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Sedimentary characteristics of *Dermochelys coriacea* nests and their effects on hatching success in the central coast of Oaxaca, Mexico

Características sedimentarias de los nidos de *Dermochelys coriacea* y sus efectos sobre el éxito de eclosión en la costa central de Oaxaca, México

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ABSTRACT

Background: For sea turtles, temperature and moisture inside the nest are known to be crucial for successful embryonic development during incubation period, and both are influenced by the physical and chemical characteristics of the sand that constitute the incubation chamber; therefore, sediment size influences sea turtle nesting and hatching success. Until now, no other studies have been done in *D. coriacea* to associate sand characteristics with the hatching success in Mexican protected hatcheries, and the consequences that this has on reproductive success are not fully understood. **Objective**: During 2016/2017 leatherback-breeding season, we evaluated the sedimentary characteristics of *Dermochelys coriacea* nests in two hatcheries in the central coast of Oaxaca, by associating the sediment properties of the incubation chambers with hatching success and incubation duration. **Results**: The sand of San Juan Chacahua hatchery showed a dominance of medium sands, with the medium-sand fraction. Palmarito showed a dominance of medium sand sediment too. All samples have similar grain-size spectra and were classified as mesokurtic. Clutch size exerted a positive effect on hatching success in both hatcheries, and sediment grain size did not affect it. Hatching success is mostly affected by clutch size and grain size, whilst the incubation duration is influenced by nesting date, clutch size, and grain size. **Conclusions**: San Juan Chacahua hatchery has fine grain size with major pH values, more incubation days with more clutch size than Palmarito hatchery. More studies about the effect of physical properties of sand in hatcheries are needed to establish a criterion for determine the best areas to set up this.

Key Words: Clutch size, grain size, incubation chamber, leatherback turtle, Sand.

RESUMEN

Antecedentes: En las tortugas marinas, se sabe que la temperatura y humedad dentro de los nidos son cruciales para el desarrollo embrionario exitoso durante el periodo de incubación; y ambos están influenciados por las características físicas y químicas de la arena que constituye la cámara de incubación; por tanto, el tamaño del sedimento influye la anidación de las tortugas marinas y el éxito de eclosión. Hasta ahora, ningún estudio ha sido realizado en *D. coriacea* para asociar las características con el éxito de eclosión in campamentos mexicanos, y las consecuencias que esto tiene sobre el éxito reproductivo no están completamente entendidas. **Objetivo**: Durante la temporada de reproducción 2016/2017 de la tortuga laúd, evaluamos las características sedimentarias de los nidos de *Dermochelys coriacea* en dos campamentos de la costa central de Oaxaca, asociando las propiedades sedimentarias de las cámaras de incubación con el éxito de eclosión y duración de la incubación. **Resultados**: La arena del campamento San Juan Chacahua presentó una dominancia de arenas medias, con la fracción de arena media. El campamento Palmarito presentó un dominancia de arenas medias también. Todas las muestras tuvieron un espectro de tamaño de grano similar, y fueron clasificadas como mesocúrticas. El tamaño de nidada ejerció un efecto positivo sobre el éxito de eclosión en ambos campamentos, y el tamaño de grano de la arena no lo afectó. El éxito de eclosión está principalmente afectado por el tamaño de la nidada y el tamaño de grano de arena, mientras que la duración de la incubación está influenciada por la fecha de anidación, el tamaño de nidada y el tamaño de grano. **Conclusiones**: El campamento de San Juan Chacahua tiene un tamaño de grano más fino con un valor de pH mayor, presenta más días de incubación con mayor tamaño de nidada en comparación con el campamento Palmarito. Un mayor número de estudios acerca de los efectos de las propiedades físicas de la arena en los campamentos son necesarios para establecer criterios que ayuden a determinar las mejores áreas para establecerlos.

Palabras clave: Cámara de incubación, tamaño de nido, tamaño de grano, tortuga laúd.

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INTRODUCTION

Sea turtles are experiencing severe declines worldwide due to anthropogenic disturbances, such as egg exploitation (Mortimer, 1991; Chan 2006; Pike, 2013), marine pollution (Chan 2006; Lazar & Gracan, 2011), incidental capture in fishing gear (Chan *et al*., 1988), and habitat loss (Chan, 2006; Pike, 2013). Particularly, the leatherback turtle, *Dermochelys coriacea* (Vandelli 1761) is globally listed as vulnerable species under the International Union for the Conservation of Nature (IUCN) criteria (Wallace *et al*., 2013), and trends and status in the Pacific Ocean basin have declined precipitously during the last several decades, including declines of more than 90% in Mexico (Sarti *et al*., 2007; The laud OPO Network 2020).

Taking it into account, most conservation efforts of sea turtle populations are concentrated at nesting beaches where the aim is to hatch and survive the greatest number of hatchlings in each nest as is possible (García *et al*., 2003; Stewart *et al*., 2019). This strategy includes the protection of nesting beaches where regular patrols against human poaching are done; finding nests on the beach and relocated them to protected hatcheries; as well as other general actions, such as a complete ban on the exploitation of turtles and their eggs (García *et al*., 2003).

For leatherback turtle and under a dynamic metapopulation approach, specific conservation efforts have been focused on four index beaches (Mexiquillo, Tierra Colorada, Cahuitán and Barra de la Cruz) selected due to their intense nesting activity (more than 100 nests per season) that are monitored regularly over long-term to provide us insights of population trends (Sarti *et al*., 2007; Santidrían *et al*., 2017). Nevertheless, there are secondary beaches where turtles of the same subpopulations nest regularly with lower intensity (less than a hundred nests per season) compared to the index beaches (Santidrían *et al*., 2017). In all these beaches, as soon as the clutches are laid by the female, they are relocated to protected hatcheries aiming to increase the hatchling success.

Bearing this in mind, the hatching success, incubation duration, embryonic development and hatchlings' characteristics, are directly influenced by the microenvironmental characteristics of the place where the incubation of the clutch occurs (Ackerman, 1991; Packard & Packard, 1988; Mohd Salleh *et al*., 2021). Those features refer to the physical, chemical and biological properties of the substrate that make up the microhabitat in the incubation chamber (Abella 2010).

Particularly, temperature and moisture inside the nest are known to be crucial for successful embryonic development during incubation period (Bodensteiner *et al.,* 2015; Maloney *et al*., 1990; Hewavisenthi & Parmenter, 2001) influencing over embryogenesis, phenotype, performance, and survivorship of hatchlings (Booth, 2017; Lolavar & Wyneken, 2020). At the same time, temperature and moisture in the nest microenvironment are influenced by the physical and chemical characteristics of the sand that constitute the incubation chamber; therefore, sediment size influences sea turtle nesting and hatching success (Mortimer, 1990; Speakman *et al*., 1998; Chen *et al*., 2007, 2010; Yalçin-Özdilek *et al*. 2007; Fuentes *et al*., 2010).

Until now, we know that there is only one study that analyzed the effect of incubation temperature in relation to sex determination in this species (Benabib 1984), but we are aware of no other studies that have been done in *D. coriacea* to associate sand characteristics with the hatching success in Mexican protected hatcheries, and the consequences that this has on reproductive success are not fully understood. With this background, the objective of this study was to assess the sedimentary characteristics of *Dermochelys coriacea* nests in two protected hatcheries in the central coast of Oaxaca, by associating the substrate properties of the incubation chambers with hatching success and incubation duration.

MATERIAL AND METHODS

Study area — The study took place in San Juan Chacahua and Palmarito beaches in the Central Pacific Coast of Oaxaca, Mexico (Fig. 1). San Juan Chacahua beach (15°57'48"N, 97°40'52"W — 15°58'54"N, 97°46'54"W) is 12 km long, and is part of the Lagunas de Chacahua National Park; while Palmarito (15°52'23"N, 97°06'23"W — 15°55'36"N, 97°13'58"W) is about 16 km long, extending from San José Manialtepec River on the northwest to Punta Colorada on the southeast. The climate is tropical, hot, humid, and characterized by well-defined dry and rainy seasons. Mean annual temperature is 27.5 °C and mean annual rainfall is 800 mm, concentrated between July and October; the dry season can last 8 months, from November to June (Trejo, 2010).

Hatcheries and nocturnal surveys — This study was carried out from October 2016 through May 2017 (eight months), comprising only one leatherback-breeding season. In each beach there are community groups that protect and relocate the nests to increase the hatching success (García *et al.,* 2003; Vannini & Rosales, 2009; Vannini *et al.,* 2011). The enclosed hatchery locations were constructed with a total area of 80 m^2 (10 X 8 m), which was sufficient to accommodate 100 nests and far enough away from the tide flood. The distance between nests was set at 1 m to reduce interaction, follow the NOM-162-SEMARNAT-2012 instructions (Diario Oficial de la Federación 2012), and to allow hatchery personnel to walk without stepping onto the nests. Likewise, to protect them from the intensity of the sun, the hatcheries were covered by a shading net at a height of 1.5 m during all breeding seasons. Hatcheries have been moved from site year after year, following the protocols established by the Mexican NOM-162 to avoid accumulation of bacteria and other kinds of contamination (Diario Oficial de la Federación 2012).

Community groups (three people per group) patrolled both beaches at night from 21:00 to 06:00 h, using an all-terrain vehicle ATV, to record any sea turtle activity. All found nests were recorded, numbered, cluth size counted, and transported in clean plastic bags to the enclosed hatcheries; and these sites were monitored daily for threats by natural predators. All relocated nests were buried at a depth of 80.0 cm in the hatchery (García-Grajales *et al*., 2019), the mean depth of leatherback nesting activity reported among Pacific populations (Benson *et al*., 2015).

Environmental characteristics of nest incubated in hatcheries — To evaluate microhabitat characteristics associated with eggs incubation, a total of 20 sand samples from 20 nests were collected from both San Juan Chacahua and Palmarito hatcheries (10 nest for each hatchery). Sand samples were collected from the middle of the nest during the nest digging in the hatcheries, 250.0 g sand samples were weighed using a 300.0 g scale (Mod. Pesola), each individual sand sample was tightly sealed in a plastic bag and transported to the Laboratory of Soils in the Universidad del Mar.

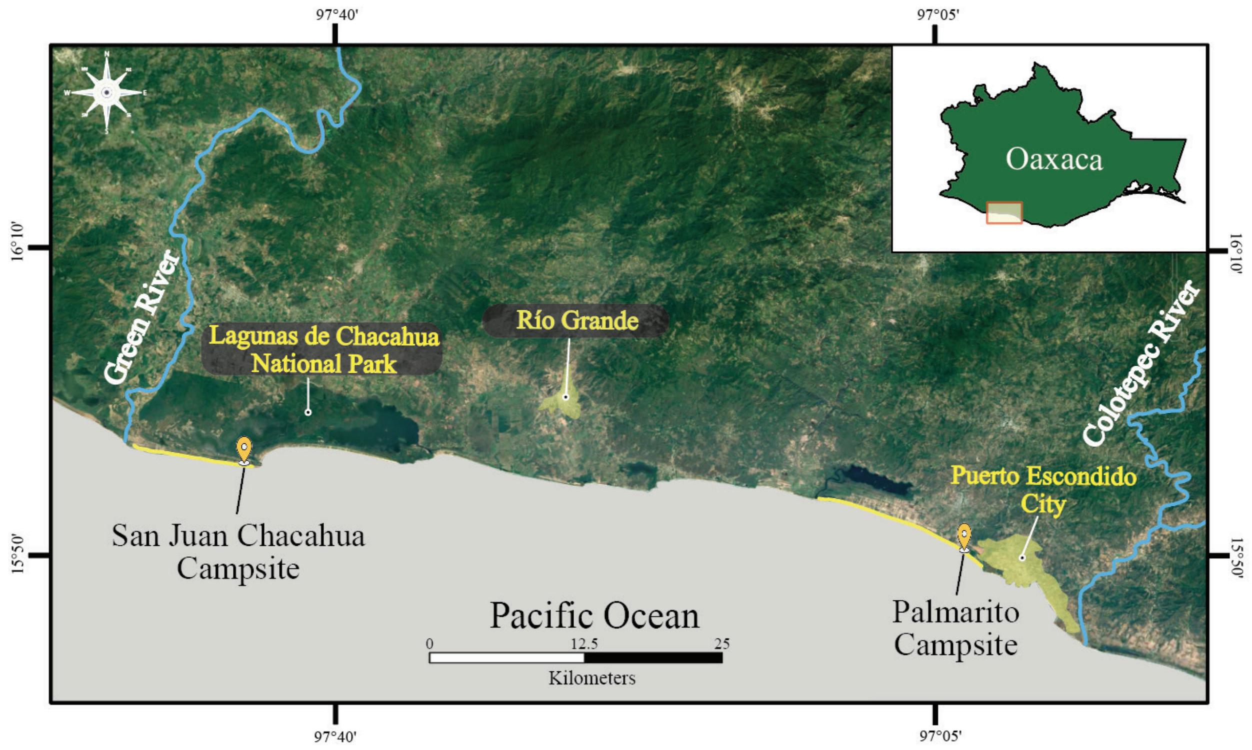

Figure 1. Location of hatcheries in the central coast of Oaxaca, Mexico.

At the laboratory we dried the sand samples by exposure to the sun during a lapse of 72 hours, stirring the samples every 12 hours to uniformly dry them. Then, we followed the Foley *et al.* (2006) protocol, which consists in separating the sand into its various-sized components by sieving 200 g of the sand through the following series of sieves: 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm, and 0.063 mm. The sand retained by each sieve was dried for 24 hours at 105ºC and weighted. We then calculated the percent of sand by each size class and determined the grain size (mean particle diameter) by weight according to the following formula (Hillel, 1980):

$$X = \sum_{i=1}^{n} x_i w_i$$

Where x_i was the mean diameter of any size range of particles separated by sieving, and w_i was the weight of the particles in that size range as a fraction of the total dry weight of the sample analyzed.

Other 40.0 g of each sand sample was treated with distilled water and sodium hexametaphosphate as dispersant agent (Borja *et al.*, 2015). Then, we evaluated the particle size distribution through hydrometer method (Gee & Bauder, 1986) and used the Udden-Wentworth grain-size classification (Wenwhorth 1922), whose notation (phy Ø) is funded on the base 2 algorithm of the sediment particle diameter ($Ø = -\log_2 D_{mm}$).

The remaining dried sand (10 g) was immersed in plastic vials holding 10 ml of deionized water for determination of pH, using a Ph meter (Mettler S400).

Data analysis — When assumption of normality or homogeneity of variance were not met, the Kruskal-Wallis's test was used to perform a nonparametric analysis.

Differences among the hatcheries, including the biological (clutch size) and physical (grain size) features were further investigated using one way ANOVA, followed by the Bonferroni Post-hoc test. Variables expressed as percentage (grain size, hatching success) were transformed by arcsine of its square root, to achieve data normalization (Zar 2008). When necessary, data normality was tested by K^2 and data homoscedasticity was tested by Bartlet X^2 (Zar, 2008).

The statistical analysis of the size distribution for each sample was determined using the formula from Folk & Ward (1957) obtained. Also, the classifications of skewness (Sk), and kurtosis (K), were done also based on the description defined by Folk & Ward (1957). Statistical measurements included measure of degree of sorting, kurtosis, the degree of peakedness, and skewness, which are described in Table 1.

The total number of eggs (clutch size) laid into the nest and the hatching success were calculated by counting unhatched eggs, dead embryos in eggs, and dead hatchlings in nests, and by excluding the shelled albumen globes (SAGs, Patiño-Martínez *et al.*, 2010, 2012). The hatching success (HS) for each nest was calculated as the percentage of hatchlings in the clutch and was determined using the following formula: [(total eggs – unhatched eggs)/total eggs] X 100 (Hitchins *et al.*, 2004). Mean hatching success was calculated by hatchery. A one-way ANOVA test was used to examine differences between hatcheries. The incubation period (IP) per nest was determined as the numbers of days from the date of eggs lying to the date of the first hatchling emergence (Yalçin-Özdilek *et al.,* 2007).

Table 1. Physical parameters and classification of grain size using the logarithmic Folk and Ward (1957) graphical measures.

Sorting (σ)		Skewness (Sk)		Kurtosis (KG)	
Very well sorted	<0.35	Very fine skewed	0.30 to 1.00	Very platykurtic	<0.67
Well sorted	0.35 - 0.5	Fine skewed	0.10 to 0.30	Platykurtic	0.67 - 0.90
Moderately well sorted	0.7 - 1.00	Symmetrical	0.10 to 0.10	Mesokurtic	0.90 - 1.11
Poorly sorted	1.00 - 2.00	Coarse skewed	0.10 to 0.30	Leptokurtic	1.11 - 1.50
Very poorly sorted	2.00 - 4.00	Very coarse skewed	0.30 to 1.00	Very leptokurtic	1.50 - 3.00
Extremely poorly sorted	> 4.00			Extremely leptokurtic	>3.00

To evaluate the influence of substrate properties, nesting date and cluth size on incubation duration and hatching success, a stepwise backward multiple regression was made, taking the number of days of incubation period and the proportion of hatching success as independent and dependent variables, respectively. The goodness of fit for regression residuals to the normal distribution was tested by K^2 (Fadini *et al.*, 2011).

Canonical Correspondence Analysis (CCA) was performed to analyze the relation between physical properties of grain size and biological properties of nests. All *P*-values were compared to an alpha level of 0.05, and all variables of the nests sedimentary characteristics were evaluated with Gradistat software (Blott & Pye, 2001), and all analyses (including normality tests) were conducted with XLStat software v. 2018.1 (Addinsoft, Inc.).

RESULTS

The physical parameters and classification of grain size of 20 *D. coriacea* nests from both hatcheries are presented in Table 1. The sand of San Juan Chacahua hatchery showed a dominance of medium sands, with the medium-sand fraction comprising, on average, over 80%, while the remaining categories together accounted for 8%. Palmarito showed a dominance of medium sand sediment too, on average, over 70%, and the remaining categories together brought 2%.

The descriptive statistics of grain size (mean particle diameter) for the two hatcheries are presented in Table 2a. Sediment taken from *D. coriacea* incubation chambers in hatcheries was moderately well sorted in both sites. Data shows that all samples have similar grain-size spectra, where all sediments are in the region of sand. Also, all samples were symmetrical skewed, and all samples were kurtosis classified as mesokurtic.

Incubation duration was significantly different between hatcheries (F = 6.012, $P<0.05$), but hatching success was the same for both sites (See Table 2b).

The dominant grain size was between 0.25 mm and 0.125 mm for both hatcheries. There were significant differences for >1 mm size ($P<0.05$), 0.05 mm ($P<0.05$), 0.25 mm ($P=0.003$), and 0.125 mm ($P= 0.03$) between the two hatcheries; but no differences were found for 2 mm and 0.063 mm grain sizes between the two hatcheries (Table 3).

Both hatcheries were analyzed collectively, as if they represented a single hatchery site, and hatching success correlated to clutch size and grain size. So, clutch size exerted a positive effect on hatching success in both hatcheries, and sediment grain size did not affect it (Table 4).

Incubation duration was affected by the nesting date, clutch size, and grain size (Table 5). For both hatcheries, nesting date presented a negative correlation with incubation duration, and in the same way, clutch size showed a negative correlation with incubation duration. However, incubation duration correlated positively with the all-grain size.

According to CCA ordination, the first and second axes accounted for 74.79% of the data variability. Medium Sand (MS) was the abiotic factor with positive higher influence in the hatching success of *Dermochelys coriacea*; however, all abiotic factors showed a strong positive correlations, with distribution along the positive region of both axes (Fig. 2).

DISCUSSION

The incubation substrate by variation in chemical composition and particle size can influence the microenvironment within the nest (Stewart *et al.*, 2019). Leatherback and other sea turtles often nest successfully on beaches with widely variable particle diameters (Carr & Ogren, 1959; Hendrickson & Balansingam, 1966; Pritchard, 1971; Mortimer, 1982), suggesting sand particle size alone is not likely a cue to which nesting turtles are particularly responsive (Roe *et al.*, 2013).

Both hatcheries showed similarities in the distribution of sand grains despite their geographical distances, with a dominance of medium-sized sediments, emphasizing the absence of very coarse sand. Whit this in mind, it is hard to explain the variables that determine success of hatching or incubation time. But our results have interesting implications, for example coarse-grained substrates are more permeable to respiratory gases (Ackerman, 1977, 1980), as the open structure allows for more air to be trapped (Speakman *et al.*, 1998) and therefore enhance respiratory gas exchange between the embryos and the atmosphere. However, high substrate permeability also decreases the moisture content of the nest (Bustard & Greenham, 1968) and increases the chance of egg desiccation (Ackerman, 1977; Mortimer, 1990). Conversely, compacted grain fine-grained substrates favor greater water retention (Chen *et al.*, 2010) and impedes the diffusion of respiratory gases (Yalçin-Özdilek *et al.*, 2007; Chen *et al.*, 2010; Fuentes *et al.*, 2010; Cheng *et al.*, 2015; Stewart *et al.*, 2019). In addition, larger grain sizes typically have poorer thermal conductivity as they cannot transfer heat as effectively and are thus expected to be cooler overall if heat for incubation comes predominantly from heating on the beach surface (Speakman *et al.*, 1998; Fuentes *et al.*, 2010). Therefore, sediment size plays a direct role on hydraulic conductivity, total porosity, air-filled pore space, salinity (Foley *et al.*, 2006) and heat transfer (Souza & Vogt, 1994) with consequences to embryo survival.

Table 2. Descriptive statistics of *Dermochelys coriacea* nests during 2016-2017 reproductive season in two hatcheries of Oaxaca. SD = Standard deviation, * denote significance at 0.05 level. MWS: Moderately well sorted; Sim: symmetric; Msk: Meso-kurtic.

	San Juan Chacahua hatchery			**Palmarito hatchery**				
	n	**Mean ± SD**	**Min - Max**	**n**	**Mean ± SD**	**Min - Max**	**Parameter**	**p**
a) Grain size:								
Very coarse sand *	10	0	-	10	0	-	-	-
Coarse sand *	10	4.72 ± 3.55	0.98 - 9.21	10	12.58 ± 8.11	4.28 - 21.41	H = 16.26	< 0.05
Medium sand *	10	86.95 ± 8.32	72.59 - 97.71	10	76.25 ± 9.44	63.72 - 86.69	H = 113.21	< 0.05
Fine Sand *	10	7.44 ± 2.16	4.98 - 9.97	10	10.44 ± 8.45	1.82 - 19.62	H = 24.53	< 0.05
Very fine sand *	10	0.89 ± 0.29	0.35 - 1.13	10	0.73 ± 0.22	0.47 - 1.08	H = 8.56	< 0.05
pH	10	9.63 ± 0.39	8.76 - 11.43	10	8.69 ± 0.24	7.98 - 9.18		
Descriptive statistics:								
Sorting (σ) classification		MWS			MWS			
Skewness (Sk) classification		Sim			Sim			
Kurtosis (K) classification		Msk			Msk			
b) Incubation duration (days)*	10	58.50 ± 4.60	50.2 - 65.72	10	56.30 ± 3.63	50.35 - 60.21	F = 6.012	< 0.05
Hatching success (%)	10	77.24 ± 8.11	64.73 - 88.72	10	81.70 ± 9.50	69.34 - 93.81	H = 7.43	0.059
Clutch size	10	100.4 ± 14.53	82.74 - 119.83	10	105.50 ± 10.88	92.73 - 121.72	F = 0.820	> 0.05

The influence of nesting site on hatching success greatly varies among the sea turtle species and the sites themselves (Miller *et al.*, 2003). Previous studies have suggested that sediment size influences sea turtle nesting and hatching success (Mortimer, 1990; Speakman *et al.*, 1998; Chen *et al.*, 2007; Yalçin-Özdilek *et al.*, 2007). For instance, we know that leatherback tend to nest on high-energy, dynamic beaches that are free of offshore obstructions, with steeply sloping shorelines and offshore depth profiles (Pritchard, 1971; Mrosovsky, 1983; Eckert 1987). In addition, Roe *et al.* (2013) found that leatherback nesting was positively correlated with sand in intermediate size class (0.025 mm diameter) at Playa Grande, Costa Rica. In our study, the contribution of medium to fine sediments in both beaches are, probably, due to the nearby presence of the Rio Verde (one of the main rivers in the coastal region) and to the constant connection between the sediments of the lagoons of Chacahua with the sea (Espinoza-Ayala *et al.*, 2011). Additionally, changes in grain size distributions of sands in such areas are determined by coastal geomorphology (erosional and depositional features associated with waves and tides), longshore transport, winds, tidal regimes, river discharges near the beach, and sand composition, among other factors (Kasper-Zubillaga & Carranza-Edwards, 2003; Kasper-Zubillaga *et al.*, 2007; Davidson-Amott 2010).

Table 3. Comparison of sand grain size separately for San Juan Chacahua (PSJ) and Palmarito (PMO) hatcheries. n = 10 for all samples. * denote significance at 0.05 level.

Mesh Size	**Hatcheries**	**Mean ± SD**	**Min**	**Max**
2 mm	PSJ	0.16 ± 1.56	0.14	1.98
	PMO	0.25 ± 0.10	0.08	2.14
1 mm *	PSJ	1.16 ± 0.65	0.34	2.11
	PMO	1.65 ± 0.94	0.58	2.96
0.05 *	PSJ	2.68 ± 1.02	1.66	3.28
	PMO	4.37 ± 1.78	1.19	7.54
0.25 *	PSJ	44.73 ± 12.34	28.83	72.29
	PMO	61.27 ± 14.23	38.56	80.64
0.125 *	PSJ	25.68 ± 9.95	13.97	39.73
	PMO	35.13 ± 11.36	20.16	49.57
0.063	PSJ	1.18 ± 0.92	0.13	2.75
	PMO	2.04 ± 1.21	0.52	4.31

Table 4. Stepwise backward multiple linear regression for hatching success of *Dermochelys coriacea* in both hatcheries and in each individually.

	Variable	Coefficient	Standard Coefficient	p
Both hatcheries ($r^2 = 0.9525$)	Clutch size	0.072	0.732	< 0.0001
	Medium sand	0.654	0.284	< 0.0001
	Coarse sand	0.843	0.175	< 0.0001
	Fine sand	1.257	0.119	< 0.0001
San Juan Chacahua hatchery ($r^2 = 0.9314$)	Clutch size	0.093	1.093	< 0.001
	Medium sand	1.478	0.573	0.012
	Fine sand	4.543	-1.645	0.045
Palmarito hatchery ($r^2 = 0.925$)	Clutch size	0.035	0.304	0.0385
	Medium sand	0.668	0.428	0.001
	Coarse sand	0.482	0.273	0.007

Both hatcheries showed carbonated (alkaline) sands, and this is perhaps related to null rain activity during the Leatherback nesting season and a little percolating soil (medium sands). All of that will cause a build-up of calcium cations increasing the carbonation as product of water evaporation (Moreno Ramon, 2023).

Average grain size and sorting values obtained here are like those reported by Carranza-Edwards (2001), who mentions that the coastal zone of the Mexican Pacific shows medium sands to fine sands. Particles in the sand (~0.1-64 mm) are cohesionless and readily set in motion by waves, meanwhile larger particles are also cohesionless, but their large size also requires very large waves for the transport (Davidson-Amott, 2010). The influence of waves and currents might generate sands with different "peaks" in their grain size distributions. In the Mexican Pacific coast, beach face slopes of 4º are characterized by medium to fine sands (Shepard 1973), probably due to the piling-up effect of finer grains transported during the run-up in wide coastal plains (Davidson-Amott 2010). In addition to this, Rio Verde discharges, to a lesser extent, deposits sediments with grain size distributions due to the dam construction landwards and the use of river tributaries for irrigation purposes (Espinoza-Ayala *et al*., 2011; Kasper-Zubillaga *et al*., 2007) in the case of San Juan Chacahua beach while the San José Manialtepec River does the same for Palmarito beach.

Grain size and nesting date have been selected as one of the main factors influencing nest temperature and embryonic development rate, having a direct effect on incubation duration (Fadini *et al*., 2011; Hawkes *et al*., 2007; Pike *et al*., 2006). Sites with coarse sand have higher temperatures, shortening incubation duration; by contrast, the lower temperatures characteristics of fine sands increase incubation duration (Ferreira Junior & Castro, 2003). In addition, water availability and respiratory gas (oxygen and carbon dioxide) concentrations (Ackerman, 1997; Erb *et al*., 2018) also influence embryonic development and hatchling quality.

Thus, incubation duration is longer in the beginning of the reproductive season of sea turtles, because the mean daily temperature is lower, and in subsequent months, days become longer and warmer, and the nest temperatures tend to increase, shortening the incubation duration (Baptistote *et al*., 2003; Hewavisenthi & Parmenter, 2002). Although this correlation has not been demonstrated in the reproduction areas of the leatherback turtle in the Mexican Pacific coast, it has been demonstrated in other geographical areas of Mexico with *Caretta caretta* (Öz *et al*., 2004).

Considering the success of eclosion as dependent variable, a greater relationship with sand size was undeniable for both sites of study; however, in separate models, San Juan Chacahua showed greater value in the coefficients of fine sands and medium sands, while Palmarito showed higher value in the coefficients of medium and fine sands.

Table 5. Multiple linear regression for incubation duration of *Dermochelys coriacea* in both hatcheries.

	Variable	Coefficient	Standard Coefficient	p
Both hatcheries ($r^2 = 0.9325$)	Nesting date	-0.0753	-0.1963	0.005
	Clutch size	-0.1319	-0.1467	0.008
	Medium sand	10.165	0.0488	< 0.001
	Coarse sand	14.037	0.1413	0.005
	Fine Sand	12.853	0.1846	0.027
	Very fine sand	16.871	0.0923	0.019

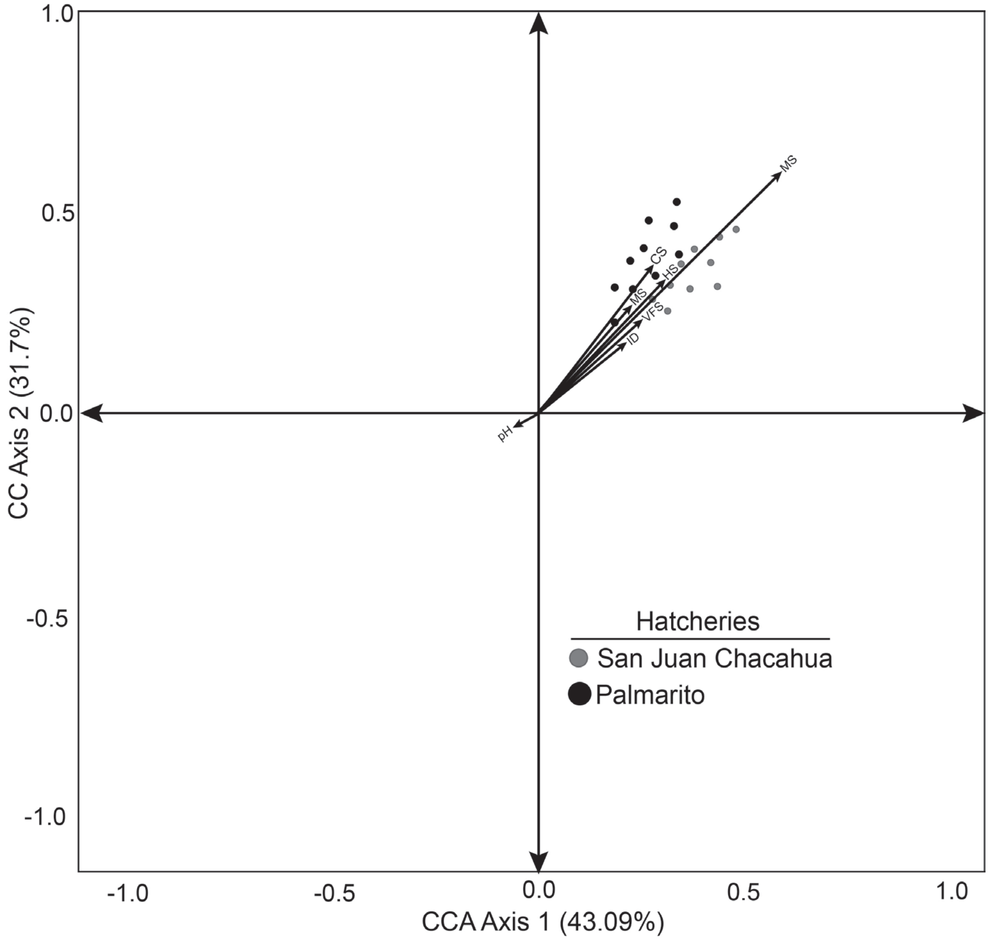

Figure 2. Biplot of axes 1 and 2 of the Canonical Correspondence Analysis (CCA) between physical properties of grain size and biological properties of *Dermochelys coriacea* nests. VFS= Very fine sand, FS= Fine sand, MS= Medium sand, HS= Hatching success, IP= Incubation period, CS= Clutch size, pH= Potential of hydrogen.

Interpreting the CCA ordination, medium grain size was the most important abiotic variable that influence in the hatching success; however, through stepwise backward linear regression the clutch size exerted a positive effect on hatching success. This is not contradictory, as the clutch size also presented a strong correlation on both axes demonstrating that both factors may have simultaneous effects. Maybe, some factors that influence sand characteristics are seasonal patterns of rainfall and the geological position and close to river openings of the beaches (Sönmez *et al*., 2013).

As product of coastal development, many managed sea turtle nesting beaches use hatcheries that follow a standard procedure regarding clutch relocation techniques, such as ensuring the new egg chamber at the same depth as the natural nest, and choosing a new site with the optimal characteristics for incubation and protection (Tanabe *et al*., 2021); however, most hatcheries are built based on easy access and without considering the characteristics of the substrate. For more than two decades these efforts have been focused on Mexico (García *et al*., 2003) with a lack of detailed understanding of which effects have the nests sedimentary characteristics in hatcheries on hatching success and incubation duration. In the current study, the influence of nesting date and some nest sediment characteristics of incubation chamber in hatcheries make it possible to highlight the importance of conducting this type of analysis for the selection of the sites where the hatcheries will be established. Clearly, more detailed studies on the effect of physical properties of sand in hatcheries are needed to establish a criterion for determine the best areas to set up this.

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