Estimating the consequences of the 2006-07 *Morbillivirus* epizootic on the long-finned pilot whales in the Strait of Gibraltar

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ABSTRACT

This study investigated the consequences of the 2006-07 Morbillivirus epizootic on the population of long-finned pilot whale (*Globicephala melas*) in the Strait of Gibraltar. Photo-identification techniques allowed estimating the basic life parameters of the pilot whale population in the Strait of Gibraltar. Survival rates were found to be 0.985 for adults in 1999-06 with a total abundance of 345 animals in 2006. Secondly, 2006-07 Morbillivirus epizootic was estimated to induce a 21.2% reduction in the survival rate (decreasing to 0.776) and to cause a total of 78 deaths (including natural mortality) between summer 2006 and summer 2007. This study assessed for the first time the consequences of a Morbillivirus infection and mortality on a well known population of pilot whales in the Strait of Gibraltar.

KEYWORD
Epizootic, disease, monitoring, trends, Mediterranean Sea

INTRODUCTION

Since 1999 the long-finned pilot whale (*Globicephala melas*) population of the Strait of Gibraltar has been studied by the research group CIRCE (Conservation, Information and Research on Cetaceans) through photo-identification technique. From 1999 to 2005, mark-recapture methods applied to photo-identified individuals estimated that summer population size was constant around 216 individuals through the years, with a survival rate of 0.982 (SE: 0.008; 95% CI: 0.955-0.993) and an annual population growth rate of 1.055 (SE: 0.017; 95% CI: 1.021-1.089) (Verborgh *et al.,* 2009). It was a unique situation where a population status had been known before possible problems could occur. In winter 2006-07 an unusual high mortality was detected with an increase of stranded pilot whales in the region. This was established based on 10 well known stranded pilot whales during a 5 months period while the average during 1998-2006 was 0.9/year (Fernández *et al.,* 2008), but nothing is known about the consequences of this epizootic on the population.

The Strait of Gibraltar is inhabited by a population of long-finned pilot whales found in the deepest and sloppiest part of the strait during summer (de Stephanis *et al*., 2008a, In press). De Stephanis *et al.* (In press) indicated that the number of long-finned pilot whales present in the strait between winter and summer varied but stayed within the 95% confidence interval. Furthermore they found that the same individuals were observed year round, and 95 % of the animals seen in winter were also seen during the summer and 92% of the pilot whales were seen for more than one year in the Strait. According to the authors, these observations strongly suggested that the pilot whales observed in the Strait of Gibraltar are part of a year round resident population.

Although previous *Morbillivirus* outbreaks in pilot whales had never been reported, antibodies to *Morbillivirus* have been reported in 86% of 2 species of pilot whales (*Globicephala melas* and *G. macrorhynchus*) in the western Atlantic (Duignan *et al.,* 1995). Fernández *et al.* (2008) reported the first epizootic of a lethal *Morbillivirus* infection of long-finned pilot whales in the Mediterranean Sea from the end of October 2006 through April 2007: more than 27 long-finned pilot whales were found stranded along the southern Spanish Mediterranean coast and Balearic Islands; 10 of these stranded in the Strait of Gibraltar area from the end of October 2006 through early February 2007 before the number of stranding animals spread eastwards. The virus involved in the present pilot whale epizootic differs from pilot whale *Morbillivirus* (PWMV) (Taubenberger *et al.,* 2000), which supports previous evidence that different strains of cetacean *Morbillivirus*es may be infecting dolphins and whales (Taubenberger *et al.,* 1996). The viruses isolated from the 1990-1992 and 2006-07 stranded striped dolphins in Mediterranean waters and the 2006-07 stranded pilot whales of the Strait of Gibraltar are closely related phylogenetically. Therefore, Fernández *et al.* (2008) think that interspecies transmission should be considered, especially because of the spatial and temporal association of the 2006-07 striped dolphins and pilot whale deaths. The authors found that all stranded pilot whales were adults or subadults, except 2 that were juveniles, and that *Morbillivirus* was detected in the brains, lymph nodes and the lungs but the central nervous and lymphatic systems were the most severely affected tissues. Possible explanations for how and why the disease starts are, among others, pollutants (Aguilar and Borrell, 1994), the high intensive chronic anthropogenic effects in the Strait of Gibraltar area (de Stephanis *et al.*, 2005), a DMV entering a naive pilot whale population, or a progressive decrease of humoral immunity against the virus in these populations (Van Bressem *et al.*, 1991; Raga *et al.*, 2008).

The main objective of this study was to assess the impact of the 2006-07 epizootic on this population.

MATERIALS AND METHODS

Photo-identification

During the sightings of pilot whales, pictures of their dorsal fins were taken using a photo-identification protocol. Under this protocol, the photographers took pictures of completely exposed left side dorsal fins of all pilot whales surfacing in the vicinity of the research vessel. The left side is the most accessible as the animals are swimming most of the time against the predominant east current and is therefore facing south where it is best lit by the sun to take good pictures. A catalogue was made only for the left side of their dorsal fins as analyses are using only one side. All the individuals in the sighting were photographed irrespective of their level of marking in order to have the same probability of captures for all individuals. In 1999-2000 the pictures were taken from whale watching boats until CIRCE acquired a research vessel in 2001. 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 EF100-400mm objective with image stabilizer was used with a Canon EOS-3 camera. Since 2004, this objective was used with a digital camera Canon 10D (6.3 Megapixels). The same protocol was implemented since 1999; therefore, all the pictures can be used and analysed in the same 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 renamed with the sighting and picture number. All the slides were looked at with an 8x 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 database. Data consisted of general information: sighting, roll, picture, total number of animals in the picture, number of each individual analysed on the picture; and information on each individual: exposure of the fin (in/out of the water), angle of the fin (every 30°), individual fin image quality (on a scale from 0 (worst) to 2 (best)), code name of the individual in the catalogue, proportion of back exposed and the age class (calf, juvenile, adult).

The angle starts from 0° when the animal is seen directly in front and goes around the animal every 30° (Figure 4). This allows a selection of only the animals seen on the left side (from 240° to 300°) for the analyses.

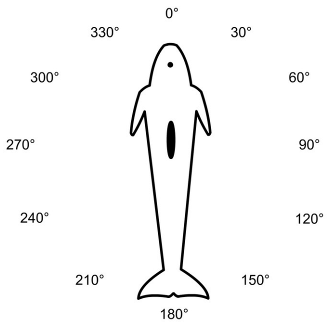

Figure 1: Angle description around the dorsal fin. The angle number is the position of the photographer.

A quality rating (*Q*) on a scale of 0 to 2 (poor to excellent) is assigned to each fin image based on image suitability in terms of five characteristics: focus, size, orientation, exposure, and the percentage of the fin that was visible in the frame:

Q 0: individual dorsal fin when its representation is blurred, too far away or if the angle is between 330 and 30° or 150° and 210° (see Figure 4).

Q 1: medium quality representation of part of or the entire dorsal fin

Q 2: high quality representation of the entire dorsal fin

A code name is given to each individual identified in the catalogue. The dorsal fin close-ups allowed "marked" individuals to be identified based on the natural features or marks of the dorsal fins (shape, notches and nicks) (Bigg, 1982; Ottensmeyer and Whitehead, 2003). Matches with previously identified individuals were made by comparing each new photograph with all the others in the catalogue. Marked animals that could not be matched but could be positively identified on high quality pictures (Q2) were given a new identification number.

Abundance and survival rates of the population

Pollock's closed robust design (Pollock, 1982) with Pradel's population growth estimator (Pradel, 1996) was used to calculate annual survival rates, abundance estimates, and population growth rate of the portion of the population that was marked using eight summers (1999-2007) of capture-resighting data. Secondary sessions' closure means that no birth, death, immigration or emigration is allowed within a secondary session (summer); however, these events are allowed between primary sessions, which are consequently considered open.

During secondary sessions a closed population model was used to estimate the capture probabilities. Because heterogeneity of capture between individuals was suspected, models with heterogeneity were used. Heterogeneity was modeled using a finite mixture model (Pledger, 2000) with two groups of individuals, one with high probability of capture (p_{high}) and the other with low probability of capture (p_{low}). The mixing parameter π(pi) indicated the proportion of the population in one of the two groups of individuals. It implies having only two probabilities of capture for each year. The mixing parameter can either differ between years or not.

As capture-recapture models were used to estimate the number ($\hat{N}$) of only marked individuals in the population from 1999 to 2007, the total population size ($\hat{N}'$) needed to be corrected by the correction factor $\hat{c}$ (see Equation 1, 2, 3 and 4):

Equation 1:

$$\hat{N}' = \hat{N} \times \hat{c}$$

The same was applied to the 95% Confidence Interval (CI) limits estimated:

Equation 2:

$$L.CI(\hat{N}') = L.CI(\hat{N}) \times \hat{c}$$

$$U.CI(\hat{N}') = U.CI(\hat{N}) \times \hat{c}$$

where *L.CI* is the lower 95% CI and *U.CI* is the upper 95% CI.

The proportion of all marked individuals in the population was estimated in order to correct the estimation made by mark-recapture models in the program *MARK*:

Equation 3:

$$\hat{c}_i = \frac{\text{number of good quality fin images (Q2) of marked and unmarked indiv.}}{\text{number of good quality fin images (Q2) of marked indiv.}}$$

This estimate assumed that, on average, the same number of best quality photographs were taken of marked individuals as of unmarked ones (Ottensmeyer and Whitehead, 2003).

Each primary period was divided into multiple secondary periods, each consisting of 15 consecutive days of sampling. All sightings of an individual within a secondary period were considered as one sighting. The number of secondary sessions varied among years. The following parameters were estimated for the intervals between primary periods:
phi(t), the probability that a member of the population in period *t* survives and is still a member of the population in period *t*+1;
L(t), the population growth rate based on both survival and recruitment in the population between primary periods.

Model selection started with the general model where survival (phi), population growth rate (lambda), heterogeneity (pi) and capture probability p varied between primary periods. Then, several hypotheses were tested : first, the effect of using slide (1999 to 2003) or digital pictures (2004 to 2007) on the capture probability (p(Slide+Digital)) and the effect of working from whale watching boats in 1999 and 2000 and a research boat from 2001 to 2007 (p(WW+Research)). A "*Morbillivirus* effect" on p, noted p(99-06+07), was also tested. Then the photographic effort was integrated in the capture probability using the standardized number of fin images analyzed in Q1 and Q2 per year as a covariate named effort (p(t(effort))). Finally, more parsimonious models were built by constraining parameters to be constant.

All mark-recapture analyses were run on Mark 5.1 (White and Burnham, 1999). Within ΔAICc ≤ 5, models were first selected to test more hypotheses. In the end, models within a ΔAICc ≤ 2 were considered to be well supported by the data (Burnham and Anderson, 1998).

All fin images of poor quality (Q0) were not used in the analyses in order to decrease the possibility of misidentification of poorly marked individuals.

RESULTS

Photo-identification

A total of 16 245 dorsal fin pictures were analysed in summer 1999-2007, rising to 24,909 fin images when including winters 2004-2006 (last sighting on 1st November 2006). Without bad quality pictures (Q0), the total of pictures of quality Q1 and Q2 was reduced to 11,158 fin images for the summer period.

Abundance and survival rates of the population

The first model considered was the general model {phi(t) L(t) pi(t) p(t)} where all the parameters were time dependent (Table 1).

Table 1. Model selection

	Model	AICc	ΔAICc	AICc Weights	Num. Par	Deviance
1	**{phit(99-06+07) L(t) pi(t) p(t(effort))}**	**1 163.2**	**0.0**	**0.42**	**39**	**1 083.7**
2	**{phit(t) L(t) pi(t) p(t)}**	**1 163.4**	**0.3**	**0.37**	**44**	**1 073.5**
3	{phit(99-06+07) L(t) pi(t) p(t)}	1 165.6	2.4	0.13	37	1 090.2
4	{phit(t) L(t) pi(t) p(t(effort))}	1 167.3	4.1	0.05	44	1 077.4
5	{phit(t) L(t) pi(t) p(99-06+07)}	1 168.9	5.7	0.02	31	1 105.9
6	{phit(t) L(t) pi(t) p(.)}	1 171.1	7.9	0.01	29	1 112.3
7	{phit(99-06+07) L(99-06+07) pi(t) p(t)}	1 172.5	9.3	0.00	33	1 105.4
8	{phit(99-06+07) L(99-06+07) pi(t) p(t(effort))}	1 172.8	9.6	0.00	35	1 101.6
9	{phit(t) L(99-06+07) pi(t) p(t)}	1 176.6	13.4	0.00	37	1 101.2
10	{phit(t) L(t) pi(t) p(WW+Research)}	1 182.4	19.2	0.00	30	1 121.5
11	{phit(t) L(t) pi(t) p(Slide+digital)}	1 227.1	63.9	0.00	31	1 164.2
12	{phit(t) L(t) pi(t) p(WW+Slide+Digital)}	1 235.2	72.0	0.00	31	1 172.2
13	{phit(t) L(t) pi(.) p(t)}	1 246.9	83.7	0.00	39	1 167.4

The two best models (model 1 and 2) had a ΔAICc=0.3 (Table 1). Therefore, a model averaging method was used to estimate the survival rate for marked individuals between summer 2006 and summer 2007 as well as summer 2007 abundance of marked individuals. The mean of 1999-06 yearly survival rates was calculated. Finally a correction factor was calculated.

Table 2. Survival rate phi and $\hat{N}$ abundance of the marked individuals and $\hat{N}'$ abundance of the entire population

Survival rate	phi	**Abundance**	$\hat{N}$ (95% CI)	$\hat{c}$	$\hat{N}' = \hat{c} \cdot \hat{N}$
1999-06	0.985	2006	235 (211-259)	1.489	350(314-386)
2006-07	0.776	2007	192 (187-198)	1.545	297(289-306)

Table 2 summarizes the estimate survival rate for the marked individuals in 1999-2006 and 2007. The *Morbillivirus* epizootic apparently caused a 21.2% reduction in the survival rate. In order to assess how many individuals died between 2006 and 2007, survival rate for 2006-07 phi=0.776 was applied to 2006 abundance estimate. The abundance estimated with the correction factor lead to the death of 78 individuals (350*(1-0.776)).

DISCUSSION

Assumptions of the Robust design

A number of assumptions were required by the robust design. First, population closure was assumed within each primary period. Given the life span, reproductive rate and social organization of pilot whales, the population was assumed effectively closed, (i.e., without mortality, births, emigration and immigration within primary periods) because only sightings during summer months were used for each year (Verborgh *et al.*, 2009). According to de Stephanis *et al.* (2008a), the distribution of pilot whales is mainly limited to the central deep channel of the Strait. Therefore, their geographical distribution is most probably closed as well.

Second, naturally marked individuals were assumed identified without errors. All the individuals in the sighting were photographed irrespectively of their level of marking. An effort was also made to make sure that all the individuals had been well photographed so that there was at least one good picture of each individual. Nicks on the dorsal fin were conserved for years but could evolve for example when new nicks appeared around an older one. Nicks were not lost over the study period as individuals identified in 1999 still had the same marks in 2007.

Survival rates and abundance estimates

The hypothesis on same probability of capture for all the animals was modelled to take into account time variation and heterogeneity between individuals. Model without heterogeneity were tested but as the abundance estimates were lower than for models integrating heterogeneity, they were discarded (Hammond, 1986).
In Pradel's closed robust design, only the marked individuals, that were mostly adults, were considered. Thereby it was assumed that there was no photographic bias between individuals. Moreover, nicks were not lost over the study period as marked individuals identified in 1999 still had the same nicks in 2007.

The two best models were selected and the *model averaging* method was used to estimate the survival reduction for the marked individuals. As this reduction could not be estimated for each age class or for the unmarked individuals, it was then assumed that survival reduction was the same for all the individuals, *i.e.* that the *Morbillivirus* could kill equally all the individuals in the population irrespective of their age or level of marking. However, Fernández *et al.* (2008) reported that most stranded animals were adult, so the assumption may overestimate the survival reduction on the whole population; nonetheless calves and juveniles under a certain age may not survive if their mother died, what could be considered as additive deaths indirectly caused by the epizootic.

This potential extra mortality was most probably not due to emigration because many individuals were reported missing in their social groups although all their mates were sighted, while the same individuals had been seen together every year between 1999 and 2005 (de Stephanis *et al.*, 2008b). Indeed, if emigration had occurred, the whole social group would have been expected to emigrate altogether.
Abundance estimate for 2007 is higher than 2006 abundance estimates minus 78 animals that were estimated to have died. This can be explained by the number of births (18 calves estimated in 2007). With a quick calculation (350-78+18), 291 individuals should be present in the population in summer 2007, which is consistent with 2007 estimation of 297 individuals (95% CI: 289-306).

Only 10 out of 78 estimated dead animals, *i.e.* 12.8%, were found stranded in the Strait of Gibraltar, what shows the importance of long-term studies on live individuals. The population should remain under supervision to further monitor the consequences of the *Morbillivirus* disease and how the population reacts to this high mortality in terms of birth intervals and survival rate for the different age classes.

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