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Long-term social structure of long-finned pilot whales (*Globicephala melas*) in the Strait of Gibraltar

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Abstract The Strait of Gibraltar is inhabited by around 216 pilot whales, which are present all year round, and nothing is known about their social structure. The aim of this study is to analyse the inter-individual association patterns within this pilot whales community to (1) provide an insight on their long-term social system and (2) to assess the relationship between sexes within this social system. Between 1999 and 2006, 23,004 km was sampled in the Strait of Gibraltar, and 4,887 images of dorsal fins of pilot whales were taken from 226 groups. The sex of 56 of the individuals could be determined genetically. The strength of the behavioural relationships between dyads of individuals was calculated, and the temporal aspects of the social structure were evaluated, showing in a non-random social structure made by constant companions. The preferred associations between individuals consisted in associations of males–females. Eight long-term units could be found with different degrees of association rates. Consequently, we propose that, in the Strait, the pilot whales exhibit a hierarchical social system composed of a population encompassing several clans of pilot whales each containing several pods. Pods will then be formed by several line units, similar to killer whale matrilineal units.

Keywords Pilot whales · Social structure · Strait of Gibraltar · Cetacean

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Introduction

The study of the social structure of a species usually refers to the results obtained from observation of interactions or associations between individuals (Hinde 1976; Whitehead 1997). The social structure has been defined as relationship patterns between individuals or the sum of social behaviours of an individual (Gosling and Petrie 1981). Usually, mammals display geographic or social dispersion of males, females or both (Greenwood 1980). Marine mammals display a high variability in their social systems. On one hand, we have the fusion–fission social structures of bottlenose dolphins (*Tursiops truncatus*), where the associations between individuals show definite patterns of association that vary according to age and gender (Connor 2000), and on the other hand, killer whales (*Orcinus orca*), that spend all their life in closed groups, but with temporary interactions between them, exhibiting a true hierarchical structure (Bigg et al. 1990; Barrett-Lennard 2000).

The social organisation of resident killer whales off Vancouver Island, British Columbia has been studied intensively and a natal 'pod' philopatry of both sexes has been found, a pattern not previously documented among mammals (Bigg et al. 1990; Baird and Whitehead 2000). In this kind of structure, the killer whales form matrilines, where family members stay in the group their entire life, and they leave it only for short periods for reproductive activities with other groups. Those matrilines can form a sub-pod of closely related matrilineal groups that almost always (>95%) travel with one another. Most sub-pods contain one or two matrilineal groups (Bigg et al. 1990). These authors defined pods as groups of closely related matrilines that travel, forage, socialise and rest with each other at least 50% of the time, and predicted that pods, like matrilines, would be stable over many generations. However, Ford and Ellis (2002) showed that inter-matrilines association patterns in the northern residents have changed during the 1990s, such that some of the pods identified by Bigg et al. (1990) now fail to meet the 50% criterion. Their analysis suggests that pods are best defined as transitional groupings that reflect the relatedness of recently diverged matrilines. Each resident pod has a unique dialect made up of approximately a dozen discrete calls (Ford 1989, 1991). These dialects can be distinguished, providing each pod with a unique acoustic signature. Dialects are learned from mothers and other associated kin, and are highly stable over time (Ford et al. 2000). Pods that share one or more calls belong to a common clan. Resident killer whales that share a common range and that associate at least occasionally are considered to be members of the same community or population. There are two communities of resident killer whales in British Columbia: the northern residents and the southern residents. They have not been observed interacting and genetic studies have revealed that the two populations rarely, if ever, interbreed (Barrett-Lennard 2000).

It has been suspected that two species of pilot whales, long-finned pilot whale (*Globicephala melas*) and short-finned pilot whales (*Globicephala macrorhynchus*) could present the same type of behaviour (Heimlich-Boran 1993; Amos et al. 1991a, b, 1993a, b; Fullard 2000; Fullard et al. 2000). For short-finned pilot whales, only behavioural studies have been carried out (Shane and McSweeney 1990; Heimlich-Boran 1993), and there is no genetic evidence that could confirm this hypothesis. In the case of long-finned pilot whales, studies carried out on the North Atlantic drive fisheries analysed genetic samples (Andersen 1988; Amos et al. 1991a, b, 1993a, b, Caurant et al. 1994; Fullard 2000; Fullard et al. 2000). Those studies show that no genetic interchanges between the groups caught at the same time in the Faroese fisheries seem to exist. Analysis on polymorphic proteins (Andersen 1988, 1993), organochlorine concentrations (Aguilar et al. 1993), trace metals (Caurant et al. 1993, 1994) and intestinal parasite communities (Balbuena and Raga 1994) also showed differences between the groups caught. Nevertheless, those studies were made on individuals or groups that were forced to the coast by whaling operations; therefore, no information regarding a possible behavioural social system is available (Connor 2000). Only the study by Ottensmeyer and Whitehead (2003) analysed the possible social structure of this species in Nova Scotia, Canada. This study reveals that pilot whales of this area would form groups between 11 and 12 individuals, showing a social system based on casual acquaintance and constant companions, for at least 5 years.

The Strait of Gibraltar is the only natural connection between the Mediterranean Sea and the Atlantic Ocean. Seven cetacean species have been described in its waters (de Stephanis et al. 2008). Among them, the long-finned pilot whale is probably the best studied (de Stephanis 2008, de Stephanis et al. 2008; Verborgh et al. 2008). A community of approximately 216 long-finned pilot whales was identified (Verborgh et al. 2008). The social system of this community has not been studied until now. The aim of this study is to analyse the inter-individual association patterns within this pilot whales community to (1) provide an insight on their long-term social system and (2) to assess the relationship between the sexes inside this social system.

Materials and methods

Study area and surveys

The study area is the Strait of Gibraltar and contiguous waters, comprising between 5° and 6° longitude west. All waters lying from the Spanish coast to the Moroccan territorial waters were surveyed for pilot whales. The bathymetry of the Strait is characterised by a west to east canyon, with shallower waters (200–300 m) found on the Atlantic side and deeper waters (800–1,000 m) on the Mediterranean side (Fig. 1).

Survey transects were conducted from whale watching boats belonging to the companies Firmm, Whale Watch España and Aventura Marina between 1999 and 2000, and from the CIRCE research motorboat, *Elsa*, between 2001 and 2006, surveying the study area through the whole year. Transects were conducted without any predefined track for each of these surveys but were designed to cover the whole bathymetric range through the entire year, crossing the isobaths as perpendicularly as possible. The sampling strategy was identical throughout the survey period and onboard the different boats (SEC 1999). The area was surveyed at an average speed of 5.3 knots. Searching effort stopped when a sighting of pilot whales was contacted and started again when the sighting was ended, with a return to the course previously established. When surveys were conducted simultaneously by several boats (between 1999 and 2000), no contacts were made between vessels, so transects were considered independent from each other.

Two trained observers occupied an observation platform, 4 m above sea level, in 1-h shifts during daylight with visibility over 3 nm (5.6 km), assisted with 8 × 50 binoculars, covering 180° ahead of the vessel. Sighting

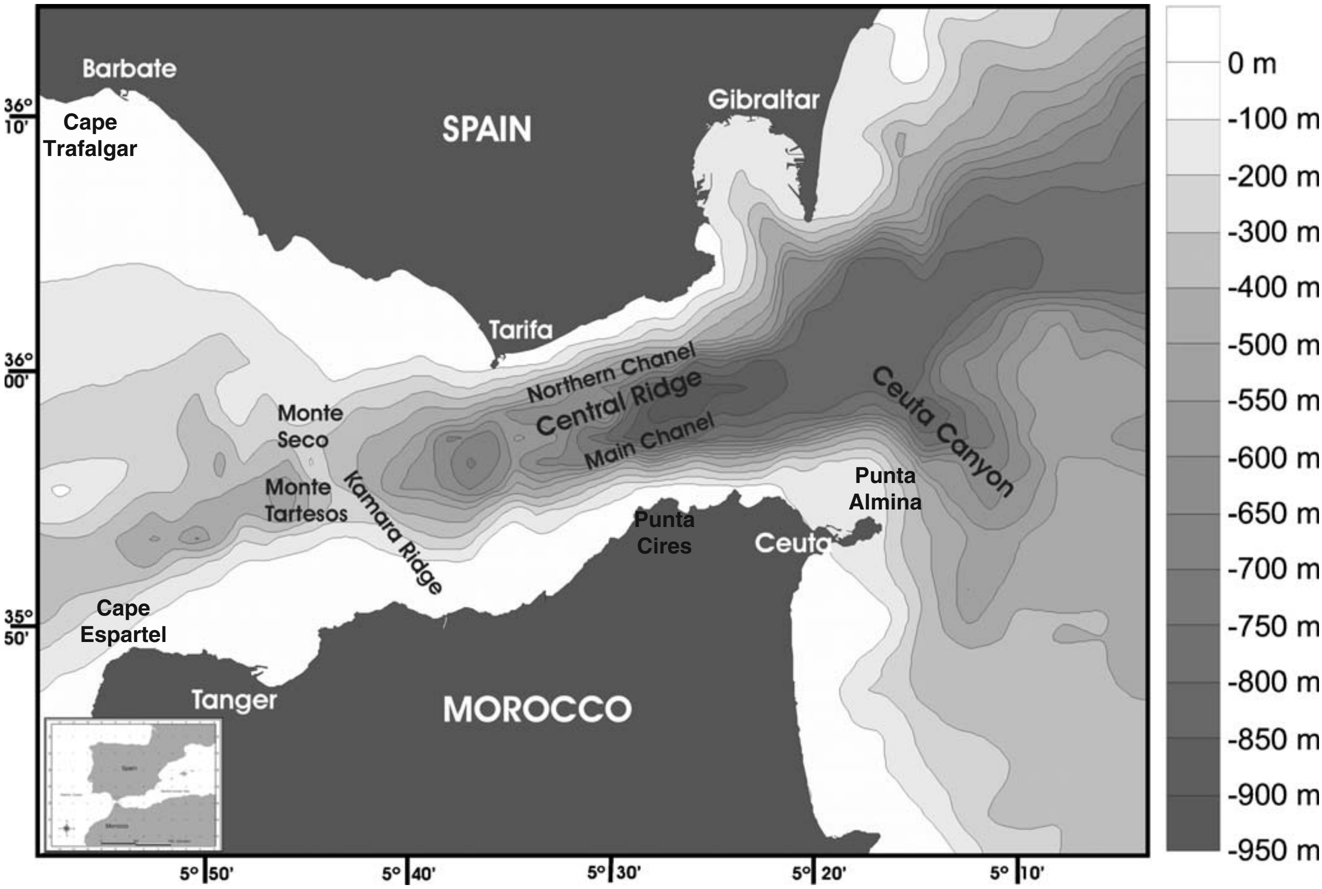

Fig. 1 Research area and bathymetry of the Strait of Gibraltar (from Sanz et al. 1988)

effort was measured as the number of kilometres travelled with adequate sighting conditions (i.e. with sea state Douglas less than 4 and two observers on the lookout post). The geographic position of the ship was recorded every minute on the ship's computer from a GPS navigation system logger using the International Fund for Animal Welfare Data Logging Software Logger 2000 version 2.20 (available at http://www.ifaw.org/ifaw/general/default.aspx?oid=25739) between 2001 and 2006. During 1999 and 2000, the GPS position was taken every 10 min, or for any change of course, weather and effort conditions. A group was defined as a compact aggregation of long-finned pilot whales seen at the same time, showing similar behaviour and in which the maximum distance between two individuals was less than 5 m (one body length). When a group of animals was first seen, the location of the ship, the approximate distance and bearing of the cetaceans were recorded to be able to localise during approach. The location of the animals was also recorded when they were approached by the vessel (i.e. less than 100 m of the animals). All animals observed from the beginning to the end of an observation in which animals were approached were considered to belong to the same group.

Photo-identification and groups

The methodology used to organise the photo-identification catalogue and to select the groups that were used in the subsequent analyses followed that of Ottensmeyer and Whitehead (2003) and Jankowski (2005) so that the social structure of the long-finned pilot whales observed in the Strait of Gibraltar could be compared with those observed elsewhere, and in particular in northern Nova Scotia, Canada. During the encounter with a group of pilot whales, pictures of their dorsal fin were taken using a photo-identification protocol. Under this protocol, the photographers took pictures of the completely exposed dorsal fins of all pilot whales surfacing at the vicinity of the research vessel with the best possible magnification. The animals were approached as close as possible to take pictures of the left side of the dorsal fin. The left side is the most accessible as they are swimming most of the time against the predominant east current to remain approximately in the same area (Verborgh et al. 2008). Therefore, the left side is oriented southward and best lit by the sun to take good pictures; consequently, a left side catalogue was built up for conducting the analyses. All individuals encountered were photographed irrespective of their level of marking. The dorsal fin close-ups allowed individual identification according to their natural features or "marks" of the dorsal fins (shape, notches and nicks; Bigg 1982; Ottensmeyer and Whitehead 2003; Auger-Méthé and Whitehead 2007). From 1999 until 2002, pictures were taken with a Nikon F-810 camera equipped with a 100–300-mm objective. The films used were Fujichrome Sensia 100 ASA colour slides. From 2002 to 2004, a Canon EF100–400-mm image stabiliser lens was used on a Canon EOS-3 camera. Since 2004, this lens is mounted on a digital camera Canon 10D (6.3 megapixels).

On many pictures, more than one individual or dorsal fin was photographed due to the compact grouping behaviour exhibited by pilot whales. The term 'fin image' will be used in this study to refer to the representation of a single dorsal fin in a picture that can contain many others. The same method was applied from 1999; therefore, all pictures were used and analysed in an identical way.

All slide pictures taken from 1999 to 2003 were labelled according to the sighting, roll and picture number. Since 2004, all pictures were digital and labelled according to the group and picture number. All slides were studied with an ×8 magnifying eyepiece on a light table, while digital pictures were examined on a computer screen. Each picture was analysed and data entered in a related Access® database. Data consisted of general information with sighting, roll, picture, total number of individuals in the picture and a number given to each individual present on the picture according to its position on the picture (from the closest to the furthest one, and from left to right) of each individual analysed on the picture. Information on the exposure of each individual present on the picture were: fin (out of the water or not), angle of the fin (every 30°), regardless of the level of marking of the fin and a fin image quality level ranging from 0 (the worst) to 2 (the best) was attributed, a code name for identified individuals, proportion of the back exposed and behaviour.

An alpha-numeric code name was given to each individual formally identified (seen and identified from two different sightings) which were then included in the photo-identification catalogue. Matches with previously identified individuals were made by comparing each new photograph with all the others in the catalogue. Animals that could not be matched and positively identified on more than two high quality pictures were given a new identification code and were entered in the photo-identification catalogue. The best slide of each individual for each sighting was scanned with a Nikon Coolscan III scanner, in a 2,700-dpi resolution which allowed easier matching with digital pictures.

Only individuals indexed in the catalogue were ranked according to their marking level (M) going from M0 (little marks) to M3 (highly marked). Individuals without any distinctive marking were categorised as unmarked and excluded from the catalogue.

Once we estimated to have photographed all individuals present in the group, the photo-identification effort stopped, and the search effort started again, with a return to the course previously established.

During the photo-identification sessions of the groups, one observer counted every 2 min the number of individual present in the group and its composition (i.e. the number of adults, juveniles, calves and newborns). The respective size of individuals was assessed according to three age-class categories: adults were large animals with a dark coloration, juveniles were between 1/3 and 2/3 the length of adults and calves were less than 1/3 the length of adults. Newborns were individuals with fetal fold marks on their body (SEC 1999; Cañadas and Sagarminaga 2000).

Groups with poor photographic coverage were excluded (i.e. the number of photographs taken was smaller than the number of individuals estimated to be present in the group, either from counts or according to the minimum number of pilot whales present simultaneously on a picture). Furthermore, we excluded groups for which we failed to photograph all individuals. The remaining groups are designated as "coverage > 0" (Ottensmeyer and Whitehead 2003). Analyses that excluded groups based on the poorest coverage were likely to give a more consistent assessment of individual presence or absence within a pod than analyses including all groups. However, such selection criteria could bias our results towards smaller groups, since it is difficult to photograph all individuals in very large groups. To improve the quality of our assessment of individual presence or absence, we selected encounters in which the number of photographs taken was at least twice the estimated group size. These encounters are designated "coverage ≥ 2".

The proportion of marked individuals in the population was estimated from 1,000 samples of 270 random fin images of high quality (Q2) for the whole study period (1999–2006). We estimated the inverse of the proportion of identifiable individuals (noted $\bar{\hat{c}}$) with the mean of the ratios from Eq. 1 calculated for the 1,000 samples. This estimate was named "correction factor" and noted $\bar{\hat{c}}$.

$$\hat{c} = \frac{\text{number of good quality fin images (Q2) of unmarked and marked individuals}}{\text{number of good quality fin images (Q2) of marked individuals}} \quad (1)$$

This estimate assumes that, on average, the same numbers of best quality photographs (Q2) were taken of well-marked individuals the same as that of lightly marked or unmarked ones (Ottensmeyer and Whitehead 2003; Auger-Méthé and Whitehead 2007; Verborgh et al. 2008). The confidence interval (95%) was also calculated. To have an idea of the variability of the individuals marked in each group photo-identified, a correction factor was also calculated from the pictures taken in each identified group. To have this metric, all the pictures were taken into consideration in each group, and no permutation was possible due to the low number of images made per group.

Pilot whales sampling strategy

To determine the sex of the pilot whales, a genetic analysis was performed from skin samples obtained from the animals. Skin biopsies were performed using a 67-kg draw crossbow (Barnett Wildcat XL) at distances ranging from 5 to 15 m from the animal, and the dart was fired at the mid-lateral region near the dorsal fin of the whale. A stop collar attached to the tip of the bolt prevented penetration deeper than the biopsy tip and caused the bolt to rebound upon impact with the whale. The darts were designed to float and were collected using a dip net. The skin biopsies collected included epidermis and dermis layers with biopsy arrows with tips 1.5 cm long and 0.6 cm in internal diameter. Both the tips and the arrows were designed and fabricated by Finn Larsen of the Danish Institute for Fisheries Research, Charlottenlund, Denmark. Samples were obtained under a permit issued by the Spanish Ministry of Environment of Spain. The skin was preserved at −20°C in a solution of 20% dimethylsulphoxide in saturated salt (Amos and Hoelzel 1991).

The animals were biopsied during two periods: the first one between 25 and 27 October 2005, and the second one between 25 July and 2 August 2006. Only adults were biopsied. The animals were biopsied only if they were found travelling (never if they were seen foraging, milling, resting or socialising). The biopsies were only attempted if the sea conditions of the sea were between 0 and 1 Douglas, and the wind conditions were between 1 and 0 Beaufort. In each biopsy session, the crew of the *Elsa* made sure that there was no calf present in the area before firing the crossbow. Prior to the biopsy, the dorsal fin of the individual to be sampled was photographed, and its identity confirmed onboard with the photo-identification catalogue to avoid double samplings. In each attempt of biopsy, information regarding the reaction of the animals was annotated. The number of animals that reacted to the biopsy and the type of reaction (none, dive, dive and evasion and tail slap) were recorded in each attempt of biopsy.

Sex determination

The DNA was purified from the biopsy taken with the Qiagen DNeasy blood and tissue sample kit. The sex of the 56 individuals sampled was assessed genetically by polymerase chain reaction (PCR) of introns within the *Zfx* and *Zfy* genes (Shaw et al. 2003). To double check the results obtained with the *Zfx*/*Zfy*-specific primers and avoid false positives, we reamplified the same samples with primers specific for males only, targeting the *SRY* gene (Rosel 2003). DNA was extracted and purified from a 20-mg aliquot of skin with the Qiagen DNeasy blood and tissue kit. PCR was performed on 30 ng of purified DNA in a 20-µl reaction volume otherwise containing: 2 µl of 10× *Taq* polymerase reaction buffer (supplied by the manufacturer), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.1 mg/ml BSA, 1U of *Taq* polymerase (Invitrogen), and either 0.2 µM of primers LGL331-forward and LGL335-reverse (for *Zfx/Zfy* amplification), or 0.2 µM of primer *SRY*-forward and 0.04 µM of primer *SRY*-reverse (for *SRY* amplification). The PCR reactions were performed as follows: 1 cycle at 94°C for 3 min, then 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 52°C (for *Zfx*/*Zfy* primers) or 56°C (for *SRY* primers) and amplification at 72°C for 45 s. Then, each sample was loaded on a 1% agarose gel and the fragments were separated by electrophoresis. *Zfx*/*Zfy*-specific primers allowed for the differentiation between males (two bands, ~930 bp for the X-specific band and ~1,000 bp for the Y-specific fragment) and females (one band). A confirmation of the results was obtained with the primers specific for the *SRY* gene which is positive for males (one band, ~350 bp) and negative for females (no band). The sequences of the primers are presented in Table 1.

Associations

The strength of the behavioural relationships between dyads was initially calculated using the half-weight association index and the simple ratio association index (Cairns and Schwäger 1987; Ginsberg and Young 1992; Ottensmeyer and Whitehead 2003). However, as the inferences drawn were the same for the two indices, only values of the half-weight association index are presented in this paper. Individuals photographed in the same pod at least once during a day were considered associated for the day (the sampling period). The association patterns of the most commonly encountered pilot whales were represented using average-linkage cluster analyses (Manly 1994) using animals sighted on at least four different days and only groups with a “coverage > 0” were included.

Permutation tests (Bejder et al. 1998; Whitehead 1999) were implemented to test whether the association patterns

Table 1 Sequence of the primers used to determine sex of the pilot whales genetically from skin biopsies

Primers	Sequence
LGL331-forward	5′-CAAATCATGCAAGGATAGAC-3′
LGL335-reverse	5′-AGACCTGATTCCAGACAGTACCA-3′
SRY-forward	5′-ACCGGCTTTCCATTCGTGAACG-3′
SRY-reverse	5′-CATTGTGTGGTCTCGTGATC-3′

observed were different from a random association calculated for a population size and residency patterns identical to our study population. The absence of overlap between standard deviation interval of the pairwise association indices between the observed data set and the association indices randomly generated were used to identify preferred or avoided associations between two individuals (Whitehead 1999). All groups with "coverage > 0" were included, and 20,000 permutations were generated for each test. To ensure that p values were stable, six runs of the permutation test were generated using the simple ratio and half-weight association indices of three runs each (Ottensmeyer and Whitehead 2003).

To model temporal aspects of the social structure, we used the standardised lagged association rate (SLAR; Whitehead 1995, 1997). The SLAR is the average probability, given that individuals A and B are associated at time 0, that B will be identified later in a photograph of a randomly chosen group member at a time lag τ when A is also present (Whitehead 1995). The SLAR is estimated by calculating

$$\hat{g}(\tau) = \frac{\sum_A \sum_t c(A,t,\tau)}{\sum_A \sum_t N(A,t) \cdot N(A,t+\tau)} \quad (2)$$

where $c(A, t, \tau)$ is the number of associates that were seen with individual A at both time t and time $t + \tau$, and $N(A,t)$ is the number of associates seen with individual A at time t. The SLAR is compared at all time lags with the null association rate, i.e. the SLAR expected if all individuals are associating at random, given the population size:

$$\hat{g}(\tau) = \frac{1}{(P-1)}$$

where P is the number of well-marked individuals in the analysis. Sampling periods were individual days, and in this analysis, we restricted to only those groups with coverage ≥2, and the individuals sighted on six or more occasions. All individuals photo-identified within a group were considered to be associated. Standard errors were estimated by jackknife methods (Efron and Gong 1983). Mathematical models representing simulated social structures (Whitehead 1995) were fitted to the SLAR (Table 1). According to Whitehead (1999), the best fit model was chosen as the one that minimised Akaike's (1974) Information Criterion or the Quasi Akaike's (1974) Information Criterion. The preceding analyses of association patterns were using the compiled version of SOCPROG (Whitehead 1999).

Sex influence on association indexes

The maximum association index ratios of individual of the same sex and different sexes were compared. The term "best companion" was defined as the individual B with which another individual A has the highest association index rate.

Unit membership

To investigate the stability of groups over time, we defined the term "unit" following the procedure proposed by Christal et al. (1998) in sperm whales and Ottensmeyer and Whitehead (2003) for long-finned pilot whales. Individuals sighted in at least five different years, each observation of the individual in a group separated from the preceding one by a gap of at least 180 days, were identified as "key individuals". Individuals sighted at any time in the same group as the "key individual" during a day for at least 5 days, each observation of the group separated from the preceding one by a gap of at least 60 days and in almost two different years, were selected as constant companions of the key individual. A "unit" was defined as a key individual and all of its constant companions (if a unit consisted of more than two individuals, all individuals had to be constant companions of at least two of the others). Once the units were defined, the dendograms and the SLAR of individuals selected as constant companions were calculated in the groups with more than three individuals.

Results

Observed group size

A total observation effort representing 23,004 km searched in the Strait of Gibraltar was realised between 1999 and 2006, allowing the assessment of 225 groups of pilot whales. On 136 occasions, the age class of the individuals in the group could be noted. The mean observed group size data are given in Fig. 2 and Table 2. Group size was significantly larger in spring compared to summer and autumn (Kruskal–Wallis = 23.77, $p < 0.0001$; Bonferroni < 0.05). Calves or newborns were observed in 67% of the groups, while newborns were only observed in 17% of the groups.

Photo-identification

A total of 10,932 pictures representing 19,015 pilot whale individuals (a given picture can include several individuals), of which 14,096 were fin images, have been analysed. From these, 5,364 were fin images of quality Q0, 4,542 of Q1 and 4,190 of Q2. From 1999 to 2006, 234 individuals have been identified and included in the catalogue with one individual marked M0, 135 marked M1, 73 marked M2 and 25 marked M3. A total of 4,887 fin images of good quality could be associated to a group. Of those, 2,887 were of individuals marked M2 or M3, and represented a total of 69 individuals

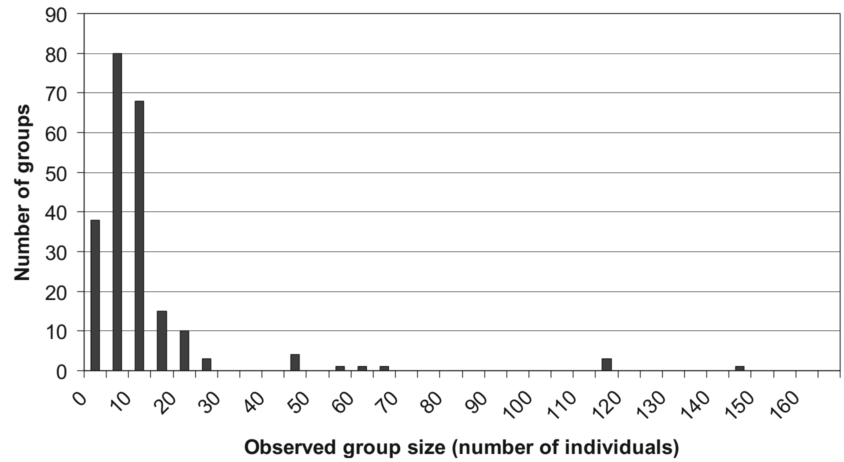

Fig. 2 Observed group size of long-finned pilot whales between 1999 and 2006. Bins for group sizes include the values of the label (e.g. 1–5, 6–10, etc.)

marked M2 and 25 individuals marked M3. For the complete data set, the correction factor $\bar{c}$, after performing 1,000 randomisations, was estimated to be 1.42, (95% CI = 1.32–1.52). For groups, the mean correction factor was 1.52 (SD = ±0.75, range = 1–7; Fig. 3).

Associations of individuals

The standard deviation of the observed pairwise association indices was significantly higher than those from permuted data (simple ratio index $p < 0.0001$; half-weight index $p < 0.001$), so the null hypothesis of a random association of individuals was rejected. The cluster diagram (Fig. 4; cophenetic correlation coefficient = 0.84) indicates that most individuals sighted repeatedly (on at least four different occasions, i.e. days) were sighted with preferred companions. Fifty percent of them were seen at least 70% of the days with the same companion, and 88% were linked to another individual at a half-weight index of ≥0.5. The SLAR (Fig. 5) stabilised at a level higher than the one expected if individuals were associating at random (null association rate = 0.0238) at a time scale ranging between 10 to 2,275 days. The social system model that best fit the curve is described by the model including the constant companion hypothesis over this period (Table 3).

Biopsies reaction

A total of 68 biopsies were attempted. Nine percent of the attempts were negative and did not impact on the pilot whale, and 91% of the attempts were positive and hit the whale. From the positive attempts, in 10% of the occasions, no sample was obtained, leaving a total of 56 biopsies performed on the pilot whales. When the arrow did not hit the animal, in 17% of the occasions, no reaction was observed, and in 83% of the occasions, all the groups reacted, making a dive and evading the ship. When the arrow hit the whale, in 34% of the occasions, the whale or group of whales did not react; in 31% of the occasions, a reaction of all the group was recorded (21% tail slapped, 68% dived, and 10% dived and avoided the boat); and in 35% of the occasions, only the whale biopsied reacted (27% tail slapped, 68% dived and 45% dived and avoided the boat).

Sex determination and association between sexes

From the 56 individuals genetically sexed, 23 were females and 33 were males. In this subsample, male–female relationships showed significantly higher maximum association rates than male–male relationships (ANOVA

Table 2 Mean group size, standard deviation, median, mode and range of the groups of pilot whales observed in winter, spring, summer, autumn and annually in the Strait of Gibraltar between 1999 and 2006

	Winter (January–March)	Spring (April–June)	Summer (July–September)	Autumn (October–December)	Annually
Mean group size	15	25	13	11	14
Standard deviation	4	25	18	4	18
Median	15	15	10	10	10
Mode	15	13	10	12	10
Range	8–20	3–120	2–150	5–20	2–150
n	9	29	166	21	225

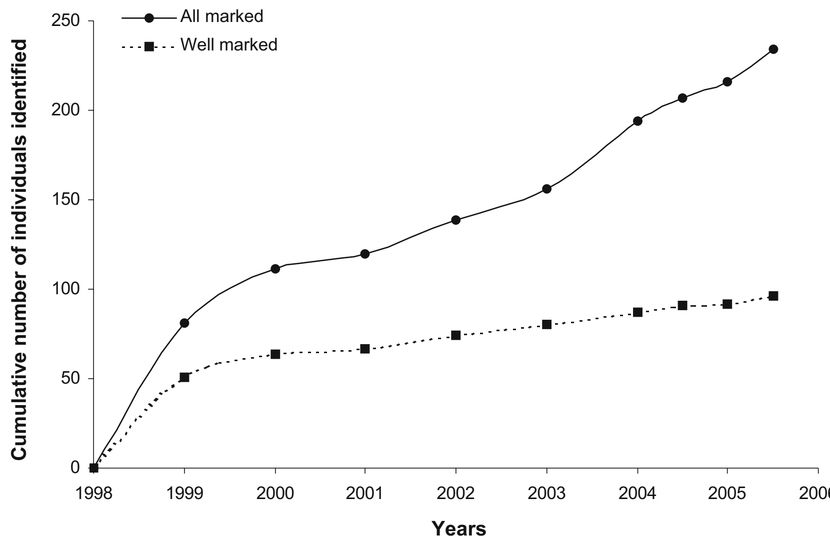

Fig. 3 Discovery curve of all marked (M0, M1, M2 and M3) and well-marked (M2 and M3) individual pilot whales in the Strait of Gibraltar

F = 3.19, df = 2, p < 0.05; LSD p < 0.05). In 76% (19 occasions) of the cases, the best companion was an individual of another sex with a mean association rate of 0.79 (SD = 0.18); in 16% (four occasions) of the cases, best companions were both females with a mean association rate of 0.51 (SD = 0.21); and in 8% (two occasions) of the cases, best companions were both males with a mean association rate of 0.91, SD = 0.05.

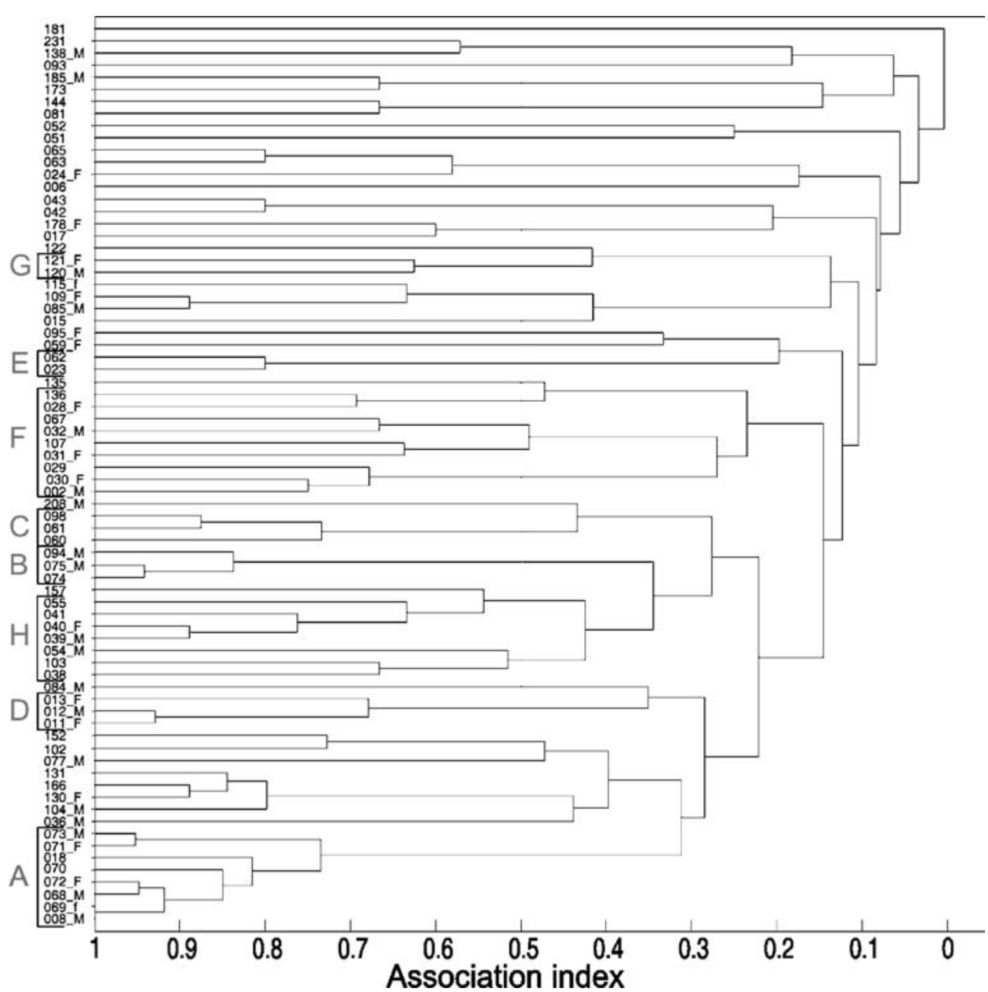

Fig. 4 Average-linkage cluster analyses of association (half-weight association index) between well-marked (M2 and M3) individuals seen on ≥4 days, using only groups with coverage >0 for long-finned pilot whales in the Strait of Gibraltar (cophenetic correlation coefficient=0.84). The sex is marked after the individual code with *F* Female and *M* Male. The code *f*, associated to the individual 069-f and 115-f, indicates that the animal was sexed visually. The units defined in Fig. 6 are also shown in this figure with their corresponding letter

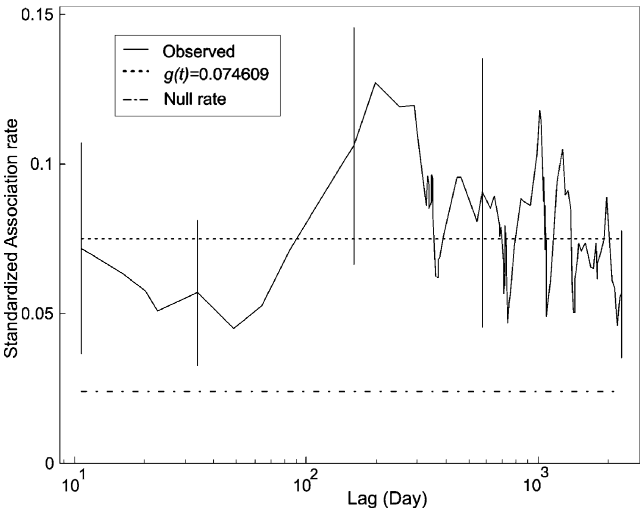

Fig. 5 Standardised lagged association rate (SLAR) for all well-marked individuals sighted in groups with coverage ≥2. Approximate error bar were generated by jackknife techniques (+/− 1 standard error). The moving average over 5,400 possible associations is shown. The maximum-likelihood best fit model represents association with constant companions. The null association rate represents the theoretical SLAR if individuals associated randomly (y=0.0135)

Unit membership

A total of 32 key individuals and eight units could be defined (Fig. 6). Three of the units (A, F and H) had more than three individuals. Units A, F and H had mean association index rates of 0.82 (SD = 0.1), 0.39 (SD = 0.18) and 0.60 (SD = 0.23), respectively (Table 4). Their dendograms and SLAR are plotted in Fig. 7. In all three occasions, the social system model that best fit the curve describes constant companions and is displayed in Fig. 7.

Discussion

If we observe the types of reaction of the whales to the biopsy sampling strategy, we can see that there were more reactions to the biopsy attempt when the arrow did not hit the whale than when the arrow hit the whale. This can be explained by the noise that the arrow will do when impacting the sea surface. This noise will probably scare the group of animals, as it is not a natural noise, and therefore, the group will avoid this source of noise. This study is one of the first using this kind of methodology in a group of pilot whales. If we observe the number of groups that reacted avoiding the ship (only 5% of the occasions), we can conclude that the whale did not relate the impact of the arrow to the ship or researchers, and therefore, the methodology does not seem to impact negatively on the behaviour of the whale. One possible explanation could be that the whale could associate the impact of the arrow to a physical interaction with another whale. Therefore, one of the most important components to be taken into account using this methodology is that the attempts need to be well aimed most of the times. An experienced researcher and really good sea conditions are crucial for a correct biopsy sampling strategy.

This study shows that individual long-finned pilot whales of the Strait of Gibraltar exhibit long-lasting relationships among themselves. The mean observed group size of the groups was around 14, ranging between 2 and 150. The observed group size varied by season, with significantly larger groups in the spring, nearly twice as large as those observed in summer, fall and winter. As the population is closed (de Stephanis 2008; Verborgh et al. 2008), these results suggest that behavioural changes take place in spring, with an increase in the association of

Table 3 Fit of social system models to the standardised lagged association rate (SLAR) of the clan of long-finned pilot whales of the Strait of Gibraltar

Description of model	Model formula	Maximum likelihood values for parameters	Jackknifed standard errors for parameters	No. of parameters	QAIC
Constant companions (CC)	$g(\tau)=a_1$	a_1=0.074609	0.017291	1	<u>3,374.9</u>
Casual acquaintances (CA)	$g(\tau) = a_2 e^{(-*a_1.\tau)}$	$a_1=1.06\times10^{-4}$	1.11×10^{-4}	2	3374.5
		a_2=0.082288	0.024052	2	
CC + CA	$g(\tau) = a_2 + a_3 e^{(-*a_1.\tau)}$	a_1=2.2866	4.4948	3	3,378.3
		a_2=0.074898	0.017348	3	
		a_3=−49.2973	55,759,928.3	3	
Two levels of CAs	$g(\tau) = a_3 e^{(-*a_1.\tau)} + a_4 e^{(-*a_2.\tau)}$	a_1=0.19236	0.88798	4	3,378.4
		$a_2=1.10\times10^{-4}$	1.35×10^{-4}	4	
		a_3=−0.018486	1.0482	4	
		a_4=0.0827	0.026682	4	

The value underlined is for the best fit model according to the QAIC (Cuasi Akaike Information Criteria).

Fig. 6 Sightings of the units of all key individual and their constant companions between 1999 and 2006 (see definition in "Materials and methods"). Key individuals are marked in *bold*. The individuals 098-, 062-, 067-, 038- and 055- are constant companions but not key individuals. Individual 67- is not a constant companion, but is included due to its relationship with the rest of the group. The individual 060- and 013_F included in units C and D, respectively were seen alone and not in the group formed by its two constant companions on the days 13/08/2004 and 20/10/2005, respectively and so are marked with a *O* instead of an *X* for those 2 days. For the rest of the sightings the rest of the days, the individuals were seen with the rest of the constant companions marked with an *X*

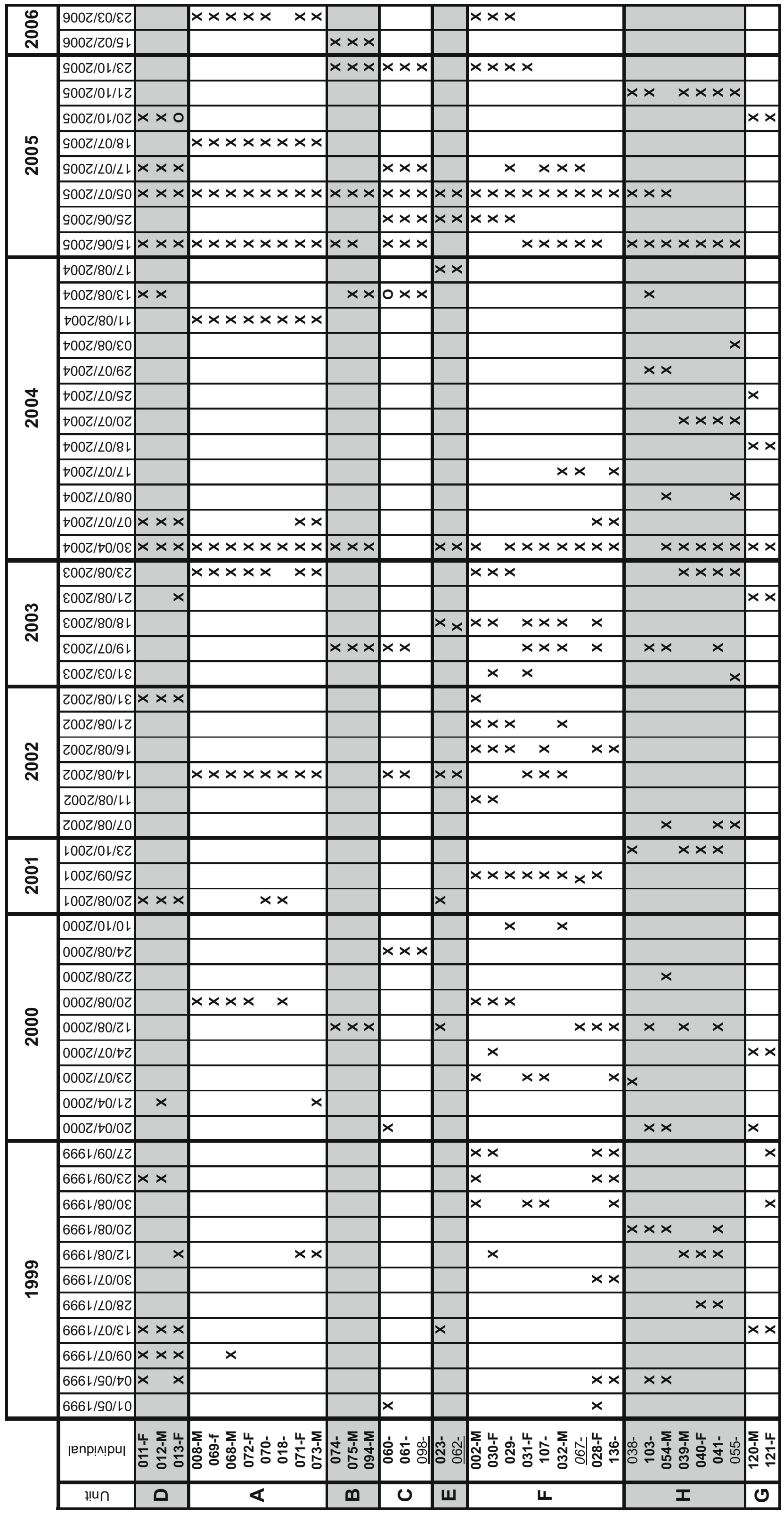

Table 4 Description of the long-finned pilot whales Units, their mean and mean of the maximum association index rate (AIR) in the Strait of Gibraltar

Unit	Number of individuals	Mean association rate (SD)	Mean maximum AIR
A	8	0.82 (0.05)	0.94 (0.06)
B	3	0.87 (0.04)	0.92 (0.04)
C	3	0.78 (0.05)	0.85 (0.04)
D	3	0.76 (0.07)	0.86 (0.12)
E	2	0.80 (0)	0.80 (0)
F	9	0.39 (0.04)	0.69 (0.04)
G	2	0.63 (0)	0.63 (0)
H	7	0.53 (0.06)	0.73 (0.12)

groups which tend to travel separately the rest of the year. The reason for such regrouping is unknown but could be linked to a shift in foraging behaviour (prey), as evidenced by the change in spatial distribution of the long-finned pilot whale between seasons in the Strait of Gibraltar (de Stephanis 2008; de Stephanis et al. 2008). As an alternative hypothesis, this could be linked to breeding behaviour, with sub-groups regrouping to mate, but so far, we are lacking any evidence. Only paternity tests will allow us to assess if the fathers all belong to the 234 individuals resident to the Strait of Gibraltar or are unknown. Cañadas and Sagarminaga (2000) found an opposite trend in the group size of the long-finned pilot whales in the Alborán Sea, with smaller groups in spring (April–June) compared to summer (July–September). If we compare the observed group size with the results presented by Ottensmeyer and Whitehead (2003) and Jankowski (2005) in Nova Scotia (Canada), both the mean and median size of the observed group size were close to the mean and median size observed in this study (mean = 14.5 SD = 12, median = 11 for Nova Scotia vs. mean = 14 SD = 18, median = 10 in this work).

The results of this research also show that pilot whales in the Strait exhibit long-lasting relationships and the best model fitting the data is the one with constant companions, between 10 days and 6.4 years. In Nova Scotia, the social system was described as being of casual acquaintances and constant companions (Ottensmeyer and Whitehead 2003; Jankowski 2005). A mix of casual acquaintances and constant companions was described to occur for around 4 days. In our case, because of the restrictions to fit the social systems (coverage $\geq$ 2 and individuals sighted on more than six occasions), the group size used to analyse the SLAR were almost never bigger than 21 individuals. In 10% of the occasions, the observed group size was bigger than 21 individuals, with a maximum size of 150 individuals. This suggests that, in some occasions, the different groups defined in this study would meet for short time periods (less than 10 days). The data of this study are fitted for more than 10 days; therefore, if a casual acquaintance social component is also present in the social structure of the pilot whales of the Strait, as it can be inferred observing the maximum size of the groups seen in this study, our data set will not allow the model to detect it.

This work also shows that small units, which we will refer to as line units, can be detected. Units B, C, D and E, formed by two or three well-marked individuals, and unit A, formed by eight well-marked individuals, have high mean and mean maximum association rates. The individuals belonging to unit A were seen together 82% of the time and their SLAR also fit a constant companion model (Table 4 and Figs. 4, 6 and 7). According to the high differences in the correction factor between groups (c = 1.52 SD = 0.75, range 1–7), the line units formed by two or three individuals are probably composed of more but unidentified individuals (like unit A).

Units F and H would be formed by several line units, constant in the time (as revealed by their SLAR), and with strong social relationship. Several long-term studies have been carried out on different cetacean species. Sperm whales usually form matrilineal groups of young individuals without adult males (Christal and Whitehead 2001). Killer whales have been found to be the only mammal showing natal ‘pod’ philopatry of both sexes (Bigg et al. 1990; Baird and Whitehead 2000). Stable pods of individuals, associating along matrilines, are quite large, averaging about 12 and ranging from three to 50 individuals (Bigg et al. 1990). The social structure of pilot whales described in this study seems to be similar to the one described for the resident type of killer whales from the eastern North Pacific. The line units defined in this work would be the matrilines as proposed for the killer whales system, and the groups with a mean estimated size of 14 individuals would be similar to the pods described in killer whales and would be formed by several line units as is the case for killer whale pods with matrilines. The pilot whale community found in the Strait of Gibraltar would then consist of several pods that form large temporary aggregations (up to 150 individuals). The different sex best companions of the unit lines of our study could be explained by two scenarios. The first would be that these are stable breeding partnerships. This hypothesis is unlikely according to what was observed in pilot whales, as studied by Amos et al. (1991a, b, 1993a, b) and Fullard (2000) unless there is an inordinate intraspecific variability. These authors showed that usually the fathers of the individuals belonging to the grinds taken around the Faeroe Islands were not present in the same grind, but could be present in other grinds, taken at another time and location.

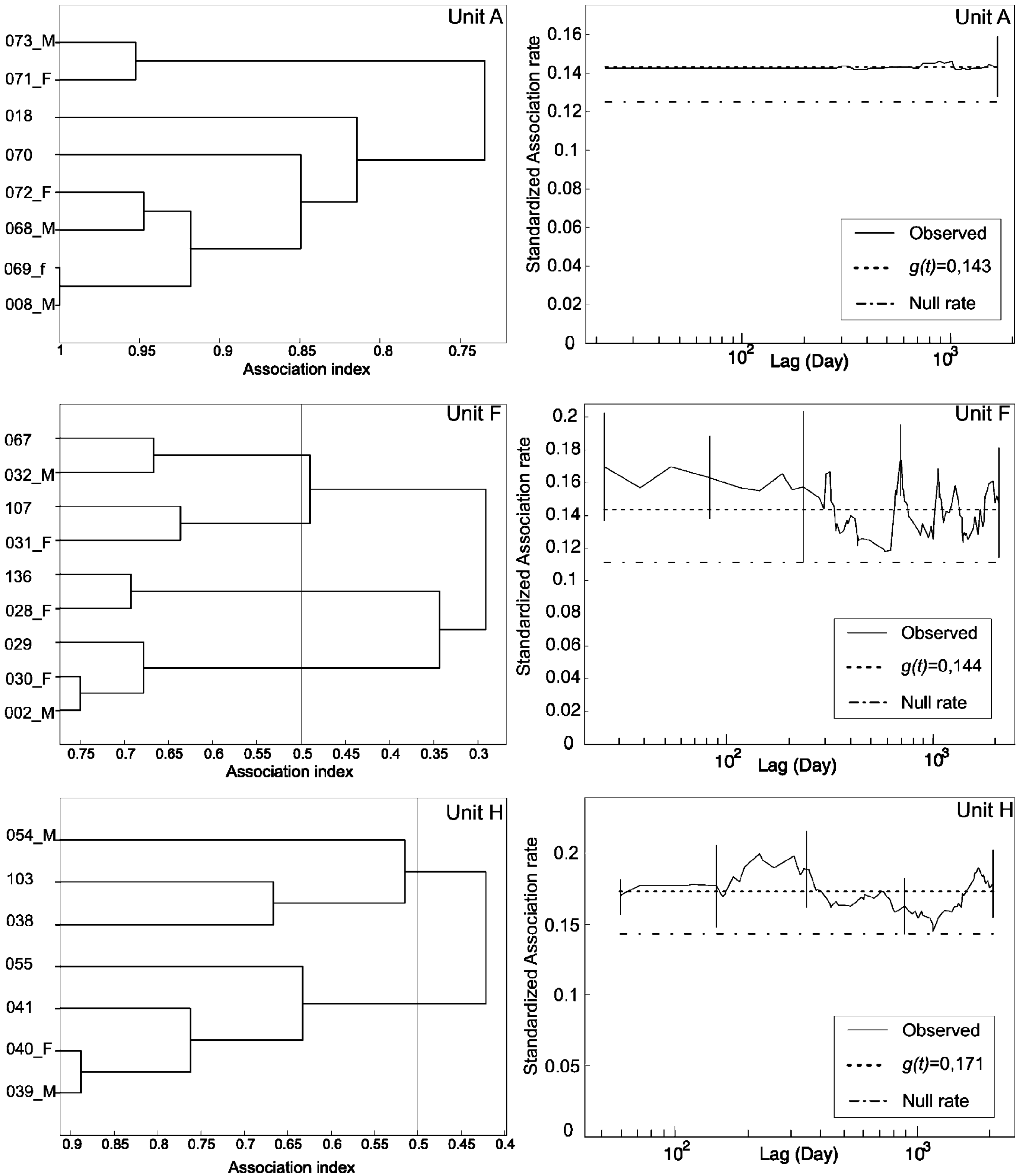

Fig. 7 Average-linkage cluster analyses of association (half-weight association index) between individuals included in units A (cophenetic correlation coefficient (CCC)=0.89), F (CCC=0.82) and H (CCC=0.86), using only groups with coverage > 0 and their SLAR using groups of coverage ≥ 2. The maximum-likelihood best fit model for each unit and their null association rate are also represented

A more probable scenario is that these long-term male–female associations reflect mother–son relationships and this should be further investigated with a genetic approach.

There is evidence that long-finned pilot whales caught in the Faeroe Islands showed a matrilineal structure (Amos et al. 1991a, b, 1993a, b; Fullard 2000). In the Faroese fisheries, between 1729 and 1992, the mean size of the groups caught was of 149.8 whales (n = 1,629 grinds; Zachariassen 1993), and nothing was known about their structure at sea when they were caught. Cañadas and Sagarminaga (2000) described groups of pilot whales as aggregations of animals separated by more than 1,000 m. These sightings included several sub-aggregations of pilot whales. Therefore, grinds taken in the Faeroes, or those sightings in the Alborán Sea could be formed by different pods as defined in this work, and those pods would form the so-called aggregations of the Alborán Sea, or the so-called grinds of the Faeroe Islands that we can refer to as a clan (i.e. pods which associate regularly).

The pilot whale pods encountered in the Strait of Gibraltar form a clan, which is known to leave the Strait by the end of the spring possibly to breed (de Stephanis 2008). It is presently unknown if pilot whales belonging to the same clan interbreed. Based on the results obtained in this study, we hypothesise that, as in the Faeroe Islands, pilot whales encountered in the Strait of Gibraltar form a grind or clan (as defined in this work), possibly mating with other clans living out of the Strait of Gibraltar. Although we have never observed the moment when the clan leaves the Strait, the same species shows an inverse trend in the Alborán Sea (Cañadas and Sagarminaga 2002), with an increment of encounter rates of pilot whales at the end of the spring. This allows us to speculate with the possibility of mating behaviours with the long-finned pilot whales of this area, but this issue requires further investigation. Consequently, we propose that the pilot whales encountered in the Strait of Gibraltar exhibit a social system based on a clan formed by pods. This clan will belong to a population encompassing several clans of long-finned pilot whales each containing several pods. Pods will then be formed by several line units, similar to killer whale matrilineal units. This type of social structure could also apply for the same species in other areas of the Atlantic or the Mediterranean Sea.

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