

anoxic. The local active fishing fleet totals about 360 boats and is composed exclusively of small-scale fishing boats working primarily with set nets (i.e. trammel and gill nets), targeting mainly small pelagic/epipelagic fish and shrimp *Penaeus kerathurus*.

The Gulf of Ambracia, also referred to with its Greek name, “Amvrakikòs Kolpos”, is a shallow, semi-closed embayment of 405 km² whose only communication with the open Ionian Sea is through the Preveza Channel, a narrow (minimum width of 370m) and shallow (2–12 m) 3 km-long corridor (see map on page 11). The Gulf of Ambracia is partially included in Natura 2000 and Ramsar sites and is protected by national, European and international legislation. In 2008 it was designated as a ‘National Park’ in accordance with the Greek national legislation (11989/08 KYA).

Bottlenose dolphins *Tursiops truncatus* in the Gulf Ambracia constitute a geographically and otherwise distinct unit with little demographic exchange that is exposed to high levels of pollution, mostly derived from local agriculture (i.e., pesticides). Based on photographic mark-recapture estimates, 134 animals (CV = 0.11) resided in the Gulf in 2015 (Gonzalvo et al., 2016).

Criterion A - Species or Population Vulnerability

The Mediterranean subpopulation of common bottlenose dolphin *Tursiops truncatus* is assessed as Vulnerable in the IUCN Red List of Threatened Species - A2cde and based on a suspected population decline of at least 30% over the last 60 years (Bearzi et al., 2012). The bottlenose dolphin is listed in the Appendix II (Mediterranean subpopulation) of the Convention on the Conservation of Migratory Species of Wild Animals (CMS), in the Appendix II (Strictly Protected Fauna Species) of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention), and in the Annexes II and IV of the EU Habitats Directive (Council Directive 92/43/EEC).

While local density of bottlenose dolphins is among the highest recorded anywhere in the Mediterranean Sea, this is not indicative of favourable conservation status or pristine habitat. On the contrary, the viability of bottlenose dolphins in the Gulf of Ambracia may be at risk due to their likely reproductive isolation, small population size and small extent of occurrence, as well as acute and growing anthropogenic impacts in their semi-closed shallow habitat. The data used to provide evidence on the importance of the Gulf of Ambracia for the Mediterranean population of bottlenose dolphins comes primarily from intensive fieldwork conducted throughout the 2006-2015 study period (Gonzalvo et al., 2016; Gonzalvo et al., 2015a, 2015b).

Criterion B: Distribution and Abundance

Sub-criterion Bi: Small and Resident Populations

The Gulf of Ambracia hosts a small population of bottlenose dolphins that lives in the Gulf year-round. Bottlenose population estimates between 2006-15 mostly fell between 130 and 170 with CVs averaging about 10%. Confidence that the true size of the population lies within this range is therefore high. The estimated trend in population size over those 10 years was a decline of 1.6% per year but this was not significant and there is insufficient power in the data to detect a decline of this magnitude over 10 years.

A population size estimated to number fewer than 250 mature individuals can be classified as Endangered under criterion D (IUCN, 2012). The most robust estimates of the total number of bottlenose dolphins in the Gulf of Ambracia never exceeded 170 individuals and even the uppermost 95% confidence limit did not exceed 250.

Bottlenose dolphins in the Gulf of Ambracia showed high levels of year-round site fidelity throughout the 10-year study period. Dolphin groups encountered in the southwest portion of the Gulf, in areas closer to the Preveza channel, were followed for periods of up to

several hours, but they never entered the narrow and shallow corridor leading to open seawaters. Three individuals first photo-identified in the Gulf of Ambracia and regularly observed between 2003-2008, were subsequently found in the Inner Ionian Sea archipelago and in the Gulf of Corinth, not to be seen ever again in Ambracian waters (Gonzalvo, unpublished data). Based on photographs of their genital area, all three animals were males. This is consistent with the hypothesis that males are more wide-ranging than females, and they may therefore be the primary vectors of genetic exchange. Such observations indicate some small degree of emigration, but no immigration into the Gulf has been recorded so far.

Criterion C: Key Life Cycle Activities

Sub-criterion Ci: Reproductive Areas

Bottlenose dolphins live year-round in the semi-enclosed waters of the Gulf of Ambracia where they also reproduce and complete their natural life cycle. Boat surveys were conducted between 2006 and 2015 based on predefined routes (i.e., transects) designed to guarantee a uniform effort coverage of the whole Gulf of Ambracia on a monthly basis. Survey conditions were considered as “positive” under daylight and good visibility, sea state ≤ 3 Beaufort (large wavelets, crests beginning to break and scattered whitecaps) and with, at least, two observers scanning the sea surface looking for dolphins. When spotted, dolphin groups were approached at low speed, progressively converging with their routes and avoiding sudden changes of speed and directionality to minimize potential disturbance. A group was defined as ‘that composed of dolphins within approximately 100 m radius of each other that were observed in apparent association, moving in the same direction and often, but not always, engaged in the same activity’. Group size estimates and composition were recorded. The latter included four age categories (i.e., newborn, calf, juvenile and adult) based on visual assessment of sizes in relation to average adult size. During each dolphin

sighting, photo-identification effort was conducted to obtain as many good images as possible of every individual present in our focal group throughout the duration of the observation. Photo-identification was consistently based on long-term natural marks such as notches and nicks in the dolphins’ dorsal fins, as well as on any additional marks in other body parts.

Capture histories for each individual bottlenose dolphin identified in the Gulf of Ambracia were constructed based on the photo-identification work allowing also to follow the life-cycle as well as their reproductive success. Bottlenose dolphins in the Gulf seem to concentrate their breeding season during the summer months, when the sea temperature is higher and the probability of survival of their offspring is higher.

Criterion D: Special Attributes

Sub-criterion Di: Distinctiveness

The dolphins of the Gulf of Ambracia are genetically differentiated from the surrounding Mediterranean populations (Gonzalvo et al., 2016). The genetic diversity observed for the Gulf of Ambracia population was the lowest when compared with the rest of populations analysed in this study and with previous studies found in the literature, which suggests that the bottlenose dolphin Ambracian population is highly isolated. The genetic uniqueness of the dolphins of the Gulf of Ambracia, as shown by low mtDNA diversity and the presence of unique haplotypes, demonstrates low connectivity with neighbouring Adriatic Sea and Ionian Sea and could be explained by historical events, and/or low gene flow among populations.

Supporting Information

*Bearzi, G., Agazzi, S., Bonizzoni, S., Costa, M., Azzellino, A. 2008. Dolphins in a bottle: abundance, residency patterns and conservation of bottlenose dolphins *Tursiops truncatus* in the semi-closed eutrophic Amvrakikos Gulf, Greece. Aquatic*

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