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Comparison of reproductive output of hybrid sea turtles and parental species

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Abstract Globally, sea turtle hybridization has been reported at very low frequencies. However, in Brazil, a high incidence (>40% of morphologically assigned hawksbills) of hybridization between loggerheads and hawksbills has been reported. To the best of our knowledge, this is the first analysis of the effect of hybridization on the reproductive output of sea turtle hybrids. We used nuclear and mitochondrial markers to assign a status of hawksbill (*Eretmochelys imbricata)*, loggerhead (*Caretta caretta)*, or hybrid to 146 females that deposited 478 nests. Hybrids do not appear to be at either a reproductive advantage or disadvantage relative to their parental species based on the parameters analyzed (female curved carapace length, clutch size, emergence success, incubation period, hatchling production, observed clutch frequency, and observed breeding frequency). Although emergence success is lower in hybrids, hatchling production per clutch, as well as clutch frequency and breeding frequency, is similar among the three groups. These results show that hybrids may persist in this region. Further research on hybrid survival rates at different life stages, as well as growth rates and their ecological roles, will be fundamental to predict the fate of hybrid turtles. Sea turtle populations that overlap with other sea turtle species in space and time on nesting beaches should be screened for hybridization with the appropriate genetic markers.

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Introduction

Interspecific hybridization is widespread across a large range of taxa (Mallet 2005). Although often viewed as a deleterious process leading to reduced reproductive output and hybrid breakdown, consistent with the biological species concept, interspecific hybridization can also lead to speciation, promote hybrid vigor and create permanent hybrid zones (Rhymer and Simberloff 1996; Allendorf et al. 2001; Abbott et al. 2013). Among sea turtle species, hybridization was first suggested for loggerheads (*Caretta caretta*) and hawksbills (*Eretmochelys imbricata*) by Garman in 1888. Kamekazi (1983) and Frazier (1988) also reported on hybrids between the two species based on morphological features. The first biochemical assays to show hybridization really occurred between sea turtles was by Wood et al. (1983) involving a green turtle (*Chelonia mydas*) and a hawksbill. Conceicao et al. (1990) were the first to confirm loggerhead/hawksbill hybrids in Brazil. Since then, hybridization has been confirmed between all

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species of the Cheloniidae family, except for the flatback turtle *Natator depressus* (Bowen and Karl 2007; Reis et al. 2010a, b; Vilaça et al. 2012; Kelez et al. 2016). Outside of Brazil, these reports of hybrids have been limited to very few individuals.

In Brazil, there is a high incidence of hybrids in the main hawksbill and loggerhead nesting area. Lara-Ruiz et al. (2006) reported that 42% of the morphologically assigned hawksbills ($n = 119$) had loggerhead mtDNA. Vilaça et al. (2012) analyzed the same dataset with 12 nuclear markers and confirmed that the majority of hybrids was first generation (i.e., heterozygous to species-specific alleles). This is evidenced that most hybridization events in this region are recent, estimated to be around ~30 years old, coincident with the major decline of both species' populations in Brazil.

The coast of Bahia has the largest rookeries in the country for both loggerheads and hawksbills with overlapping spatial and temporal distributions. Both populations were heavily depleted before 1980 and now show an increasing population trend, based on nest abundance over the past 20 years (Marcovaldi and Chaloupka 2007; Marcovaldi et al. 2007). In the late 1980s, the numbers of nests for both species, especially hawksbills (Marcovaldi and Laurent 1996), were low. Thus, a very small number of individuals were assumed to be in the area, which could yield lower mating probabilities. These factors (spatial and temporal overlap and low mating encounters) may have contributed to the hybridization process (Vilaça et al. 2012).

Genetic analyses in loggerhead turtles in Bahia have been limited to mitochondrial DNA (mtDNA) (Bowen et al. 2005; Reis et al. 2010a; Shamblin et al. 2014). Although this marker is a great tool to investigate connectivity and population structure, it is inappropriate to determine hybridization, because mtDNA is strictly maternally inherited. Thus, detecting hybridization is not possible when the mtDNA haplotype matches the species morphological assignment. To resolve this and correctly genotype these turtles, the population was evaluated with mtDNA and the appropriate nuclear markers that can show the female and male genetic contributions.

We had three main objectives. First, we evaluated the turtles' morphological assignments with mtDNA and nuclear markers to determine the extent of hybridization and our ability to detect it. Second, we determined the spatial and temporal nest distribution of genetically assigned loggerheads, hawksbills, and hybrids (hereafter "groups") to evaluate the extent of spatial and temporal reproductive overlap among groups, from which we might infer the extent of opportunities for interspecific mating. Third, we evaluated whether the three groups vary in aspects of their reproductive output and, if so, whether hybrids are at a reproductive advantage or disadvantage. These analyses can provide insights on whether hybrids might persist along with hawksbills and loggerheads, replace one or both of the pure species, or disappear. For each of the three groups, we assessed body size (curved carapace length) and other reproductive parameters.

Methods

Data collection and sampling

Projeto TAMAR researchers (The Brazilian Sea Turtle Conservation and Research Program) patrolled nesting beaches in the State of Bahia, from September through March (1999–2014) every night to encounter nesting females. They measured curved carapace length (CCL) from the anterior point of the mid line (nuchal scute) to the posterior tip of the supracaudals (Bolten 1990). TAMAR collected skin samples from 146 nesting sea turtles between 1999 and 2012. Samples were taken between the first and second scales on the front flippers or on the neck region with a 6-mm disposable biopsy punch and stored in 70% ethanol. Researchers tagged individual females with Inconel flipper tags (National Band and Tag Co., style 681) to avoid re-sampling. In the mornings, TAMAR revisited the same nesting areas to collect data on incubation period (IP—days between oviposition and first hatchling emergence) based on observation of hatchling tracks on the nest surface and to excavate every nest to assess clutch size (CS—number of eggs in a clutch), emergence success (ES—the proportion of eggs that produced live hatchlings reaching the beach surface), and hatchling production (HP—product of clutch size and emergence success). For more details on the methodology, please see Marcovaldi and Marcovaldi (1999).

Nesting females were identified in the field by experienced biologists, based on TAMAR's protocol. Nesting females were always assigned to a species, never as hybrids. If any female had mixed traits that could suggest hybridization, these were noted in the observation field. Among the sampled females, there were 82 morphologically identified as loggerheads and 64 as hawksbills.

We retrieved all nesting records for each of the 146 females from TAMAR's 35-year database. For body size, we used only the first CCL record (the first time a female was encountered) because females essentially stop growing after they become sexually mature (Bjorndal et al. 2013). We used data from both in situ nests (left in their original site) and transferred nests for CS, observed clutch frequency (OCF—average number of clutches laid by individual turtles during a single nesting season), and observed breeding frequency (OBF—number of breeding seasons for individual turtles during a given time frame). Because moving clutches can affect emergence success, only in situ

nests were used for ES, IP, and HP. Due to variable monitoring efforts before the 2008 nesting season, we could only use data from 2008 through 2014 to analyze OCF and OBF. Sample size for each group varied according to the parameter that was analyzed because not every parameter was available for all of the 478 nests in our dataset belonging to the 146 genetic assigned females.

In Brazil, the nesting season spans two calendar years (e.g., starts in September 1999 and ends in March 2000). For readability, in this paper, we use a single year designation based on the year of the start of the nesting season (e.g., 2000/2001 = 2000).

Genetic analyses and species assignment

All females were assigned to groups by the combined use of mtDNA and nuclear markers. Forty-five turtles were genetically assigned by techniques described in Vilaça et al. (2012), and the remaining 101 females were assigned as follows.

We first extracted genomic DNA using a DNeasy Blood and Tissue Kit (QIAGEN Inc.) following the manufacturer's protocol. We then amplified a ~830 bp fragment of the mtDNA encompassing the D-loop of the control region and the adjacent $tRNA^{Thr}$ and $tRNA^{Pro}$ with primers LCM15382 and H950 developed by Abreu-Grobois and colleagues, as cited in Proietti et al. (2014a). We conducted 25 µl PCR which included 50 ng of genomic DNA, 12.5 µl of NEB One Taq Hot Start Master Mix (M0488L, New England Biolabs, Inc.), 9.5 µl of sdH20 and 1 µl of each primer, with the following PCR conditions: 95 °C for 5 min; 35 cycles of 95 °C for 30 s; 50.5 °C for 60 s; 72 °C for 30 s; and a final extension of 72 °C for 10 min. After amplification, the PCR products were confirmed by running 1.5% agarose gels stained with GelRed (Biotium). The PCR products were purified using ExoSAP-IT (USB Corporation) according to the manufacturer's instructions. Samples were Sanger sequenced for both strands using both amplification primers at the DNA Analysis Facility at Yale University run in the Thermo Fisher Scientific 96-capillary 3730xl DNA Analyzer.

We amplified and Sanger sequenced four autosome markers previously used by Vilaça et al. (2012) (RAG1, RAG2, R35, and CMOS) for 25 nesting females morphologically assigned as 15 loggerheads and 10 hawksbills. We conducted PCR as described above with the following PCR cycling parameters for RAG1 and R35: 95 °C for 5 min; 35 cycles of 95 °C for 30 s; 61.7 °C for 60 s; 72 °C for 30 s; and a final extension of 72 °C for 10 min. The PCR cycles for RAG2 were: 95 °C for 5 min; 35 cycles of 95 °C for 30 s; 64.4 °C for 60 s; 72 °C for 30 s; and a final extension of 72 °C for 10 min. Finally, for CMOS, the parameters were: 95 °C for 15 min; 35 cycles of 95 °C for 30 s; 66 °C for 60 s; 72 °C for 30 s; and a final extension of 72 °C for 10 min. After amplification, PCR products were confirmed by running 1.5% agarose gels stained with GelRed (Biotium). PCR products were then purified using ExoSAP-IT (USB Corporation) according to the manufacturer's instructions. Samples were Sanger sequenced for both strands using either of the amplification primers at the DNA Analysis Facility at Yale University.

We aligned haplotypes for each marker against known loggerhead and hawksbill haplotypes as previously determined by ACCSTR (http://accstr.ufl.edu/files/accstr-resources/cclongmtdna.pdf) and Vilaça et al. (2012). We used the software Geneious R8 (Kearse et al. 2012) with default Geneious alignment algorithm parameters. Hybrids were assigned when the chromatograms for the nuclear markers clearly showed the presence of both species alleles at all diagnostic polymorphic nucleotides within the locus for each gene.

The remaining 76 samples were genotyped only for RAG2 (following the same methodology described above). Our analyses showed that this marker was the most polymorphic and best diagnostic for hybridization.

Data analyses

We used generalized additive models (GAM) as in Zarate et al. (2013) to statistically model the following response variables: body size (CCL), clutch size (CS), emergence success (ES), incubation period (IP), hatchling production (HP), observed clutch frequency (OCF), and observed breeding frequency (OBF). Possible covariates were CCL, groups, year, day of nesting season the nest was laid (DONS), IP, and CS. In GAM analyses, each covariate is conditioned on all other covariates.

The first models were designed with a general formula: GAM (response variable ~ appropriate covariates (except CCL), cubic smoothing splines, and error function). The GAM models included group and other appropriate covariates, according to the response variable. After we ran each GAM model, if the covariate "groups" was significant, we added CCL to the model as a covariate to determine whether the group effect was due only to differences in body size. Our final model dropped any nonsignificant covariates. The significance of the contribution of each covariate to the overall model fit was evaluated with *t*-ratio statistical inference. The value of R^2 was calculated as (null deviance—residual deviance)/null deviance (Chaloupka and Limpus 1997). Model selection for nested GAMs was based on analysis of deviance. All GAM models had a robust quasi-likelihood error function except the ES GAM model, which was a binomial model.

All GAM analyses were conducted in S-Plus software (TIBCO Spotfire S + Version 8.2.0), and all other statistical analyses were run in Excel 10.0 (data analyses package). We used alpha = 0.05 for all analyses.

Results

For the 82 morphologically assigned loggerheads, the mtDNA and the nuclear marker RAG2 analyses revealed that 81 of them had only loggerhead haplotypes for both markers. The remaining individual had a hawksbill mtDNA haplotype and was heterozygous for hawksbill and loggerhead at RAG2. For the 64 morphologically assigned hawksbills, there were 31 hawksbills and 33 hybrids based on our DNA analysis. All 33 of these hybrids had loggerhead mtDNA haplotypes and were heterozygous for species-specific alleles at RAG2.

The nesting distribution of hybrids overlaps those of the parental species both spatially (Fig. 1a) and temporally (Fig. 1b). However, hybrid nests are temporally intermediate between the loggerheads, which are the first to arrive on the nesting beaches, and the later hawksbills (Fig. 1b). Thus, there are ample opportunities for interspecific hybridization.

Hybrids and parental species varied for many traits. Values for body size (CCL) and reproductive parameters are summarized for the three groups in Table 1. Hybrids are larger than both parental species (i.e., greater CCL; Tables 1, 2). Clutch size (CS) was similar for hybrids and hawksbills, which both produced larger clutches than loggerheads when CCL was not a covariate. However, when CCL was accounted for, clutch size of hybrids was significantly smaller than that of hawksbills, but not smaller than loggerheads (which also had significantly smaller CS than hawksbills). The other covariates for this model [year and day of the nesting season (DONS)] were nonsignificant with respect to CS (Table 2 and Fig. 2).

For emergence success (ES), the GAMs showed that hybrids had a significantly lower ES than either hawksbills or loggerheads, and hawksbills had a significantly lower ES than loggerheads. CCL did not have significant effect on ES, but year, DONS and incubation period (IP) did (Table 2 and Fig. 3). Hatchling production (HP) was not significantly different among any of the groups. The only covariate with a significant influence on HP was DONS (Table 2).

Incubation period (IP) was significantly different among the three groups. Loggerheads had the shortest IP, followed by hybrids and hawksbills. DONS had a significant effect on IP, but year and CS did not (Table 2 and Fig. 4).

The GAM analyses for observed clutch frequency (OCF) showed that hawksbills and loggerheads were significantly different from each other but not from hybrids. The difference between hawksbills and loggerheads is a result of body size; when CCL is added to the model, the species difference between hawksbills and loggerheads disappears. The other two covariates—year and CS—had no effect on OCF. Observed breeding frequency (OBF) showed no effect of groups, indicating that hawksbills, loggerheads, and hybrids were not significantly different for this reproductive parameter (Table 2).

Discussion

Comparison of reproductive parameters

We had a rare opportunity to evaluate and compare the reproductive output of sea turtle hybrids and their parental species. Hybrids had the largest body size (CCL). This

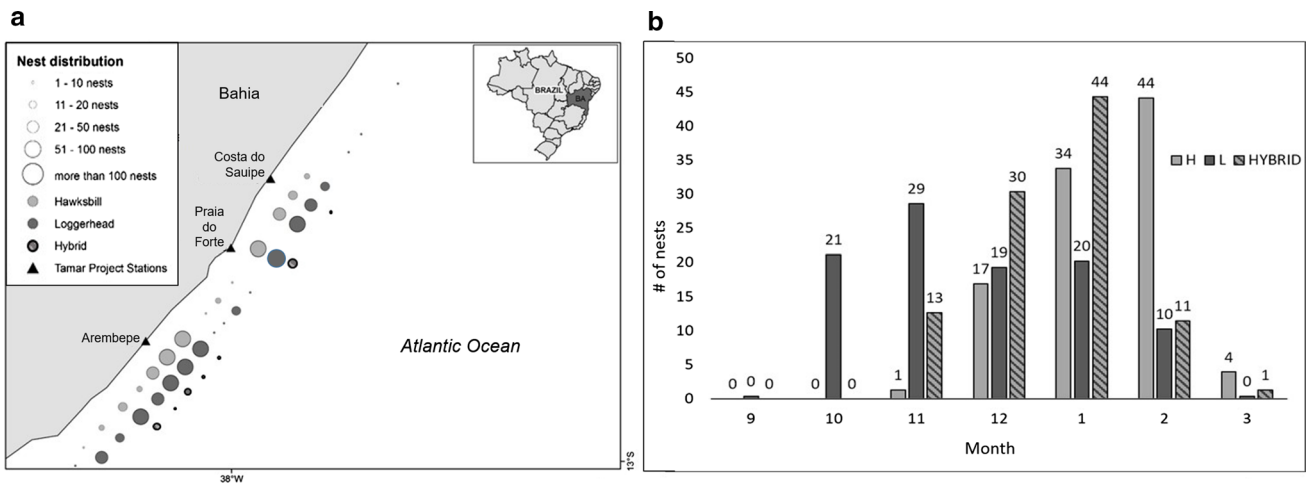

Fig. 1 Spatial distribution of morphologically assigned hawksbills and loggerheads and genetically assigned hybrids (**a**) and temporal distribution (**b**) of genetically assigned hawksbills (H—*light gray bars*), loggerheads (L—*dark gray bars*), and hybrids (*light*/*dark gray* diagonal pattern *bars*)

Table 1 Summary statistics for morphological and reproductive parameters

		Hawksbill	Loggerhead	Hybrid
CCL (cm)	# of females	30	79	33
	Mean (SD)	92.3 (4.8)	99.8 (4.8)	103.7 (4.4)
	Range	78–100	90–114	98–113
CS (# eggs)	# of clutches	77	322	79
	Mean (SD)	136.0 (25.5)	121.9 (25.7)	137.2 (26.9)
	Range	44–189	44–189	53–187
ES (%)	# of clutches	66	269	56
	Mean (SD)	51.1 (24.2)	62.5 (22.9)	52.2 (22.2)
	Range	2.2–99	0–98.8	5.5–89.3
IP (# days)	# of clutches	63	247	56
	Mean (SD)	55.3 (3.2)	50.7 (3.2)	53. 4 (3.2)
	Range	48–65	46–65	48–63
HP (# individuals/clutch)	# of clutches	66	269	56
	Mean (SD)	69 (34)	75.7 (31.8)	68.7 (5)
	Range	2–139	0–152	9–131
OCF (# of clutches/year)	# of annual OCF records	33	136	19
	Mean (SD)	1.7 (0.9)	2.2(1.3)	1.8 (1.2)
	Range	1–4	1–6	1–5
OBF (# of clutches—2008/2014)	# of females	21	78	11
	Mean (SD)	1.6 (0.7)	1.7 (1.0)	1.7 (0.7)
	Range	1–3	1–6	1–3

Female *CCL* curved carapace length, *CS* clutch size, *ES* emergence success, *IP* incubation period, *HP* hatchling production, *OCF* observed clutch frequency, *OAP* observed annual production, *OBF* observed breeding frequency, *OTP* observed total production

size difference could result from hybrids taking longer to become reproductively mature, or, if maturing at the same age, having faster growth rates than the parental species (Bjorndal et al. 2013).

For individual clutch measures—clutch size (CS), incubation period (IP), emergence success (ES), and hatchling production (HP)—hybrids had intermediate values between hawksbills and loggerheads for CS and IP. Hybrids had the lowest ES values, but all groups had equal HP. When we integrated reproductive output over a nesting season, the three groups had equivalent observed clutch frequency (OCF). Thus, all groups should have similar annual hatchling production, given their equal HP and OCF. When we integrated reproductive output over a 7-year period, observed breeding frequency (OBF) was equivalent among the three groups. Thus, again, the total hatchling production for this time frame should not differ among the three groups.

In our analyses, we first compared hybrids and their parental species without CCL as a covariate. This was important to evaluate how each reproductive parameter is influenced by the species alone, on an individual level. We then added CCL to the models to evaluate the extent to which size accounts for differences in reproductive parameters among the three groups. Female body size, clutch size, and egg size are inter-related because the volume of eggs in a clutch is constrained by the volume within the hard shell of the female (Ehrhart 1995). In our study, when CCL was accounted for, hybrids had smaller clutch sizes compared to hawksbills, and both had larger clutches than loggerheads. These differences in clutch size may be a result of egg size or differences in the resources available to invest in clutch size among the groups. Egg size values reported for other hawksbill and loggerhead populations (Van Buskirk and Crowder 1994) indicate that hawksbills eggs are significantly smaller than loggerhead eggs. Unfortunately, we do not have egg size data for our populations. Data on egg size in all three groups should be collected in the future.

Nesting temporal distribution (Fig. 1b) shows that loggerhead females nest earlier than both hybrids and hawksbills. This corroborates Vilaça et al. (2012) explanation of the directionality of the cross observed in F1 individuals. Most hybrids have hawksbill morphology but loggerhead mtDNA, likely the result of early arriving hawksbill males having an opportunity to mate with loggerhead females whereas most loggerhead males have already left the breeding grounds by the time the hawksbill females arrive.

Hybrids have an intermediate incubation period. Incubation period is affected by the time within the nesting season when the eggs are deposited because of changes

Table 2 Summary results for final GAM models for each response variable and covariates

Response variable	Residual df	Covariates								R^2	Comparison among groups
		CCL	*Groups*			*Year*	*DONS*	*IP*	*CS*		
			H × L	H × HYBRID	L × HYBRID						
CCL	139	–	**<0.001**	**<0.001**	**<0.001**	–	–	–	–	0.41	H < L < HYBRID
CS	457	**<0.001**	**<0.001**	**<0.001**	**<0.001**	NS	NS	–	–	0.32	L < HYBRID < H
ES	350	NS	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	–	0.15	HYBRID < H < L
HP	384	–	NS	NS	NS	NS	**<0.001**	–	–	0.11	H = L = HYBRID
IP	359	–	**<0.001**	**<0.001**	**<0.001**	NS*	**<0.001**	–	NS*	0.5	L < HYBRID < H
OCF	169	NS	**<0.05**	NS	NS	NS	–	–	NS	0.28	H < L; H = HYBRID; L = HYBRID
OBF	95	–	NS	NS	NS	–	–	–	–	0.12	H = L = HYBRID

NS are nonsignificant values for covariates not in the final GAM model. *NS** are nonsignificant values for covariates that were maintained in the final model based on analyses of deviance. Dashes represent covariates not tested for the response variable. *CCL* curved carapace length, *CS* clutch size, *ES* emergence success, *HP* hatchling production, *IP* incubation period, *OCF* observed clutch frequency, *OBF* observed breeding frequency, *DONS* day of the nesting season, *H* hawksbills, and *L* loggerheads

Significant *p* values are presented

Bold values indicate the covariates that are significant on each response variable

in temperature over the season. Hybrids have a temporal nesting distribution that overlaps with both parental species (Fig. 1b). Incubation period is an indirect method to estimate sex ratios in hatchling sea turtles (Glen and Mrosovsky 2004). Sex ratios of hawksbills (Godfrey et al. 1999; Marcovaldi et al. 2014) and loggerheads (Marcovaldi et al. 1997) in Bahia are very female biased (>90%). Based on these findings and the incubation periods for hybrids, we predict that hybrid hatchlings are also female biased. Studies should be conducted to confirm this prediction.

Emergence success was lowest for hybrids, suggesting lower embryonic viability that could lead to lower reproductive output. This difference in ES is not a result of differential ability of hatchlings to escape their nest because the difference between ES and hatching success (the proportion of eggs that produced live hatchlings inside the nest) is the same for all groups. However, HP per clutch was similar among all groups. Therefore, when reproductive output is integrated over time (either for a nesting season or for a 7-year period), hybrid reproductive output would not be different from those of the parental species. Thus, based on the parameters of reproductive output that we measured, hybrids are apparently not at a reproductive advantage or disadvantage over the parental species.

Comparison of sea turtle hybrids with other hybrid groups

There are few quantitative studies of wild hybrids that address reproductive success with which we can compare our results. Hoffman et al. (1978) showed that gull hybrids (*Laurus glaucescens* × *L. occidentalis*) had greater reproductive success than their parental species. Pairs composed of conspecifics had smaller clutch size (CS) and hatchling success than those pairs composed of hybrids or by a hybrid and one of the parental types. Flockhart and Wiebe (2009) found no difference in reproductive success (clutch size and hatching success) among two northern flicker subspecies (*Colaptes auratus auratus and C. a. cafer*) and their hybrids. These findings differ from the results for the turtle hybrids given that hawksbill individuals had the largest clutch sizes, and hybrids in our study had the lowest emergence success. Alatalo et al. (1982) reported that offspring production of collared and pied flycatcher hybrids (*Ficedula albicollis* × *F. hypoleuca*) was intermediate between the parental species, whereas there was no difference in hatchling production of sea turtle hybrids in our study.

Endangered Florida panthers *(Puma concolor cory* and *P. c. stanleyana)* were bred with Texas panthers in an attempt to increase genetic diversity in a very inbred and depleted population. The resulting hybrids have been studied by several researchers who showed that hybrids

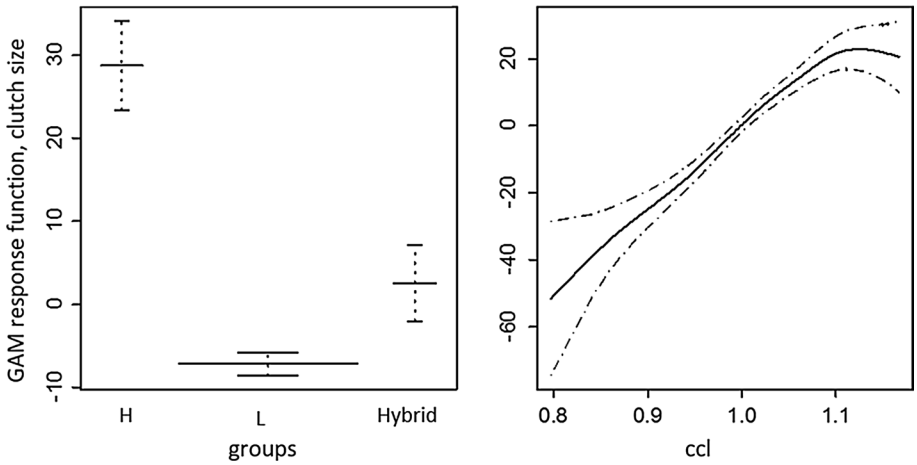

Fig. 2 Graphical summary of the general additive model (GAM) analysis of clutch size covariates of: groups and curved carapace length (measured in meters) (CCL). The response variable (clutch size) is shown on the y-axis as a centered smoothed function scale to ensure valid pointwise 95% confidence bands. Solid lines are the cubic smoothing spline fits for each covariate conditioned on all other covariates in the analysis (Table 2). Dashed lines are pointwise 95% confidence lines around the fits

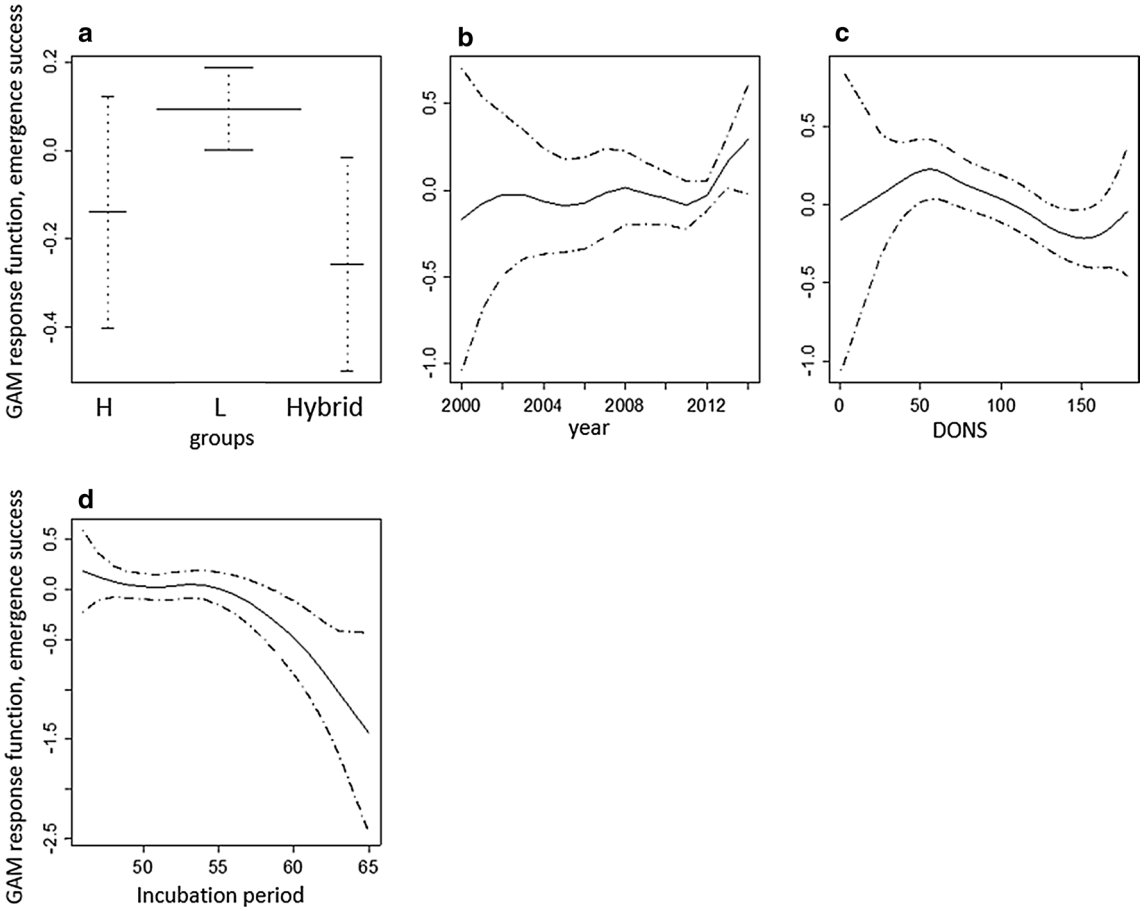

Fig. 3 Graphical summary of the general additive model (GAM) analysis of emergence success covariates of: **a** groups, **b** year, **c** day of nesting season (DONS), and **d** incubation period. The response variable (emergence success) is shown on the y-axis as a centered smoothed function scale to ensure valid pointwise 95% confidence bands (**b**, **c**, **d**). *Solid lines* are the cubic smoothing spline fits for each covariate conditioned on all other covariates in the analysis (Table 2). *Dashed lines* are pointwise 95% confidence lines around the fits

had larger litter sizes (Ortego et al. 2007), greater life time reproductive success (Slate et al. 2000; Ferreira and Amos 2006), and increased age-specific survival probabilities (Hostetler et al. 2010; Benson et al. 2011). However, in later research with a larger sample size, more detailed ancestry categories and a novel method for modeling litter size Hostetler et al. (2012) concluded that the hybrids had no advantage over parental types.

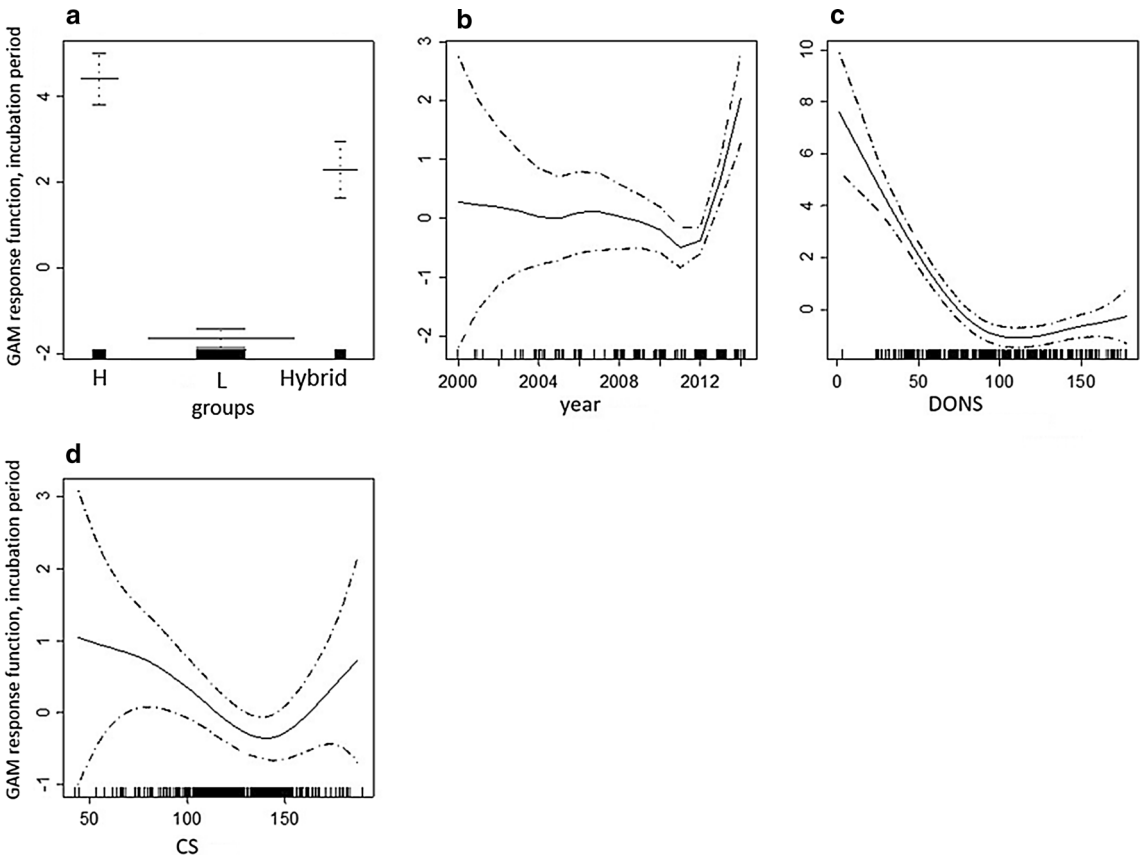

Fig. 4 Graphical summary of the general additive model (GAM) analysis of incubation period covariates of: **a** groups, **b** year, **c** day of nesting season (DONS), and **d** clutch size (CS). The response variable (incubation period) is shown on the y-axis as a centered smoothed function scale to ensure valid pointwise 95% confidence bands (b, c, d). *Solid lines* are the cubic smoothing spline fits for each covariate conditioned on all other covariates in the analysis (Table 2). *Dashed lines* are pointwise 95% confidence lines around the fits

Although not all reproductive parameters are comparable among these studies and ours, reproductive output of hybrids in various taxa varies greatly (Arnold and Hodges 1995). As demonstrated by the various studies cited in the previous two paragraphs, hybridization can cause infertility or lower viability, can result in higher reproductive outcomes with hybrid vigor, or can have no impact. The first two results would tend to destabilize hybrid zones, whereas the last would tend to stabilize hybrid zones.

Conclusions and future research

Our study shows for the first time the effect of hybridization on sea turtle reproductive output. Our results indicate that hybrid individuals exhibit neither hybrid vigor nor evidence of hybrid breakdown in those measures of reproductive output that we evaluated. Thus, hybrids in this area could persist. Sea turtles have a slow rate of molecular evolution (Avise et al. 1992), have the same karyotype (Bickham 1981) and as shown in this manuscript, can interbreed despite the millions of years of isolation. Loggerheads and hawksbills diverged 10–20 MY (Zangerl 1980; Naro-Maciel et al. 2008). These traits may help to explain why speciation has been rare in their evolutionary history. This hybridization phenomenon observed in Brazil could be of evolutionary importance and requires long term monitoring of this population.

Sea turtles take at least 30 years to reach reproductive maturity, so in this study we have only evaluated the historic hybridization process. Therefore, the question remains; given that both parental species have increased in abundance over the last several decades, is hybridization continuing? A study of contemporary hybridization through analysis of hatchling genotypes is needed. Furthermore, sea turtles can have multiple paternity within a clutch of eggs (Bowen and Karl 2007). Therefore, the effect of the direction of hybridization (i.e., the species identity of sire and dam) on hatchling survivorship should be evaluated.

Demographic parameters (e.g., survivorship, growth rates, age to maturity) of the parental species and hybrids should be compared for different life stages to see if hybrids are at an advantage or disadvantage. Hybrid

juveniles are found on the foraging areas of juvenile loggerheads along the south coast of Brazil and in Argentina waters, but have not been found on juvenile hawksbill foraging grounds (Vilaça et al. 2013; Proietti et al. 2014a, b; Prosdocimi et al. 2014). Thus, these groups are exposed to different selective pressures in different areas throughout their life stages. These demographic parameters are critical for evaluating the persistence of hybrids in these populations and should be a focus of future research.

Hybridization has important implications for the study of ecology, behavior, and demography of sea turtles in Brazil. Based on our results, before morphologically assigned animals are included in such studies, they should be screened genetically to exclude hybrids. This is particularly important for hawksbills, where >50% of the morphologically assigned individuals were hybrids. We suggest that other sea turtle populations worldwide be screened for hybrids with appropriate genetic markers, particularly in regions where multiple sea turtle species overlap spatially and temporally during their breeding activities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the University of Florida and Projeto TAMAR-ICMBio at which the studies were conducted. This research was approved by the Institutional Animal Care and Use Committees at the University of Florida (201101985) and conducted under SISBIO permit 28938-3 from the Brazilian Ministry of the Environment. Samples were exported under CITES permit 13BR010456/DF and were imported into the USA under CITES permits 13US724540/9 (Archie Carr Center for Sea Turtle Research).

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