Darwin Project DPLUS034
"Akrotiri Marsh Restoration: a flagship wetland in the Cyprus SBAs"

Conservation study of the Mediterranean Killifish Aphanius fasciatus in Akrotiri Marsh (Akrotiri SBA, Cyprus) Final Report

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Submitted to BirdLife Cyprus

15/2/2017

Conservation study of the Mediterranean Killifish *Aphanius fasciatus* in Akrotiri Marsh, (Akrotiri SBA, Cyprus) - Final Report

by Stamatis Zogaris

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Cover page: *Aphanius fasciatus* from the Episkopi Bay wetlands (Photo: C. Makris) and a medieval map of Akrotiri showing the extensive lagoon formations during the period that the Aliki Lagoon was open to the sea.

To be cited as:

Zogaris, S. 2017. Conservation study of the Mediterranean Killifish Aphanius fasciatus *in Akrotiri Marsh, (Akrotiri SBA, Cyprus) - Final Report. D*arwin Project DPLUS034 "Akrotiri Marsh Restoration: a flagship wetland in the Cyprus SBAs BirdLife Cyprus". Nicosia Cyprus. Unpublished final report, 64 pp.

Table of Contents

Executive Summary

Aphanius fasciatus, the Mediterranean Killifish, is a native inland waters fish on Cyprus. It is known to be found in only three areas on the island (Famagusta, Morphou and Akrotiri). This small fish (usually 4 to 6 cm in total length) feeds mainly on planktonic animals often devouring large numbers of the eggs, larva and aquatic life-forms of mosquitoes and midges. *Aphanius* is a protected species and a Mediterranean basin endemic; it is listed in Annex II of the Habitats Directive and the Bern Convention. Although assessed as "Least Concern" in the IUCN red list it is locally threatened in many parts of its range; relatively few island populations exist and in many places they have become locally extinct or extirpated.

This study suggests that at the Akrotiri Wetlands *Aphanius* has a "contraction-expansion" distributional behaviour depending on the Salt Lake's fill-re-fill annual hydrology. Fish survive the long drought in refugia (traditionally at Zakaki, Akrotiri Marsh, the north shores of the Salt Lake and Episkopi Bay quarry pits). After the winter rains and marine storms raise water levels, fish disperse to the Salt Lake and enter various marshy areas along its shores. By late spring they are breeding and locally found in high concentrations, but especially so in brackish and saline waters where there is no competition with Eastern Mosquitofish *Gambusia holbrooki*.

The *Aphanius* population of the Akrotiri Wetlands seems to have recently changed. *Aphanius* used to be found in the Akrotiri Marsh (until the early 2000s) but no longer inhabits this area, although its alien competitor, *Gambusia*, seasonally thrives there. The species is no longer common at Zakaki as it was before 2012 (it was not recorded anywhere at Zakaki or the eastern part of the Salt Lake during surveys in 2015-2017). At Zakaki, this may be a result of hydrological changes and unforeseen impact of the new Lemesos storm water drainage system which now feeds increased freshwater runoff directly into Zakaki Marsh. Furthermore, the now fresher/slightly brackish conditions at Zakaki Marsh have promoted a seasonal super-abundance of *Gambusia*. Circumstantial evidence also points to competitive exclusion as at least one main reason for the *Aphanius's* dramatic recent decline at other parts of the Akrotiri Wetlands; special research is required to confirm and monitor this.

This report proves for the first time that the artificial gravel-pit pools/ponds of the Episkopi Bay Wetland are currently the most important refugium for *Aphanius* during drought periods at Akrotiri. This is further enhanced by the total absence of *Gambusia* in these saline pools. During drought years, as in 2016, *Aphanius* survive in at least seven gravel pit ponds and in some their population density is locally high. Identified threats to the *Aphanius* of Akrotiri Wetlands are the following: a) total population collapse after extreme drought periods; b) expansion of *Gambusia* in fresh-brackish aquatic refugia and predatory fish introduction in the Episkopi Bay Wetland gravel pits; and c) hydrological fragmentation among different wetland units that may block winter dispersal of *Aphanius*.

Proposals of the study include: a) a scientific conservation ecology-monitoring study of *Aphanius* and other fishes at the Akrotiri Wetlands is required and must begin immediately in incremental steps, i.e. through a practical action plan and adaptive monitoring focusing on *Aphanius* restoration; b) connections among mosquito biocontrol, biodiversity conservation, water management and education should be promoted and supported by local institutions and stakeholders; c) re-introduction through assisted migration into areas such as Akrotiri Marsh should be organized in 2017 based on an action plan developed as soon as possible; d) *Aphanius* is potential flagship species for the research and understanding of the aquatic environment of the Akrotiri Wetlands and should be used to sensitize and educate the public and promote holistic wetland management and restoration actions.

1. Research rationale and specifications

Fishes are very important in wetland management and restoration. They help structure and support food webs that affect ecosystem structure and functioning and ultimately influence limnological conditions. Many fishes are key-stone species in aquatic ecosystems, such is the case in many large coastal wetland ecosystems in the Mediterranean. Conservation research for fishes has been especially scarce in Cyprus. The ecology of the Mediterranean Killifish *Aphanius fasciatus*, one of the few native fishes in the Island's inland waters, is poorly known. Scientific awareness of the population of *Aphanius* is seen in very few instances in the published literature. Interest in the species at Akrotiri Wetland and specifically at Akrotiri Marsh begun with incidental sightings and evidence of its wider distribution in the area in the early 2000s. Questions arose about its ecology, conservation status, and management needs by environmental scientists at the SBA and increasing interest has developed by members of the public. This short-term base-line study is the first to explore conservation questions directly concerning *Aphanius fasciatus* within Akrotiri Marsh and the wider area of the Akrotiri Wetlands. It goes without saying that this report is part of a desk study and simple "search-find survey" during a very short research project; it can only stand as a beginning to scientific base-line research on the ichthyology of the area.

History and study scope

The study was awarded to Dr. Stamatis Zogaris (Researcher at Hellenic Centre for Marine Research) by BirdLife Cyprus on the 22nd of February 2016. Research and field work was co-developed with Vassiliki Vlami (PhD cand. Univ. Patras); several experts and naturalists contributed and assisted during field visits. Field visits were conducted in February, May and June of 2016 and in January 2017. In total, ten days were invested in field work, interviews and meetings on site.

Study specifications

1. *Aphanius fasciatus* distribution in Akrotiri Marsh and the wider wetland area of the Akrotiri peninsula; first complete survey using standardized sampling methods.
2. Relevant aspects of *Aphanius fasciatus* conservation biology, including appropriate conditions and resources of the species' requirements and the fish community description based on sampled assemblages.
3. Information in the life-cycle of *Aphanius fasciatus.* Life-cycle and mosquito biology to explore killifish use to control mosquito breeding.
4. Relationship of *Aphanius fasciatus* with *Gambusia holbrooki*- evidence of potential pressures on *Aphanius fasciatus.*
5. Re-introduction of *Aphanius* in wetlands. Possible implication to other species from the re-introduction of *Aphanius* in the Akrotiri wetlands (observation and bibliographical evidence on invertebrates and other aquatic animals) with emphasis on the potential impact on the wetland ecosystem.
6. Restoration options for Akrotiri Marsh with respect to the need of native fishes.
7. Monitoring recommendations.
8. Overall conclusions, priorities for further research and follow-up actions.

2. Akrotiri Mash and *Aphanius fasciatus* conservation

Akrotiri Marsh[1] is a unique natural wetland in Cyprus covering an area of approximately 150 hectares (or 1.5 Km^2). It is part of the wider Akrotiri Wetlands, the largest natural wetland complex in Cyprus, covering roughly 25 Km^2. The marsh is part of a Ramsar site, an Important Bird Area (IBA) and a specially designated Special Protection Area (SPA), equivalent to the EU designation, according to the mirror law (26/2007) of the Cyprus Sovereign Base Areas (SBAs)[2].

Compared to other large Mediterranean wetlands, Akrotiri Marsh and the wider Akrotiri Wetlands, have been rather poorly studied at the ecosystem and landscape scale; only recent studies promoting management measures for biodiversity have been developed (see Cox 2009; AP Marine 2012 and references therein). Ground water hydrology is better studied; but surface water hydrology is generally poorly described. Anthropocentric water over-abstraction or water control structures promote mostly small-scale agricultural irrigation and military-relevant land-use (i.e. drainage schemes in telecommunication structure areas). Poor management and or unintentional mismanagement in terms of biodiversity values has resulted in some environmental changes; such as an "overexpansion" of reeds and consequent loss of certain habitat conditions and resources and a decline of plant diversity in Akrotiri Marsh. Also, until recently, the Akrotiri Marsh's hydrology is poorly managed and not regulated within a framework that includes biodiversity conservation or concerns for ecosystem-specific restoration to conditions of high ecological integrity (or in an effort to enhance specific habitats and scarce species assemblages). Aquatic conditions in Akrotiri Marsh are variable based primarily on meteorological events, drainage management and water over-abstraction for irrigated agriculture. Some years high water levels have been artificially elevated (notably during the first years of the 2000s) while during drought years over-abstraction in the wider area influences and lowers ground-water levels and the marsh becomes seasonally desiccated. Seasonal desiccation is to some extent a natural phenomenon but its affects have been poorly described. Although a system of drainage canals connect the marsh directly with the nearby Akrotiri Salt Lake, during drought years the water flow from the Marsh to the Salt Lake completely dries out. During most years some water remains within the canals hosting refugia for aquatic biota.

Akrotiri Marsh is part of the wider Akrotiri Wetlands protected area so it must be managed within the scope of a broader conservation and monitoring effort which has a broader vision for conservation and sustainable management of the Protected Area. Akrotiri Marsh is part of a hydrological system receiving underground waters from the Kouris river basin. Akrotiri Marsh's immediate surroundings include a fertile traditional agricultural landscape (Fasouri), a windswept tombolo beach and salt marsh system (the Episkopi Bay Wetland) and Akrotiri Salt Lake, the dominant landscape feature (which is bounded by Zakaki, Lady's Mile Beach and the

[1]Akrotiri Marsh is also known as Fassouri Marsh or perhaps more correctly Livadi Marsh; all locals interviewed refer to it as "Livadi".

[2] In this report, we use the term Akrotiri Wetlands to cover all the wetland areas of the Akrotiri Peninsula similar to the Special Protection Area (SPA) delineation Akrotiri Wetlands ("Υγρότοποι Ακρωτηρίου" site). Interestingly, this term is rarely used and is sometimes poorly defined or delineated (see Fig. 2 below). In this report when we use the term "Protected Area" we refer to the SPA delineation.

"island" of the southern part of the Akrotiri Peninsula). Any conservation or ichthyological study must promote conservation within this broader framework since the aquifer-agriculture-wetland complex and coastal zone dynamically interact in a hydrological, biological and ecological sense.

The present ichthyological study

This ichthyological survey takes place within a wider ecosystem-based conservation project in combination with public engagement actions that started in April 2015 to restore Akrotiri Marsh and its biodiversity. The 2-year project (April 2015 to March 2017) with the title 'Akrotiri Marsh Restoration: a flagship wetland in the Cyprus SBAs' is funded by the Darwin Initiative through UK Government funding (Darwin Plus, the Overseas Territories Environment and Climate Fund). The project is implemented with BirdLife Cyprus as a lead partner and in collaboration with the Akrotiri Environmental Education Centre and the RSPB (BirdLife partner in the UK).

This research project focuses on *Aphanius fasciatus*[3]. This small fish (usually 4 to 6 cm, Total Length) is a protected species at Akrotiri and on Cyprus. It is listed in Schedule 2 to the Nature Ordinance, requiring protection and management through SAC designation. It is listed in the Habitats Directive (Annex 2). Because Aphanius fasciatus is considered as a locally threatened species in many parts of its range and receives international protection since it is listed in Appendices II and III of the Bern Convention (the Convention on the Conservation of European Wildlife and Natural Habitats) and has protected species status in many EU states as well. Since *Aphanius* is a cyprinodontiform killifish species we should mention that many other killifish species are threatened in the Western Palearctic as well. In fact, killifishes are ranked just behind sturgeons as the most threatened fish group of Western Palearctic freshwater fishes. The high "threat levels" are due to killifishes high vulnerability to alien species- especially *Gambusia holbrooki*- which is now "almost omnipresent in killifish habitats"; and the fact, that many killifishes are restricted to a few or just one refuge areas such as springs, lakes or lagoons in arid or semiarid climate regions that are vulnerable to total desiccation (Freyhof 2014).

Aphanius fasciatus is an endemic of the Mediterranean basin, found nowhere else in the world. It is fairly widespread but with localized occurrences in inland wetland and transitional waters of the coast, primarily in the central and northeastern Mediterranean. Due to its relatively widespread global range and its local abundance at several large wetland sites the species is designated as "Least Concern" by IUCN. However there is documentation of a serious decline and many extirpation events of many local populations have occured (e.g. local extinctions have been well documented in many areas in Italy, France, Malta, Greece, and Israel). The species is very sensitive to habitat degradation of coastal wetlands. *Aphanius*

[3] *Aphanius fasciatus* has several common names in English; it is often called Mediterranean toothcarp, Southern European toothcarp, because it belongs to the toothcarp family, Cyprinodontidae. We now use the name "Mediterranean Killifish" for the species in this report (FishBase uses a new and scarcely used name "Mediterranean Banded Killifish"). The term killifish is now more widely used to include any of various cyprinodontiform fish (including families Cyprinodontidae, Aplocheilidae, Fundulidae, Profundulidae and Valenciidae). Killifish should not be confused with guppies (Poeciliidae family). The rather similar-looking eastern mosquitofish (*Gambusia holbrooki*) which is also in the Poecilid family.

fasciatus furthermore is found in very few Mediterranean Islands[4] and some island populations may have become extinct where it formally did have populations (for example, there is no recent evidence of the species on Crete (Bianco 1996)). On Crete for example, an exhaustive search has not been made or its absence represents a recent extinction (e.g. contradictory information exists such as the mention of existence of the species in Crete in Kottelat and Freyhof, 2007). Under harsh conditions, this species (as other *Aphanius* spp.) sometimes persists in very small numbers in wetlands and may be very hard to find. Many researchers feel that there is a real danger and threat that small isolated insular populations may be threatened with extinction (and we feel the IUCN or Red Listing process does not adequately represent the threats this species is facing at the regional scale).

Because the species is relatively widespread in several Mediterranean wetlands there have been only a few local actions for its restoration (i.e. projects focusing on translocation and re-establishment of populations and habitats, and constituent ecosystems). Most notably in Malta and Italy efforts have been made and in Greece there is a single volunteer-based initiative (Alyki Lagoon, Aigaion[5]). However, the species is also hardy, a good survivor, once it becomes established (or re-established). In Spain for example, a new non-indigenous population have developed in the Ebro Delta (suspected to have been released by aquarists).

Prior to the present study there is only scant knowledge of *Aphanius fasciatus* on Cyprus or for Akrotiri SBA. Mention of *Aphanius fasciatus* in Cyprus is found in Demetropoulos and Neocleous (1969) and there is to our knowledge only scant mention in the published literature at both the local level or island-wide level before 2008. No island-wide search for the species has ever been properly conducted and there are contradicting statements about its status (native or not; for example). The species is definitely a neglected part of the island's biodiversity.

At Akrotiri interest in the species by workers at Akrotiri Environmental Education Center holds from before 2000. The Author did collect and observe *Aphanius* on several visits since 2008 (Zogaris et al. 2012a,b, Zogaris 2012). Also, immediately before the study began an independent "survey visit" was conducted at Akrotiri by Zogaris's collaborators, C. Englezou and M. Ford with the help of Akrotiri Environmental Education Centre personnel, in late December 2015. The current project aims to shed light on the potential for conservation and restoration with respect to this fish species and its ecological influence and requirements both at Akrotiri Marsh and on the wider area's wetland ecosystems.

3. Delineation of the Study Area

<u>Operational hydrobiological unit delineation</u>

[4]Populations exist in a few coastal islands of the Adriatic and Ionian (Kerkyra, Lefkada), Sicily, Sardinia, Corsica, Malta and a few in the Aegean (notably Euboea, Cos, Samos, Lesvos).

[5] Costas G. Papaconstantinou and volunteers of the Hellenic Ornithological Society dug trenches during a prolonged drought period, during the summers of the early 1990s, to protect the isolated population of *Aphanius fasciatus* at Alyki Aegaion Lagoon, the only known population of the species in the southern part of the Corinthian Gulf.

This ichthyological work focuses on Akrotiri Marsh but with equal regard for the Akrotiri Wetlands Protected Area as a whole. Akrotiri Marsh is of course hydrologically and biologically connected to the wider wetland complex and it is a relatively small part of the wider Akrotiri Wetlands; it covers about 22% of the Akrotiri Wetlands in areal extent. As explained previously, ichthyological work must explore a broader area to express conservation relevant knowledge and understanding. And there are serious gaps in ecological knowledge.

Based on a hydrobiological and landscape perspective and especially with respect to the habitat patterns influencing aquatic biota, the Akrotiri Wetlands can be divided into what we term here "operational hydrobiolological units" (see Fig. 1, Table 1). Criteria for defining these units focuses on the following elements: Hydrogeomorphological structure and topography; dominant vegetation patterns (i.e. wetland plant communities); and homogenous landscape features defining distinct and discrete areal units relative to their surrounding wetland, terrestrial and marine areas. This initial sketch mapping exercise may become better defined in the future for other uses as well; its use here is for ichthyological and conservation-relevant information management and survey work.

Fig. 1. Sketch map of eight arbitrary delineated operation "hydrobiological units" with respect to aquatic biota in the Akrotiri Wetlands: 1 Akrotiri Marsh; 2 Kouris river corridor; 3 Episkopi Bay Wetland; 4 Akrotiri Salt Lake; 5 Zakaki Marsh; 6 Lady's Mile; 7 Artificial water bodies of Bishop's pool and quarry pool; 8 Agios Georgios upland quarry marshes.

#	Hydrobiological Landscape Unit	Area (Km^2)	Perimeter (Km)
1	Akrotiri Marsh	1.39	5.72
2	Kouris River Corridor	0.8	5.1
3	Episkopi Bay Wetland	5.59	14.4
4	Akrotiri Salt Lake	18.2	16.9
5	Zakaki Wetland	1.17	5.21
6	Lady's Mile	3.13	12.7
7	Bishop's pool and Quary Pool	0.5	<1
8	Agios Georgios Upland Quarry Marshes	0.5	<1

Table 1. Preliminary areal cover figures of the operational units of Akrotiri Wetlands as defined in this study. This preliminary delineation gives a total "wetland unit" area of 31.6 Km^2. The delineations were made using Google Earth Pro and not the Fig.1 sketch map. The total wetland area delineation is very close to the roughly 25 km^2 often given to cover the wetland area of Akrotiri Wetlands. Of course, there are important caveats in this delineation here: wetland riparian buffer areas are included in the delineation, these include dunes, beaches, even Eucalyptus plantations on former wetland areas, within these general hydrobiological landscape-scale units.

Preliminary delineations

1. Akrotiri Marsh: Dominated by a large *Phragmites australis* reedbed and surrounding wet-meadows; this wetland has a variable flooding regime with respect to artificial and human-modified flooding (particularly high water levels in the early 2000s). The wetland has been impacted by drought during the last few years. It is crossed by a system of canals and ditches, a major canal leading downstream to Akrotiri Salt Lake holds water during most summers. During the long summer-autumn drought there is no connection with the surrounding salt marshes but during winter flooding the Akrotiri Marsh is hydrologically connected at a few locations with ditches in the northern sector of the Episkopi Bay Wetland and with the Salt Lake as well (see below). Four fish species have been documented: *Aphanius fasciatus*, Mugillid Grey Mullet species, *Anguilla anguilla*, *Gambusia holbrooki* (only *Gambusia holbrooki* was found during this study) (See Annex B).

2. Kouris River Corridor: The former Kouris river mouth and its deltaic plain - a wide braided river channel and riparian zone. There is no longer regular surface flow after the creation of the Kouris dam and only in exceptionally wet years is the former river bed partially wet (it can be described as a degraded "artificially ephemeral river"). Fish no longer exist here; in the past at least one fish species was present; *Anguilla anguilla* (see Annex B). The Kouris river aquifer is the main source of subterranean water feeding Akrotiri Marsh and the ground water table along the north shore of the Akrotiri Salt Lake.

3. Episkopi Bay Wetland: Backed by the high tombolo cobble barrier beach this is a large marshland area, dominate by salt marshes, salt steppe, and several ponds located South and West of Akrotiri Marsh. In winter the salt marshes are usually flooded. Flooding occurs due to rainwater run-off and especially from seawater surges during storms (the timing, degree, flow and contribution of marine storm water input has not been described). About a dozen very

small and three large gravel pit ponds were excavated in the area in the past and now create permanent/near permanent aquatic refuges. Three of these ("Ai Yorkis Pond", "Navagio Pond" and "Paraga Pond"[6]) are deep (2+ m) and host several marine fishes as well. The gravel pits are of outstanding value as refuges for *Aphanius fasciatus* since they maintain brackish-to-saline water throughout the year, even in periods of extreme drought. At least seven fish species have been documented: *Aphanius fasciatus*, Mugillid Grey Mullet species, *Mugil cephalus*, *Gambusia holbrooki, Atherina boyeri, Gobio cobitis, Dicentrarchus labrax* (See Annex B).

4. Akrotiri Salt Lake: A huge coastal lagoonal basin; desiccated completely during the summer-autumn period giving the impression of a large "salt lake" (or huge salina, Alyki in Greek). It is actually a brackish-to-hypersaline coastal lagoon. The wider lagoon basin covers 940 ha and its deepest part is at 2.7 m below sea level. This shallow lagoon is filled by brackish waters for about 6 months of the year; varying brackish in winter, hypersaline during spring, and early summer (salinity ranging from 5 -100‰). Storms bring in marine waters primarily during winter from the both the western tombolo (Episkopi Bay) and the eastern beach area (Lady's Mile) and run-off waters flow in from Akrotiri Marsh (and the Phassouri area) and the wider Garylis basin (through Zakaki Marsh and associated drainage ditches-storm water runoff systems). Sand spit development on the east side of Akrotiri gradually closed off lagoon's the opening to the sea (at a point on Lady's Mile) a few hundred years ago. A visitor to the area in 1589 (Villamont, in Heywood 1982) noted that, “fish entered the lake from the sea ‘through one little entrance” implying that spit development was nearly complete by the end of the sixteenth century (cited in AP Marine 2011). Four fish species have been documented: *Aphanius fasciatus*, Mugillid Grey Mullet species, *Mugil cephalus*, *Gambusia holbrooki* (See Annex B).

5. Lady's Mile: Long low-sloping sandy beach with sand flats, extensive dunes, dune slacks, and extensive salt marshes with wide shallow pools during the wet season. The low sandy "barrier beach" breaks during storms and provides irregular inflows into the shallow pools and marine water flow to the adjacent Akrotiri Salt Lake basin. Lady's mile was open in the middle ages, functioning as a lagoonal spit. Sand spit development on the east side of Akrotiri gradually closed off the opening to the sea, thereby closing the lagoon. At least three fish species have been documented in modern times: *Aphanius fasciatus*, Mugillid Grey Mullet species, *Mugil cephalus*; in the distant past *Sparus aurata* and other species entered the Salt Lake from this area (See Annex B).

6. Zakaki Marsh: Formerly a small marshy area dominated by salt marshes near the Garylis river-mouth west of the Port of Lemesos. The ecological history of the ponds and marshes is poorly described. A pond-like waterbody dominates the marsh area; it is called Lake Makria in some reports. Today the area has been altered by increased freshwater run-off (part of a water-drainage scheme of the Water Authority of Lemesos); this also probably increases freshwater run-off into the adjacent Akrotiri Salt Lake as well. Recent satellite photos show an

[6] These place names were given by contributors in this study, they have not been named in other published research work; "Paraga" refers to the wooden shack built next to one of the pools.

increase in common reed in the last few years. After Akrotiri marsh this area has the largest Common reedbed habitat in the Akrotiri Wetlands. Four fish species have been documented: *Aphanius fasciatus*, Mugillid Grey-Mullet species, *Gambusia holbrooki, Anguilla anguilla* (See Annex B).

7. Bishop's Pool and Quary Pool: Artificial permanent lentic bodies. These are hydrologically isolated water bodies with fresh-slightly brackish conditions. Two fish species have been documented: *Cyprinus carpio, Gambusia holbrooki* (See Annex B).

8. Agios Georgios Quarry Marshes: Artificial water bodies on upland wet meadows and seasonal marshes in quarry areas south of Ai Yorki (Agios Georgios Church) near Akrotiri. These anthropogenic very small micro-wetland basins are hydrologically unrelated to Episkopi Bay Wetlands since they are on the upland terraces of the paleao-island of Akrotiri. Small rush-beds and temporary ponds are created at the bottom of the quarry features. No fish have been documented.

Not all of the above wetland areas are protected (see delinations in Fig. 2).

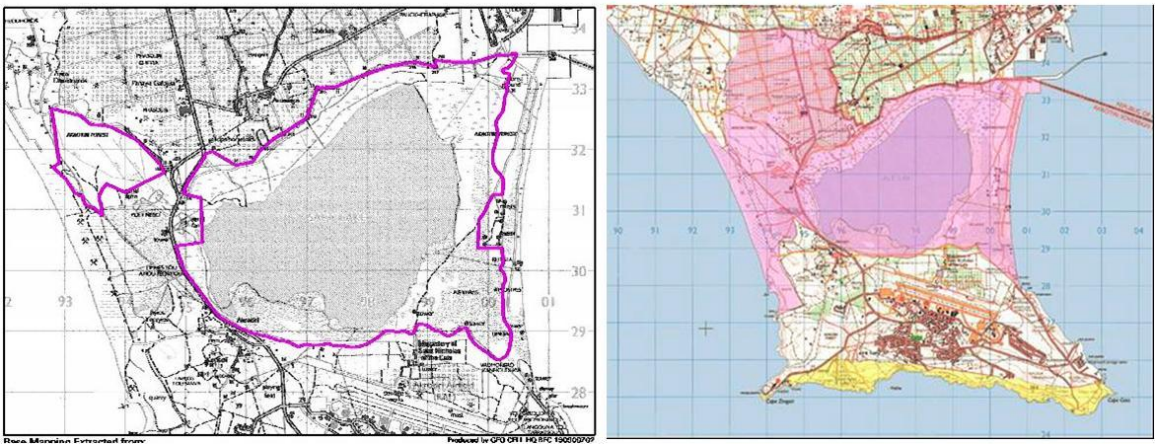

Fig. 2. Wetland protected area designations; the Ramsar Site in purple (at Left); it does not include most of the Episkopi Bay Wetland. The "Akrotiri Wetlands" SPA site in pink (at Right); includes a large area of the agricultural land in Fasouri as well.

<u>Akrotiri Marsh and Akrotiri Wetlands Hydrology (an initial sketch for conservation biologists)</u>

Akrotiri Marsh has always had a connection to the Kouris Aquifer and it seems to be fed by ground waters since it topographically slopes off and descends rather abruptly into the Akrotiri Salt Lake basin (at 2.7 m. below sea level). This is why there has for a long time been a large "wet meadow area" with "springs" and a network of drainage canals. Canals and ditches are probably dated at least since the middle ages. Grazing and agricultural use was defined by water levels in the past. Elders in Akrotiri village have remarked with nostalgia that fish and crabs (and turtles) abundant in these drainage canals before 1970 (See annotated fish list in Annex A). Today a series of sluices manage water flowing from the marsh (which is also fed by run-off from the surrounding Fasouri agricultural area in winter). There are a few ditches in the western part of the Marsh that have a connection to the Episkopi Bay Wetland (the salt marshes in this large area periodically flood after autumn due to marine storm surges). The Episkopi Bay Wetland probably had a greater amount of sea-water influence in the past since a

major anti-flooding barrier was created along the entire Episkopi Bay beach a few decades ago (around 1970; as remarked by Akrotiri elders). This barrier now breaks/breaches primarily in the southern end of the beach (near the Ai Yorki and Navagio areas).

There is evidence that reed beds dominated by *Phragmites australis* have flourished and expanded in Akrotiri Marsh during the last two decades (Fig 3.). This remarkable change and the problem of reed-bed management requires further research.

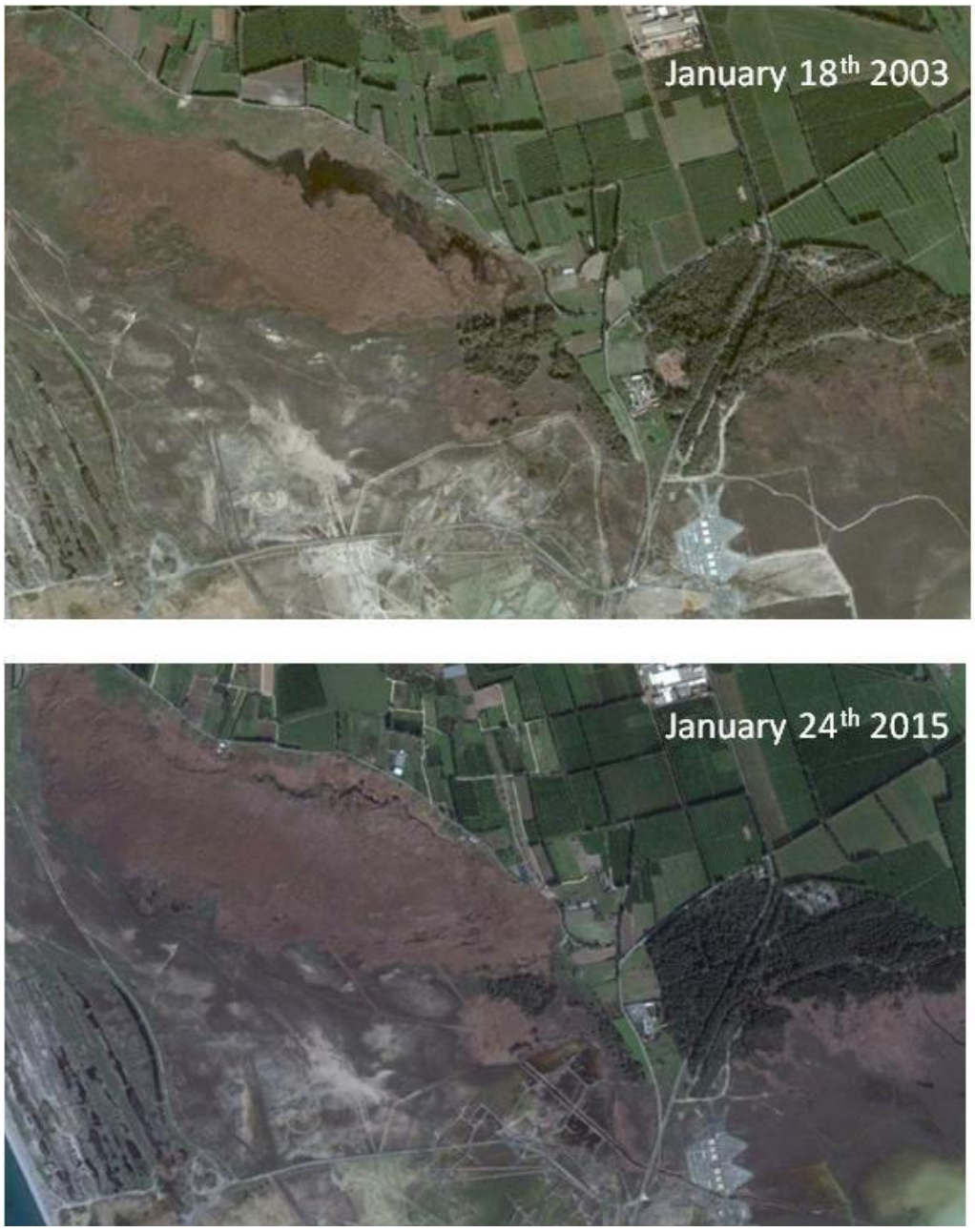

Fig. 3. Changes in Akrotiri Marsh during recent years. Recent Google Earth images show the local expansion of *Phragmites australis (light buffish-red colour)* and decline of both open water areas and wet meadows in Akrotiri Marsh. Akrotiri Marsh immediately borders on the Episkopi Bay Wetland (at lower Left). There are roads and embankments that may create permanent or semi-permanent barriers to saline waters and fish movement between these two adjacent wetland areas. Akrotiri Marsh's connection to Akrotiri Salt Lake is via a major

canal (Enetiki Tafros) that flows downstream to the lagoon basin (diagonally at Lower Right). In the lower part of the photographs human-induced changes are evident in roads, off-road trampling and vegetation clearing for construction and extensive military installations. Water levels are also artificially regulated both by sluice-gates and the variable water ground water levels, which are influenced by water abstraction and agriculture in the wider Fasouri area. From an ichthyological perspective several important problems are evident for fish survival and movement: habitat changes, salinity and water quality issues, desiccation events, lack of permanent aquatic refuges, barriers to connectivity among water bodies among others.

Finally, based-on the existing literature, and on interviews with elders and knowledgeable naturalists, an initial hypothesis has been developed here to describe changes of water flow regimes of the Akrotiri wetlands. These changes may have both a broader effect and a specific effect on the Akrotiri Marsh biota as well. The presumed changes can be summarized as follows and hypotheses are also presented (and sketched in Fig 4).

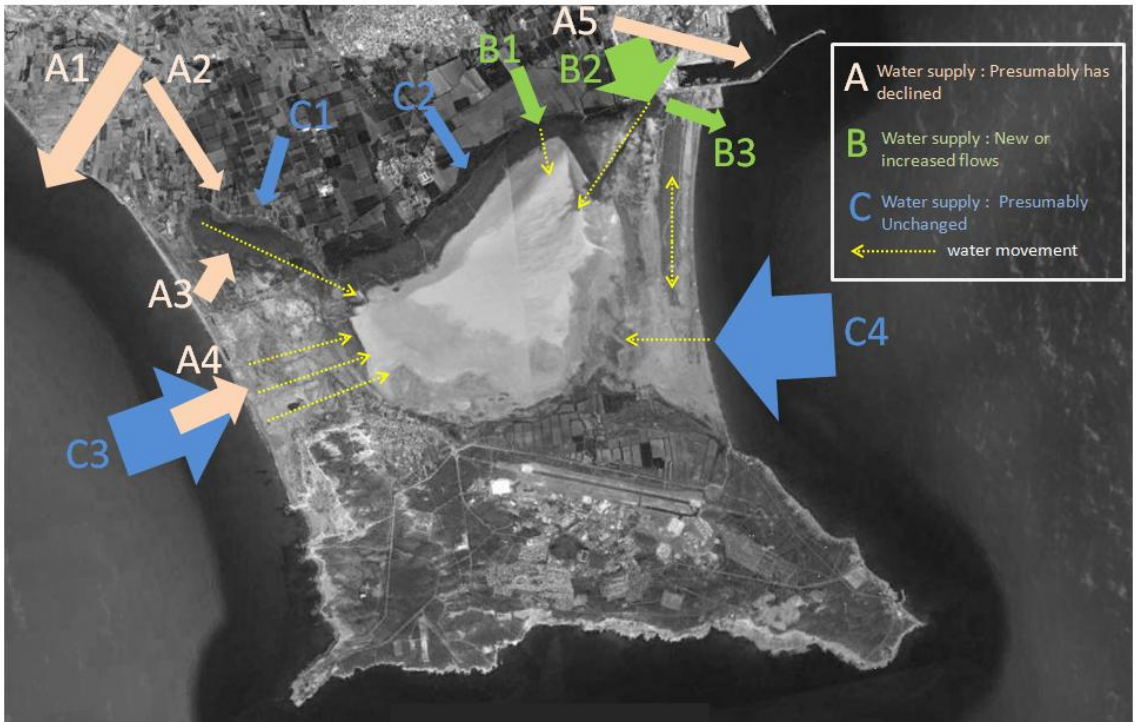

Fig. 4. An heuristic sketch of hypothesized surface water hydrological changes in Akrotiri Wetlands during since 1970. Beige arrows refer to decreasing water inputs (A); Blue arrows refer to unchanged water inputs (B); Green arrows refer to increasing freshwater inputs (C). Size of arrows represent hypothetical importance of relative water inputs as surmised/presumed based on literature and interviews. Dotted yellow arrows show key water movement flows (See text below for details).

The above sketch (Fig. 4) provides the following information and interpretation:

A (Beige in Fig. 4). Water flow from above ground has been reduced in: The Kouris basin (Kouris Dam and Water Diversion) (A1), and perhaps also from the Fassouri Plantations (A2). Marine storm surges in the northern part of the Episkopi Bay beach are now harnessed by a low anti-flooding embankment which is breached mostly in the southern part of the beach

(A4); and perhaps importantly this embankment does not allow frequent saline waters to enter Akrotiri Marsh (A3). On the eastern part of Akrotiri Wetlands water flow and sediment movement from the Garyllis has ceased due to the Lemesos port expansion (A5); however this has been compensated for by increased urban and storm water runoff directly into Zakaki (B2).

B (Green in Fig. 4). Freshwater input has artificially increased, and perhaps especially so after 2012 in the NE part of Akrotiri Wetlands with the construction of the Lemesos new water drainage scheme from run-off and storm water from Lemesos (B1, B2). This has definitely increased freshwater conditions in the area of the Zakaki Marsh/ Makria Lake and the drainage ditch leading into the Salt Lake proper and the new outlet to the sea (B3). This situation has seen an increase in common read (*Phragmites australis*) in the Zakaki area and locally at the other alternative inflow site on the northeast shore of the Salt Lake (B1). As documented later in this study, this freshwater increase has probably proven detrimental to the *Aphanius* population refuge at Zakaki (i.e increasing freshwater conditions may have helped a super-abundance and higher population densities of the very competitive *Gambusia*).

C (Blue in Fig. 4). There is still some flow of waters from drainage ditches from the Fasouri (C1) and Assomatos area (C2). Marine storm flows into the area are probably important for the water balance of Episkopi Bay Wetland (C3) (which in the past may have been directly connected to Akrotiri Marsh). The literature does not mention the importance of marine water movement into the area but locals are very much aware and the "flooding problem" is the reason for the embankment at Episkopi Bay Beach. All locals interviewed mentioned that flooding was much more prevalent in the past and it "brought in" fishes (grey mullets, sand-smelts etc.) that were traditionally harvested as water levels dropped in spring-summer. Specifically with regard to Akrotiri Marsh no mention has ever been made of marine water flooding affecting it (in the Literature). One hypothesis may be that Akrotiri Marsh periodically flooded with marine waters increasing salinities and thus affecting common reed growth. (The current drainage canals drain most of Epsikopi Bay Wetland directly to the Salt Lake leaving Akrotiri Marsh more-or-less isolated from the surrounding Episkopi Bay Weltand). And finally more locals are aware of the storm water inflows from Lady's Mile (C4) [7].

[7] *Please note*: the above sketch of "hydrological changes" is partially substantiated by the key literature (Cox 2009; AP Marine 2012, Mer Lab 2013, 2014 and hydrological studies therein) and by interviews (see Annex B); however there are still serious knowledge gaps concerning the history of recent hydrological changes, the effects of modern water discharge patterns and the specific hydrological reference conditions of the constituent ecosystems. Surface water flow and water level regimes vary among years due to extremely heterogeneous meteorological conditions, variation in flow conditions and patterns. The particular hydroperiod regime and how it has been influenced by human-induced changes/barriers to water movement etc. is still poorly documented. As a result this initial sketch is a heuristic outline in order to provide a basis for further discussion and research.

4. Research Methods

Fish sampling

A search-find method was adopted for this initial screening level survey. Based on local conditions sampling was either conducted using dip-nets or a short seine net. The sampling protocol follows the IMBRIW field form applied in Cyprus during the last years (See IMBRIW-HCMR 2012). In this case identical tools and protocols were used as in BirdLife Cyprus's Lake Oroklini study (described in detail in Zogaris 2013).

Standardized fish population and certain biologically-relevant data were collected at each sampled site. Photographs and video were compiled. Efforts to standardize survey approach were made at each site in order to test the development of a Catch per Unit Effort protocol (at least 3 sampling seine net pulls at each site; or 10 ten dips). Difficulty to stream-line sampling tools and applications exist due to the very different habitats surveyed; different seasonal conditions etc. (i.e. reed-swamp, shallow pools, deep ponds, ditches etc.).

The following attributes are noted either through seine netting or dip-netting:

- Specimens identified to species level.
- Size classes (generalizations of age stages). For small-sized fishes the following categories are set: <20 mm, 21-40 mm, >41 mm.
- Signs of disease and deformities (DELTS) documented.
- Environmental conditions, habitat conditions.

Juveniles were defined as all killifish individuals up to a maximum size of 2.1 cm, below which the typical colouration of the adult fish was absent. This broadly follows Zammit-Mangion and Deidun (2010) who established the juvenile cut off at 2.8 cm. Below about 3 cm it is difficult to record sex from rapid field-based screening, so only the larger fishes are sexed.

Habitat and microhabitat measurements were recorded at each sampling area?. Six variables related to habitat structure and presence of surrounding fish refuge were recorded:

- water depth, mean and max in sampled area (cm),
- distance to the nearest shore (cm),
- submerged vegetation cover (%),
- submerged vegetation density (in categories: rich, intermediate, sparse)

The assessment of submerged vegetation cover and density was made visually by two observers. Other data on hydrology, anthropogenic pressures and other organisms observed where also noted. Water samples were not collected consistently from most sites; salinity was sometimes recorded using a hand-held device on site (unfortunately during one of the expeditions the hand-held device was shown to be damaged and collected data were not reliable).

Research timing and effort

Research was timed to include late Spring and mid-Winter; in this way a two season result is produced. It should be said that although 30 site samples where gathered in total many other sites were visually assessed for fish without using sampling gear (in many cased in shallow, non-turbid conditions it is easy to see fish using binoculars).

Work done specifically for this project during four visits by S. Zogaris. Table 1 refers to the specific work done during this project so far.

Date	Description	Field work
February 22-23 2016	S. Zogaris and V.Vlami survey entire area; First meeting at Environmental Education Center Meeting at Environmental Center	Pilot survey of the site and planning for sampling protocol.
May 19-21 2016	S. Zogaris, V.Vlami and V. Michael survey. Meeting at Environmental Education Center	Late spring/summer surveys. Optimal time for investigation.
June 3rd & 22nd 2016	S. Zogaris and Chris Englezou independently survey with V. Michael.	Continuation of summer surveys. Optimal time for investigation.
January 12-15 2017	S. Zogaris, V.Vlami and V. Michael survey. Other experts visit as well. Meeting at Environmental Education Center Meeting at Environmental Center	Rains where late in coming and flooded the area; fish had not yet dispersed beyond refugia. Poor time for investigation

Table 2. Field work time sheet during this project.

Bibliographical research and survey

A survey of the literature, including grey literature was attempted in this project. This survey was not exhaustive but certainly includes all modern works (after 1970) and some older studies as well. Historical archives were not investigated.

Interviews

In order to record historic species distributions and environmental change in the study area interviews were made with a 10 local residents and local naturalists (mostly Akrotiri and Lemesos residents). These are unstructured and the information is given within the text stating the specific interviewed sources (see Annex B for details).

5.Results

A. Aphanius fasciatus **distribution at Akrotiri Marsh and the wider wetland area of Akrotiri peninsula**

A1. Historical information

Mention of *Aphanius fasciatus* in Cyprus is found in Demetropoulos and Neocleous (1969). Surprisingly we found no older references in a casual review of published sources, however the species is easily overlooked as proven by many recent range-expansion records published in recent year. The species is found in large wetlands that host areas that foster excellent aquatic refugia during summer (Akrotiri Wetland, Pediaios-Delta/Famagusta Bay , Morphou Bay). Former survey data from investigations by HCMR/WDD from 2008 to 2015 have been reviewed as have incidental data gathered by visiting naturalists and members of the Akrotiri Environmental Education Center of the SBA. No historical or published mention of *Aphanius fasciatus* was documented for Akrotiri Marsh before 2000. Locals mention "small fishes" but there is no reference to the species in particular (all small fishes go by the collective name of *Atherina*). It is presumed that the species is native and has largely been overlooked by researchers. Figure 7 shows the potential distribution of *Aphanius fasciatus* in the Akrotiri Wetland and the hypothetical dispersal pattern and drought-period refugial survival in the area.

A2. Field surveys

Current field survey information is based on implementation of plans organized by S. Zogaris with the cooperation of the Akrotiri Environmental Education Center and SBA authorities (specifically the Joint Services Health Unit, BFC RAF Akrotiri, Cyprus). Table 3 and Annex A provide a summary of all sampled sites; figures 4,5,6 provide distributional details of all compiled recent data.

Survey results

Thirty site samples were collected in standardized field forms. Of these only two were repeated surveys at exactly the same sites and areal cover (Paraga Pond, Cistern at Zakaki); some were very close to the same sampled sites but are regarded as separate site samples (Table 4). Effort was made to investigate as many sites as possible both in the spring-summer and winter periods. 16 sites were investigated in winter, 14 in summer. Many other sites were observed (visually inspected) but not recorded as sampled sites. The research focus was specifically targeting *Aphanius* habitats so this work does not represent a complete ichthyological survey. Deep-water habitats in the quarry pits of Episkopi Bay Wetlands and several other habitats (Bishop's pool, Salt Lake remnant waters etc) were not investigated. During the summer most investigated sites had fishes; in the winter only four sites had fishes. Generally, in the shallow water habitats investigated, two species dominated (*Gambusia* and *Aphanius*). In total, 1255 *Aphanius fasciatus* and 1989 *Gambusia* were recorded in the above samples. Three other fish species recorded are: *Gobio cobitis, Atherina boyeri, and Anguilla anguilla* (See Annex B for a detailed annotated list).

Site Name	Coordinates N	Coordinates E	Date	Sampling	Vegetation	Depth Mean	Depth Max	Aphanius <2cm	Aphanius 2-4 cm	Aphanius >4 cm	Total Aph.	Gambusia <2cm	Gambusia 2-4 cm	Gambusia >4 cm	Total Gambusia	Other fish	Unit	Comments
Louki	34°35'55.74"B	32°56'6.54"A	19.05.2016	Seine	Sparse	25	50	31	18	2	51	0	0	0	0	None	Episkopi Bay Wetland	Small turbid pool, created by road and storm erosion
Navagio	34°36'14.74"B	32°55'58.09"A	19.05.2016	Seine	Rich	23	50	3	17	0	20	0	0	0	0	None	Episkopi Bay Wetland	Large permenant quarry-pit pond
Ai Yorkis Casual	34°36'22.96"B	32°56'19.36"A	19.05.2016	Seine	Sparse	8	25	861	68	2	931	0	0	0	0	None	Episkopi Bay Wetland	Salt marsh pools drying-up; super-abundance of Aphanius.
Limni Ai Yorki	34°36'20.07"B	32°56'9.99"A	19.05.2016	Seine	Sparse	70	120	9	130	3	142	0	0	0	0	Present	Episkopi Bay Wetland	Large permenant quarry-pit pond
Livadi Pinakida	34°37'54.09"B	32°55'55.07"A	20.05.2016	Seine	Intermediate	28	50	0	0	0	0	105	80	380	565	None	Akrotiri Marsh	Reedswamp
Red Canal	34°37'31.48"B	32°55'59.81"A	20.05.2016	Dip net	Intermediate	30	70	0	0	0	0	0	0	0	0	None	Akrotiri Marsh	Reedswamp canal, in recent burn
Symvoli	34°37'30.13"B	32°56'16.60"A	20.05.2016	Dip net	Rich	30	60	0	0	0	0	20	20	20	60	None	Akrotiri Marsh	Reedswamp canal
Alyki Canal	34°37'9.55"B	32°57'3.88"A	20.05.2016	Dip net	Intermediate	22	40	0	0	0	0	0	0	0	0	None	Akrotiri Salt Lake	Canal in Akrotiri Salt Lake
Enetiko-gef	34°37'14.76"B	32°56'38.30"A	20.05.2016	Dip net	Rich	40	100	0	0	0	0	92	55	1	148	None	Akrotiri Marsh	Canal Akrotiri Marsh
Paraga Pond	34°36'53.04"B	32°55'35.50"A	20.05.2016	Seine	Sparse	50	110	12	0	0	12	0	0	0	0	Gobius cobitis (6)	Episkopi Bay Wetland	Large permenant quarry-pit pond
Zakaki Canal Alyki	34°37'49.00"B	32°59'25.10"A	03.06.16	Dip net	Rich	22	40	0	0	0	0	0	0	0	0	None	Zakaki Marsh	Akrotiri Salt Lake near outlet of Zakaki Canal.
Zakaki Canal 2	34°37'50.02"B	32°59'23.39"A	03.06.16	Dip net	Rich	15	40	0	0	0	0	2	1	0	3	None	Zakaki Marsh	Akrotiri Salt Lake near outlet of Zakaki Canal.
Zakaki Overflow Cistern	34°38'37.40"B	33° 0'12.70"A	03.06.16	Dip net	Sparse	50	90	0	0	0	0	197	296	101	594	None	Zakaki Marsh	Concrete pool water flood control structure
Near Zakaki Hide	34°38'33.00"B	33° 0'11.00"A	03.06.16	Dip net	Rich	41	60	0	0	0	0	171	358	90	619	None	Zakaki Marsh	Zakaki Reedswamp
Paraga Pondw	34°36'53.04"B	32°55'35.50"A	12.01.17	Seine	Intermediate	50	110	13	3	0	16	0	0	0	0	Gobius cobitis (2), Atherina cf boyeri (70)	Episkopi Bay Wetland	Large permenant quarry-pit pond
Birdlife Pond	34°37'37.08"B	32°56'17.08"A	13.01.17	Dip net	Intermediate	50	150	0	0	0	0	0	0	0	0	None	Akrotiri Marsh	Reedswamp canal
Livadi PinakidaW	34°37'54.09"B	32°55'55.07"A	15.01.17	Dip net	Rich	28	50	0	0	0	0	0	0	0	0	None	Akrotiri Marsh	Reedswamp
Ai_Yorki_rivulet	34°36'18.90"B	32°56'11.77"A	15.01.17	Seine	Sparse	10	15	72	11	0	83	0	0	0	0	None	Episkopi Bay Wetland	Shallow waters near quarry pit pool.
Ennetiko_GefW	34°37'14.61"B	32°56'39.15"A	15.01.17	Dip net	Rich	20	40	0	0	0	0	0	0	0	0	None	Akrotiri Marsh	Canal Akrotiri Marsh
Livadi West	34°38'0.08"B	32°55'12.01"A	14.01.17	Dip net	Sparse	20	40	0	0	0	0	0	0	0	0	None	Akrotiri Mar	Inermittent Ditch Akrotiri Marsh
Dasonomio1	34°37'35.40"B	32°57'11.79"A	14.01.17	Dip net	Sparse	20	30	0	0	0	0	0	0	0	0	None	Akrotiri Salt Lake	Eucalyptus forest, flooded roads.
Dasonomio2	34°37'37.49"B	32°57'37.50"A	14.01.17	Dip net	Sparse	20	45	0	0	0	0	0	0	0	0	None	Akrotiri Salt Lake	Flooded roads in rush-swamp
Near Ai Yorki	34°36'30.74"B	32°56'27.68"A	15.01.17	Dip net	Sparse	20	45	0	0	0	0	0	0	0	0	None	Episkopi Bay Wetland	Flooded salt marsh
North Inflow	34°38'27.69"B	32°59'22.58"A	14.01.17	Dip net	Intermediate	35	55	0	0	0	0	0	0	0	0	None	Akrotiri Salt Lake	Artificial freshwater input from Water authority canal of Lemsos
Zakaki Inflow	34°38'39.18"B	33° 0'10.08"A	14.01.17	Dip net	Intermediate	35	55	0	0	0	0	0	0	0	0	None	Zakaki Marsh	Inflow area of Waer Authority Canal of Lemesos.
Zakaki Overflow Cistern	34°38'37.20"B	33° 0'13.00"A	14.01.17	Dip net	Sparse	50	90	0	0	0	0	0	0	0	0	Eel (1)	Zakaki Marsh	Concrete pool water flood control structure
Zakaki Hide	34°38'32.64"B	33° 0'12.24"A	13.01. 17	Dip net	Rich	25	90	0	0	0	0	0	2	1	0	None	Zakaki Marsh	Zakaki Reedswamp
Zakaki Outflow	34°38'28.05"B	33° 0'40.10"A	14.01.17	Dip net	Sparse	10	20	0	0	0	0	0	0	0	0	None	Lady's Mile	Concrete canal mouth with a trickle of water exiting into sea.
Lady's Mile Pool	34°37'54.81"B	33° 0'14.48"A	15.01.17	Seine	Sparse	23	40	0	0	0	0	0	0	0	0	None	Lady's Mile	Temporary pool
Alyki South	34°36'14.89"B	32°58'50.35"A	15.01.17	Seine	Seine	25	45	0	0	0	0	0	0	0	0	None	Akrotiri Salt Lake	Salt lake recently flooded.

Table 3. 30 sampled sites surveyed during the project; In red are "spring-summer" samples (2016) and in blue are "winter" samples (2017). Exact location maps are shown in Annex A.

A3. Results of the survey work: Distributional data

Aphanius fasciatus was absent from Akrotiri Marsh and this was confirmed through several site samplings and visual observations at many locations throughout the Marsh during both the winter and spring-early summer seasons 2016-2017. Because of this, effort was applied to provide explanations and interpretations in a geographically broader area, i.e. throughout the Akrotiri Wetlands. The results of all available surveys and search-find data are shown below (Figures 4,5, and 6).

Fig. 4. Recent survey results (December 2015, by Englezou, Ford, Michael): Green: *Aphanius* present; White: No fish; Pink: *Aphanius* present from recent introductions by SBA authorities; Blue: *Gambusia* present; Yellow: Dry site; Red: *Aphanius* absent although present in 2011; Dark Blue: Site inaccessible.

Fig. 5. Current study survey results, First Phase (May-June 2016): Green: *Aphanius* present; White: No fish; Blue: *Gambusia* present; The observations at the artificial ponds at and near Bishop's pool were not based on samples, only casual visual documentation. Note the summer expansion of *Gambusia.* (Records based solely on work in this study by Zogaris, Englezou, Vlami, Michael).

(Fig. 5)

Fig. 6. Current study survey results, Second Phase (January 2017): Green: *Aphanius* present; White: No fish; Blue: *Gambusia* present; Yellow: Eel. Note the retraction of the *Gambusia* distribution; similar but not as pronounced as in Fig. 4. (Records based solely on work in this study by Zogaris, Vlami, Michael).

2016 was a drought year at Akrotiri and the distributional data are perhaps not typical of the distribution pattern that should usually be observed. In fact, one may be drawn to the conclusion that the *Aphanius* population is extremely localized and under immediate threat of local extinction at the site based solely on the survey's results.

Through the historical review in this study we provide evidence for an *expansion-contraction distributional pattern* for *Aphanius* at Akrotiri Wetlands. *Aphanius* is not a migratory fish, but it will disperse beyond its resident areas (presumably when swept by water flow (storm, tidal and run-off waters) as observed at Akrotiri and at the Pediaios system at Famagusta[8]). This study has shown that at the Akrotiri Wetlands *Aphanius's* distributional pattern expands and contracts depending on meteorological conditions and seasonal drought effects on the local populations. The pattern is presumed to be as follows: a) Fish survive the long drought and desiccation in refugia (traditionally at Zakaki Marsh, Akrotiri Marsh and particularly at the Episkopi Bay Wetland; but perhaps also on the north shores in canals waters leading from Asomatos to the Salt Lake). b) After the winter rains and marine storms raise water levels at Episkopi Bay Wetland and in the Salt Lake, fish disperse via drainage ditches from the refugia sites to the Salt Lake and enter various marshy areas along the shores of the Salt Lake and beyond. By late spring they are breeding and locally abundant at certain areas, but especially so in saline-brackish and saline waters where there is no competition with alien mosquito fish (*Gambusia holbrooki*). After the long summer drought many *Aphanius* die (mass fish-kills have been observed in Salt Lake as well on several occasions in recent years and there is geo-referenced photographic evidence of this (P. Charilaou, pers. com). c) The fish that have survived in refugia will be the population sources for future expansion events. During drought years the expansion phase is severely restricted and this has occurred in 2016.

Gambusia also has an expansion phase during the summer months at Akrotiri but it shuns polysaline or hypersaline waters. As a result it flourishes only in fresh and brackish waters; but at some sites populations may rise to remarkable densities. Gambusia populations sources in the wider aquatic network are poorly known. Metapopulations may exist in ditches and irrigation works in the Fassouri area and they may be source populations for conlonization after winter or drought. During cold winters (such as December 2016-January 2017) *Gambusia* may be difficult to observe. Gambusia are also routinely stocked on Cyprus for biocontrol (although SBA authorities no longer do this). We are aware of people activley stocking

[8] At the Pediaios Delta Wetland Complex at Famagusta/Ammochostos there are two small populations of *Aphanius a*t two separate wetlands ("Silver Beach" near Salamis on the river-mouth wetland pools and Glapsides where there is a lagoon-like waterbody south of the Pediaiow river floodplain). We presume that these two sites are re-colonized by fishes using the marine shoreline to re-colonize. A recent paper recorded *Aphanius* from marine waters at Famagusta bay as well; so dispersal should be considered possible via marine water in some cases. *Aphanius* is not a marine fish and from work in Greece we know that population survive in marine waters only locally (such as Amvrakikos Gulf, for example); when in marine waters they are only in the very shallow open lagoonal or shallow bays near river mouths (since the species has no defense against marine predators and is adapted to living in marshes).

Gambusia from reservoirs to volunteer efforts towards mosquito control; we have no evidence of this at Akrotiri.

Fig. 7. Potential distribution of *Aphanius* populations in the Akrotiri Wetland (dotted yellow line) and actual distribution confirmed in 2016 and early 2017 ("yellow area"). Presumed areas that are important as natural refugia for the species during times of drought as documented by past research and observations shown by "yellow stars". Due to natural desiccation of the Salt Lake and its surrounding salt marshes aquatic habitats with permenant aquatic habitats remained both in the western part (Akrotiri Marsh/ Episkopi Bay Wetland) and in the eastern part (Zakaki Marsh/Makria Lake). Major re-colonization movements are possible via downwards water flow from Episkopi Bay Wetland towards the Salt Lake ("yellow arrow"). Since 2016 was a drought year the dispersal to the Salt Lake was either minimal and serverly restricted. Furthermore conditions at Zakaki and Akrotiri Marsh areas are suboptimal due to the high population density of *Gambusia*.

The refugial survival areas for *Aphanius* seem to be decreasing or have become less viable for the species during recent years. Due to increased freshwater flow at Zakaki Marsh after 2012 (through the new Lemesos storm water project) *Aphanius* presence has declined there. *Aphanius* is negatively influenced primarily by completion with *Gambusia*, not primarily by the salinity changes. There is circumstantial evidence that the *Aphanius* may no longer be able to penetrate into the long-lasting aquatic refugium of Zakaki Marsh since it is actively displaced by summer-abundant *Gambusia*. *Aphanius* will survive in freshwater refugia during summer (and they have been abundant in Akrotiri Marsh in the recent past). In the summer of 2016 Akrotiri Marsh retained water but there was a local super-abundance of *Gambusia* (not a single *Aphanius* was spotted or collected). So it is possible that the same distributional pattern observed at Zakaki Marsh also currently precludes re-colonization by *Aphanius* to Akrotiri Marsh. The same may be taking place at the northern shores of Akrotiri Salt Lake- in the Eucalypt plantation ditches south of Asomatos (where there are some run-off ditches with

permanent water patches in most years - also hosting rare freshwater plant communities, including *Cladium mariscus*). There is video footage of *Gambusia* competing and showing agonistic behaviour towards Aphanius during summer at this area in recent years (P. Charilaou, pers. com). During the drought year between Dec. 2015 and January 2017 we saw no fishes south of Asomatos in the Eucalypt plantation areas. Was it just drought that extirpated both fishes? Or were *Aphanius* extirpated first due to the *Gambusia* competition, and as conditions became more saline in shrinking water patches, the *Gambusia* could also no longer could survive? Interestingly, we do not know how re-colonization by *Gambusia* takes place here, is it primarily human-mediated stocking or drift of metapopulations that survive in the Fassouri irrigation-water network?

Based on expert knowledge of the habitat requirements and dispersion pathways at Akrotiri wetlands, the potential range of *Aphanius* was estimated. The potential distributional range of *Aphanius fasciatus* in the Akrotiri Wetland covers the entire wetland complex (and former wetland area) from the coastal marshes south of the Kouris river-mouth to Lemesos harbour, originally near the Garylis river mouth (Fig.7). The Aphanius population is now distributed solely within the SBA. The species is no longer present in the "free-areas" of the Republic of Cyprus (it technically may have been present in the wider Zakaki area before road works and water drainage works in the early 2010s; but this was never confirmed). This study, identifies a potential negative impact from the new Lemesos storm water drainage works at Zakaki against the *Aphanius fasciatus* population for the first time.

To summarize this pattern: In recent years two refugial population centers are documented, that is, areas where the population survives during extreme drought or prolonged drought). These are: A) in the salt-marsh and artificial quarry pit ponds of Episkopi Bay Wetland (formerly including Akrotiri Marsh as well); and B) the eastern part of the Salt Lake shores, primarily the salt and brackish marshes in and around Zakaki (which also hold water summer). The Salt Lake basin completely dries during summer-autumn (July to October) and during this period all fishes trapped in remaining hot hypersaline waters die. Fish-kills in such conditions, involving *Aphanius* and mugilids, have also been reported in analogous salina-like lagoon systems in the Mediterranean and have also been observed in Famagusta (in the Pediaios Delta and saline lagoons (see Zogaris, internet sources, 2012).

The Salt Lake is a dispersal and nursery area for *Aphanius* but they need surrounding summer refugia to survive (i.e. sites connected to the Salt Lake that provide year-round water). In a series of drought years *Aphanius* may be confined to very limited areas of the Akrotiri Wetland (such as during 2016). *Aphanius* also need dispersal pathways (hydro-morphological connectivity among wetland areas). They may disperse via the shallow marine waters if during floods they are swept in the marine shallows but this is highly unlikely in Akrotiri peninsula conditions. However, the species is generally considered a poor disperser (and especially prone to predation by predatory marine fishes in the marine environment as well). The species also disperses over-land (i.e. using marsh channels and flooded salt marsh flats) during extreme winter floods. *Aphanius* eggs are known to survive for up to two weeks in dry mud - so it is possible that birds can carry off eggs to nearby wetlands (see Thienemann 1950) but this has not been researched in recent times.

Finally, along with *Aphanius*, the *Gambusia* population is also stressed by drought/desiccation and especially by salinity barriers (*Aphanius* in contrast does not have salinity barriers to survival or dispersal). The situation is very interesting with respect to re-establishment/restoration of *Aphanius* in Akrotiri Marsh. Due to a reed swamp burn in March 2016 and an extremely long drought during most of the year we feel that *Gambusia* may have been extirpated from nearly all parts of Akrotiri Marsh in 2016 (although source populations may exist in ditches in and around Fassouri). If this is the case, the vacant niche conditions, with no competitors against *Aphanius*, may exist in the Spring of 2017 in Akrotiri Marsh. This provides optimal conditions for human-assisted dispersal of *Aphanius* from the potentially burgeoning late spring populations in Episkopi Bay Wetland to Akrotiri Marsh. It is imperative that no stocking of *Gambusia* take place anywhere in the Akrotiri Wetlands or in Fassouri, but especially in such sensitive fresh/slightly brackish refugia such as Akrotiri Marsh.

A4. Current understanding of past presence/distribution of the species

There is documented evidence that during high-water level years (e.g. in 2004) *Aphanius* was very widespread while in 2015-2017 there has been a significant areal decline in its distribution (evidence in Zogaris et al. 2012b and personal observations by several other investigators as well). *Aphanius* will survive well in fresh and slightly brackish conditions (documented in the bibliography). In nearly all cases of observed high density *Aphanius* populations *Gambusia* is absent. Both *Aphanius* and *Gambusia* populations and their distributions fluctuate at Akrotiri; current understanding documents that in habitats with increased freshwater conditions *Gambusia* will outcompete *Aphanius.* Unlike *Aphanius, Gambusia* cannot tolerate saline conditions for long periods.

Aphanius became extirpated sometime after 2004 from Akrotiri Marsh; *Gambusia* was abundant there since 2008 (Zogaris pers obs.). After 2012 the Zakaki *Aphanius* population was affected by changes brought about the storm water channel development with a resultant increase in freshwater input (both Lake Makria and the channel leading to the Salt Lake proper). This presumably negatively affected the *Aphanius* population which was not present in the area in 2016. The resultant increase of *Gambusia* with the decrease of salinity in Zakaki was not foreseen as a potential threat in the Environmental Impact Assessment (Planning Partnership LTD, 2011). Moreover, the philosophy of "dirty water is better than no water" seems to have some value (for general biodiversity conservation[9]) but implications with alien species such as *Gambusia* were not foreseen or described in Environmental Impact Studies (Planning Partnership LTD, 2011). Water management planning for biodiversity is definitely not simple or static. The issue does need further study, but here we provide observational and circumstantial evidence that an increase in freshwaters did benefit conditions for *Gambusia* which seem to be the major reason for *Aphanius* extirpation at Zakaki Marsh. Routes of *Gambusia* colonization have not been investigated; stocking by local residents is possibly one important route.

[9] Several freshwater and brackish water plants and habitat types thrive in increased freshwater conditions (as do several bird species, notably Ferruginous Duck and many other waterfowl).

B. Relevant aspects of *Aphanius fasciatus* conservation biology, including appropriate conditions and resources of the species' requirements and the fish community description based on sampled assemblages

B1. Genetics of the Cyprus populations

Work by Hellenic Centre for Marine Research (HCMR) collaborators is ongoing and the genetics of the *Aphanius* specimens from Akrotiri are being investigated. There is now evidence that *Aphanius fasciatus* should be considered native to Cyprus. Initial barcoding data[10] show that the specimens from Cyprus are different from Aegean populations and are more similar to a Southern Anatolian population[11]. This will be reported in detail in an upcoming publication.

B2. Species requirements, etc.

Data from sampling of the fish community at each site (30 samples) has produced some first insights. Conservation-relevant aspects of the biology of the species are being compiled and evidence is also being compiled from work done by Cypriot colleagues who have kept the species in captivity (e.g. S. Michaelides). The populations of Akrotiri seem to be and act such as typical of *Aphanius fasciatus* populations in other Central Northern Mediterranean wetlands (however, this does require further research for a full life-history description). *Aphanius* thrive in slightly brackish and saline inland marsh and lagoonal waters and can survive quite well in freshwaters, although there is some evidence they prefer to reproduce in slightly brackish or brackish conditions (or at least in freshwaters with high electrical conductivity values). They form large concentrations in small brackish pools in salt marshes and disperse among pools during winter-spring flooding events, but generally they are considered poor dispersers. *Aphanius* forms dense concentrations when waters shrink in spring and summer; they can survive in very crowded conditions and in very warm waters.

C. Information in the life-cycle of *Aphanius fasciatus*. Life-cycle and mosquito biology to explore killifish use to control mosquito breeding

C1. Data for *Aphanius fasciatus* life-cycle etc.

Spawning

Kottelat & Freyhof (2007) state that *Aphanius fasciatus* spawns in the benthic submerged vegetation, with a spawning period from March to September, depending on the geographic position of the population. Populations in Italy spawn from March to June, in Greece from April

[10] "DNA barcoding" has been used as a way to identify species since 2003. Barcoding uses a very short genetic sequence from a standard part of the genome the way a supermarket scanner distinguishes products using the black stripes of the Universal Product Code (UPC). Two species may look very similar to the untrained eye, but in both cases the barcodes are distinct.

[11] Work undertaken at Hellenic Centre for Marine Research (HCMR) with post-doc Dr. Maarten Vanhove has shown that the species in the Cypriot populations from Famagusta/Ammochostos and Akrotiri are definitely part of the nominate species, *Aphanius fasciatus*. This work is the first to identify the species from genetic material. Morphological examination and meristic analysis under the supervision of R. Barbieri have also shown no real divergence from other Mediterranean populations (unpublished results not to be cited; released here for conservation-relevant use only).

to July and in Corsica from April to September (Leonardos & Sinis 1998, Marčić et al. 2015). Spawning takes place on the bottom and in submerged vegetation. The species is short-lived and maturity is reached within less than a year. Reproductive strategies of *Aphanius* show the typical adaptations to the unstable and unpredictable environment of transitional waters, such as batch spawning and early sexual maturation (Leonardos and Sinis, 1998). Observational evidence shows that they reproduce in spring and/or early summer at Akrotiri.

Aphanius fasciatus is a rather hardy species that is fairly adaptable. *Aphanius fasciatus* from different wetland sites (Cavraro et al. 2014) showed differences both in demographic and life history. Adult mortality and local environment were the main factors influencing life history behavior of the species. In natural salt marshes, adult mortality was higher, fish attained smaller sizes and showed higher fecundity compared to those from artificial sites with lower predator pressure. As would be expected, different levels of food availability were related to fish density and growth rate (Cavraro et al. 2014).

Predation

Aphanius is a relatively slow-swimming fish, it is easy prey for marine fishes. The sea bass *(Dicentrarchus labrax)*, eel and several birds (e.g. *Egretta garzetta*, *Ixobrychus minutus, Alcedo atthis* and even some species of waders (*Tringa nebularia*)) are known to actively prey on *Aphanius fasciatus*. Some species, especially fish, are known to significantly influence *Aphanius* population structure due to predation. Several researches have documented this and Cavraro et al. (2014) showed that "predation intensity seemed to mainly influence fish size. Fish in low predation sites, probably due to a longer life expectation, attained a bigger size than fish in high predation sites, which suffered higher mortality rates". At Akrotiri it is very interesting to note that within the two deepest quarry pits (Paraga and Ai Yorki) predatory fishes have established populations and presumably as a result, the *Aphanius* is found in remarkably low density and small size-classes dominate (relative to elsewhere). This is especially remarkable at Paraga Pond. In 2011 and 2012 Zogaris's research team (2012b) sampled at Paraga (termed "Phassouri Pits" in the report) and found very large numbers with three size classes; in contrast during this study only one, juvenile size class was recorded at Paraga Pond and very low population densities both in 2016 and 2017. Local interviewee say that Paraga has recently been stocked with Sea Bass and other marine fish, and this would explain the remarkable change that was documented in this study. Of the seven to ten quarry pit ponds hosting Aphanius we assume predatory fish (mainly sea bass) have been released in Paraga, Ai Yorki and perhaps Navagio ponds.

Diet

Aphanius fasciatus are micropredators. They are known to feed a wide variety of zooplanktonic animals; on insect larvae, small aquatic crustaceans, worms, and other macroinvertebrated though algae and other plant material is also taken at times. In similar lagoonal habitats in Greece the species is known to feed on juveniles of shrimps, Isopods, Branchiopod, Bivalves, eggs of invertebrates, mosquitoes (adults and larvae) and diatoms. An ontogenetic diet shift with an increase in mean prey size with fish length was observed, younger fish feed on much smaller items (Leonardos 2008 and references therein). Work on the feeding ecology of the species has not been published for Cyprus, but

visual observations confirm that it feeds both in midwaters (water column) and near the surface on zooplankton.

Since it is known that the species often devours large numbers of mosquitoes and midges, it is often referred that it could help locally reduce mosquito numbers. The closely related *Aphanius dispar* has been reported on several occasions to suppress mosquitoes both in natural and anthropogenic habitats (wells etc.) in the tropics and the Middle East (Chandra et al. 2008), so there is definitely research interest in this area.

C2. Data for mosquito issues, populations life-cycle, relevant to biological control using fishes

The widespread and persistent use of DDT between 1948 and 1978 created serious problems for aquatic biodiversity on Cyprus, and there is evidence of many species becoming extirpated and shrinking remarkably in population and distribution - this includes birds, reptiles, amphibians, fish and invertebrates (Zogaris et al. 2014). In order to exterminate malaria a "silent spring" effect was created; after the ceasing of DDT poisoning there is evidence of a come-back of many aquatic species. Mosquitoes are still a problem. Mosquitoes breed in shallow waters both fresh and brackish conditions. Violaris et al. (2009) show that Cyprus hosts twenty-three species belonging to 6 genera; about fifteen species have been recorded at Akrotiri (Martinou, pers. com.). The use of biological and more environmentally-friendly techniques for mosquito control is being pursed in the SBA and in the territory controlled by the Republic of Cyprus as well. Empirical evidence from Cyprus is scant but some positive aspects exist. Here we relate this issue to *Aphanius fasciatus* conservation.

Mosquito Species	Reproductive habitat	Adult activity period	Salinity and other conditions favoured	Comments
Ochlerotatus detritus	Salt marshes	February-July	High salinity tolerance	Nuisance species, vector in other countries
Ochlerotatus caspius	Salt marshes	February-July	High salinity tolerance	Nuisance species vector in other countries
Culex pipiens	Stagnant water, fresh, artificial man made habitats	Present all over the year	Water in high organic matter	Nuisance species, WNV in many parts of the world

Table 5. Life cycle and mosquito biology of some important species (Source: A.F. Martinou, unpublished information).

Table 5 summarizes current knowledge of nuisance or potentially vector-concern mosquitoes at Akrotiri. *Ochlerotatus detritus* and *Ochlerotatus caspius* are nuisance species in Cyprus but

in other countries they are also disease vectors. They reproduce in the salt marshes and have high salinity tolerance. *Culex pipiens* is also present and since it is a WNV in other countries concern focuses on this species too. It reproduces in anthropogenic habitats, lives closer to humans and in fresh, non-saline water, in stagnant waters with high organic matter.

Akrotiri SBA has taken the following steps to control mosquitoes (Martinou, pers. com.):

- 1950s: Mainly petroleum, DDT was also widely used in the malaria control programme.
- 1970s: petroleum and malathion
- 1980s: temephos (organophosphate larvicide), *Gambusia holbrooki as a* biological control agent
- 2000: methoprene insect growth regulator
- 2015: *Bacillus thuringiensis* israelensis and *Aphanius fasciatus*
- Currently RoC is also using *Bt*i

The issue of mosquito control is important and may affect stakeholders (public, military, institutions etc.) and their perception of wetlands, aquatic biodiversity etc. There is always a potential threat of vector-borne diseases (mainly arboviruses carried by birds) since there are potential vectors present. Of course in order to have a disease you need vectors (mosquitoes in this case) in high numbers. A successful vector management program can keep mosquito numbers very low and reduce possibilities for disease introduction to null. There is always a risk that invasive mosquito species will enter Cyprus e.g. *Aedes aegypti* and *Aedes albopictus.* Therefore surveillance programs are imperative all over the island. Also a few years ago malaria re-emerged in Greece and there is a risk for diseases like malaria returning to the island.

Weather conditions such as precipitation and temperature affect mosquitoes. The saltmarsh mosquitoes lay their eggs also in wet soil and not in constant aquatic environments like most mosquito species. These eggs remain in the soil till it rains again and the conditions are optimal for their development (Martinou, pers. com.). They can sustain desiccation for a couple of years if it does not rain for very long periods.

Mosquito treatment at Akrotiri

Current mosquito control efforts focus on suppressing the mosquito populations whilst they are still at the larval stage. In order to achieve this, all breeding sources for mosquitoes are identified during surveillance and mapped. The area is then treated with methoprene a juvenile hormone inhibitor that prevents the mosquito larvae from becoming adults and in parallel it has lower impact on aquatic insects than conventional pesticides. An additional future asset for JSHU would be purchasing also *Bacillus thuringiensis israelensis* (Bti) a biological product that can also be used as a larvicide in order to have an alternative product for the control of the larval stages and apply the latter larvicide in the most vulnerable habitats. Control of the adult mosquitoes is not a preferred method globally for the control of nuisance mosquitoes therefore it is undertaken occasionally only in urban areas when the nuisance levels are high by using a pyrethroid (deltamethrin). This chemical is toxic to fish under laboratory conditions and therefore its use around the salt lake and salt marshes is prohibited. Current and recent methods show effectiveness at surpassing mosquito

populations at Akrotiri. Laboratory and semi field trials testing the efficacy of different products and the susceptibility of different mosquito species regularly take place at the JSHU headquarters. Field observations are also taking place regularly after application of the biological larvicide.

Biocontrol

Biological control of mosquitoes by using predators is attractive; e.g. the use of fish is considered one of the "most desirable methods for mosquito control" as it is environmentally friendly and it can also be economical compared to repeated pesticide treatments. Biocontrol efforts started early on in Cyprus. *Gambusia holbrooki* was widely introduced in the 1940s; and routinely stocked. It can survive in various habitats (present in man-made reservoirs, wells, marshes, ditches, dams, lakes, ponds and streams). However, *Gambusia* due to is highly predatory and territorial/agonistic habits, is characterized as one of the 100 worst invasive species in the world by IUCN -ISSG (http://www.issg.org/) and due to its invasiveness status and its negative effects to biodiversity and ecosystem services this fish should no longer be used for biocontrol on Cyprus. Current SBA efforts focus on studying the population dynamics of *Aphanius* throughout the year and mapping the habitats where it occurs. The SBA also attempts to breed the fish in natural confinement and in an artificial one. The SBA's aim is to learn as much as possible about *Aphanius* in order to be able to breed and release it in the mosquito problematic areas but also in the environmentally vulnerable areas where pesticide use is not advisable. One of the problems encountered is that many workers from the Republic of Cyprus public authorities do not know the differences between *Aphanius fasciatus* and *Gambusia holbrooki* and deliberately or accidentally release *Gambusia* to water bodies. Also many members of the public do not know the problems caused by *Gambusia* on native species and associated ecosystems. This stocking practice should stop as *Gambusia holbrooki* can adversely affect many other aquatic species given its invasive status. Native fishes can be used cost-effectively, especially if there is also community participation and in this study there is evidence to show that *Gambusia* should be replaced by *Aphanius* as a biocontrol agent.

D. Relationship of *Aphanius fasciatus* with *Gambusia holbrooki* - evidence of potential alien invasive pressures on *Aphanius fasciatus*.

D1. Evidence of completion caused by *Gambusia*

The literature is consistent about the aggressive effects of *Gambusia holbrooki* on the genus *Aphanius* and other related small-sized native fishes in various countries (Pyke 2005 and references theirin; Kalogianni, 2012, 2014).

Incidental observations show that *Gambusia* probably directly competes, is aggressive towards, and actively displaces *Aphanius* at the Akrotiri Wetlands. Observational evidence from Cyprus both from observations in the wild and within aquaria show that *Aphanius* and the *Gambusia holbrooki* aggressively compete; *Gambusia* actively show agonistic behaviour against *Aphanius* (i.e. biting, fin-clipping, etc.). These behaviors have been documented on video in the wild at Akrotiri in places where *Gambusia* and *Aphanius* coexist (e.g. on the north shore pools of the Salt Lake, documented by members of the Akrotiri Environmental Education Center).

Salinity and water temperature are known to mediate the competitive interactions between *Gambusia* and *Aphanius* species (and other threatened small-sized native fishes). *Gambusia* do not thrive in cold water or in water with salinities greater than 20 ‰. Also the rapid decline and local extirpation of *Aphanius* from Zakaki is considered to be related to altered salinity and consequent *Gambusia* invasion- this taking place once brackish conditions altered to "slightly brackish" conditions (due to freshwater inputs from the new storm water works project at Zakaki). *Gambusia* directly displaces *Aphanius* in many small aquatic areas, however *Aphanius* is much more tolerant of higher salinity levels and may also be able to survive in colder waters (i.e. survive cold winter spells). In Greece, the competition among *Gambusia* and small native fishes was shown also by prey overlap (Kalogianni et al. 2012, 2014). *Aphanius* and *Gambusia* are both micropredators of zooplankton so their trophic requirements and niches overlap.

In coastal wetlands, a gradient of salinity levels enables *Aphanius* to survive and thrive in waters that are above 20‰ while *Gambusia* is usually abundant and often "super-dominant" in waters below 5‰. This pattern is repeated in many Mediterranean wetlands which host both *Gambusia* and *Aphanius* spp.. *Gambusia* does survive much higher salinities for short period of time and can disperse even through waters that are above 30‰, but it usually does not survive for long or reproduce effectively in waters above 20‰ salinity (for work in Spain, see Alcaraz 2006). This makes *Gambusia* an important "survivor" in salt marsh conditions and increases the threat of co-occurrence/competition with *Aphanius*. In other countries evidence has shown that *Gambusia* (as aliens) are aggressive, fin-nipping harassers of other fish, and pose a serious threat to native fish and aquatic fauna (e.g in Australia, Mediterranean states etc). *Gambusia* also have the potential to competitively displace *Aphanius* and other fishes through interference competition. Negative impacts by *Gambusia* on invertebrate species and at least one frog species have been documented (even in the early part of the last century, in works by Th. Stephanides on Corfu). Compounding the issue, *Gambusia* have the ability to thrive in many different environmental conditions which are usually lethal to other fish species (so their survival potential is greater than native species living in harsh conditions caused by anthropogenic or aquatic stresses). Despite the accumulating evidence condemning *Gambusia* one of the world's worst alien invasives, on peculiar insular ichthyofaunas such as in New Zealand (where there are not direct competitors to be affected by *Gambusia*) a more ambivalent stance has been recorded with respect to its impact on other fishes (Ling et al. 2004). However, this is not the case in areas inhabited by *Aphanius* in Cyprus and all other Mediterranean states where the *Gambusia* coexist with small-sized native fishes.

In summary, there is evidence from many other similar habitats that shows: a) higher salinity is a "refuge" for *Aphanius* and a restriction/barrier to *Gambusia*; and this is hypothesized to take place at Akrotiri as well; b) as salinities near polysaline conditions (20‰) *Gambusia* reproduce and feed in very poor condition and do not compete as actively with *Aphanius* (Alcaraz 2006); so under these conditions co-existence is possible (i.e. as in the recent past at Zakaki Marsh). Observational evidence in lagoons in Greece shows that *Gambusia* outcompete or produce seasonally overabundant situations below a threshold salinity of around 6‰ (e.g. at the closed lagoon of Koumoundourou Lake, see Mentzafou et al. 2016). *Gambusia* usually gain highest densities in stagnant warm waters under 5‰. In conditions such as these they outcompete and actively displace small native fishes when there are no serious predatory pressures on them; c) *Gambusia* should not be used for stocking in Cyprus as a biocontrol mechanism

against mosquitoes. *Aphanius* could definitely have inhabited habitats were this species is now stocked or thrives from previous introductions and *Aphanius* would be a good candidate species for biocontrol.

D2. Relevant issues concerning *Gambusia*

Gambusia holbrooki is a small, light-colored fish with semitransparent fins that looks very similar to a colourless form of guppy (*Poecilia reticulata*). Very young fishes can be confused off-hand with *Aphnaius fasciatus*. The females usually have a black stripe near their eye area and light spots can be seen on the caudal and dorsal fins of both sexes. Generally, males reach 3.8 cm and females 6.4 cm. Unlike *Aphanius*, they are a livebearer species; the females are larger and more rounded than the males. Pregnant females are recognizable by their gravid spot (a darker area on their bellies where they hold the fry). *Gambusia* is considered a planktivorous species which consumes invertebrate fauna but also consumes algae and detritus. Feeding habits change based on season, on maturity and mating conditions and perhaps other factors. *Gambusia* will, if need arises, switch food sources (as do many small fish species -the species has a "feeding generalist" trait). With an increase of competition, this species will switch from a diet rich in plankton, algae, and detritus to one consisting of zooplankton, other invertebrates, the larvae of many species, and plant-associated animals. The main source of competition for *Gambusia* seems to be an increase of its own species and other planktivorous species. The females tend to not specialize on one prey, and consume all evenly, whereas the males and juveniles may sometimes specialize on one prey type. However, males, females, and juveniles all consume detritus at similar rates (Pyke et al. 2005).

There has been no single study of *Gambusia* on Cyprus, although the species is used widely as a biocontrol and is stocked in natural ecosystems and reservoirs (and stocking is known to be perpetuated by local citizens as well). *Gambusia* may be more hardy than *Aphanius* but this has not been tested in natural/semi-natural conditions in Cyprus. The detrimental effects of *Gambusia* on aquatic biota are very poorly researched on Cyprus. Evidence-based approaches for interpreting impacts are needed.

D3. Proposals for further investigations on the *Aphanius-Gambusia* relationship

Despite anecdotal observations, and much evidence from abroad, little is known about the relationship between *Aphanius* and *Gambusia* at Akrotiri (or anywhere on Cyprus) and the impacts of the *Gambusia* on the ecosystem as a whole. More research would assist in the following conservation-relevant aims:

- Explore the history and current trends in stocking *Gambusia* in Cyprus.
- Explore diet, abundance and distributional characteristics of *Gambusia*.
- Establish the physicochemical, other environmental and biological constraints to *Gambusia* invasion;
- Initiate a campaign to exterminate *Gambusia*. Use natural conditions to help locally exterminate *Gambusia* (i.e. winter-cold, desiccation events, salinity increases);
- Explore aspects of impacts *Gambusia* may have on other aquatic biota. Do *Gambusia* ingest or disturb amphibian eggs and larvae?

- Gain evidence to ban or modify ad hoc *Gambusia* introductions (i.e. based on knowledge that there are detrimental or ecosystem altering effects of *Gambusia* - as has been described in some areas abroad).
- Explore, in tandem, options of re-introducing or introducing *Aphanius* to replace *Gambusia* as a biocontrol agent.

Many other research questions, affecting *Gambusia* and *Aphanius* can be developed with extra care for their utilization in conservation and restoration actions at the Akrotiri Wetlands.

E. Re-introduction of *Aphanius* in wetlands

E1. Background

In the Oroklini Lake ichthyological study (Zogaris 2013) it is hypothesized that *Aphanius* may once have had a much wider distribution on Cyprus and various human-induced pressures are probably responsible for its decline. DDT poisoning and desiccation-prone coastal wetland degradation are probably enough to extirpate these fishes from many areas; even larger wetlands and lowland river systems. A similar situation has been observed in other Mediterranean coastal ecosystems (France, Italy, Malta) so the issue of the decline of this "poor-disperser" and wetland-specialist should be better researched and more widely promoted as an urgent conservation theme. If it can be proven that *Aphanius* was once more widespread and can survive in both river, wetland and transitional water environments a framework for biodiversity-driven restoration can be developed.

Once the conceptual framework for re-introduction is established, the only solution is a re-introduction action plan (Zogaris et. al 2016). In the case of *Aphanius*, the species is potentially important as a replacement for *Gambusia* for mosquito biocontrol and this gives an added incentive for serious study of various options. Obviously this conceptual framework may affect interest beyond the Cyprus SBA and should be of interest for stakeholders and institutions in the Republic of Cyprus as well.

E2. Current routine and protocol

Already the Akrotiri Environmental Education Center is helping stock quarry-pit pools in the Episkopi Bay Wetlands area from adjacent pools (artificial quarry pit refugia). The simple action of introducing/re-introducing fairly large numbers of fishes in nearby pools/quarry pits in the Episkopi Bay wetlands may easily help boost surviving populations within a said hydrobiological unit (i.e. Episkopi Bay Wetlands). This is easily done during late winter and spring when large numbers of *Aphanius* are present in some quarry pits of Episkopi Bay Wetland. Transferring fishes a few hundreds of meters to new quarry pits or deep pools in this area is a simple and practical method to protecting the stock at the Episkopi Bay Wetland area. However, a scheme to promote the wider dispersal of the species needs careful consideration.

Research and discussions with specialists were developed in this project to prepare a preliminary plan and consider aspects of conservation genetics, interspecies competition, limnological conditions, and potential risks with respect to re-introduction planning for both the Akrotiri Mash in particular and the wider Akrotiri Wetlands.

E3. *Aphanius as a potential competitor in local food webs*

Issues relating to the food competition between fish and zooplankton-feeding birds (such as flamingos) have been placed as a concern. The A.P. Marine (2012) study is actually one of the few studies to explore all available data with the goal of building an Akrotiri Peninsula Management Plan. The study expounds on the fragmented nature of available biological and hydrological (surface waters) information. Among other important results, the study documents the food web base created by the halophilic fairy shrimp, *Phallocryptus spinosa* (a critically important food source for the large numbers of wintering flamingos and other planktivorous birds at Akrotiri Salt Lake). However, the A.P. Marine study (2012) produced a provocative statement concerning the effect *Aphanius* "introduction" may have on the Salt Lake system: "The introduction (assisted or natural) of *Aphanius* to the Salt Lake during the months when the low salinity is triggering the hatching of the first generation of *Phallocryptus*, will eventually cause an unknown but most probably, significant impact on the Salt Lake's food web. It could be a text book case of disruption and alteration of ecological webs" (p. 56). This statement provides a useful warning against tampering with food web dynamics, however we feel there is little evidence that *Aphanius* can be such a threat within the Akrotiri Wetlands context.

Aphanius are poor dispersers and they slowly build their populations in the Salt Lake only after winter movements from refugia (e.g. from Episkopi Bay Wetlands). The dispersal of *Aphanius* takes place regularly from the existing refugia, following natural flooding of the constituent marshes and the increase of run-off water flows from the refugia downwards toward the Salt Lake Lagoon basin. The expansion-contraction of the *Aphanius* population thus happens on most years even if rainfall is sparse; since extensive flooding in the Episkopi Bay wetland is often caused by winter storms (prevailing westerly winds create very high storm-wave surges and flood the area with marine waters on most years). But flooding of this nature is a winter-time phenomenon[12] and the *Aphanius* increase their densities only after reproduction in the shoreline salt marshes of the huge expanse of the Salt Lake (i.e. in late spring).

The current conceptual idea of the *Aphanius* population build-up in the Salt Lake points to the following: a) The population moves into the Salt Lake in winter initially in very small numbers and the numbers build only after reproduction in spring (after March); b) Population densities in the Salt Lake are thus always very low in winter when *Phallocryptus* have already hatched and matured. *Aphanius* numbers are definitely very low or non-existent in many parts of the Salt Lake in winter (observational evidence exists for December 2015, February 2016 and January 2017). c) As a result, there can really be no spike in *Aphanius* population influx during late autumn-winter when the salinity levels are low the peak *Phallocryptus* is hatching and

[12] Empirical evidence and monitoring is lacking so it is difficult to make sure that the said dispersal pattern is the only one that holds; but the sea-storm flooding phenomena is well known among local elders and naturalist observers. And the runoff phenomenon was clearly observed during this study in January 2017. Fishes were found in "rivulets" formed by flowing waters moving down towards the Salt Lake basin in January 2017 and there was a direct surface water connectivity between Episkopi Bay Wetlands and the Salt Lake. In the Salt Lake itself no *Aphanius* were found - the dispersal movement does take time since the fish do not actively migrate.

numbers of panktivorous birds are at their peak in the Salt Lake. Since *Aphanius* does not reproduce until spring any disruption to food webs by the gradual dispersal of relatively few adults from the refugia into the Salt Lake during winter cannot really be enough of a predatory pressure to force tipping-point that will alter the *Phallocryptus*-based food web.

As mentioned in the study of A.P. Marine (2012), the food web dynamics in the Salt Lake must be also strongly affected by the annual and interannual variations in the onset of the flooding (changes in salinity and nutrients) and the consequent algal blooming, hatching of *Phallocryptus*, and arrival of predators. Complex variations are to be expected due to frequent internal and seasonal changes of water levels, flood timing and salinity producing strong influences in the dominating trophic levels and dynamics. Many piscivorous birds, gather during spring at the Salt Lake (egrets etc.) and they are important predators on *Aphanius*- little has been said about their contribution to the food web. We do not feel there is any real threat of "disruption and alteration of ecological webs" from the gradual increased dispersal of *Aphanius* (especially since it is late in winter and water levels are usually at their highest making *Aphanius* population densities very low, in any case). In fact, the populations of *Aphanius* may have been much higher in the past when there were more refugia and the expansion-contraction population movement was more pronounced. Finally, before *Gambusia* infestation at Zakaki Marsh and elsewhere in the Akrotiri Wetlands, *Aphanius* populations where much higher than today but even then their influx into the Salt Lake during winter was presumably gradual and much later than the *Phallocryptus* hatching period.

E4. *Caution in translocation practices required*

Several scientists mention the use of *Aphanius* for biocontrol and are discussing actions to promote dispersal and translocation across Cyprus. The authors of this report insist that no translocation of *Aphanius* from Akrotiri Wetland take place without a concrete plan and a specific and standardized re-introduction/ introduction method based on scientific justifications geared with respect to biodiversity conservation. Any such project requires an action plan (taking *Gambusia* invasion and mosquito biocontrol into consideration), rigorous scientific monitoring and an adaptive management framework to be effective.

However, we feel that efforts must be made for *Aphanius* stocking within the Episkopi Bay Wetland and within Akrotiri Marsh. At Akrotiri Marsh the goal would include an attempt to exterminate *Gambusia* and replace it with *Aphanius*. We believe these efforts will have no significant change to the Akrotiri Salt Lake food web (as discussed above) and should benefit the ecological integrity of Akrotiri Wetlands as a whole.

Lastly it goes without saying that as the complexity of water management, mosquito control, aquatic biodiversity restoration and management become apparent, a serious foundation for policy-relevant research, monitoring, knowledge management and decision-making must be developed. Actions promoting fish (or birds) should not be isolated from wider ecological understanding and goal-setting.

6. Restoration options for Akrotiri Marsh with respect to the needs of native fishes

6A Theoretical framework

Effective conservation of a threatened fish species must take many factors into account and must be based on a firm scientific knowledge foundation. Modern conservation science lays weight on: a) empowering individuals and institutions, b) systematic conservation assessment, and c) securing effective actions (Fig. 8). Although our interests for this fish species may be ambitious (i.e. biocontrol, alien species control), this study and its environmental snap-shot survey provides only a few of the knowledge baselines required for effective conservation planning. Our goal is to help establish evidence-based basic goals and further requirements in order to support future plans for effective management, adequate reporting and scientifically-led conservation actions.

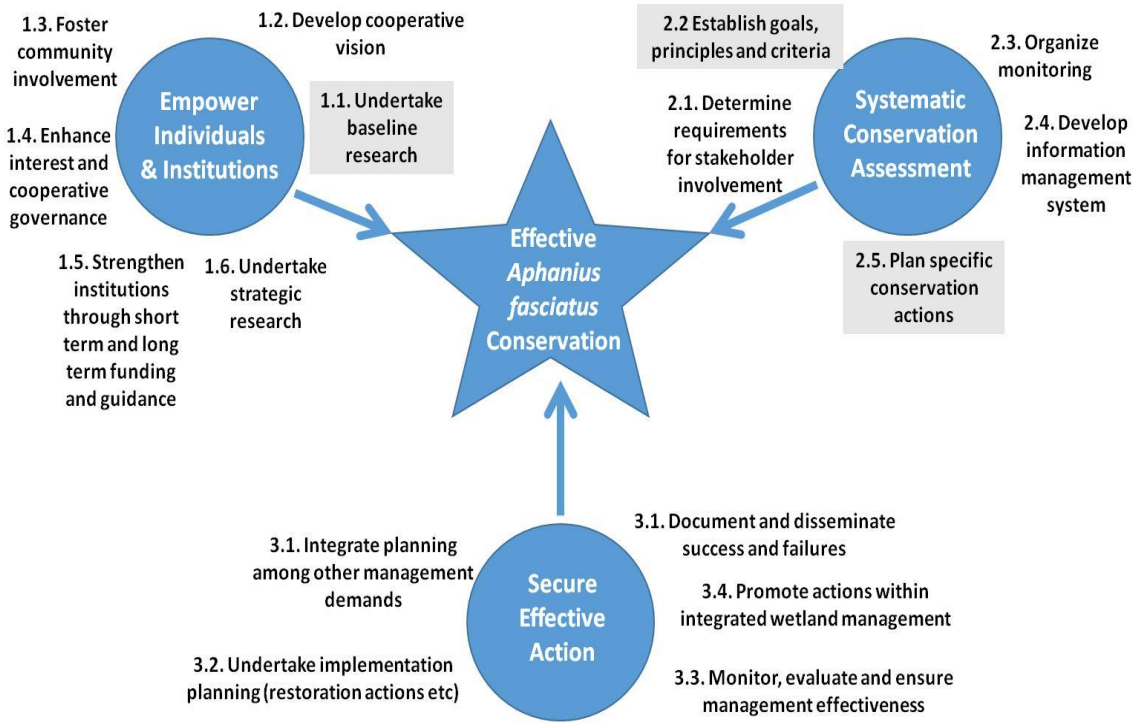

Fig. 8. An operational model for pragmatic conservation planning. (Adapted and modified from Knight et al. 2006). In grey blocks are aspects that have been developed to some degree within the present study. It is obvious that the present study created only a baseline outline of needs within an effective conservation project.

6B Restoration options defined

From the experience at Akrotiri, we are convinced that assisted dispersal by population translocations is a low-risk and straightforward technique that can be promoted and continue to be utilized by SBA authorities within the Akrotiri Wetlands. However, there is a lack of holistic ecosystem-based study of the Akrotiri Wetlands and this unmet need for better organized research and knowledge management may provide for uncertainties (i.e. as expressed in the A.P. Marine (2012) study). Caution is called for and a precautionary approach requires careful science-led research, monitoring and adaptive management actions.

It has been repeatedly stated that there is an interest in promoting the re-establishment of *Aphanius fasciatus* in Akrotiri Marsh (and in other parts of its former habitat on Cyprus as well; see Zogaris 2013, Zogaris et al. 2016). The ecological reasoning and requirements for this at Akrotiri Wetland and Akrotiri Marsh were investigated in this study. This study shows that assisted migration actions are scientifically justified. Local institutions such as the Akrotiri Environmental Education Center and SBA authorities confirm the need for the assisted dispersal of the fish (an important reason is mosquito biocontrol). Assisted migration/dispersal is easily done and has already shown to be effective among wetland elements of the Episkopi Bay Wetland (transfers from one quarry pit to another during spring when numbers and densities of *Aphanius* are locally high). This study promotes the continuation of assisted dispersal but aims to promote specific and practical targets as well.

Aspects that negatively impact *Aphanius* populations within the Akrotiri Marsh and wider Akrotiri Wetlands include the following:

1. The locally high population densities of *Gambusia holbrooki.* This invasive fish is a serious competitor and it will actively harass and displace *Aphanius.* Perhaps the severe drought and reedswamp fire of 2016 may have helped extirpate *Gambusia* from most parts of Akrotiri Marsh - thus providing a rare opportunity for restoration action with no or little competition.
2. *Gambusia* stocking. It is imperative that no stocking of *Gambusia* take place anywhere in the Akrotiri Wetlands, but especially in such sensitive fresh/slightly brackish refugia such as Akrotiri Marsh and the north shores of the Salt Lake (Asomatos - Dasonomio etc).
3. Connectivity among *Aphanius* populations surviving in the Akrotiri Salt Lake basin and the Episkopi Bay wetland. Connectivity exists but the issue of natural dispersal requires better data and understanding (i.e. surface flow patterns, specific obstacles to movement, the influence of marine vs precipitation flooding etc.)
4. Akrotiri Marsh hydrology and habitat conditions have changed. The wetted period and the aquatic conditions (which have become progressively dominated by freshwater and dense *Phragmites* reed encroachment).
5. Aquatic chemical and physico-chemical conditions. These may also be influenced by fire in the reed-bed or other disturbances in the reed swamp that may impact physico-chemical conditions.
6. The effects of seasonal desiccation (i.e. the extent of "summer-kill" of *Aphanius* populations within Akrotiri Marsh and in the surrounding areas).
7. Indtroduced predatory fishes. Sea Bass have been introduced in two or three quarry pits and since they are the largest and most permenant of the quarry pit ponds at Episkopi Bay Wetland they are a very serious threat to the *Aphanius* population.

A serious problem is the dominance of *Gambusia* within the deeper and permanent water areas of Akrotiri Marsh (until 2016). It is "nearly certain" that competition displaces *Aphanius* and this is especially true in areas where the *Gambusia* have very high densities (i.e. what is often called a "super-abundance" condition). *Gambusia* may be exterminated by managing units of the marsh and taking advantage of the drought-induced "summer-kill" (as has occurred in 2016). Exterminating *Gambusia* is an important part of any *Aphanius* re-introduction into fresh or slightly brackish waters. It is also important to stop any act of *Gambusia* stocking. A single stocking event can totally destroy all efforts at re-introduction of *Aphanius*. The invasive dispersion act cannot be reversed.

Due to altering wet-dry periods in Akrotiri Marsh, *Aphanius* need to move to survive - even though the species is a poor disperser. A connectivity among the Akrotiri Marsh basin and adjacent areas where *Aphanius* is present or may persist even during harsh drought periods is important but there are anthropogenic barriers to fish dispersal here. A ring-road creates a boundary between the Akrotiri Marsh and the Episkopi Bay Wetland. Research is needed to design connectivity into the local landscape and this requires specific restoration planning and adaptive management approaches.

With respect to artificial water bodies such as new ponds being constructed in the Akrotiri Marsh (i.e. Darwin Iinitiative funded restoration actions www.akrotirimarsh.org) for restoration the prospects of permanent refuges for the fishes arises. Attempts must be made to re-introduce *Aphanius* in such cases - however this must be done in conjunction with *Gambusia* extermination and anti-stocking information campaigns.

2017 may be an opportune year for attempting and initial phase of *Aphanius* re-introduction into Akrotiri Marsh. Due to a long drought and the effects of reed-bed fire *Gambusia* numbers may be extremely small and/or locally extirpated. These conditions may be optimal for *Aphanius* re-establishment. The campaign is relatively simple: large numbers of *Aphanius* (of both sexes) must be transferred from the dense thriving populations in the quarry pit ponds of Episkopi Bay Wetland in Spring. Results must be monitored throughout 2017. A *Gambusia* anti-stocking information campaign is immediately required.

The following restoration goals identified in this study are scored based on priority and urgency (as: moderate => Important=>very important=>urgent; in that order of significance)

- Develop Annual Action plan and personnel training for specific actions, data gathering and monitoring in 2017. PRIORITY: URGENT
- Take steps to re-establish at a refugium at Akrotiri Marsh in 2017. PRIORITY: URGENT.
- Monitor the ichthyfauna of newly dug canals and "ponds" within Akrotiri Marsh.
- Provide hydrological connections among Episkopi Bay Wetland-Akrotiri Marsh-Salt Lake lagoonal basin. PRIORITY: IMPORTANT.
- Combat *Gambusia holbrooki* stocking/dispersal and survival (especially in freshwater parts of the system (Akrotiri Marsh, Zakaki, North shore of Salt Lake). PRIORITY: URGENT
- Combat predatory fishes that have been introduced into Quarry pit ponds of Episkopi Bay wetlands. PRIORITY: URGENT

- Explore food web implications of increasing *Aphanius* populations. i.e. Set up natural experiments/adaptive management frameworks etc. PRIORITY: MODERATE

6C Educational, promotional prospects and the aquarium trade

As is made clear from conservation requirements for a poorly-appreciated fish, it is important nowadays to respect societal needs and the interests of local stakeholders and institutions.

We feel there has been a recent rising interest in *Aphanius* and much of this owes interest to efforts by the Akrotiri Environmental Education Center (were the species is also displayed in captivity). However, we feel it is still not effectively promoted as a flagship. A cyberspace search engine screening survey does show a moderate amount of interest in the species at Akrotiri, considering that it is a rather cryptic aquatic denizen (Table 6).

Killifish are important for education and sensitization of the public and local and wider society at Akrotiri for the following reasons, among others:

- The species detracts from a focus on just birds or typical wetland attractions; it enriches a more integrative wetland learning. Wetlands need understanding of ecosystem and landscape (drainage basin) water needs; the fish is a good indicator and is threatened by both human-induced changes, meteorological and climate issues and the simple harshness of Mediterranean wetland conditions. Its "story" of contraction -expansion related to dispersal provides a rich educational and conceptual platform.

-The waters of the wetlands, including the history of wetland ecosystem change, both at geological and recent time scales is interesting and important since knowledge is poorly recorded and often in "incomplete" form. Fish again provide a re-focus on poorly explored and poorly researched issues. Fish will assist in providing more investigative approaches to management.

-Killifish feed on mosquitoes and other dipterans (flies and midges). They provide an important ecosystem service. The mosquito biocontrol issue is scientifically justified and can be of use in education and as an adaptive management procedure. The issue can also dispel some of the myths of "ecosystem disservices" given to wetlands.

-Killifish have fierce alien invasive competitors; the *Gambusia*. The problem of continually stocking Mosquitofish is a serious ecosystem-altering issue on Cyprus. The Killifish again provides an educational platform and a symbol of one of the most harmful invasive alien species.

-Killifish are colourful and beautiful species that belong to one of Eurasia's most threatened fish groups, the Cypridontiformes. The issue of extinction, extirpation and restoration can be showcased through this representative of one of Eurasia's most threatened fish groups.

Species with name "Akrotiri"	Description	Google Hits
"Caretta caretta" Akrotiri	Sea turtle	34,200
"Monachus monachus" Akrotiri	Mediterranean Monk Seal	10,300
"Ophrys" Akrotiri	Genus of ground orchids	2,510
"Phoenicopterus roseus" Akrotiri	Flamingo	2,490
"Falco vespertinus" Akrotir	Threatened Red-footed falcon	879
"Aythya nyroca" Akrotiri	Threatened duck species	787
"Phanourios minutus" Akrotiri	Extinct pygmy hippo	323
"Macrovipera lebetina" Akrotiri	Widespread viper species	286
"Aphanius fasciatus" Akrotiri	!!	257
"Hyla savignyi" Akrotiri	Widespread tree frog species	217
"Hemiechinus auritus" Akrotiri	Common and widespread hedgehog	178
"Cladium mariscus" Akrotiri	Rare and threatened saw-sedge	145
"Percnon gibbosus" Akrotiri	Invasive and now abundant marine crab	3

Table 6: How well known is *Aphanius* at Akrotiri? A quick Google search-engine survey using scientific names provides a comparison to other supposedly higher or lower profile plant and animal species. Scientific names are used for consistency; similar surveys can be done using common names in various languages. (Screened on February 20th 2017).

Fig. 9. Logo of the Killifish Conservation Project, Malta. Killifish can be used as flagships (or symbolic mascots) to spearhead aquatic and wetland biodiversity conservation.

Finally, *Aphanius fasciatus* is a rather attractive fish for aquarium displays. Several members of the public have collected fishes at Akrotiri and they have shown the fishes on websites. Since it is a protected species its collection and reproduction in captivity must be discouraged since this may lead to unwanted or poorly planned introductions (or failed introductions). Also, poorly monitored reproduction in captivity may lead to genetic bottlenecks and other genetic disturbances in captive sub-populations. Any display of the fishes in public aquaria should note that the species is protected by law and its conservation is a priority.

7. Draft monitoring scheme: initial foundations

Monitoring is critical for gaining knowledge of ecosystem structure and functioning in a large and dynamic wetland-coastal system such as Akrotiri Wetlands. It is also vital for adaptive management and effective conservation of aquatic and semi-aquatic systems where efforts are being implemented to protect and enhance biodiversity values. Changes are taking place and are driven by a multitude of natural and human-induce factors both in the Akrotiri Marsh and the surrounding wetland area. Surveillance and understanding of the impacts of these changes must be organized and streamlined for long-term biodiversity conservation and Protected Areas sustainability.

In order to monitor for the *Aphanius* population, work must obviously be undertaken in the wider Akrotiri Wetland area and a monitoring plan should be organized based on specific research conservation aims and restoration initiatives. In lieu of a complete monitoring plan an Action Plan may be developed to accompany restoration initiatives and within the Action Plan specific monitoring actions can be set and developed.

The structure for such a scheme requires the development of specific conservation targets and participation by key stakeholders. During the January 2017 meeting among stakeholders and institutions working in the Darwin project “Akrotiri Marsh Restoration: a flagship wetland in the Cyprus SBAs” the following relevant items were expressed as a result of this study:

a) Akrotiri Marsh is immediately connected and a part of the Akrotiri Wetlands and any effort to monitor fishes there requires coordination with a larger monitoring effort.

b) Base-line knowledge of many hydrobiological / hydrobiological issues is lacking at Akrotiri Wetlands. It is evident in the recent literature (and grey literature) that even baseline attributes of the structure and workings of the ecosystem (or constituent ecosystem units) are poorly described. In this respect, the author of this study feels that focus should be aimed at completing baseline studies first and developing/refining a monitoring scheme within an adaptive process (i.e. investigative and adaptive monitoring). In fact, part of this study provided a springboard for investigative monitoring and much was learnt in the process.

c) Fish are very important key-stone species and may alter and affect limnology and wetland ecology. They must be taken seriously in any relevant conservation, restoration and monitoring effort; this has never before been attempted and shows a serious neglect on the part of previous research in the area (as explained in the Annotated checklist of Annex B, this neglect is of course unintentional and is commonly a problem associated with a "shifting baseline syndrome", i.e. fish were thought not to exist...).

It is not possible in this study to draft a final monitoring plan for *Aphanius fasciatus* and this should not be done without the planning and prospects of a long-term management or special conservation-orientated plan. We therefore propose a new study within a restoration-centered action plan that must focus on the fishes of Akrotiri Wetlands and provide a monitoring scheme both within the targeted action area (Akrotiri Marsh) and for the entire site. Such developments may be done within increments and within the collaborative arena of academic institutions.

We will however provide elements of the main issues that must be investigated, surveyed and monitored. The key aims for further investigation and monitoring of fishes at Akrotiri Wetlands should include at least the following:

a) Distribution and relative population density of *Aphanius* and *Gambusia* (spatio-temporal monitoring with close regard to habitat and physico-chemical parameters).

b) Presence of other fishes especially natives and predatory species and their impact on *Aphanius*.

c) Regular snap shots of hydro-morphological, hydrological and habitat conditions in all major hydrobiological units of the Akrotiri Wetlands.

d) Water physico-chemical conditions must include important attributes among others such as water depth, water flow, electrical conductivity, salinity, pH, turbidity. The monitoring must provide the ability to show seasonal and inter-annual patterns of flow, depth, desiccation date, re-flooding dates, water movement processes, marine connections, run-off inputs and outputs in the entire Akrotiri Wetlands system.

e) Ability to warn and signal problems (i.e. drastic population losses or human-induced problems).

Basics for fish monitoring

A. For small sized fishes such as *Aphanius, Gambusia*, Mugilids, Atherinids, it is possible to consider a rather simple monitoring scheme based on the following procedures:

-A simplified survey protocol will follow what has been practiced during this study. It is vital to combine the use of both sturdy dip nets with a small seine net as basic sampling tools. Physico-chemical parameters that are important are salinity(‰), electrical conductivity, and temperature and water depth, flow (and flow direction) and substrate/vegetation characteristics.

-About 30 sites should be monitored at least three times per year for *Aphanius* (wet season winter, late spring, and dry late summer-autumn) throughout the Akrotiri Wetlands.

-Investigative surveys and monitoring should also focus on any new sites where the species has been introduced/re-introduced (or were metapopulations of *Gambusia* exist, such as Fasouri and near the Lemesos port area ditches).

-Monitoring should be reviewed and over-seen by and expert or expert professional body (academic institute or other).

B. For Eels

- Traps must be used for a wide-ranging survey. Eel traps are available for this and are widely used in the UK.

C. For other larger fishes (Predatory fishes in the Gravel Pits)

-Standing gill-nets must be used (typically used by gill-net fishermen in marine waters in Cyprus). The fishes being caught will range from 15 cm to 35 cm. This entails and operation requiring a small boat and careful casting in order not to have dumped obstacles stuck in the net or to damage the net.

8. Summary of major findings

Field research has begun based on surveys using dip-nets and seine nets throughout Akrotiri Marsh and the wider area of Akrotiri Peninsula. Work done in December 2015 (before contract), in February, May, June 2016 and January 2017 is indicative of the current situation. During these visits the work crew established the current distribution throughout the Akrotiri Wetlands and the conditions within Akrotiri Marsh. Major findings are outlined here.

Distribution

- This is the first time a scientifically documented descriptive study of the species distribution patterns in Akrotiri Wetlands has been completed. Former published knowledge or data on distribution and status of *Aphanius* is scant and much of the knowledge has a high level of uncertainty or lacks scientific field sampling.

- Little is apparently known about the past status of the species at Akrotiri. The few elders interviewed at Akrotiri mentioned the existence of "small fish" in Akrotiri Marsh (they mentioned what they call "Atherina" as well). Atherina could refer both to sand-smelt (*Atherina* sp.) which still enters parts of Akrotiri Wetland from the sea (after storms) or could be a catch-all term for any small "bait-fish" species that may include *Aphanius*. No local or colloquial name for *Aphanius* was recorded in the few interviews conducted. The colloquial/vernacular Greek names (Zacharias, Zabarola) are not used at Akrotiri. In Cyprus, during the last few years the name Aphanius (Αφάνιος) is commonly used among scientists, educators, naturalists and various stakeholders. Historical research on the species in Cyprus has not been adequately explored; and the present study certainly does not provide a thorough historical survey.

Taxonomy and Provenance

- Even the provenance of the species has been questioned since so many fishes have been introduced to the island for various uses or unintentionally by humans. Some have asked if this species is native or not to Cyprus (although most scientists consider it native). The species' extant populations on Cyprus are disjunct and inhabit only the largest areas of brackish water wetlands where lagoonal water bodies are present (natural and artificial). Currently *Aphanius fasciatus* is known to be found only in three locations on the island: Akrotiri, Famagusta and Morphou Bay. The species is absent from potentially suitable habitats in many other coastal wetlands (e.g. Larnaka wetlands, Oroklini Lake and several small river delta wetlands which provide presumably suitable habitats). The scientists of this study feel the species absence is probably due to human-induced extirpation since many of these wetlands have suffered from both water abstraction and desiccation, severe drought effects, and former DDT poisoning campaigns (till about 1978). The smaller the wetland, the greater the potential for extirpation; this may be the reason the species has survived only in the island's largest wetlands and at sites where definitive lagoonal waters exist during even the most extreme drought.

- Recent genetic work (by the HCMR team and collaborators, unpublished) shows that the *Aphanius* of Cyprus belongs to the typical *Aphanius fasciatus* taxon; it shows very little genetic

differentiation among central Mediterranean populations (but is clearly distinct from Aegean populations). It is probably most closely related to the fishes found in southern Anatolia. Morphologically the specimens from Cyprus show typical Mediterranean *Aphanius fasciatus* characteristics (but again they differ from Aegean populations). The initial screening barcoding analyses provides evidence to an ecoregional connection to the Southern Anatolian basins and this supports evidence that the populations are native to Cyprus. Further work is needed on the conservation genetics of the Cypriot populations. It is important to trace where and when the species first colonized the island; one hypothesis is during the Messinian Salinity Crisis (5.9 to 5.3 million years ago) when the island was periodically connected/ or very close to the opposite mainland coasts. Grounds for human-assisted introduction to Cyprus are further considered unlikely due to geo-political isolation of Cyprus from Southern Anatolia. Furthermore there is no reason or potential for *Aphanius fasciatus* to have been brought from southern Turkey (especially after the British controlled the island in the late 19th Century). Furthermore, British and Israeli anti-malarial teams in the Middle East have not to our knowledge used *Aphanius fasciatus* for mosquito control but they have used a closely related widespread Middle Eastern Arabian killifish, *Aphanius dispar*, as an effective anti-mosquito fish in the Middle East and Africa. But *Aphanius dispar* has never been found in Cyprus so there is little reasoning in relating to *Aphanius fasciatus* as a potential introduction by anti-malarial campaigns. In Israel, introduced *Aphanius dispar* and it has hybridized and displaced native *Aphanius fasciatus* along the coast.

Habitat and dispersal

- *Aphanius* inhabits similar habitats as it does in other Southern European states and islands at Akrotiri. It can survive in fresh and slightly brackish waters (and can thrive in saline and hypersaline conditions), and it does show mass death/fish kill events after waters desiccate in summer. Refugia are critically important for summer survival as elsewhere in Mediterranean lagoon systems.

- *Aphanius* is not a migratory fish, but it will disperse beyond its resident areas (presumably when swept by water flow (storm, tidal and run-off waters) as observed at Akrotiri. This study has shown that at the Akrotiri Wetlands *Aphanius* has a "contraction-expansion" distributional pattern depending on meteorological conditions and drought effects on the local populations. Fish survive the long drought and desiccation in refugia (traditionally at Zakaki, Akrotiri Marsh/Livadi and particularly at the Episkopi Bay Wetlands). After the winter rains and marine storms raise water levels at Episkopi Bay Wetland and in the Salt Lake, fish disperse to the Salt Lake and enter various marshy areas along the shores of the Lake. By late spring they are breeding and locally abundant, but especially so in saline-brackish and saline waters where there is no competition with alien *Gambusia holbrooki*. Due to increased freshwater flow at Zakaki, there is circumstantial evidence that the Aphanius are no longer able to penetrate into this long-lasting aquatic refugium since they are displaced by *Gambusia*. *Aphanius* will survive in freshwater refugia during summer (and they have been abundant in Akrotiri Marsh in the recent past). In the summer of 2016 Akrotiri Marsh retained water but there was a local super-abundance of *Gambusia* (not a single *Aphanius*).

- Based on expert knowledge of the habitat requirements and dispersion pathways at Akrotiri Wetlands, the potential range of the species was estimated for the first time in this study. The potential distributional range of *Aphanius* included nearly the entire Akrotiri Wetlands (and former wetland area) from the coastal marshes south of the Kouris river-mouth to Lemesos harbour, originally near the Garylis river mouth. The species is no longer present in the free-areas south of the dividing line of the Republic of Cyprus. This report, identified a negative impact from the water drainage works at Zakaki against the *Aphanius* population for the first time.

- In recent years refugial population centers have been documented at Akrotiri (i.e. areas where the population survives during drought). These are: A) in the salt-marsh and artificial quarry pit areas of Episkopi Bay Wetland (formerly including Akrotiri Marsh as well); and B) the Eastern part of the Salt Lake shores, primarily the salt marshes and brackish reedswamp ponds in and around Zakaki Marsh (which also hold some water during most summers). The Salt Lake basin completely dries during summer-autumn (July to October) and during this period all fishes trapped in remaining hot hypersaline waters die. Fish-kills in such conditions, involving *Aphanius*, have also been reported in analogous salina-like lagoon systems in the Mediterranean and have also been observed in Famagusta (in the Pediaios Delta and saline lagoons; see Zogaris (Internet sources, 2013)). Mass deaths have been observed in Salt Lake as well (P. Charilaou, pers. com). The Salt Lake is a dispersal and nursery area for the fishes but they need surrounding refugia to survive. In a series of drought years *Aphanius* may be confined to very limited areas of the Akrotiri Wetland (such as during 2016). *Aphanius* also need dispersal pathways (connectivity among wetland areas).

- Based on surveys between 2008 and 2015 by the author (and confirmed by independent investigations by collaborator Chris Englezou) the species' range has recently significantly contracted in the wider area of Akrotiri. However, periodic contraction-expansion phases for *Aphanius* populations are typical in Mediterranean wetlands and this is principally affected by seasonal drought phases. Severe or prolonged drought could create local extirpation for some years (i.e. during the last three years). Drought negatively affects summer survival and re-colonization. However there is evidence to support that during the last few years important human-induced changes have taken place that may make the *Aphanius* population vulnerable and may limit future dispersal ability (i.e. changes in hydrological conditions due to the new Lemesos storm water works at Zakaki Marsh). Long term study and monitoring is needed to explore dispersal mechanisms and the influence of multiple stresses such as competition with *Gambusia*.

- Two anthropogenic changes may affect the area's *Aphanius* refugia in the near future: A) Important changes have taken place within the Akrotiri Marsh during the last few years. Since 2004 when water levels where artificially elevated the Marsh is in a drying phase. Fire has recently burned areas of the marsh and this has locally affected conditions in the aquatic environment as well (temporary chemical pollution from ash-fall in stagnant waters may alter conditions). Severe drought makes fish re-colonization difficult. The result is that most of the surveyed areas of the marsh had no fish present (although the competitive *Gambusia holbrooki* did survive until the Summer of 2016 in unburned deeper parts of the marsh and

along parts of the major canal connecting the marsh with the Salt Lake (Enetiki Tafros). B) The *Aphanius* population in the eastern part of the Salt Lake basin and specifically within the Zakaki Marsh area has to our knowledge "recently collapsed". Last documented evidence for *Aphanius* in the area was during 2012 (however it is possible that the fishes survived in this area in more recent times in severely reduced numbers). After a series of water work engineering changes took place this resulted in an increase in freshwater run-off entering the former salt marsh (and flowing towards the Salt Lake basin). *Gambusia* are now found in super-abundant densities in all investigated sites since conditions with lowered salinity favour this invasive species. This anthropogenic change in hydrology may affect *Aphanius* dispersal and may block its access to brackish waters where *Gambusia* thrives.

- During the last few years *Aphanius* has not been recorded in Akrotiri Marsh. This may be primarily due to an infestation of *Gambusia holbrooki*. Proposals and specific steps are outlined to promote the re-establishment of the species in Akrotiri Marsh.

- In winter *Gambusia* numbers and densities drastically drop; they rapidly increase in freshwaters during the spring-summer to the point of super-abundant densities in areas that are fresh-to-slightly brackish (areas influenced by freshwater inputs). These super abundant densities were observed in 2016 within Akrotiri Marsh, Zakaki Marsh and Zakaki Canal (the canal adjoining Zakaki Marsh with the Salt Lake). Due to increased salinity during late Spring *Gambusia* does not survive in remaining waters within the Salt Lake or at the shallow pools behind Lady's Mile.

- This study proves for the first time that the Episkopi Bay Wetland (specifically about half a dozen gravel pits) are of outstanding value as refuges for *Aphanius fasciatus* since they maintain water throughout the year, even in periods of extreme drought. These are anthropogenic habitats but they do mimic wave-scoured storm depression pits or deeper dune slack ponds that may have existed on this storm-swept coast. And they hold a very interesting biodiversity that has been poorly explored/documented.

- *Aphanius* populations in the Episkopi Bay Wetland (quarry pits) vary in numbers depending on particular pool/pond. In some pools they are in good and moderate condition, particularly since conditions are saline and/or brackish and no *Gambusia* establish in these areas. The Episkopi Bay Wetland populations are currently the sole source for re-colonization of the entire Akrotiri wetland complex (this occurred in just two years of drought and with changing conditions at Zakaki). Akrotiri Environmental Education Center officers have made efforts to re-stock or stock small pools in this area - and these are good and simple stocking efforts. These simple actions mimic natural dispersal phenomena since they are from "nearest-neighbor" water bodies and fairly large numbers are introduced/re-introduced (so no bottle neck is formed). This management practice may continue, it poses no harm to *Aphanius* and there is some evidence that it may provide a bio-control pressure on mosquito populations as well. However, at Ai Yorki and especially at Paraga Pond the *Aphanius* populations is "unhealthy" and particularly depressed, probably primarily due to predation; and this is a serious issue that needs further investigation (see below).

- A new threat to *Aphanius* populations has been identified in the Episkopi Bay quarry pits during this project: The anthropogenic introduction of predatory marine fishes (notably sea bass *Dicentrarchus labrax*). *Aphanius* population and age-group dynamics were unusually skewed within the two larger quarry pits in both summer and winter observations/samplings at Ai Yorki Pond and Paraga Pond. In fact, in contrast to conditions documented in Paraga Pond in 2011 and 2012 (Zogaris et al. 2012b), *Aphanius* was extremely scarce in spring and summer 2016 (and only a few juvenile fishes were collected!). Anecdotal evidence points to the introduction of Sea Bass and other fishes for un-regulated fish stocking / fishing purposes by a local resident who is keen to stock these fishes for private consumption from a nearby fish farming unit. Further investigation into this threat must be made as soon as possible through a rigorous sampling/monitoring project through the use of gill-nets to investigate the predatory fishes of these two deep ponds. If the predatory fishes are present they must "fished-out" as soon as possible. The issue is significant since the introduction of sea bass to more quarry-pitponds would mean an over-all crippling of the species' reproductive success at the Akrotiri Wetland as a whole. This totally unforeseen pressure and threat on *Aphanius* gives further urgency to the issue of a special study and action plan development.

Management

- The Akrotiri Basin and aquifer geology, hydrological budget, and water management scenarios and conditions have been studied extensively but little regard has been given to the aquatic biota and particularly wholly aquatic species such as fishes. More integrative and transdisciplinary research and monitoring is called for in order to water management and conservation planning to become more integrated and effective. As the complexity of water management, mosquito control, aquatic biodiversity restoration and management become apparent, a serious foundation for policy-relevant research, knowledge management and decision-making must be developed. Specific proposals are made on behalf of fishes for the first time in this study.

- One study (A.P. Marine 2012) has posed a management question concerning a potential "threat" by the hypothetical increase of *Aphanius* in the Salt Lake with regards to the *Phallocryptus spinosa* fairy shrimp food web, which involves the Greater Flamingo and other planktivorous birds populations. Adult *Aphanius* probably will and do feed on these shrimp and strong competitive interactions between fish and flamingos could exist if the Salt Lake did not dry out completely. Current knowledge of the *Aphanius* distribution and potential distribution suggests that there is probably no such threat. This is proven by new knowledge concerning the *Aphanius* timing and mode of dispersal towards the Salt Lake (a saline lagoonal basin that always is desiccated during summer). Available evidence shows that *Aphanius* move slowly into the Salt Lake primarily after winter flooding of their peripheral refugia perhaps in mid or late winter, usually many weeks after the hatching of most of the *Phallocryptus*. Since *Aphanius* begin spawning in March/April their populations develop only much later in late spring (when they become apparent due to a rapid lowering of water levels in the Salt Lake, as well). All searches for *Aphanius* in the early and mid part of the last three winters (Dec-Jan) have shown no results in the Salt Lake (confirming that the trickle of dispersing fish from the refugia is very slow and densities in the Salt Lake are usually extremely low in winter). The

observed pattern corroborates the expansion-contraction hypothesis of the species distributional pattern at Akrotiri Wetlands (expansion takes place as fish drift from the refugia to the Salt Lake only late in winter after water levels rise, and they breed late, perhaps beginning in March). In our opinion, the timing and infinitesimal low density of *Aphanius* arrival in the Salt Lake in winter which usually hosts booming populations of *Phallocryptus* could not cause a tipping-point effect on the food web. Even if *Aphanius* numbers are restored by human stocking to more refugia sites (peripheral year-round wet areas), their gradual drift towards the Salt Lake cannot conceivably create a problem for the shrimp or planktivorous birds (i.e. food source competition with the wintering Greater Flamingo). By mid spring most planktivorous birds should have left. Since remarkable variability in hydrology and species-movement connectivity exists, monitoring is essential for long-term understanding of food-web structures and key-stone species influences.

- The SBA authorities are keen to used *Aphanius* as a mosquito biocontrol and this is scientifically justified. This study proposes the continuation of stocking based on transplanting populations in the Episkopi Bay Wetland and in Akrotiri Marsh; this should continue in 2017. However, a rigorous monitoring project is required. The project requires, hypothesis-led research questions, and training and quality control aspects in protocol development, data gathering and data management. Efforts for biocontrol must be combined with the requirement of exterminating *Gambusia holbrooki* (in order to replace them with *Aphanius*) at Akrotiri Marsh.

- 2017 may be an opportune year for attempting and initial phase of *Aphanius* re-introduction into Akrotiri Marsh. Due to a long drought and the effects of reed-bed fire *Gambusia* numbers may be extremely small and/or locally extirpated. These conditions may be optimal for *Aphanius* re-establishment. The campaign is relatively simple: large numbers of Aphanius (of both sexes) must be transferred from the dense thriving populations in the quarry pit ponds of Episkopi Bay Wetland to Akrotiri Marsh. Results must be monitored throughout 2017.

The use and attractiveness of the species for aquarists

- *Aphanius fasciatus* is an attractive fish for amateur aquarists and hobbyists however it is rarely kept and not traded. Several members of the public have collected fishes at Akrotiri. Since it is a protected species its collection and reproduction in captivity must be discouraged since this may lead to unwanted or poorly planned re-introductions (or failed introductions). Also, poorly monitored reproduction in captivity may lead to genetic bottlenecks and other genetic disturbances in captive sub-populations.

Environmental education and promotion

- Evidence is provided that based on the contributor's expert opinion that *Aphanius* should be used as a flagship to promote wetland conservation research, education, sensitization and awareness. Promoting a more integrated hydrobiological and mosquito control investigation is important for wetland management at Akrotiri and *Aphanius* is a good mascot for this.

Other Ichthyological data gained from this project

- This study provides the first ichthyofaunal list for the Inland waters of Akrotiri SBA (See Annex B). At least 10 species of fishes are recorded within the inland waters of Akrotiri. The list is still unpublished and has some uncertainties that need attention (introduced marine fish populations etc.). The study added one new fish species the inland waters fauna of Cyprus: the common marine goby *Gobius cobitis* - discovered in Paraga Pond in Episkopi Bay wetlands on June 20th 2016 (and then again in January 2017). *Gobius cobitis* has been observed in inland transitional waters in small coastal lagoons in Greece and elsewhere in the Mediterranean (Zogaris pers obs.).

Fig 10. Compilation of photos from field work (Summer 2016). **A**. *Gobius cobitis* a new species of marine goby found for the first time in inland waters in Cyprus at Paraga Pond (June 2016). B. Young-of-the-year *Aphanius fasciatus* at Episkopi Bay Wetland in June 2016 (shown here with a marine gastropod that inhabits Paraga Pond). C. Seine net fishing at Paraga Pond, one of the largest quarry pits ponds at Episkopi Bay Wetland. D. Female *Gambusia holbrooki* at Akrotiri Marsh ("Livadi Pinakida" site; the only recorded fish species in Akrotiri Marsh during the project.

Fig 11. Compilation of photos from field work (2016). **A**. "Alyki_Canal" site in the north west part of the Salt Lake where the Ennetiko Canal enters the basin from Akrotiri Marsh (no fish recorded here on 20.05.16). **B.** "Red Canal" site in Akrotiri Marsh on 20.05.16 (the reed-bed was regenerating after a burn in March 2016). **C.** "Limni_Ai_Yorki" site at Episkopi Bay Wetland, the largest and deepest quarry pit (19.05.16). **D.** Ennetiko Canal expels very little water from Akrotiri Marsh during the midst of a draught year (*Phragmites* clogs channel on 22.02.16). "Louki" site, southernmost distribution of *Aphanius* at Episkopi Bay Wetland (19.05.16). **F.** *Gambusia holbrooki* super-abundance at "Livadi Pinakida" site (20.05.16). **G.** *Aphanius* male with deformity/injury on pectoral fin at "Ai_Yorki_rivulet" site (19.05.16). **H.** Dense populations of *Aphanius* at "Ai Yorkis Casual" (19.05.16) in shallow pools among salt marshes that were drying rapidly.

Fig 12. Compilation of photos from field work (2017). **A**. "Livadi PinakidaW" with new flooding after a long drought (no fish here on 15.01.2017). **B.** Seine net sampling at "Alyki South" in the recently filled Salt Lake basin, 15.01.2017 (rains and storms were late in coming in winter 2016/2017). **C.** Sheets of clear shallow water cover huge expanses north of "Paraga PondW", 12.01.17. **D.** "North Inflow" is a site where the new Lemesos storm water discharge has been created in the northeast shores of the Salt Lake; waters were fresh with breeding green toads *Bufotes viridis* here on 14.01.17. **E.** *Atherina boyeri* and a few *Aphanius* at "Paraga PondW" site on 12.01.17. The *Atherina* presumably entered from storm water surges over the Episkopi Bay beach. **F.** "Dasonomio1" site is a potential refugium where fresh irrigation waters may enter the northern shores of the Salt Lake south of Asomatos village; *Phragmites* and *Acacia* in recently flooded runoff waters (14.01.17). **G.** Eel at "Zakaki Overflow Cistern" (14 & 15.01.17). **H.** "Zakaki Inflow" where the Lemesos new storm water discharge connects with Zakaki Marsh.

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Acknowledgements

Much of this work was a cooperative venture with the key contributors being: Vassiliki Vlami (PhD Cand. Univ. Patras), Varnavas Michael (SBA Akrotiri), Chris Englezou (Freshwater Life Project, UK), Matt Ford (Freshwater Life Project, Spain), Angeliki F. Martinou (Joint Services Health Unit, BFC RAF Akrotiri), Melpo Apostolidou (BirdLife Cyprus) and Pantelis Charilaou (SBA Akrotiri).

The Principle Investigator wishes to thank the members of the staff of the Akrotiri Environmental Education Centre and especially Melpo Apostolidou from BirdLife Cyprus for all assistance. We are especially indebted to Pantelis Charilaou for efforts made to provide help and coordination of field research. For research information and advice on *Aphanius fasciatus* we thank the following experts: Brian Zimmerman (ZSL, London), Jὸrg Freyhof (Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Germany), Eleni Kalogianni (HCMR, Athens) and Roberta Barbieri (HCMR, Athens). Work with Maarten Vanhove (Belgium) has also helped to reveal much about the conservation genetics of the Cypriot *Aphanius* populations and their parasites as well (ongoing research). Genetic barcoding was undertaken on Cypriot specimens by Jὸrg Freyhof for the first time in 2016. George Minos and his associates provided taxonomic confirmation (and genetic screening) of the *Atherina boyeri* from the Episkopi Bay Wetland for the first time in 2017. In Cyprus we thank members of Department of Fisheries and Marine Research (DFMR) for assistance on ichthyological/limnological research at Akrotiri and especially Savvas Michaelides for his contributions. We thank Athina Papatheodoulou, Gerald Dὸrflinger, Kostas Aristeidou and Iakovos Tzortzis for advice and assistance as well.

Annex A.

Results of the sampling surveys (location maps)

(Note: Yellow sites are "summer" samples; blue are "winter" samples; site names given as on summary of sampling results list; Table 4, p.20)


Zakaki Canal Alyki2
Lady's Mile Pool
Zakaki Canal Alyki
Dasonomio2
Dasonomio1
Akrotiri Salt Lake
&
Lady's Mile
Alyki_canal
Alyki South
Image © 2016 DigitalGlobe
Google Earth


Zakaki Marsh
Zakaki Inflow
Zakaki Overflow CisternS
Zakaki Overflow Cistern
Near Zakaki Hide
Zakaki Hide
North Inflow
Zakaki Outflow
Image © 2016 DigitalGlobe
Google Earth

Annex B.

A preliminary annotated Ichthyofauna list of Akrotiri Wetlands

The ichthyofauna of Akrotiri Wetlands has never before been scientifically documented through field sampling and historical surveys. Although this rapid survey is not exhaustive it provides evidence that the Akrotiri Wetlands host at least 10 fish species. There is a serious lack of "completeness" in the knowledge baselines concerning fishes in the Akrotiri Wetlands. The lack of scientific reference to fishes in the literature represents a "shifting baseline syndrome" among recent researchers who have unintentionally neglected the "presence" of fishes both in recent and current times in the wetlands. More research is needed both to explore the reference conditions, the assemblage ecology and hydrology-biology of the constituent systems of the Akrotiri Wetlands and finally to plan for adequate conservation/restoration approaches required with respect to the ichthyofauna[13].

Interviews with locals and experts

During the study period (2016-early 2017) short discussions were made where knowledge of fishes was discussed in unstructured interviews. Key individuals in these interviews are: 1) Mr. Haris (Owner of Polis Tavern), 2) Mr. Thomas Hatzikyriakou(AEEC), 3) Christodoulos Makris (Lemesos), 4) Mrs Ifigenia (the basket weaver, Akrotiri resident), 5) Mrs Ifigenia's friend (Akrotiri resident), 6) Mrs Varvara (AEEC), 7) Varnavas Michael (AEEC), 8) Pantelis Charilaou (AEEC), 9) Neophytos (Health Services of SBA), 9) Kostas Aristeidou (WDD), 10) Savas Michaelides (DFMR).

Preliminary Annotated ichthyofaunal List

1. ***Anguilla anguilla*** / European Eel (Native; Former catadromous migrant from marine waters; now probably scarce; poorly studied species)

There is very little reference to eels within the Akrotiri Wetlands in recent times (Zogaris 2012a). However eels are known to inhabit or have been fished and observed in the Old Harbour of Lemesos where the Garyllis river has its rivermouth and eels were common in the Kouris river in the past (interviews during WDD projects by S. Zogaris 2012a, 2012b). Eels enter freshwaters usually when they are attracted by a freshwater plume that pushes out to marine waters in winter through spring; the young glass eels /elvers swim up these river mouths during this time. Because of this situation - since the Salt Lake no longer has a regular outlet to the sea, one would not imagine eels easily entering the Salt Lake. However there are other storm-water and run-off outfalls that may connect with the wetlands surrounding the Salt Lake. Eels are important indicators of natural connectivity among wetlands.

Most young locals that were interviewed did not have recent experience with eels; one or two mentioned that very long ago they were aware that Eels did inhabit "Livadi" (Akrotiri Marsh) and the canals in Fasouri. The older generation, interviewees (#1, #4, #5) who are older than 65 years old, however are very much aware of eels and their culinary values. They speak of

[13] As of writing, in February 2017, the data gathered and interpretations represent the opinion of the Principle Investigator (S. Zogaris). Knowledge and understanding will change as more information is acquired; the gaps in knowledge are still significant and they are showcased in this report. Of the ten taxa featured here there is only anecdotal evidence for three of them (their names in brackets [ ]). It is possible that more species may be found (at least two mugilid species, for example) if efforts are made to study and monitor fishes in the Akrotiri Wetlands.

eels with fondness and nostalgia. Eels once inhabited and may have been seasonally abundant (or at least regular) in Livadi-Akrotiri Marsh (and older residents know that the eels were there since their parents collected them in the irrigation ditches). The ditches were watered from abstraction of waters from the Kouris - so it is possible these eels migrated this way to Phasouri and Livadi-Akrotiri Marsh (i.e. hypothesized route: Kouris river-mouth - abstracted waters - irrigation ditches- Akrotiri Marsh- Salt Lake-Zakaki-Marine waters). It was made quite clear that eels were common and much sought after. They were cooked fried (skinned first) and grilled or over open fire (with skin on). Interestingly, one interviewee (#1), mentioned that in the Livadi-Akrotiri Marsh drianage ditches there use to be both crabs and turtles and tiny little fishes as well. After the Kouris surface waters were withdrawn (and perhaps due to DDT spraying in the past) after the building of the Kouris Dam, all of this vanished.

On 14.01.2016 a single adult silver eel (large enough to be ready to move back to marine waters for the long migration to reproductive grounds) was recorded and photographed in the overflow cistern at Zakaki, "Zakaki Overflow Cistern" site (S. Zogaris, G. Dòrflinger). The specimen was approximately 60 cm. and photographed (see fig. 12G). The eel was seen again the next morning at the same position as well. This fish has survived in inland waters for at least 6 to 8 years (i.e. before the new storm water construction of the Water Board of Lemesos was created). One hypothesis is that there is a flow of water connecting Zakaki with the sea - even thorough artificial sewers and ditches- and some elvers swim up during winter flooding to reach Zakaki Marsh. The large eels head out to sea often during full moon phases and stormy humid weather - conditions at the time of this observation. This observation is the first recent record of the species in Akrotiri Wetlands. Since the species is assessed as **IUCN Critical** it is important to explore the status of the population in the protected area.

2. ***Aphanius fasciatus*** / Mediterranean Killifish (Native resident)

Recent studies (unpublished, outlined in the present report) show that this is a native species on Cyprus. The Akrotiri Wetlands hold Cyprus's largest known and most secure population (the others being in the fragmented and degraded wetland of the Pediaios river delta at Ammochostos/Famagusta and near Syrianochori on Morphou Bay (both sites in north of the dividing line of Cyprus). In recent years (after 2012) the population at Akrotiri Wetlands has declined and receded to a single refuge area, the Episkopi Bay Wetland during summer; however, in winter the species probably usually disperses to nearly the entire Akrotiri Salt Lake basin (in most years). After the Salt Lake's summer desiccation most fish undergo a mass deaths. Fishes seam to re-colonize the Salt Lake from Episkopi Bay Wetland via ditches draining to the Lake. In recent years (2015-17) the species has not been recorded in Akrotiri Marsh (Livadi) or Zakaki Marsh (Markia Lake) which hold fresh-to-slightly brackish waters in spring and polysaline waters during most summers; these refugia formerly sustained the species in abundance during summer. Circumstantial evidence points to the potential negative effect of interspecies competition between *Aphanius* and *Gambusia holbrooki* in freshwater/slightly brackish water areas of the system (i.e waters holding <10‰). Since some of these seasonally slightly brackish-to-brackish waters are dominated by a super-abundance of *Gambusia holbrooki* where once both species existed; it is hypothesized that salinity plays a primarily role in holding back *Gambusia* invasion. Timing and extent of seasonal desiccation and connectivity among wetland areas is also important for *Aphanius* survival and dispersal. Episkopi Bay Wetland holds populations in at least half a dozen artificial quarry pit ponds that maintain polysaline-eusaline conditions (>25‰) throughout the year and do not have any *Gambusia holbrooki*. A new threat in at least two of these quarry pit ponds in the recent anthropogenic introduction of predatory sea bass and other marine fish that prey on *Aphanius*. Conservation and restoration (through assisted migration) and *Gambusia* extirpation campaigns is required. Finally due to the small and localized population of the species at Akrotiri, this population

should be considered at the very least as "vulnerable" to extinction and requires targetted conservation management to ensure its survival.

3. ***Mugil cephalus*** / Striped Grey Mullet (Native marine migrant; incidental transient populations from marine waters; reproducing only in marine waters; perhaps more common in before 1970 within Akrotiri Wetlands)

   This species naturally would enter lagoon waters and does so during storm surges (most probably more frequently from the East side of the Peninsula, through the "aplostra" at Lady's Mile). One adult measuring at least 50 cm was recorded by S Michaelides in Salt Lake on January 23 2013. Several small Mugil cephalus were found by Zogaris's team at Paraga Pond (in 2011-12)(in Zogaris et al. 2012b).

   The species was said to be much more common in the past and several interviewees mention its presence in both the Salt Lake, Zakaki and Akrotiri Marsh as well (they all mention that during the lowering of the waters people would walk out to find grey mullets; they may be referring to fishes such as *Mugil cephalus*; and/or other species of Mugillids as well).

   We are not sure if this was the most prominent and most common grey mullet species to enter the lagoon or wetland areas of Akrotiri and include all "reports" and mention of "kefaloi/kefalopoula" in the Mugillidae text below.

4. **[Mugillidae sp.]** / Grey mullet species (Native marine migrant; incidental transient populations from marine waters; reproducing only in marine waters; more common in before 1970 within Akrotiri Wetlands)

   The grey mullets Mugillids, perhaps of the genus Chelon also enter the Salt lake area and are known to congregate at Zakaki even in recent years (V. Michael, pers. obs), where they are fished sometimes using gilnets by locals. These fishes most probably include both Mugil cephalus and various species of *Chelon* (for taxonomy as Barbieri 2015).

   Interviews in Akrotiri village show that at least until the late 60s Mugilids were widespread, common and very well known among the village-folk. When the waters of Salt Lake dropped the locals of Akrotiri would walk out and stun the larger mugilids with wooden clubs (as mentioned by Interviewees #4 and #5). After the construction of the anti-flooding embankment on Episkopi beach it was said that the infiltration of marine transient fishes declined (but this need further research).

5. ***Atherina boyeri*** /Big-scaled sandsmelt (Native marine migrant; incidental transient populations from marine waters)

   The species has been collected at Paraga Pond by S. Zogaris in two occasions (2011-12 and 2017). In January 2017 specimens were taken in ethanol solution for genetic examination. The January 2017 specimens seem very thin (probably due to lack of food). Among the Akrotiri locals this species was well known (interviewees #1, #7), collected from Livadi and the marshes, and Akrotiri Salt Lake. It was eaten deep-fried. (Mediterranean atherinids are sometimes difficult to identify in the field and may need laboratory or genetic examination. (The 2017 specimens were collected and identification was confirmed as this taxon by Dr. George Minos, a species expert, in Thessaloniki).

6. ***Gobius cobitis*** /Giant Goby (Native; emigrant from marine waters; probably reproduces locally in saline/slightly brackish conditions of artificial lagoon-like gravel pit).

   Collected for the first time from Paraga pond in the summer of 2016 and then again in January 2017; this is the first documented case of this marine gobiid species in inland waters (albeit lagoonal marine/brackish conditions) in Cyprus. Varius size classes caught- providing possible indication that the species does reproduce in the pond. The species is also known to inhabit inland transitional waters in Greece (e.g. Agios Andreas Lagoon, Samothraki).

7. ***Gambusia holbrooki*** /Eastern Mosquitofish / (Alien introduced; resident populations; reproduces in fresh and brackish waters)

   Gambusia holbrook was first introduced on the island in the 1939 from stock of Syrian origin (the species was spread into the Mediterranean countries originally from the Southeastern USA). The species is still stocked for mosquito control in many parts of Cyprus and is one of the most widespread and seasonally most abundant inland fish species on the island. It is seasonally the most abundant fish during the summer period in the Akrotiri Wetland- particularly in incoming fresh-brackish waters of the north shore of Akrotiri Salt Lake (Assomatos area -Eucalyptus forest); Zakaki Wetland; Akrotiri Marsh and at Bishops Pool. In winter after the rains and during a cold spell the species is surprisingly scarce and population densities seem to drastically drop. Interestingly, during January 2017 the species was spotted only in one location, Zakaki Marsh (from Zakaki Hide), yet it was more widespread in Dec. 2015 (before the rains). Populations are affected by seasonal desiccation and salinity (is not a migrant but disperses faster than the similar sized *Aphanius*). The species can survive and reproduce even in polysaline conditions (c. 20 ‰) as stated in the bibliography; however it also capable of surviving for short periods in high salinity conditions, even sea water concentrations (38‰) and can disperse through sea water for very short periods. The species survives in polluted stagnant waters as well where other fishes do not; it can build dense populations in such waters and degrade limnological conditions by feeding on all macroinvertebrates (plankton etc), fish and amphibian eels and larvae. It is known to be aggressive towards several species of small fishes (such as *Aphanius*) and will bite and nip fins off these fishes. In low population densities it can coexist with native fishes. Its predators include other large fishes (including eels) and several birds.

8. **[*Dicentrarchus labrax*]** / European Sea Bass (Translocated; possibly framed stock; reproduces only in marine waters)

   In the distant past perhaps this species may have found its way into the wetland of the Salt Lake along with mullets, Sea-smelts and Sparids. However nowadays the populations known to exist at Paraga Pond and Ai Yorki Pond are introduced (most probably from then nearby fish farming unit at the Kouris river mouth, as stated by three interviewees). The fishes have not been seen by any ichthyologist but the introduction is "common knowledge" among many residents at Akrotiri village. Intersingly the population of *Aphanius* at Paraga Pond was found to incredibly low in density (as opposed to seine net sampling there in by the author).

9. ***Cyprinus carpio*** /Common Carp (Alien introduced; resident population)

   Photographs exist of the fish in the eutrophic pond of Bishop's Pool. The sightings have been confirmed by Mr. V. Michael and there is video footage from recent years. This introduced fish is known to alter the limnological conditions of pools and ponds (increasing water turbidity among other impacts) and survives only in freshwater conditions.

10. **[*Sparus auratus*]** /Gilt-head Bream (Former marine migrant; now introduced into quarry pit ponds at Episkopi Bay Wetlands)

    Natural populations used to live and where professionally harvested from the Salt Lake Lagoon during Ottoman times. Accounts published in the 16th century by travellers visiting the area, indicate that there was no salt production from the lake at the time but the lake was a very important fishing ground. It was used as a huge fishery for this species known as “dorade” (tsipoura) and water was brought into it from the sea by a channel. The species has now been introduced within the Ai Yorki Pond (V. Michael pers. observation; but no photographic documentation exists). It should also be mentioned that another Sparid species (Sparus sp.) has probably been introduced within the Ai Yorki Pond (V. Michael pers. observation); from the description it may be *Sondyliosama cantharus*).