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Ecological and biometric analysis techniques on two contiguous populations of green toad (*Bufotes balearicus*) within the Sentina Regional Natural Reserve (San Benedetto del Tronto, AP)

Stage report

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Abstract

La finalità di questo stage è volta allo studio della popolazione di rospo smeraldino (*Bufotes balearicus,* Laurenti, 1768) durante il periodo di fregola all'interno della Riserva Naturale Regionale della Sentina. Nel corso dei monitoraggi, avendo rinvenuto una consistente popolazione di questa specie nel vicino torrente Ragnola, a 2,2 km di distanza dalla riserva, quest'ultimo è stato incluso nel monitoraggio. Il rilevamento dei dati biometrici (lunghezza e peso) ha coinvolto anche gli altri membri della batracofauna della riserva, onde avere un quadro della situazione più chiaro. È stata rilevata la presenza abbondante di *Pelophylax bergeri* kl. *Hispanicus* (Bonaparte, 1839), e una più modesta di *Hyla intermedia* (Boulenger, 1882), oltre quella del rospo smeraldino, il quale all'interno della riserva sembra soffrire della situazione di instabilità del livello d'acqua dei canali e del regime agricolo attualmente presente. Al torrente Ragnola la popolazione e l'attività di fregola è più stabile. La virtuale assenza del rospo comune, specie euriecia per eccellenza, è degna di nota.

Introduction

The present study is an analysis of the herpetofauna in the Regional Natural Reserve of Sentina. In particular, our attention has focused on an ecological and biometric analysis of two adjacent populations of green toads (*Bufotes balearicus*, Laurenti, 1768), a species protected by the Habitat Directive, Annex IV.

This work has been conducted in the context of a broader need for monitoring the amphibian populations of the area as bioindicators. The geographic and anthropic boundaries of this nature reserve, constituted to the south by the mouth of the Tronto river, to the west and north by roadways and to the east by the coastline, facilitate the collection of data on the demography of the species and on the reproductive biology within

the Reserve and outside this (Ragnola brook). This opens a window on the consistency and potential of the population of amphibians, sensitive to the anthropic disturbance factor, such as the green toads, permanently present in the area.

This stage aims to create the conditions for learning monitoring techniques, biometrics and ecological analysis of a particular type of fauna, the batrachofauna, characterized by significant conservation value and difficult to study due to the highly cryptic nature, nocturnal habits and reduced ability to spread and recolonize environments.

The collection of data on these amphibians began in March 2019 and ended in May 2019, proximate to the peak of the breeding season. The green toad is recognised as a species which reproduce early in the year, (Lanza, 1983), and it is mainly linked to soft sandy soils and brackish waters.

The specimens were sampled manually, using predetermined transects within the Reserve. The installation of falling traps was thought inadviseable due to the dense reedbush vegetation surrounding the two main water mirrors.

1. General characteristics of Amphibians

The word "amphibian" comes from the ancient greek αμφιβιον, in which αμφι means "double" and βιος "life", in reference to their ability to live both in aquatic and terrestrial environments. These small vertebrates are strongly linked to the aquatic environment, fundamental for their reproduction. In most cases they present a complex biphasic life cycle, in which an aquatic larval stage is fallowed by one of amphibian sexual maturity. They belong to the *phylum Chordata* and are today represented by the Orders of *Urodela* (salamanders and newts), *Anura* (frogs and toads) and *Apoda* (*Cecilidae*). All three of these orders appear to descend from the sub-class of the *Lissanphibia*, the other two classes of amphibians (*Leposopondyli* and *Temnospondyli*) being completely extinct.

Adaptation to terrestrial life brought with it challenges: dehydration, gravity, aerial respiration and sperm migration towards the egg in dry conditions.

The transition from fish to tetrapod dates back to the Devonian (about 370 million years ago). The appearance of the lungs in bony fishes is found early in evolution as an adaptation to anoxic environments or to areas where the water level inconstant (Graham, 1997). The amphibians derive from a group of fish with muscular and robust fins (*Crossopterigi*), and the evolutionary process has led to an increasing capacity to walk, starting from the pectoral and ventral fins, thus forming the first group of vertebrates that colonized the earth. The intermediate forms we can observe through to palaeontology are represented by the genera *Tiktaalik*, *Eusthenopteron* and *Acanthostega*, among others.

The class of amphibians globally includes around 7600 species, most of which belong to the *Anura* order (6800). In Italy it is represented only by the order of the *Anura* (*Ranidae, Bufonidae, Discoglossidae, Bonbinatoridae* and *Hylidae*, with 26 species) and that of the *Urodela* (*Salamandridae, Plethodontidae* and *Proteidae* with 19 species), being *Apoda* distributed only in of tropical climates.

Amphibians are tetrapod (with the exception of *Apoda* species), with sub-equal legs in the *Urodela* order or a greater hind legs development in the *Anura* order. With a few exceptions, the life cycle is biphasic, presenting an aquatic larval stage and a metamorphosed amphibian stage. The larvae of the *Cecilidi* and *Urodela* are morphologically similar to the adult form, although they have external branchial apparatuses, and in the *Urodela* the hind limbs are poorly developed. The *Anura* larvae have an oval bodily with an elongated and flattened tail to facilitate swimming. The hind legs develop before the front legs during the metamorphosis process. The food regime goes from herbivore to insectivore with metamorphosis. The integumentary system is kept moist thanks to glands that secrete mucus and, sometimes, poisonous substances as protection from predators. Skin moisture is particularly important in amphibians, as most of the gas exchanges take place through the latter, which is highly

vascularized (the genus *Plethodontidae* is completely devoid of lungs). This confines most of the amphibians to live in wetlands.

For the most part oviparous, among the amphibians we also find larviparous species (*Salamandra salamandra*, Linnaeus, 1758) or viviparous (most of the *Cecilidi* and some *Urodela*, including *Salamandra atra*, Laurenti, 1768). The majority of the *Anura* lay eggs in the thousands and have a high prolificacy; the *Urodela* deposit eggs in the tens or hundreds and the *Cecilidi* are the least prolific of all. There are some exceptions to this: for example, the *Genus Stefania* among the anurans is not very prolific, as a result of the parental investment of caring for the young until egg hatching and metamorphosis.

As regards the conservation of this genera, the number of endangered species is increasing more rapidly than is the case with mammals and birds (Stuart et al., 2004). This phenomenon is caused by a series of factors: first of all the fragmentation and reduction of habitats, linked to human activity, in which the wet and vegetated areas where amphibians live are most affected. Moreover, the effect that climate change has is becoming increasingly evident, inducing early reproduction and the alteration of some basic physiological functions of these animals that are highly sensitive to changes in temperature and humidity.

Another effect that seems partly related to global warming is the varied dynamics of some diseases, including kithridiomycosis, caused by *Batrachochytrium dendrobatidis* (Sodhi *et al.*, 2008), which already brought to extinction some species and is decimating hundreds of others.

1.1 *Anura* characteristics

Anura are ectothermic animals, like all amphibians. They are characterized by the absence of tail in the adult phase (from the ancient Greek an + oura = without tail). The structure of the legs varies widely between species, depending on morphological adaptation to the type of locomotion: jumping, walking, swimming or gliding. However, a strong

dimorphism is always maintained between hind legs, which are more robust and elongated, and front ones.

JUMPING STRUCTURE: Several adaptations in the body shape lead most anurans to be good jumpers. The body is short and stocky, presenting a non-flexible vertebral column, formed usually by 9 pre-sacral vertebrae and a urostyle (several vertebrae fused together). Radium and ulna are fused together in the forelimbs, giving a greater mechanical resistance in dampening the fall, as well as the tibia and fibula in the posterior ones, giving greater strength in pushing to jump. The morphological key of the ability to jump, however, can be sought in the structure of the tendons of the hind limbs, which constitute an elastic structure which, combined with the ability to lengthen the tarsus, allows some species to make very long jumps (up to 6 m). The rib cage is reduced, so as to further lighten the skeletal structure.

RESPIRATORY SYSTEM AND HYOID APPARATUS: The lungs are poorly vascularised, and the diaphragm and thoracic muscles that allow breathing are missing. This occurs through rhythmic movements of the mouth and throat in the inhaling phase, and through the contraction of the trunk muscles in the expiratory phase (bucco-pharyngeal respiration). A role in respiration is also played by the hyoid apparatus, which together with the opening and closing of the nostrils (valve-like function) allows inhalation and exhalation. The hyoid apparatus is cartilaginous and formed by two processes. These constitute the anchoring surface of the tongue, allowing its extrusion. It allows also the expansion of vocal sacs in males.

MOUTH: The skull has a triangular shape, with the back wider than the front. The mouth is large and, with some exceptions, has keratin teeth with the function of keeping a firm grip on the prey, which is swallowed whole.

EYES AND SIGHT: The eyes are large and mobile and have three eyelids: upper and lower one, not very mobile, and the middle one with a moistening function in sub-aerial environment and protective function during the submersion period. The eyes are positioned on either sides of

the head, which allow a very wide field of view. The sight is well developed, and it has recently been shown that they can distinguish colours, even at low brightness levels (Kelber *et al.,* 2017).

EARS: The anurans have no external ear, and the tympanic membranes are externally exposed; they are able to perceive sounds both in the subaqueous and aerial environment.

SEXUAL DIMORPHISM: This is expressed through different characteristics: (1) males are commonly smaller than females; (2) presence of nuptial pads in males, chiefly evident during the reproductive season. They are dark-coloured corneal excretions on the first three toes of the front limb with the function of holding the grip on the partner during the copulatory embrace. (3) a degree of chromatic dimorphism in some

PHOTO 1 - Nuptial pad in male green frog

species; (4) the ability to croak through the expansion of the vocal sacs, which is restricted to males. The sound of crocking is species-specific and diagnostic.

REPRODUCTION: Fertilization is external, and courtship involves calling, which is also a territory definition mean, and at the breading site, a tight embrace. The type of embrace (axillary, inguinal or cephalic embrace) and the way eggs are laid (cords, spherical masses, anchoring to plants, etc.) is characteristic of each species or group of species.

LARVAL STAGE: In most anurans lasts for 2 or 3 months. It presents a morphology and a mode of life very different from the adult form. The skeleton is completely cartilaginous;, the life mode is purely aquatic; the

diet is herbivorous. Tadpoles have internal gills and only in the genus *Pipidae* we observe two wattles with sensory function (similar to catfish whiskers). The metamorphosis process has been extensively studied and is induced by the release of the hormone thyroxin, involving the appearance of limbs, first posterior and later anterior, the calcification of the skeleton and the atrophy of the tail and gills, with simultaneous formation of lungs.

BIOGEOGRAPHY AND ECOLOGY: The *Anura* have colonized most of the emerged lands, with few exceptions regarding the extremely rigid climate zones of the Sahara desert and the arctic climate zones of Greenland and Antarctica. They have dimensions ranging from 7.7 mm of the *Paedophryne amauensis* to 30 cm of the *Conraua goliath* (Boulenger, 1906).

The *Anura* are voracious predators of small arthropods and invertebrates and in some cases even larger species (e.g. *Rhinella marina*) as other amphibians, saurians, and micro-mammals. They are in turn preyed upon by a wide variety of fish, reptiles, birds and mammals. They are fully integrated into the trophic network, of which they are an essential link.

Many species are also used as biological indicators in environmental quality studies, especially for the identification of the presence of pollutants from heavy metals (Simon *et al.*, 2012).

1.1.1 Family *Bufonidae* (Gary, 1825)

Widely distributed in all continents except Australia and Antarctica, the *Bufonidae* are known in English as true toads. It is the only family which includes exclusively species commonly identified as toads. Its members all have the characteristic of a squat body, wrinkled skin, short legs, absence of teeth, horizontal pupils and the Bidder organ, which in case of malfunctioning testicles in the male, may become an ovary. The members of the *Bufonidae* family are also endowed with parotid glands that secrete a variety of alkaloid toxic substances, collectively known as bufotoxins,

which vary in toxicity from species to species. This family includes about 500 species, and the systematic are complex and subject to frequent revisions and updates.

In Italy there are four species, the common toad (*Bufo bufo,* Linneaus 1758), the Italian green toad (*Bufotes balericus,* Boettger 1880), the Sicilian green toad (*Bufotes suculus,* Stöck *et al.,* 2008) and the European green toad (*Bufotes viridis,* Laurenti 1768).

In this study we will deal with *B. balearicus*, with a brief analysis on the presence of *Hyla intermedia* (Boluenger, 1882) and *Pelophylax bergeri kl. hispanicus* (Günther, 1986) and *B. bufo*, whose presence within the reserve was found during the study.

A) *Bufo bufo* (Linnaeus, 1758) – common toad

Known as common toad, this amphibian is the most ubiquitous of all European amphibians, and is found throughout the subcontinent, with the exception of Iceland and the cold parts of Scandinavia. The colour ranges from grey to brown, and females may reach up to 15 cm in length. The male is devoid of vocal sac, and during the breeding season it exhibit nuptial pads in the first three fingers of the front paws. A diagnostic feature of this species are the slanted parotid glands. Populations of common toads are known to occupy a diverse range of habitats, even relatively arid and strongly anthropic ones. Outside the breeding season this species is exclusively terrestrial. It has crepuscular and nocturnal activity. Less euryhaline than the green toad, eggs can however hatch at a concentration of 15 g/L of NaCl (Lanza, 1983).

The *International Union for Conservation of Nature* (IUCN) list places the common toad within the Least Concern(LC) category, stating the stable trend of its population. The main threats are represented by invasive/problematic species and diseases and pollution form agriculture.

B) *Bufotes balearicus* (Boettger, 1880) – green toad

PHOTO 2 - *B. balearicus,* female

It has a fairly large range, which includes most of the Italian regions (except the Valle d'Aosta region). Its dimensions rarely reach 10 cm, ranging more usually around 7 cm.

It is characterized by the trill call, audible especially in the warm evenings of the reproductive period, which serve females in selecting their partner. The frequency of the call varies among different individuals and it's related to size. Large-sized individuals have low-frequency calls and are more easily chosen by females. Large size indicates not an older age, but the timing of sexual development (Giacoma *et al.*, 1997), both in males and females. In females a large size also indicates better efficiency in reproductive investment and the production of higher quality offspring (Castellano *et al.,* 2004). Sexual maturity is normally reached around 4 years of age (Lanza, 1983).

For reproduction the green toads look for stagnant or weakly flowing waters, river mouths and small basins, even of temporary nature. They prefer areas with sandy soil and are common along the coasts. They are characterized by being strongly euryhaline and more thermophilic than *Bufo bufo* and fear less than the latter drought. The breeding period is relatively prolonged and can last from March to August. It has a crepuscular and nocturnal behaviour.

As for the sexual dimorphism, in males we observe (1) a smaller size than that of females, (2) the presence of black nuptial pads on the first three fingers which is accentuated during the breeding season, (3) front legs which are more robust than in the female and hind limbs more extendedly webbed than the females.

The larval form appears in the classic aspect of a tadpole and lasts 2-3 months, depending on water temperature. The females lay between 5000 - 13000 eggs, which normally hatch within 1-2 weeks.

The *B. balearicus* is part of the LC class (Least Concern) of the IUCN list (2008), although a decline in population due to agriculture, pollution, habitat reduction and fragmentation is reported.

1.1.2 Family *Ranidae* (Rafinesque, 1814)

It is the largest family in the world, with the exception of Antarctica. The size varies from 5 cm to 35 cm, with *Conraua Goliath* (Boulenger, 1906). It is represented by around 50 genera, with over 720 species. In Italy there are the genera *Rana* (Linnaeus, 1758), *Pelophylax* (Fitzinger, 1843) and *Lithobates* (Spix, 1824) (with a single species, the bullfrog (Lithobates catesbeianu) introduced from North America).

PHOTO 3 - Livrey variation in *P. bergeri* kl. hispanicus

Pelophylax bergeri (Günther 1985) / *kl. hispanicus* (Bonaparte, 1839) – green frog

Commonly known as edible frog or green frog, it usually lives in colonies. It produces a vast assortment of croaks, which have the

purpose of attracting females and marking the territory. It presents a variable range of livery, from bright green to brown to turquoise, with a yellowish white line on the middle part of the back. It feeds on insects, annelids and molluscs and is tightly linked to water. It lives in lakes, ponds or slow-flowing rivers with rich riparian vegetation. In the IUCN list it is included in the LC class, excluding it from the endangered species. However, a decline in populations is recorded, linked to habitat loss, presence of invasive species, diseases, pollution and human capture for food purposes.

1.1.3 Family *Hylidae* (Rafinesque, 1815)

The *Hylidae* family is cosmopolitan and includes over 600 species; it is not found south of the Sahara or in Madagascar. It is commonly known as the tree frog. The vast majority are arboreal frogs, characterized by a good ability to climb, with dilated toes that present adhesive discs at the apex. It usually has a good capacity to withstand semi-arid conditions. Even the *genera* that have returned to terrestrial life, such as Acris and Pseudacris, retain atrophied disks. Only the genus *Hyla* is part of the European fauna. This is characterized by being equipped with only one vocal sack under the throat. It is distributed in Eurasia, North-West Africa and in America. In Europe there are 4 species, all found in Italy.

Hyla intermedia (Boulenger, 1882) – Italian tree frog

Known with the common name of Italian tree frog, it's widespread throughout the national territory, excluding Sardinia, Puglia and Liguria and many alpine areas.

It is predominantly a climbing species, with medium-small dimensions (4-5 cm) and a marked resistance to aridity; when it is not the breeding season it is common to find it far away from watercourses. During the period of activity it spends most of the time on shrubs or among tree branches. It is mainly a nocturnal species, and feeds primarily on arthropods. It lays eggs in roundish masses that fall to the bottom of the

PHOTO 4 - *H. intermedia*

water body or anchor themselves to the aquatic vegetation.

Very similar to *H. arborea*, it is distinguished from the latter by the black lateral strip, which extends up to the intersection with the lower limb. Due to its small size, it may be difficult to distinguish males and females through the observation of the nuptial pads. However in this species sex is immediately detectable through the observation of the vocal sac, which gives to males a yellowish colour to the underside of the throat, otherwise immaculate.

The IUCN list places it in the LC class with a stable population trend. The main risks turn out to be habitat loss and water pollution.

2. Characteristics of the study area: The San Benedetto Coastline

The study area is located in the southern border of the Marche coast. The climate in this area is Mediterranean, with mild winters and hot summers, with an average annual temperature of 15 ° C. The annual rainfall ranges around 700 - 800 mm. This is a densely populated area and an important tourist and fishing centre on the Adriatic coast.

2.1 The Regional Natural Reserve Sentina

PHOTO 5 - Satellita view of the Regional Natural Reserve Sentina

Established in 2004, it covers 180 hectares, and presents itself as one of the last remaining wetlands between the Po delta and the Gargano. It is also one of the very rare areas along the Adriatic coast with a sandy beach with a hinterland which is not built upon,; it is in fact annexed to the Natura 2000 network and as Z.P.S. IT5340022 (Zona di Protezione Speciale, Special Protection Area) that like S.I.C. IT5340001 (Sito di Interesse Comunitario, *Site* of Community Interest), as well as I.B.A. (Important Birds Area) cod. 087 criterion C6 for the high biodiversity of aquatic birdlife. Despite its small size, the environmental value of this area is very high.

FLORA: we find a rich diversity, composed of over 400 species, of which numerous are rare. Among the most significant plant species worthy of mention are : the *Suaeda maritima* and the *Salicornia patula*, which together form what is called the "salt steppe", a particularly rare habitat in which we can also find the sea aster (*Aster tripolium*) and

liquorice (G*lycyrrhiza glabra*). Other characteristic plants are notgrass (*Polygonum maritimum)*, t smooth cordgrass (*Spartina alterniflora)* and the opposite-leaved saltwort (*Salsola soda*), which, together with other psammophilous plants, form the dunes environment of the backshore. In the "flat dune" environment we find the association *Echinophoro spinosae-Elymetum farcti*, which is often found embryonic dune areas. A wide area of the reserve is used for agricultural purposes, and is therefore devoid of spontaneous plants. Projects for floral reintroduction begun in 2012, with the Re.S.C.We project (Restoration of Sentina Costal Wetland), implemented within the frame of LIFE+ Nature and biodiversity projects. The reintroduction regarded in particular sea lavender (*Limonium narbonense*), ravennagrass (*Erianthus ravennae*), *Plantago cornuti* and *Arthemisia caerulescens*. Eradication of invasive species is also taking place.

BIRD FAUNA: There are many noteworthy bird species a significant number of which are protected. There are about 180 of them recorded, including 69 species belonging to passerines and 14 of ciconiformes. Among the most important is the Black-winged stilt (*Himantopus himantopus*, Linnaeus 1758), symbol of the reserve; the Kentish plover (*Charadrius alexandrinus*, Linnaeus 1758), a small cosmopolitan shorebird of the *Charadriidae* family and a wintering species in Italy; the ringed polver (*Charadrius hiaticula*, Linnaeus 1758), whose national wintering population amounts to only 100-200 individuals; the kingfisher (*Alcedo atthis*, Linneaus 1758) and the western marsh harrier (*Circus aeruginosus*, Linnaeus 1758) among many others. The reserve has a particular significance for birds, both for its value as an "island" within a heavily populated area, for its location along the Adriatic migration route of many water birds, birds of prey and passerines, and finally also due to the presence of wetlands, now so rare..

MAMMAL FAUNA: The biodiversity of this class is less abundant then that of birds, amounting to little over 20, of which six are included in Annexes II and IV of the Habitat Directive. Worthy of mention are: the hedgehog (*Erinaceus europeanus*, Linnaeus 1758), the crocidura

(*Crocidura* sp., Wagler 1832) and the beech marten (Martes foina, Erxleben 1777). Of particular interest is the presence of *Chiroptera*, including the greater vespertilium (*Myotis myotis*, Borkhausen 1797) and the vespertilium of Daubenton (*Myotis daubentonii*, Kuhl 1817).

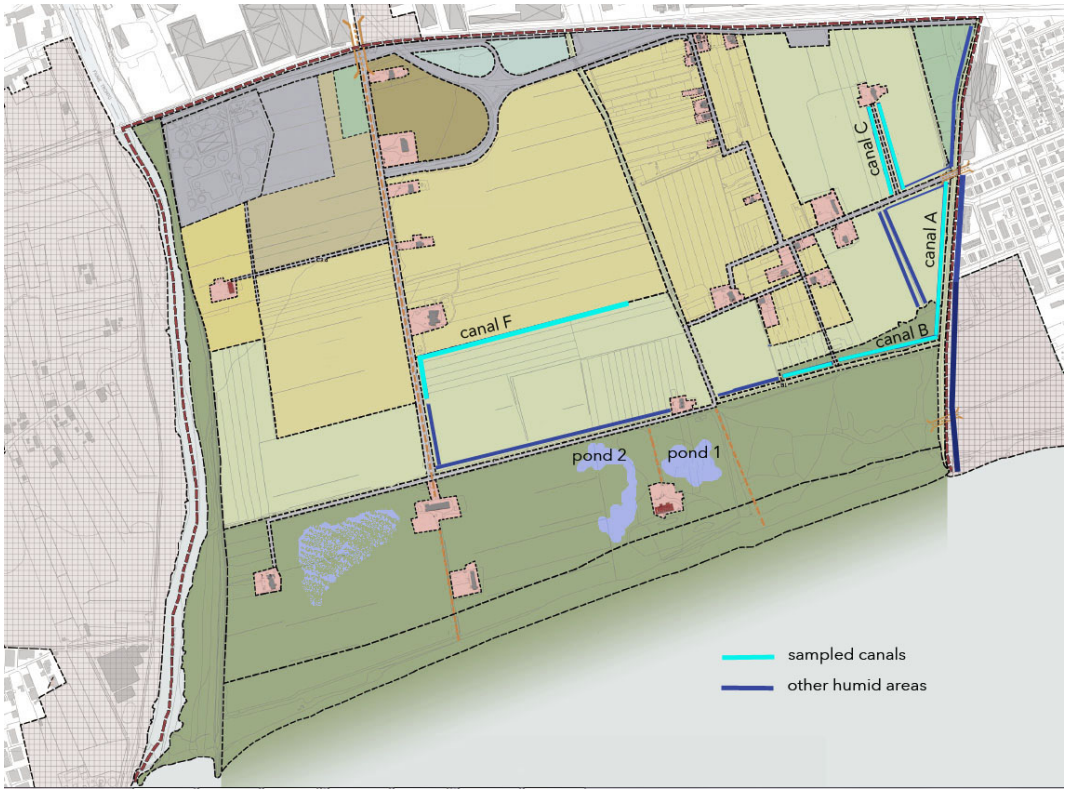
HERPETOFAUNA: a study conducted in 2009 (Marini *et. al.*, 2010) shows the presence of four species of amphibians and eight of reptiles. Of the amphibian species present, *B. balearicus* and *H. intermedia* are included in Annex IV of the Habitat Directive, wile *P. bergeri kl. hispanicus* and *B. bufo* are excluded. All four species appeared to be negatively affected by intensive agriculture and the poor management and maintenance of canals. Only of *B. balearicus* and *P. bergeri* the conservation state is considered satisfactory.

Within the reptile group we find the presence of (1) the common wall lizard (*Podacis muralis,* Laurenti 1768), with a good state of conservation although negatively affected by agricultural activity; (2) the ruin lizard (*Podacis siculus,* Rafinesque 1810), which would greatly benefit from a reduction in anthropogenic contribution to the area to improve its conservation status; (3) the green whip snake (*Hierophis viridiflavus,* Lacépède 1789) and (4) the western green lizard (*Lacerta bilineata,* Deudin 1802*)* are both negatively affected by intensive agriculture, which strongly affects their conservation status; (5) the common wall geko (*Tarentola mauritanica,* Linnaeus 1758) and (6) the mediterranean house geko (*Hemidactylus turcicus,* Linnaeus 1758), are particularly affected by predation by the domestic cat, although their conservation status is considered satisfactory; (7) the grass snake (*Natrix helvetica,* Linnaeus 1758), strongly linked to the humid environments for its trophic activity and finally, specimens of loggerhead sea turtle (*Caretta caretta,* Linnaeus 1758) have been sporadically found beached. A re-introduction project of European pond turtle (*Emys orbiculris*, Linneaus 1758) is currently underway within the reserve.

LAND USE: As previously stated, intensive agriculture is a predominant aspect of the Sentina reserve. As can be seen from the satellite view (PHOTO 5), only the area closest to the coastline preserves

the natural humid environment. The current agricultural system no longer supports the agricultural ecosystem that had developed decades earlier through traditional cultivation practices, which included the presence of farmland hedges and crop rotation as well as a less weighty use of fertilizers and pesticides. A constraint determined by modern crops is caused by the timing of water supply provided by the Tronto Land Reclamation Authority (TLRA). The irrigation of these croplands is linked to the seasonal needs of arable land and crops; this involves an additional supply of water to the wetlands of the reserve (ponds and canals) starting only from April. The coastline adjacent to the reserve has shown strong erosion phenomena over the years which strongly compromises the conservation of the retrodunal environment (Acciarri *et al.,* 2017) and the dune system itself is strongly at risk both from the morphological and vegetation point of view; despite sand replenishment interventions, in the interval 1985-2007 there has been a loss of 8 hectares of territory of reserve, a reduction in the continuity of the vegetation cover, the disappearance of the water bodies naturally present within the reserve and a greater vulnerability to saline wedge intrusion (Bisci *et al.*, 2010).

2.2 Sites characterization (reserve)



MAP 1 - Map of sampled areas

Canal A is 330 m long, presents very steep vegetated banks. The level of water is quite stable, ranging around the 50 cm of depth, even when the water from the Tronto river is not poured in the Reserve. It is thickly vegetated from aquatic plants and algae, and reeds grow in the middle of the canal.

Canal B is a stretch of 450 m where the banks are less steep and less densely vegetated than in canal A. The bed comprises cemented stretches [contains cemented stretches? Comprises cemented stretches?]which cover most of it, therefore the aquatic vegetation is absent. It is sided from the footpath on one side and reeds on the other. It is subject to desiccation when the input from the waters of the Tronto river is closed.

Canal C is a double canal of 195 m in length (390 m of canal in total) located in the middle of a cultivated field and with a footpath that runs

along the middle of it. It is characterised by a shallow water depth, and it is not connected to the main water network. Thick aquatic vegetation is present. The soil around it is predominantly clayey. Its only water supply derives from rainfall, making it a highly unstable environment subject to desiccation, variable salinity and temperature. Furthermore, from what can be deduced from the black colour of sediment and the sulphuric smell, it is a highly anoxic environment.

Canal F is the canal that presents the greatest depth. It stretches for 350 m in the middle of intensively cultivated fields (in the period of study 3 cultures fallowed one another), bordered by steep banks which are scarcely vegetated. The canal itself presents scarce aquatic vegetation. The soil alternates between sandy and clayey, and the level of water gradually decreases towards the end of the canal, creating an environment of shallow pools. It is the living habitat also of coypu (*Myocastor coypus,* Molina 1782).

Pond 1 is the deeper pond that can be found within the reserve. It was created during the Re.S.C.We project in 2012 with the use of a clay impermeable layer. It has a depth that ranges from 60 to 100 cm. The aquatic vegetation is thick, as is the reed vegetation along the banks. The water level before the input from the Tronto river was shallow, uncovering in places the aquatic vegetation.

Pond 2 in fed by the excess water of pond 1, and is not equipped with an impermeable layer, creating an environment favourable to limicolous birds, but sensitive to salinity intrusion; before the opening, by the TLRA, of the Tronto river waters, it was very close to complete desiccation. It presents no aquatic vegetation and the amphibian fauna began populating it only after the water level rose.

2.3 The Ragnola Brook

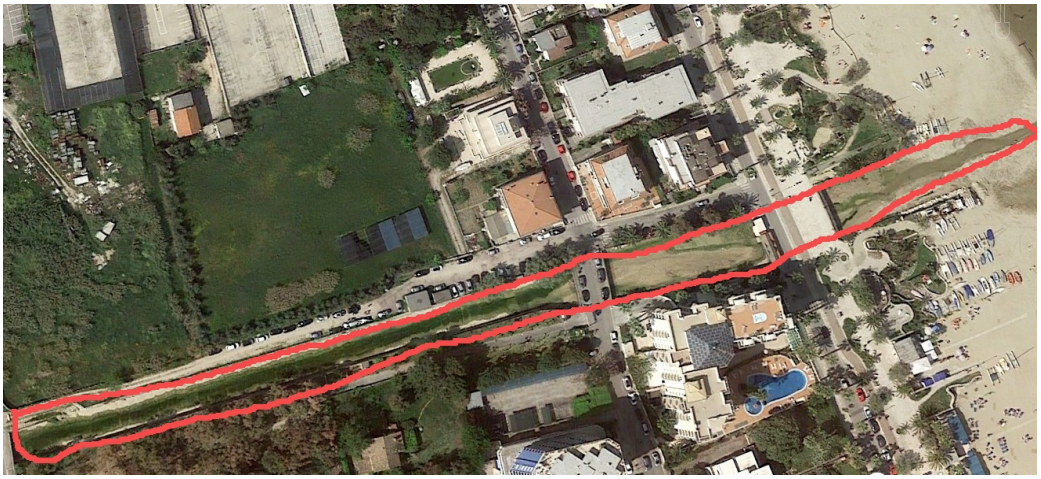


PHOTO 6 - Ragnola brook

The Ragnola brook is located 2.2 km north of the Sentina reserve; this site was also taken in consideration, even though it is outside the reserve (2,2 km from northern boundary), due to the consistent green toad population found there. It has an upbrook section which is not cemented, with steep vegetated banks and shallow pools. Downbrook there is a cemented stretch of 270 m in length, with a shallow water depth of about 10 cm, and a thick lining with filamentous algae (*Chladophora* sp.). The brook flows into the sea through the sandy beach, where it cuts a channel of about 80 m in length and with a maximum depth of 1 m. it is surrounded by a sandy shore, palm trees and, on the right bank, a retaining embankment.

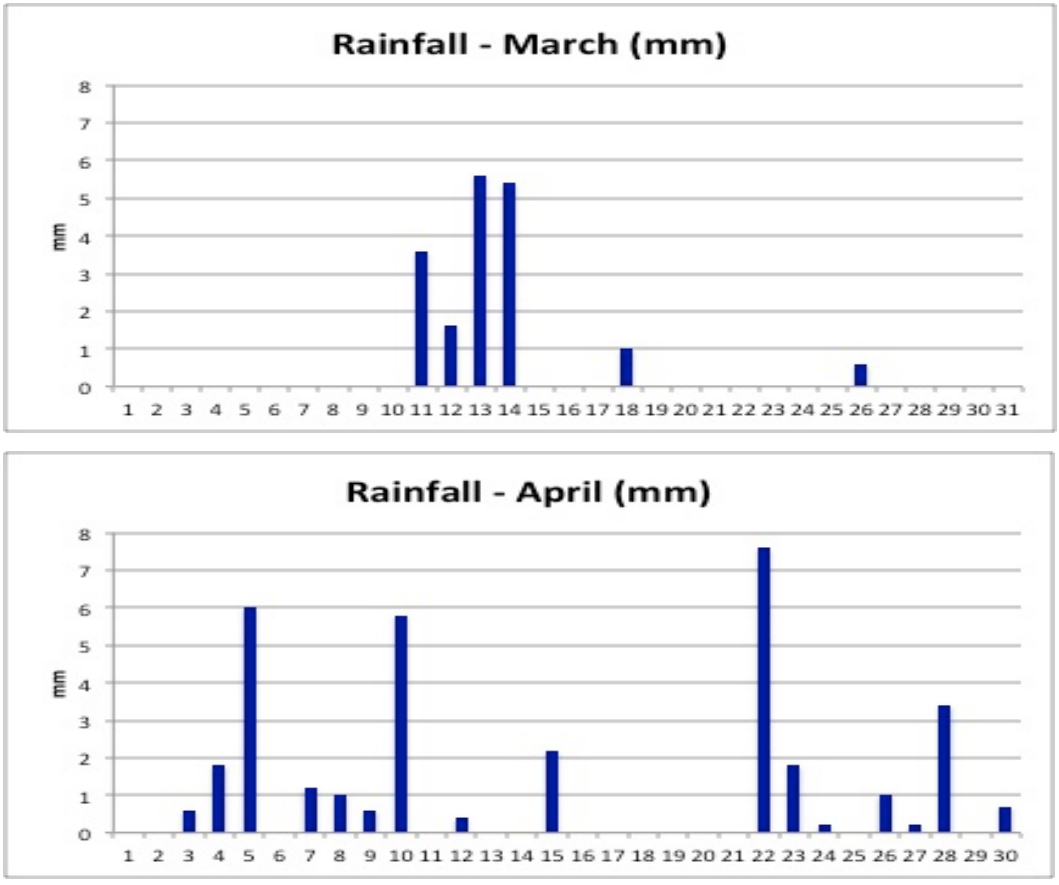
Site	Mean water conductivity (µS/cm)	Mean water temperature (°C)
Ragnola brook	1140	15,1
Canale A	670	15,5
Canale C	1775	15,8
Canale F	>2000	17,6
Brook at Sentina edge	670	15,1
Albula brook	/	14,5
Pond 1	534	15,4
Pond 2	1270	/

TABLE 1 – Mean conductivity and water temperature in sampled sites

2.4 Spring 2019 climatic data

To better frame this study, it is necessary to mention the unusual climatic conditions that prevailed during March and April. Spring 2019 was a particularly dry season and slightly colder than usual. The mean temperature of 11,8 °C of the survey period is slightly lower than the mean temperature for the 2010-2015 period of 12,5 °C. The cumulative rainfall for March and April 2019 is of 52,3 mm, against a mean of 138,4 mm for the 2010-2015 period. This drought negatively affects the breeding and feeding activity of amphibians (United States Geological Survey, 2013).

In such a year it is particularly evident the importance of constant water input managed by the TLRA. The variation linked with the beginning of water supply, which leads to a variability and fluctuation of the water level, salinity and temperature in canals and ponds, is a problematic factor.



GRAPH 1 and 2

3. Materials and methods

The study period extended from the beginning of March 2019 to the end of Aprll 2019, focusing on the breeding period of the green toad. During this period we monitored the beginning of the reproductive period of the green toad and collected data on the other amphibian species present with weekly surveys at the reserve. Once green toads' spawning activity had begun and the breeding sites were located, surveys took place every night, until enough data was collected to have a clear vision of the structure of the population and of its reproductive range.

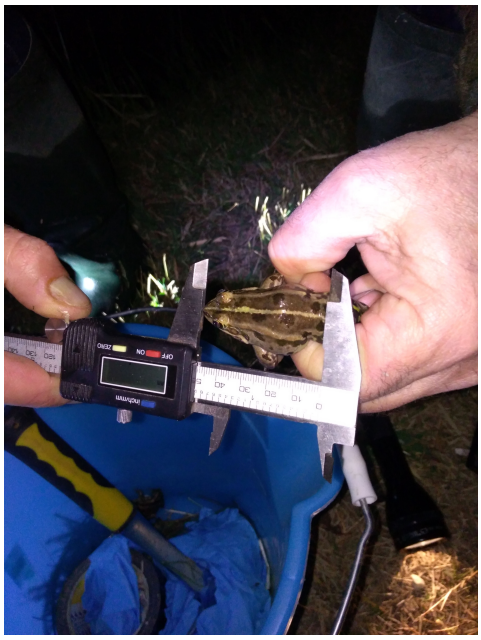


PHOTO 7 - Measurement with digital caliper of green frog

PHOTO 8 - Weight measurement of green frog with digital scale

Surveys were conducted after nightfall. A routine path was developed for the control of the canal and the ponds. Particular attention was paid to the identification of hiding places of the green toad. Several methods of sampling were considered, and after inspection on, site the use of pitfall was excluded, due to the thick ground and reed vegetation and the wide area to cover. The sampling of specimens was carried out by direct capture, on land and in water, using a hand net when necessary.

Environmental data (conductivity of water, air temperature, water temperature) was collected on every night of the survey.

The biometric data for all amphibian specimens was collected, recording the following parameters:

- Species
- Sex
- Snout-vent length (L)
- Weight (W)

The measurements for the length were taken using a digital caliper with the precision of 0.01 mm (PHOTO 7); a digital portable scale was used to measure weight, with the precision of 1 g (PHOTO 8).

Unpublished data on the 2001 green toad population of the Ragnola brook was available. This, together with the collected data, was statistically analysed with the support of the software Statistical Package for Social Science (SPSS) performing the fallowing tests:

- Two way ANOVA test (univariate analysis of variance)
- Test of significance considering two factors (sex and year) and two independent variables (L and W)
- 95% confidence interval
- Pearson correlation index

4. Results

Bufo bufo - Common toad

Only two specimens of common toad were found at the beginning of March. The virtual absence of common toad was surprising and unexpected, as its presence had been stated in the study on the reserve's herpetofauna, held by Marini *et al.* (2010).

Hyla intermedia - Italian tree frog

In the surveys led in March, the Italian tree frog was nether found nor heard. In the study area this species is active starting from April. On the 4th of April the first two specimens were found on the edge of canal B, near the reeds. From then on, an increasing presence within the reserve was observed at every survey, with several specimens performing the breeding call. The occurrence of Italian tree frog is strictly linked to the water body riparian vegetation. At the Ragnola brook, no specimen was found.

Pelophylax bergeri kl. *hispanicus* - Common green frog

P. bergeri population was abundant in canal A and pond 1, presenting a calling behaviour since early March. In warmer evenings specimens were found also on land, along the footpaths, and one also on the beach. Canal B showed a less abundant population, as the water level was often very low and at times completely dry. Until middle of April the TLRA did not proceed towards the input of water from the Tronto river in the reserve. Pond 2, not being lined with an impermeable layer, was almost dry and had a strong saline concentration. At Ragnola brook specimens were observed only upbrook, outside the cemented area.

Bufotes balearicus - Green toad

	canal C	canal F	canal B	B. Ragnola	B. Albula
11-apr	38				
13-apr				12	
17-apr		1		26	
18-apr		10		21	1
19-apr	2	3		9	
20-apr		20			
23-apr	28		1		

TABLE 2 - Samplings per day, per site.

	Sentina		C. Ragnola
	canal C	canal F	
total	102		68
	68	34	
Male	94		54
	61	33	
Female	8		14
	7	1	
Sex Ratio (M:F)	11,8		3,9
	8,7	33,0	

TABLE 3 - Number of samplings and sex ratio per site.

The first green toad breeding site was located on the 11th of April in canal C, outside the area of the ponds and canals we had been regularly controlling. Unfortunately for our study, the breeding season started rapidly, and spawning activity had begun, with numerous egg masses in the water. We were not able to count them due to the numerous strings overlapping. Over half of canal C's length was covered by them. We sampled 38 individuals, of which only 2 females. One female was embraced by 3 males at the same time (PHOTO 9) wrestling among themselves, putting at risk the females life (Lanza, 1983). On the 13th of April we returned on site and not even one individual was detected. On the same evening, a survey was conducted in the Ragnola brook, 2,2 km north to the Sentina Reserve. Twelve specimens were sampled, with 7 egg masses counted. Calling

PHOTO 9 - Three green toad males embracing one female

and spawning activity was observed. The 17th of April canal C was drying up and no green toads were found. 27 individuals were sampled at the Ragnola brook breeding site, of which eight were females. The breeding site at canal F was individuated. It was scarcely populated, with only three males calling, but it showed thousands of tadpoles and no egg mass. Within the reserve, no other breeding sites were found. An inspection of canal C on the 19th of April revealed that the left canal had almost completely dried out, and there was no sign of egg mass or tadpoles on the right one, in which the water level was also greatly reduced, increasing salinity, temperature and fostering an increasingly anoxic environment; *P. bergeri,* however, was always present in the site. The 3 breeding sites (canal C, canal F and Ragnola brook) were monitored for several days in a row, registering a stable spawning activity at the Ragnola brook and a progressive return to terrestrial life in the other two sites. The observational effort at the Ragnola brook was also aimed at locating the shelter sites of green toad, being this site heavily lined with concrete. One burrowing site was identified, via observation of the footprints on sand, in the embankment present at the right side of the Ragnola brook, on the sandy area of the beach (PHOTO 10 and 11). Numerous footprints were observed departing from there and leading to the water (an essay was later conducted to verify the type of footprints green toads leave. One specimen was released on sand to verify the type of footprints it left on this substrate). The green toad population of the Ragnola brook occupied exclusively the cemented and beach area. Egg mass and specimens were found up to 20 m from the shoreline.

On 18th of April, in order to gain a clearer vision of the green toad range, a further investigation was carried out in the Albula brook, 4,8 km north of the reserve. The brook bed here there is heavily lined with concrete, and the water channelled, so that the current is rather rapid. Only one large female was found in the sandy area at the mouth of the river.

A survey on the 23rd of April, a day after a rainfall event, registered an increase in the number of individuals at canal F and C sites. However on that day several individuals were found on the road perpendicular to canal C and a male was located on the footpath near canal B, an area where individuals were never found. This dispersal from breeding site indicates a shift in focus from reproductive to trophic activity. On a further survey carried out the 21st of May, after some weeks of rain, several tadpoles were found in canal C; canal F was populated by tadpoles at an advanced stage of development and young tadpoles too. The Ragnola brook had numerous tadpoles, no egg mass were found, but males were still calling

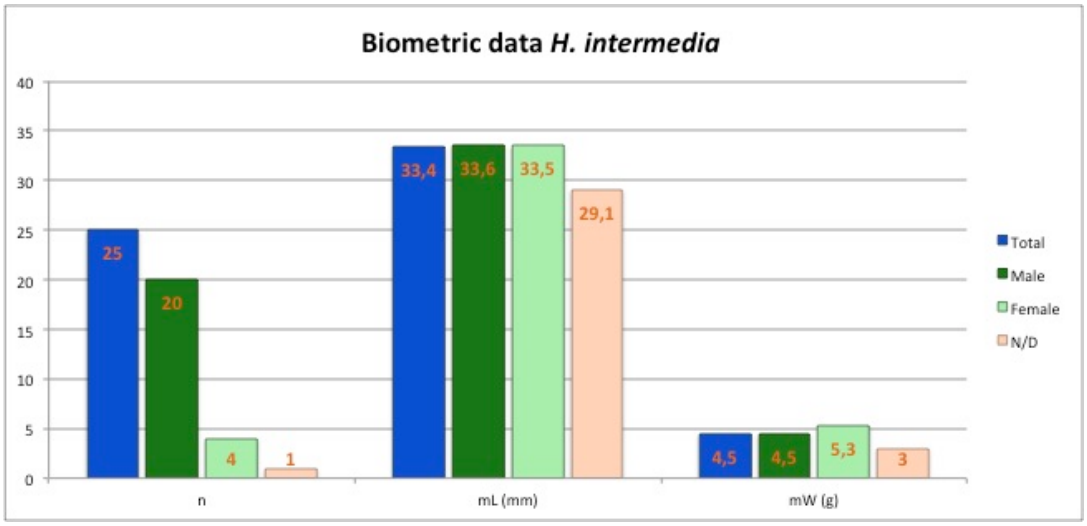
PHOTO 10 - Walking green toad footprints on sand

PHOTO 11 - Hopping green toad footprints on sand

6. Data Analysis

The data collected on the 16 nights of survey was analysed using a Windows Excel spreadsheet.

Hyla intermedia – Italian tree frog



GRAPH 3

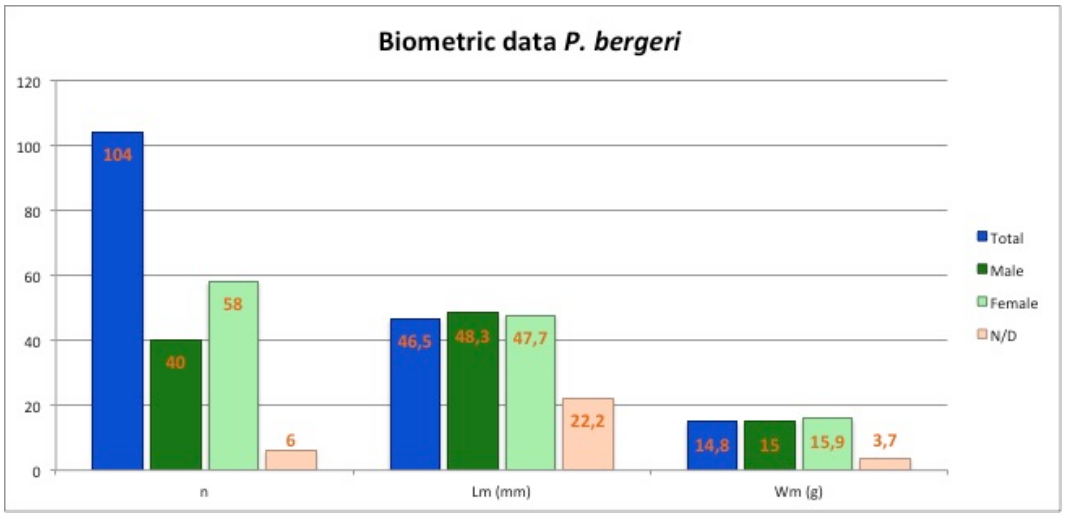
The individuals sampled in total were 25: 20 males, four females and a young individual whose sex was not identifiable (N/D). The sex ratio is 5 (M:F). The species was ubiquitous within the reserve, even though it was less abundant than the common green frog. Specimens frequented canal and pool edges. The reproductive period for this species had not yet begun at the time of survey, even though calling behaviour was frequently observed.

Specimens within the reserve showed a maximum length of 37,8 mm and a mean length (mL) of 33,4 mm. The maximum weigh (maxW) registered was 7 g, and the minimum weight (minW) 3 g. The mean weight (mW) being 4,5 g.

H. intermedia	n	mL (mm)	mW (g)	Lmax (mm)	Pmax (g)	Lmin (mm)	Pmin (g)	Sex ratio
Total	25	33,4	4,5	37,8	7,0	29,1	3,0	5,0
Male	20	33,6	4,5	36,8	7,0	30,2	3,0	
Fermale	4	33,5	5,3	37,8	6,0	31,5	4,0	
N/D	1	29,1	3,0	29,1	3,0	29,1	3,0	

TABLE 4 - Biometric data *H. intermedia*

Pelophylax bergeri kl. *hispanicus* – green frog



GRAPH 4

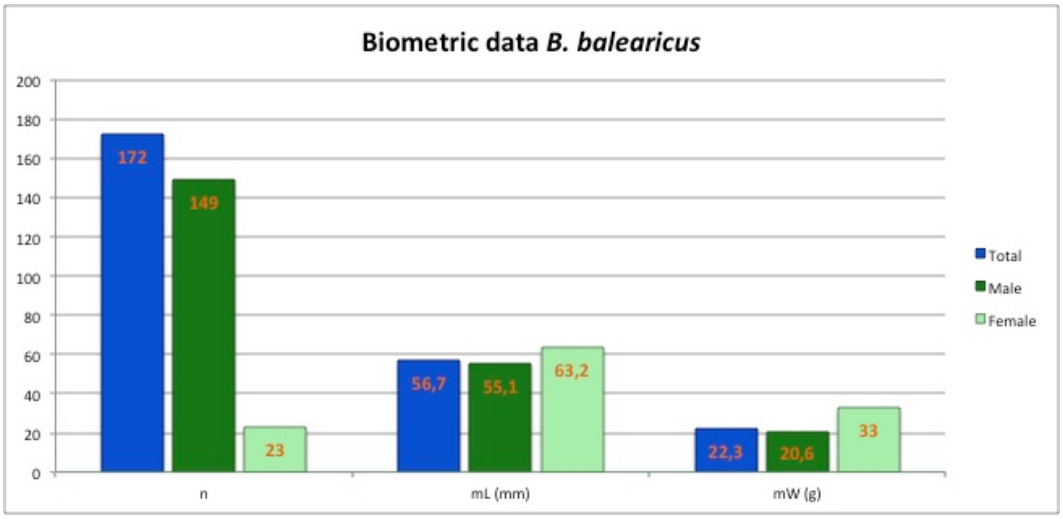
The presence of *Pelophylax bergeri* was well established and ubiquitous within the reserve boundaries. A total of 104 individuals were sampled, and their sex ratio was 0,7 (M:F). The maximum L (maxL) of specimens found was 73,5 mm, and minimum L (minL) 14,4 mm; the mL of sampled individuals is 46,5. For what regards weight the mW is 14,8 g. Further collected data is indicated in the table below.

Reproductive period for this species had not yet begun at the time of our survey even tough calling behaviour has been observed.

Bufotes balearicus – green toad

P. bergeri	n	Lm (mm)	Wm (g)	Lmax (mm)	Wmax (g)	Lmin (mm)	Wmin (g)	Sex ratio
Total	104	46,5	14,8	73,5	51,0	14,4	2,0	0,7
Male	40	48,3	15,0	58,6	26,0	35,1	5,0	
Female	58	47,7	15,9	73,5	51,0	26,4	4,0	
N/D	6	22,2	3,7	35,0	5,0	14,4	2,0	

TABLE 5 - Biometric data *P. bergeri*



GRAPH 5

A total of 172 specimens of *B. balearicus* were sampled (149 males and 23 females), of which 102 within the reserve area. The only breeding sites were located at canal C and canal F. The surrounding crop field gives burrowing space within clay or sand clods. Two specimens were found in clayey crevice near canal C.

PHOTO 12 - Female green toad in crevice

As previously reported, within the reserve it was not possible to count the number of egg masses. In canal C the density was too thick to allow counting without ruining them. In canal F the finding of the site was too late. On the other hand, while sampling a total of 19 egg

masses were counted at the Ragnola brook. Numerous tadpoles have been found both at canal F and at the Ragnola Brook. On a later survey on the 21st of May, also canal C presented tadpoles.

B. balearicus		n	mL (mm)	mW (g)	Lmax (mm)	Pmax (g)	Lmin (mm)	Pmin (g)	Sex ratio M:F
T. Ragnola	Total	68	59,5	23,4	80,7	54,0	49,4	13,0	11,8
	Male	54	58,4	21,6	78,2	38,0	49,4	13,0	
	Female	14	65,9	34,1	80,7	54,0	58,1	21,0	
Sentina	Total	103	54,2	20,7	69,2	54,0	36,9	8,0	3,9
	Male	95	53,9	20,0	69,2	38,0	39,6	12,0	
	Female	8	60,9	31,4	68,2	54,0	36,9	8,0	

TABLE 6 - Biometric data *B. balearicus* per site

Unpublished data on the Ragnola green toad population of 2001

Available data from a survey conducted during the reproductive period of *B. balearicus* in 2001 collected by M. Marconi at the Ragnola brook has been used in order to have a comparison tool to better evaluate the trend of the Ragnola population.

Data Ragnola 2001	n	mL (mm)	mW (g)	Lmax (mm)	Pmax (g)	Lmin (mm)	Pmin (g)	Sex ratio M:F
Total	63	*61,9*	20,7	*75,8*	35,7	*50,5*	10,8	9,5
Male	57	*61,4*	20,5	*75,8*	35,7	*50,5*	10,8	
Female	6	*65,9*	22,5	*72,9*	32,7	*57,9*	11,7	

TABLE 7 - Biometric data of the 2001 *B. balearicus* population present at Ragnola brook

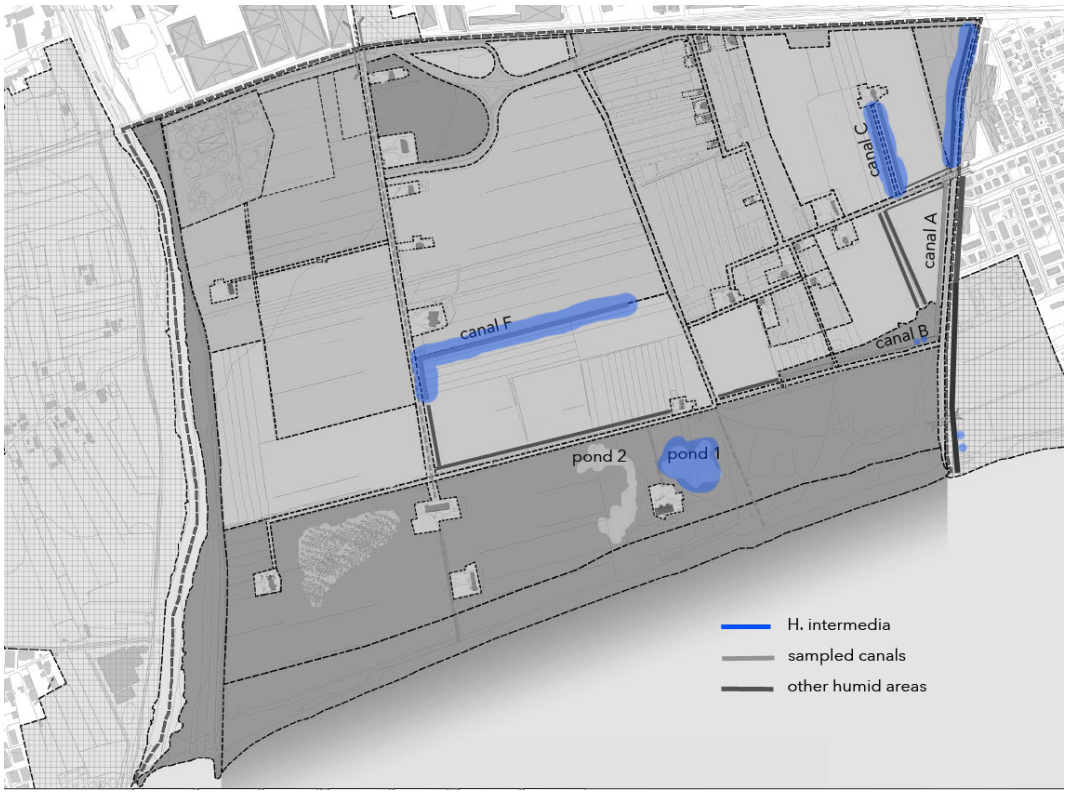
7. Discussion

Bufo bufo - common toad

A possible hypothesis about the virtual absence could be that spawning in this area may be particularly early (ie before the period of the survey), however no egg masses, tadpoles or adults in trophic

activity were observed during the two months of surveys. In order to make a statement on the virtual absence of the common toad, a species which is present in a wide variety of environments and whose presence within the reserve was confirmed by Marini *et al.* (2010), further investigation is needed.

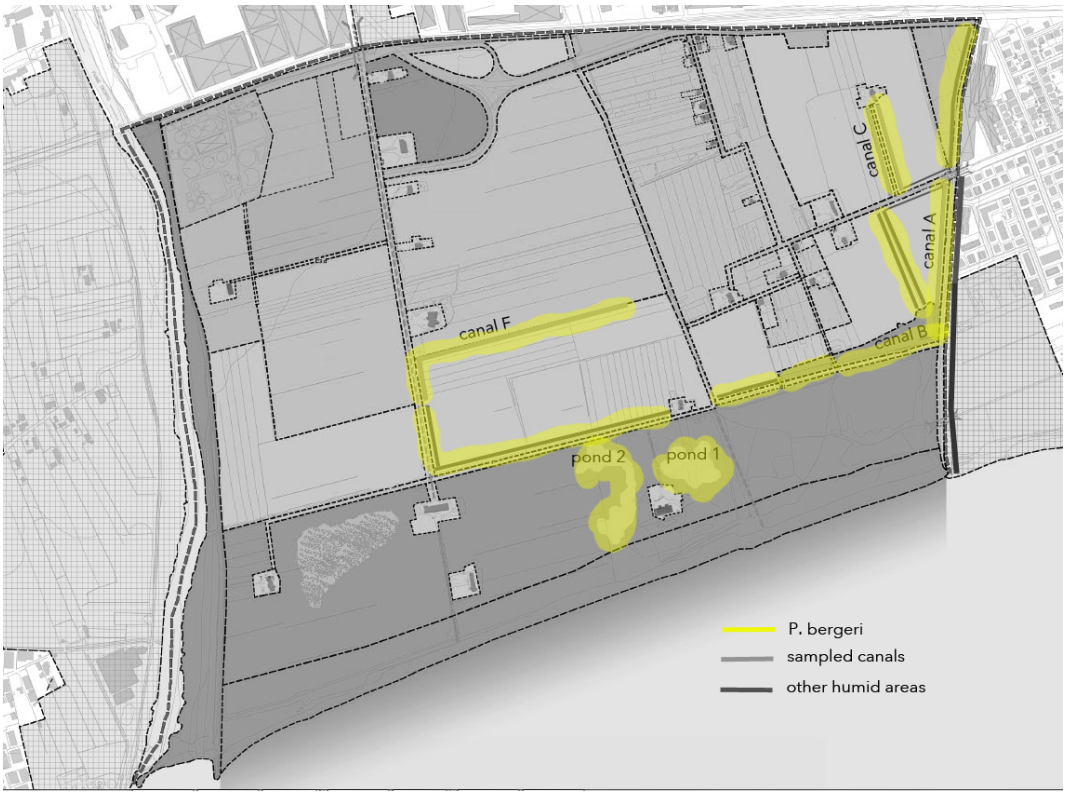
Hyla intermedia – Italian tree frog



MAP 2 - *H. intermedia* range within the reserve

The presence of this species was widely confirmed both via observation and sampling. On pond 2 no specimen was sampled nor observed, however during the survey period it was almost dry, reacquiring a good water level only after TLRA water supply was effective. No specimen was found at the Ragnola brook, probably due to the absence of riparian vegetation. The population present at the Sentina reserve is well established, however not abundant.

Pelophylax bergeri kl. *hispanicus* – common green frog



MAP 3 - *P. bergeri* range within the reserve

The common green frog showed, through observation and sampling, an ubiquitous distribution within the reserve and an abundant population. At the Ragnola brook no specimen was observed along the segment covered in concrete of the water course, while upstream some were observed.

We observed the individuals found in canal A and B were smaller, with respect to Umbria's and Tuscany's population (Ragni *et al.,* 2006, Vanni & Nistri, 2006). The maxL of specimens sampled is of 73,5 mm against 115 mm reported indicated by Ragni *et al.* (2006) and Vanni & Nistri, (2006). The abundance the common green frog is confirmed also by the occurrence of its predators such as *Natrix Helvetica*, observed twice swimming in the canal in hunting activity.

Bufotes balearicus – green toad

MAP 4 - *B. balearicus* range within the Sentina reserve

The survey revealed the areas of reproduction of *B. balearicus* within the reserve. The areas chosen by the green toad had in common shallow water and sandy or soft sediment surrounding. In canal F, which presented a segment with a deep water level and another with shallower water, where pools alternated with dry ground, specimens were always sampled in the shallow water area or on the ground. This two conditions: (1) shallow water and (2) soft sediment are both present at the Ragnola brook, where the water level is constant and shallow, and the beach area gives burrowing space, as confirmed by footprints found right after dusk (PHOTO 10, 11). Spawning activity proved more successful at the Ragnola brook, where egg masses were more vital and proceeded successfully towards hatching (PHOTO 13). This is due to the stable environment at this site and constant water supply. In canal C egg masses did not reach hatching due to the drying out of the canal, wile in canal F, although numerous tadpoles were

found, spawning activity did not proceed in a constant manner as at the Ragnola brook, and no egg masses were ever found there.

PHOTO 13 - Recently hatched tadpoles at Ragnola brook.

The fluctuating reproductive activity of the green toad is in accordance with Kovács & Sas (2010), which describes this species as being an opportunistic breeder with a long breeding period (up to 7-8 months) where terrestrial and reproductive activity alternate, using for the latter periods of rainfall. This behaviour is the only option for reproductive success, given the choice of temporary water bodies for reproduction. This consideration is confirmed by the observations done in canal C. The environment here is not constant and depends entirely on rainfall, specimen number fluctuated fallowing the rainfall pattern, alternating trophic activity with reproductive one.

Analysis of biometric data revealed the mean length of the green toad populations as being undersized with respect to data reported in literature. The mL of the green toad population sampled is of 54,2 mm for the Sentina population and of 59,5 mm for the population at the Ragnola brook, while literature (Ragni *et al.,* 2006, Vanni & Nistri, 2006) reports a length that ranges between 60 and 80 mm in males and up to 100 mm in females. Sinsch *et al.* (2007) suggests that size may be significantly influenced by human land use. In areas with intensive land use individuals will mature earlier, have a smaller size and a shorter life cycle than those in undisturbed areas. On the other hand Kutrup *et al.* (2005) report a correlation between body size and climatic conditions, where lowland populations of *B. viridis* in Turkey have a smaller body

size than those at higher altitudes. However, the lowest mL reported in this study (63,5 mm) is higher than that found at the Sentina reserve.

Ragnola brook: 2001-2019 comparison:

The statistical comparison of data relative to the green toad Ragnola's population revealed some facts.

Variable: weight

Tests of Between-Subjects Effects

Dependent Variable: peso

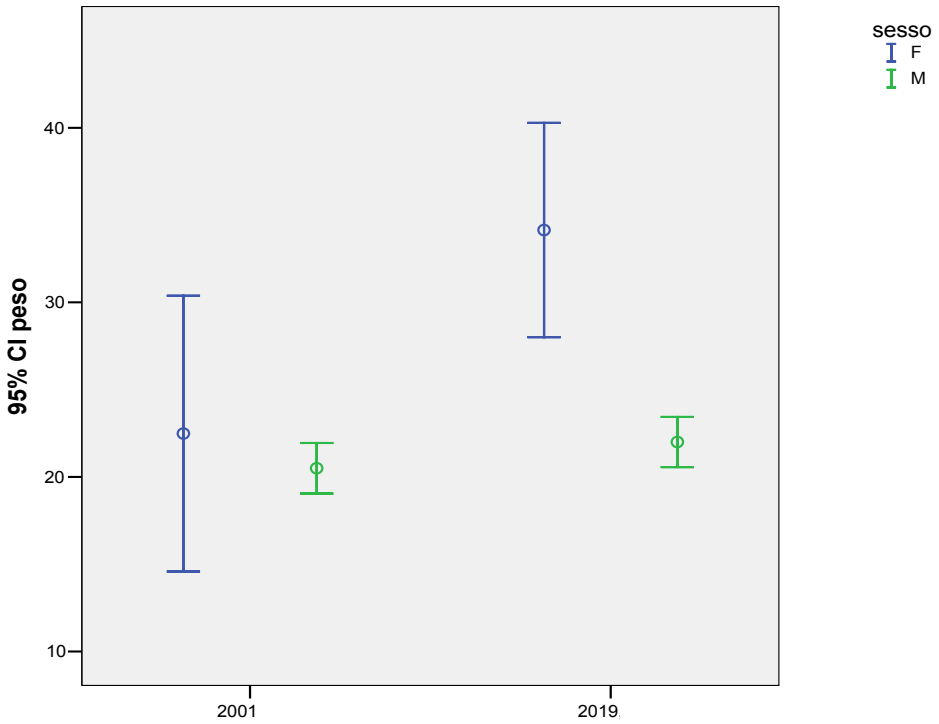
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2136,407[a]	3	712,136	18,465	,000
Intercept	35838,419	1	35838,419	929,235	,000
sesso	728,189	1	728,189	18,881	,000
Anno	631,958	1	631,958	16,386	,000
sesso * Anno	376,250	1	376,250	9,756	,002
Error	4898,094	127	38,568		
Total	74334,252	131			
Corrected Total	7034,501	130			

a. R Squared = ,304 (Adjusted R Squared = ,287)

TABLE 7 - Significance for the dependent variable weight

The difference in weight between males and females is significant, and so is the difference in weight sampled in 2001 compared to that of 2019.

The confidence interval (GRAPH 6) is greater for females than for males, due the small number of data available for females. However the mean weight of females is significantly lower in 2001 than in 2019, while not so for the length variable. This could be connected to a year of lower food availability.



GRAPH 6 - 95 % confidence interval for waight variable

Variable: length

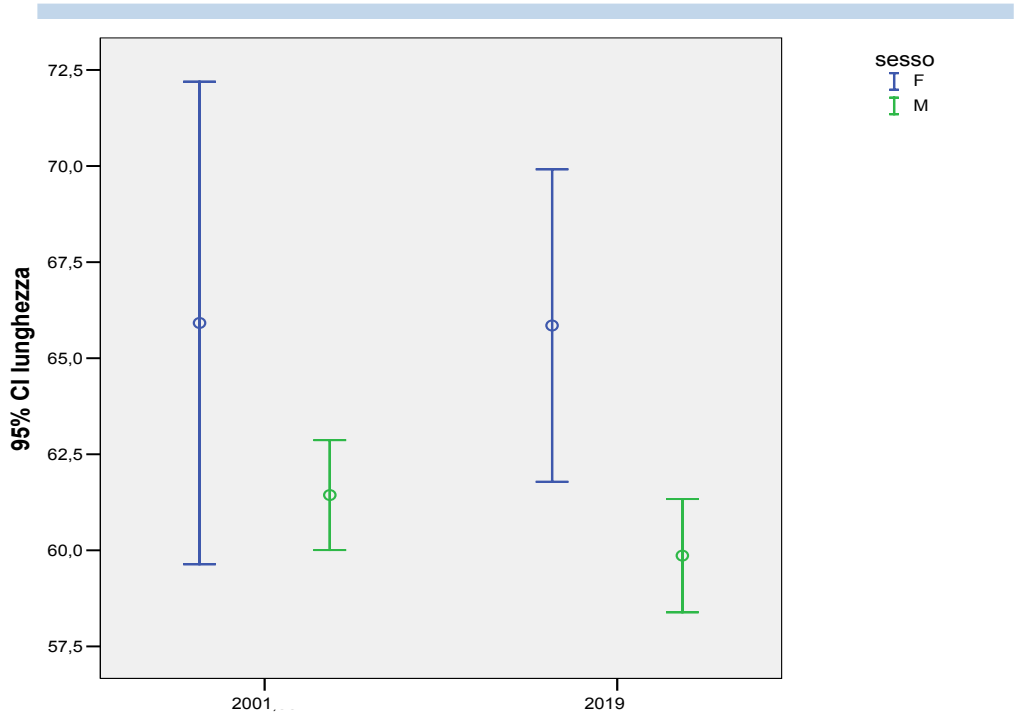
Tests of Between-Subjects Effects

Dependent Variable: lunghezza

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	527,053[a]	3	175,684	5,586	,001
Intercept	233597,057	1	233597,057	7427,579	,000
sesso	399,641	1	399,641	12,707	,001
Anno	9,856	1	9,856	,313	,577
sesso * Anno	8,322	1	8,322	,265	,608
Error	3994,145	127	31,450		
Total	499427,850	131			
Corrected Total	4521,197	130			

a. R Squared = ,117 (Adjusted R Squared = ,096)

TABLE 8 - Significance for the dependent variable length



GRAPH 7 - 95% confidence interval for length variable

While the difference in length is significant when comparing sex, it is not so when comparing data relative to the two different years. Again, the confidence interval for females is wider than for males, given the scarce amount of data due to the unbalanced sex ratio, but the fluctuation from year to year is not significant.

The Pearson correlation index reveals a very strong correlation between L and W, as it could be expected.

8. Conclusions

Through this survey it was possible for us to assess the presence and conditions of the amphibian populations within the Sentina Reserve and at the Ragnola brook. The overall situation that emerges for each species analysed is the fallowing:

- **Common toad:** further investigation is needed to better understand the reason for the virtual absence of this species.
- **Common green frog:** the population is abundant, the situation is of least concern.
- **Italian tree frog:** the population is present yet not abundant.
- **Green toad:** within the reserve the population is present, however it suffers from the unstable conditions of the water bodies chosen for reproduction. There may be a negative impact from the agricultural regime, connected both to the chemicals used and to the tilling of the earth, where this species takes shelter.

  At the Ragnola brook the population was more stable and abundant.

The conditions within the reserve underline the necessity of taking action to stabilize the water input and to reduce drastically the impact caused by intensive agriculture, in order to improve the life of the batrachofaune and the conservation of the breeding sites in this area.
Another action that could be taken involves the creation of underpasses for crossing the road that is within the boundary of the Reserve, which is subject to the traffic of the people living there. Such underpasses have been elsewhere considered very effective for the conservation of migrating amphibian species (Schmidt & Zumbach, 2008)

BIBLIOGRAFIA

BISCI C., BOVINA G, CANTALAMESSA G., CAPPUCCI S., CONTI M., SINATRA A., VALENTINI E., 2010 – Caratteristiche sedimentologiche e morfologiche, tendenza evolutiva della costa della Riserva Naturale di Sentina (Marche) e ruolo dell'Habitat dunale per la riqualificazione ambientale – Studi Costieri 2010 – 17: pp.149-164

CASTELLANO S., CUCCO M., GIACOMA C., 2004 – Reproductive investment of Female Green Toads (*Bufo viridis*) – Copeia, pp.659-664.

GIACOMA C., ZUGOLARO C., BEANI L., 1997 – The advertisement calls of the green toad (*Bufo viridis*): variability and role in mate choice – Herpetologica 53(4), 454-464.

GIUSTIN M., MARINI G., 2011 – L'Avifauna della Riserva Naturale Regionale Sentina – Tipografia media prina 2000

GRAHAM J. B., 1997 – Air breathing fishes, evolution, diversity and adaptation – Academic press, pp. 6-7.

GRIZIMEK B.(ed.), 1971 – Grizimek; Vita degli animali, volume 5 Pesci (*secondo*) e anfibi – Bramante editrice.

KOVÁKS E., SAS I., 2010 – Aspects of breeding activity of *B. viridis* in urban habitat: a casa study in Oradea, Romania – Biharean Biol. 4, 2010.

KUTRUP B., BULBUL U., YILMAZ N., 2005 – Effects of the ecological conditions on morphological variations of the green toad, *Bufo viridis*, in Turkey – The Ecological Society of Japan

LANZA B., 1983 – Anfibi, rettili; guida per il riconoscimento delle specie animali delle acque interne Italiane - Consiglio nazionale delle ricerche.

MARINI G., DI MARTINO V., PICCININI G. B., FIACCHINI D., 2010 – Anfibi e rettili della Riserva Naturale Regionale Sentina (Marche) – Atti VIII congresso Nazionale *Societas Herpetologica Italica*, pp.63-68.

RAGNI B., DI MAURO G., SPILINGA C., MANDRICI A., GHETTI L., 2006 – Anfibi e rettili dell'Umbria – Petruzzi editore.

REPORT Re.S.C.We Restoration of Sentina Costal Wetland- Layman's report - Life09 NAT/IT/608, www.life-rescwe.it

RODRIGUES A. S. L., FISHMAM D. L., WALLER R. W., 2004 – Status and Trends of Amphibian Declines and Extinctions Worldwide – Science 3 dec 2004 vol. 306, issue 5702, pp. 1783-1786.

SHDHI N. S., BICKFORD D., DIESMOS A. C., LEE T. M., KOH L. P., BROOK B. W., *et al.,* 2008 – Measuring the Meltdown: Drivers of Global Amphibian Extinction and Decline - *PLoS ONE*, 3 (2) , e1636 ·https://doi.org/10.1371/journal.pone.0001636

SIMON E., BRAUN M., TOTHMERESZ B., PUKY M*.,* 2012 – Frogs and Toads as Biological Indicators in Environmental Assessment. (In): Murray J. L. (ed.). Frogs: Biology, Ecology and Uses. – Nova Science Publishers. 2012 pp.141-150.

SINSCH U., LESKOVAR C., DROBIG A., KÖNIG A., GROSSE W., 2007 – Life-history traits in green toad (*Bufo viridis*) populations: indicators of habitat quality – Can. J. Zool. 85: 665-673.

SCHMIDT B. R., ZUMBACH S., 2008 – Amphibian road mortality and how to prevent it: a review – Zurich Open Repository and Archive, University of Zurich, http://www.zora.uzh.ch .

UNITED STATES GEOLOGICAL SURVEY, 2013, May 18. Frogs, salamanders and climate change. ScienceDaily. www.sciencedaily.com/releases/2013/05/130518153747.htm

VANNI S., NISTRI A., 2006 – Atlante degli anfibi e dei rettili della Toscana – Edizioni Regione Toscana.

VITT L. J., JANALEE P. CALDWELL, 2014 - Herpetology: an introductory biology of Amphibians and Reptiles – Elsevier