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Peter Dutton[1] et al.

[1] NOAA-Fisheries, Southwest Fisheries Science Center, La Jolla, USA.

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Status and Genetic Structure of Nesting Populations of Leatherback Turtles (*Dermochelys coriacea*) in the Western Pacific

Peter H. Dutton[1], Creusa Hitipeuw[2], Mohammad Zein[3], Scott R. Benson[1], George Petro[4], John Pita[5], Vagi Rei[6], Levi Ambio[7], and Jacob Bakarbessy[8]

[1]*NOAA-Fisheries, Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, La Jolla, California 92037 USA [peter.dutton@noaa.gov];*
[2]*World Wide Fund for Nature-Indonesia, Jl. Mega Kuningan Lot 8-9/A9, Maga Kuningan Jakarta, Indonesia [chitipeuw@wwf.or.id];*
[3]*Pusat Penelitian dan Pengembangan Biologi, Lembaga Ilmu dan Pengetahuan Indonesia (LIPI)-Cibinong, Bogor, Indonesia;*
[4]*Wan Smolbag Theatre, Port Vila, Vanuatu, PO Box 1024, Port Vila, Vanuatu [gpetroster@gmail.com];*
[5]*Ministry of Natural Resources, Dept. Forestry, Environment and Conservation, PO Box G24, Honiara, Solomon Islands;*
[6]*Office of Environment and Conservation, PO Box 6601, Boroko, National Capital District, Papua New Guinea;*
[7]*Kamiali Integrated Conservation Development Group, PO Box 3339, Lae, Morobe Province, Papua New Guinea [kicdg@global.net];*
[8]*KSDA II Papua, Forestry Department, Jl. Sorong, Indonesia*

Abstract. – A group of researchers, managers, and tribal leaders with extensive local knowledge from Papua New Guinea, Solomon Islands, Vanuatu, and Papua, Indonesia, provided new information on the status of leatherback nesting populations in the western Pacific Ocean. Twenty-eight nesting sites were identified, of which 21 were previously unknown or poorly described. Although data are still incomplete, we estimate a total of ca. 5000–9200 nests currently laid each year among these 28 sites, with approximately 75% of this nesting activity concentrated at 4 sites along the northwest coast (Bird's Head Peninsula) of Papua, Indonesia. Genetic analysis by using mitochondrial deoxyribonucleic acid sequences identified a total of 6 haplotypes among the 106 samples analyzed for Solomon Islands, Papua, and Papua New Guinea, including a unique common haplotype that is only found in these western Pacific populations. There was no significant difference in haplotype frequencies among these rookeries, which suggests that they represent a metapopulation composed of a single genetic stock. Further work is needed to define the demographic structure within this metapopulation.

Key Words. – Reptilia; Testudines; Dermochelyidae; *Dermochelys coriacea*; sea turtles; threats; conservation; stock structure; mtDNA; western Pacific; Papua; Indonesia; Papua New Guinea; Solomon Islands; Vanuatu

There has been great uncertainty over the status of leatherback turtles (*Dermochelys coriacea*) in the western Pacific Ocean. Aerial surveys of the entire coast of Mexico and Central America carried out annually since 1995 identified all the nesting sites in the eastern Pacific (Sarti-Martínez et al. 2007). In addition, consistent monitoring at the major rookeries in Mexico and Costa Rica yielded reliable estimates of annual nesting abundance and confirmed the severe decline of these eastern Pacific stocks (Spotila et al. 1996, 2000; Sarti et al. 1996; Sarti-Martínez 2002). The catastrophic decline of the rookery at Terengganu, Malaysia, is also well documented (Chan and Liew 1996). This population plummeted from over 3000 nesters per year in the late 1960s to less than 20 per year by 1993. In the last decade, only 2 or 3 leatherbacks nested each year (Liew 2002). The only large population of leatherbacks that was identified in the western Pacific is the rookery at Jamursba-Medi on the north Vogelkop coast (also known as Bird's Head Peninsula) in Papua, Indonesia, where ca. 300–900 females nest annually (Hitipeuw et al. 2007). These estimates are similar to those in the 1990s (Spotila et al. 1996), although it appears that there has been a long-term decline since the 1970s (Hitipeuw et al. 2007). However, monitoring has not been consistent, and recent surveys indicate a sizable rookery at Wermon, adjacent to Jamursba-Medi, that may have been overlooked (Hitipeuw et al. 2007). Little is known about the location, abundance and trends of other rookeries in the western Pacific. Although leatherbacks have been reported to nest in the Solomon Islands, Papua New Guinea (PNG), New Ireland, New Britain and Vanuatu (Spring 1982; Quinn and Kojis 1985; Bedding and Lockhart 1989; Hirth et al. 1993; Petro et al. 2007; Fig. 1), the population stock structure and sizes of these rookeries are unknown. The most recent global population assessment estimated the total nesting abundance of leatherbacks in the western Pacific from limited published reports as 700 females nesting annually (Spotila et al. 1996). Because many of the rookeries were not included and the available data were incomplete for this region, this number is certainly an underestimate.

Genetic studies of maternally inherited mitochondrial deoxyribonucleic acid (mtDNA) have been useful in understanding the population structure of leatherbacks

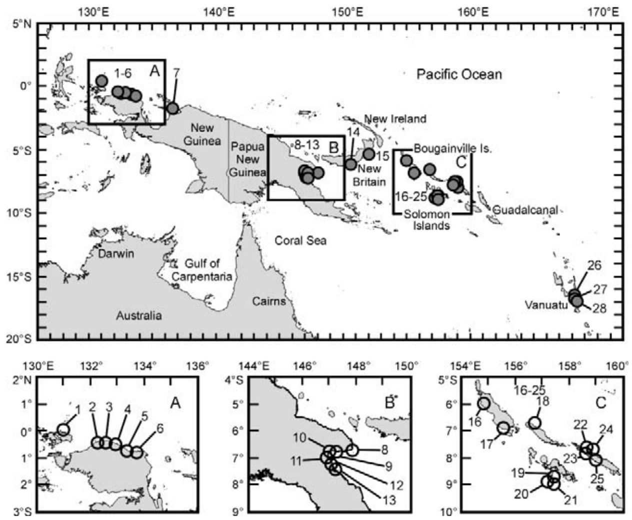

Figure 1. Locations of significant (>20 nests/season) nesting sites for leatherbacks identified in the western Pacific. Names of the nesting sites are given by the corresponding numbers of the locations listed in Table 1.

and in demonstrating the existence of distinguishable stocks for management purposes (Moritz 1994; Dutton et al. 1999). A global survey of leatherbacks identified an eastern Pacific genetic stock, consisting of rookeries in Mexico and Costa Rica, that was distinct from the single rookery sampled in the western Pacific in the Solomon Islands and the Indo-Pacific rookery at Terengganu, Malaysia (Dutton et al. 1999). Because the other regional nesting sites were not sampled in this previous study, the extent of stock structuring among the western Pacific leatherback rookeries remains unknown. An understanding of genetic stock structure is necessary to accurately define management units for conservation (Moritz 1994).

The objective of this paper is to update information on leatherback nesting in the western Pacific by 1) identifying all known leatherback nesting beaches and 2) providing minimum estimates of current rookery sizes. For each of these sites, we assess current threats and indicate where gaps in information exist. Finally, we use mtDNA analysis to examine the degree of genetic population structuring among the key rookeries in the western Pacific. This paper lays the groundwork for future research and describes the geographic extent of the western Pacific leatherback stock.

METHODS

Population Status and Threats. — In May 2004, a working group (WG) convened that included representatives from Papua (Indonesia), PNG, Solomon Islands, and Vanuatu (Kinan 2005). This WG consisted of researchers, managers, and tribal community leaders with extensive local knowledge. The WG reviewed collective knowledge and mapped out nesting sites that were either documented or believed to have more than 20 nests per season. The WG drew on several sources of information from internal reports, gray literature in local languages, field notes, and personal observations to compile a matrix of information on population size and threats (Kinan 2005). Where possible, the WG attempted to estimate the number of nests laid per season at each site. Given the inherent error in these estimates, in addition to the annual variability in numbers of nests laid characteristic of marine turtles, a range was given for numbers of nests laid each year based on observations since 1999. The intent was to provide a minimum estimate for the number of nests laid annually by leatherbacks in the western Pacific as a basis for comparison to the previous estimate reported in Spotila et al. (1996). To make the estimates comparable, we divided the number of nests by 5 to estimate the number of females per year as described in Spotila et al. (1996). This approach makes the assumption that the average number of clutches laid by leatherbacks in the western Pacific is five. However, the number of nests laid per females is unknown for these populations. It is important to emphasize that we are only using this simplistic approach to provide a conservative population estimate for comparative purposes.

Genetic Population Structure. — Skin samples were collected from nesting females or salvaged from dead hatchlings from Jamursba-Medi (Wembrack, Warmamedi, and Batu Rumah; Fig. 1), and Wermon in Papua; from Kamiali in PNG; and from Solomon Islands (Sasakolo; Dutton et al. 1999; Fig. 1). Hatchling samples came from nests after emergence, taking care not to sample more than 1 nest per female. Skin samples were preserved in a 20% dimethyl sulphoxide solution saturated with laboratory grade salt, as described in Dutton et al. (1999). DNA was isolated by using either standard phenol/chloroform extraction techniques (Sambrook et al. 1989) or by using the Fast Prep DNA isolation kit (Bio101®). Amplification of mtDNA was performed by polymerase chain reaction (PCR) by using the primers HDCM2 and LTCM2, designed to target 496 bp at the 5′ end of the control region of the mitochondrial genome (see Dutton et al. 1999). Template DNAs were amplified in 50 μL PCR reactions on a Perkin Elmer 480 thermocycler by using the following profile: initial denaturation at 94°C for 2 minutes, followed by 36 cycles of 1) DNA denaturing at 94°C for 50 seconds, 2) primer annealing at 52°C for 2 minutes, and 3) primer extension at 72°C for 1 minute 30 seconds, concluding with a final primer extension for 5 minutes at 72°C. The size of the amplified products were determined by using electrophoresis in a 2% agarose gel stained with ethidium bromide. PCR products were then purified by using the Qiaquick PCR Purification Kit (Qiagen, Valencia, CA) and stored at 4°C. Direct cycle sequencing reactions of the light strand were performed on 2 μL purified PCR product combined with 2 μL ABI Prism dRhodamine Terminator Cycle Sequencing Kit, 3 μL primer LTCM2, and 5 μL purified water. The labeled extension products were then purified via ethanol precipitation and analyzed with an Applied Biosystems model 377 or 310 genetic analyzer. The sequences were edited by using Gene Codes Sequencher 4.1. Haplotype frequencies were compared between the sampled nesting sites at PNG and Papua, and with published data from Solomon Islands and Malaysia (Dutton et al. 1999) by using a χ^2 test to determine genetic homogeneity. Monte Carlo procedures were used to correct the probabilities obtained by the χ^2 test for multiple tests and small samples (Roff and Bentzen 1989).

RESULTS AND DISCUSSION

Nesting Distribution and Abundance. — Several previously undescribed nesting sites were identified. In most cases, there was local knowledge of leatherback nesting; however, no census work was carried out, and the number of nests could not be estimated (Table 1, Fig. 1). We estimate an approximate total of 5000–9100 leatherback nests are laid each year among all the beaches identified in the western Pacific (Table 1). The rookery at Papua, Indonesia, remains the largest and best studied (Hitipeuw et al. 2007), with 3 beaches at Jamursba-Medi containing the bulk of the nesting. However, there are several sites along the northwest coast of Papua that had historic reports of high-density nesting, where the status is now unclear. An aerial survey conducted in July 2005 only detected relatively low densities of nests along the stretch of coastline northwestward from Jamursba-Medi to Sorong (S.R. Benson, unpubl. data). In addition, Wermon appears to have much greater nesting activity than previously thought (Hitipeuw et al. 2007). Little is known about the vast, isolated coastline stretching southeastwards from these beaches to the border of PNG (Fig. 1), thereby underscoring the need for aerial surveys to identify unknown leatherback nesting sites along the coastline from the Bird's Head (Vogelkop) Peninsula of Papua to the PNG border. In PNG, there is scattered nesting along the north coast and around the Island of New Britain and Bougainville (Table 1; Fig. 1). This has been confirmed by recent aerial surveys (Benson et al. 2007) that identified Buang-Buasi and Kamiali as 2 areas of higher-density nesting. We recommend establishing these 2 areas as index sites for long-term monitoring to determine nesting abundance trends in PNG. The other sites listed for PNG in Table 1 can essentially be considered beaches that are used by the same nesting population that nests in the Huon Gulf. The estimates of total nests laid annually at all the sites in the Huon Gulf range from 500 to 1150 (Table 1). This range reflects the annual variability in nests and is based on preliminary data from 3 years of aerial surveys (S.R. Benson and V. Rei, unpubl. data). There was sporadic monitoring of nesting within a 4-km stretch of beach demarcated at the Kamiali Wildlife Management Area (KWMA), and 40–89 nesters were tagged annually between 1999 and 2005 (Kisakao 2004; Kinan 2005; Benson et al. 2007; N. Pilcher, unpubl. data). Nest counts from this effort underestimate the total nesting activity for PNG and the Huon Gulf, and we consider the numbers provided in Table 1 from the aerial surveys as the most reliable estimates currently available. More recently, beach monitoring was initiated at the sites in Labu-Tale, Buang-Buassi, and Paiawa (Table 1; N. Pilcher, *pers. comm.*), and it is hoped that this expanded monitoring will provide more accurate estimates of annual abundance in the future.

Satellite telemetry suggests that nesters tagged at Kamiali tend to lay subsequent clutches in the same season at other sites, as well as within and adjacent to the KWMA. There is also an indication of movement by nesters between Kamiali and Bougainville (Benson et al. 2007). However, Bougainville is closer to the Solomon Islands than the Huon Gulf, and it is possible that some leatherbacks nesting in the Solomon Islands tend to also lay clutches on Bougainville. This would account for the variability in nest counts reported for Bougainville (Table 1), however, the extent of within-season movement between nesting sites is unknown. The Solomon Islands are more important than previously thought, with scattered nesting reported from several sites in the western province of Isabel (Table 1; Fig. 1). Surveys have been incomplete, and it is likely that nesting activity described in Table 1 underestimates the true population size. Vaughan (1981) reported leatherback nesting at 61 beaches in Isabel and the western province but many with just a few scattered nests. In 1996, 40 leatherbacks were sampled over a 7-day period for the genetic study at Sasakolo (Dutton et al. 1999; D. Broderick, *pers. comm.*). Given the scattered nesting around the islands in the region, it is possible that the size of this nesting population is on the order of hundreds, rather than tens of females. Extensive aerial surveys in conjunction with complete monitoring and tagging at multiple sites should be a priority for a more accurate assessment of leatherback population abundance in the region.

By using the numbers given in Table 1, we estimated a total of 1113 females nesting annually (FNA) following methods reported in Spotila et al. (1996). This number might be larger, because there are still areas where undocumented nesting occurs throughout the Island of New Guinea and beyond, such as in Thailand and Vietnam. This minimum estimate is larger than that of Spotila et al. (1996) who estimated 700 females for the western Pacific rookeries. Use of these new estimates would have produced a regional population estimate of 2782 breeding females in the western Pacific by applying the same simplified methods Spotila et al. (1996) used (multiplying FNA by 2.5) to derive their 1996 estimates for the regional populations. This is likely a conservative estimate and depends on the assumption that the average number of nests laid per female is 5 (Spotila et al. 1996). If leatherbacks lay fewer nests on average then the estimated number of females derived from the nest counts will be greater and vice versa, so that estimates would range from 2100–5700 females based on estimates ranging from ca. 840-3200 FNA (Table 2). This illustrates the problem of drawing conclusions on population status from estimates

Table 1. Western Pacific leatherback (*Dermochelys coriacea*) nesting sites identified as having more than 20 nests annually.[a]

	Nesting season by month												
Beach	J	F	M	A	M	J	J	A	S	O	N	D	No. nests
PAPUA													
1. Raja Ampat								x	x				?
2. Jamursba-Medi	X	x	X	X	X	XX	XX	XX	X	X	x	x	1865–3601[b]
3. Wermon	XX	XX	X	X	x	x	x	x	x	x	X	XX	1508–2760[b]
4. Mubrani-Kaironi													20–25[c]
5–6. Sidey-Wibain													20–25[c]
7. Yapen Isl.													?
PNG													
Huon Gulf	XX	XX	X	x						x	X	XX	500–1150[d]
8. Finschhafen													
9. Bukaua													
10. Labu-Tale													
11. Buang Buassi													
12. Kamiali													
13. Paiawa													
New Britain	XX	XX	X	x						x	X	XX	140–260[d]
14. Fulleborn													
15. Korapun													
Bougainville	XX	XX	X	x						x	X	XX	160–415[d]
16. Empress Augusta Bay													
17. Tokuaka													
SOLOMON ISLANDS													
Choisel	XX	XX	X	x						x	X	XX	50[e]
18.Vachu River													
Western Province	XX	XX	X	x						x	X	XX	123[e]
19. Baniata													
20. Havila													
21. Quero													
Isabel	XX	XX	X	x						x	X	XX	640–717[e]
22. Rakata Bay													
23. Sasakolo													
24. Lilika													
25. Katova Bay													
VANUATU	XX	XX	X	x						x	X	XX	31–50[f]
26. Malakula													
27. Votlo													
28. Southern Epi													
TOTAL													5067–9176

[a] Location of sites in Fig. 1 indicated by number in column 1. Nesting beaches are grouped regionally to identify geographic areas that are believed to be part of the same population. The nesting season is shown for each of these regional populations, with relative level of activity indicated by symbols (XX = peak; X = moderate; x = low). The current estimated number of nests laid each year is based on information since 1999.
[b] Hitipeuw et al. 2007; H. Suganuma, *pers. comm.*
[c] Locals report at least 20–25 nests per year.
[d] Benson et al. 2007, unpubl. data; Kisakao 2004; I. Kinan unpubl. data.
[e] Pita 2005; Pita and Ramohia, unpubl. data.
[f] Petro et al. 2007.

Table 2. Estimates of minimum females nesting annually (bold) and total number breeding females (*italics*)[a] in the western Pacific leatherback metapopulation by assuming 4, 5, or 6 nests laid on average by each nester per season. Estimates based on upper and lower range of nests given in Table 1.[b]

No. nests/female	5067 nests	9176 nests
4	**1266**	**3294**
	3165	*5735*
5	**1113**	**1835**
	2782	*4587*
6	**844**	**1529**
	2110	*3822*

[a] NBF = FNA × 2.5 as described in Spotila et al. (1996).
[b] FNA = females nesting annually; NBF = number breeding females.

of numbers of nesters derived from nest counts and the importance of further research to determine other demographic parameters, such as numbers of nests laid per female and the extent of movement between nesting beaches. Given the high level of uncertainty, we suggest evaluating the population size in terms of numbers of nests, rather than attempting to derive numbers of females.

Genetic Population Structure. — A total of 6 haplotypes were identified among the 106 samples analyzed for Solomon Islands, Papua, and PNG (Table 3). All of these haplotypes were previously identified and described in Dutton et al. (1999). Haplotype I was the most common haplotype in all the rookeries sampled. There was no significant difference in haplotype frequencies among

Table 3. mtDNA haplotype frequencies at major rookeries in the western and Indo-Pacific.[a]

	Haplotype					
Rookery	A	D	E	F	H	I
PNG-Kamiali	1	1	1			15
Papua-Jamursba-Medi	1	1	1			28
Papua-War Mon						9
Solomon Islands[b]	2	1	1	1	3	40
Malaysia-Terengganu[b]	3	2	3		1	

[a] mtDNA = mitochrondrial deoxyribonucleic acid.
[b] Data for Terengganu and Solomon Islands from Dutton et al. (1999).

all 4 populations sampled ($\chi^2 = 7.363$, $p = 0.972$). Given this lack of differentiation, further statistical analysis was not pursued. The lack of genetic differentiation between Papua, PNG, and Solomon Islands leatherback rookeries could indicate ongoing gene flow among this metapopulation or could also be the result of the inability of these mtDNA markers to resolve fine-scale population subdivisions (Dutton et al. 1999). Further analysis by using multiple nuclear markers (microsatellites) may help resolve this (Dutton et al. 2000). The demographic connection between PNG and Solomon Islands is plausible, given recent telemetry data that indicate leatherbacks tagged at Kamiali (PNG) may also nest on Bougainville during the same season. Further tagging and telemetry studies will help resolve this.

The common "I" haplotype for the western Pacific rookeries in our survey was not found in the eastern Pacific stock (Costa Rica and Mexico; Dutton et al. 1999), nor was it found in the limited sample from Malaysia (Dutton et al. 1999; Table 3). This haplotype, therefore, can be used to identify individuals of western Pacific stock origin encountered at foraging areas and in fisheries by-catch (Dutton et al. 2000, 2006). The published haplotype frequencies for Terengganu, Malaysia, were significantly different from the 4 western Pacific rookeries from this study ($\chi^2 = 49.346$, $p = 0.002$), indicating that this Indo-Pacific stock was distinct from the western Pacific stock that consisted of the rookeries reported in this study.

Conclusion and Recommendations

Our results show that the western Pacific harbors some of the last remaining nesting aggregations of significant size in the Pacific and that this metapopulation may consist of approximately 1100–1800 FNA or a total of 2700–4500 breeding females when following methods reported in Spotila et al. (1996). This is greater than the previous estimate of 700–750 FNA and 1775–1900 breeding females, because it includes information on additional nesting sites that were not previously available and is not an indication of actual abundance or trend. These numbers should not be used for management purposes, because the numbers of FNA are derived from nest counts, and reliable data on the number of nests per female for this regional population are not available. In addition, there is an unknown degree of uncertainty associated with the nest counts themselves. A regionwide aerial survey, repeated periodically throughout the year, would be the most effective way to rapidly identify nesting sites and provide a basis to plan follow-up ground monitoring. Once monitoring projects are established, it will be important to further define management units (Dutton et al. 2002) by using a combination of approaches, including:

1. expanded genetic sampling and analysis with multiple nuclear markers (Dutton 1996);
2. simultaneous tag-recapture studies at multiple beaches throughout the region, with a focus on new sites in the Solomon Islands, Vanuatu, and PNG; and
3. telemetry studies to determine internesting movement and the extent of nesting areas used by individual females.

The preliminary genetic results presented here suggest that leatherbacks nesting in the western Pacific consist of a metapopulation made up of small aggregations scattered throughout the region, with a dense focal point on the northwest coast of Papua, all belonging to a single genetic stock. The degree to which the aggregations nesting at these different areas are demographically independent remains to be determined, because mtDNA data alone are most likely insufficient for determining fine-scale population structure in leatherbacks (Dutton et al. 1999).

Although the numbers of leatherbacks nesting at these western Pacific rookeries are larger than previously reported, there are some indications of a long-term decline (see Hitipeuw et al. 2007). Although large-scale egg harvest has been eliminated at key rookeries like Jamursba-Medi and conservation efforts to reduce subsistence harvest of eggs is underway in PNG (Kisokao 2004; Kinan 2005; Senego 2005; N. Pilcher, *pers. comm.*), predation and natural beach erosion continue to suppress hatchling production throughout the region (Suganuma 2005; Tapilatu and Tiwari 2007). In addition, competing economic activities, such as logging and mining, threaten to compromise the nesting habitat vital to sustain these populations. There are opportunities to immediately enact conservation measures through community-based initiatives on the nesting beaches to dramatically increase hatchling production before these populations collapse. This is a priority that has been highlighted in a recent regional action plan for leatherbacks in the Pacific (Worldfish Center 2004), as well as in other regional agreements such as the Indian Ocean and Southeast Asia Memorandum of Understanding, and the Tri-National Agreement signed in September 2006 between Indonesia, PNG, and Solomon Islands to coordinate conservation of leatherbacks. Other threats, like traditional taking of adults and juveniles on foraging areas in the Kei Islands (Suarez and Starbird 1996) and incidental capture on high-seas and coastal fisheries are also of concern and need to be addressed as part of a broad suite of measures to achieve population recovery in the long run (Dutton and Squires, in press). Long-term monitoring at key index sites in Papua, PNG, Solomon Islands, and Vanuatu is crucial for understanding demographics, status, and trend, and for measuring the efficacy of various conservation measures.

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