

Direct genetic evidence for reproductive philopatry and associated fine-scale migrations in female blacktip reef sharks (*Carcharhinus melanopterus*) in French Polynesia

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Abstract

Conservation of top predators has been emphasized as essential in an ecosystem due to their role in trophic chain regulation. Optimizing conservation strategies for these endangered marine top predators requires direct estimates of breeding patterns and connectivity as these are essential to understanding the population dynamics. There have been some attempts to investigate breeding patterns of reef sharks from litter reconstruction using molecular analyses. However, direct fine-scale migrations of female sharks for parturition as well as connectivity at a medium scale like between islands remain mostly unknown. We used microsatellite DNA markers and a likelihood-based parentage analysis to determine breeding patterns of female blacktip reef sharks in Moorea (Society Islands, French Polynesia). Most females gave birth at their home island but some migrated to specific nursery areas outside the area they are attached to, sometimes going to another island 50 km away across deep ocean. Our analysis also revealed that females migrated to the same nursery for every birthing event. Many offspring showed a high level of inbreeding indicating an overall reduced population size, restricted movements and dispersal, or specific mating behaviour. Females represent the vectors that transport the genes at nursery grounds, and their fidelity should thus define reproductive units. As females seem to be philopatric, males could be the ones dispersing genes between populations. These results highlight the need to conserve coastal zones where female reef sharks seem to exhibit philopatry during the breeding season.

Keywords: *Carcharhinus melanopterus*, inbreeding, migration, nursery grounds, parentage analysis, philopatry

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Introduction

Connectivity is a major process for marine organisms that determines the rates of exchange among populations and has effects on individual behaviour as well as population dynamics, and in turn influences evolution, persistence and resilience of populations (Hastings & Harrison 1994; Cowen & Sponaugle 2009). Understanding patterns of dispersal in marine organisms is also critical in conservation biology for the design of effective networks of marine protected areas (MPAs)—the

main tools used in marine conservation plans (Sale *et al.* 2005; Botsford *et al.* 2009; McCook *et al.* 2009).

Life history, dispersal and connectivity of sharks differ from most marine teleost fishes for which MPAs are usually designed. Sharks do not have a dispersing larval stage and migration only occurs during the subadult or adult stage (Musick *et al.* 2004). Further, the scale of dispersal of numerous species of sharks (e.g. transoceanic migration; Bonfil *et al.* 2005) is wider than dispersal range of marine teleost fish larvae (≤ 1000 km; Jones *et al.* 2009). Many species of sharks are threatened by overfishing around the world (Stevens *et al.* 2000; Baum *et al.* 2003; Robbins *et al.* 2006; Myers *et al.* 2007) as demonstrated by declines of more than 75% for some large coastal species in some regions

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during the last decade (Baum & Blanchard 2010). Sharks are particularly vulnerable to over-exploitation because of their K-selected life history strategy (e.g. slow growth, late sexual maturity, long lifespans and low fecundity), and once overfished, most populations of sharks take decades to recover (Stevens *et al.* 2000). Impacts of chronic overfishing of sharks have received focus because of their role in the trophic chain and the cascading effect on the ecosystem balance (Myers *et al.* 2007). Local and global threats to sharks are decreasing population size and can increase fragmentation and isolation of populations (Allendorf *et al.* 2008). Declines in shark populations have propelled sharks to the top of the worldwide conservation effort (i.e. numerous species in the red IUCN list), and knowledge of most essential biological parameters to understand the potential resilience of their diminishing populations is lacking or is incomplete.

Where coastal sharks give birth as well as whether they show philopatry to specific nursery sites (i.e. reproductive philopatry) has important implications for the spatial scale of their management and conservation. Data for juvenile reef sharks indicate they generally remain in restricted areas (Wiley & Simpendorfer 2007) and can return to these areas after experimental displacement (Edren & Gruber 2005). Once leaving nursery sites, subadults become much more mobile (Feldheim *et al.* 2001; Chapman *et al.* 2009). It remains uncertain whether juveniles will return to their natal sites for reproduction once they reach maturity, and whether they will keep returning to a particular site over multiple years as in the case of marine turtles (Meylan *et al.* 1990). Female reproductive philopatry has been previously found in shark species like lemon sharks *Negaprion brevirostris* (Feldheim *et al.* 2004), sandbar sharks *Carcharhinus plumbeus* (Portnoy *et al.* 2010) and bull sharks *Carcharhinus leucas* (Tillett *et al.* 2012).

One way to examine the fidelity to particular birthing places some shark species exhibit is through mark-recapture or manual and remote tracking methods (Hueter *et al.* 2005). Tagging approaches are efficient for understanding short-term to medium-term behavioural ecology of individuals (i.e. limited to the tag attachment or the 3–4 years of battery life). However, they often are of little assistance in understanding population-level patterns and processes occurring over an individual's lifespan over 25 years, and are not applicable in understanding processes across generations (Planes & Lemer 2011). The extent of reproductive philopatry in sharks has usually been tested by comparing the diversity of two genetic markers (mitochondrial DNA and microsatellites) in juveniles sampled from nurseries (for a review, see Dudgeon *et al.* 2012; Portnoy & Heist 2012).

The capacity to distinguish individuals using genetic markers also makes it possible to follow an individual's genotype across generations (Dudgeon *et al.* 2012).

Individual-based genetic identification has been mostly used in the context of parentage analysis (Planes & Lemer 2011). Patterns of parentage can help resolve some key ecological and evolutionary processes in population dynamics (Jones *et al.* 2010), and this technique has been used to address questions of dispersal and connectivity for coral reef fish with pelagic larvae (Jones *et al.* 2005; Planes *et al.* 2009; Saenz-Agudelo *et al.* 2009, 2011; Christie *et al.* 2010). In the case of sharks, this approach can provide new insight to fine-scale reproductive patterns and connectivity. Parentage analysis can also help test for and determine levels of reproductive philopatry by assessing whether particular males or females produce offspring in multiple reproductive episodes at a single nursery site (Feldheim *et al.* 2002; DiBattista *et al.* 2008).

For many shark species, juveniles tend to show nursery-site fidelity for at least the first few years (Wiley & Simpendorfer 2007; Chapman *et al.* 2009). Assignment of juveniles to putative parents from the adult population may help determine the migrations and connectivity patterns occurring during shark birthing events. The aim of this study is to apply parentage analysis based on hyper-variable microsatellite DNA markers to investigate mating patterns, as well as other processes of recruitment and connectivity in the blacktip reef shark (*Carcharhinus melanopterus*) in Moorea, French Polynesia. The approach was based on the identification of the parent-offspring relationship within the island populations. The migration of females when they give birth can be deduced if the location of adults and juveniles is known, or can be assumed. This is due to the site attachment and restricted movements of adults (Mourier *et al.* 2012) and the sedentary behaviour of juveniles within their nursery. Data presented provide the first direct estimates of the fine-scale breeding patterns and migrations of females in a shark species.

Methods

Study species and location

The blacktip reef shark (*Carcharhinus melanopterus*, Quoy & Gaimard 1824) is a common shark species of Indo-Pacific coral reefs (Compagno *et al.* 2005). It inhabits shallow reefs and sand flats both at atolls and high islands (Hobson 1963; Nelson & Johnson 1980; Stevens 1984; Papastamatiou *et al.* 2009a, 2010; Mourier *et al.* 2012). While abundant around French Polynesian islands, many aspects of its basic natural history remain poorly documented. Adult blacktip reef sharks show

high degrees of site attachment (Stevens 1984; Papastamatiou *et al.* 2009a) characterized by small daily home ranges of *c.* 0.5 km² (Papastamatiou *et al.* 2009a) and maximum known movement distances of about 3–10 km (Stevens 1984; Mourier *et al.* 2012), while juveniles seem to occur in shallow-water nursery areas (Papastamatiou *et al.* 2009a). Mating occurs between November and March with parturition occurring between September and January in French Polynesia (Porcher 2005). Each viviparous female follows an annual gestation cycle of about 300 days (Porcher 2005). During parturition, resident females appear to leave their adult resident small home range (Porcher 2005) to give birth in specific nursery habitats. Nursery habitats already have been described, but the origin of females giving birth in a specific location was previously unknown. The present study location encompassed two islands in the Society Islands, French Polynesia: the island of Moorea (17°30'S; 149°51'W) and the atoll of Tetiaroa (17°00'S; 149°33'W) located about 50 km north of Moorea (Fig. 1), selected mainly because of local facilities (Mourier *et al.* 2012).

Sampling

Carcharhinus melanopterus specimens were caught at different locations around Moorea from 2007 to 2010. Adults and subadults were caught from a boat using a fishing rod with barbless hooks within and outside the lagoon at different locations around the island (Fig. 1). They were brought alongside the boat where they were inverted and placed in tonic immobility while biological data and tissue samples were collected, and their dorsal

fin photographed for individual identification. Both sides of the dorsal fin as well as other distinctive marks such as scars, notches and dots on the body were recorded via photo-identification (more details in Mourier *et al.* 2012). Juveniles were caught from the shore in their nursery area with gillnets positioned perpendicular to the shoreline (Fig. 1). Each individual was measured, sexed and a small fin clip was removed from the second dorsal fin or anal fin. Samples were preserved in 95% ethanol and returned to the laboratory for subsequent genotyping. Although adults were sampled from all coasts around Moorea, sampling effort was greater on the north shore where a parallel monitoring survey was conducted. Between 2008 and 2009, several field trips to sample juvenile sharks were conducted at Tetiaroa.

For subsequent analyses, shark samples were divided into three categories according to their size. The first category consisted of juveniles (<70 cm), the smallest ones being newborn sharks still showing apparent umbilical scars. The remaining individuals were then divided into 'subadults' (70 < TL < 110 cm) and 'adults' (>110 cm) according to the average size at maturity known for this species (Papastamatiou *et al.* 2009b; J. Mourier, unpublished data). A total of 264 individuals were sampled including 247 sharks at Moorea (187 adults, 14 subadults and 46 juveniles) and 17 sharks at Tetiaroa (two adults, 15 juveniles). We caught 103 adult males and 86 adult females. Sampling of adult sharks was exhaustive in the north of Moorea and incomplete in the west and east coast while sampling was exhaustive in the west coast for juveniles but not on the other coastlines.

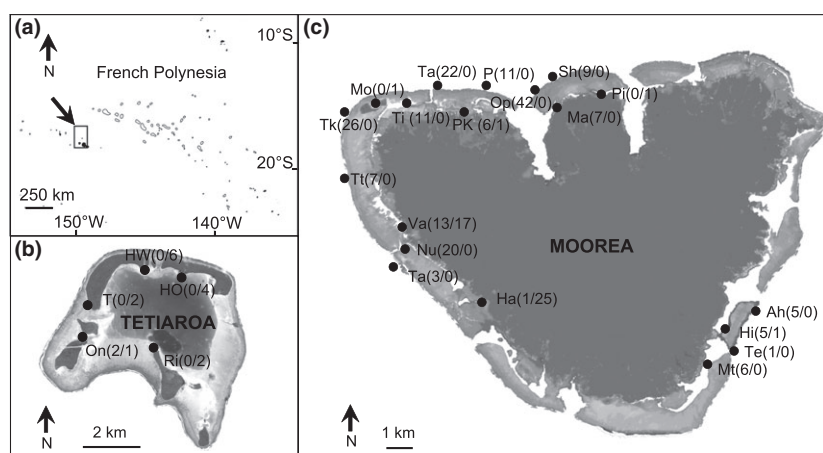


Fig. 1 Location of Moorea and Tetiaroa in French Polynesia (a). Location of sampling sites at Tetiaroa Atoll (b) and Moorea Island (c). The numbers of sampled adult and juvenile sharks at each site are indicated in brackets. Site abbreviations are as follows: Tiki (Tk), Motu (Mo), Tiahura (Ti), Taotoi (Ta), PK23 (PK), Papetoai (P), Opunohu (Op), Mareto (Ma), Sheraton (Sh), Pihaena (Pi), Ahi (Ah), Hitiaa (Hi), Teruapu (Te), Maatea (Mt), Haapiti (Ha), Tauotara (Ta), Nuurua (Nu), Varari (Va), Tetaiuo (Tt), Hoa Williams (HW), Hoa Oroatera (HO), Tiaraunu (T), Onetahi (On) and Rimatuu (Ri).

Genotyping

DNA samples were extracted following PUREGENE™ DNA Purification Kit procedure for 5–10 mg fresh tissue. After DNA extraction, five multiplex polymerase chain reactions (PCRs) were performed per individual, using fluorescently labelled primers to screen 17 microsatellite loci (Table 1). Microsatellite markers were adapted from Feldheim *et al.* (2001, 2002), Keeney & Heist (2003), Chapman *et al.* (2004), Ovenden *et al.* (2006) and Portnoy *et al.* (2006) and optimized for our target species, *Carcharhinus melanopterus*. PCR products were processed on a Beckman Coulter sequencer CEQ 8000 Genetic Analysis System, and the resulting electropherograms were scored manually using the program CEQ™8000. Uncertainties were reconciled by reamplification and comparison. Alleles were scored as PCR product size in base pairs. Allelic frequency and expected heterozygosity under Hardy–Weinberg equilibrium were calculated for each locus in GENALEX version 6 (Peakall & Smouse 2006). The presence of null alleles was investigated using MICROCHECKER (van Oosterhout *et al.* 2004). Tests for Hardy–Weinberg and linkage disequilibrium were conducted using GENEPOP 3.4. (Raymond & Rousset 1995), and significance levels were adjusted with sequential Bonferroni corrections for multiple tests with $P < 0.05$. In addition of photo-identification (Mourier *et al.* 2012), GENALEX version 6 (Peakall & Smouse 2006) was used to detect potential identical genotypes belonging to the same individual captured in two sampling events. Every identical multilocus genotype was considered to be the same individual, and only one of each was retained for subsequent analysis.

We investigated potential population substructure using Bayesian method with MCMC estimation implemented in STRUCTURE version 2.3.3 (Pritchard *et al.* 2000). An admixture model with correlated allele frequencies between clusters was run ten times for a given number of inferred clusters, K , from $K = 1$ –4 using burn-in lengths of 5×10^4 and run lengths of 5×10^5 Monte Carlo Markov Chain steps. The optimum number of clusters, K , was determined following guidelines from Evanno *et al.* (2005).

Parentage analysis

Parentage analyses were performed using FAMOZ (Gerber *et al.* 2003). The algorithm in this software calculates log of the odds ratio (LOD) scores for parent–offspring relationships and constructs statistical tests for parentage assignment. Tests are based on simulations that generate offspring from genotyped parents (H_0 : the most likely parent is the true parent), or from allele frequencies in the population (H_1 : the most likely parent is not the true parent). For each analysis, allelic

frequencies were estimated from the 264 genotyped individuals assumed to match the true allele frequencies in the population. Then, simulations of sets of 10^4 juveniles were carried out under the two possible hypotheses (H_0 and H_1 above), and subsequent statistical tests were constructed to decide whether a given parent would be selected as the true parent or true parent pair. The distribution of the simulated LOD scores under the two hypotheses was plotted, and the intersection between these distributions was designated as the threshold decision value (individuals with LOD scores above the threshold value were accepted as true parents). FAMOZ also allows for the introduction of an error rate in the LOD score calculation that takes into account genotyping errors and null alleles (Gerber *et al.* 2000). Introduction of this error, even if it underestimates the real error rate, can reduce type I and II errors related to the parentage tests (Gerber *et al.* 2000; Morrissey & Wilson 2005). We evaluated four different error rates and chose the best compromise between introduced error and type I and II statistical errors. An error rate of 10^{-3} yielded the lowest statistical type I and II errors (4.7 ± 0.6 and $6.3 \pm 0.6\%$, respectively) and was used for all further parentage analyses. There was only one missing allele at one locus for one juvenile over the all genotypic database.

We tested the presence of full-sibling among females that gave birth in the same nursery. Although an absolute determination of kinship cannot be made using these markers only, it is possible to estimate a relatedness index and use a likelihood approach to decide whether the observed relatedness measures are true or not (Weir *et al.* 2006). For each pair of females using the same nursery ground, we calculated the likelihood ratio for the hypotheses of full-siblings (primary hypothesis) vs. unrelated (null hypothesis) with KINGROUP v.2 (Konovalov *et al.* 2004). The likelihood of a specified alternative relationship of the null hypotheses was obtained by simulations. Individual pairwise likelihood ratios were assessed in KINGROUP v. 2.0 (Konovalov *et al.* 2004) following Goodnight & Queller's (1999) algorithm. The rates of Type I errors (rate of false positive) and Type II errors (rate of false rejection of the primary hypothesis) were calculated using 10 000 simulations at $P < 0.05$ significance level. Female pairs with ratios that corresponded to P -values < 0.05 were considered full-sibs.

Inbreeding measures

While parentage analyses and pedigree reconstruction provide insights on inbreeding (Keller & Waller 2002), these analyses require extensive data sets and long-term studies that are difficult to achieve in species with a

Table 1 Description of the 17 microsatellite loci used to genotype blacktip reef sharks

Locus	Primer sequence 5'-3'	Repeat type	Ta	N	H ₀	H _E	k	F _{is}	H-W	Source
Cs08	F: GGCCATCAGTTTGCTTA R: AATCCAGTTCATCTTCAATA	(CA) ₂₈	57	264	0.795	0.811	11	0.020	NS	Ovenden <i>et al.</i> (2006)
Cpl169	F: TGACACAACCAATTAATCCACAG R: GGTTCCTTGAGTGAAAGAGAGAGC	(TG) ₄₂	57	264	0.292	0.278	4	-0.048	NS	Portnoy <i>et al.</i> (2006)
Cs02	F: GGCTCCATAAAAAAGTTGGTA R: GGCTCTGTTTAAATGATGAATGTA	(GT) ₂ (GC)(GT)(GC)(GT) ₁₁	57	264	0.780	0.798	14	0.022	*	Ovenden <i>et al.</i> (2006)
Chi102	F: GACTGGCTGACCTAACTAAGC R: ATCCTGTGGTCTCTATC	(GA) ₉	57	264	0.436	0.438	3	0.006	NS	Keeney & Heist (2003)
Chi107	F: GGATTCACAACACAGGGAAC R: CTCATTCCTTAGTTCCTCCG	(GT) ₁₄	57	264	0.481	0.479	3	-0.004	*	Keeney & Heist (2003)
LS75	F: TGTTACTGGCACAATAATTC R: GAGTTATCTTTCTGTGTAGT	(TC) ₁₁ (AC) ₁₁ AG(AC) ₁₀	57	264	0.610	0.606	3	-0.007	NS	Feldheim <i>et al.</i> (2002)
Chi108	F: TCACTGGGTTAGACACTCC R: CCACAGTCAGAAAAAATG	(GT) ₁₂	57	264	0.723	0.679	6	-0.065	NS	Keeney & Heist (2003)
Chi111	F: ACTTACGAACGTGTGCTAACTC R: GGGAGATAAACGACAAATGTG	(GT) ₂₈	57	264	0.902	0.906	19	0.005	NS	Keeney & Heist (2003)
LS53	F: GCCTCATCTGCTCTGTGTTT R: CACATAACCTCCTCTGCTCC	(AC) ₁₄	64	264	0.939	0.937	28	-0.003	NS	Feldheim <i>et al.</i> (2001)
LS32	F: TTAAGTCAGGTAATGFGACTCGT R: GCTTGCTTTCACACCTACCCATTT	(AC) ₄ (AG) ₂ (AC) ₇	64	264	0.511	0.455	3	-0.124	*	Feldheim <i>et al.</i> (2001)
LS54	F: TTGGAAAACCGTGGAGTGAA R: GGGGAAAAAGAACIGGGACTAAATCC	(CT) ₁₀ (CA) ₈	57	264	0.466	0.468	3	0.004	NS	Feldheim <i>et al.</i> (2002)
LS20	F: AAGTCCTGGAGCACAGCCTTCAAG R: AAATCCCAATGGAAATCGTGG	(AC) ₈ GC(AC) ₃	57	264	0.303	0.308	2	0.015	NS	Feldheim <i>et al.</i> (2002)
Chi103	F: GCTTCATCCATGAGAG R: ITTCTCTGCTCTGGTITTC	(GA) ₅ (GA) ₁₄ (GA) ₇	57	264	0.436	0.489	2	0.109	NS	Keeney & Heist (2003)
Pg102	F: ACCCGACTGCCAGGATTCACT R: CCCGATCACTCACCCG	(GA) ₁₀	63	264	0.640	0.629	5	-0.017	NS	Chapman <i>et al.</i> (2004)
Chi2	F: CTTTGAGGAAGTTGGTACTGATG R: GCCACTCTGTCTGAATTTTCCG	(GA) ₁₀	63	264	0.261	0.244	2	-0.070	NS	Keeney & Heist (2003)
Chi12	F: TCCCAGTCACATTTACACATGC R: GGAAGACCAATGAACCCAATC	(GT) ₉	63	264	0.303	0.544	6	0.443	*	Keeney & Heist (2003)
Cpl128	F: GCTGTGATCTTCTGATGAGC R: GGATGGTGGATGTGGATTTG	(CA) ₁₃ TA(CA) ₁₃	60	264	0.848	0.808	18	-0.050	NS	Portnoy <i>et al.</i> (2006)

Ta: annealing temperature (°C); N: number of individual scored; H₀: observed heterozygosity; H_E: expected heterozygosity; k: number of alleles; F_{is}: inbreeding coefficient; H-W: exact test for departure from Hardy-Weinberg.

H-W: significance tests (*P < 0.05 after Bonferroni correction, NS: not significant).

long lifespan like sharks. We therefore calculated internal relatedness (IR) of 61 sampled juvenile sharks (offspring or young juvenile; o-IR) as a measure of how related their parents were to assess the prevalence of inbreeding (Amos *et al.* 2001). IR expresses the proportion of typed loci that are homozygous in an individual scaled by population allele frequencies, so that homozygotes with rare alleles are given more weight than homozygotes with common alleles. IR provides a measure of allele sharing between parents due to recent common ancestry. Inbred individuals will exhibit high IR, whereas outbred individuals will exhibit IR close to or below zero. IR was calculated for all juvenile blacktip reef sharks in the program STORM v. 1.0 (Frasier 2008).

We tested statistical divergence of internal genetic diversity from random expectations using a Monte Carlo simulation implemented in STORM v. 1.0 (Frasier 2008). This approach generates o-IR values expected from the gene pool if mating is random with respect to parental relatedness. In such a process, we generated simulated o-IR measures by sampling random males ($n = 103$) and females ($n = 86$) with replacement. Each random mating pair produces a simulated offspring whose internal relatedness is measured (o-IR). Sixty-one simulated offspring were generated, their simulated o-IR values were averaged and then this average of simulated o-IR was recalculated 10 000 times. To test significance, the observed mean o-IR was compared with the distribution of average simulated o-IR.

The relatedness coefficient of both parents can be estimated for parent pairs assigned from parentage and compared to 10 000 simulated parent pairs built from the gene pool of sampled adults. Overall, this analysis tests whether individuals are avoiding inbreeding, or if they tolerate or favour inbreeding (i.e. less vs. more than expected).

Results

Microsatellite summary statistics

Across all individuals of *Carcharhinus melanopterus* collected for this work, polymorphism varied from 3 to 28 alleles per locus. Observed heterozygosity ranged from 0.261 to 0.939 per locus and expected heterozygosity from 0.244 to 0.937. Significant heterozygote deficiencies were found in four loci (Cs02, Cli12, Cli107 and LS32; with all $P < 0.05$ following Bonferroni standard correction). Null alleles and scoring errors were not detected for these loci using MICRO-CHECKER software. All 17 loci were therefore included in our analysis (Table 1). Significant heterozygote deficiencies were found in the

global population with all loci included ($P < 0.001$), suggesting that the population is not in the Hardy–Weinberg equilibrium.

STRUCTURE did not detect structured variance within samples and the hypothesis that $K = 1$ consistently exhibited a higher probability than $K = 2, 3$ or 4. Individuals were assigned to each of the 2, 3 or 4 populations (where K was set to 2, 3 or 4, respectively) with approximately equal proportions (i.e. apparently at random). This suggests that the population harboured a single gene pool and that Wahlund effects are not a cause for deviation from Hardy–Weinberg equilibrium which in turn may be due to inbreeding.

Finally, no identical genotypes were found within the collection indicating that no individual was sampled twice. This result confirms that photo-identification for this species using fin patterns is highly reliable because genetic sampling was coupled with photo-identification of individuals through their fin patterns (Mourier *et al.* 2012) to avoid double sampling.

Parentage assignment

Parentage analysis assigned 43 juveniles of 61 that were genotyped to at least one parent or a pair of parents among 189 sampled adult sharks (Table S1, Supporting information). From these 43 juveniles, 19 (44.2%) were assigned only to a female, 18 (41.8%) only to a single male and 6 (14.0%) to a pair of parents. Assignments of juveniles to females were also supported by underwater observations of gestation for some individuals (Fig. S1, Supporting information). We did not observe evidence of multiple paternity in our data set.

Although multiple paternity has not been documented in this species, the absence of evidence does not mean absence of the process and small litter size in this species or incomplete sampling of litters may have limited our ability to detect multiple paternity.

Nursery and dispersal

From the 61 juveniles sampled over 3 years (2007–2009), seven juveniles were assigned to the same female over multiple years. In fact, female RF11 gave birth to the same nursery (Haapiti) to at least two juveniles sampled in 2007 (H4-07 and H5-07) and one juvenile sampled in 2009 (H11). Female Tuao3 gave birth in 2007 (juvenile H2) and in 2009 (juvenile H21) at Haapiti (Fig. 2, Table S1, Supporting information), and female Op36 gave birth in 2007 and 2008 at a nursery of Tetiaroa (juvenile i12 and i14, respectively, Fig. 3 denoted by '×2' arrow) following a 1-year reproductive cycle. Putative juveniles from females RF11 and Tuao3 were not found in 2008, possibly due to low sampling effort on

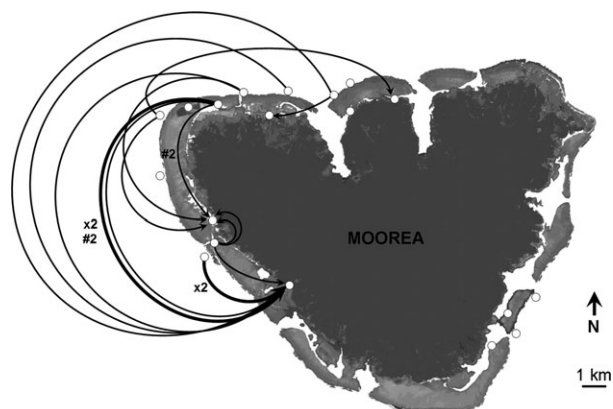


Fig. 2 Migrations of females for parturition in Moorea as determined by parentage analyses. Circles indicate sampling locations. Arrows show the travel from the female's home range or its capture site to the nursery ground where the juvenile it was assigned to was caught. Annotation 'x2' indicates that a female gave birth at the same site two different years, and annotation #2 indicates that two offspring of the same cohort were assigned to the female. For more details about parentage assignment, see Table S1 (Supporting information).

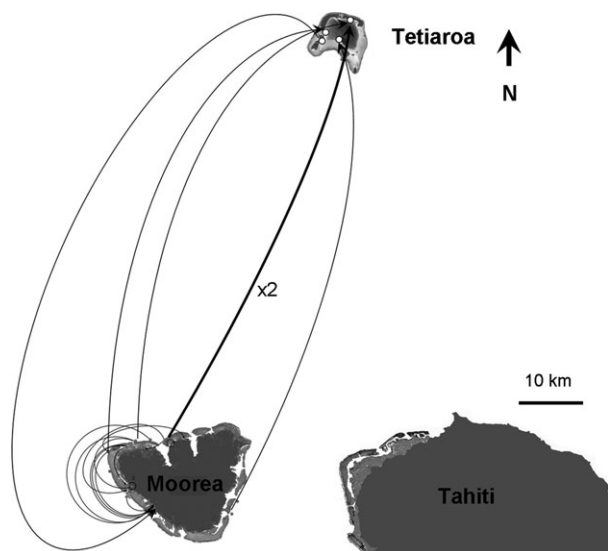


Fig. 3 Migrations of females for parturition between Moorea and Tetiaroa inferred from parentage analysis. Annotation 'x2' indicates that a female gave birth at the same site on two different years.

juveniles in 2008 (Table S1, Supporting information). Both females could have given birth in 2008, although they also could have failed to breed. Of the 24 assigned fathers, no male contributed genes to more than one litter. Also, only 30% of males that fathered pups were caught near their offspring nursery, and four of six parent couples were comprised of individuals from the same coastline of Moorea (Table S1, Supporting infor-

mation) refuting the idea of potential male philopatry for this species in this location.

Of 21 mothers determined by parentage analyses, only three of them gave birth close to their home range (Fig. 2). Migrations of females to reach their nursery ground occurred primarily in a north to west direction at Moorea (also biased by the sampling). No migrations have been found from the east coast, but we have few samples from that area (six females and one juvenile).

At Moorea, two nursery areas (Varari and Haapiti, Fig. 1) were regularly followed and sampled. Using parentage analysis, we found that five females used Varari while nine females used Haapiti for parturition. Kinship was tested between these females using KINGROUP (Konovalov *et al.* 2004). Of the nine females using Haapiti as a nursery, two groups of two full-siblings (T45-Tuao3 and Op35-RF11, $P = 0.037$ and $P = 0.039$, respectively) were identified. Additionally, of five females using Varari as a parturition site, one full-sibling pair was found (Mot14-T1-07, $P = 0.02$).

Interisland connectivity associated with parturition

Among the 21 female-juvenile assignments, 16 gave birth at the island where they were caught in Moorea and five gave birth at Tetiaroa. Four females caught and observed at Moorea gave birth at Tetiaroa, and one female caught at Tetiaroa gave birth at Moorea (Fig. 3). One female caught at Moorea made two migrations to Tetiaroa (2007 and 2008) to give birth at the same location. Among the six male-juvenile assignments in Tetiaroa, all males came from Moorea (Table S1, Supporting information). The unique parent pair assigned to a juvenile of Tetiaroa (Op36-Tuao1; Table S1, Supporting information) was also comprised of two individuals from Moorea. This suggests that the migration to the nursery in Tetiaroa occurred postmating.

Inbreeding

The mean internal relatedness of the 61 sampled offspring (o-IR) was not significantly higher than that expected under random mating (Monte Carlo randomization procedure ($\times 10\,000$): mean o-IR \pm 95% confidence interval = 0.038 ± 0.025 and mean simulated o-IR \pm 95% confidence interval = 0.005 ± 0.002 , $P = 0.06$). However, maximum o-IR value was 0.595 ($IR > 0.5$) that corresponds to full-sibling or parent-offspring mating and was high compared to maximum o-IR from simulated data (max = 0.094). Overall, 8% (5/61) of all offspring showed o-IR values higher than 0.25 (i.e. the value expected for offspring of half-sibling mating), and in 38% of the cases (23/61), the o-IR was larger than 0.125 (i.e. the value expected for offspring of cousins

mating) demonstrating some reproduction between relatives. Parentage analyses have assigned six juveniles to parent pairs. The mean relatedness of the seven was significantly higher than that expected under random mating (Monte Carlo randomization procedure ($\times 10\,000$): mean $r_{xy} \pm 95\%$ confidence interval = 0.15 ± 0.12 and mean simulated $r_{xy} \pm 95\%$ confidence interval = -0.013 ± 0.001 , $P = 0.0015$). This result suggests nonrandom mating or small population size. One parent pair corresponds to half-sibling ($r_{xy} > 0.25$), and four of seven parent pairs correspond to cousins ($r_{xy} > 0.125$). We did not observe any significantly higher average IR values in juveniles than in adults (mean o-IR $\pm 95\%$ confidence interval = 0.038 ± 0.025 , mean a-IR $\pm 95\%$ confidence interval = 0.013 ± 0.025 , d.f.: 201, $P = 0.23$) suggesting that the entire population was equally prone to inbreeding. Interestingly, five pairs were made of individuals that did not share the same home range (i.e. caught and surveyed at different locations, cf Mourier *et al.* 2012).

Discussion

This study describes new insights regarding reproductive migration patterns, nursery utilization and interisland connectivity for a reef-associated shark species. Our results provide evidence that (i) at least some females are philopatric to specific nursery areas where they return every year to give birth, (ii) parturition occurs mainly outside of their home range and (iii) this species is able to migrate over open deep ocean to reach birthing places. These are key elements to understanding the mechanisms underlying population dynamics.

We assigned parentage to 43 of 61 juveniles. This high success rate in parentage assignment suggests that the population is relatively small and closed in Moorea. The lack of parentage assignment of 18 of the 61 sampled juveniles is likely due to the incomplete sampling of potential parents in Moorea and Tetiaroa, or because some of the parents may belong to other islands not sampled in this work. While some attempts previously have been made to understand the breeding dynamic of sharks, they primarily relied on litter reconstruction from sampled juveniles (Feldheim *et al.* 2002, 2004; Di-Battista *et al.* 2008) or on determining reproductive periodicity via underwater gestation monitoring (Porcher 2005; Bansemer & Bennett 2009). Our work based on parentage analysis provides new and essential insights regarding migration and connectivity; we show movement of females to give birth in specific nursery grounds within or outside the island they inhabit on a regular basis. Knowing the range of the females, we have been able to infer for the first time where and how far they travel for parturition. We also observed sibling females using the same nursery area. Despite

these patterns of dispersal, it appears that mating site selection did not result in complete avoidance of inbreeding.

Long-distance reproductive migrations

Females travelled surprisingly long distances for a shark species characterized by restricted movements (Papastamatiou *et al.* 2009a). Most females gave birth in nursery areas located several kilometres (from 10 to 50 km) away from their home range (Mourier *et al.* 2012), and some made long journeys across the open sea to give birth at another island. Overall, eight females from the north coast migrated to nurseries along the west coast (Fig. 2), while four females from Moorea crossed deep ocean (up to 2000 m depth) to give birth at Tetiaroa (Fig. 3). One female from Tetiaroa made the opposite journey (Fig. 3). Such interisland travel was certainly unexpected based on the biological and behavioural characteristics of this species. Blacktip reef sharks are known to display limited movements inside lagoons or along the reef, rarely exceeding tens of kilometres (Stevens 1984; Papastamatiou *et al.* 2009a, 2010; Mourier *et al.* 2012). As demonstrated by Mourier *et al.* (2012) while studying the same population along the north coast of Moorea, blacktip reef sharks show strong patterns of site fidelity and restricted movements. Such patterns were also confirmed by telemetry data on this population around Moorea (J. Mourier, unpublished data) showing that sharks annually spent up to 70% of their time within an area of about 0.3 km². The present results demonstrate that interisland migration is not a rare event and is likely to happen regularly among nearby islands, at least during birthing events. In the case of Tetiaroa, we question how many of the juveniles belong to Moorea's females and/or other nearby islands like Tahiti (that was not sampled) as we only have large samples from the Moorea adult population.

Although movements by males and females were observed in our system during the mating season (Mourier *et al.* 2012; J. Mourier, unpublished data), our results conflict with a recent long-term study conducted at Palmyra atoll (Pacific ocean) using both satellite and acoustic telemetry on blacktip reef sharks. Large-scale movements by blacktip reef sharks or migrations across open sea were not demonstrated at Palmyra (Papastamatiou *et al.* 2010). Only a few reef shark species have shown evidence of interisland migrations. In the Hawaiian Islands, tiger sharks *Galeocerdo cuvier* move regularly between islands as far as 320 km apart (Meyer *et al.* 2009). One individual lemon shark, *Negaprion brevirostris*, travelled 1000 km between the Bahamas and Florida (Feldheim *et al.* 2001). However, these two coastal species are larger (reaching 3 m for the lemon

shark and 5 m for the tiger shark) than blacktip reef sharks and are likely better adapted for long-distance travel across open sea. Medium-sized reef sharks (between 2 and 3 m total length) have been reported to migrate between two islands separated by deep depths. Examples include the grey reef shark *Carcharhinus amblyrhynchos* travelling 134 km across open sea in Australia (Heupel *et al.* 2010), and Caribbean reef sharks, *Carcharhinus perezi*, travelling between two atolls separated by 50 km (Chapman *et al.* 2005). However, blacktip reef sharks are rarely longer than 150 cm and open sea migrations were believed to be rare events (maybe with drifting floats) for this small species, even if infrequent dispersal has been deduced from low population genetic structure in a similar size species *Triaenodon obesus* (Whitney *et al.* 2012b) with sedentary behaviour (Whitney *et al.* 2012a).

The relatively high frequency of these migrations was unexpected, and the present small sample size in this study (15 juveniles sampled for two adults from Tetiaroa and 46 juveniles from Moorea) suggests that the frequency of interisland migration is likely to have been underestimated. Our study supports recent work on the global population genetic structure of blacktip reef sharks in French Polynesia, revealing that sharks at Moorea, Tetiaroa and Rangiroa form a unique population (T. Vignaud, unpublished data).

Female philopatry to nursery grounds

Such large migrations were believed to be infrequent in small sedentary reef shark species, and most movement occurs within a limited home range (Papastamatiou *et al.* 2010; Whitney *et al.* 2012a). Whitney *et al.* (2012b) argued that natal homing is unlikely for sharks that disperse from isolated oceanic islands, mostly because long-distance travels require finding a suitable habitat. Opposite to this general view, our study demonstrates that such migrations between oceanic islands are common over the life cycle of blacktip reef sharks, at least in French Polynesia. Regular migrations between Polynesian islands, especially those involving philopatry, suggest that blacktip reef sharks can orient themselves in open sea to locate islands in a fragmented environment without using physical landmarks. Philopatry, defined as the successive use of native habitats throughout the life cycle of an organism, has been primarily studied in marine turtles (Meylan *et al.* 1990). Marine turtles return to the same sites to give birth after long migrations, and it has been suggested that geomagnetic fields are used to orient and return to sites from great distances (Lohmann *et al.* 2008). Sharks have been shown to be able to detect changes in geomagnetic fields (Meyer *et al.* 2005) and to swim accurately

towards a target island over large spatial scales. This suggests that they have the ability to memorize and integrate cognitive maps of their environment (Papastamatiou *et al.* 2011). Our results show that at least some females return to the same nursery ground to give birth (i.e. philopatry). In fact, newborn sharks from different cohorts were assigned to the same female while being sampled at the same nursery. As an example, female Op36 from Moorea gave birth in 2007 and 2008, at exactly the same nursery at Tetiaroa. Similarly, female RF11 from Moorea gave birth in 2006 and 2009 at the same site at Moorea (Haapiti). Similar to some other marine animals like salmon and marine turtles (Meylan *et al.* 1990; Neville *et al.* 2006), natal homing (the return to its birthplace to give birth) has been suspected in sharks (Feldheim *et al.* 2002; DiBattista *et al.* 2008), but has never been demonstrated.

In our study, of the nine females that gave birth at Haapiti nursery, two groups of full-siblings were identified, and at Varari nursery, one pair of full-siblings have been found from five females. This is the first time that full-sibling pairs of female sharks have been found to give birth at the same nursery site. Although this could have occurred simply at random, two hypotheses can be drawn from this observation. First, we can propose that the blacktip reef sharks form long-term, stable associations among adults from an early stage (Mourier *et al.* 2012). These long-lasting stable communities do not imply they are kin based. The presence of inbreeding in this population would only result from the occurrence of some relatives within the gene pool combined with patterns of site attachment and restricted movements (Mourier *et al.* 2012). This pattern will be driven by some kin-relative females occurring together as adults and following their associate to their specific nursery to give birth and, after a favourable parturition experience, fixing on that site (the 'social facilitation hypothesis' of philopatry; Meylan *et al.* 1990). Second, the previous pattern may result from natal homing behaviour with kin-relative females of the same litter returning to their natal nursery to give birth. Natural selection can lead to this behaviour if parturition in natal areas increases juvenile survival. This reproductive strategy could be advantageous for blacktip reef sharks because females produce a small number of young that have been heavily invested in. A number of studies claimed to have found evidence of natal homing through tagging (Pratt & Carrier 2001; Sims *et al.* 2001; Sundström *et al.* 2001) or global genetic population structure (Hueter *et al.* 2005; Keeney *et al.* 2005).

Although natal homing can be indirectly inferred with mtDNA (Meylan *et al.* 1990; Keeney *et al.* 2005), only one direct test can be carried out, and that is by

tagging (physically or genetically) a shark at birth and then recapturing that individual as an adult giving birth at the same site. Future monitoring of the population over multiple reproductive cycles and across generations will enable us to confirm our natal homing hypothesis.

Female fidelity to specific nurseries may define reproductive units that are reinforced when females return to their birth place. Natal homing or even philopatry to coastal areas may make the population more vulnerable to anthropogenic factors (i.e. habitat degradation or fishing pressure). Beyond the originality of this concept for the ecology of sharks, this notion highlights the need for conservation and additional information about potential natal homing in sharks.

Inbreeding

A large proportion of offspring in this population is considered to be the result of inbreeding. While mean offspring internal relatedness was relatively low ($o\text{-IR} = 0.038$), maximum internal relatedness reached up to a maximum $o\text{-IR}$ of 0.595. Overall, 8% of all offspring corresponded to the outcome of half-sibling mating and 38% were the outcome of cousins mating. This result is unexpected in the case of a mobile top predator capable of interisland migrations. In addition, litter size is relatively small in this species since females only give birth to 2–4 pups for a given reproduction year (Compagno 1984). Thus, relatedness values within the population were not expected to be high. Such a high degree of inbreeding is even more unusual in a mobile, free-living marine species, even if the fragmented habitat tends to isolate populations and reduce the opportunity to find unrelated mates. There was no significant difference between IR values of adult and juvenile sharks, suggesting that inbred sharks were not removed from the population earlier than outbred conspecifics and there is no evidence of any inbreeding depression. In this case, it remains difficult to determine if inbreeding is a consequence of small population size, social behaviour or the sedentary nature of this species at Moorea. Wahlund effects are a common explanation given for observed heterozygote deficiencies (Wahlund 1928). This occurs when two or more subpopulations are inadvertently sampled as a single population. However, no population subdivision was detected in our study, even when including individuals from Tetiaroa, suggesting that inbreeding is likely to explain the observed heterozygote deficiencies in the population. It is unknown whether inbreeding represents a deliberate choice of individuals to maximize fitness or the failure of inbreeding avoidance mechanisms like kin recognition. In natural populations, the development of

inbreeding is expected when avoidance costs are higher than those of tolerance. Inbreeding has been found in marine predators like dolphins that are able to recognize their kin (Frère *et al.* 2010). However, no studies have found any evidence of inbreeding in any elasmobranch species, even in critically endangered smalltooth sawfish (mean IR = -0.02 ; Chapman *et al.* 2011). Chapman *et al.* (2011) suggested that life history and reproductive behaviour of elasmobranchs may reduce their susceptibility to inbreeding. Contrary to these findings and hypotheses, we show that a relatively high proportion of consanguineous mating occurs within a population of sharks not currently in decline or subjected to any fishing pressure. While the causes of the inbreeding remain obscure, the evidence of inbreeding we present suggests the subject shark population(s) from isolated oceanic islands may be vulnerable to a decrease in population size, which could further increase inbreeding.

Sex contribution to dispersal patterns

Our study showed directly the migration patterns of some female sharks, but male dispersal is less clear from our parentage analyses. However, the level of inbreeding found in our study suggests limited male dispersal in our system. We previously observed that during the mating period, males appear to shift social community and join females outside of the group but not distant from their usual range (Mourier *et al.* 2012). This pattern was also found in the sicklefin lemon shark *Negaprion acutidens* in Moorea (Clua *et al.* 2010), with resident males observed at a provisioning site disappearing for the mating period. This suggests that males migrate during the mating season as an attempt to avoid inbreeding. From our parentage analyses, it is difficult to detect males' contribution to the dispersal process in this population as parentage assignment is conducted on juveniles born following a female migration.

Several species of sharks exhibit sex-biased dispersal (Pardini *et al.* 2001; Daly-Engel *et al.* 2012; Portnoy & Heist 2012). For example, contrasting mtDNA and microsatellite inheritance revealed male-biased dispersal between South Africa and Australia in great white sharks (*Carcharodon carcharias*; Pardini *et al.* 2001); however, mature females have also been shown to migrate between locations (Bonfil *et al.* 2005). Males and females may have the same migratory route, but female migrations include reproductive philopatry whereas male migrations only provide an opportunity for long-distance gene flow. Likewise, female lemon sharks (*Negaprion brevirostris*) show philopatry to specific sites for parturition (DiBattista *et al.* 2008), but may move extensively between breeding seasons while

males appear to roam and mate in different pools (Feldheim *et al.* 2002). In several studies, male-mediated dispersal and gene flow have likely facilitated the contemporary connectivity observed among global shark populations, while population genetic differences between sites are enhanced by females requiring coastal habitat (Feldheim *et al.* 2002; Keeney *et al.* 2005; Schultz *et al.* 2008; Portnoy *et al.* 2010; Daly-Engel *et al.* 2012).

Further genetic analyses are required to investigate any potential sex-biased gene flow in this small, site-attached reef shark species. Our study highlights, however, the capability of this species to undertake long-distance migrations.

Conclusion

This study advances our knowledge of shark breeding behaviour. Using parentage assignment between adult and juvenile sharks caught in two nearby islands, we were able to demonstrate that most females migrate to specific nursery areas outside their home range, sometimes even going to another island. Analysis also revealed that at least some migrate to the same nursery over two birthing events. Meanwhile, offspring showed some level of inbreeding. Inbreeding is most likely to occur if individuals do not disperse far, and reproduce close to their site of birth, but it seems unlikely in our population because sharks are able to undergo long migration. Rather, inbreeding in our study likely indicates an overall reduced population size or oriented mating choice. Females represent the vector that transports genes at nursery sites, and their fidelity should define reproductive units. As they are philopatric, males could be the ones dispersing genes between populations. These results emphasize the need for conservation plans for coastal zones where female reef sharks seem to exhibit philopatry during the breeding season. We encourage local vs. broad-scale management of shark nurseries in vulnerable coastal habitat of oceanic islands under development. Effective protection of these essential habitats is critical as human activities may drastically alter or degrade coastal areas commonly used by sharks for pupping.

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Data accessibility

Sampling locations and microsatellite data: DRYAD entry doi:10.5061/dryad.gk7nh.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Parentage assignment of the 61 juvenile blacktip reef sharks with FAMOZ software.

Fig. S1 Example of gestation monitoring of an individual female using the photo-identification technique (Mourier *et al.* 2012).

Supporting Information for online publication only

Supplementary Material 1: Parentage assignment of the 61 juvenile blacktip reef sharks

with FAMOZ software.

Cohort	Juvenile ID	Island	Nursery	Mother ID	Sampling site of the mother	Father ID	Sampling site of the father	Parent pairs
2006-2007	H1-07	Moorea	Haapiti	Tao23	Taotoi			
2006-2007	H2-07	Moorea	Haapiti					
2006-2007	H3-07	Moorea	Haapiti	i1	Tetiaroa (Onetahi)			
2006-2007	H4-07	Moorea	Haapiti	RF11	Tiahura			
2006-2007	H5-07	Moorea	Haapiti	RF11	Tiahura			
2007-2008	H1	Moorea	Haapiti	PT10	Papetoai			
2007-2008	H2	Moorea	Haapiti	Tauo3	Tauotara			
2007-2008	T5	Moorea	Varari			RF3	Tiahura	
2008-2009	Lmot1	Moorea	Motu			PT4	Papetoai	
2009-2010	Pi1	Moorea	Pihaena	Mot21	Tiki			
2009-2010	P6	Moorea	PK23	Op27	Opunohu			
2009-2010	H4	Moorea	Haapiti			Op14	Opunohu	
2009-2010	H5	Moorea	Haapiti	RF4	Tiahura	Tet7	Tetaiuo	RF4-Tet7
2009-2010	H6	Moorea	Haapiti					
2009-2010	H7	Moorea	Haapiti	T45	Nuurua			
2009-2010	H8	Moorea	Haapiti			Tao12	Taotoi	
2009-2010	H9	Moorea	Haapiti	PP1	Mareto	PP7	Mareto	PP1-PP7
2009-2010	H10	Moorea	Haapiti			Mot5	Tiki	
2009-2010	H11	Moorea	Haapiti	RF11	Tiahura			
2009-2010	H12	Moorea	Haapiti			PP2	Mareto	
2009-2010	H13	Moorea	Haapiti					
2009-2010	H14	Moorea	Haapiti	Op35	Opunohu	Op28	Opunohu	Op35-Op28
2009-2010	H15	Moorea	Haapiti					
2009-2010	H16	Moorea	Haapiti					
2009-2010	H17	Moorea	Haapiti					
2009-2010	H18	Moorea	Haapiti					
2009-2010	H19	Moorea	Haapiti			Op3	Opunohu	
2009-2010	H20	Moorea	Haapiti					
2009-2010	H21	Moorea	Haapiti	Tauo3	Tauotara	T10	Varari	Tauo3-T10
2009-2010	T20	Moorea	Varari			Mot23	Tiki	
2009-2010	T21	Moorea	Varari	Tao3	Taotoi			
2009-2010	T22	Moorea	Varari	Mot14	Tiki			
2009-2010	T23	Moorea	Varari					
2009-2010	T24	Moorea	Varari			Mot20	Tiki	
2009-2010	T25	Moorea	Varari	RF1	Tiahura			
2009-2010	T26	Moorea	Varari					
2009-2010	T27	Moorea	Varari			T7	Varari	
2009-2010	T28	Moorea	Varari			She1	Sheraton	
2009-2010	T29	Moorea	Varari			Tet2	Tetaiuo	
2009-2010	T30	Moorea	Varari					
2009-2010	T31	Moorea	Varari	T1-07	Varari			
2009-2010	T32	Moorea	Varari	RF1	Tiahura	Op41	Opunohu	RF1-Op41
2009-2010	T33	Moorea	Varari			T39	Nuurua	
2009-2010	T34	Moorea	Varari			P5	PK23	

2009-2010	T35	Moorea	Varari	T40	Nuurua			
2009-2010	Teru4	Moorea	Hitiaa					
2006-2007	i7	Tetiaroa	Tiaraunu	Mot6	Tiki			
2007-2008	i2	Tetiaroa	Hoa Williams					
2007-2008	i3	Tetiaroa	Hoa Williams			Tao7	Taotoi	
2007-2008	i4	Tetiaroa	Rimatuu					
2007-2008	i5	Tetiaroa	Rimatuu					
2007-2008	i6	Tetiaroa	Hoa Oratera			Mot11	Tiki	
2007-2008	i8	Tetiaroa	Hoa Williams			She3	Sheraton	
2007-2008	i9	Tetiaroa	Hoa Williams					
2007-2008	i10	Tetiaroa	Onetahi	Ma6	Maatea	Tet6	Tetaiuo	
2007-2008	i12	Tetiaroa	Hoa Oratera	Op36	Opunohu			
2008-2009	i13	Tetiaroa	Hoa Oratera	Tao19	Taotoi			
2008-2009	i14	Tetiaroa	Hoa Oratera	Op36	Opunohu	Tao1	Tautara	Op36-Tao1
2008-2009	i15	Tetiaroa	Hoa Williams					
2008-2009	i16	Tetiaroa	Hoa Williams			Teru2	Hitiaa	
2008-2009	i17	Tetiaroa	Hoa Williams					

Supplementary Material 2: Example of gestation monitoring of an individual female using the photo-identification technique (Mourier *et al.* 2012). It was observed with mating scars on March 2009. Then parturition for this female (Op27) occurred between November 25th of 2009 when it was observed pregnant for the last time and January 13th of 2010 when it reappeared empty in its usual home site. One juvenile (P6) that was sampled in its nursery (PK23) on January 1st of 2010 has been assigned to Op27, supporting the information of underwater observations.

