

Who's your daddy? Using genetics to count male turtles at Sandy Point NWR, St. Croix.



Photo: Jeremy Smith



**Kelly Stewart - The Ocean
Foundation and Marine Mammal and
Turtle Division, NOAA-SWFSC
La Jolla, CA, USA**

**WIDECAST AGM
3.5.2015
Maunabo
Puerto Rico**



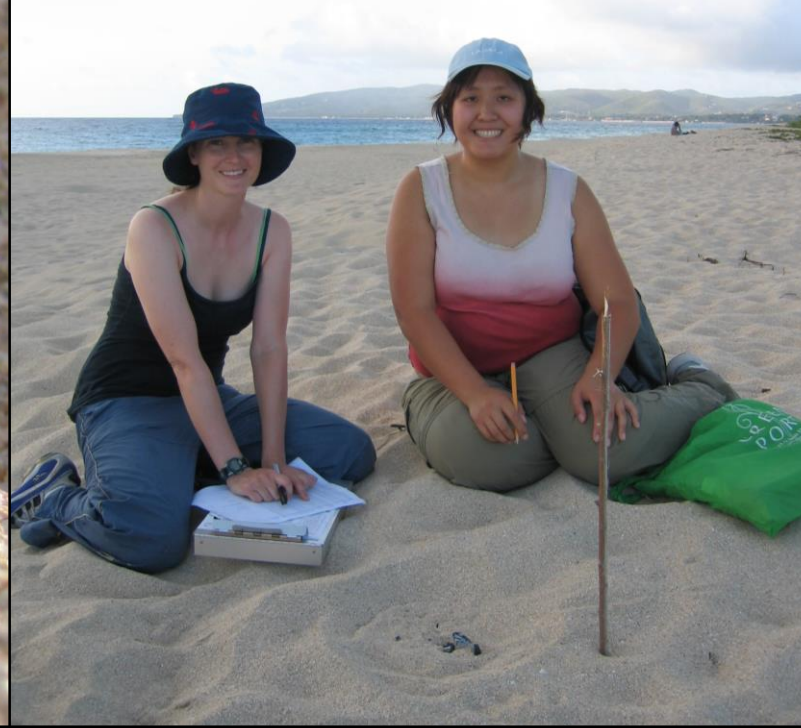
Photo: Jeremy Smith

Individual Identification

1. Genetic tagging (age to maturity, survival rates)
2. Multiple paternity (mating systems, demography)
3. Operational sex ratios (life tables, modeling, life history strategies)
4. Reproductive success (male contributions to populations, gene flow)



Methods



Methods

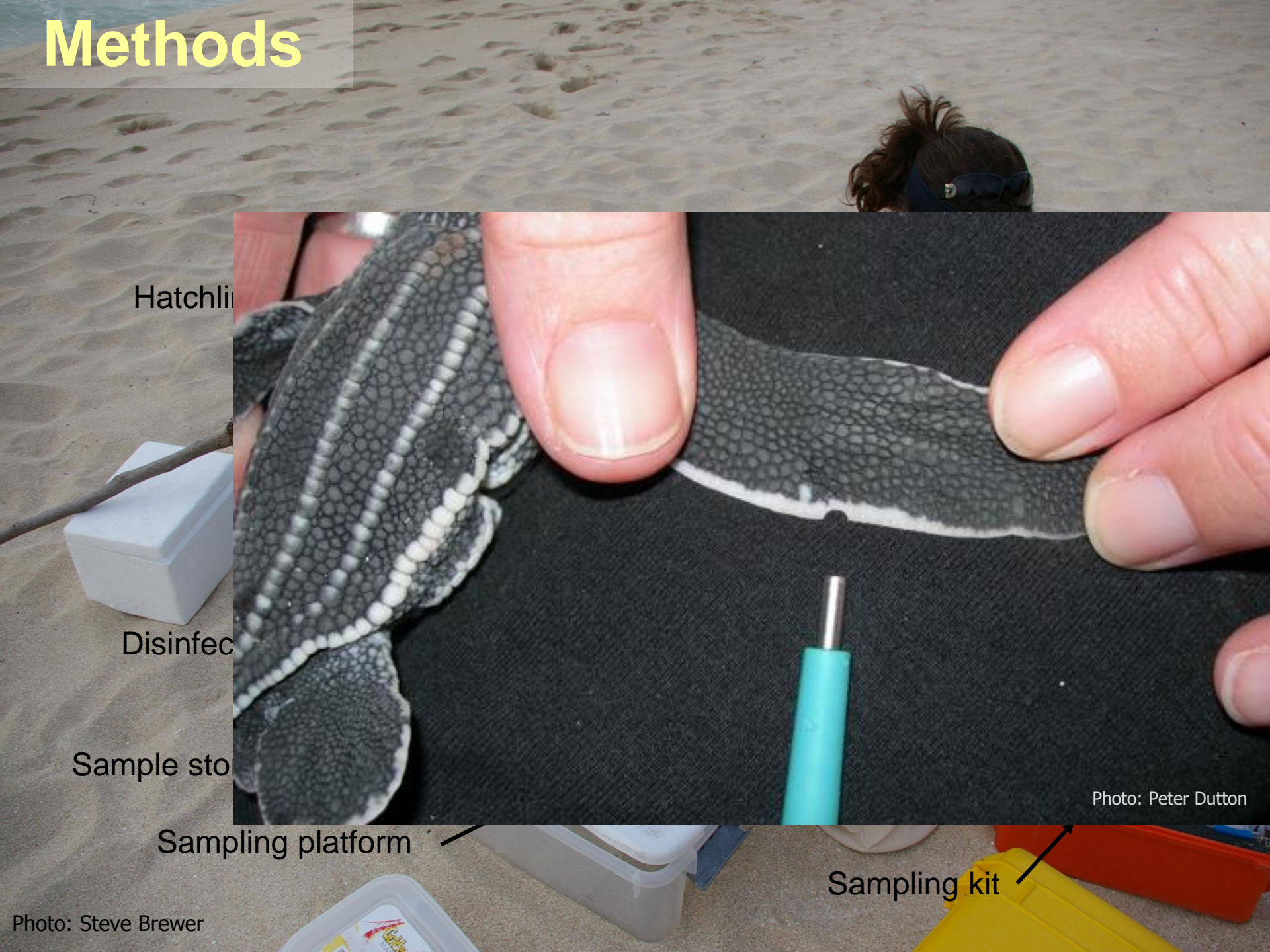


Photo: Peter Dutton

Photo: Steve Brewer

Expected benefits of genetic tagging

30,363 samples

**Age to
maturity?**

Thousands of leatherbacks that already have an identity

~ 5 to 25+ years?

Survival rates



Individual Identification

1. Genetic tagging (age to maturity, survival rates)
2. Multiple paternity (mating systems, demography)
3. Operational sex ratios (life tables, modeling, life history strategies)
4. Reproductive success (male contributions to populations, gene flow)



Microsatellites

nuclear DNA

- contained in all cells in the body, inherited from both parents
- i.e., a locus has 2 alleles (one from each parent)
- specific sequence of alleles at a # of loci = **fingerprint**



Locus	D1	14-5	LB141	LB142	LB145
PPQ311	235.239	216.224	180.194	234.238	145.149

Methods

- 12 females chosen from 122 nesters
- 3 or 4 clutches each (38 nests total)
- 1,019 hatchlings



Paternal Genotype Reconstruction

	D1		5H7		5C8	
Maternal	219	259	224	232	288	288
Hatchling ID						
96401	219	231	232	236	288	292
96402	219	231	232	236	288	292
96403	231	259	232	236	288	292
96404	231	259	208	232	288	292
96405	231	259	208	232	288	292
96406	247	259	232	236	288	292
96407	219	231	208	232	288	292
96408	219	247	208	224	288	292
96409	219	247	224	236	288	292
96410	247	259	208	232	288	292
Paternal	231	247	208	236	292	292

- Sampling the mother is very important.
- Identify maternal alleles.
- Leftover alleles belong to the father.

Benefits of Multiple Paternity Studies



1. We found 17 DIFFERENT males were responsible for mating with 12 females
2. Mating took place before the first nest was laid (both fathers represented in all clutches): 17 males and 12 females created 38 nests

Stewart and Dutton. 2011. Paternal genotype reconstruction reveals multiple paternity and sex ratios in a breeding population of leatherback turtles (*Dermochelys coriacea*). Conservation Genetics.

Individual Identification

1. Genetic tagging (age to maturity, survival rates)
2. Multiple paternity (mating systems, demography)
3. Operational sex ratios (life tables, modeling, life history strategies)
4. Reproductive success (male contributions to populations, gene flow)



Operational Sex Ratio

1. Evaluated 46 nesting females
2. Identified 47 unique males
3. Polyandry (multiple males)
4. Polygamy (multiple females)
5. Ratio = 1.01 males to 1 female

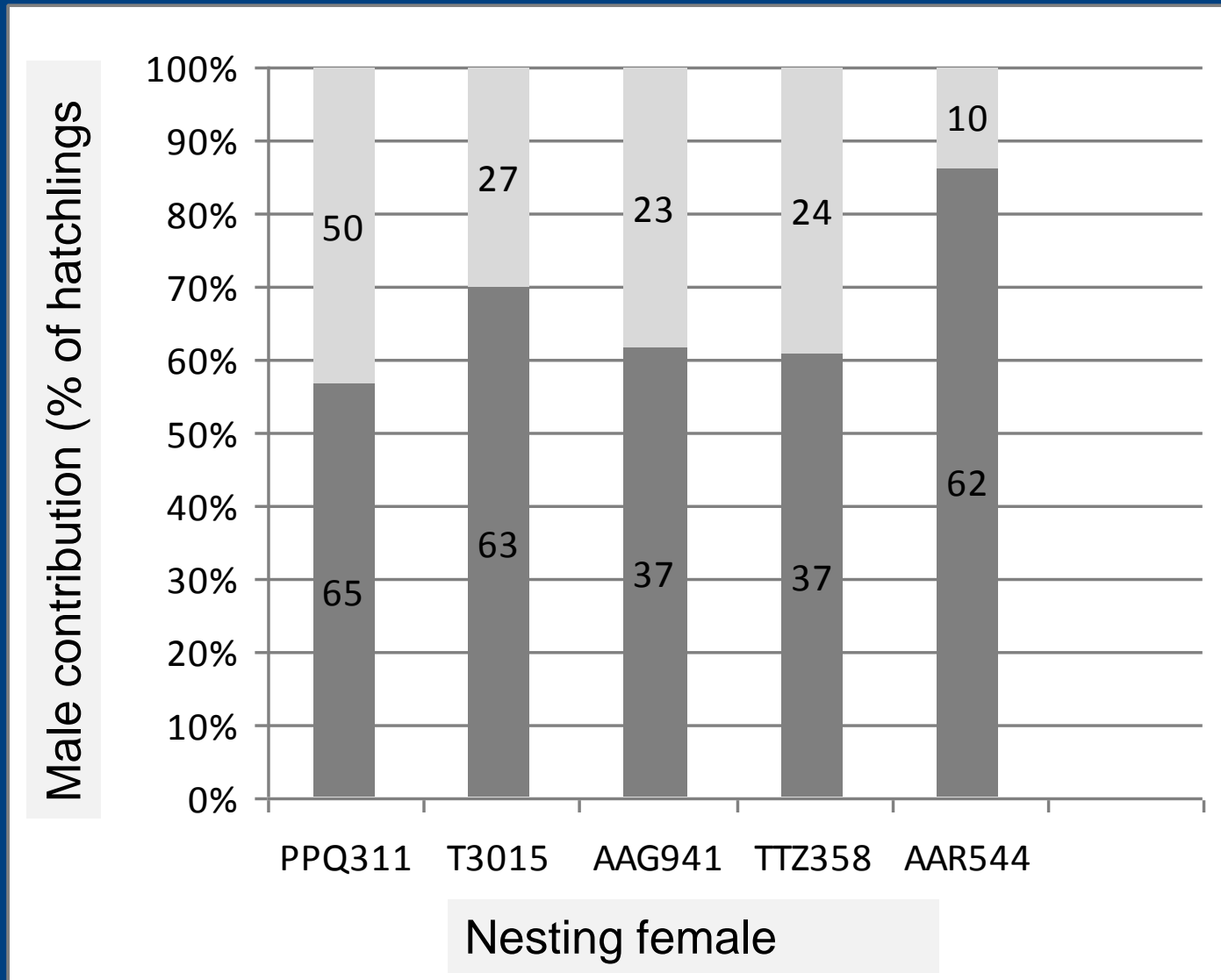


Individual Identification

1. Genetic tagging (age to maturity, survival rates)
2. Multiple paternity (mating systems, demography)
3. Operational sex ratios (life tables, modeling, life history strategies)
4. Reproductive success (male contributions to populations, gene flow)



Contributions by multiple males



Female-biased hatchling sex ratios

1. Hatchlings: Female-biased, often highly so
2. Juveniles: Generally female-biased
3. Adults: What is the operational sex ratio?

How many breeding males exist in the population? Are there enough?

Conclusions



1. Males may be as plentiful as females
2. Sex ratios on nesting beaches are important
3. Life history of turtles – female bias not detrimental?

Applications

- Identification of thousands of turtles around the world
- Define important life history parameters (survival etc.)



- Account for male contributions to populations
- Explore population dynamics in more detail
- Improve recovery plans and goals for delisting

Acknowledgements

Funding: NOAA-SWFSC,
TOF, Lenfest, STAR-
CalPoly, volunteer funds.

STX: Mike Evans, Claudia
Lombard – USFWS;
WIMARCS; Geographic
Consulting.

Permits: USFWS #41526-
2014-002; DPNR STX-039-
2014

Supplies: Grant Long - Port Plastics,
San Diego, Cottages by the Sea (Paul
Benedict), and Armstrong's Ice Cream!



Field Work: Suzanne Roden,
Robin LeRoux, Erin
LaCasella, Amy Frey, Amy
Lanci, Justin Perrault, Dana
Tomlinson, Amy Semple,
Mike McKay, Jeremy Smith,
Vicki Pease, Shane Morales,
Violet Campbell, Claire
Gonzales, Elvis Liburd,
Jamé Frey, Tim and Rachelle
Kent, Lori Jackson, Shannon
Haché, and Peter, Diana,
Emma, Donna Dutton.



**LENFEST
OCEAN
PROGRAM**







	D1		14-5		LB99		LB133		LB141		LB142		LB145	
PPQ311	235	239	216	224	124	124	175	177	180	194	234	238	145	149
Male #1	243	259	206	206	122	124	167	179	168	188	228	228	129	149
Male #2	227	247	206	206	122	124	167	167	174	188	230	234	129	129
Hatchlings	227	235 (11)	206	216 (58)	122	124 (60)	167	175 (41)	168	180 (18)	228	234 (29)	129	145 (49)
	227	239 (13)	206	224 (60)	124	124 (57)	167	177 (43)	168	194 (20)	228	238 (34)	129	149 (39)
	235	243 (21)					175	179 (13)	174	180 (9)	230	234 (19)	145	149 (13)
	235	247 (13)					177	179 (16)	174	194 (8)	230	238 (10)	149	149 (12)
	235	259 (9)							180	188 (21)	234	234 (11)		
	239	243 (13)							188	194 (25)	234	238 (8)		
	239	247 (11)												
	239	259 (16)												
	235	263 (1)^												
	219	243 (1)*												
243	247 (1)*													
Evidence of > 5 hatchling genotypes at > 1 locus														
T3015	223	235	206	206	122	130	169	177	188	188	228	232	125	129
Male #1	247	259	204	206	122	132	167	177	188	188	228	230	125	149
Male #2	239	247	204	212	124	132	167	177	180	188	228	232	145	149
Hatchlings	223	239 (6)	204	206 (54)	122	122 (16)	167	169 (22)	180	188 (6)	228	228 (22)	125	125 (13)
	223	247 (20)	206	206 (30)	122	124 (10)	167	177 (18)	188	188 (77)	228	230 (11)	125	129 (16)
Conclusion: 2 males mated with PPQ311 based on genotypes of 119 hatchlings!														
mispl	227	247 (1)												145 (7)
	223	263 (1)^												149 (21)
														145 (6)
														149 (28)
AAG941	243	251	206	206	124	124	167	177	168	168	228	232	129	145
Male #1	223	255	206	216	124	130	167	177	174	180	230	234	129	145
Male #2	223	255	206	214	122	124	157	177	174	180	228	228	129	145
Hatchlings	223	243 (16)	206	206 (29)	122	124 (12)	157	167 (3)	168	174 (26)	228	228 (15)	129	129 (21)
	223	251 (16)	206	214 (11)	124	124 (31)	157	177 (7)	168	180 (21)	228	230 (7)	129	145 (24)
	243	255 (15)	206	216 (15)	124	130 (18)	167	167 (13)			228	232 (10)	145	145 (17)
	251	255 (13)					167	177 (26)			228	234 (7)		
	223	235 (1)*					177	177 (13)			230	232 (9)		
	235	255 (1)*									232	234 (9)		