónoma de Sinaloa, Culiacán, Sinaloa 80040, Mexico

ABSTRACT: Despite being the most abundant sea turtle in the world, the olive ridley turtle *Lepidochelys olivacea* is classified as Vulnerable by the IUCN. There is evidence of congenital malformations in hatchlings, and the associated causes are multifactorial, with both genetic and environmental sources. Santuario Playa Ceuta (SPC) is a sanctuary for the olive ridley, located at the northernmost region of its nesting range in the Mexican Pacific. The objective of this study was to identify and quantify the prevalence and severity of congenital malformations in olive ridley embryos/hatchlings in SPC during the 2017 nesting season. We collected 62 907 eggs from 643 relocated nests that were moved to a hatchery, of which 4242 eggs with obvious development did not hatch and were analyzed for this study. Hatching success was 53.9 %, with 22.5 % of nests (n = 145) and 0.54 % of eggs (n = 344) showing embryos or hatchlings with malformations. The nest severity index was 2.4 (range: 1–10) malformed embryos or hatchlings per nest, and the organism severity index was 1.4 (range: 1–7) malformations per malformed embryo or hatchling. Leucism was the most prevalent malformation (34.4 %; 170/494 total observed), with the craniofacial region showing the greatest diversity of malformations (17/35 types). Given the geographical position of SPC, extreme environmental conditions (e.g. cold, heat, and dryness) could be one of the main causes of teratogenesis in this species. However, more studies are needed regarding the presence of contaminants, genetic factors, health assessments of nesting females, and malformation rates of nests that remain *in situ* versus those that are relocated.

KEY WORDS: Wildlife diseases · Hypopigmentation · Albinism · Teratogenic agents · Marine turtles

1. INTRODUCTION

The field of teratology explores abnormal development and congenital malformations, which are defined as structural defects that are present at birth and involve the intrinsic alteration of the affected tissue (Smith 1975, Trigos et al. 2000). Research on teratogenesis has primarily been carried out in humans and model organisms (Guest et al. 1994, Ujházy et al. 2012, Kameoka et al. 2014). Wild, cap-

*Corresponding author: vicente.olimon.uas@gmail.com

tive, and domesticated animals have also been examined (Rousseaux & Ribble 1988, Donnai & Winter 1995, Rojas-Lleonart et al. 2010, Martín-del-Campo et al. 2019a). Malformations can appear early on in embryonic development and can be lethal, depending on the affected organ and level of severity (Needham et al. 2008, Moore et al. 2016). The etiology of teratogenesis can be multifactorial, including genetic and environmental agents (e.g. UV radiation, hyperthermia, pollutants, nutritional defects or viruses), and can involve complicated interrelations among these factors (Smith 1975). The incidence of congenital malformations depends on the species, embryonic stage, and the time and dose of exposure to the teratogenic agent (Edwards 1968, Upfold et al. 1989, Alwan & Chambers 2015). In reptiles, the presence of congenital malformations has been previously reported (Martín-del-Campo et al. 2019a), with associated causes considered to be both genetic and environmental, among which are exposure to chemical agents (Bishop et al. 1991), abnormal temperatures (high or low), embryonic anoxia (Wallach 2007), absence or excess of humidity, insufficient nutrients, and radiation (Martín-del-Campo et al. 2019a).

The presence of congenital malformations has been reported in all species of sea turtles (Martín-del-Campo et al. 2021), and although most are lethal (Bárcenas-Ibarra et al. 2015), those that occur in late embryonic development do not always result in mortality (Martín-del-Campo et al. 2021). Bárcenas-Ibarra et al. (2015) suggested that olive ridley sea turtles *Lepidochelys olivacea* nesting in northwestern Mexico (El Verde Camacho, Sinaloa) have a higher incidence (2%) of congenital malformations compared to other species of sea turtles (e.g. 0.2% in green sea turtles *Chelonia mydas*) and can present multiple malformations per individual. Santuario Playa Ceuta (SPC), also in northwestern Mexico, is a nesting beach for *L. olivacea* with more than 40 yr of nesting records (Sosa-Cornejo et al. 2021). This beach is located at the northernmost limit of the species' nesting area on the mainland (Campista-León et al. 2019), although sporadic nesting may occur above this latitude (Seminoff & Nichols 2007). Records from this beach indicate that hatchlings can have congenital malformations and often exhibit deficient pigmentation; in some of those cases, the turtles were able to hatch and reach the ocean (F. Enciso-Saracho unpubl. data). Thus far, no study has been conducted regarding the incidence of congenital malformations and the occurrence of pigmentation defects in embryos of the SPC population.

Leucism (i.e. hypopigmentation) is a congenital condition characterized by lack of pigmentation in certain parts of the body (van Grouw 2013). Whereas albino animals show a complete lack of pigmentation, including eyes, skin, and claws, leucistic animals often have some parts of the body that are normally pigmented (Brito & Valdiviesso-Bermeo 2016, Izquierdo et al. 2018). Leucism has been associated with several factors, such as genetic mutations (van Grouw 2013), inbreeding (Bensch et al. 2000), and contaminant exposure (Møller & Mousseau 2003, van Grouw 2013) in birds and insufficient nutrition in mammals (Brito & Valdiviesso-Bermeo 2016). Leucism has been reported in all sea turtle species and is typically not lethal when other malformations are not present (Martín-del-Campo et al. 2021); however, leucism can affect the fitness of individuals since coloration is an important component for survival and reproduction (Møller & Mousseau 2003, Krecsák 2008, Madeira et al. 2020).

Several studies have been carried out on congenital malformations in the olive ridley sea turtle (Galván 1991, Gularte 2000, Trejo 2000, Ruiz 2002, Bárcenas-Ibarra & Maldonado-Gasca 2009, Bárcenas-Ibarra et al. 2015). In the present study, we aimed to identify and quantify the prevalence and severity of congenital malformations in olive ridley embryos and hatchlings in SPC, Sinaloa, Mexico — a previously unexplored population. Based on the information on the malformations in this population, the stage of embryonic development (Miller 1985, Kaska & Downie 1999) in which these alterations occurred can be inferred.

2. MATERIALS AND METHODS

2.1. Study area

SPC is located in the central region of the state of Sinaloa, Mexico, within the municipality of Elota, between the Cospita River to the north (24° 05′ 42″ N, 107° 11′ 38″ W) and the Elota River to the south (23° 52′ 43″ N, 106° 55′ 52″ W) (Fig. 1). The beach area is 37 km long and is part of the Quevedo peninsula. The Autonomous University of Sinaloa began monitoring nesting in SPC in 1976; a decade later, it was designated as a Reserve Zone and Refuge Site for the Protection, Conservation, Repopulation, Development and Control of Various Species of Sea Turtles (DOF 1986). The area was designated as a Sanctuary for the olive ridley turtle nesting in 2002 (DOF 2002).

Fig. 1. Study area along the coast of Mexico. Yellow area: the municipality of Elota; red: Santuario Playa Ceuta, where olive ridley sea turtle nesting data were collected

2.2. Nest collection and incubation

Nests were collected during nightly surveys between July and December 2017 using the methodology proposed by Schroeder & Murphy (1999) and SWOT Scientific Advisory Board (2011). A Honda 250 all-terrain vehicle was used to patrol the beach looking for nests, and eggs were collected in a plastic bag and transported in a bucket. Nests were transferred to the SPC camp after a period not exceeding 4 h and incubated using one of 2 techniques: (1) in polyurethane boxes or (2) in a hatchery (protected beach area). Nest protection and relocation occur because of temperature fluctuations that are recorded during the season that can cause embryonic mortality (Mortimer 1999), and due to poaching and predation. Each nest was labeled with a serial number as well as its incubation date and number of eggs.

Once the eggs hatched (mean ± SD: 45 ± 3 d), nests were excavated and inventoried, which consisted of counting the number of shells, live and dead hatchlings, and eggs with and without obvious embryonic development (EED and EWED respectively) to estimate the hatching success (Fowler 1979, Caut et al. 2006). Eggs with blood vessels present in the yolk or a white circle on the outside of the eggshell were considered EED. The circle indicates the adherence of the shell membranes to the shell, which occurs during early incubation of fertile eggs (Whitmore & Dutton 1985). If the yolk or shell did not present the above-mentioned features, the egg was classified as EWED and considered not fertilized. For some EEDs, we were unable to identify the development stage because development was stopped too early to detect the corresponding stage. EEDs were analyzed, and each dead embryo or hatchling with a malformation was collected in a 250 ml bottle with 90% alcohol and transferred to the Zoology Laboratory of the Faculty of Biology of the Autonomous University of Sinaloa. A stereomicroscope and an

Olympus lens digital camera (Olympus FE-370) were used to identify and take pictures of the malformations. Hatching success and incidence of malformations between the 2 incubation techniques were compared using a Mann-Whitney *U*-test.

2.3. Malformation analysis

For the identification of malformations, we used nomenclature proposed by Frye (1991) and Makris et al. (2009). Malformations were classified according to anatomical region, including whole body, carapace, limbs and tail, and craniofacial structures (skull, eyes, nostrils, and jaw). The frequency of each of the identified malformations was estimated as well as their prevalence and severity indices (Margolis et al. 1982, Fajardo-Gutiérrez 2017). The prevalence index measures the proportion of nests or organisms in which at least one congenital malformation was observed relative to the total number of nests (nest prevalence [NP]; Eq. 1) or eggs (egg prevalence [EP]; Eq. 2) examined. The nest severity index (NS) indicates the number of malformed organisms per nest (Eq. 3), and the organism severity index (OS) indicates the number of congenital malformations per organism relative to the total number of organisms with congenital malformations (Eq. 4) (Bárcenas-Ibarra et al. 2015).

$$NP = \frac{\text{No. of nests with malformed organisms}}{\text{Total no. of examined nests}} \times 100 \quad (1)$$

$$EP = \frac{\text{No. of malformed organisms}}{\text{Total no. of examined eggs}} \times 100 \quad (2)$$

$$NS = \frac{\text{No. of malformed organisms}}{\text{Total no. of nests with malformed organisms}} \quad (3)$$

$$OS = \frac{\text{No. of congenital malformations}}{\text{Total no. of malformed organisms}} \quad (4)$$

NP, EP, and NS were also measured for each type of malformation.

3. RESULTS

A total of 643 nests were collected, of which 30% (191 nests) were incubated in polyurethane boxes and 70% (452 nests) in a hatchery, resulting in 62 907 eggs. A hatching success of 53.9% was calculated, with 32 783 live hatchlings and 1145 dead hatchlings. Hatching success did not differ significantly between incubation techniques (55.09 ± 14.72% for polyurethane boxes, 50.72 ± 16.39% for hatchery; $p > 0.05$). Of the unhatched eggs, 24 737 were EWED and 4242 were EED. Of the 643 nests recorded,145 had at least one malformed organism (NP = 22.5%), 13 of which included fully formed hatchlings (not embryos). A total of 344 organisms (328 embryos and 16 hatchlings) were malformed (EP = 0.54%), with 494 total observed malformations (i.e. some organisms had multiple malformations). Incidence of malformations did not differ significantly between incubation techniques (0.60 ± 1.78% for polyurethane boxes, 0.50 ± 1.61% for hatchery; $p > 0.05$). NS was 2.4 (range: 1–10) malformed embryos or hatchlings nest^{-1}, and OS was 1.4 (range: 1–7) malformations per malformed embryo or hatchling.

A total of 35 different types of malformations were identified, of which 6 impacted the whole body (Fig. 2b–f): partial leucism, characterized by partially reduced pigmentation (Fig. 2b); full leucism, characterized by reduced pigmentation throughout the body (Fig. 2c); anasarca, characterized by an accumulation of interstitial fluid in the subcutaneous connective tissue (Fig. 2d); twins, characterized by 2 embryos joined at the dorsal or posterior region of the head, at the umbilical cord, or embryo attached to a parasitic twin, whose body is smaller (Fig. 2e); and dwarfism and gigantism, characterized by embryos of smaller or larger than normal size, respectively (Fig. 2f).

Eight malformations were identified in the carapacial region (Fig. 3a–h): supernumerary scutes, characterized by more scutes than typical (Fig. 3a); subnumerary scutes, characterized by fewer scutes than typical (Fig. 3b); deformed scutes, characterized by abnormal shape of scutes (Fig. 3c); scoliosis, charac-

Fig. 2. Normal development and congenital malformations of the entire body in olive ridley sea turtles from Santuario Playa Ceuta. (a) Normal hatchling; (b) partial leucism; (c) total leucism; (d) anasarca; (e) twins; (f) gigantism and dwarfism

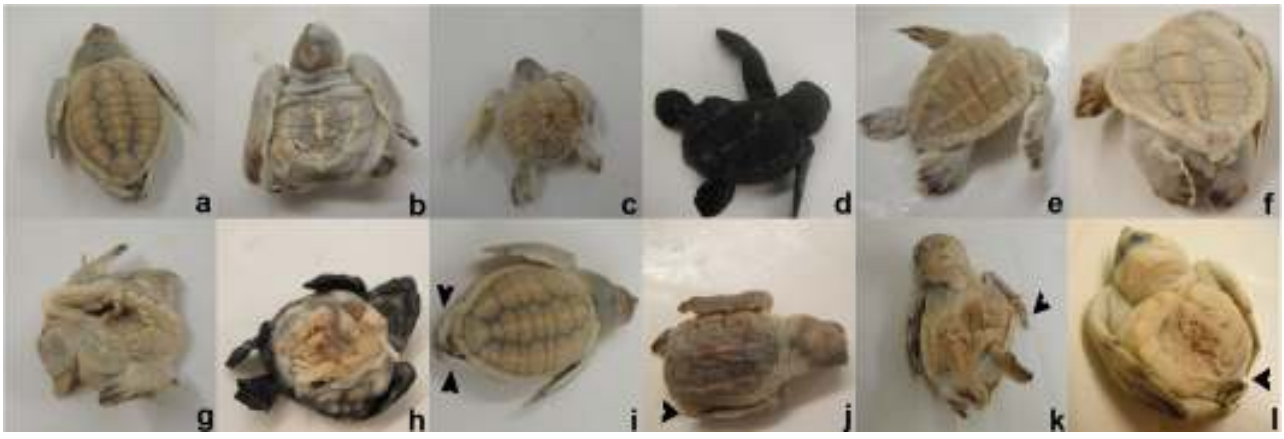

Fig. 3. Congenital malformations in the carapace, limbs, and tail in olive ridley sea turtles from Santuario Playa Ceuta. (a) Supernumerary scutes; (b) subnumerary scutes; (c) deformed scutes; (d) scoliosis; (e) kyphosis; (f) osteo-agenesis; (g) misshapen bones; (h) plastron metaplasia; (i) micromelia; (j) amelia; (k) brachydactyly; (l) anury. Arrowheads show malformation

terized by lateral curvature of the spine (Fig. 3d); kyphosis, characterized by abnormal curvature of the spine of dorsal convexity (Fig. 3e); osteo-agenesis, characterized by absence of any bone (Fig. 3f); misshapen bones, characterized by abnormal shape of the bones (Fig. 3g); and plastron metaplasia, characterized by abnormal tissue in the plastron (Fig. 3h).

Four malformations were identified in the limbs and tail (Figs. 3i–l): micromelia, characterized by disproportionately short or small flipper(s) (Fig. 3i); amelia, characterized by complete absence of one or more flipper(s) (Fig. 3j); brachydactyly, characterized by shortening of the digits (Fig. 3k); and anury, characterized by absence of the tail (Fig. 3l).

In the craniofacial region, 17 malformations were identified, of which 5 were identified in the skull (Fig. 4a–e), 6 in the eyes (Fig. 4f–k), 4 in the jaws (Fig. 4l–o), and 2 in the nostrils (Fig. 4p–q). For the

Fig. 4. Craniofacial malformations in olive ridley sea turtles from Santuario Playa Ceuta. (a) Anencephaly; (b) exencephaly; (c) encephalocele; (d) acephaly; (e) microcephaly; (f) anophthalmia; (g) cyclopia; (h) sinophthalmia; (i) macrophthalmia; (j) microphthalmia; (k) eyelid dysplasia; (l) agnathia; (m) prognathia; (n) laterognathia; (o) brachygnathia; (p) arhinia; (q) rhinoschisis. Arrowheads show malformation

skull region, these were anencephaly, characterized by absence of the cranial region of the head with brain absent or reduced in size (Fig. 4a); exencephaly, characterized by the brain protruding outside the skull due to absence of all or part of the cranial vault (Fig. 4b); encephalocele, characterized by sac-like protrusions of the brain and membrane covering through openings in the skull (Fig. 4c); acephaly, characterized by absence of a head (Fig. 4d); and microcephaly, characterized by disproportionately small head (Fig. 4e). In the ocular region, the following malformations were identified: anophthalmia, characterized by absence of eyes (Fig. 4f); cyclopia, characterized by the presence of only one eye (Fig. 4g); sinophthalmia, characterized by the eyes being fused into one (Fig. 4h); macrophthalmia, characterized by enlargement of the eyes (Fig. 4i); microphthalmia, characterized by eyes being reduced in size (Fig. 4j); and eyelid dysplasia, characterized by abnormal eyelids (Fig. 4k). For the region of the jaws: agnathia, characterized by an absence of the jaw (Fig. 4l); prognathia, characterized by enlarged or protruding jaw compared to its normal position in the skull (Fig. 4m); laterognathia, characterized by the lower jaw turned to the right or left (Fig. 4n); and brachygnathia, characterized by the presence of small jaw (Fig. 4o). For the nostrils: arrhinia, characterized by absence of the nares (Fig. 4p); and rhinoschisis, characterized by vertical separation of the nares into 2 equal parts (Fig. 4q).

The craniofacial region had the highest number of malformations (17/35 malformation types) compared to other regions of the body. The most prevalent malformations were total leucism (n = 114/494 total observed malformations; 23.1%), followed by partial leucism (n = 56/494 total observed malformations; 11.3%), and both were frequently accompanied by craniofacial malformations (eyes, jaws, and nostrils; see Table 1). Total leucism was the malformation with the highest nest prevalence (NP = 17.7%) and severity indices (NS = 1.58 malformations nest^{-1}) (Table 1). The lowest NP (0.31%) was observed for anasarca, gigantism, anury, and brachydactyly (Table 1).

Regarding severity, microphthalmia — despite being a malformation with low frequency (n = 9/494 total observed malformations; 1.8%) and low NP (1.4%) — was the malformation that showed the greatest NS (2.25 malformed organisms nest^{-1}), followed by scoliosis (1.95 malformed organisms nest^{-1}) (Table 1). The lowest NS (1.0 malformed organisms nest^{-1}) was observed for anasarca, dwarfism, gigantism, supernumerary scutes, osteo-agenesis, micromelia, amelia, brachydactyly, anury, microcephaly, cyclopia, macrophthalmia, and rhinoschisis (Table 1).

4. DISCUSSION

In SPC, the presence of congenital malformations was identified in all anatomical regions of olive ridley embryos and hatchlings, with leucism being the most prevalent malformation (34.4%). The craniofacial region exhibited the greatest diversity of malformations (17/35 malformation types). Hatching success (53.9%) of olive ridleys nesting in SPC was similar to that reported in the hatchery by Bárcenas-Ibarra et al. (2015) for El Verde Camacho nesting beach (58%), Sinaloa, also in northwestern Mexico. Other studies on relocated olive ridley nests have reported hatching success ranging from 75–85% (Galván 1991, Trejo 2000, Ruiz 2002, Bárcenas-Ibarra & Maldonado-Gasca 2009); however, these studies were conducted in more southern latitudes of the Mexican Pacific, where the environmental conditions are not as extreme as in the north of the Mexican Pacific. Higher hatching success has been reported for other species, such as hawksbill *Eretmochelys imbricata* and green sea turtles (Bárcenas-Ibarra et al. 2015) from the Yucatan Peninsula, Mexico, possibly because in other species incubation regularly occurs *in situ*, while for the olive ridley, solitary nests are regularly artificially incubated or relocated in hatcheries to avoid poaching and predation (da Silva et al. 2007, Maulany et al. 2012, Bárcenas-Ibarra et al. 2015, Hart et al. 2018, Kurniawan & Gitayan 2020), which can affect mortality and hatching success (Mrosovsky 1982).

In this study, it is important to consider the effect of nest handling. SPC has a length of 37 km, and the eggs are subject to physical stressors associated with transfer time, relocation, and manipulation, all of which can affect hatching compared to nests left *in situ* (Eckert & Eckert 1990, Pintus et al. 2009). In fact, during nest relocation management in SPC, the greatest losses occur during the incubation and egg collection phase (Sosa-Cornejo et al. 2022); therefore, it is necessary to monitor and improve both phases as well as carry out a study on malformation rates of nests that remain *in situ* versus those brought to the hatchery. Furthermore, hatching success depends on multiple environmental (e.g. humidity, temperature, sand grain size) and individual variables (e.g. clutch, seasonality, maternal factors) that can influence hatching success (Ditmer & Stapleton 2012, Perrault et al. 2012). The optimal range for

Table 1. Distribution, abundance, and prevalence and severity indices of malformations in olive ridley sea turtle *Lepidochelys olivacea* embryos and hatchlings from Santuario Playa Ceuta during the 2017 nesting season. NP: nest prevalence; EP: egg prevalence; NS: nest severity

Region	Malformation	Observed frequency	Proportion (%)	NP (%)	EP (%)	NS (no. of malformed organisms nest^{-1})
Full body	Partial leucism	56	11.3	8.71	0.089	1.10
	Total leucism	114	23.1	17.7	0.181	1.58
	Twins	11	2.23	1.71	0.017	1.22
	Anasarca	2	0.40	0.31	0.003	1.00
	Dwarfism	11	2.23	1.71	0.017	1.00
	Gigantism	2	0.40	0.31	0.003	1.00
Carapace	Supernumerary scutes	3	0.61	0.47	0.005	1.00
	Subnumerary scutes	8	1.62	1.24	0.013	1.60
	Deformed scutes	9	1.82	1.40	0.014	1.29
	Scoliosis	41	8.30	6.38	0.065	1.95
	Kyphosis	37	7.49	5.75	0.059	1.76
	Osteo-agenesis	5	1.01	0.78	0.008	1.00
	Misshapen bones	7	1.42	1.09	0.011	1.17
	Plastron metaplasia	6	1.21	0.93	0.010	1.20
Limbs	Micromelia	3	0.61	0.47	0.005	1.00
	Amelia	4	0.81	0.62	0.006	1.00
	Brachydactyly	2	0.40	0.31	0.003	1.00
Tail	Anury	2	0.40	0.31	0.003	1.00
Craniofacial						
Skull	Anencephaly	4	0.81	0.62	0.006	1.33
	Exencephaly	11	2.23	1.71	0.017	1.38
	Encephalocele	11	2.23	1.71	0.017	1.83
	Acephaly	6	1.21	0.93	0.010	1.20
	Microcephaly	3	0.61	0.47	0.005	1.00
Eyes	Anophthalmia	14	2.83	2.18	0.022	1.27
	Cyclopia	3	0.61	0.47	0.005	1.00
	Sinophthalmia	11	2.23	1.71	0.017	1.57
	Macrophthalmia	7	1.42	1.09	0.011	1.00
	Microphthalmia	9	1.82	1.40	0.014	2.25
	Eyelid dysplasia	12	2.43	1.87	0.019	1.71
Jaws	Agnathia	4	0.81	0.62	0.006	1.33
	Prognathia	9	1.82	1.40	0.014	1.13
	Laterognathia	9	1.82	1.40	0.014	1.13
	Brachygnathia	29	5.87	4.51	0.046	1.45
Nostrils	Arhinia	25	5.06	3.89	0.040	1.39
	Rhinoschisis	4	0.81	0.62	0.006	1.00
Total	35	494				

embryonic development in sea turtles is 25–35°C (Ackerman 1997). However, Sandoval-Espinoza (2012) reported that for some periods during the incubation in the hatchery of SPC, temperatures are outside this range (≤25 or ≥35°C), which may partially explain the low hatching success observed in the present study. Regarding high temperatures and dry conditions, some management strategies have been proposed, including the use of shade structures and water supplementation in the hatcheries (Fuentes et al. 2012, Jourdan & Fuentes 2015), measures that have been implemented at SPC since 2014. Low temperatures occur late in the nesting season, and solving this problem is more challenging; however, few nests are impacted by low temperatures. Humidity during the incubation period is closely related to temperature and has been associated with the presence of anomalies in the carapace (e.g. supernumerary scutes) (Zimm et al. 2017).

Regarding congenital malformations in olive ridley relocated nests only, our NP (22.5%) was lower than the values shown in other studies (Bárcenas-Ibarra & Maldonado-Gasca 2009, Bárcenas-Ibarra et al. 2015). Similarly, EP in the present study (0.54%) was lower

than reported in other olive ridley studies (>1%) (Bárcenas-Ibarra & Maldonado-Gasca 2009, Bárcenas-Ibarra et al. 2015), except for olive ridleys in Guatemala that have a reported EP of 0.5% (Gularte 2000). When discussing EP, it is important to consider the risk of misidentification of EWED. In our study, we identified a considerable number of EWED (n = 24 737); however, their development might have been arrested in early stages when the embryo was not visible to the naked eye. The risk of misidentification of non-evident embryonic development with the possible presence of malformations can influence the results of the malformation prevalence index (Galván 1991). Thus, it is important to compare embryonic development in turtles from the hatchery to *in situ* nests, since relocation could be a cause of early embryonic death. The NS value (2.4 malformed embryos or hatchlings nest^{-1}) in the present study was similar to that previously reported for olive ridleys (Bárcenas-Ibarra et al. 2015). Compared to hawksbill and green turtles, olive ridleys have a higher prevalence of malformations; however, in both species, the nests have remained *in situ* (Bárcenas-Ibarra et al. 2015). The incidence of malformations reported among different species of sea turtles may be due to dissimilar distributions and ecological niches in addition to potential resilience to malformations (Martín-del-Campo et al. 2021). Furthermore, sampling design and calculation of malformation rates must be standardized across studies for accurate comparisons globally.

The craniofacial region presented 37.6% of all malformations and was the anatomical region with the greatest diversity of malformation types (17/35), similar to other Mexican populations of olive ridleys (Bárcenas-Ibarra et al. 2015). However, the most prevalent malformation was leucism (both partial and total; 34.4%), which was regularly accompanied by other craniofacial malformations (Hewavisenthi 1990, Galván 1991, Bárcenas-Ibarra & Maldonado-Gasca 2009, Craven et al. 2019). Formation of the neural crest leads to proper development of facial structures and pigment cells, so failure of the neural crest during early development could lead to the presence of both malformations (Lapedriza et al. 2014). However, craniofacial structures are formed in early development whereas pigmentation is formed at the end of embryonic development, so it is possible that embryos that present severe craniofacial malformations do not reach term and regularly present leucism (Miller 1985). If leucism is not accompanied by other malformations, these individuals can survive to adulthood and even remain reproductively active, although this is rare (Restrepo & Valverde 2019). To date, no known leucistic females have been observed laying eggs in SPC, but hatchlings showing leucism have hatched and been released at this beach (H. Contreras-Aguilar unpubl. data).

Setting aside extreme environmental variations in SPC, we do not rule out that the incidence of malformations found in this study may be directly related to nest management, so *in situ* nesting studies are necessary. However, if nest manipulation is ruled out as the main problem, genetic issues and environmental contaminants could be considered as potential causes. Multiple cases of lack of pigmentation (albinism) have been reported in loggerhead and green turtles, the potential cause of which is inbreeding and genetics (Marcovaldi et al. 1995, Perrault & Coppenrath 2019, Madeira et al. 2020, Perrault et al. 2022). Environmental contaminants have also not been ruled out as a potential teratogenic cause, since metals and non-metals (e.g. arsenic, cadmium, nickel, selenium, and zinc) have been reported in tissues of olive ridleys foraging north of Sinaloa (Zavala-Norzagaray et al. 2014, Olimón-Andalón et al. 2021), and mercury and organochlorine pesticides (endosulfan, heptachlor, and dichlorodiphenyldichloroethylene) have been found in tissues from embryos with multiple congenital malformations (25%), among which included leucism (Martín-del-Campo et al. 2019b,c). In this sense, genes involved in early embryonic development (e.g. *Pax6*) in olive ridley turtles could be environmentally regulated in response to environmental toxins and hypoxia (Martín-del-Campo et al. 2018), and methylation patterns of the *Hoxb9* gene may be related to the embryonic development of olive ridleys (Martín-del-Campo & García-Gasca 2021). This is relevant since this type of epigenetic mechanism can be influenced by environmental factors (Dolinoy et al. 2007).

5. CONCLUSIONS

For the olive ridley population of SPC, the most prevalent congenital malformation was leucism, in addition to the presence of multiple malformations in the craniofacial region likely related to alterations occurring during early embryonic development. Due to the geographical position of SPC, at the northern edge of the olive ridley nesting area in the Mexican Pacific, the extreme environmental conditions (e.g. cold, heat, and dryness) sometimes experienced by the turtles in this region could be one of the main teratogenic agents for the observed abnormalities.

However, genetic influences and the presence of contaminants have not been ruled out as potential factors and could act in a synergistic manner given the multifactorial origin of congenital malformations. Therefore, it is necessary to conduct a study of malformation rates of olive ridley nests that remain *in situ* versus those that have been relocated. Doing so is important, as it could guide nest relocation efforts in a way that will hopefully reduce the number and severity of malformations in this area, thereby enhancing restoration efforts.

Acknowledgements. We thank the staff and volunteers of the Sea Turtle Program of the Autonomous University of Sinaloa (PROTORMAR-UAS) for their support in the conservation of sea turtles in northwestern Mexico. We also thank Jesús Ivan Guardado, Alejandro Ayón-Rivas, and Karla Hernández for their collaboration in the analysis and collection of information as well as the City Council of Elota, Sinaloa, for their support and vigilance. We also thank Pavel Reid for his constructive remarks on this paper.

LITERATURE CITED

Ackerman RA (1997) The nest environment and the embryonic development of sea turtles. In: Lutz PL, Musick JA (eds) The biology of sea turtles. CRC Press, Boca Raton, FL, p 371–380

Alwan S, Chambers CD (2015) Identifying human teratogens: an update. J Pediatr Genet 4:39–41

Bárcenas-Ibarra A, Maldonado-Gasca A (2009) Malformaciones en embriones y neonatos de tortuga golfina (*Lepidochelys olivacea*) en Nuevo Vallarta, Nayarit, México. Vet Mex 40:371–380

Bárcenas-Ibarra A, de la Cueva H, Rojas-Lleonart I, Abreu-Grobois FA, Lozano-Guzmán RI, Cuevas E, García-Gasca A (2015) First approximation to congenital malformation rates in embryos and hatchlings of sea turtles. Birth Defects Res A Clin Mol Teratol 103:203–224

Bensch S, Hansson B, Hasselquist D, Nielsen B (2000) Partial albinism in a semi-isolated population of great reed warblers. Hereditas 133:167–170

Bishop CA, Brooks RJ, Carey JH, Ng P, Norstrom RJ, Lean DRS (1991) The case for a cause–effect linkage between environmental contamination and development in eggs of the common snapping turtle (*Chelydra s. serpentina*) from Ontario, Canada. J Toxicol Environ Health 33: 521–547

Brito J, Valdivieso-Bermeo K (2016) First records of leucism in eight species of small mammals (Mammalia: Rodentia). Therya 7:483–489

Campista-León S, Beltrán-Espinoza JA, Sosa-Cornejo I, Castillo-Ureta H, Martín-del-Campo-Flores JR, Sánchez-Zazueta JG, Peinado-Guevara LI (2019) Haplotypic characterization of the olive ridley turtle (*Lepidochelys olivacea*) in northwest Mexico: the northernmost limit of its distribution. Anim Biodivers Conserv 42: 113–126

Caut S, Guirlet E, Jouquet P, Girondot M (2006) Influence of nest location and yolkless eggs on the hatching success of leatherback turtle clutches in French Guiana. Can J Zool 84:908–915

Craven KS, Sheppard S, Stallard LB, Richardson M, Belcher CN (2019) Investigating a link between head malformations and lack of pigmentation in loggerhead sea turtle embryos (*Caretta caretta*, Linnaeus, 1758) in the southeastern United States. Herpetol Notes 12:819–825

da Silva ACCD, de Castilhos JC, Lopez GG, Barata PCR (2007) Nesting biology and conservation of the olive ridley sea turtle (*Lepidochelys olivacea*) in Brazil, 1991/1992 to 2002/2003. J Mar Biol Assoc UK 87:1047–1056

Ditmer MA, Stapleton SP (2012) Factors affecting hatch success of hawksbill sea turtles on Long Island, Antigua, West Indies. PLOS ONE 7:e38472

DOF (Diario Oficial de la Federación) (1986) Decreto por el que se determinan como zonas de reserva y sitios de refugio para la protección, conservación, repoblación, desarrollo y control, de las diversas especies de tortuga marina, los lugares en que anida y desova dicha especie. http://dof.gob.mx/nota_detalle.php?codigo=4815894&fecha=29/10/1986

DOF (2002) Acuerdo por el que se determinan como áreas naturales protegidas, con la categoría de santuarios, a las zonas de reserva y sitios de refugio para la protección, conservación, repoblación, desarrollo y control de las diversas especies de tortuga marina, ubicadas en los estados de Chiapas, Guerrero, Jalisco, Michoacán, Oaxaca, Sinaloa, Tamaulipas y Yucatán, identificadas en el decreto publicado el 29 de octubre de 1986. https://www.dof.gob.mx/nota_detalle.php?codigo=723470&fecha=16/07/2002#gsc.tab=0

Dolinoy DC, Weidman JR, Jirtle RL (2007) Epigenetic gene regulation: linking early developmental environment to adult disease. Reprod Toxicol 23:297–307

Donnai D, Winter RM (1995) Congenital malformation syndromes, 6th edn. Chapman & Hall Medical, London

Eckert KL, Eckert SA (1990) Embryo mortality and hatch success in *in situ* and translocated leatherback sea turtle *Dermochelys coriacea* eggs. Biol Conserv 53:37–46

Edwards MJ (1968) Congenital malformations in the rat following induced hyperthermia during gestation. Teratology 1:173–177

Fajardo-Gutiérrez A (2017) Medición en epidemiología: prevalencia, incidencia, riesgo, medidas de impacto. Rev Alerg Mex 64:109–120

Fowler LE (1979) Hatching success and nest predation in the green sea turtle, *Chelonia mydas*, at Tortuguero, Costa Rica. Ecology 60:946–955

Frye FL (1991) Biomedical and surgical aspects of captive reptile husbandry. Krieger Publishing, Miami, FL

Fuentes MMPB, Fish MR, Maynard JA (2012) Management strategies to mitigate the impacts of climate change on sea turtle's terrestrial reproductive phase. Mitig Adapt Strategies Glob Change 17:51–63

Galván PVH (1991) Estudio de la mortalidad embrionaria de *Lepidochelys olivacea* en nidos incubados seminaturalmente en el Playón de Mismaloya, Jalisco, México. BSc dissertation, Universidad de Guadalajara

Guest I, Buttar HS, Smith S, Varma DR (1994) Evaluation of the rat embryo culture system as a predictive test for human teratogens. Can J Physiol Pharmacol 72: 57–62

Gularte WE (2000) Deformities in hatchling olive ridleys. In: Mosier A, Foley A, Brost B (eds) NOAA Tech Memo NMFS-SEFCS-477:156

Hart CE, Maldonado-Gasca A, Ley-Quiñonez CP, Flores-Peregrina M and others (2018) Status of olive ridley sea turtles (*Lepidochelys olivacea*) after 29 years of nesting rookery conservation in Nayarit and Bahía de Banderas, Mexico. Chelonian Conserv Biol 17: 27–36

Hewavisenthi S (1990) Abnormal hatchlings of green and olive ridley turtles. Mar Turtle Newsl 50:15–16

Izquierdo L, Thomson RL, Aguirre JI, Díez-Fernández A, Faivre B, Figuerola J, Ibáñez-Álamo JD (2018) Factors associated with leucism in the common blackbird *Turdus merula*. J Avian Biol 49:e01778

Jourdan J, Fuentes MMPB (2015) Effectiveness of strategies at reducing sand temperature to mitigate potential impacts from changes in environmental temperature on sea turtle reproductive output. Mitig Adapt Strategies Glob Change 20:121–133

Kameoka S, Babiarz J, Kolaja K, Chiao E (2014) A high-throughput screen for teratogens using human pluripotent stem cells. Toxicol Sci 137:76–90

Kaska Y, Downie JR (1999) Embryological development of sea turtles (*Chelonia mydas*, *Caretta caretta*) in the Mediterranean. Zool Middle East 19:55–69

Krecsák L (2008) Albinism and leucism among European Viperinae: a review. Russ J Herpetol 15:97–102

Kurniawan N, Gitayan A (2020) Why did the population of the olive ridley turtle *Lepidochelys olivacea* (Eschscholtz, 1829) increase in Alas Purwo National Park's beach, East Java, Indonesia? Russ J Mar Biol 46: 338–345

Lapedriza A, Petratou K, Kelsh RN (2014) Neural crest cells and pigmentation. In: Trainor PA (ed) Neural crest cells: evolution, development and disease. Academic Press, Amsterdam, p 287–311

Madeira FM, Patrício AR, Indjai B, Barbosa C, Regalla A, Catry P, Rebelo R (2020) High number of healthy albino green turtles from Africa's largest population. Mar Turtle Newsl 160:19–22

Makris SL, Solomon HM, Clark R, Shiota K and others (2009) Terminology of developmental abnormalities in common laboratory mammals (version 2). Birth Defects Res B Dev Reprod Toxicol 86:227–327

Marcovaldi M, Paes-e-Lima E, Penteado R (1995) Albino sea turtles hatchlings in Brazil. Mar Turtle Newsl 69:10

Margolis L, Esch GW, Holmes JC, Kuris AM, Schad GA (1982) The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). J Parasitol 68:131–133

Martín-del-Campo R, García-Gasca A (2021) Methylation patterns in the 5′ flanking region of the *Hoxb9* gene in sea turtle embryos with congenital malformations. Am J Biomed Sci Res 12:420–426

Martín-del-Campo R, Bárcenas-Ibarra A, Sifuentes-Romero I, Llera-Herrera R, García-Gasca A (2018) Methylation status of the putative *Pax6* promoter in olive ridley sea turtle embryos with eye defects: an initial approach. Mech Dev 154:287–295

Martín-del-Campo R, Sifuentes-Romero I, García-Gasca A (2019a) *Hox* genes in reptile development, epigenetic regulation and teratogenesis. Cytogenet Genome Res 157:34–45

Martín-del-Campo R, Bárcenas-Ibarra A, Lund G, Rodríguez-Ríos D, Yong-Villalobos L, García-Hernández J, García-Gasca A (2019b) Mercury concentration, DNA methylation and mitochondrial DNA damage in olive ridley sea turtle embryos with schistosomus reflexus syndrome. Vet Pathol 56:940–949

Martín-del-Campo R, Bárcenas-Ibarra A, Betancourt-Lozano M, García-Gasca A and others (2019c) Presencia de plaguicidas organoclorados en embriones y crías de tortuga golfina con malformaciones congénitas en la playa de anidación El Verde Camacho, Sinaloa, Mexico. In: Cuevas-Flores EA, Guzmán-Hernández V, Guerra-Santos J, Rivas-Hernández GA (eds) El Uso del Conocimiento de las Tortugas Marinas como Herramienta Para la Restauración de Sus Poblaciones y Habitats. UNACAR, Ciudad del Carmen, p 251–255

Martín-del-Campo R, Calderón-Campuzano MF, Rojas-Lleonart I, Briseño-Dueñas R, García-Gasca A (2021) Congenital malformations in sea turtles: puzzling interplay between genes and environment. Animals (Basel) 11:444

Maulany RI, Booth DT, Baxter GS (2012) Emergence success and sex ratio of natural and relocated nests of olive ridley turtles from Alas Purwo National Park, East Java, Indonesia. Copeia 2012:738–747

Miller JD (1985) Embryology of marine turtles. In: Gans C, Billett F, Maderson P (eds) Biology of the Reptilia. John Wiley & Sons, New York, NY, p 269–328

Møller AP, Mousseau TA (2003) Mutation and sexual selection: a test using barn swallows from Chernobyl. Evolution 57:2139–2146

Moore KL, Persaud TVN, Torchia MG (2016) The developing human: clinically oriented embryology, 10th edn. Elsevier, Philadelphia, PA

Mortimer JA (1999) Reducing threats to eggs and hatchlings: hatcheries. In: Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M (eds) Research and management techniques for the conservation of sea turtles. IUCN/SSC Marine Turtle Specialist Group Publication, Washington, DC, p 175–178

Mrosovsky N (1982) Sex ratio bias in hatchling sea turtles from artificially incubated eggs. Biol Conserv 23: 309–314

Needham LL, Calafat AM, Barr DB (2008) Assessing developmental toxicant exposures via biomonitoring. Basic Clin Pharmacol Toxicol 102:100–108

Olimón-Andalón V, Valdés-Flores J, Ley-Quiñonez CP, Zavala-Norzagaray A and others (2021) Essential and trace metals in a post-nesting olive ridley turtles (*Lepidochelys olivacea*) in Ceuta beach, Sinaloa, Mexico. Environ Sci Pollut Res Int 28:29998–30006

Perrault JR, Coppenrath CM (2019) Albinism in Florida green turtle (*Chelonia mydas*) hatchlings: ratio-based evidence of basic mendelian recessiveness. Mar Turtle Newsl 156:38–40

Perrault JR, Miller DL, Eads E, Johnson C, Merrill A, Thompson LJ, Wyneken J (2012) Maternal health status correlates with nest success of leatherback sea turtles (*Dermochelys coriacea*) from Florida. PLOS ONE 7: e31841

Perrault JR, Page-Karjian A, Morgan AN, Burns LK and others (2022) Morphometrics and blood analytes of leatherback sea turtle hatchlings (*Dermochelys coriacea*) from Florida: reference intervals, temporal trends with clutch deposition date, and body size correlations. J Comp Physiol B 192:313–324

Pintus KJ, Godley BJ, McGowan A, Broderick AC (2009) Impact of clutch relocation on green turtle offspring. J Wildl Manag 73:1151–1157

Restrepo J, Valverde RA (2019) Leucistic adult female green sea turtles (*Chelonia mydas*) successfully nesting at Tortuguero, Costa Rica. Mar Turtle Newsl 159:23–25

Rojas-Lleonart I, Silveira-Prado EA, Rojas-Borroto L, Oramas-Torres E (2010) Malformaciones congénitas: consideraciones sobre su presentación fenotípica. Rev Electrón Vet 11:1–13

Rousseaux CG, Ribble CS (1988) Developmental anomalies in farm animals: II. Defining etiology. Can Vet J 29:30–40

Ruiz VM (2002) Evaluación del avivamiento y proporción de sexos en embriones y neonatos de tortuga golfina (*Lepidochelys olivacea*), en condiciones supervisadas en el campamento tortuguero de Manzanillo, Colima. BSc dissertation, Universidad de Colima

Sandoval-Espinoza S (2012) Proporción sexual en crías de tortuga *Lepidochelys olivacea* en corrales de incubación del Pacífico Mexicano. PhD dissertation, Instituto Politécnico Nacional, La Paz

Schroeder B, Murphy S (1999) Population surveys (ground and aerial) on nesting beaches. In: Eckert KL, Bjorndal KA, Abreu-Grobois FA, Donnelly M (eds) Research and management techniques for the conservation of sea turtles. IUCN/SSC Marine Turtle Specialist Group Publication, Washington, DC, p 45–55

Seminoff JA, Nichols WJ (2007) Sea turtles of the Alto Golfo: a struggle for survival. In: Felger RS, Broyles B (eds) Dry borders: great natural reserves of the Sonoran Desert. University of Utah Press, Salt Lake City, UT, p 505–518

Smith WD (1975) Classification, nomenclature, and naming of morphologic defects. J Pediatr 87:162–163

Sosa-Cornejo I, Martín-del-Campo R, Contreras-Aguilar HR, Enciso-Saracho F and others (2021) Nesting trends of olive ridley sea turtles *Lepidochelys olivacea* (Testudinata: Cheloniidae) on two beaches in Northwestern Mexico after 30 and 40 years of conservation. Rev Biol Trop 69:1124–1137

Sosa-Cornejo I, Peinado-Guevara LI, Contreras-Aguilar HR, Enciso-Saracho F, Sandoval-Bautista M, Enciso-Padilla I, Campista-León S (2022) Evaluation of nest management phases for *Lepidochelys olivacea* at two beaches in Northwest Mexico. Environ Monit Assess 194:130

SWOT Scientific Advisory Board (2011) Minimum data standards for nesting beach monitoring, version 1.0. The State of the World's Sea Turtles (SWOT), Arlington, VA

Trejo RJA (2000) Mortalidad embrionaria en nidos protegidos de tortuga golfina Lepidochelys olivacea en La Reserva 'Playón de Mismaloya' Jalisco, México. MSc dissertation, Universidad de Colima

Trigos MI, Herrán MFS, Saavedra OMD (2000) Reconsideraciones sobre la terminología de los defectos congénitos. Cir Plast 10:119–121

Ujházy E, Mach M, Navarová J, Brucknerová I, Dubovický M (2012) Teratology: past, present and future. Interdiscip Toxicol 5:163–168

Upfold JB, Smith MSR, Edwards MJ (1989) Quantitative study of the effects of maternal hyperthermia on cell death and proliferation in the guinea pig brain on day 21 of pregnancy. Teratology 39:173–179

van Grouw H (2013) What colour is that bird? The causes and recognition of common colour aberrations in birds. Br Birds 106:17–29

Wallach V (2007) Axial bifurcation and duplication in snakes. Part I. A synopsis of authentic and anecdotal cases. Bull Md Herpetol Soc 43:57–95

Whitmore CP, Dutton PH (1985) Infertility, embryonic mortality and nest-site selection in leatherback and green sea turtles in Suriname. Biol Conserv 34:251–272

Zavala-Norzagaray AA, Ley-Quiñónez CP, Espinosa-Carreón TL, Canizalez-Román A, Hart CE, Aguirre AA (2014) Trace elements in blood of sea turtles *Lepidochelys olivacea* in the Gulf of California, Mexico. Bull Environ Contam Toxicol 93:536–541

Zimm R, Bentley BP, Wyneken J, Moustakas-Verho JE (2017) Environmental causation of turtle scute anomalies *in ovo* and *in silico*. Integr Comp Biol 57: 1303–1311

Editorial responsibility: Thierry Work, Honolulu, Hawaii, USA
Reviewed by: K. Craven and 2 anonymous referees

Submitted: November 27, 2021
Accepted: October 13, 2022
Proofs received from author(s): November 12, 2022