COMMENT

Baiting sharks for marine tourism: Comment on Clua et al. (2010)

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ABSTRACT: A recent study by Clua et al. (2010; Mar Ecol Prog Ser 414:257–266) that looks at the behavioural response of sicklefin lemon sharks *Negaprion acutidens* to underwater feeding for eco-tourism purposes has a number of methodological and semantic problems that complicate the evaluation of the results and raise questions about the conclusions. Main issues are the lack of a control, the use of non-defined terms to characterize observed behaviours, and statements not supported by data. Unwarranted conclusions include the notion that behavioural changes were caused by the human interference, the link between intraspecific aggression and the feeding process, and the loss of genetic variability as a consequence of the aggregating effect of shark feeding.

KEY WORDS: Shark feeding · Behaviour · Intraspecific aggression · Marine tourism · Food provisioning

Attracting elasmobranchs with food for the purpose of marine tourism is a highly controversial issue. There is general agreement that feeding or attracting wildlife with food can have a variety of effects (Orams 2002). In the case of sharks, quantifying the magnitude of these effects has proven difficult because critical baseline data remain scarce (Laroche et al. 2007, Meyer et al. 2009). Whereas using biomarkers to measure physiological responses to provisioning-tourism and effects on health is relatively straightforward (Semeniuk & Rothley 2008, Semeniuk et al. 2009), measuring behavioural responses by directly observing the animals is much more challenging. Clua et al. (2010) have taken on this challenge and present data from a group of sicklefin lemon sharks *Negaprion acutidens* in the South Pacific. Here we point out fundamental methodological and semantic issues with this study that we think complicate the evaluation of the study's results and raise questions about the conclusions.

(1) The collection of presence/absence, abundance or behavioural data of mobile fish using underwater visual census and observation techniques is prone to bias (Samoilys & Carlos 2000, Edgar et al. 2004, Ward-Paige et al. 2010), but this is not acknowledged in Clua et al. (2010). For example, direct observation data of shark behaviour can typically only be collected during a few hours each day, and therefore the majority of the behaviour is not recorded. The use of proxies such as mating scars or bite wounds to measure or quantify behaviour is possible (e.g. Porcher 2005), but relating these proxies with confidence to spatio-temporal causative intra- and interspecific behaviours is challenging, requiring adequate caution when interpreting the results.

(2) The frequent and arbitrary use of non-defined terms is a problem in Clua et al. (2010). Terms such as 'natural behaviour' (p. 257, 258), 'real provisioning' (p. 258), 'atypical dominance behaviour' (p. 262), 'pivotal role' (p. 262), 'exacerbated competition' (p. 263), 'dominant' (p. 263), and 'natural conditions' (p. 263) are neither defined nor referenced. It remains unknown, for example, what the authors consider typical

dominance behaviour and, as a consequence, irreproducible why an observation should be 'atypical dominance behaviour' (p. 262).

(3) It remains unclear how Clua et al. (2010) measured and quantified 'increased aggression' (p. 262), 'M05, which appeared to be old' (p. 262), 'Since males M07 and M18 were dominant in 2005' (p. 263), and 'Aggression increased significantly' (p. 263) and, if at all, against what baseline they compared their observations.

(4) A fundamental problem in Clua et al. (2010) is the lack of a control. Without a comparable group of sharks that are not being fed the authors cannot postulate with confidence that any observed behavioural changes were caused directly by the human interference.

(5) Several statements and conclusions in Clua et al. (2010) are based on anecdotal observations and not evidenced. (a) There are no data that 'link' intraspecific aggression to the feeding process (p. 263). Therefore, the anecdotal observation of intraspecific aggression cannot be interpreted as 'deviant behaviour' (p. 263). (b) The statement of 'an increase in shark abundance over time' (p. 263) is not supported by results. (c) The authors repeatedly mention that there may be loss of genetic variability as a consequence of the aggregating effect of shark-feeding (p. 263, 264). However, these speculations are unsupported; the data do not address any such link. In fact, the authors themselves state that the number of males decreased during the reproductive season (p. 259), suggesting 'temporary migration for mating with females that do not belong to the studied population' (p. 262).

The debate over baiting sharks for marine tourism is largely based on inference, opinion and anecdote, and therefore research on the effects of food-provisioning tourism is much needed. Thus Clua and his coworkers are to be acknowledged for bringing science to this controversial topic. In our opinion, however, the presentation and discussion of the results in Clua et al. (2010) adds more to the debate than to the objectification of the public discourse.

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REPLY COMMENT

Effects of provisioning on shark behaviour: Reply to Brunnschweiler & McKenzie (2010)

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ABSTRACT: Brunnschweiler & McKenzie (2010; Mar Ecol Prog Ser 420:283–284) expressed reservations over the findings of Clua et al. (2010; Mar Ecol Prog Ser 414:257–266), mostly related to the lack of a reference site or a control group in the methodology. In our study, we distinguished between 39 individuals of sicklefin lemon sharks *Negaprion acutidens,* mainly based on photo-identification. Our study was based on the field-survey approach, with time (a continuous variable) as the source of variation, and thus a control group was not necessary. We provide here additional data that support the notion that abundance of lemon sharks on the provisioning site was increasing, both in their number and fidelity. We maintain our conclusion that sicklefin lemon shark provisioning off Moorea Island can continue, but should be more intensely controlled.

KEY WORDS: Field survey approach · Lack of control site · Shark abundance · Site fidelity · Shark conservation

We are grateful to our colleagues J. M. Brunnschweiler and J. McKenzie for their interest in our article about the behavioural response of sicklefin lemon sharks *Negaprion acutidens* to underwater provisioning in French Polynesia (Clua et al. 2010). To their major issues we respond as follows.

(1) Methodology for assessing shark behaviour

Applying reliable methods for assessing shark behaviour is a challenge. We assured the quality of our observations by identifying each individual lemon shark through photo-identification, as detailed in a separate paper (Buray et al. 2009). Our study comprised a relatively low number of sharks (39), while photo-identification allows independent and reliable diagnoses for much larger numbers, e.g. 159 whale sharks *Rhincodon typus* (Meekan et al. 2006) or 194 nurse sharks *Gynglymostoma cirratum* (Castro & Rosa 2005). In that context, the references to Samoilys & Carlos (2000) and Edgar et al. (2004) made by Brunnschweiler & McKenzie (2010, p. X) are irrelevant as those authors discuss biases linked to underwater visual censuses (UVC) of several small-sized, highly mobile and hard to distinguish coral reef finfishes. There is no logical or factual link between the potential biases discussed in these studies and the individual identification of large sharks moving in a restricted area, as in our study. When discussing the biases in

shark observation methodology, Ward-Paige et al. (2010, p.5) even state 'When the goal of a scientific study is to examine relative spatial and temporal differences in the density of a single species surveyed under homogenous sampling conditions, non-instantaneous UVC count data may produce satisfactory information'. This is actually the case for our study.

(2) Arbitrary use of non-defined terms

'Real provisioning' refers to any anthropogenic provisioning of sharks with a sizeable amount of food that they can acquire, and does not rely only on the olfactory attraction of food, usually described as 'chumming' or 'baiting'.

We anticipated difficulties in understanding of some of our statements, when no previous references are available. Therefore, we defined what we considered to be 'atypical dominance behaviour' (Clua et al. 2010, p. 262) with the following sentence: 'As the study progressed, in addition to a strong residency pattern, this shark showed increasing aggression towards its male and female conspecifics and, to a lesser degree, toward divers (N. Buray pers. obs.).' We think this explanation is clear.

For a better understanding of the imminent risk of long-term feeding, we referred to the observed behaviour of lemon sharks over time, see Section (4). Unfortunately, there are no scientific studies fully describing the natural behaviour of lemon sharks, so we had to rely on statements by our team of experienced scientific divers who have accumulated thousands of hours of underwater observations.

(3) Justifying and quantifying 'increased aggression'

At its adult stage, *Negaprion acutidens* cannot be considered to be a gregarious species (Stevens 1984). In contrast to other sharks with a solitary behaviour but a capacity to aggregate and compete, such as white shark *Carcharodon carcharias* (McCosker 1985) or tiger shark *Galeocerdo cuvier* (E. Clua pers. obs.), aggressiveness over food does not occur in the wild for lemon sharks (see Nelson & Johnson 1980). Therefore, we maintain that provisioning sicklefin lemon sharks may create a non-natural source of intra-specific aggressiveness (Clua et al. 2010, p. 263). We are willing to modify this statement only if evidence is presented that *N. acutidens* are prone to natural intraspecific aggressiveness by reason of food in the wild.

The 'lack' of a base line for assessing and quantifying the increasing aggression is a particular aspect of the critique discussed in Section (4) below.

(4) Lack of a control site

Many experimenters compare their 'treatments' with 'controls' and evaluate their data by analysis of variance (ANOVA). Field survey ecologists may use another approach: the observations are carried out along one or several gradients represented by continuous variables, so that there is no need for an independently-set control to draw conclusions (Hurlbert 1984, Legendre & Legendre 1998). In our study, we used this field survey approach with 'time' as a continuous variable. We demonstrated an increase in shark aggregation by plotting the number of shark sightings as a function of time and computing linear regressions (see Fig. 4 in Clua et al. 2010). There is a significant increase in the number of sightings of individual sharks in Groups A, C and D across the 44 mo of the study. As natural aggregation of sicklefin lemon sharks does not exist in the wild (see Section 3), it was not possible to carry out a study at a control site.

(5a) Provisioning and intraspecific aggression

As stated in Section (3), there is no evidence that lemon sharks are aggressive over food in the wild. Therefore, artificial provisioning is most likely adding another cause for intraspecific aggression to the existing competition for mating. Clua et al. (2010) do not directly link the feeding process to an increased competition for mating. However, provisioning, which produces artificial aggregation, during or right after the mating season may raise the level of intra-specific aggression, and may be the cause of obvious wounds on male lemon sharks which are more likely to fight for dominance (N. Buray pers. obs.). Fig 5b in Clua et al. (2010) gives an example of a wounded male; the total number of such wounds, however, was not anecdotal. In the context of our study over 44 mo, which did not focus on intraspecific dominance, we presented the accumulated information on the increased aggression amongst males after the mating season as an incidental result. It was a qualitative analysis that compared observations from different seasons, rather than a conclusion based on quantitative data.

(5b) Increase in shark abundance

In the context of Clua et al. (2010), the term 'abundance' can be understood in different ways: (1) as a purely quantitative term (number of shark *sightings*, not regarding the number of different individuals); (2)

as a purely qualitative concept (number of *different* sharks involved in a given number of sightings); and (3) as a mix of these 2 concepts. We agree that the accuracy of our data does not allow us to use the term 'abundance' in the second or third sense. However, our results (see Fig. 4 in Clua et al. 2010) allow us to use the term for the number of shark sightings. We are pleased to provide here complementary data, not included in our original article, showing that, in addition to an increase in site fidelity, our data also support our hypothesis of a significant increase with time in the number of distinct sharks involved in the sightings (Fig. 1). Both graphs show that the provisioning site was increasingly frequented. From this observation we derived our statement that 'In the case of lemon sharks, their increased site fidelity can have a negative effect on gene flow' (Clua et al. 2010, p. 263).

(5c) Loss of genetic variability

At the end of our discussion (Clua et al. 2010, p. 264), we cautiously stated that 'Because the studied population is small, daily aggregations at the same location could result in increased social interactions and increased mating between close relatives, reinforcing the risk of inbreeding.' We were not saying that there was any present loss. We think readers will be able to appreciate the difference between a 'risk', which includes a probability for not happening, and a 'fact', which does not allow any room for an alternative, and needs to be supported.
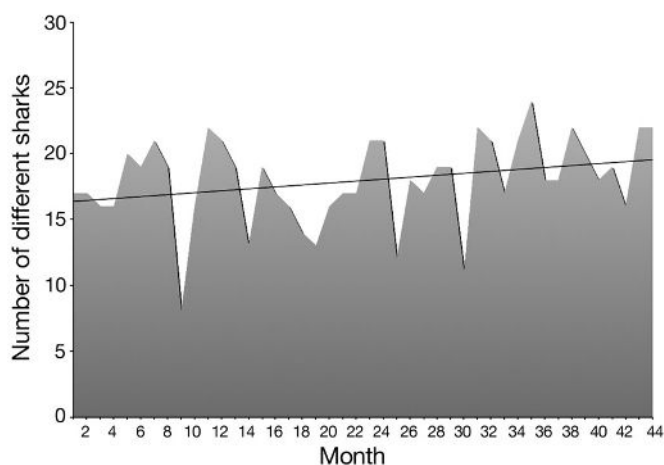

Fig. 1. *Negaprion acutidens*. Number of different sharks contributing to the sightings during the course of the study (44 mo). The line representing the linear trend supports the hypothesis of a significant increase (at the usual 5% significance level) in the number of sharks at the feeding site (1-tailed *t*-test of significance of the slope, p = 0.029).

CONCLUSIONS

Clua et al. (2010) provided (1) reliable information (2) in a specific case (3) about a controversial subject. The consideration of provisioning as an asset for conservation of endangered species (Bookbinder et al. 1998, Halpenny 2003), however, does not automatically allow the results of Clua et al. (2010) to be generalised to other feeding sites or shark species. We concluded that sicklefin lemon shark provisioning off Moorea Island can continue but should be better controlled. Our recommendations were specifically addressed to the French Polynesian management authorities (Clua et al. 2010, p. 266). At this stage, we are not advocating a ban on shark feeding. We are currently assessing the direct income generated by lemon shark ecotourism and its importance for the local economy of Moorea Island. This information should convince fishermen of the benefits of shark conservation (Clua et al. unpubl.). However, shark-feeding management still requires studies addressing a variety of subjects and situations, hopefully including further contributions by our colleagues J. M. Brunnschweiler and J. McKenzie.

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Underwater photo-identification of sicklefin lemon sharks, *Negaprion acutidens*, at Moorea (French Polynesia)

by

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ABSTRACT. - Shark feeding is a controversial recreational activity that may alter shark behaviour. In order to investigate possible behavioural changes at the level of the individual, it is necessary to recognise each shark underwater and in a non-intrusive way. In this study, we tested a protocol based on natural marks on fins, and coloured spots and scars on the body to differentiate individual sicklefin lemon sharks. We found that a feeding group, aggregated for 26 months at a northern location off Moorea Island, comprised 32 animals (19 females and 13 males), identified from 2589 observations made over 541 dives. Post-dive photo-identification of individual sharks was a reliable technique, whereas a high level of skill was required to ensure an instantaneous identification underwater. However, direct underwater identification of individual sharks can be of potential use in shark behavioural studies.

RÉSUMÉ. - Photo-identification des requins limon faucille, *Negaprion acutidens*, à Moorea (Polynésie française).

Le nourrissage des requins (communément appelé 'shark feeding') est une activité récréative controversée qui peut altérer le comportement des requins. Afin d'étudier les possibles changements de comportement des individus, il est nécessaire d'être capable de reconnaître chaque requin en plongée sans leur porter préjudice. Dans cette étude, nous testons un protocole utilisant les marques naturelles sur les nageoires, ainsi que des taches colorées et des cicatrices présentes sur le corps des requins afin de différencier chaque requin limon faucille, *Negaprion acutidens*, à Moorea en Polynésie française. Nous avons observé qu'un groupe de requins s'étant rassemblé pendant 26 mois sur un site de nourrissage situé dans la partie nord de l'île de Moorea, était composé de 32 animaux (19 femelles et 13 mâles) identifiés à partir de 2589 observations réalisées à l'issus de 541 plongées. L'analyse des clichés de retour de plongée s'est avérée une technique efficace, alors que des compétences spécifiques ont été requises pour garantir une identification instantanée en plongée. Cependant, une identification directe en plongée de chaque individu peut permettre d'effectuer des études sur le comportement des requins évoluant dans leur milieu naturel.

Key words. - Carcharhinidae - *Negaprion acutidens* - Sicklefin lemon shark - French Polynesia - Moorea Island - Shark feeding - Sighting-resighting - Visual identification.

Although the Pacific lemon shark *Negaprion acutidens* (Rüppell, 1837) was described before the Atlantic species *Negaprion brevirostris* (Poey, 1868), very little information about its biology and behaviour are available, except some data on growth and reproductive biology (Stevens, 1984; Dulvy and Reynolds, 1997) and nutrition (Salini *et al.*, 1992; White *et al.*, 2004). Meanwhile, the Atlantic species has been specifically and intensively studied over the past twenty years, in particular by Gruber and collaborators both in Caribbean (Gruber *et al.*, 1988; Dibattista *et al.*, 2007) and Brazilian waters (Freitas *et al.*, 2006). The sicklefin lemon shark is a widely distributed Indo-Pacific coastal shark that ranges from Eastern Africa to French Polynesia. The species is of long-standing commercial interest for human consumption (Compagno, 1984), but more recently it has also become one of the most impressive focal species in shark feeding activities in the central and eastern Pacific. Except for a study by Scharfer (2003) there are no scientific studies that deal with the controversial recreational activity, particularly in relation to critical issues such as increased potential risk of attacks on humans and the effect of feeding on the biology of shark populations. For countries such as French Polynesia that are concerned by the controlled development of feeding as an attractive tourist activity, but also by the potential effects on shark populations, lemon sharks in particular, there is a need for objective data. Collection of these data depends to a large extent on the capacity to identify individual sharks. Photo-identification has shown great potential, despite some inconveniences linked to its technical implementation (Cailliet, 1996). This technique was used for marine mammals such as humpback whales (Katona *et al.*, 1979), dolphins (Mizroch and Bigg, 1990; Markowitz *et al.*, 2003; Mazzoil *et al.*, 2004), for basking sharks (Sims *et al.*, 2000) and recently for marine crustaceans (Frisch and Hobbs, 2007). Shark photo-

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identification is usually based on spot or colour patterns and scars on specific areas of the body, such as recently described for white sharks (Domeier and Nasby-Lucas, 2006), and whale sharks (Meekan *et al.*, 2006; Graham and Robert, 2007). Bansemer and Bennett (2008) recently tested the use of spots on flanks of *Carcharias taurus* to identify individuals and to monitor shark populations. Compared to these species, the lemon shark presents the inconvenience of being of a relatively uniform yellow colour. However, Castro and Rosa (2005) faced the same problem with nurse sharks in Brazil and used natural marks on fins for successful estimation of their population.

In this paper, we test whether natural marks on fins, plus coloured spots and scars on sicklefin lemon sharks are suitable for photo-identification of individual shark in a feeding-related aggregation off Moorea Island (French Polynesia). If successful, this first step will allow useful data to be collected and used in a potential behavioural study of this population.

MATERIAL AND METHODS

Study area

The study was conducted at Moorea Island, French Polynesia (17°30' S; 149°51' W). Moorea authorities implemented a Management Plan for Marine Environment (Plan de gestion de l'espace maritime - PGEM) in October 2004 which included zoning different human activities. Two zones
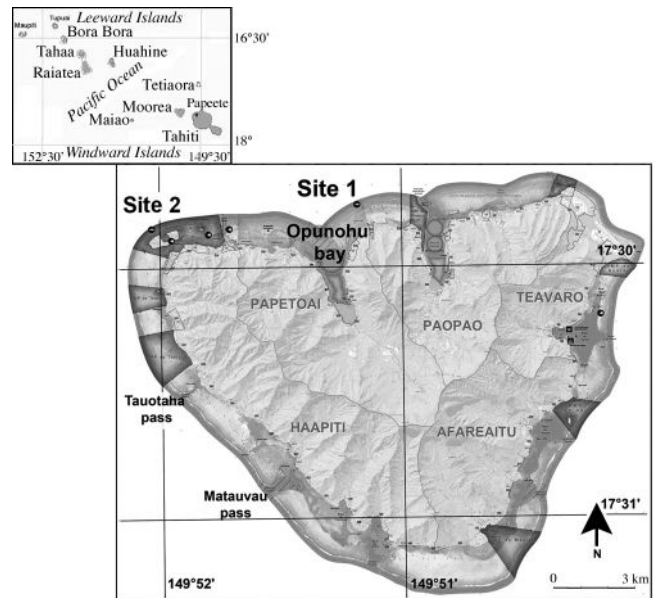

Figure 1. - Location of shark feeding sites in Moorea Island (French Polynesia). *[Localisation des sites de nourrissage des requins sur l'île de Moorea (Polynésie française).]*

were defined in Papetoai (site 1) and Haapiti (site 2) for shark feeding activities (Fig. 1). Our specific study area is site 1 which encompasses a surface of 2.2 km in length (149°50.67' to 149°51.39' W) and 100 m in width, on the outer slope from 5 to 25 m of depth. Shark feeding activities in Moorea started in 1999 with three diving centres, and only one was still operating from 2004 to 2007.
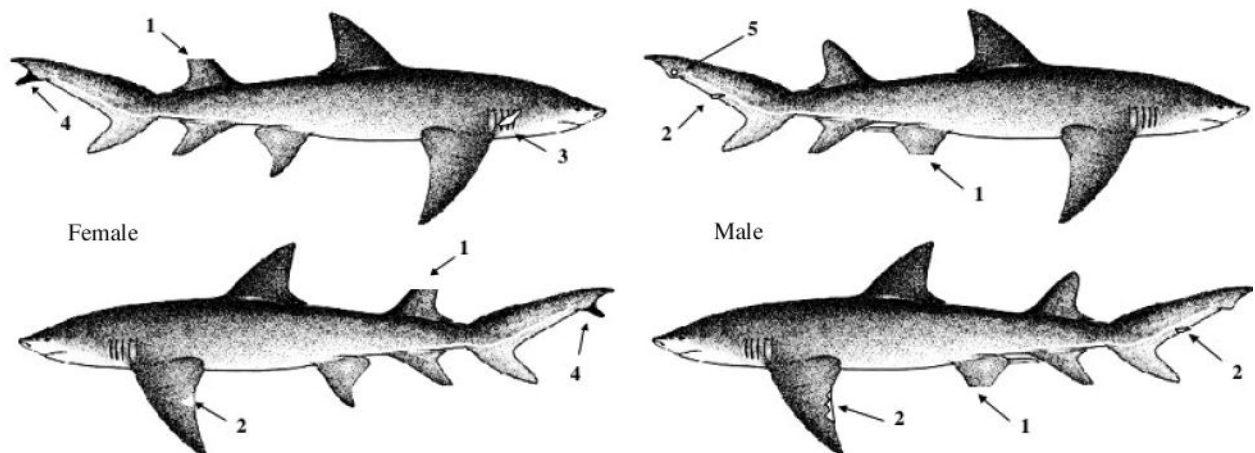

Figure 2. - Example of individual schemes for visual identification of sharks on predefined templates. Left: female with (1) a truncated apex of the second dorsal fin, (2) a notch on the left pectoral fin, (3) a scar on gills on the left side and (4) an extended subterminal notch on the caudal fin. Right: male with (1) a truncated apex of the left pelvic fin, (2) cuts in the median zone of the posterior margin of the caudal fin and on the posterior margin of the left pectoral fin, and (5) a white spot on the right side of the subterminal notch of the caudal fin. *[Exemple de fiches individuelles pour l'identification visuelle des requins sur des patrons prédéfinis. A droite: une femelle avec (1) l'apex de la seconde nageoire caudale tronqué, (2) une encoche sur la nageoire pectorale gauche, (3) une cicatrice au niveau des fentes branchiales gauches et (4) une expansion de l'encoche subterminale sur la nageoire caudale. A gauche: un mâle avec (1) l'apex de la nageoire pelvienne gauche tronqué, (2) des coupures sur la zone médiane de la marge postérieure de la nageoire caudale et de la nageoire pectorale gauche, et (5) une tache blanche sur le côté droit de l'encoche subterminale de la nageoire caudale.]*

Table I. - Criteria for visual and photo-identification of the lemon shark population at site 1 off Moorea Island. RF: Right fin; LF: Left fin; RS: Right side; LS: Left side; BS: Both sides; PM: Posterior margin; AM: Anterior margin; D1: First dorsal fin; D2: Second dorsal fin; SN: Subterminal notch; SE: Skin excrescence; TA: Truncated apex; MA: Missing apex; CF: Caudal fin; AF: Anal fin; PF: Pelvic fin; MZ: Median zone; UZ: Upper zone; LZ: Lower zone. [*Critères d'identification visuelle et photographique de la population du requin limon faucille sur le site 1 sur la pente externe de l'île de Moorea. RF : Nageoire droite ; LF : Nageoire gauche ; RS : Côté droit ; LS : Côté gauche ; BS : Des deux côtés ; PM : Marge postérieure ; AM : Marge antérieure ; D1 : Première nageoire dorsale ; D2 : Seconde nageoire dorsale ; SN : Encoche subterminale ; SE : Excroissance cutanée ; TA : Apex tronqué ; MA : Apex manquant ; CF : Nageoire caudale ; AF : Nageoire anale ; PF : Nageoire pelvienne ; MZ : Zone médiane ; UZ : Zone dorsale ; LZ : Zone ventrale.*]

ID	Criterion 1		Criterion 2				Criterion 3	
	Gender	Length (m)	Caudal fin	Dorsal fins	Pectoral fins	Anal and pelvic fins	Spots	Scars
F01	FEMALES	2.8			RF 2 cuts PM	TA on AF		RS 1 vert. on caudal peduncle x 20 cm
F02		2.4	RS SE in MZ		LF 2 cuts PM		LS white MZ	
F06		2.4					LS white after gill slits + RS belly MZ	RS 1 on gill slits
F08		2.3		RF black spot MZ				RS 1 on labial furrows
F11		2.8					BS white CF on SN	LS 1 on eye
F13		2.8		D1 concave PM			RS white flank MZ	
F15		2.4		D2 MA	RF 1 cut PM			
F16		2.6	Notch in LZ		LF 1 cut PM			
F17		2.6		D2 MA + RS black spot	LF 2 cuts PM			
F20		2.5					BS white CF on SN	
F21		2.7		D2 MA	LF 1 cut PM			
F22		2.3					RS 2x2 superposed black spots (before and after gill slits)	
F25		2.5	TA		RF 3 cuts PM			
F26		2.2	SE under SN + cut in MZ		LF 1 cut PM			
F27		2.5					RS white CF UZ	LS flank 1 vert. x 30 cm
F29		2.1		D2 square apex				
F30		2.7		D2 1 vert. 20 cm cut (scar) on Apex			BS white CF on SN	
F32		2.2	Extended SN					
F33		2.7	TA and LS SE in MZ	D2 2 vert. cuts on apex	LF 3 cuts PM			
M03	MALES	2.4		D1 flat apex		RS 3 cuts PM PF		
M04		2.5	Cut median		Bite on LP	LS MA on PF	RS of CF white	
M05		2.5			LF cut PM	LS cut PM PF		BS on gills
M07		2.5	LS 1 SE in LZ		RF SE AM		BS white CF on SN	
M09		2.5	2 SE under SN				LS white CF on SN	
M10		2.3		D2 MA	RF 1 cut PM		BS white CF on SN	
M12		2.5	MA on SN + extra notch below					
M14		2.3	SN missing					
M18		2.5			LF 1 cut PM proximal zone			
M19		2.1	1 SE under SN		LF 3 cut PM			
M28		2.5	Sharp upper apex					
M31		2.2	Round upper apex		RF irregular PM			
M34		2.4	RS 1 SE under SN		RF 1 cut PM	RS PF concave		RS 1 on first gill

Feeding activity

Feeding sessions in site 1 (Fig. 1) were implemented through dives at a depth of 20 to 25 m, which lasted from 60 to 100 min depending on the breathing mixture (either normal air or 33% Nitrox). A feeding session comprised the placement of a small cage on the substrate that enclosed tuna discards, such as heads and fins from 1.5 to 2.5 kg fish. The cage prevented the sharks from reaching and eating the food too quickly, and allowed divers to observe the sharks from a distance of about two to five metres from the cage. The distance between divers and sharks was dependent on the shyness of the sharks. The food was released 15 min before the end of the dive.

Criteria for identification

The first criterion for underwater shark identification was both the gender and the size of the animal. The second criterion relied on characteristics of the fins (caudal, dorsal, pectoral, pelvic and anal). These characteristics can be one or several scars or excrescences of tissue (such as warts) on the surface of the fin, or a marginal loss of tissue on the margins of the fin (after a bite), which evolves into a truncated apex or a single cut depending on its severity. Specific attention was given to the particularities of the subterminal notch located on the top posterior margin of the caudal fin. The third criterion relied on scars or coloured spots on the body of the shark (excluding fins). Each criterion was compiled on waterproof sheets with shark scheme template during the underwater observation, as shown in figure 2. The side of the body was also recorded, as unilateral observations may not provide enough information to identify a particular individual.

Data collection

The data were collected during 541 feeding sessions which were implemented during 26 months between October 2004 and February 2007. The diver in charge of providing the food to the sharks was equipped with a 3 megapixel digital camera (SONY and Ikelite housing). Following an opportunistic approach, images of the entire body and of specific areas of the sharks were taken during each dive.

RESULTS

The dives at site 1 allowed 2589 observations. 32 sharks were distinguished, of which 19 were females and 13 were males, based on morphological characteristics in order of priority: caudal fins, dorsal fins, pectoral fins, anal and pelvic fins, plus the potential presence of coloured spots or scars on the body (Tab. I). Specific and visual examples of the criteria used such as truncated apex, notches, scars, spots or skin excrescences are given in figure 3.

DISCUSSION

Photo-identification strictly based on colour patterns has been shown to be reliable if the focal animals have at least two distinct colours on specific areas of their bodies, such as the design of the white colouration on the ventral side of the fluke in sperm whales (Katona *et al.*, 1979), the extension of the white colouration on the gill flaps of white sharks (Domeier and Nasby-Lucas, 2006) or the spots and stripes near the posterior gill slit of whale sharks (Meekan *et al.*, 2006). Data analysis with software that uses information-theoretic scores of match parsimony provides a very reliable method for mark-recapture studies without resorting to invasive marking techniques (Speed *et al.*, 2007). However, automated spot-recognition programs used for analysis of photo-identification require natural, significant and extended differences in the colour patterns of the studied animals. In the current study, white or black spots did occur on what were otherwise homogeneously coloured lemon sharks, but the frequency of occurrence of spots was low and precluded their use as the main basis for shark identification as it was used for other species with uniform body colouration (Bansemer and Bennett, 2008). In addition, although these spots were useful on a temporal scale of several weeks or months, they showed a poor resilience over years. The efficiency of our method mainly relied on the observation of natural marks, similar to the approach used to assess a population of nurse sharks in Brazilian waters (Castro and Rosa, 2005). Therefore, in contrast to most photo-identification methodologies so far implemented (Domeier and Nasby-Lucas, 2006; Meekan *et al.*, 2006; Speed *et al.*, 2007), colour considerations were only a complementary and secondary criterion.

The majority of marks, other than natural spots, were consistent with shark-inflicted bite injuries, considered to result primarily from intra-specific interactions, and external injuries inflicted by hooks. These marks were observed to vary with time. Given the ability of sharks to regenerate tissue (Reif, 1978; Domeier and Nasby-Lucas, 2006), in particular carcharhinids (Heupel *et al.*, 1998), the quantity of tissue lost is of major importance in the longevity of visible wounds useful for shark identification. There were several examples observed in our study that showed this regenerative characteristic of sharks. A male lemon shark was photographed in October 2004 with a 20 cm vertical laceration, but with no loss of tissue, on the second dorsal fin. This injury had healed significantly within two months by December 2004 and was difficult to distinguish a year later in October 2005 (Fig. 4). However, based on our study we found that when there was a significant tissue loss, such as fin notches and truncated apices of fins, these did not change as markedly over time and they were reliable features for individual shark recognition over extended periods of time. This obser-
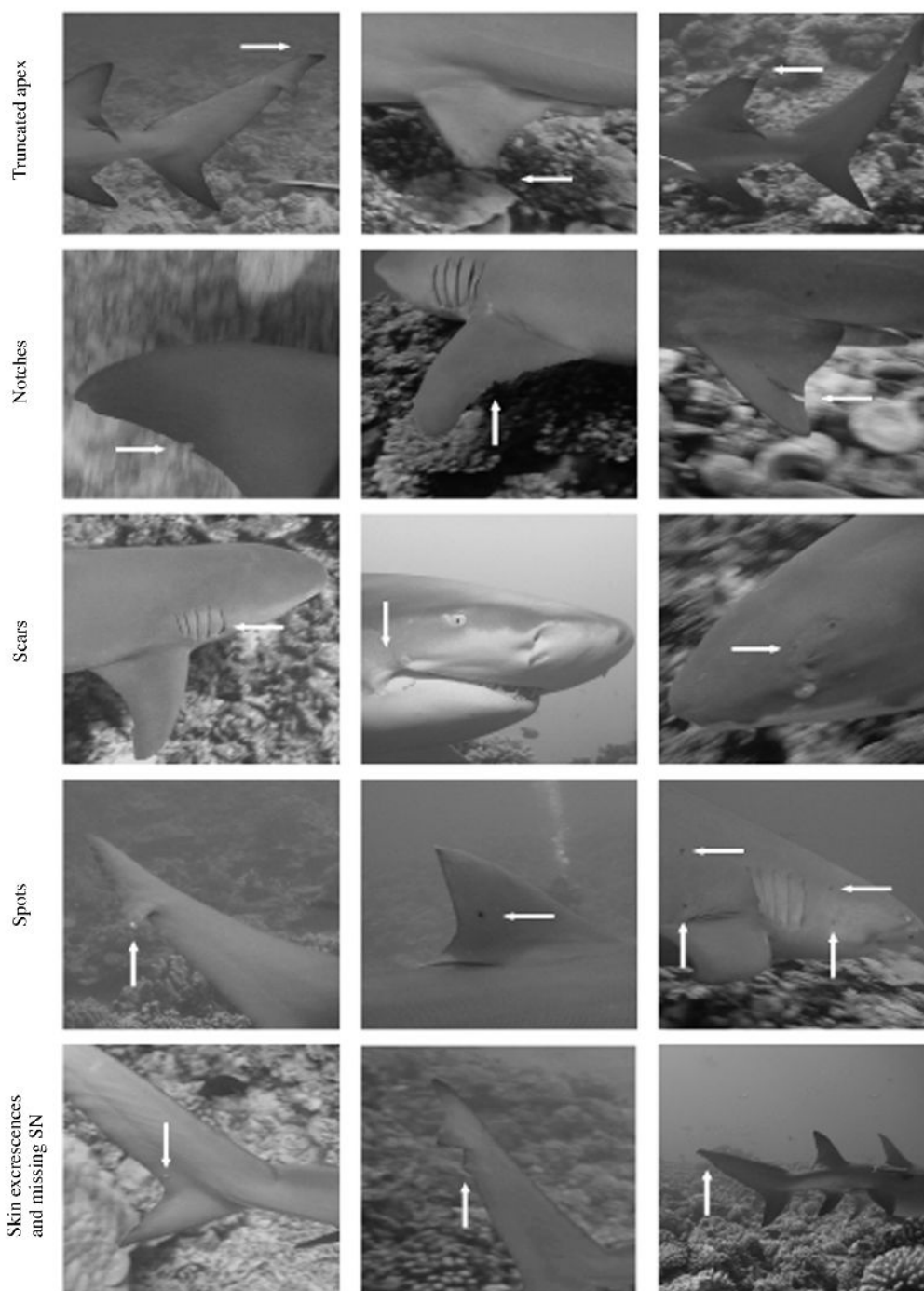
Figure 3. - Examples of visual characteristics used for shark identification. From top to bottom and left to right : Truncated apex : F25 (Caudal fin), M04 (Left pelvic fin), F15 (Second dorsal fin); Notches on fins : F02 (Left pectoral fin), M05 (Left pectoral fin), M05 (Left pelvic fin) ; Scars : M31 (Gills , left side), F08 (Mouth, right side), F21 (Top of the head, left side); Spots : F20 (White on subterminal notch, right side), F08 (Black on dorsal fin, right side), F22 (Black around gills, right side); Others : F02 (Skin excrescence on the median zone of the caudal fin, right side), M19 (Skin excrescence below the subterminal notch, posterior margin), M14 (Absence of the subterminal notch). *[Exemples de caractéristiques visuelles utilisées pour l'identification des requins. De haut en bas et de gauche à droite : Apex tronqué : F25 (Nageoire caudale), M04 (Nageoire pelvienne gauche), F15 (Seconde nageoire dorsale); Encoches sur les nageoires : F02 (Nageoire pectorale gauche), M05 (Nageoire pectorale gauche), M05 (Nageoire pelvienne gauche) ; Cicatrices : M31 (Fentes branchiales , côté gauche), F08 (Bouche au niveau du sillon labial, côté droit), F21 (Au dessus de la tête, côté gauche); Taches : F20 (Blanche sur l'encoche subterminale, côté droit), F08 (Noire sur la nageoire dorsale, côté droit), F22 (Noire autour des fentes branchiales, côté droit); Autres : F02 (Excroissance de peau sur la zone médiane de la nageoire caudale, côté droit), M19 (Excroissance de peau sous l'encoche subterminale, marge postérieure), M14 (Absence d'encoche subterminale).]*
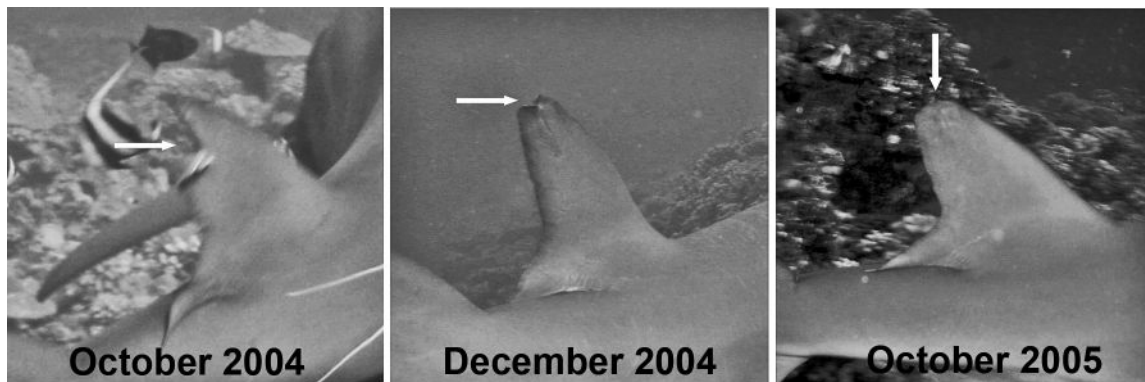
Figure 4. - Progressive and rapid healing of a 20 cm vertical cut on the second dorsal fin of the male lemon shark M10 from October 2004 to October 2005. *[Cicatrisation progressive et rapide d'une entaille verticale de 20 cm sur la seconde nageoire dorsale du requin limon faucille mâle M10 d'octobre 2004 à octobre 2005.]*

vation is in contrast to that of Pratt and Carrier (2001) who found that the natural marks in nurse sharks healed rapidly, grew or changed in time.

Our results suggest the recognition of individual sicklefin lemon sharks was best achieved primarily through the use of wound occurrence, particularly to the fins, augmented by the occurrence of natural spots on the body. Unambiguous identification of individual sharks was generally possible after a dive, based on analysis of images. The ability to quickly identify any of the 32 lemon sharks while underwater at our study site was also possible, but required a high level of experience. As behavioural data collection necessitates instantaneous recognition of individuals underwater the specific skills that our team has acquired, coupled with the possibility to regularly observe these sharks throughout the year, provides us with an excellent opportunity to assess the long term effects of feeding on a coastal shark population.

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Business partner or simple catch? The economic value

of the sicklefin lemon shark in French Polynesia

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Abstract

Most arguments invoked so far by the scientific community in favour of shark conservation rely on their ecological importance, which have little impact on management policies. During a 57-months study, we were able to individually recognize 39 sicklefin lemon sharks which support a shark-feeding ecotourism activity in Moorea Island, French Polynesia. We calculated the direct global revenue generated by the provisioning site, based on the expenses of local and international divers. The total yearly revenue was around US$5.4 million and the 13 sharks most often observed at the site had an average contribution around US$316,699. Any one of these sharks represents a potential contribution of US$2.64 million during its life span. We hypothesize that publicizing economic values per individual will be more effective than general declarations about their ecological importance for convincing policy-makers and fishers that a shark alive is more valuable than a dead shark for the local economy. Studies monitoring the potential negative ecological effects of long-term feeding of sharks should however be conducted to ensure these are also considered. Besides declarations about the non-consumptive direct use value of sharks, as promoted by ecotourism, the calculation of their other economic values should also benefit shark conservation.

Introduction

There is a general consensus that the ongoing decimation of shark populations by commercial fishing is putting many species in danger of extinction (Baum *et al*. 2003; Clarke *et al*. 2006; Ferretti *et al*. 2008). The situation is alarming because of the critical role that sharks play as top predators in marine ecosystems and the potential consequences of their depletion through cascading effects (Stevens *et al.* 2000; Sergio *et al.* 2006, Heithaus *et al*. 2008, Baum and Worm 2009). As an example, the decline in abundance of 11 shark species along the east coast of North America has increased cownose ray (*Rhinoptera bonasus*) populations, which significantly impacted scallops on which they feed (Heithaus *et al.* 2008). In reef systems, the absence of apex predators has a significant impact on the total fish biomass and the structure of the trophic pyramid, altering the health of the ecosystems (Friedlander and De Martini 2002). In spite of this well-demonstrated role, and the arguments of the scientific community in favour of shark conservation, the situation of their populations around the world has not improved. At the moment, 17% of the shark and ray species are listed as Critically Endangered, Endangered, and Vulnerable categories of the International Union for Conservation of Nature Red List (IUCN 2008) and a further 47% are listed as "data deficient" (Polidaro *et al*. 2008).

The extractive activity of fishing relies on the market value for consumption, mainly motivated by the shark fin trade (Brierley 2007). Estimates of the world annual revenues from the fin trade (including the sale of shark carcasses and other by-products), ranges from US$400 million to US$550 million (Clarke *et al*. 2007). However, sharks have other attractive economic values as was shown through the development of ecotourism of the whale shark, *Rhincodon typus*, in the Republic of Seychelles (Rowat and Engelhardt 2009), and in Australia where the world's first whale shark tourism industry was established in 1989. In 2006, tourists' expenditure in the region of Ningaloo reef (Western Australia) was US$758 per trip, and the total expenditure was US$5.9

million (Catlin *et al*. 2009). Recreational activities in South Africa, focussing on snorkelling in the Aliwal Shoal region (KwalaZulu Land) with tiger sharks *Galeocerdo cuvier*, generated around US$1.62 million per year (Dicken and Hosking 2009). In the Republic of the Fiji Islands, Brunnschweiler (2009) showed that, in addition to the direct income of the two diving centres involved in the ecotourism activity, a shark feeding site was providing the eligible Fijian villages around Beqa Island (South Viti Levu) with around US$60,000 per year in compensation for not using the marine reserve where bull sharks, *Carcharhinus leucas*, are aggregated. However, in these studies, the economic valuation was conducted globally, with no specific references to the number of sharks, and no study has ever been done of the economic value of individual animals.

Marine mammal conservation is supported by the worldwide whale-watching industry; the economic value of that industry was estimated at US$160 million in the US in the late 1990s (Hoyt 2001). By attaching economic value to wildlife and educating tourists about the need for conservation, researchers can use wildlife tourism as leverage for the conservation of vulnerable and endangered species (Bookbinder *et al*. 1998; Higginbottom *et al*. 2003). Nowadays, whale-watching takes place on all continents and in more than 500 communities in over 70 countries, targeting over 600 regional populations of cetaceans (Hoyt 2007). Any information promoting a similar development for the benefit of shark conservation appears timely. In French Polynesia, shark fishing was officially banned in 2006 (Arrêté 396/CM, 28 April 2006), but poaching and attempts to cull sharks by local fishers are still seen (see Accessory Publication). This process could jeopardize the ecotourism activity, as occured in the Republic of Maldives where fishers caused diver operators to suspend their activity, resulting in a loss of US$500,000 per year for the local economy; the fishers removed about 20 grey reef sharks *Carcharhinus amblyrhynchos* which had a market value of only about US$1,000 (Anderson and Waheed 1999). Given the audience of fishers and resource managers, this goal can be better met by providing information for individual sharks instead of a shark population in general.

In this paper, we calculated the direct revenues to Moorea Island, French Polynesia, derived from tourist divers visiting a sicklefin lemon shark *Negaprion acutidens* feeding site. Based on a long-term study where 39 lemon sharks involved in ecotourism activity were individually identified and followed through time, we were able to estimate the contributions of individual sharks to the overall income, and to assess their potential contribution throughout their life span.

Materials and Methods

Study of the shark-feeding activity in Moorea Island

Shark-feeding consists of using a small amount of natural bait to attract and aggregate animals at a given site where divers can observe them. This type of activity began in Moorea Island, in the Society Islands Archipelago, French Polynesia, in the late 1980s. Under public pressure regarding the risk of increasing aggressiveness toward humans, local authorities limited shark-feeding activities to two zones on the outer slope of the reef, and banned provisioning of sharks in the lagoon where most human activity is concentrated. The two zones where provisioning is allowed are located in the northern part of the island, close to several international seaside resorts. Our specific study area was at the diving spot called "Opunohu" (17°30' S; 149°51 W), on the outer slope off Opunohu Bay, which was selected for its abundance of sicklefin lemon sharks *Negaprion acutidens*. This tropical coastal species is very attractive in the context of ecotourism because its large size impresses tourists; *N. acutidens* can reach 3.5 m in total length (TL) (Compagno 1984). Although they usually display a shy and solitary behaviour, lemon sharks can aggregate in significant numbers through feeding (up to 15 animals in French Polynesia) (see Accessory Publication). Five different diving centres bring tourists daily to the Opunohu site, while only three feed the sharks. For almost five years (57 months), from January 1st, 2005 to

September 31st, 2009, we monitored the feeding sessions implemented by the TopDive Center in the presence of tourist divers, at depths of 20 to 25 m; each session lasted 60 to 100 min. Sessions consisted of hiding a small cage containing tuna heads and flesh on the substrate to lure and keep the sharks around to ensure close observation. The food was released at the end of the dive. Data on presence/absence of individual sharks were recorded for each dive using photo-identification, the size and gender, and natural marks on shark bodies such as scars, notches, missing tissues or coloured spots (Buray *et al*. 2009).

Calculation of the direct non-consumptive use value of sharks

After the seminal study of Costanza *et al*. (1997) valuing the world natural capital and ecosystem services, the concept of Total Economic Value (TEV) was developed, in particular through the Millenium Ecosystem Assessment (MEA 2005). Following this methodology, the economic value of an entire ecosystem or a single species can be divided between use and non-use values. The former can be split into non-consumptive and consumptive direct use values, indirect use values, and option values. The non-use values are shared between existence and bequest values (Fig. 1). While fisheries focus on the consumptive direct use value of sharks, the development of ecotourism activities involving sharks fits into the direct non-consumptive use values. For assessing this value, we collected data on the total expenditures that can be directly linked to the purchase of a dive allowing visitors to watch lemon sharks in their natural environment. Divers were divided in two groups composed of local divers (who do not need to come to Moorea since they live there) and international divers (who must pay at least for accommodation and food in addition to the diving fees). For the local divers group, the expenditure is limited to the diving fees. For the international divers group, expenditures encompass the average cost for a diving session (which includes two dives), one night before and one night after diving, four meals and, if

they came to Moorea purposely to see sharks, a return flight ticket between Tahiti Island (where international flights land) and Moorea Island. Based on this approach, we obtained a total expenditure that we combined with lemon shark information in three ways: we first calculated an average value per shark by dividing this amount by the number of sharks that contributed to the attractiveness of the Opunohu site; we then did a more accurate calculation by dispatching this same amount per individual shark, based on the visitation of the Opunohu site by individual sharks; and finally, based on the sicklefin lemon shark life history traits, we calculated the average economic value of a shark for its life span, with a yearly discount rate of 8%.

Assessment of the diving activity in Moorea Island and the attractiveness of sharks for divers

French Polynesia is a well-known destination for tourism, with a strong reputation in the specific market of "honeymooners"; in 2009, 160,447 tourists visited this French territory which has a total resident population of 259,600 people (IEOM 2010). To obtain an accurate number of dives per year at the Opunohu site, where the lemon sharks are aggregated through provisioning, we contacted the five diving centres that take divers to this location. We requested figures about the total number of dives at the Opunohu site between 2005 and 2008 (four years), including information about the origin of divers (either local or international). We then calculated the average number of dives as the mean for the four years. The importance of the lemon sharks in the divers' decision to purchase a dive from one of the five diving centres was assessed through a survey conducted by sending emails to over 300 divers who had had shark experiences with the TopDive Center asking: did the potential presence of sharks during the dive play a positive role in: i) your choice for coming purposely to dive in Moorea? ii) purchasing a dive at the Opunohu site while you already were in Moorea (for another reason than to specifically watch sharks)? iii)

the quality of your dive experience? The divers were also asked to mention their country of residence. If they were living in Moorea, they were asked to answer only the last two questions.

Results

Direct income generated by lemon sharks

The five diving centres bringing divers to the Opunohu site for the four years totalled 61,050 person-dives in this location, for an average of 15,262 divers per year (SD = 3,158). A total of 151 persons answered the email survey, comprising 26% of local divers and 74% of international divers. Amongst the international divers, 27% of them had came purposely to Moorea for shark watching and 68% of the remaining ones did not originally come to Moorea for sharks but made the decision to dive with sharks during their stay; 100% of the local divers declared that sharks had significantly contributed to the decision of purchase a dive. Based on these proportions, we estimated a total of 3,968 local divers, 3,049 international divers per year who came purposely to Moorea to see sharks, and a total of 5,606 international divers who made the decision in Moorea to go and dive with sharks.

A diving session comprises two dives, and costs US$150 for an international diver and US$125 for a local diver. The average cost for a night in Moorea is around US$186 (including breakfast), an average meal (either lunch or dinner) costs US$26 and the two-way ticket from Tahiti to Moorea costs US$62. The cost of transportation on the island (taxis, car rental) is not included in our calculations. The average expenditure for an international diver who purposely came for shark watching is around US$688; it drops down to US$626 if the choice of diving for shark watching was made in Moorea (as we do not include the cost of airfare). If we multiply these figures with the number of international and local divers mentioned above, we obtain a total annual generated

income around US$248,015 for local divers and US$5,156,349 for international divers, respectively. The total amount is US$5,404,365.

Individual and life-span economic value of sharks

We studied 1,013 diving sessions that allowed 6,415 observations of 39 individual lemon sharks. The contribution of each shark to the total revenue is estimated by the relative importance of its participation to the feedings. It varied from 8.77% (563 sightings) of the total occurrences for the most regular animal, down to 0.02% for a female shark that showed up only once (Fig. 1). The 13 most commonly occurring animals, which represent 33% of the population, accounted for 73% of the occurrences and were considered to be resident sharks. Based on the total revenue of US$5.404 million, their decreasing contributions depending on their sightings range from US$474,304 to US$180,286 (Table 1). These 13 sharks at the site had an average contribution of US$316,699.

In the absence of accurate data on the life history of the sicklefin lemon shark, we used the life history traits of the Atlantic lemon shark, *Negaprion brevirostris*. A growth curve gives an asymptotic maximum age of 27 years for this species, with sexual maturity reached around 11 years of age (Brown and Gruber 1988), corresponding to the average size of 2.4 m TL. Given that the youngest sharks observed at the feeding site were around 2.1 m TL (which corresponds to an age of about 7 years), we hypothesize that a shark could remain associated with to a feeding site for 20 years. Based on the average contribution of a shark in the present study of US$138,573 (equal to the mean yearly financial contribution of the 39 sharks), a lemon shark would represent a direct non-consumptive use value for its entire life of US$2.64 million in the present context of Moorea Island.

Discussion

Individual and life-span economic values

Diving with sharks is estimated to have contributed millions of dollars to local and regional economies for decades. The annual revenues per site or country range from US$2.2 million to US$7.4 million depending on year and region (Table 2). Our findings of US$5.4 million for Moorea, a single island of French Polynesia, are consistent with these figures. However, in the previous studies reported in Table 2, the economic valuation was globally conducted, with no specific reference to the number of sharks. No study had ever been done of the economic value of individual sharks. Identification of individual sharks allowed us to estimate their individual contributions to the total revenue. A parallel study on the effects of shark feeding on the behaviour of these sicklefin lemon sharks allowed us to demonstrate a significantly increasing residency pattern, in particular for the 13 most commonly sighted sharks on which the diving tourism relies (Clua *et al*. 2010). In addition to assessing a total revenue for Moorea Island, we were able to show that a single resident lemon shark can generate up to US$474,000 per year in revenue and is worth up to US$2.64 million for the local economy during its entire life. We believe that this type of information is critical, and more efficient than total figures, in the process of advocating shark conservation, in particular for local and coastal fishers. The objective is to convince them and other resource users that a shark alive is more valuable than a dead shark for the local economy, which can obtain continuous direct and indirect income from live sharks. This process will never be fully successful until some compensatory mechanism is established that allows all stakeholders, in particular fishers, to benefit indirectly from ecotourism revenues (Wilson 2003; Dobson 2006).

There are two other islands with similar international reputations for shark diving tourism in French Polynesia: Bora-Bora and Rangiroa Islands. Sharks and their potential for attracting tourists appear to be an important asset to be preserved, mainly in the context of a declining tourism industry, such as in French Polynesia which faced a drop of 29% in activity between 2006 and 2009 (IEOM 2010). The shark tourism industry in the Polynesian islands and elsewhere should benefit from our findings.

Monitoring the potential negative effects of feeding sharks

The calculation of the economic value of sharks will help demonstrate that the non-consumptive wildlife tourism in better assisting the conservation of vulnerable and endangered species, by attaching economic value to wildlife, which speaks to decision makers, and by educating tourists about the need for conservation (Halpenny 2003; Higginbottom *et al*. 2003; Topelko and Dearden 2005). However, care must be taken to anticipate and alleviate possible negative effects of shark diving ecotourism on shark behaviour, habitat and ecology (Orams 2002; Walker 2002). Operators who specialize in wildlife-based tourism tend to be characterized by small and medium-sized enterprises that are often locked into dependence relationships with external creditors. Therefore, issues of profitability will often take precedence over issues of environmental protection (Issacs 2000; Williams and Montanari 1999). In the best cases, the balancing of anthropocentric and biocentric concerns in the management of shark-based tourism seems to often result in compromises that do not provide full support to conservation (Dobson 2006). Given the lack of knowledge on these issues, which has been one of the major pitfalls in the assessment of marine ecotourism (Garrod and Wilson 2004), studies about the effects of tourism activity upon the health and well-being of the target species must be developed, to ensure that the entertainment of tourists will not prevail on the well-being and reproduction of wild animals (Newsome and

Rodger 2008). Our preliminary results from Moorea Island in 2005 show a risk of behavioural shift for the lemon sharks (Clua *et al*. 2010). In spite of some movements of males and females, probably related to mating, the general trend is that residency significantly increased during the study, showing that inbreeding is at least a risk due to the reduction of shark mobility, in particular for males. The lack of genetic variability would decrease the resilience of the lemon shark population. This issue leads to rejecting any proposal or compromise on the part of fishers to keep alive the resident sharks and harvest the others. Intra and inter-specific aggressiveness was also witnessed during the study, leading to an induced risk of potentially severe bites on humans, which led us to suggest a yearly closure period during the mating period in French Polynesia to decrease shark behavioural modifications due to long-term uninterrupted shark-feeding activities (Clua *et al*. 2010).

Assessment of other values composing the Total Economic Value of sharks

In a world dominated by the concept of *Homo oeconomicus* (Smith 1986), it appears critical to transfer the issue of shark conservation from the ecological domain that of the economy. In this respect, our efforts should go further than the single calculation of the direct non-consumptive use of sharks (Fig. 1) mainly promoted by ecotourism. In that perspective, the value of the direct consumptive use, mainly based on fisheries, cannot be demonstrated (see Clarke *et al*. 2007). Given its consumptive nature, this value is not supporting shark conservation, except for the suggestion that fisheries should be made sustainable to ensure long-term profits.

The indirect-use value (Fig. 1) mainly relies on the added value obtained by maintaining sharks in marine ecosystems. An ecosystem with apex predators leads to higher productivity and biomass (Friedlander and DeMartini 2002), and it could benefit to finfish fisheries. Through underwater visual censuses conducted on sites with a gradient of density of top predators, Sandin *et al*. (2010)

showed that fish species targeted by shark (and potentially by fishermen) tended to allocate more energy to reproduction than somatic storage; this phenomenon led to a gain in biomass based on more individuals, in spite of their smaller size compared to those which were not subjected to shark predation. This gain of biomass, that was not yet assessed in economic terms, could indirectly benefit to fishers. As a complementary approach, some information is available about the cascading effects following the depletion of sharks, including the economic consequences of the process. Following the collapse of the scallop stocks over-consumed by cownose rays which were not predated by large sharks that had disappeared from the coast of North Carolina, Myers *et al*. (2007) mention the destruction of a century-old small-scale traditional fisheries which depended on this resource. The indirect economic consequences of either keeping or making disappear sharks from marine ecosystems both advocate their conservation.

In the same spirit, it would be interesting to assess the option values of sharks (Fig. 1), based for example on the potential profit linked to the discovery or commercial use of medically active substances present in their skeleton or organs. For instance, the squalene is an antioxidant decreasing the probability of cancer. Its preventive effect on tumours was clinically proven (Kelly 1999), in particular for the colon (Rao *et al*. 1998). Extracts from shark cartilage, already marketed under the brand name of Neovastat[ND], inhibits tumour progression by limiting angiogenesis (Sheu *et al*. 1998). Shark immune systems are much more sophisticated than was previously thought and radically different from that of other vertebrates (Streltsov *et al*. 2004). Sharks seem to retain a non-specific immune response throughout their lives, allowing them to detoxify many potentially harmful compounds quickly without need of prior exposure. Criscitiello *et al*. (2010) just obtained the first cellular identification of T-Cell Receptor-expressing lymphocytes in a cartilaginous fish, the nurse shark *Ginglymostoma cirratum*. These studies may produce critical improvements of the mediated human immune response to viruses such as HIV.

Based on these examples in the domain of health, it seems obvious that the option values of sharks should also be considered from the perspective of avoiding their extinction.

The non-use value is also far from negligible for sharks, which have a privileged status in several cultures, especially in the Pacific. The bequest value refers to the importance that current generations place on ensuring the presence of sharks to future generations. In the Melanesian culture, the shark often has the status of a *totem* animal which hosts the soul of ancestors; people never cull it since it is holder of the destiny of the tribe (Riesenfeld 1950). The existence value relies on the willingness of people to maintain sharks as part of the shared natural heritage, without any intention of using them now or in the future. A study conducted in Canada assessed the willingness by four groups of the population to pay to ensure the conservation of endangered aquatic animals, including the right whales *Eubalaena* species, the leatherback turtle *Dermochelys coriacea* and the porbeagle shark *Lamna nasus*. The study showed that the youngest segment of the population, in spite of ranking it at the end of the list, was ready to spend about US$15 per person per year to save this shark (Rudd 2007).

Any attempt to calculate non-use values seems legitimate; not taking these values into consideration, in addition to the use values, would underestimate the real value of marine wildflife (Whitehead 1993). However, there is controversy about the reliability of the contingent valuation method (Cameron and Carson 1989) which appears to be the most widely used approach for assessing non-use values, as applied for example by Rudd (2007). Limitations include the subjectiveness of human perceptions involved in the process, which prevents one from being able to accurately replicate a study, as required for any scientific method (Boardman *et al*. 2001; Bateman *et al*. 2002). In this context, we suggest a focus on the calculation of use values, which are less subject to criticism and are powerful enough to support objective arguments in favour of shark and other wildlife conservation.

Acknowledgements

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Tables captions

Table 1 - Differential individual income generated by the 13 most regular lemon sharks based on their presence at the Opunohu shark-feeding site. The total occurrences are the sums of the sightings through 1,013 diving sessions during 57 months. NB: There is a slight difference between this group of 13 sharks and the group of resident sharks in Clua *et al.* (2010). Sharks F06 and F13 in this table replace sharks F24 and F29.

Table 2 – Comparative yearly revenues of several shark-based ecotourism activities around the world. These activities (individual sites or whole countries) were chosen based on the similarities of the calculated global revenue for one year.

Figures captions

Fig. 1 - Description of the different components of the Total Economic Value (TEV), following the methodology developed by the Millennium Economic Assessment (MEA 2005). TEV is an aggregate of the use and non-use values. The use values are an aggregate of Direct use, Indirect use, and Option values. The non-use values are an aggregate of Existence and Bequest values.

Fig. 2 – Contributions, in percentages, of the 39 observed lemon sharks to the total sightings along 57 months (from January 2005 to September 2009) of observation at the Opunohu feeding site. The ecotourism diving activity in Moorea Island, French Polynesia, significantly relies on this activity. Sharks are identified by a letter referring to their gender (F for females and M for males) and a number.

Accessory publication - Wounds inflicted by humans are regularly observed on lemon sharks in Moorea; here (left) a resident male shark (M04) with a loose hook and several wounds, following a killing attempt, probably with a knife, by a local fisherman. Lemon sharks are usually shy and solitary animals; in French Polynesia, they can however aggregate in large numbers, as in this photograph (right) showing 9 sicklefin lemon sharks together at the Opunohu feeding site. Photos: N. Buray.

Table 1

Shark ID	Number of total occurrences	Average presence per month (day)	Contribution to total revenue (%)	US$
F11	563	9.8	8.77	$474 304
M10	472	8.3	7.35	$397 640
F15	469	8.2	7.31	$395 113
F20	457	8.0	7.12	$385 003
M07	414	7.3	6.45	$348 777
M18	405	7.1	6.31	$341 195
M31	366	6.4	5.70	$308 339
M03	363	6.4	5.65	$305 812
M04	356	6.2	5.54	$299 915
F06	347	6.1	5.40	$292 333
F23	247	4.3	3.80	$208 087
F13	214	3.8	3.33	$180 286
F25	214	3.8	3.33	$180 286

Table 2

| Country	Specific sites	Activity	Period	Shark species	Global annual revenue (million)	Year	Reference
Maldives	All sites	Diving	12 months	Carcharhinids	US$2.3	1992	Anderson and Ahmed 1993
Australia	Ningaloo park	Snorkeling	8 weeks	Whale shark *Rincodon typus*	US$4.5	1995	Davies *et al.* 1997
Australia	Ningaloo park	Snorkeling	8 weeks	Whale shark *Rincodon typus*	US$5.9	1997	Topelko and Dearden 2005
South Africa	Gansbaai	Diving	12 months	White shark *Carcharodon Carcharias*	US$1.6	1997	Anonymous 1998
Belize	Gladden	Snorkeling	12 months	Whale shark *Rincodon typus*	US$1.5	2001	Graham, Comm. Pers.
Seychelles	All sites	Snorkeling	14 weeks	Whale shark *Rincodon typus*	US$1.2	2002	Rowat and Engelhardt 2007
Maldives	All sites	Diving	12 months	Carcharhinids	US$7.4	2002	Martin and Abdoul Hakeem 2006
South Africa	Aliwal shoal	Snorkeling	12 months	Tiger shark *Galeocerdo cuvier*	US$1.6	2007	Dicken and Hosking 2009

Figure 1
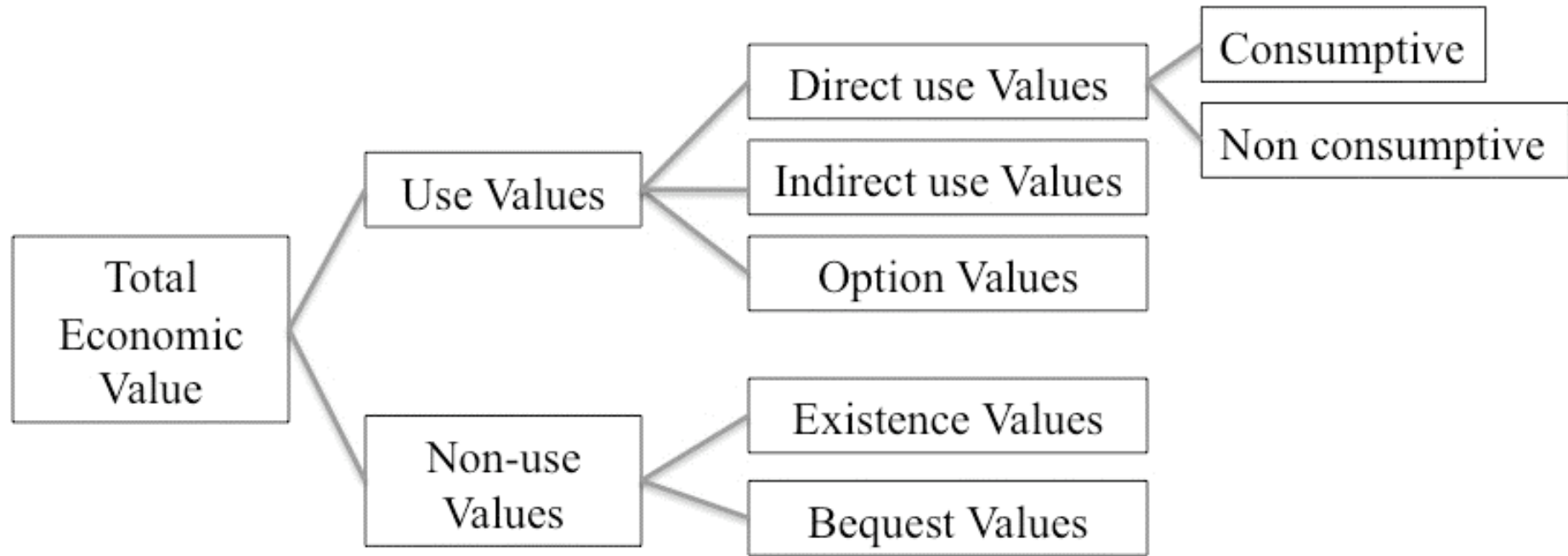
Figure 2
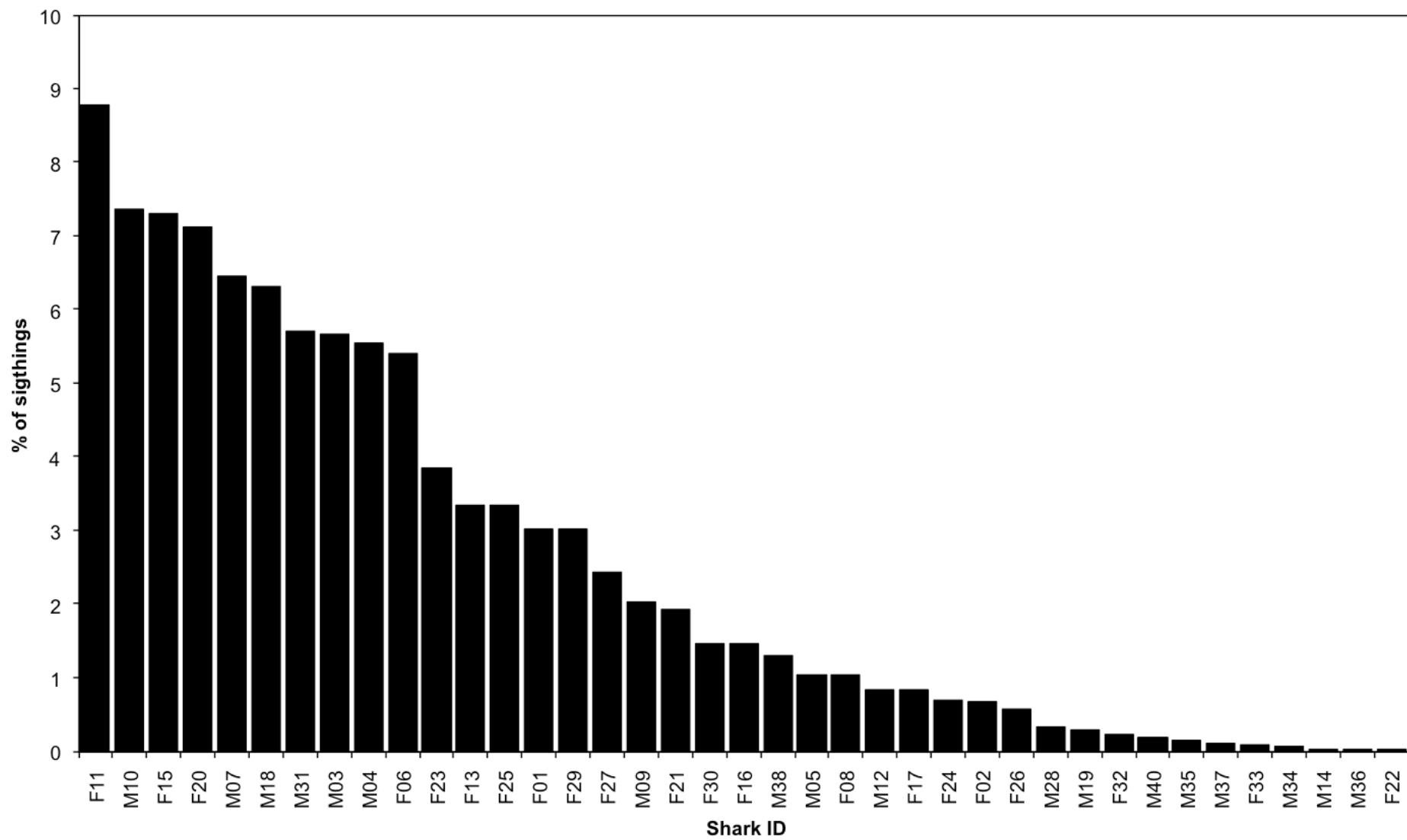
Behavioural response of sicklefin lemon sharks *Negaprion acutidens* to underwater feeding for ecotourism purposes

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ABSTRACT: The feeding of marine predators is a popular means by which tourists and tour operators can facilitate close observation and interaction with wildlife. Shark-feeding has become the most developed provisioning activity around the world, despite its controversial nature. Amongst other detrimental effects, the long-term aggregation of sharks can modify the natural behaviour of the animals, potentially increase their aggression toward humans, and favour inbreeding. During 949 diving surveys conducted over 44 mo, we investigated the ecology and residence patterns of 36 photo-identified adult sicklefin lemon sharks *Negaprion acutidens*. The group contained 20 females and 16 males. From this long-term survey, we identified 5 different behavioural groups that we described as 'new sharks' (7), 'missing sharks' (4), 'resident sharks' (13), 'unpredictable sharks' (5) and 'ghost sharks' (7). In spite of movements in and out of the area by some males and females, which were probably related to mating, the general trend was that residency significantly increased during the study, particularly in males, showing a risk of inbreeding due to the reduction of shark mobility. Intra- and interspecific aggression was also witnessed, leading to an increased risk of potentially severe bites to humans. Our findings suggest the need for a revision of the legal framework of the provisioning activity in French Polynesia, which could include a yearly closure period to decrease shark behavioural modifications due to long-term shark-feeding activities.

KEY WORDS: Shark-feeding · Provisioning · Human disturbance · Behaviour · Site residence

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INTRODUCTION

Large predators, which are potentially dangerous to humans and often feared, account for a substantial proportion of ecotourism activities based on animal sightings. However, because of their generally elusive nature and locally low population densities, such predators are often difficult to observe. Sharks are shy animals (Bres 1993), and provisioning is necessary to produce reliable and impressive aggregations of animals. The last decade has seen tremendous development of ecotourism based on the sighting of top marine predators (Orams 2002, Topelko & Dearden 2005). The practice of shark-feeding is widespread throughout the tropical and subtropical seas of the world, e.g. in the Bahamas, Fiji, South Africa, Australia and French Polynesia, and it is becoming controversial, with little consensus about how it should be managed. Deliberate and long-term shark-feeding is suspected to generate problems for both animals and humans (Dobson 2006, Newsome & Rodger 2008). It may alter the natural behavioural patterns of sharks, generating biological (for the animal themselves) and ecological (for the ecosystem) effects. Provisioning may cause habituation to human contact and increase aggression towards humans by associating divers with food (Burgess 1998,

Orams 2002). However, feeding wildlife can be a positive tool for assisting in the conservation of vulnerable and endangered species, through attaching economic value to wildlife and educating tourists about the need for conservation (Bookbinder et al. 1998, Halpenny 2003); it can also increase the probability of a shark encountering a partner as a result of aggregation (Orams 2002). Despite the controversy, few, if any, comprehensive reports have measured the impact of shark-feeding, which is now widespread and growing around the world.

To date, studies have been conducted on the effect of chumming on white shark *Carcharodon carcharias* in South Africa (Johnson & Kock 2006, Laroche et al. 2007), as well as sandbar *Carcharhinus plumbeus* and Galapagos *C. galapagensis* sharks in Hawaii (Meyer et al. 2009). These studies all concluded that moderate levels of provisioning of cage-diving ecotourism probably had a minor impact on the behaviour of the sharks and no risk of increased attacks on humans in adjacent areas. In South Africa, Johnson & Kock (2006) showed that conditioning only arises if white sharks gain significant and predictable food rewards, which only happens if operators contravene permit regulations prohibiting intentional feeding of sharks. White sharks are lured to the boat with baits (typically, mashed sardines and fish oil; Laroche et al. 2007) that are significantly different from their usual prey in the area, Cape fur seals *Arctocephalus pusillus pusillus* (Ferreira & Ferreira 1996). In Hawaii, Meyer et al. (2009) showed that cage-diving activities did not increase the number of attacks on humans, probably due to the fact that the shark tours use a small amount of fish scraps, mimicking the activities of crab fishing vessels which have been operating in the same area for over 40 yr. In both cases, while some food is used to attract sharks to the cages for observation and photography, the quantities involved are small, so this activity cannot be considered as real 'provisioning'. Light baiting is also used at Aliwal Shoal (South Africa) for attracting tiger sharks *Galeocerdo cuvier* and allowing encounters with snorkelers in open water (Dicken & Hosking 2009). However, the available scientific data focus on the economic value of the recreational activity, and do not address its effects on the behaviour of these potentially dangerous sharks (ISAF 2010). Bull sharks *Carcharhinus leucas*, another dangerous species (ISAF 2010), have been attracted to an ecotourism site in Beqa (Fiji Islands) since 2002 through a real feeding and conditioning process based on the release of several tuna heads during each dive (E. Clua pers. obs.); here again, however, the only data provided are socio-economic (Brunnschweiler 2010), with no reference to the biological issues of provisioning of carnivorous animals. Given the controversial nature of shark-feeding, there is a critical need for empirical studies that focus on potentially dangerous sharks, and address both the potential disruption of their natural behaviour, which underpins their resilience, and the increasing risk of fatal attacks on humans (Garrod & Wilson 2006).

In French Polynesia, sharks are fed daily during diving activities. The main species involved, the sicklefin lemon shark *Negaprion acutidens*, can reach over 3 m in length and is considered to be potentially dangerous to humans (Maillaud & Van Grevelynghe 2005, ISAF 2010). This coastal shark is widely distributed in the Indo-Pacific, from Eastern Africa to French Polynesia. However, very little is known about the ecology of the sicklefin lemon shark in the Central Pacific. Despite its commercial value (Compagno 1984), only a few studies have been conducted in the Indian Ocean (Stevens 1984) and in Western Australia (White et al. 2004) besides a recent global genetic study (Schultz et al. 2008). The ecology of its sister species, the Atlantic lemon shark *N. brevirostris*, has been well documented during past decades (Gruber 1982, Chapman et al. 2009), mostly in the central Western Atlantic Ocean. However, while its early life has been extensively studied (Morrissey & Gruber 1993, DiBattista et al. 2007), very little is known about the adult stages of *N. brevirostris* and even less about *N. acutidens*.

Moorea Island (French Polynesia) is among the few locations worldwide where it is possible and feasible to have daily encounters with several wild adult sicklefin lemon sharks in their natural environment. This characteristic provided us with an opportunity to investigate the behaviour and residency pattern of an adult population of this reef shark species through daily underwater observations at a provisioning tourism location. Here, we describe the population size and structure of this species, aggregated for ecotourism purposes at a site on the northern outer reef of Moorea Island. We divided the population into co-occurrence groups and describe the residence patterns and behaviour of these groups. We also tested the hypothesis that shark-feeding increases the fidelity of lemon sharks to the site, and discuss the potential long-term effects on population resilience and behaviour, including the risk of increased interactions with humans.

MATERIALS AND METHODS

Study implementation. The study was conducted at Moorea Island (17° S, 149° W) in the Society Islands Archipelago, French Polynesia. Shark-feeding activities started there in the late 1980s, in the lagoon, passes and outer slope of the barrier reef. In October 2004, Moorea authorities implemented a Management Plan for the Marine Environment (Plan de Gestion de

l'Espace Maritime, PGEM) that restricted shark-feeding activities to 2 zones. Our specific study area was located at Papetoai on the outer slope of the reef (from 149° 50′ 670″ to 149° 51′ 389″ W); it was selected for its abundance of sicklefin lemon sharks (Buray et al. 2009). At this site, 3 different diving centres feed the sharks between 08:00 and 10:30 h. Our feeding sessions were conducted in the presence of tourist divers through dives at depths of 20 to 25 m, starting at 09:00 h and lasting 60 to 100 min. Sessions consisted of placing a small cage containing tuna discards on the substratum at the beginning of each dive to lure and aggregate the sharks in the area. The food was released at the end of the dive for the benefit of 1 or sometimes 2 sharks. Data on the presence or absence of sharks were recorded on each dive using natural identification marks on their bodies (Buray et al. 2009), photographed with a digital camera when necessary. Part of the identification process included the determination of sex from the presence or absence of claspers, and total length, estimated visually. We cross-checked the reliability of this visual assessment through a laser measurement of some individuals, based on the projection of 2 laser light spots, 43 cm apart, onto the flank of the shark as it was photographed (Bansemer & Bennett 2008). DNA sampling with a biopsy probe mounted on a spear gun was also conducted on 80% of the sharks for paternity analysis (the subject of a complementary study), which also allowed us to assess the reliability of the photo-identification process through genetic fingerprinting.

The data analysed in the present paper comprise 36 sicklefin lemon sharks observed during 949 dives spanning 1338 d, or more than 3.5 yr. The study started on 2 January 2005, and ran until 31 August 2008. The animals are numbered F01 to M38 (F: female, M: male). Sharks numbered 14 and 22 are not included in the present study as they were photo-identified only once in the provisioning area.

Statistical methods. Females F32 and F33 and males M34 to M38 arrived at the study site late in the study and were seldom seen (2 to 19 times each, for a total of 51 sightings of these 7 ind.), and never in groups. The earliest sighting was on Day 692 of the study (animal M34). These 7 ind. were excluded from the following analyses; they were considered as a separate group.

For the 29 remaining sharks, we computed a square (29 × 29) matrix showing how many times each pair of animals was observed during the 949 dives. That value is usually called a in descriptions of binary similarity indices like the Jaccard and Sørensen coefficients (Legendre & Legendre 1998); and we follow this usage in this paper. This statistic can be tested for significance against the null hypothesis H_0 that there is no association between these 2 sharks. We developed an

R function to carry out the test of a by permutation, following the method originally proposed by Raup & Crick (1979) and detailed by Legendre & Legendre (1998, p. 273). The function produced 2 outputs: a (29 × 29) matrix of coefficients a and a (29 × 29) matrix of p values (after 9999 random permutations) associated with the coefficients.

We used the matrix of p values to delineate groups of lemon sharks. An initial total of 406 tests of significance were computed. The Holm (1979) correction for multiple testing was applied to the p values to obtain an experiment-wise error rate of 5%. After correction, the 52 pairs of animals that had a coefficients with original p values of 0.0001 or less remained significant. Agglomerative clustering methods were not useful for this study because the groups were not clearly isolated from one another and some individuals belonged to 2 groups. We therefore examined the connections among animals on a graph obtained by principal coordinate ordination of the matrix of significant p values (Gower 1966, Legendre & Legendre 1998).

We used simple linear regression analysis to relate the abundances of the sharks, globally and in groups, to days since the beginning of the survey, in order to determine which group, if any, displayed increased fidelity to the site. The regression lines were plotted on graphs showing how many sharks of each group were observed during each dive.

RESULTS

Population size and structure

The 36 observed sharks comprised 20 females (55.5%) and 16 males (44.5%). Total length (TL) of the identified sicklefin lemon sharks ranged from 230 to 310 cm, with a mean of 273 ± 24 cm (95% confidence interval, Fig. 1). The sex ratio was slightly in favour of females all year long but varied during any given year, with the number of males decreasing during the reproductive season, around October (Fig. 2). Overall, this population was made up of adults larger than 230 cm, which are assumed to be sexually mature at that size (Stevens 1984).

Residence patterns and grouping

Fig. 3 presents the co-occurrence links between sharks in the principal coordinate ordination plot. The first 2 principal coordinate axes accounted for 21% of the variance in the matrix of p values, which is sufficient for such a representation. One can make out 5 groups, with the largest possibly containing 2 subgroups.
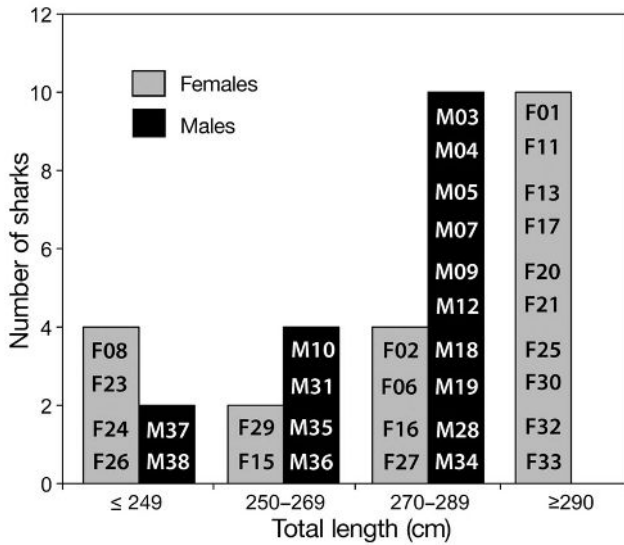
Fig. 1. *Negaprion acutidens*. Size distribution of the 36 male and female sicklefin lemon sharks at the Moorea shark-feeding site. Individual sharks are identified in the histogram bars
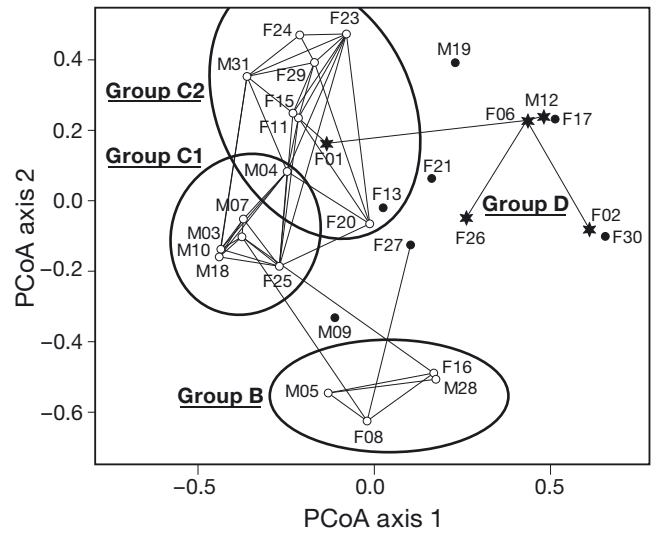

Fig. 3. *Negaprion acutidens*. Principal coordinate analysis (PCoA) ordination showing the co-occurrence links among sharks with a p value of 0.0001. The first 2 PCoA axes together account for 21 % of the variation in the matrix of p values among the 29 sharks. Groups B, C1 and C2 are identified by ellipses, and Groups D and E by asterisks and black dots, respectively

Group A (51 sightings in total), designated 'new sharks', comprised females F32 and F33 and males M34 to M38. They arrived at the site late in the study (first sighting on Day 692). These individuals were seldom observed, and no more than one was seen during a dive (Fig. 4A), as described in the statistical methods above. Because of their peculiar time distrib-

ution, they displayed a strong significant increase with time (Table 1). This grouping was more a result of late occurrence than any real interaction grouping. However, it demonstrates a renewal of the pool that gained 7 new individuals (20 %) in a single year.
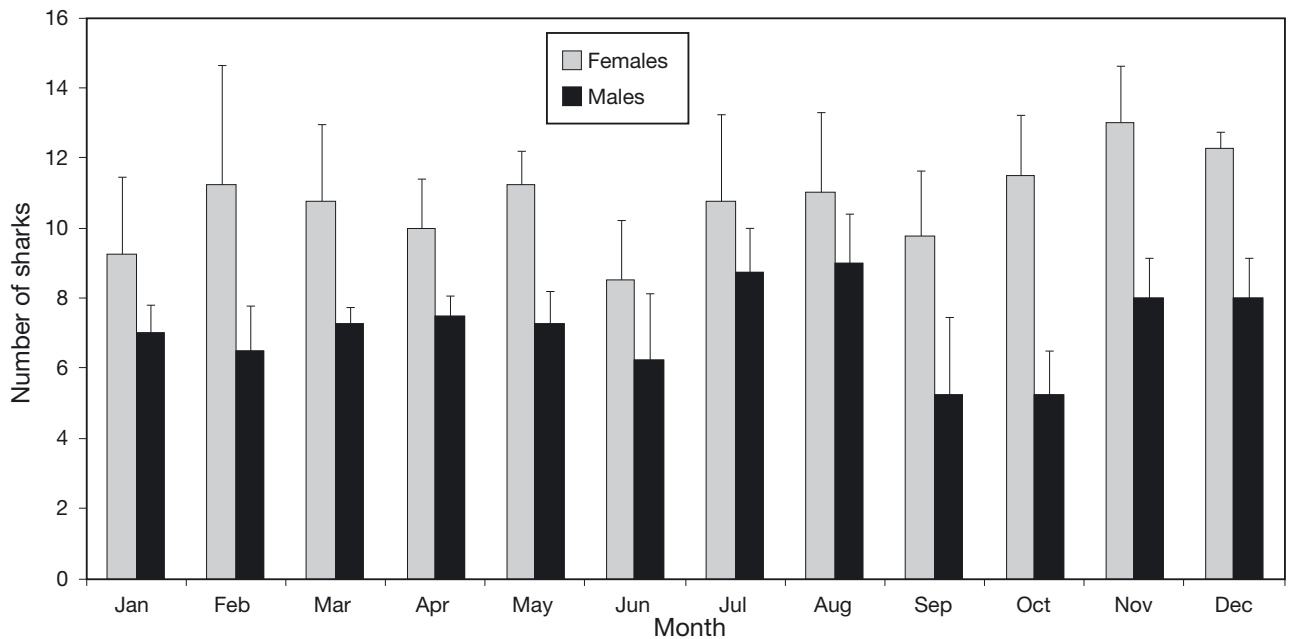

Fig. 2. *Negaprion acutidens*. Mean number of male and female sharks in each month of the year after 44 mo of observation with 95 % confidence intervals (error bars)
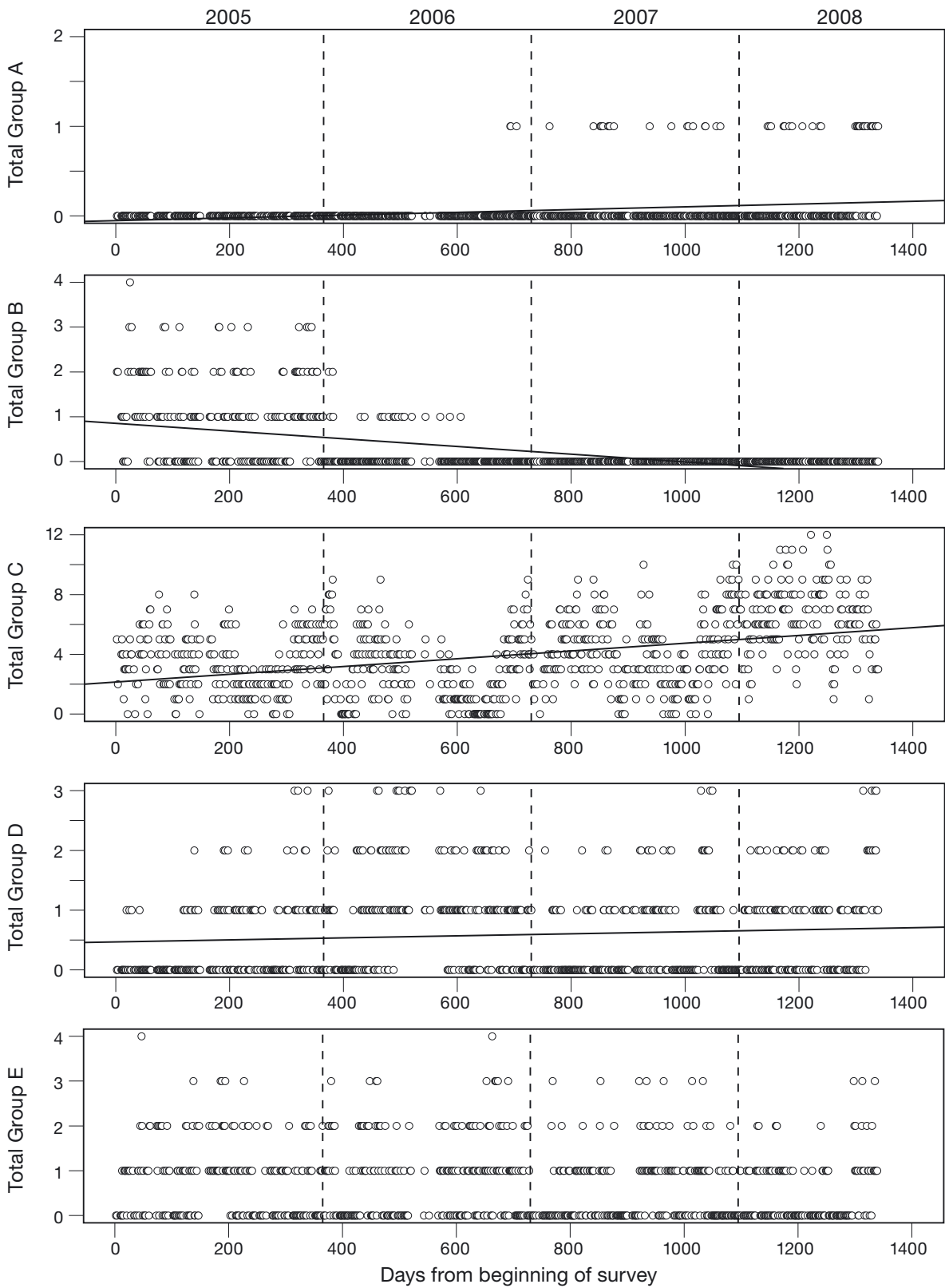
Fig. 4. *Negaprion acutidens.* Total number of sharks in a group (A to E are the group identifiers) observed during the 949 dives (days from the beginning of the survey along the abscissa). The linear regression line is shown in each graph, except for Group E. Vertical dashed lines are year divisions (2005, 2006, 2007, 2008)

Table 1. *Negaprion acutidens*. Simple linear regression results for the relationships between the number of sharks, globally and in groups (except Group E), observed during 949 dives, and the number of days since the beginning of the survey. The slope values are very small because the day numbers range from 2 to 1339

	Slope	p-value	Interpretation
All sharks (n = 36)	0.00176	2.05×10^{-12}	Strong significant increase
Group A	1.542×10^{-4}	3.68×10^{-16}	Strong significant increase
Group B	-8.588×10^{-4}	$<2 \times 10^{-16}$	Strong significant decrease
Group C	0.00260	$<2 \times 10^{-16}$	Strong significant increase
Group C1	0.00033	0.0151	Slight significant increase
Group C2	0.00252	$<2 \times 10^{-16}$	Strong significant increase
Group D	1.677×10^{-4}	0.0103	Slight significant increase

Group B (246 sightings), called 'missing sharks', contained the 4 strongly interconnected sharks (M05, F08, F16, M28) at the bottom of Fig. 3. F27 was not included in that group for 2 reasons: it was only associated with F08 and it was seen at the site during the whole study, whereas the members of Group B were only observed up to Day 606 (Fig. 4B). This is the only group that displayed a strong significant decrease (Table 1): it was present in 2005 but disappeared during 2006.

Group C (3739 sightings), designated 'resident sharks', was the largest group, with 13 ind. (M03, M04, M07, M10, F11, F15, M18, F20, F23, F24, F25, F29, M31), and was composed of 2 subgroups. The pivotal male M04 belonged to both subgroups C1 and C2. This male showed some atypical dominance behaviour. As the study progressed, in addition to a strong residency pattern, this shark showed increasing aggression towards its male and female conspecifics and, to a lesser degree, toward divers (N. Buray pers. obs.).

Subgroup C1 (1877 sightings) contained 6 sharks (M03, M04, M07, M10, M18, F25), all of which were males except F25. It showed a slight but significant increase in sightings over time (Table 1).

Subgroup C2 (2137 sightings) included 8 sharks (M04, F11, F15, F20, F23, F24, F29, M31), all of which were female except M04 and M31. It showed a strong significant increase in sightings over time, particularly in the last 2 yr (Table 1).

Group D (556 sightings), designated 'unpredictable sharks', contained 5 loosely interconnected sharks (F01, F02, F06, M12, F26) of which all were female except M12. Female F06 played a pivotal role in this group. The group showed a slight but significant increase in sightings over time (Table 1).

Group E (617 sightings), designated 'ghost sharks', contained 7 ungrouped individuals (M09, F13, F17, M19, F21, F27, F30). All except F27 had no co-occurrence link at the 0.0001 significance level.

DISCUSSION

This is the first detailed study to address the effects of provisioning on sharks by providing observational data which describe the response of these predators to a multi-annual daily feeding in a natural environment. It provides complementary information about a different type of feeding (conducted underwater), on a different species (lemon shark), than previous studies which addressed the effects of surface chumming on white sharks in South Africa (Laroche et al. 2007), or cage diving on Galapagos and sandbar sharks in Hawaii (Meyer et al. 2009).

Our statistical analysis allowed us to classify the 36 sharks into 6 groups (A, B, C1, C2, D, E), based on the affinity between sharks and their fidelity to the site. Groups A and E, each composed of 7 sharks, were of limited interest, as they comprised sharks that were either too late in coming to the feeding site to determine any significant pattern, or displayed unpredictable behaviour with no clear pattern. However, it is interesting to notice a clear turn-over in the population with the arrival of new individuals that became established. The 4 sharks composing Group B had a resident pattern in 2005 but disappeared from the study site during 2006. This may be explained by death (i.e. M05, which appeared to be old), stress due to shark intraspecific interactions (see below) or just temporary disappearance (i.e. M28, which re-appeared in 2008 after a 2 yr absence).

Group C, which comprised 13 'resident' sharks, showed a strong pattern of sexual segregation. Subgroup C1 was mostly composed of males (5 males and 1 female), and subgroup C2 was mostly composed of females (6 females and 2 males), with male M04 showing strong affinities with both subgroups. This spatial and temporal sexual segregation is commonly encountered in carcharhinids (Klimley 1987, Economakis & Lobel 1998) and other shark families (Bansemer & Bennett 2008, Mucientes et al. 2009). Male sharks of Subgroup C1 (1877 sightings) showed strong residency during all 4 yr; their presence rate decreased strongly in October–November, corresponding with the mating period (Stevens 1984). This trend may be due to a temporary migration for mating with females that do not belong to the studied population. In fact, reproduction has been recognised as a driving factor for spatial segregation between sexes in other studies (Economakis & Lobel 1998). Sharks from Subgroup C2 (2137 sightings) mostly comprised females which seemed to aggregate at the

feeding site and leave for only a few days for parturition (easily detected by external shape of the belly), as witnessed between August and October in 2005 and 2007 (for F11, F15 and F20), in September 2007 (F23) and in August and October 2008 (F24 and F29; N. Buray pers. obs.).

We considered the 'unpredictable' sharks from Group D, comprising 4 females and 1 male (556 sightings), as 'non-residents'. This term is based mainly on the consideration of the cumulative number of days at the site; it does not refer to a pattern of regular yearly presence at the feeding site during the extended mating period (July to November). This pattern can be seen as the inverse of the disappearance of the C1 sharks in October–November. As some 'resident' males were leaving the study site for mating, some females may have arrived for the same purpose. Genetic investigations on the lemon shark *Negaprion brevirostris* in the Bahamas (Feldheim et al. 2002) have shown that to avoid inbreeding problems within their relatively small populations, they appear to have developed a mating strategy. Whereas female lemon sharks return to their natal grounds each year, males remain nomadic, only infrequently returning to the same mating group. In our study, we found a similar pattern of 'mixing population' in *N. acutidens*, mainly with females potentially coming back to their natal grounds; however, unlike *N. brevirostris* in the Bahamas, males showed a strong residency and site attachment over the years. Assuming similarity in the natural behaviour of these 2 sister species, our findings could be linked to an aggregating effect of shark-feeding, which decreases the mobility of animals, mainly the males, and may contribute to increased inbreeding. This trend may lead to long-term loss of genetic variability in the Polynesian lemon shark populations, even though natural philopatry in *N. acutidens*, which would have been a detrimental factor, seems to be low (Schultz et al. 2008).

Increasing residency was a general trend for the shark population. For all groups except Group B, which was composed of animals that disappeared, the linear regressions had positive slopes (Fig. 4), indicating an increase in shark abundance over time, and their site fidelity increased over the 44 mo, particularly for the 'resident' subgroups, C1 and C2 (Table 1). This means that, despite some sharks leaving and others arriving, the number of days with sharks present and the number of sharks at the site both increased. This trend is explained by the increased attraction of sharks by provisioning, suggesting that learning plays a strong role in optimising their food search (Guttridge et al. 2009). Our findings are consistent with similar situations where other elasmobranchs (rays) learned to associate specific locations with food rewards, with detrimental effects on their behaviour, and indirect effects on the surrounding marine ecosystems, leading to the concept of an 'ecological trap' (Corcoran 2006, Gaspar et al. 2008, Semeniuk & Rothley 2008). In the case of lemon sharks, their increased site fidelity can have a negative effect on gene flow, as mentioned previously, and can also affect their role as top predators in the area, as shown for top terrestrial predators such as dragons *Varanus komodoensis* in the Komodo National Park, where provisioning was eventually banned (Walpole 2001).

Among the negative effects, we observed intraspecific interactions generated by the provision of a limited amount of food. Not all sharks present during a dive acquired food, and this resulted in exacerbated competition among the animals. This pattern can lead to increasing the number of intraspecific dominance actions and the aggression of sharks to acquire food (Ritter 2001), as shown for rays (Semeniuk & Rothley 2008). Dominance is often driven by the size (length) of the sharks in social groups (Allee & Dickinson 1954, Myrberg & Gruber 1974). During several feeding sessions, the largest resident male, M04, appeared to be the most inquisitive, approaching the divers closer than any other individual did. Since males M07 and M18 were dominant in 2005, M04 definitely acquired increasing dominance behaviour with respect to other individuals, which turned into deliberate aggression towards other males when several of them were present. As was previously observed in 2005 for its 2 predecessors, from 2006 onwards M04 often arrived in the morning with fresh scars or notches that can be attributed to intraspecific fights (N. Buray pers. obs.). Aggression increased significantly when resident males came back to the feeding site after the mating period, probably in the context of a reorganisation of the hierarchy, as shown by serious wounds on males that were quite different in their severity and locations from those inflicted on females during mating (Fig. 5). In natural conditions, sicklefin lemon sharks cannot be considered a gregarious species (Stevens 1984), except during the mating period, and animals usually feed separately. Therefore, intraspecific aggression linked to the feeding process, even though natural among carnivorous animals, can be interpreted as deviant behaviour, exacerbated by human activity. Although managers may consider this process of increasing intraspecific aggression to be acceptable among sharks, it represents a real issue regarding the safety of divers for whom the risk of accidental bites has increased critically (Burgess 1998). Between 1979 and 2001, 47% of shark bites in French Polynesia were experienced in the context of shark-feeding activities (Maillaud & Van Grevelynghe 2005). Although anecdotal, this was confirmed by a serious bite by shark
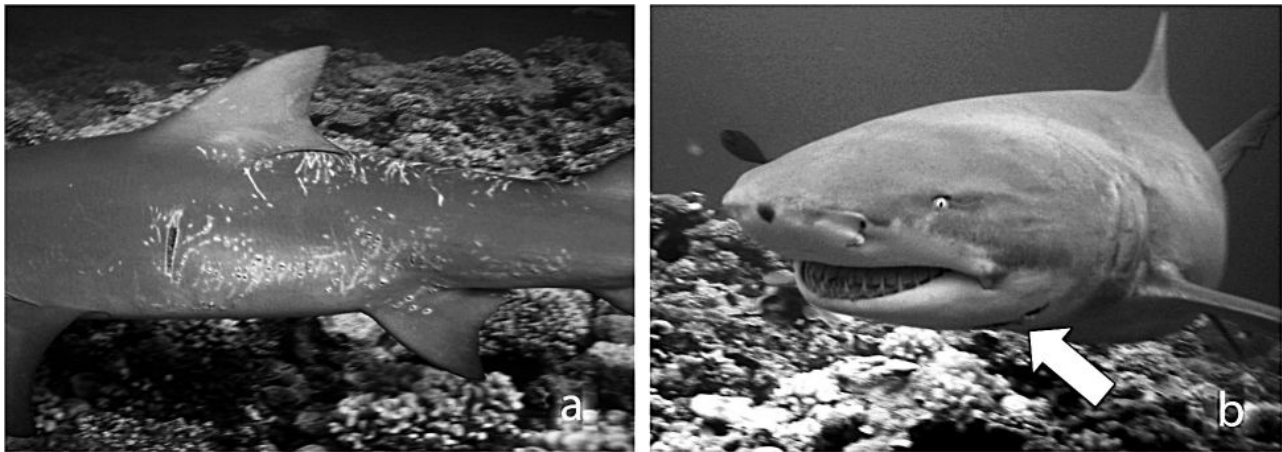
Fig. 5. *Negaprion acutidens*. (a) Mating scars (white lines and dots) in the middle and posterior part of the body of a female lemon shark, and (b) a bite wound located on the throat (arrow) of a male lemon shark, inflicted during intraspecific male–male fighting for dominance (photos by N. Buray)

M04 on the left hand, which was not holding any food, of the diver doing the feeding in January 2006 (N. Buray pers. obs.).

The results of this study indicate that in spite of the provisioning activity, several male and female sicklefin sharks seem to have left the study site while others came back to it for mating. This positive aspect from the perspective of maintaining gene flow between this shark population and adjacent ones is mitigated by the increasing pattern of residency for the overall population during the study. At present, the population seems to be a balanced mix of resident and non-resident individuals, which favours population mixing. However, if the resident sharks increase their numbers and their attachment to the feeding site, group living can generate costs for animals which are normally solitary foragers, such as injuries, predation, increased stress hormone levels and exposure to parasites due to increased transmission rates between individuals (Semeniuk & Rothley 2008). If supplemental feeding can be perceived as an artificial support to sharks by providing easy-to-access resources (Milazzo et al. 2006, Laroche et al. 2007), and can allow increasing energy allocation to other fitness-related activities such as rest and reproduction (Orams 2002), long-term unnatural aggregation can also have long-term fitness consequences for the population. Because the studied population is small, daily aggregations at the same location could result in increased social interactions and increased mating between close relatives, reinforcing the risk of inbreeding. As lemon sharks are known for their polyandry (Feldheim et al. 2004), the potential negative effect on gene flow linked to the increasing residency pattern might be buffered by the multiple pater-

nity process; this needs to be thoroughly monitored. This factor, added to the development of aggression and incremental risk of accidental bites to divers, should lead managers to seriously consider a revision of the regulations on shark-feeding in French Polynesia in order to reduce these risks. An annual cessation of the feeding activity for several months, preferably encompassing the mating period, is an obvious solution. Whereas our study allowed us to draw these preliminary conclusions, additional field investigations are required to better understand the long-term effects of provisioning on shark populations. Further work may also enable us to better understand the risks induced by feeding predators.

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BRIEF COMMUNICATION

Plasma cortisol and 11-ketotestosterone enzyme immunoassay (EIA) kit validation for three fish species: the orange clownfish *Amphiprion percula*, the orangefin anemonefish *Amphiprion chrysopterus* and the blacktip reef shark *Carcharhinus melanopterus*

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Commercially available enzyme immunoassay (EIA) kits were validated for measuring steroid hormone concentrations in blood plasma from three fish species: the orange clownfish *Amphiprion percula*, the orangefin anemonefish *Amphiprion chrysopterus* and the blacktip reef shark *Carcharhinus melanopterus*. A minimum of 5 µl plasma was required to estimate hormone concentrations with both kits. These EIA kits are a simple method requiring minimal equipment, for measuring hormone profiles under field conditions. © 2010 The Authors

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Key words: elasmobranch; ELISA; haematocrit; hormone; steroids; teleost.

Steroids are a hormone group produced in the gonads and in the interrenal gland. They are produced in response to a plethora of environmental cues and stimulate changes in cellular metabolism, resulting in a variety of effects on important life-history traits. Steroid hormones, such as testosterone, have important consequences for reproduction, while corticosterone is produced in response to stressful conditions (Sapolsky *et al.*, 2000). Due to their trade-offs with immune function, steroid hormones have concomitant effects on other life-history traits such as reproduction and survival, and as such are important physiological variables that can be used to understand an organism's overall fitness (Grossman, 1985; Folstad & Karter, 1992; Mills *et al.*, 2010).

Plasma steroid levels can be measured using multiple methods including radioimmunoassay (RIA) (Mills *et al.*, 2007, 2008, 2009), high performance liquid chromatography (HPLC) with UV detection, isotope dilution gas chromatography spectrometry (De Brabandere *et al.*, 1995) and enzyme-linked immunosorbent assay

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(ELISA) (Barry *et al.*, 1993; Ackerman & Iwama, 2001; Sink *et al.*, 2008). The choice of technique is often based on expense, equipment and licence limitation, relative speed and ease of use and suitability to the study species. HPLC with UV detection and isotope dilution gas chromatography spectrometry require specialized equipment, while RIA involves radioactive substances (Sink *et al.*, 2008). ELISA kits, on the other hand, contain no radioactive agents, a spectrophotometer is the only specialized equipment required and the simple protocol favours their use. Enzyme immunoassay (EIA) kits, however, usually employ human plasma-based standards that are not always suitable for comparison with other non-mammalian species, therefore validation of the kit with each study species is a necessity before use.

Clownfishes and anemonefishes, *Amphiprion* spp., are model species used to answer multiple and disparate questions ranging from connectivity (Jones *et al.*, 2005), cooperative breeding (Buston, 2004), sex change (Godwin & Thomas, 1993), size hierarchies (Buston, 2003), mutualism (Holbrook & Schmitt, 2005) and sound production (Parmentier *et al.*, 2007). Clownfishes and anemonefishes are also highly valued in the aquarium trade and are used to promote diving tourism. Other key species for diving tourism include sharks, yet their populations are under threat due to fisheries overexploitation (Stevens *et al.*, 2000; Baum & Myers, 2004; Campana *et al.*, 2006; Robbins *et al.*, 2006; Myers *et al.*, 2007; Heupel *et al.*, 2009). In order to understand the physiological processes associated with anemonefish behaviour, reproduction and survival, and the impacts of overfishing and tourism on shark physiology and life-history traits, it is necessary to measure steroid hormone levels. The aim of the present study was to validate commercially available EIA kits in order to measure plasma 11-ketotestosterone and cortisol in *Amphiprion percula* (Lacepède) and *Amphiprion chrysopterus* Cuvier and 11-ketotestosterone in the blacktip reef shark *Carcharhinus melanopterus* (Quoy & Gaimard).

Twenty *A. percula* (Lautän Production; www.lautanproduction.com) were kept in aquaria at EPHE, Perpignan, for 10 days at $24°$ C and on a 12L:12D photoperiod. The mean $\pm$ S.E. wet mass of the experimental fish was 1.21 ± 0.03 g (range $= 0.67 - 3.06$ g) and the mean $\pm$ S.E. standard length (L_S) was 35.05 ± 0.11 mm (range $= 26.03 - 45.07$ mm). The fish were placed for $3-4$ min in a 2 l seawater anaesthetizing tank containing 1 ml of 10% Eugenol (Merck; http://www.merck.de) diluted in 95% ethanol. Blood samples were taken laterally from the caudal vein using heparinized (Sigma H-0878; www.sigmaaldrich.com) 1 ml plastic syringes fitted with a 30 gauge needle (Godwin & Thomas, 1993).

Thirty *A. chrysopterus* were caught by divers with hand-nets from their host, the magnificent sea anemone *Heteractis magnifica* on the north shore of Moorea ($17°$ $30'$ S; $149°$ $50'$ W) in French Polynesia in June 2008. The mean $\pm$ S.E. total length (L_T) of the 30 individuals was 93.6 ± 8.9 mm (range $= 46.0 - 154.0$ mm). Individuals were tagged (fin-clipped) and released back on their anemones within $5-10$ min after capture. Blood ($100-250$ μl) was drawn from the caudal vein underwater using a heparinized 25 gauge needle and 1 ml plastic disposable syringe. Syringes were kept on ice on the boat.

Twenty-five male *C. melanopterus* were caught from a boat inside and outside the lagoon on the north and west shores of Moorea ($17°$ $30'$ S; $149°$ $50'$ W) in French Polynesia in 2008 and 2009. The mean $\pm$ L_T was 120.12 ± 9.75 cm (range $= 93.0 - 134.0$ cm). Individuals were tagged (fin-clipped) and released back to the sea within $5-10$ min after capture. A minimum of 250 μl of blood was drawn

from the caudal vein using a heparinized 15 gauge needle and 10 ml plastic disposable syringe. Syringes were kept on ice on the boat.

Individual blood samples were transferred to 75 µl haematocrit capillaries and centrifuged (Beckman Coulter TJ-25 centrifuge; www.beckmancoulter.com) at 10 000 g for 5 min at 4° C and the haematocrit (% of red blood cells in the blood) were measured. The mean ± S.E. haematocrit values were $16·5 ± 0·4$, $46·5 ± 4·3$ and $29·0 ± 1·8\%$ for *A. percula*, *A. chrysopterus* and *C. melanopterus*, respectively. The supernatant, a yellow plasma layer, was collected without disturbing the white buffy layer or the blood cells. Twenty, 30 and 25 samples were pooled for *A. percula*, *A. chrysopterus* and *C. melanopterus*, respectively and the pools were stored at $-80°$ C.

Plasma 11-KT was measured using an EIA kit (11-keto Testosterone EIA Kit, No. 582751, Cayman Chemicals, SPI BIO; www.spibio.com). Fifty microlitres of the eight standards or 50 µl of the blood plasma samples were added with 50 µl of 11-KT-acetylcholinesterase (AChE) conjugate and 50 µl of 11-KT-specific rabbit antiserum to a 96-well plate. During 18 h at 4° C, 11-KT AChE and sample or standard 11-KT competed for a limited number of 11-KT-specific rabbit antiserum-binding sites, whose complex was attached to the mouse monoclonal anti-rabbit IgG antibody previously attached to the well. The plate was washed five times to remove any unbound reagents and 200 µl of Ellman's reagent, that contains the substrate to AChE, was added to the wells. The plate was placed on an orbital shaker in the dark for 55 min. The intensity of the yellow colour was measured spectrophotometrically (Beckman Coulter AD 340 Spectrophotometer) at 405 nm and was proportional to the amount of 11-KT AChE bound to the well, which was inversely proportional to the amount of free 11-KT present.

Plasma cortisol was measured using an EIA kit (Cortisol EIA Kit, No. 582121, Cayman Chemicals, SPI BIO). The methods were identical to those used for the 11-KT assay except that cortisol-acetylcholinesterase (AChE) conjugate was used and the rabbit antiserum binding sites were cortisol-specific. The plate was placed on an orbital shaker in the dark for 80 min.

Sample 11-KT and cortisol concentrations were determined by interpolation from the standard calibration curve using a common functional model for calibration curves. The data were plotted as % maximum bound (% B Bo^{-1}) $v.$ log concentration using a logit-log curve fit as recommended for these kits (www.caymanchem.com/analysis/eia).

Validation of both kits comprised 1) parallel displacement of serially diluted plasma to the standard curve, 2) accuracy from spike recovery and 3) precision from intra- and inter-assay variabilities.

Parallelism was evaluated by measuring 11-KT and cortisol concentrations in pooled plasma samples, serially diluted in EIA buffer provided with the kits. For *A. percula*, two sets of dilution ratios were prepared, both were used for validation of the 11-KT assay, whereas only the first set was required for validation of the cortisol assay. The first dilution set ratios were 1:10, 1:32, 1:100, 1:302, 1:585, 1:1210, 1:3732 and 1:12 109 and the second dilution set ratios were 1:3, 1:6, 1:10, 1:18·5, 1:32, 1:59, 1:100, 1:185, 1:302, 1:585 and 1:1216. For *A. chrysopterus* and *C. melanopterus*, one set of dilution ratios was prepared for both species: 1:1·8, 1:3·18; 1:6, 1:10, 1:31·875, 1:100, 1:318·75 and 1:1000. The % BBo^{-1} for each sample dilution and for the standards was plotted against their relative log dilution

and the shapes of the resulting curves were compared. These curves must be parallel to support the assumption that the antibody-binding characteristics of standard and sample are similar enough to allow the determination of antibody levels in the diluted plasma sample. An ANCOVA was carried out to determine the homogeneity of slopes between the sample dilutions and those of the kit's standards. In addition, regression analysis of the diluted sample was used to determine the dilution factor that corresponds to 50% of antibody bound.

Accuracy was assessed by the determination of spike recovery performed by adding a pre-determined hormone concentration (see Table I for each concentration per species and per kit) to each of four plasma sample dilutions, in duplicate. The per cent recovery is the concentration of the spiked sample minus the spike concentration, as a proportion of the unspiked sample. Spike recovery detects errors due to either sample manipulation, which may sometimes result in the loss of an analyte leading to erroneously low measured values, or the introduction of compounds by the sample or sample matrix that interfere with accurate measurement. Therefore, spike recovery assists in accounting for the per cent loss of an analyte and may detect interfering substances.

Precision was assessed by examining intra- and inter-assay variability of samples with different hormones levels. Intra-assay variability was determined by evaluating between four and 23 plasma samples (according to species and assay; see Table II) in duplicate within the same run of the assay. Inter-assay variability was determined by evaluating between three and eight samples (according to species and assay; see Table II) in two or three runs of the assay. Variabilities or coefficients of variation (C.V.) of repeated measures of samples were assessed. C.V. (y) was calculated according to the formula: $y = 100z\,\overline{x}^{-1}$, where $z = $ S.D. A kit was considered to have good precision if the C.V. was $<20\%$ as per the guidelines, for example Plikaytis *et al.* (1994) and Sukovaty *et al.* (2006).

Pooled plasma from the three species was screened with eight dilutions of the 11-KT kit's standards. A characteristic S-shaped curve was observed for 12 dilutions of *A. percula's* pooled plasma [Fig. 1(a)]. As only the linear part is of interest, however, the curve using seven dilutions of pooled plasma was used and was found to run parallel to that obtained using standards provided with the 11-KT kit [Table I and Fig. 1(b)]. Five dilutions of pooled plasma from *A. chrysopterus* and five dilutions of pooled plasma from *C. melanopterus* were screened with the 11-KT kit's standard curve. The curves obtained were parallel to those using 11-KT standards [Table I and

TABLE I. ANCOVA on homogeneity of slopes for sample dilution v., standard dilution curves for 11-ketotestosterone (11-KT) and cortisol kits in, *Amphiprion percula, Amphiprion chrysopterus* and *Carcharhinus melanopterus*. The dilution factor (dilution) for 50% of antibody bound determined from regression analyses (Figs 1 and 2) is also given

Assay	Species	d.f.	Mean square	F	P	Dilution factor
11-KT	*A. percula*	1,47	0·002	0·180	>0·05	1:18·7 (0·054)
	A. chrysopterus	1,35	0·015	1·991	>0·05	1:8·7 (0·115)
	C. melanopterus	1,33	0·011	1·264	>0·05	1:187·5 (0·005)
Cortisol	*A. percula*	1,55	0·018	0·676	>0·05	1:425·9 (0·002)
	A. chrysopterus	1,36	0·004	0·379	>0·05	1:137·4 (0·007)

TABLE II. Percentage spike and recovery of 11-ketotestosterone (11-KT) and cortisol in *Amphiprion percula*, *Amphiprion chrysopterus* and *Carcharhinus melanopterus*

Assay	Species	n	Spike (pg ml^{-1})	Mean ± S.E. spike recovery
11-KT	*A. percula*	4	50	116·1 ± 22·1
	A. chrysopterus	4	25	89·8 ± 16·0
	C. melanopterus	4	100	123·6 ± 16·2
Cortisol	*A. percula*	4	5000	111·5 ± 14·0
	A. chrysopterus	4	1000	107·9 ± 20·5

Fig. 1(c), (d)]. Regression analyses enabled the appropriate dilution factors for 50% of antibody bound for all three species to be determined for the 11-KT kit (Table I). All three species tested with the 11-KT kit showed high accuracy determined from spike and recovery (89·8–123·6%; Table II) and high precision determined from intra- and inter-assay variabilities (8·7–14·8%; Table III).

11-KT concentrations of aquarium and field, *A. percula* and *A. chrysopterus*, based on the mean ± S.E. of the pooled plasma from 20 and 30 individuals were 0·35 ± 0·02 and 0·11 ± 0·00 ng ml^{-1}, respectively. These means are comparable to 11-KT levels previously recorded using radioimmunoassay for *Amphiprion melanopus* Bleeker (mean ± S.E. plasma 11-KT value estimated from figures: males = 0·63 ± 0·06 ng ml^{-1} and females = 0·23 ± 0·02 ng ml^{-1}; Godwin & Thomas, 1993). 11-KT concentration of field *C. melanopterus*, based on the mean ± S.E. of the pooled plasma from 25 individuals was 1·82 ± 0·10 ng ml^{-1}. This mean is comparable to values previously recorded using radioimmunoassay for adult male bonnetheads, *Sphyrna tiburo* (L.) (mean ± S.E. range of plasma 11-KT values = 0·07 ± 0·91 to 2·21 ± 0·09 ng ml^{-1}; Manire *et al.*, 1999).

Pooled plasma from the two *Amphiprion* species were also screened for parallelism with eight dilutions of the cortisol kit's standards. For *A. percula* and *A. chrysopterus*, seven and four dilutions of pooled plasma respectively, were found to run parallel to the cortisol standard curve (Table I and Fig. 2). Regression analyses enabled the appropriate dilution factors for 50% of antibody bound for both clown anemonefish species to be determined with the cortisol kit (Table I). *Amphiprion percula* and *A. chrysopterus* also showed high accuracy and precision with the cortisol kit determined from spike and recovery (107·9–111·5%; Table II) and intra- and inter-assay variabilities, respectively (9·9–19·4%; Table III).

Cortisol concentrations of aquarium and field *A. percula* and *A. chrysopterus* based on the mean ± S.E. of the pooled plasma were 18·98 ± 1·59 and 21·53 ± 2·46 ng ml^{-1}, respectively. These means are comparable with cortisol levels recorded using radioimmunoassay for *A. melanopus* (mean plasma cortisol values: males = 17 ± 4 ng ml^{-1} and females = 16 ± 3 ng ml^{-1}; Godwin & Thomas, 1993).

In conclusion, 11-KT EIA assay kits were tested on two *Amphiprion* species, *A. percula* and *A. chrysopterus* and *C. melanopterus*, and cortisol EIA assay kits were tested for use on *A. percula* and *A. chrysopterus*. The dose–response curves were parallel to the kits standards [Figs1(b)–(d) and 2(a), (b) and Table I], high accuracy was obtained from spike recovery determination (Table II) and high precision was obtained from intra- and inter-assay variabilities (<20%; Table III) for both kits with
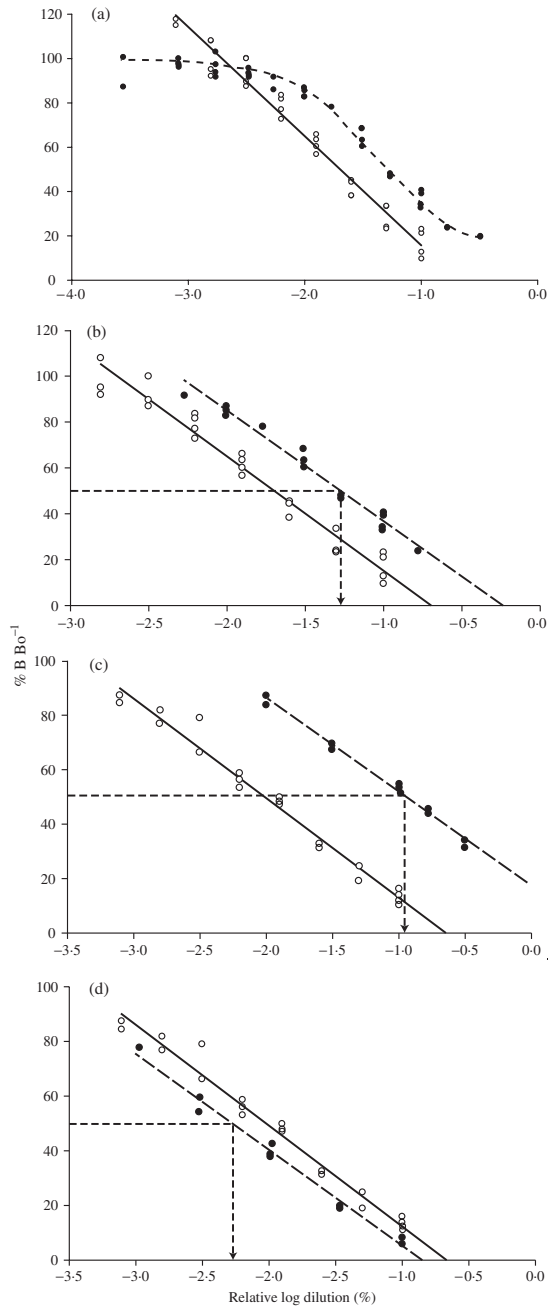
FIG. 1. Dose–response curves for 11-ketotestosterone (11-KT) obtained using eight kit standards and pooled plasma from (a) *Amphiprion percula*, (b) *Amphiprion percula* [simple linear regression: kit standards $y = -49.26x - 33.52$ ($r^2 = 0.97$, $n = 29$, $P < 0.001$) and samples $y = -48.19x - 11.27$ ($r^2 = 0.97$, $n = 17$, $P < 0.001$)], (c) *Amphiprion chrysopterus* [kit standards $y = -36.71x - 24.08$, ($r^2 = 0.98$, $n = 21$, $P < 0.001$) and samples $y = -34.32x + 17.73$ ($r^2 = 0.99$, $n = 13$, $P < 0.001$)] and (d) *Carcharhinus melanopterus* [kit standards $y = -36.71x - 24.08$ ($r^2 = 0.98$, $n = 21$, $P < 0.001$) and samples $y = -35.02x + 19.59$ ($r^2 = 0.99$, $n = 11$, $P < 0.001$)]. Dashed line and arrow represents 50% bound (see Table I for corresponding dilution factors). ●, pooled species plasma; ○, 11-KT kit standards.
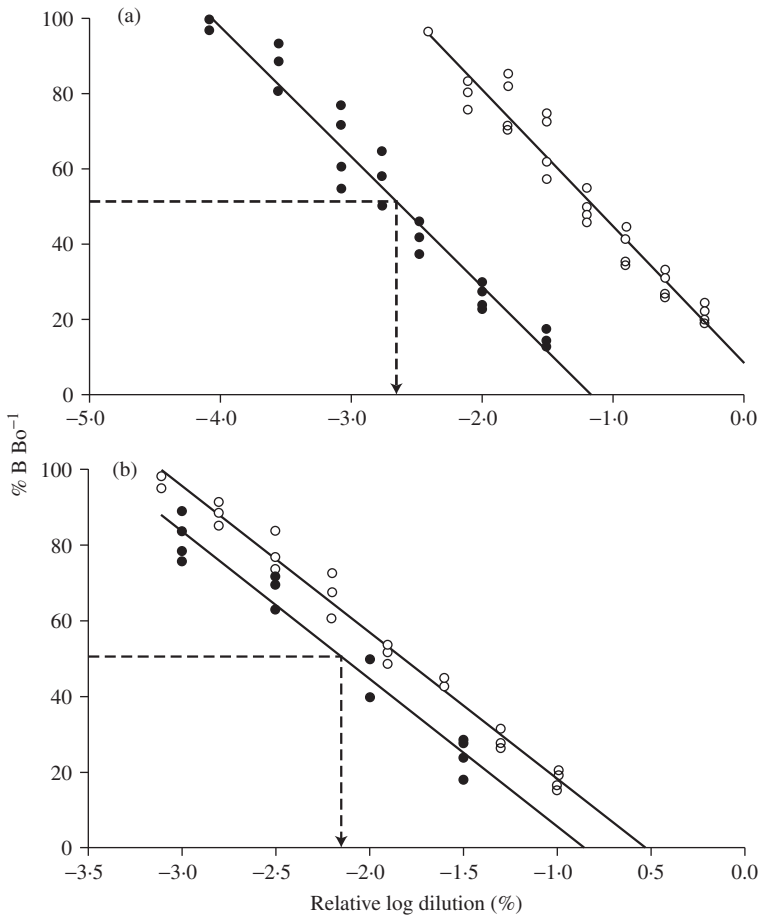
FIG. 2. Dose–response curves for cortisol obtained using eight kit standards and pooled plasma from (a) *Amphiprion percula* [kit standards $y = -36 \cdot 39x + 8 \cdot 27$ ($r^2 = 0 \cdot 95$, $n = 29$, $P < 0 \cdot 001$) and samples $y = -34 \cdot 52x - 40 \cdot 76$ ($r^2 = 0 \cdot 95$, $n = 25$, $P < 0 \cdot 001$)] and (b) *Amphiprion chrysopterus* [kit standards $y = -38 \cdot 66x - 20 \cdot 56$ ($r^2 = 0 \cdot 99$, $n = 23$, $P < 0 \cdot 001$) and samples $y = -38 \cdot 92x - 33 \cdot 22$ ($r^2 = 0 \cdot 95$, $n = 12$, $P < 0 \cdot 001$)]. Dashed line and arrow represents 50% bound (see Table I for corresponding dilution factors). ●, pooled sample plasma; ○, cortisol kit standards.

the two *Amphiprion* species, and for the 11-KT kit with *C. melanopterus*. Consequently, these kits can be confidently used for measuring 11-KT and cortisol in *A. percula* and 11-KT in *C. melanopterus*. In addition, only small quantities of blood plasma were required, the minimum quantity of blood plasma required being 5 or 7 μl if the samples are to be tested in duplicate. This is of particular interest for the small-bodied, *A. percula* (mean $L_S = 35 \cdot 05$ mm). *Amphiprion percula* has an average haematocrit of $16 \cdot 5\%$, therefore the total blood volume required is 6 and 8 μl, for measuring samples singly or in duplicate, respectively. The small volume of blood required for hormone determination is an additional advantage for using this EIA kit and furthermore, multiple sampling on the same individual may also be performed.

TABLE III. Intra- and inter-assay variabilities (coefficient of variation c.v.) for 11-ketotestosterone (11-KT) and cortisol in *Amphiprion percula*, *Amphiprion chrysopterus* and *Carcharhinus melanopterus*

Hormone	Species	Intraplate c.v. (*n*)	Interplate c.v. (*n*)
11-KT	*A. percula*	14·8 (22)	12·6 (7)
	A. chrysopterus	8·7 (6)	9·6 (3)
	C. melanopterus	14·2 (7)	14·6 (3)
Cortisol	*A. percula*	9·9 (23)	13·1 (8)
	A. chrysopterus	16·6 (4)	19·4 (4)

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