# Università degli Studi di Ferrara

## PH.D. Course
### in
### Evolutionary Biology and Ecology

In cooperation with:
Università degli Studi di Parma
Università degli Studi di Firenze

CYCLE XXXII°

COORDINATOR Prof. Guido Barbujani

# *Status of the fish communities and need for management in the Po River Delta*

Scientific/Disciplinary Sector (SDS) ____BIO/07____

| **Candidate** | **Supervisor** |
|---|---|
| Dott. Lanzoni Mattia | Prof. Fano Elisa Anna |
| *(signature)* | *(signature)* |

Year 2016/2019

UNIVERSITA' DEGLI STUDI FERRRA

DOTTORATO DI RICERCA IN

"BIOLOGIA EVOLUZIONISTICA ED ECOLOGIA"

CICLO XXXII

# *Status of the fish communities and need for management in the Po River Delta*

Coordinatore: Chiar.mo Prof. GUIDO BARBUJANI

Tutore: Chiar.mo Prof. ELISA ANNA FANO

Dottorando: Dr. MATTIA LANZONI

Anni 2016/2019

# Sommario

# ABSTRACT

The term "delta", have used as a synonym for transitional water bodies, includes the terminal stretch of rivers, estuaries, deltas, fjords and lagoons, as defined by the EU WFD directive. Wetlands are sites of high biodiversity and productivity, with great ecological interest, functional richness and great exploitation of renuwable resources. Recently, these ecosystems have suffered a serious decline worldwide due to human disturbances. The Po River delta (Adriatic Sea, Italy) and the related coastal lagoons are not an exception. In the last twenty years, extensive phenomena of eutrophication and related dystrophic crises, introduction of alien species and the development of new fishing and aquaculture practices have further contributed to the breakdown of biological communities and, in particular, of fish ones.

The aim of this study was to update the general status of the fish communities in the Po Delta of Emilia-Romagna, both in composition and trophic structure, and to identify the main problems of fisheries related to the physical evolution of the environment and the change in human activities.

The results show that freshwater and transitional water suffered from different pressures: freshwater communities are affected by a progressive invasion of exotic species, which has intensified starting from more than 30 years ago. Whereas, fish communities in transitional water do not suffer of exotic fish invasions, but their overexploitation and play a central role in fish community changes. The cases of eels and the fishery of Comacchio are presented and analized as study cases.

# INTRODUCTION

## 1.1. International scientific context

Wetlands are sites of high biodiversity and productivity (Mitsch & Gosselink, 2000), and Deltas occupy about 13% of all coastal areas of the world, areas of great ecological interest, functional richness (Franco et al., 2008) and great exploitation of renwable resources (Deegan et al., 2000; Franco et al., 2007; Malavasi et al., 2004). All deltas must be seen as dynamic systems, characterized by the presence of steep environmental gradients, which constitute a complex variety of habitats, species and communities (Pombo et al., 2005). The high spatial variability of biological assemblages is the base of their high productivity (Gamito & Erzini, 2003). The deltas provide essential services, such as maintenance of atmosphere composition, are key habitats for migratory species, and important nursery areas (Basset & Abbiati, 2004).

Humans have always benefited from deltas in terms of the goods and services they provide, first and foremost the supply of food, such as fish, molluscs, birds, algae, etc. (McLusky and Elliott, 2004), having thus an interest in preserving them in a good quality state. In particular, in the Mediterranean deltas, the various forms of fishing and aquaculture, practiced since ancient times, have been identified as the main toolsof conservation of their ecological characteristics, together with the socio-economic interest and the historical and cultural values (Cataudella et al., 2015).

Recently, these ecosystems have suffered a serious decline worldwide due to human disturbances (Shine & Klemm, 1999; Solimini et al., 2008; Stenert & Maltchik, 2007). The general worsening of water quality, and of eutrophication, and additional disturbances in the watersheds, such as the increase of population density, industrial and agricultural activities, together with loss of natural features of waterways, loss of aquatic vegetation and related ecosystem services are exposing wetlands and deltas to a progressively more svere disturbance worldwide (Drake et al., 2011). Moreover, coastal wetlands have been subjected also to reclamation and habitat change and deterioration worldwide (Goudie, 1990). For example, in most countries of western Europe more than 50% of the area converd by coastal wetlands in 1900 has been lost (Jones & Hughes, 1993).

In the Mediterranean, pollution, climate change, habitat loss due to reclamation for agriculture, climatic and physical changes have strongly changed both structure and functioning of these ecosystems (Cataudella et al , 2015), losing much of their intrinsic value for biodiversity and related ecosystem services (Perez-Ruzafa et al., 2006). The continuous environmental changes, both natural and activities, have today caused alteration or loss of most of the delta habitats, affecting the structure and abundances of the biological communities of these environments (Perez-Ruzafa A., 2011).

The Po River delta and the northern Adriatic coastal lagoons are not an exception as, in the last twenty years, extensive phenomena of eutrophication and related dystrophic crises, intruduction of invasive alien species and the development of new fishing and aquaculture practices have further contributed to the breakdown

of biological communities and, in particular, of fish ones (Lanzoni et al., 2010). These modifications and the general impairment of habitats, have not only substantial effects on fish communities, has had serious repercussions on entire ecosystems and, in turn, on the secondary production itself (Pombo et al., 2005). However, the Po Delta has recently recognised a hot spot for conservation (Abbiati & Basset, 2001; Barnes, 1999) with the declaration of Special Areas of Conservation, listed as a priority habitat type (1150*) on Annex I of the European Union Habitats Directive (Council Directive 92/43/EEC). The conservation of these habitats depends largely on the assessment of their natural characteristics, especially biodiversity, which is one of the main criteria used when elaborating wetland protection policies (Ramsar Convention Bureau, 2005).

## 1.2.    Po River Delta

The term "*delta*", here used as a synonym for transitional water bodies, includes the terminal stretch of rivers, estuaries, deltas, fjords and lagoons, as defined by the EU WFD directive (Water Framework Directive 2000/60 / EC) (Franco et al., 2008).

In the case of the Po River delta, in theis study i consider the hydraulic system of fluvial branches through which the Po river flows into the northern Adriatic Sea. Its current hydraulic structure derives from the consequences of the Ferrara earthbreaker of 1570 and from the "cut of Porto Viro", a large hydraulic work carried out by the Republic of Venice in 1604.

The delta branches of the Po currently active, which all together constitute the delta, are, from north to south:  Po di Maistra, Po di Venezia - Po della Pila which flows into the sea through three distinct vents (Busa di Tramontana, Busa Dritta and Busa di Scirocco), Po di Tolle (with the branches of Busa Bastimento and Bocca del Po delle Tolle), Po di Gnocca or Donzella (also with a terminal bifurcation) and Po di Goro.

The whole river branches and the territory among them cover a  surface of ca  786 km$^2$. The Po Delta extends over two regions, Veneto and Emilia-Romagna, 3 Provinces (Rovigo, Ferrara e Ravenna) and 19 municipalities: Adria, Alfonsine, Argenta, Ariano nel Polesine, Cervia, Codigoro, Comacchio, Corbola, Goro, Loreo, Mesola, Ostellato, Papozze, Porto Tolle, Portomaggiore, Porto Viro, Ravenna, Rosolina and Taglio di Po.

The current layout of the Po Delta, with the longest stretch of non-man-made sandy shoreline in Italy, is the product of the action of the river which, over the centuries, has continued to settle its alluvial deposits near the mouth, determining the progressive advancement of the coastline. The habitats included from the mainland to the sea configure the Po Delta as a territory with high biodiversity. I is the most important wetland in Italy, a mosaic of variable and unique environments, where it is possible to meet coastal dune systems, sandy developments, wetlands areas, fishing lagoons, sandbanks, river islands, fossil dunes, embankments, canals, river courses, coastal pine forests, riparian forests, floodplains, brackish and freshwater wetlands.

Such a diverse territory supports a very a remarkably high biodiversity of flora and fauna, in terms of species richness, and rarity.

<u>Flora</u>

The Po Delta has a high number of floristic species and a wide variety of plant associations that are very different from each other: from the scarce *Salicornia spp.* grasslands, to the lush woods of farnie, ash and alder, to the Ammophile that colonize the sands of coastal dunes to the reeds that still characterize large tracts of the landscape. Among the most widespread vegetation communities we find halophytes, plants that grow on widespread surfaces that are perpetually or seasonally flooded with salt water. Since the ecological conditions are limiting for the development of vegetation, the flora of the halophilous environments is composed of a small number of highly specialized plants, whose distribution is influenced by the characteristics of the habitat rather than by the climate: these species have characteristics of rarity and peculiarity. Another very peculiar community is that of sandy areas: the coastal dunes of the Delta host a diversified flora, while the consolidated sand dunes and ancient dune cordons present a more varied flora. There are well over a thousand species of vascular flora, of which at least thirty of orchids, distributed according to ecological needs. The most representative testimony of wood is the Gran Bosco della Mesola. The hygrophilous woods are found in the most internal area, in flood areas or near freshwater valleys. The tree cover of these environments is represented by species such as the white Poplar (*Populus alba*), white willow (*Salix alba*) and common elm (*Ulmus minor*).

<u>Fauna</u>

The fauna presents an extraordinary variability in all its components. Aquatic environments and wetlands in general host very characteristic communities, where species with restricted ecological requirements are often found and therefore strongly linked to these habitats. Evaluating over fresh, brackish and salty waters there are over 50 species of fish, among which we mention two interesting euryhaline species: the eel (*Anguilla anguilla*) and the cobice sturgeon (*Acipenser naccarii*), which migrate for reproductive purposes from the sea to inland waters and viceversa.

Among the reptiles and amphibians there are about thirty species, some particularly rare like the Lataste frog (*Rana latastei*) and the pelobate fosco (*Pelobates fuscus*). On the other hand, reptiles include the European pond turtle (*Emys orbicularis*) and Hermann's terrestrial tortoise (*Testudo hermanni*).

The Po Delta is home to over 40 species of mammals. Remarkable is the presence of the Mesola deer (*Cervus elaphus*), the only native population of peninsular Italy.

The birds of the Po Delta made this area becomes the most important Italian ornithological area and one of the most important in Europe. This extraordinary value is due to the great environmental complexity that supports almost 450 birds' species that can be observed during the year.

The delta includes the protected natural areas established in the geographical area of reference:

- The Po Delta Regional Park of Emilia-Romagna - established in 1988, but effective only since 1996, it also includes areas that are part of the water basin of other rivers (including the Reno river). It includes the southern part of the Po river delta, which geologically may be characterised as paleodelta, and a small portion of the present delta;

- The Veneto Regional Park of the Po Delta, effective since 1997, which includes almost the entire recent delta.

## 1.3. Delta fish fauna

Deltas have a central role both for marine aquatic ecosystems and for inland waters, and host marine, migratory, lagoonal and freshwater species (Elliott and Hemingway, 2002, Mc Lusky and Elliott, 2004, Rountree and Able, 2007).

The composition, structure and functionality of deltas' fish fauna, widely used to monitor the quality of these ecosystems (Whitfield and Elliott, 2002; Delpech et al., 2010), are important component of biodiversity and indicators of the ecological integrity of Mediterranean Delta's, including the northern Adriatic ones (Franzoi et al., 2010).

In deltas, fish species exploit the environment according to the wide variety and availability of resources (Elliot and Hemingway, 2002), based on the different capacity of physiological tolerance to the variations of the main environmental parameters, such as temperature and salinity, and according to the different feeding stratgy, reproduction and migratory behavior (McHugh 1967; Dando 1984; Quignard 1984; Elliott and Dewailly, 1995; Elliott et al., 2007). On the basis of these distinctions, fish species can be grouped into different ecological categories, already defined by Root (1967) as groups of species that exploit the same compartment of environmental resources in a similar way. The introduction of functional groups has been an important step in the estimation of functional diversity (Diaz and Cabido, 1997; Lavorel et al., 1997, 1999.; Walker et al., 1999; Wilson, 1999; Camminatore and Langridge, 2002).

These categories are used to provide information on the functional and hierarchical structure of the communities, on the relationships present, such as assembly and coexistence rules, trophic interactions, the redundancy of species or similarities, as well as on the influences that the environmental parameters or the perturbations of the system may involve (Elliot et al., 2007). This has made it possible to simplify the study of the most complex ecosystems (Albaret, 1999;; Lobry et al., 2003; Franco et al., 2006), and provide useful information for the strategies of management of these environments (Elliot et al., 2007). The approach to the classification of fish into categories based on the ways in which they exploit the deltas is currently applied worldwide, adapted to each individual biogeographic area and type of transitional environment (Elliot et al., 2007).

According to the type, frequency and timing of use of the delta environments by the fish species, ecological categories have been defined as ecological categories, namely Estuarine Use Functional Group (Quingrad,

1984; Elliot and Dewailly, 1995; Elliot et al., 2007; Franco et al., 2008). In the northern Adriatic the high diversity of fish species is an important component of the whole biodiversity of the coastal lagoons. Fish populations that distinguish the transitional waters of the Po Delta can be grouped into 6 different ecological categories: marine migratory (*MM* - Marine Migrators), occasional migratory (*MS* - Marine Stragglers), lagoon residents (*ES* - Estuarine Species), migratory anadromous *(A* - Anadromous species) and catadromous (*C* - Catadromous species), freshwater species *(F* - Freshwater species) (Gandolfi et al. 1985; Franzoi et al. 1989; Franco et al., 2006a, 2008a , 2008b)

Marine migratory species (*MM*) are linked to the sea for reproduction, but which perform periodic migrations in lagoon environment. (Rountree and Able, 2007). These migrations can be linked to a precise phase of the biological cycle: the species defined as juvenile migrants use the lagoon as a "nursery" area, exploiting the marine currents for the entrance of the larval stage in the delta wehere they find excellent conditions for growth. Other marine species, the seasonal migrants, occupy the transitional waters with a seasonal periodicity, entering the lagoon mainly in spring and summer months during which they find an abundance of food (Elliot et al., 2007). Among the juvenile migrants we find the sole *Solea solea*, the gilthead *Sparus aurata*, some gobies like *Pomatoschistus minutus*. In some cases, the juvenile, sub-adult and adult stages occupy the transitional waters simultaneously, making cyclical migrations between the marine area and transitional waters: these species are therefore to be considered both juvenile and seasonal migratory. In the lagoons of the Northern Adriatic this behavior is due to the flounder *Platichthys flesus*, the sea bass *Dicentrarchus labrax* and the mullets *Mugil cephalus*, *Chelon labrosus, Liza Aurata, Liza ramad*a and *Liza salies* (Franzoi et al., 1985; Gandolfi et al., 1985, 1991). *Sprattus sprattus*, *Belone belone* belong to the category of seasonal migrants. (Gandolfi et al., 1985; Franco et al., 2006a, 2008b).

Occasional migrants (*MS*) are species whose reproduction is linked to the marine environment and which occupy the transitional waters in an irregular and sporadic manner (Elliot and Dewailly 1995; Elliot et al. 2007), passing most of their life in marine waters. Present in transitional waters with few individuals, they do not show seasonal mingration and are normally found in those lagoon areas most subject to marine influence (Franco et al., 2006). In the lagoons of the northern Adriatic there are mackerel *Scomber scombrus*, *Scophthalmus rhombus*, *Sciaena umbra*, anchovy *Engraulis encrasicolus*, *Boops boops* and others. It should be noticed that euryhaline species are not part of this category, as they are regular migrants of the lagoon environment.

Lagoon residents (*ES*) spend their entire life cycle in the lagoon environment, or at least most of it. This capacity is due to their high degree of tolerance and adaptation to the changing environmental conditions ( e.s salinity and temperature, tides and oxygen concentration) The harsh environment of the lagoon coasts challenges the physiological adaptive mechanisms of fish species (especially larval and egg phases), making it difficult for many of them to remain in these environments. In fact, the number of *ES* species is generally low, compared to the total number of all the species found within the lagoons, and is strictly dependent on

the characteristics of the lagoon environment and of the community itself. (Quignard, 1984, Elliott and Dewailly, 1995, Elliott et al., 2007; Franco et al., 2008). This category includes numerous species of Gobies such as *Pomatoschistus marmoratus*, *P. canestrini, Knipowitschia panizzae, Gobius niger*, *Zosterisessor ophiocephalus*, ather species such as *Atherina boyeri*, pipefish *Syngnathus abaster* and *Aphanius fasciatus*. These species, although belonging to different families, have a life cycle with very similar characteristics (Quignard, 1984; Maccagnani et al., 1985; Franzoi et al., 1989, 1993; Franzoi et al., 1989.1993; Elliott and Hemingway, 2002). The spawning usually takes place in spring and / or summer with increasing temperatures, fixing the eggs to the aquatic vegetation or to the substratum in order to prevent the continuous inflows of spring waters and tidal changes (Dando 1984). In addition, many species belonging to the Gobidas and Singnatidas family offer parental care to the offspring, providing for the care of eggs and larvae. Species such as the dairy and Rio pipefish, althoughif they are lagoon residents, to avoid the low temperatures that characterize the lagoons, they can migrate towards the sea in the late autumn-early winter (Quignard 1984), returning then to the beginning of spring in the lagoon environment for reproduction (Franzoi et al., 1989, 1993).

Migratory anadromous *(A)* reproduce in fresh water and subsequently migrate to the sea, where they spend most of their lives. These species use transition environments as passage areas during migration between the sea and fresh water (Elliot and Hemingway, 2002). Among the anadromous migrants of the Northern Adriatic it is possible to find the *Alosa fallax*, *Petromyzon marinus*, sturgeons such as *Huso huso* and the endemic sturgeon *Acipenser naccarii* (Gandolfi et al. 1985, 1991). It should be that in Italy, in recent decades, the populations of these species have undergone a considerable demographic decline, determined by the constant lowering of water quality, the presence of dams along the main rivers such as the Po and excessive fishing pressure; not least in order of importance, among the causes of decline there is also the strong competition with the new non-native species. In the most recent version of the Red List of Freshwater Fishes of Italy (2013), 2 migratory species are considered "*extinct in Italy*": *Huso huso* and *Acipenser sturio*, two other migrants are considered "*seriously threatened*": *the Petromizon marinus* and *Acipenser naccarii* ; *Alosa fallax* is inserted as "vulnerable".

Migratory catadromas *(C)* spend great part of their life cycle in fresh water, using the transitional waters as a passage during the migration towards the sea, where they go to reproduce (Elliot et al., 2007). In the transitional waters of the Northern Adriatic, only one species is considered a catadromous migrant: the European eel (*Anguilla anguilla*) (Gandolfi et al., 1985, Franco et al., 2008b). "*glass eels*", the juvenile stage, enter in the lagoon, where they remain for most of their lives "*yellow eel*", until they reach sexual maturity at the stage of "*siylver eel*" to return to the sea, ready to play the only reproductive event of his life (Deelder, 1984).

Freshwater species *(F)* arc they occasionally enter transition environments. They can usually be found in oligoaline waters, due to the proximity of freshwater outlet, and in conjunction with the periods when the

rivers are full of water(Franco et al., 2006). Primarily of this category are the cyprinids such as *Alburnus alburnus alborella*, *Abramis brama*, *Carassius carassius*, *Cyprinus carpio*, *Pseudorasbora parva*, or predators such as *Esox cisalpinus* or *Silurus glanis.*

The available knowledge about the ecology of fish species in transition environments allows a distinction according to the functional aspects related both to food preferences and feeding strategies (Feeding mode functional group), and to the reproductive mode (Reproductive mode functional group) (Franco et al ., 2008).

Feeding mode functional group, although feeding opportunism is widely reported for fish species associated with Delta environments (Gerking 1994, Blaber 1997, Wootton 1999, Elliott and Hemingway 2002, Elliott et al., 2007), carateristics factors of a species such as morphology and behavioral restrictions define the items that can be taken from the environment as food, thus influencing the individual's ability to capture certain prey (Franco et al., 2008). External interactions (of a species or individual with the environment and with members of other communities) could also influence their diet (Elliott and Hemingway, 2002, Horn and Ferry-Graham, 2006). For example, the mode of acquisition of food resources can influence the type of prey that can potentially be included in the diet of a species (Franco et al., 2008). On the basis of this, the functional groups concerning the feeding methods are defined by Franco et al. (2008) by combining information on the predominant diet and place of feeding. In this way these groups provide information on the main types of food used by fish within the estuarial environments (eg pelagic, benthic) from which these resources are taken. The reference categories follow.

Bentivores feed mainly on invertebrates associated with the substrate, including organisms that live above sediment (iperbentos), on sediment (epifauna), and between sediment particles (infauna). They can be distingued  in Bmi and BMa in relation the prey size: Bmi if prey is smaller than 1 cm, BMa if larger . In the Northern Adriatic lagoons the taxon gobiidae *Knipowitschia panizzae*, *Pomatoschistis canestrinii* and *P. minutus*, and the singnatids *Syngnathus abaster* and *Syngnathus acus*, are Bmi; *Sparus aurata*, the *Platichtys flesus* and the *Solea solea* are BMa.

*Planctivorous (PL)* species predominantly feed on zooplankton (es. hydroids, planktonic crustaceans, eggs and larvae of fish species) and occasionally phytoplankton in the water column, mostly by filtration. Among these are  *Engraulis encrasicolus* and the sprat *Sprattus sprattus*.

Hyperbentivorous / zooplanctivorous (HZ) feed, just above the bottom, of small mobile invertebrates that live above the sediment and of zooplankton, through techniques of active capture.. The most representative species of this category in the waters of the upper Adriatic is the dairy *Atherina boyeri*.

Hyperbentivorous/ piscivorous (HP) feed mainly on the backdrop of medium and large invertebrates, which live above the substratum, and of small and medium-sized fish. Among these are the gobiid *Gobius niger* and *Gobius paganellus*, *Dicentrarchus labrax*, *Scophthalmus rhombus*, the *Belone belone*, *Psetta maxima* and t *Conger conger* .

Detritivorous species (DV) feed mainly on small organisms present within or on the surface of the particles constituting the substrate (es benthic micro algae such as diatoms, microfauna such as foraminifera and flagellates, and to a lesser extent meiofauna) besides that of associated organic material (normally of vegetal origin); they ingest relatively large volumes of sand or mud through a suction mechanism, digest the edible part and release the inorganic part. These include all the mugilidae species found in the upper Adriatic area, *Mugil cephalus*, *Liza ramada* , *L. saliens*, *L. aurata* and the *Chelon labrosus* .

Herbivorous species (HV) graze mainly on living macroalgae and macrophytes, nourishing them with parts of them or with epiplankton. There are few herbivorous species in the Mediterranean lagoons including *Parablennius sanguinolentus* and  *Sarpa salpa*,

Omnivorous species (OV) ingest both plant and animal matter, feeding mostly on macrophytes, periphytton, epifauna and algal filaments. Among these *Aphanius fasciatus*, *Diplodus annularis* and *Salaria pavo* are found in the lagoons of the Northern Adriatic.

With reference to the Reproductive mode functional group, the peculiarities linked to the choice of the site of laying of eggs and embryos in relation to the characteristics of the site and the degree of parental care are fundamental information for defining the reproductive mode in fish (Elliott and Dewailly 1995, Blaber 1997, Elliott and Hemingway 2002, DeMartini and Sikkel 2006, Elliott et al., 2007). The fish species,  can be divided into oviparous and viviparous, depending on the maternal commitment for the offspring (DeMartini and Sikkel 2006) The oviparous species differ on the basis of the characteristics of their eggs, deposition modalities and the degree of care parents who provide eggs (Franco et al., 2008).

Viviparous species (V) are those species in which fertilization is internal, with growth of the new organism given by a wide range of nutrition modalities (from strictly lecitotrophic viviparity to matrotrophic viviparity). In the lagoons of the Northern Adriatic, no caught fish species are found that have this mode of reproductive behavior (Franco et al., 2008a).

 Oviparous species (O) provide a lecitotrophic maternal supply and external fertilization; zygotes develop outside the maternal environment and are independent of further energy investments by both parents. These species can be divided into:

- Op species that produce pelagic eggs. Among these are found in the lagoons of the Northern Adriatic *Dicentrarchus labrax*, mugilids such as *Liza aurata* and *Liza ramada*, *Platichtys flesus*, *Sprattus sprattus*, *Engraulis encrasicolus* and *Umbrina cirrosa*;

- Ob species that produce eggs laid on the substrate. This category includes the *Mugil cephalus* mullet, the *Sparus aurata* and the *Scophthalmus rhombus*;

- Ov species that produce adhesive eggs that are anchored to the substrate or to the vegetation. This is the behavior of *Aphanius fasciatus*, *Atherina boyeri* and *Belone belone*;

- <u>Og species</u> in which one or the other parent protects the eggs, eg in a nest. Among these are numerous gobiids in the northern Adriatic lagoons, including *Pomatoschistus canestrinii*, *Knipowitschia panizzae* and *Go Zosterisessor ophiocephalus*, *Salaria pavo*;

- <u>Os species</u> that collect their eggs and keep them for a period in a part of their body, for example in a baby carrier or in the mouth, where they develop in the post-larval phase and in juveniles, to then be released in the surrounding waters. Among these, species belonging to the Signatidae family.

## 1.4.    Research questions

The composition, structure and functionality of fish fauna is an important component of biodiversity in transitional ecosystems and, in turn, may be used as indicator of environmental quality (Whitfield and Elliott, 2002; Delpech et al., 2010). This holds particularly true in Mediterranean deltas, including those in the northern Adriatic (Franzoi et al., 2010). Although in recent years, numerous functional aspects of these ecosystems have been studied (Bartoli et al., 2001; Castaldelli et al., 2003; Mantovani et al., 2005), the analysis of structural and functional associations of fish species of the Po Delta river of Emilia Romagna is dated or limited to specific cases for single environments and for individual fish species (Aschonitis et al., 2015). At present, it has fundamental importance to undertake a study that synergically relates the delta fish community, with the variation of physical and environmental parameters and of management practices relative to the exploitment of ecosystem services. This is urged especially in areas as the Po Delta which represents an emblematic case of integration between human exploitation and biodiversity protection, with two Regional Parks, 8 Ramsar sites and numerous Sites of Community Interest and Special Protection Areas (SIC and ZPS) of the network Natura 2000 and the recent recognization of Biosphere Reserve of the Unesco, in 2015.

The general purpose of this study is to update the general status of the fish communities in the Po Delta of Emilia-Romagna, both in composition and trophic structure, and to identify the main problems of fisheries related to the physical evolution of the environment and the change in human activities. The work comprised the taxonomic update and qualitative characterization of fish populations in functional groups, based on the use of habitat, feeding and reproductive strategy. Furthermore, work has been done to characterize growth rates and health status of single species, particularly relevant for conservation and/or commercial interest or newly introduced.

Phases of the study of fish fauna in the Po Delta of the Emilia-Romagnaincluding:

- analysis of the long term trends of the freshwater fish community in the Po River Delta in a climate change scenario

- qualitative characterization of fish species in functional groups, based on the use of habitat, on feeding and reproduction

- analysis of the structural and functional associations of fish species in the Po Delta of the Emilia Romagna
- analysis of feeding and growth performances of some species of particular conservation and/or commercial interest
- taxonomic updating for providing the first fish species inventory and management plan of the Valli di Comachio Lagoon

To answer to the aforementioned questions, different case studies are presented in sections 2 and 3. Each case study addresses a single topic relevant to this thesis and has aready been published as scientific paper.

## References

- Bartoli, M., Nizzoli, D., Viaroli, P., Turolla E., Castaldelli G., Fano E. A., Rossi R. 2001. Impact of Tapes philippinarum farming on nutrient dynamics and benthic respiration in the Sacca di Goro. Hydrobiologia (2001) 455: 203.
- Castaldelli G., Mantovani S., Welsh D. T., Rossi R., Mistri M. & Fano E. A. (2003) Impact of commercial clam harvesting on water column and sediment physicochemical characteristics and macrobenthic community structure in a lagoon (Sacca di Goro) of the Po River Delta. Chemistry and Ecology, 19(2-3): 161-171.
- Cataudella S., Crosetti D., Massa F., 2015. Mediterranean Coastal Lagoons: sustainable management and ineractions among acquacolture, capture fisheries and the environment. FAO, Studies and Reviews, n..95; General Fisheries Commission for The Mediterranean; pp.279.
- Delpech C., Courrat A., Pasquaud S., Lobry J., Le Pape O., Nicolas D., Bodt P., Girardin M., Lepage M., 2010. Development of a fish-based index to assess the ecological quality of transitional waters: the case of French estuaries. Marine Pollution Bulletin, 60 (2010), pp. 908–918
- Deegan, L.A., Hughes, J.E., Rountree, R.A., 2000. Salt marsh ecosystem support of marine transient species. In: Weinstein, M.P., In: Weinstein, M.P., Kreeger, D.A. (Eds.), Concepts and Controversies in Tidal Marsh Ecology. Kluwer Academic Press, Dordrecht, pp. 333e365.
- Elliott M., Whitfield AK, Potter IC., Blaber SJM., Cyrus DP., Nordlie FG., Harrison TD., 2007. The guild approach to categorizing estuarine fish assemblages: a global review. Fish Fish 8:241-268.
- Franco A, Elliott M.,Torricelli.P., 2007.Biodiversity and eco system functioning in coastal and transitional water Estuarine, Coastal and Shelf Science 75 (2007) 1-3.
- Franco A., Elliot M., Franzoi P., Torricelli P., 2008 a. Life strategies of fishes in European estuaries: the functional guild approach. Mar Eco Prog Ser 354: 219-228.
- Franzoi P., Franco A., Torricelli P., 2010. Fish assemblage diversity and dynamics in the Venice lagoon. Rendiconti Lincei - Scienze Fisiche e Naturali, 21 (2010), pp. 269–281
- Gaglio M., Aschonitis V., Gissi E., Castaldelli G., Fano E.A. (2016). Land use change effects on ecosystem services of river deltas and coastal wetlands: case study in Volano-Mesola-Goro in Po river delta (Italy). Wetlands ecology and management (Accepted).
- Gamito S., Erzini K., 2003. Trophic food web and ecosystem attributes of a water reservoir of the Ria Formosa (south Portugal). Ecological Modelling.Volume 181, Issue 4, 10 February 2005, Pages 509–520
- Koutrakis E.T., Tsikliras A.C. 2003. Length-weight relationships of fishes from three northern Aegean estuarine systems (Greece). J. Appl. Ichthyol. 19, 258–260.
- Lanzoni M., Castaldelli G., Caramori G., Turolla E., Fano E.A. e Rossi R., 2010. Popolamenti ittici del Delta del Po. Biologia Ambientale 24 (1): 157-166, 2010.
- Mace, G. M., Norris, K., & Fitter, A. H. (2012). Biodiversity and ecosystem services: a multilayered relationship. Trends in ecology & evolution, 27(1), 19-26.
- Malavasi, S., Fiorin, R., Franco, A., Franzoi, P., Granzotto, A., Riccato, F., Mainardi, D., 2004. Fish assemblages of Venice Lagoon shallow waters: an analysis based on species, families and functional guilds. Journal of Marine Systems 51, 19e31. Kreeger, D.A. (Eds.), Concepts and Controversies in Tidal Marsh Ecology. Kluwer Academic Press, Dordrecht, pp. 333e365.

- McLusky DS and Elliot M. 2004, The estuarine ecosystem: ecology, threats and management, 3rd edn. Oxford University Press, Oxford.
- Mantovani S., Castaldelli G., Rossi R., Fano E.A. (2005). The infaunal community in experimentally-seeded, low and high density Manila clam (Tapes philippinarum) beds in a Po River Delta lagoon (Italy). Accepted by ICES Journal of Marine Science.
- Pe´rez-Ruzafa A., Garcı´a-Charton J.A., Barcala E., Marcos C., 2006. Changes in benthic fish assemblages as a consequence of coastal works in a coastal lagoon: The Mar Menor (Spain, Western Mediterranean).Marine Pollution Bulletin 53 (2006) 107–120
- Pérez-Ruzafa, A., Marcos, C., Pérez-Ruzafa, I., 2011. Mediterranean coastal lagoons in an ecosystem and aquatic resources management context. Physics and Chemistry of the Earth, Parts A/B/C 36 (5e6), 160e166.
- Pérez-Ruzafa, A., Marcos, C., 2012. An assumed but poorly researched aspect of the ecology and functioning of coastal lagoons. Estuarine, Coastal and Shelf Science 110 (2012) 15 e 31.
- Pombo L, Elliott M, Rebelo EJ (2005) Environmental influences on fish assemblage distribution of an estuarine coastal lagoon, Ria de Aveiro (Portugal). Sci Mar 69:143–159
- V. Aschonitis, M. Lanzoni, F. Gelli, R. Rossi, E. A. Fano and G. Castaldelli, 2015. A size-age modelling technique based on bootstrapping and Bayesian approaches to assess the population dynamics of a nearly collapsed Anguilla anguilla L. population in lagoons with fully controlled migration. Ecology of Freshwater Fish 2015; DOI: 10.1111/eff.12269; 28 DEC 2015.
- Villa P., Laini A., Bresciani M., Bolpagni R. (2013). A remote sensing approach to monitor the conservation status of lacustrine Phragmites australis beds. Wetlands ecology and management, 21(6), 399-416.
- Whitfield A. K., Elliott M. 2002. Fishes as indicators of environmental and ecological changes within estuaries: a review of progress and some suggestions for the future. Journal of Fish Biology. DOI: 10.1111/j.1095-8649.2002.tb01773.x.

# 2. FRESH WATER FISH COMMUNITY OF PO RIVER DELTA

## 2.1. Recent evolution of the fish community in the lower Po in a climate change scenario

### 2.1.1. Case study 1. Long-term fish monitoring underlines a rising tide of temperature tolerant, rheophilic, benthivore and generalist exotics, irrespective of hydrological conditions.

Astract

The invasion of exotic species is one of the main threats to worldwide biodiversity and can be aided by changes in environmental conditions. We hypothesized that a temporal trend of decreasing discharge and increasing temperature might have favored the invasion of warm-adapted, lentic exotic fish species in the lower Po River, northern Italy. We used presence/absence data over a long-term period (over 20 years) to investigate the dynamics of exotic fish invasion along water temperature and discharge gradients. Mean annual discharge and temperature did not show a clear trend and did not affect exotic fish species invasion, which progressed with time irrespective of these factors. The total number of species fluctuated without a clear trend, which underlined a progressive substitution of native species with exotic ones. Perhaps surprisingly, the community composition changed over time towards more temperature tolerant but also rheophilic, benthivore and generalist fish species. These results highlight how species interactions could be one of the main factors driving the invasion. Furthermore, our data underlines a continuously rising tide of exotics, which questions the success of past control strategies. Considering the current conservation resources limitations, priority should be given to the development of prevention strategies in order to avoid new species introductions.

Introduction

Species invasions (i.e. the establishment and spread of exotic species) are undoubtedly one of the main factors affecting biodiversity worldwide (Leprieuret al., 2008). It has been widely reported that environmental factors could favor the invasion of certain species (Moyle and Light, 1996a), but it is not yet clear what the magnitude of these effects would be and whether they will equally affect all groups. Some studies have proposed that increased global temperatures could exacerbate the effects of species invasion by allowing the colonization of areas previously unsuitable for exotic species (Hellmannet al., 2008; Rahel and Olden, 2008). This could hold particularly true for poikilotherms, as they depend on external temperature more than omeotherms. Hydrological conditions could also favor the invasion of aquatic species, by providing flow conditions that are more suitable to exotics than natives (Moyle and Light, 1996b; Bunn and Arthington, 2002) but also the opposite can occur (Leprieur et al., 2006). However, since temperature or hydrological

trends can be very subtle to detect in aquatic environments (Woodwardet al., 2010), to perform such an investigation the availability long-term environmental data is of fundamental importance.

Most research on aquatic invasions has focused on their relatively short-term effects (usually <5 years), while the long-term (usually >10 years) dynamics of the invasion processes are still relatively unexplored (Strayer et al., 2006). This is partly due to the fact that exotic species research is a relatively young branch of ecology, so that long-term specific sampling programs have rarely been put in place. However, aquatic systems are especially challenging to sample, as monitoring usually involves higher equipment costs than terrestrial ecosystem sampling. Currently, it is still logistically and financially hard to collect long-term data on an invasion process, therefore the possibility to use data collected for other purposes, as long as suitable for analysis, should always be embraced.

Italy is particularly data-deficient, since fish introductions (i.e. the deliberate or accidental release of an exotic species in a new area) have been recorded (Bianco, 1998, 2014) but the dynamics and outcomes of their invasions have been rarely thoroughly analyzed (with few exceptions, see e.g. Castaldelli *et al*., 2013; Milardiet al., 2018). Yet Italian freshwaters could be a prime model to investigate aquatic invasions: recent studies have underlined that hotspots of exotic fish invasions are present in the Po River drainage in Northern Italy (Lanzoniet al., 2018) and that in this area exotic species play a strong role in shaping the abundance and distribution of native communities even after several years from initial invasion (Milardi *et al*., 2018). It has been previously hypothesized that current distributions, with native species inhabiting smaller streams at higher elevations, are mainly due to exotic species being better adapted to warmer and more lacustrine conditions (Bianco and Ketmaier, 2001), thus outcompeting natives in the lower stretches of rivers through better adaptation. However, most recent studies utilized short-term data, albeit spatially wide, and tried to resolve the results of the invasion rather than its temporal evolution. Utilizing data on fish presence and absence in the lower stretch of the Po River, collected over a long-term (over 20 years), we attempted to investigate the broad dynamics of the latest exotic fish invasions in this area. We selected this river stretch because most fish introductions in this watershed date back to the 1970s and no major habitat alteration has taken place in the last 20 years, thus allowing to analyze the outcomes of interactions and dispersal dynamics with less confounding factors. We hypothesized that a temporal trend of decreasing discharge and increasing temperature could have favored the invasion of lacustrine exotic fish species, which are adapted to warmer waters. We investigated the variation through time in both native and exotic species ecofunctional traits with the aim to detect temporal trends in community composition. We also used linear and multimetric statistical tests to analyze the relationship between species presence, river discharge and temperature. We finally examined the relationship between all species through co-occurrence analysis, and between exotic and native species through nestedness analysis.

## Methods

*Study area*

The Po River is the largest (average discharge 1540m 3s–1) and longest river in Italy (maximum distance 682 km) flowing from the northwestern Alps into the Adriatic Sea, through all northern Italy. Near the Po River delta lies the province of Ferrara (2600 km2), which has an extensive agricultural land use supported by a canal network of more than 4000 km and a mean canal density of 1.53 linear km of canals per km2 of land. From the Po River, water is abstracted and enters this irrigation canal network through water abstraction plants. These plants draw water from the river using siphons, which are opened and closed according to irrigation needs. We collected fish samples for this study from the sedimentation basin of the Berra plant, a concrete basin of 60x30 m (1800 m2) and 3 m deep, with the purpose to avoid siltation of the canals. The basin is fed water through 4 siphons with a diameter of 2 m (without grids), which abstract water by gravity from the lower stretch of the Po River. The water in the basin reaches an irrigation canal through other four siphons (equipped with 5x5 cm grids) and enters the canal network, from where it is distributed for irrigation to the north-eastern part of Ferrara Province (Fig. 1). Siphons are progressively opened between April and March, raising the water level in the sedimentation basin. Siphoning plants let little water through during winter (October-April), thus the water level in the basin is low throughout these months. During spring and through summer, the siphons act as a one-way suction sampler for fauna of the Po River that is close enough to be captured in the siphon suction. Given the diameter of a siphon, fish of all sizes are effectively transported from the river to the sedimentation basin from where individuals cannot return to the Po River due to the strong flow at the siphon. Moreover, with the exception of some small sized individuals <10 cm, the metal grill at the downstream siphons prevents downstream movement as well. Fish escape downstream was also blocked by the action of water pumps, engaged when there was a negative difference between the basin and the canal water levels. These barriers also prevented fish from the irrigation canal network from entering in the basin. During autumn and winter, the water level in the sedimentation basin drops and the basin becomes completely isolated from both the main river and the canal network, effectively trapping all the fish within.

*Fish data collection*

In order to remove the sediment that accumulated during the spring and summer, the Water Management Authority (Consorzio di Bonifica Pianura di Ferrara) completely removed all fish trapped in the basin at the beginning of each autumn (usually October) using seine nets, 1.5 m in height with a 25 m mouth and a knot-toknot mesh size of 8 mm and landing nets, with a knot-to-knot mesh size of 4 mm. Fish were captured in repeated passes and brought up from the basin bottom by using mechanical cranes. At the complete emptying of the basin no specimens were left inside. All fish were identified (according to Kottelat and Freyhof, 2007) and subsampled before being released back into canals where a suitable water level allowed

them to survive. Fish species were either classified as exotic (i.e. introduced by human action) or native (i.e. native to the Po River watershed, including its delta) according to the latest available information. We used fish presence/absence data from these operations, collected from 1994 to 2016, to build a matrix of species occurrence through the years that would be representative of the fish fauna of the lower stretch of the Po River (indicatively from the last tributary, the Panaro River about 40 km upstream of the Berra plant, to the river delta, about 30 km downstream). Unfortunately, fish data were not available for years 2002 and 2010.
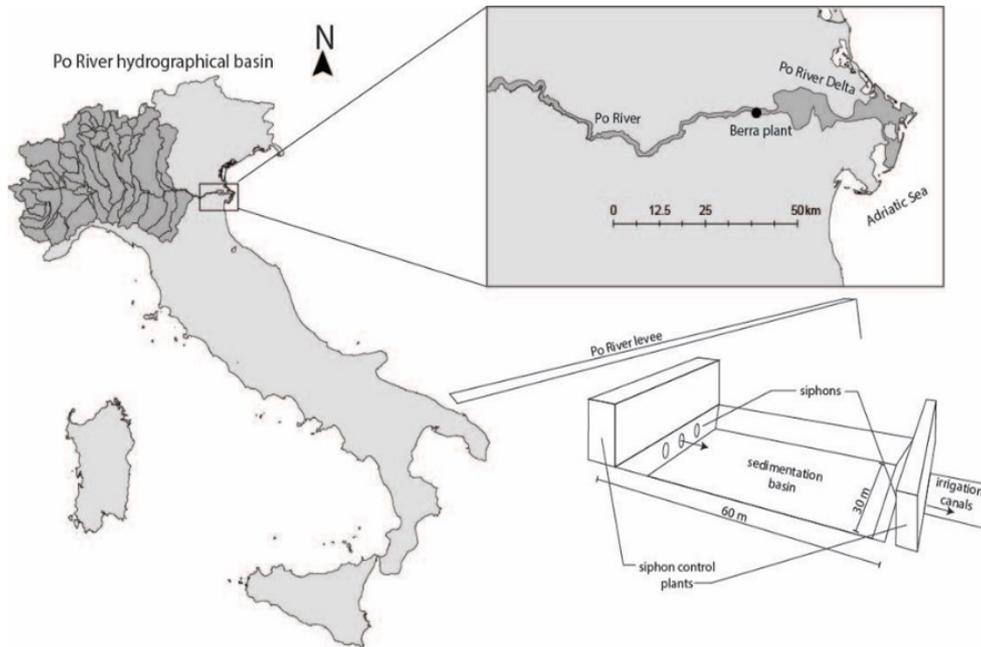


*Figure1 - Map of the study area: sampling location in the upstream section of the Po Delta (Province of Ferrara, Italy), in the Berra plant.*

*Hydraulic and thermal data*

Hydrological data on the discharge and temperature of the Po River were taken from the Regional Agency for Prevention, Environment and Energy of Emilia-Romagna (Agenzia regionale per la prevenzione, l´ambiente e l´energia dell´Emilia-Romagna - ARPAE), at a section close to the Berra plant. Since no tributary or water diversion is present between this section and the plant, the discharge and temperature were representative of the stretch of the main river near the plant. We used this data to calculate mean annual discharges and mean annual temperatures for the period 1994–2016 (discharge was not available for years 2002 and 2010 whereas temperature was not available for 2006). Daily discharge at the Berra plant was obtained from the Water Management Authority, which controls the water abstraction for irrigation purposes. We used this data to evaluate whether interannual variations in water abstraction timing and magnitude could be a disturbing factor in the analysis, using the mean abstraction and its standard deviation in a 6-years period (2011-2015). We also checked that data from this period would be representative for the whole time series (Water Management Authority, personal communication).

*Species ecofunctional traits characterization*

We characterized some ecofunctional traits of all fish species sampled (Milardi and Castaldelli, 2018), with the aim of identifying variations of community composition through time. Fish species were divided into ecofunctional guilds using the information from continuously updated online databases such as FishBase (Froese and Pauly, 2017) or Freshwaterecology.info (Schmidt-Kloiber and Hering, 2015), or through peer-review papers when available. When no information was available expert knowledge was used to fill the gaps, usually assuming that the species would share ecofunctional characteristics with the closest related species for which information was available. Within the first guild, fish were either identified as rheophils (preferring fast flowing water), limnophils (preferring slow or no current) or eurytopic (having no particular preference). Within the second guild, fish species were divided into two mutually excluding categories of tolerance/intolerance to high temperature (indicatively above 20°C). We finally used a feeding ecological function, where we considered the prevalent diet of adult individuals for the definition of categories. This was because most fish species have rather wide trophic niches and exhibit ontogenetic diet shifts. Fish were divided into herbivores (exhibiting specific adaptations for plant feeding, such as pharyngeal teeth), benthivores (exhibiting specific adaptations for bottom feeding, such as downturned mouths or barbels), invertivores (specifically adapted to or predating prevalently on insects and other invertebrates), piscivores (with specific adaptations for feeding largely on fish), and generalists (with unspecialized mouthparts and digestive systems, feeding on a broad range of items).

*Data analysis*

We used the Spearman rank test to investigate the presence of long-term correlations in mean annual discharge and temperature or number of species and share of exotic species. We further used the linear regression to identify the exact relation between variables when a correlation was found. These analyses were performed using R software (R Core Team, 2017). To assess the multivariate relationships among fish species, abiotic parameters (discharge and water temperature) and years of sampling, we considered fish species as dependent variables and abiotic parameters and years as independent ones. We used a Detrended Correspondence Analysis (DCA), in order to select the most appropriate response model for gradient analysis (Ter Braak and Smilauer, 2002; Lepš and Šmilauer, 2003). As a result of DCA, the dominant gradient length was lower than 3 and therefore we chose a Redundancy Analysis (RDA). These analyses were performed using the CANOCO 4.5 for Windows software (Lepš and Šmilauer, 2003). We tested for patterns of community co-occurrence using each year as a replicate community, without specific assumptions on exclusion patterns due to interspecific competition for shared resources (Connor and Simberloff, 1979), as there are multiple other forms of interactions possible among species (e.g. predator/prey). Cooccurrence analysis was performed using R software (R Core Team, 2017) and the "EcoSimR" package 0.1.0 (Gotelli et al., 2015). This analysis provides a so-called C-score index (both simulated and observed) which could be used to assess the level of co-occurrence by comparing it to other datasets (see e.g. Gotelli and Ulrich, 2010; Gotelli et al., 2015).

However, this metric does not distinguish between native or alien species; a low C-score (usually below 10) indicates that species are overall aggregated and its variance and skewness indicate the degree of heterogeneity in the C-scores of the different species and absence of outlier pairs that are highly segregated (positive skewness) or highly aggregated (negative skewness), respectively. In order to quantify the degree of nestedness of fish species assemblages during the period, we used the BINMATNEST software (Rodríguez-Gironés and Santamaría, 2006). Generally, the BINMATNEST provides the nestedness temperatures of the fish populations but in this study, it was used to estimate two types of rankings through the final packed matrix: the ranking of species based on their nesting capacity and the ranking of years based on species population nestedness.

## Results

### *Discharge and temperature as factors for the invasion*

Mean annual discharge and temperature were negatively correlated with each other, albeit poorly (Spearman rank coefficient P<0.05, Discharge=5234.66 (237.51×Temp), Rsqr=0.18). Both discharge and temperature were poorly correlated with time (Spearman rank P=0.352 and P=0.611, respectively); it was therefore impossible to define a clear temporal pattern or trend for these two variables (Fig. 2).
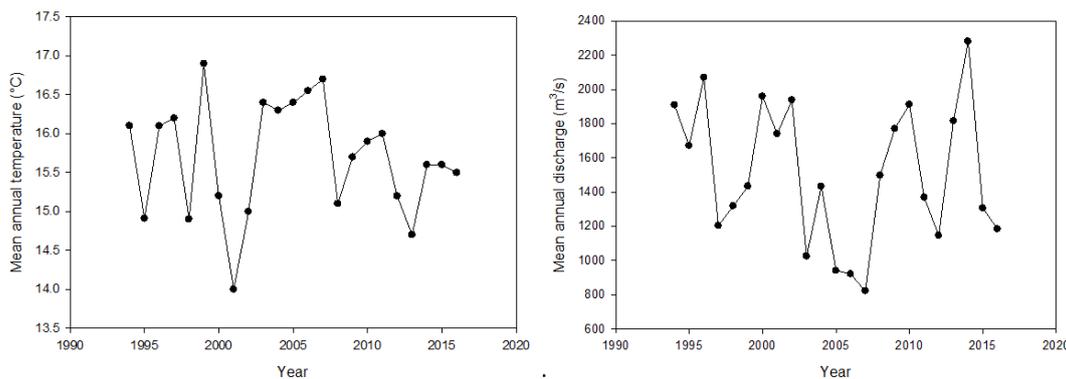


Figure 2 – Plots showing the lack of relation or temporal trends for temperature (a) and discharge (b).

Water abstraction at the Berra plant was rather uniform in timing and magnitude, with a mean annual abstraction of 16,810,720 m3year–1between 2011 and 2015 (S.D. 2,586,945 m3year–1). A matrix of presence/absence for all the fish species found in Berra over the study period was used to further analyze long-term trends in exotic and native species (Supplementary Tab. 1).

Table 1 – Nestedness of different years as derived from the presence of native, exotic and all species.

| Nestedness order | Native | Exotic | Overall | |
|---|---|---|---|---|
| 1 | 1996 | 2006 | 1996 | |
| 2 | 1998 | 2015 | 2006 | |
| 3 | 1999 | 2016 | 1997 | |
| 4 | 1997 | 1996 | 2015 | |
| 5 | 2000 | 2004 | 1998 | |
| 6 | 2006 | 2003 | 2004 | |
| 7 | 1995 | 2007 | 2007 | |
| 8 | 2007 | 1997 | 2003 | |
| 9 | 2001 | 2014 | 2016 | |
| 10 | 2003 | 1998 | 2000 | |
| 11 | 2004 | 2011 | 1999 | |
| 12 | 1994 | 2000 | 2001 | |
| 13 | 2016 | 2013 | 1995 | |
| 14 | 2005 | 2005 | 1994 | |
| 15 | 2009 | 2012 | 2014 | |
| 16 | 2013 | 2001 | 2005 | |
| 17 | 2011 | 2009 | 2013 | |
| 18 | 2015 | 2008 | 2011 | |
| 19 | 2008 | 1995 | 2009 | |
| 20 | 2014 | 1994 | 2008 | |
| 21 | 2012 | 1999 | 2012 | |

This matrix also details common and scientific names of all fish species in this study, as well as their ecofunctional traits in term of temperature, flow preference and trophic ecology. A significant increase in the share of temperature tolerant (vstemperature intolerant) species was observed through the period (Spearman Rank P<0.01, % of temperature tolerant species=-1300.27+0.69×Year, Rsqr=0.53, Fig. 3a). There was also a significant increase in the share of rheophilic species (vslimnophilic species) over the period (Spearman Rank P<0.01, % of rheophilic species=-1286.54+0.65×Year, Rsqr=0.45, Fig. 3a), while eurytopic species did not show a significant change (Spearman rank P=0.61). Finally, among trophic guilds, a decrease in the share of invertivores and an increase in the share of generalists were detected (Spearman Rank P<0.01 for all, and % of invertivores=3025.81-1.50×Year, Rsqr=0.72, % of generalists=1536.24+0.78×Year, Rsqr=0.44, Fig. 3b).
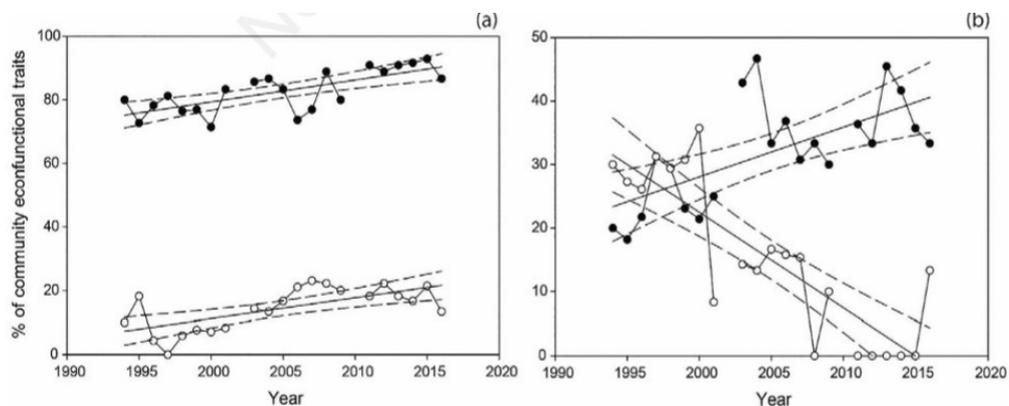


Figure 3 – Significant long-term trends in ecofunctional traits of the fish community of the lower Po River. A rising share of temperature tolerant (black circles) and rheophilic species (white circles) (a), corresponded to a decline in invertivores

(white circles) and an increase in generalists (black circles) (b). The solid lines represent significant linear regressions while the dashed lines represent 95% confidence intervals
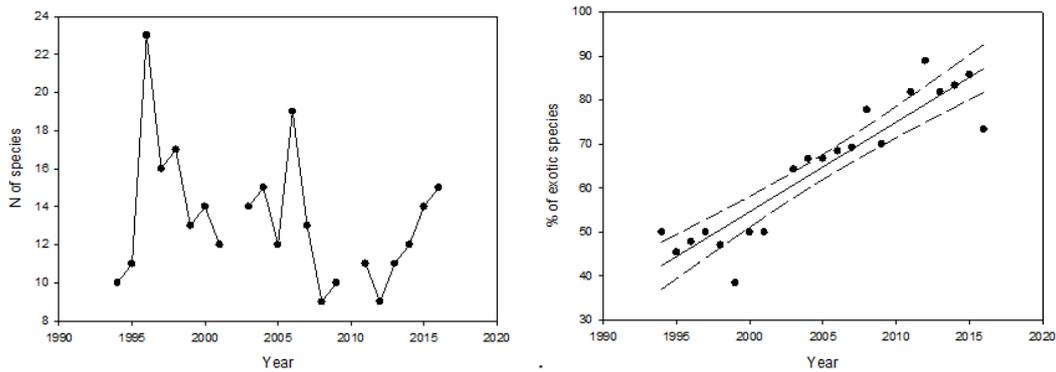


Figure 4 – Long-term trends in the total number of detected species (a) and the share of exotic species in the total (b). The solid line in (b) represents a significant linear regression while the dashed lines represent 95% confidence intervals.

An increase in the share of benthivores was also detected (Spearman Rank P<0.01, % of benthivores=-995.15+0.51×Year, Rsqr=0.32), albeit less marked, while other guilds did not show significant trends (Spearman Rank P=0.58 and 0.13 for herbivores and piscivores, respectively). The total number of fish species within the period was overall fairly stable, did not correlate with mean annual river discharge (Spearman rank P=0.30) and did not show a trend over time (Spearman rank P=0.31, Fig. 4a). Conversely, the share of exotic species showed a clear and significant increase over time (Spearman rank P<0.01, % of exotic species=-4016.76+2.04×Year, Rsqr=0.85, Fig. 4b). The share of exotic species on the total number of species was not related to either temperature or discharge (Spearman rank P=0.52 and P=0.45, respectively, Fig. 5). The results of RDA analysis confirmed that time was the main factor affecting the presence of native fish in the species assemblage (Fig. 6).
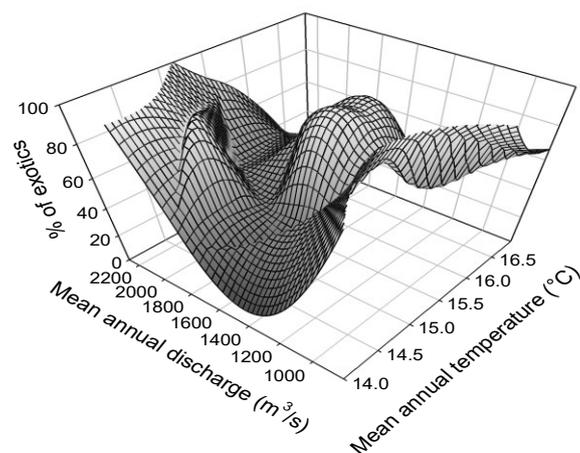


Figure 5 – Tridimensional mesh plot of the percentage of exotic species for different levels of mean annual discharge and temperature, showing no clear constant relation between these variables.
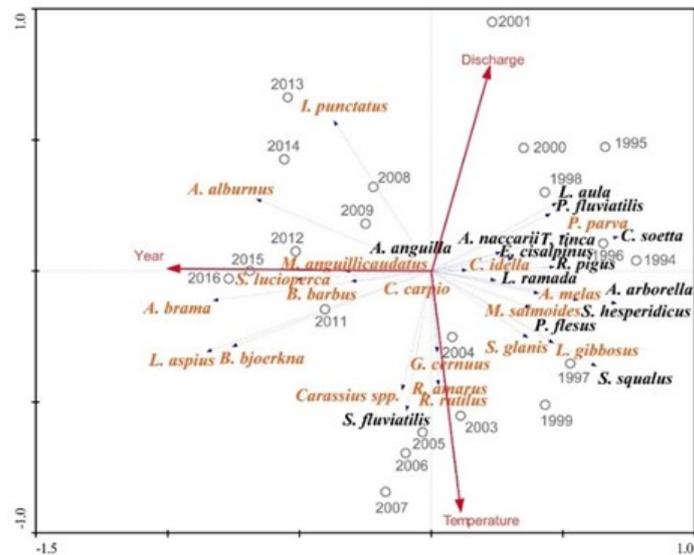
Figure 6 – RDA triplot showing the effects of time (year), discharge and temperature on the fish assemblages of the Berra plant. Sampling years indicated by gray hollow circles, native species by black and exotic species by orange scientific names

*Relationship between exotic and native fish*

There was a relatively high co-occurrence of species through the years, with an observed C-score of 8.46 and a mean simulated C-score of 7.25 (variance >0.01). There was no significant skewness in the metric. Nestedness the beginning of the time series while exotic species were mostly nested in the latter years (Tab. 1). Overall species nestedness was conversely more variable with years 2006 and 2015 ranking in the first positions together with 1996 and 1997 (Tab. 1). Among native species, *Anguilla anguilla* (Linnaeus, 1758), *Liza ramada* (Risso, 1827) and *Squalius squalus* (Bonaparte, 1837) were the most nested while Platychthys. flesus (Linnaeus, 1758), Esox cisalpinus, Bianco & Delmastro, 2011, and *Tinca tinca* (Linnaeus, 1758) the least nested (Tab. 2). Among exotic species, *Cyprinus carpio,* Linnaeus, 1758, *Carassius spp*. and *Silurus glanis*, Linnaeus, 1758, were the most nested while *Micropterus salmoides* (Lacépède, 1803), *Ameiurus melas* (Rafinesque, 1820) and Misgurnus anguillicaudatus (Cantor, 1842) the least nested (Tab. 2). Overall, *A. anguilla, C. carpio and Carassius spp*. were present in nearly all years (Tab. 2).

Tab. 2. Nestedness of native and exotic species alone and the global rank considering all species (Overall)

| Nestedness order | Native | Exotic | Overall |
|---|---|---|---|
| 1 | A. anguilla | C. carpio | A. anguilla |
| 2 | L. ramada | Carassius spp. | C. carpio |
| 3 | S. squalus | S. glanis | Carassius spp. |
| 4 | A. arborella | B. barbus | B. barbus |
| 5 | C. soetta | S. lucioperca | L. ramada |
| 6 | S. hesperidicus | A. brama | S. glanis |
| 7 | P. fluviatilis | L. aspius | S. lucioperca |
| 8 | L. aula | B. bjoerkna | A. brama |
| 9 | A. naccarii | I. punctatus | L. aspius |
| 10 | S. fluviatilis | C. idella | B. bjoerkna |
| 11 | R. pigus | A. alburnus | I. punctatus |
| 12 | P. flesus | P. parva | S. squalus |
| 13 | E. cisalpinus | L. gibbosus | A. arborella |
| 14 | T. tinca | G. cernuus | C. soetta |
| 15 | | R. amarus | S. hesperidicus |
| 16 | | R. rutilus | C. idella |
| 17 | | M. salmoides | L. gibbosus |
| 18 | | A. melas | A. alburnus |
| 19 | | M. anguillicaudatus | P. fluviatilis |
| 20 | | | L. aula |
| 21 | | | A. naccarii |
| 22 | | | G. cernuus |
| 23 | | | P.parva |
| 24 | | | R. amarus |
| 25 | | | R. rutilus |
| 26 | | | P. flesus |
| 27 | | | R. pigus |
| 28 | | | S. fluviatilis |
| 29 | | | M. salmoides |
| 30 | | | A. melas |
| 31 | | | M. anguillicaudatus |
| 32 | | | E. cisalpinus |
| 33 | | | T. tinca |

## Discussion

Prior to this study, not much was known about the fish communities of the lower Po River or their long-term dynamics. Contrarily to our initial hypothesis, it was evident from our data that exotic fish species invasion

progressed with time irrespective of environmental factors such as discharge or temperature, which had no clear trends over the long term. Ecofunctional traits of the fish community shifted towards a larger share of temperature tolerant, rheophilic, generalist and benthivore species, while invertivores declined. Our data also confirmed that the overall exclusion rate was low, but this was probably because there was a progressive substitution of native with exotic fish, whereas the total amount of species was fairly stable.

With few exceptions, native species were nested more strongly at the beginning of the period and progressively declined over time. The results of RDA and nestedness analysis underlined numerous local extinctions in the native fish community of the lower Po River over the 20-year period regardless of water temperature and discharge, while previous studies had found a relation between these abiotic variables and fish abundance or metrics (Daufresne and Boët, 2007; Daufresneet al., 2015). Although abundance or numerical data could be most useful in the assessment of species dynamics (Mertenet al., 2010), such data could not be retrieved at the Berra plant because of the intensified interactions (such as predation) forced by confining fish for prolonged time periods in a small basin with low habitat complexity. Presence absence data was thus the best available option to study species successions, even if it did not allow to explore detailed population dynamics.

While the long period considered was more than sufficient to assess longterm species dynamics, the effects of temperature increase on fish fauna were not clearly evident. Previous studies on climate change impacts on freshwater fauna suggest that a longer study period could be needed to detect temperature trends and their effects (Waltheret al., 2002; Woodwardet al., 2010), even if temperature trends were detected in another large river in northern Italy over a similar period (Salmasoet al., 2016). However, the trends revealed for the fish community could also be explained through other mechanisms not linked to discharge or temperature trends, namely the original environment adaptation of exotics and their human-mediated dispersal. Most exotic species in our study area were native of East Europe, from rivers at higher latitudes and lower average water temperature than the Po River and not warm or tropical species as suggested by other authors (Bianco, 1998; Bianco and Ketmaier, 2001; Woodwardet al., 2010; Daufresneet al., 2015). Therefore, warmer conditions in the host ecosystem could have substituted for warming trends and dispersal could have been partly human-enhanced (e.g. for those species relevant for recreational fisheries), although this aspect has never been thoroughly investigated. The rate of exotic species introduction is a phenomenon of global concern that affects freshwater communities as a whole (Nunes et al., 2015; Seebenset al., 2017), and the presence of these exotics was indeed the consequence of human activities, resulting in both unintentional (e.g. Ictalurus punctatus (Rafinesque, 1820) escaped from aquaculture) and intentional introductions (e.g. *Abramis brama* (Linnaeus, 1758) introduced for recreational fisheries or *Ctenopharyngodon idella* (Valenciennes, 1844) introduced for aquatic plant control (Milardiet al., 2015). A mean of one new exotic species was recorded every three years from 1994 to 2016 in the lower Po River. However, the vast majority of exotic species in our data were detected several years after their introduction

to this watershed and are therefore the result of dispersal and progressive invasion, rather than new introductions. Despite wide fluctuations some of which clearly evident (in 1996 and 2008), the total number of species in our study area did not show a significant trend over time. Although this matter is controversial and largely debated (Herbold and Moyle, 1986; Olden and Rooney, 2006), our data suggested that the total number of species does not increase indefinitely in aquatic ecosystems when more species are added to the community, despite the low co-occurrence typical of fish communities (Gotelli and Ulrich, 2010). On the contrary, the rising share of exotic species indicates that the native fish species were progressively displaced by exotic ones. This could also be at the origin of the detected increase in temperature tolerant, benthivore and generalist species, as several exotics are considered such in the literature and were assigned to these ecofunctional categories. Similarly, the decrease in invertivores could be due to the decline of several native species, while a progressive substitution of native with exotic predators was also observed. Counterintuitively, the increase in rheophilic species (again mostly exotics) did not correspond to a trend in river discharge, but could perhaps still be due to smallscale changes in habitat availability within the river, which cannot be gauged by broad scale parameters. While fish ecofunctional niches tend to be broad (Milardi and Castaldelli, 2018), the rise in exotics could drive the overall changes in ecofunctional traits observed in the community. At this level, it was not possible to identify the respective roles of environmental and invasion factors, so further and more detailed analysis should be used to resolve this. Besides being numerous, exotic species were particularly widespread and were previously found to be one of the most relevant factors negatively affecting the abundance and distribution of native fish communities of the lower Po River plain and its canal network (Castaldelli et al., 2013; Lanzoniet al., 2018; Milardiet al., 2018). However, due to the hydraulics of the siphons at Berra basin, some of these exotic fish have not been recorded in our data. Hypophtalmichthys spp., two pelagic planktivores present in the Po River and in the canal network (Milardiet al., 2017), were not captured. This is probably due to their very low density in the Po River and to their more epipelagic habits (Kolaret al., 2007), while the siphons draw water from a deeper water layer. However, we are confident that all other species present were sampled by the Berra siphons. In fact, species sampled at Berra plant were comparable with species found by other studies in nearby areas, where different sampling methods such as fishing nets or electrofishing were employed (Lanzoni et al., 2018).

## Conclusion

Our data underlines a continuously rising tide of exotics, which questions the success of past control strategies. Past control actions for exotic fish species have faced numerous challenges: temporal and spatial discontinuity, a large area of intervention with a complex hydrographic network, the large overall exotic biomass and also the protests of some local anglers that defend exotic species. We would advise that future control actions should be coordinated between institutions at different spatial scales to be more effective.

On the contrary, illegal fishing pressure targeting mainly exotic large size species (i.e. *S. glanisand C. carpio*) became widespread in the study area since 2010 (Milardiet al., 2015) and has probably had a significant impact on fish communities. The consequent decline of predation pressure from large-sized predators could be the reason behind the recolonization of the lower Po River and canal network by some native fish species (e.g. S. squalus orA. alborella, but careful consideration should be given to species determination as cryptic exotics, *Squalius cephalus, L., and A. alburnus*, could substitute native species and confound detection) but also by some previously less-common exotics (e.g. *I. punctatus*). Considering that conservation resources are limited and control management measures are usually expensive (Britton, Gozlan and Copp, 2011), priority should be given to the development of prevention strategies in order to avoid new species introductions. New and more effective strategies are now, more than ever, required.

## References

- Bianco PG, 1998. Freshwater fish transfers in Italy: history, local changes in fish fauna and a prediction on the future of native populations, p. 165-197. In: I. Cowx (ed.), Stocking and introductions of fishes. Fishing new book. Blackwell Science.
- Bianco PG, 2014. An update on the status of native and exotic freshwater fishes of Italy. J. Appl. Ichthyol. 30:62-77.
- Bianco PG, Ketmaier V, 2001. Anthropogenic changes in the freshwater fish fauna of Italy, with reference to the central region and Barbus graellsii, a newly established alien species of Iberian origin. J. Fish Biol. 59:190-208.
- Bunn SE, Arthington AH, 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environ. Manage. 30:492-507.
- Castaldelli G, Pluchinotta A, Milardi M, Lanzoni M, Giari L, Rossi R, Fano EA, 2013. Introduction of exotic fish species and decline of native species in the lower Po basin, northeastern Italy. Aquat. Conserv. 23:405-417.
- Connor EF, Simberloff D, 1979. The assembly of species communities: chance or competition? Ecology 60:1132-1140.
- Daufresne M, Boët P, 2007. Climate change impacts on structure and diversity of fish communities in rivers. Global Change Biol. 13:2467-2478.
- Daufresne M, Veslot J, Capra H, Carrel G, Poirel A, Olivier JM, Lamouroux N, 2015. Fish community dynamics (1985,2010) in multiple reaches of a large river subjected to flow restoration and other environmental changes. Freshwater Biol. 60:1176-1191.
- Froese R, Pauly D, 2017. FishBase. Version 03/2017. Available from: www.fishbase.org Gotelli NJ, Hart EM, Ellison AM, 2015. Co-occurrence analysis. EcosimR. Available from: http://ecosimr.org Gotelli NJ, Ulrich W, 2010. The empirical Bayes approach as a tool to identify non-random species associations. Oecologia 162:463-477.
- Hellmann JJ, Byers JE, Bierwagen BG, Dukes JS, 2008. Five potential consequences of climate change for invasive species. Conserv. Biol. 22:534-543.
- Herbold B, Moyle PB, 1986. Introduced species and vacant niches. Am. Nat. 128:751-760. Kolar CS, Chapman DC, Courtenay WR Jr, Housel CM, Williams JD, Jennings DP, 2007. Bigheaded carps: a biological synopsis and environmental risk assessment. American Fisheries Society, Bethesda: 204 pp.
- Kottelat M, Freyhof J. 2007. Handbook of European freshwater fishes. Publications Kottelat: 646 pp.
- Lanzoni M, Milardi M, Aschonitis V, Fano EA, Castaldelli G, 2018. A regional fish inventory of inland waters in Northern Italy reveals the presence of fully exotic fish communities. Eur. Zool. J. 85:1-7.
- Leprieur F, Beauchard O, Blanchet S, Oberdorff T, Brosse S, 2008. Fish invasions in the world's river systems: When natural processes are blurred by human activities. PLoS Biol. 6:e28.
- Leprieur F, Hickey M, Arbuckle C, Closs G, Brosse S, Townsend C, 2006. Hydrological disturbance benefits a native fish at the expense of an exotic fish. J. Appl. Ecol. 43:930-939.
- Lepš J, Šmilauer P. 2003. Multivariate analysis of ecological data using CANOCO. Cambridge University Press.
- Merten EC, Hemstad NA, Eggert S, Johnson L, Kolka RK, Newman RM, Vondracek B, 2010. Relations between fish abundances, summer temperatures, and forest harvest in a northern Minnesota stream system from 1997

to 2007. Ecol. Freshw. Fish 19:63-73. Milardi M, Aschonitis V, Gavioli A, Lanzoni M, Fano EA, Castaldelli G, 2018. Run to the hills: exotic fish invasions and water quality degradation drive native fish to higher altitudes. Sci. Total Environ. 624:1325-1335.

- Milardi M, Castaldelli G, 2018. A novel approach to an ecofunctional fish index for Mediterranean countries. Ecol. Indic. 89C:376-385.

- Milardi M, Chapman D, Lanzoni M, Long JM, Castaldelli G, 2017. First evidence of bighead carp wild recruitment in Western Europe, and its relation to hydrology and temperature. PloS One 12:e0189517.

- Milardi M, Lanzoni M, Kiljunen M, Torniainen J, Castaldelli G, 2015. Natural recruitment contributes to high densities of grass carp Ctenopharyngodon idella (Valenciennes, 1844) in Western Europe. Aquat. Invasions 10:439-448.

- Moyle PB, Light T, 1996a. Biological invasions of fresh water: empirical rules and assembly theory. Biol. Conserv. 78:149-161.

- Moyle PB, Light T, 1996b. Fish invasions in California: do abiotic factors determine success? Ecology 77:1666-1670.

- Nunes AL, Tricarico E, Panov VE, Cardoso AC, Katsanevakis S, 2015. Pathways and gateways of freshwater invasions in Europe. Aquat. Invasions 10:359-370.

- Olden JD, Rooney TP, 2006. On defining and quantifying biotic homogenization. Global Ecol. Biogeogr. 15:113-120.

- R Core Team, 2017. R: A language and environment for statistical computing. Vienna. Available from: www.R-project.org

- Rahel FJ, Olden JD, 2008. Assessing the effects of climate change on aquatic invasive species. Conserv. Biol. 22: 521-533.

- Rodríguez-Gironés MA, Santamaría L, 2006. A new algorithm to calculate the nestedness temperature of presence–absence matrices. J. Biogeogr. 33:924-935.

- Salmaso F, Quadroni S, Gentili G, Crosa G, 2016. Thermal regime of a highly regulated Italian river (Ticino River) and implications for aquatic communities. J. Limnol. 76:23-33. Schmidt-Kloiber A, Hering D, 2015. www. freshwaterecology. info - An online tool that unifies, standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. Ecol. Indic. 53:271-282.

- Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, Winter M, Arianoutsou M, 2017. No saturation in the accumulation of alien species worldwide. Nature Comm. 8:14435.

- Strayer DL, Eviner VT, Jeschke JM, Pace ML, 2006. Understanding the long-term effects of species invasions. Trends Ecol. Evol. 21:645-651.

- Ter Braak CJ, Smilauer P, 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). Available from: www.canoco.com

- Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJ, Fromentin J-M, Hoegh-Guldberg O, Bairlein F, 2002. Ecological responses to recent climate change. Nature 416:389-395.

- Woodward G, Perkins DM, Brown LE, 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. Phil. T. R. Soc. B 365:2093-2106.

## 2.2. The continous invasion and spread of exotic species

### 2.2.1.Case study 2. A bimodal weight-length relationship for Alburnus alburnus (Linnaeus, 1758).

Abstract

The aim of this study is to present a linearized biexponential model (LinBiExp) that identifies the specific coordinates of a probable breakpoint in the log-transformed weight-length data of fish through a bilinear curve. The analysis was performed using W-L measurements of bleak Alburnus alburnus (Linnaeus, 1758) specimens captured in freshwaters of the Province of Ferrara (Italy). The LinBiExp function identified the breakpoint coordinates in the specific dataset. The level of uncertainty in the breakpoint coordinates was analyzed based on bootstrap regression and confidence intervals analysis. The robustness of bilinearity in the log-log plot was verified through the comparison of slopes and intercepts (ANCOVA) of the two linear segments. The study showed that the LinBiExp function can be a valuable tool for detecting the absolute coordinates of a breakpoint in log-transformed W-L data, while the proposed methodology can increase the robustness of W-L analysis of fish using the typical power function.

Introduction

The most widely used function for the description of weight-length relationships (WLRs) of fish is the typical power function (W=aLb) and its respective linear form using log-transformed data (Froese 2006). Significant element of the power function is that its coefficients (a, b) or [log(a), b] for various fish species are stored in FishBase (www.fishbase.org). These coefficients have been widely used in biology, ecology and fisheries management providing significant information about growth allometry and the degree of well-being of a species in its habitat (Bolger & Connolly 1989, Lima-Junior et al. 2002, Gomiero & Braga 2005, Acarli et al. 2014).

The derivation of WLRs for fish species may seem a simple technical procedure but there are many issues associated with their derivation (Froese 2006, Froese et al. 2011). One of the most important issues is that smaller/younger specimens may present different WLR from the larger/older ones. The most important justification about this difference is the change in energy allocation between somatic growth and sexual maturation (e.g. development of reproductive organs), which can be expressed by a biphasic (Stergiou & Fourouni 1991, Simonović et al. 2000, 2011, Milardi et al. 2014, Wilson et al. 2018) or multi-phasic growth (Bervian et al. 2006, Hashiguti et al. 2018), where any change in growth rate can easily be visualized by a breakpoint in length-age (Wilson et al. 2018) or weight-length data (Lanzoni et al. 2018). However, the

intensity degree, and a possible delay or speeding up in the occurrence of a breakpoint may also be regulated by other factors such as population density, environmental and trophic state of aquatic environments, which are related to food type and availability (Lanzoni et al. 2018, Matthias et al. 2018). For example, Lanzoni et al. (2018) showed that the breakpoint occurrence in the WLR data of European eel (Anguilla anguilla, Linnaeus, 1758) from Comacchio lagoon in Italy was associated with a simultaneous initiation of sexual maturation and an ontogenetic diet shift (selection of food types of larger dimensions and of different energetic content).  Another justification for differences in WLRs between smaller/younger and larger/older specimens may be the low and not representative number of specimens due to gear/net selectivity (Bevacqua et al. 2009, Wilson et al. 2015).

A procedure to solve the issue of bi- or multi- phasic growth in weight-length (W-L) data is to describe the WLR using the typical power function after breaking the data in subsets based on visual inspection/expert judgment. An alternative methodology proposed by Lanzoni et al. (2018) for analyzing biphasic growth was the use of the linearized biexponential model (LinBiExp) of Buchwald (2007) on the log-transformed W-L data. This model describes the complete dataset and has the ability to identify the specific coordinates of a breakpoint between two linear segments at the log-transformed W-L data. The first application of the LinBiExp model for analyzing W-L data of fish was made for the European eel (*Anguilla anguilla*) (Lanzoni et al. 2018).

The aim of this study is to highlight the applicability of the LinBiExp model for analyzing weight-length data of bleak *Alburnus alburnus*, which has much smaller dimensions and completely different allometric growth from European eel. The application of LinBiExp model is followed by specific methodological steps that are used to verify the biphasic growth of the specific species.


Data

The analysis presented in this study is based on W-L measurements of 101 bleak *Alburnus alburnus* (Linnaeus, 1758) specimens. The specimens were captured in inland waters of the Province of Ferrara (Italy) (Po river, Canale Napoleonico, Canale Boicelli, C.E.R.) during the period 2014-2016. The specimens were captured using electrofishing (7.5 kW  with DC voltage of 600 V) following the protocols of fish sampling developed by ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale–HigherInstitute for Environmental Protection and Research) ([http://www.isprambiente.gov.it/files/pubblicazioni/manuali-lineeguida/metodi-biologici-acque/](http://www.isprambiente.gov.it/files/pubblicazioni/manuali-lineeguida/metodi-biologici-acque/) fiumi-fauna.pdf), which are compatible to the Directive 2010/63/EU. The species were identified according to Kottelat and Freyhof (2007). The ranges of observed length and weight were 5.5-20.2 cm and 8.4-63.5 g. The maximum observed weight exceeds the maximum weight given in FishBase for the specific species (last assessed in 8/5/2018).

## Length-weight functions

The WLR analysis was performed using the typical power function and the linearized biexponential model (LinBiExp) proposed by Buchwald (2007).

The typical power function and its respective log-transformed formula are the following:

$$W = a\,L^b \quad \text{and} \quad \log(W) = \log(a) + b\log(L) \qquad \text{(1a, b)}$$

where W: is the weight (g), L: is the length (cm) and a, b: are regression coefficients. The user can choose one of the two cases depending on the data attributes. The LinBiExp model provides smooth and fully parametrizable transitions between two linear segments maintaining a clear connection between them. The LinBiExp fitting is performed using the log-transformed W-L data and it is given by the following function (Buchwald 2007):

$$W' = f\left(L'\right) = c \cdot \log\left\{ exp\left[ a_1\left(L' - L_t'\right)/c \right] + exp\left[ a_2\left(L' - L_t'\right)/c \right] \right\} + d \quad \text{for } c \neq 0 \qquad \text{(2)}$$

where W′, L′: are the log-transformed weight (g) and length (cm), respectively, a1, a2: are coefficients that regulate the slopes of the two linear segments, c: is a parameter for adjusting the smoothness/abruptness of the transition and the orientation of the angle between the two linear segments, d: is a constant for shifting the curve along the vertical axis (log-W axis), and Lt′: is a constant that defines the break point between the two linear segments at horizontal axis (log-L axis). The transition between the two linear segments does not require a sharp break-point. It can take place along a smooth, continuously differentiable, curved portion of adjustable width (with the deviation from linearity having an exponential character). Nevertheless, a model should be considered bilinear only if it shows linearity at both ends of its considered range (Buchwald 2007). The angle degrees are regulated by the a1/a2 ratio and when it is equal or close to 1 indicates that bilinearity hardly exists. Thus, higher deviation of the ratio from value 1 indicates higher degree of bilinearity. The only difference between the two exponent factors inside Eq. 2 are the values of a1 and a2 coefficients and thus exchanging their values does not change the curve (e.g. for a1=1 and a2=4 or a1=4 and a2=1, the curve remains the same). Changing the sign of the c parameter rotates the bilinear curve by 180 degrees. The larger the absolute value of c is, the smoother is the transition between the two linear segments. The c coefficient is generally sensitive and a large variation of its values only in a positive or only in a negative range just affects the steepness in the transition between the two linear segments. For this reason, a high statistical significance for the case of c coefficient is not required but a stable sign it does.

The coordinates (x′, y′) of the breakpoint in the log(W)-log(L) plot are provided by the following functions:

for log-*L* axis: $x' = L_t'$ and for log-*W* axis: $y' = W_t' = c\log(2) + d$ (3a, b)

The respective coordinates (x, y) of the breakpoint in regular weight-length curves after removing the logarithmic transformation are provided by:

$$x = L_t = 10^{x'} \quad \text{and} \quad y = W_t = 10^{y'} \quad \text{(4a, b)}$$

*Steps of analysis*

Step 1: Assessing the coefficients of the LinBiExp and the coordinates of the breakpoint

The first step of the analysis includes simple non-linear regression (Simple-NLR) of LinBiExp (Eq. 2) for the calculation of its coefficients and the respective coordinates of the breakpoint. Identification and removal of outliers or influential points are optional but they can significantly improve the performance of LinBiExp. Simple-NLR of LinBiExp (Eq. 2) was performed based on the 101 pairs of log-transformed weight-length data. The fitting analysis together with outliers and influential points' detection was performed using StatGraphics Centurion XV software (Statpoint Technologies, Warrenton, VA, USA). Those observations with studentized residuals greater than 2 in absolute value were considered outliers (standard procedure of the aforementioned software). The influential points were assessed considering the average leverage and Cook's distance. As influential points are considered those showing five times larger value of Cook's distance from the average leverage (standard procedure of the aforementioned software). The aforementioned thresholds for identifying outliers and influential points can change depending on the data attributes and user's needs. Influential points were not detected but two outliers were detected and removed. The Simple-NLR was repeated for re-assessing the coefficients of LinBiExp. The clear bilinear response provided by the data (a1/a2=0.164) is visualized in the LinBiExp graph with 99 observed values (Fig. 1a). The visualization of LinBiExp and W-L data after removing the logarithmic transformation from all elements of Fig. 1a is also given in Fig. 1b. The coordinates of the breakpoint were Lt=12.39 cm and Wt=14.63 g.
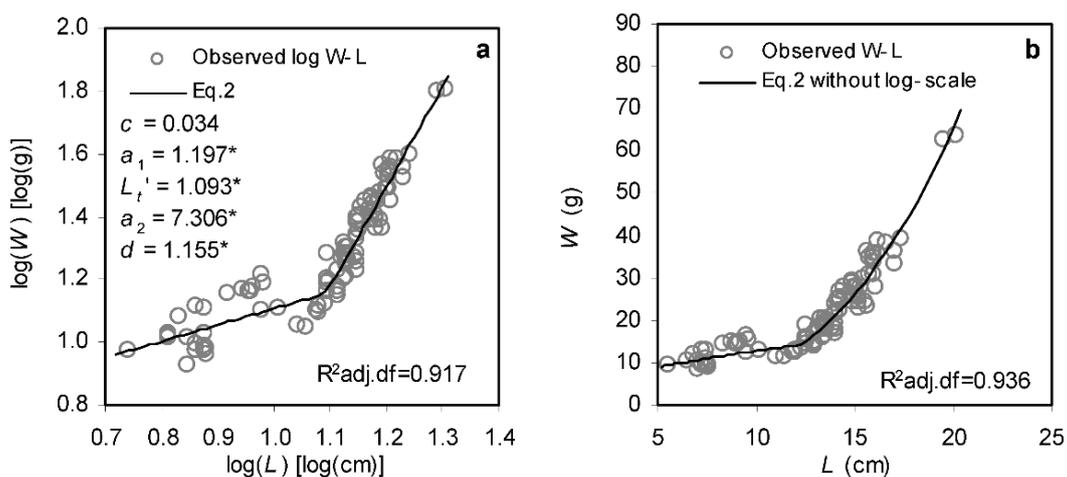


Fig. 1. (a) Simple-NLR of LinBiExp (Eq. 2) based on 99 pairs of log-transformed W-L data after excluding 2 outliers (* denotes statistical significance of the coefficients at P<0.05 ), (b) Visualization of LinBiExp (Eq. 2) versus the observed W-L data after removal of logarithmic scale from all elements of Fig. 1a.
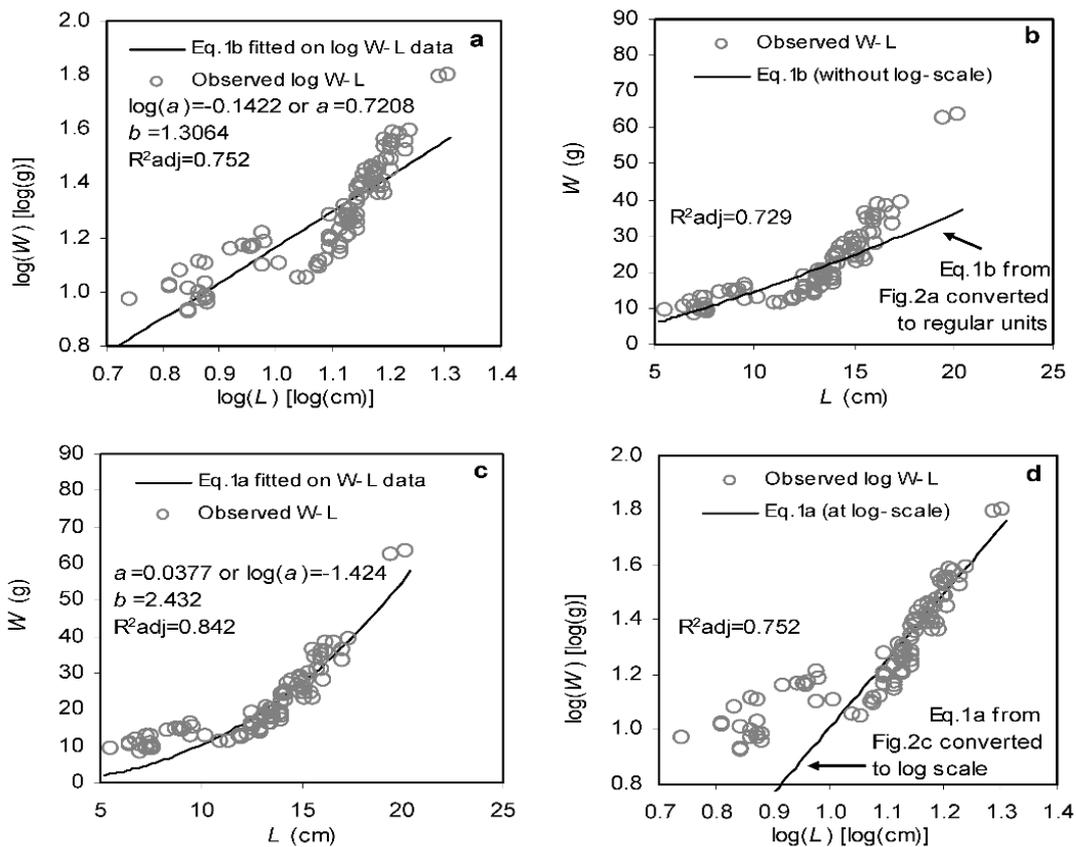
Fig. 2. (a) Eq. 1b fitted on log-transformed W-L data, (b) visualization of Fig. 2a after removing the logarithmic scale from all elements, (c) Eq. 1a fitted on W-L data and (d) visualization of Fig. 2c at logarithmic scale.

For comparative purposes, the limited performance of Eq. 1 to describe the dataset is presented in two ways: (a) using simple linear regression (Simple-LR) of Eq. 1b versus the log-transformed W-L data (Fig. 2a), which is also visualized after removing the logarithmic scale from all elements in Fig. 2b, and (b) using simple non-linear regression (Simple-NLR) of Eq. 1a versus the W-L data (Fig. 2c), which is also visualized at logarithmic scale in Fig. 2d.

Step 2: Assessing the level of uncertainty in the breakpoint attributes based on bootstrap regression of LinBiExp and confidence intervals' analysis

The second step of the analysis is performed for assessing the level of uncertainty in the bilinear response (i.e. a1/a2 ratio) and the breakpoint coordinates (Lt, Wt) based on their variance, which is estimated though bootstrap non-linear regression (Boot-NLR) and analysis of confidence intervals based on the highest posterior density (HPD) distribution. Boot-NLR is based on the generation of a large number of new datasets by randomly sampling data with replacement (Efron & Tibshirani 1994) and it is considered among the most robust methods for assessing the variability of regression coefficients.

In this study, the Boot-NLR was performed by applying the "nls. lm" function of the {minpack.lm} package (Elzhov et al. 2016) in R software. The "nls. lm" function uses the Levenberg-Marquardt non-linear least-squares algorithm. The Boot-NLR of LinBiExp (Eq. 2) was performed based on the 99 pairs of log-transformed

weight-length data for 1,000 iterations that led to a respective number of (a1, a2, c, d, Lt′) solutions. The 1,000 bootstrap sets of coefficients were then used to assess the respective values of a1/a2 ratio and the coordinates of the breakpoint (Lt, Wt) based on Eqs. 3 and Eq. 4 for each bootstrap case. The range of bootstrap estimations of the regression coefficients but also of a1/a2, Lt and Wt, was defined by the 95% confidence interval, which was estimated based on the probability distribution of their 1,000 estimations. This method was applied in order to estimate the values of the HPD distribution that indicates the 2.5% and 97.5% thresholds (HPD thresholds), which contain the central 95% of the HPD distribution. The probability interval was computed using the "p.interval" of {LaplacesDemon} package (Bernardo 2005) in R software. The statistics and the HPD intervals of a1, a2, c, d, Lt′, a1/a2, Lt and Wt are given in the Table 1. For a1/a2, Lt and Wt are also given the HPD distribution graphs (Fig. 3).

Table 1 shows that all the parameters, except c coefficient, present a restricted range of variation according to HPD thresholds, which suggests a robust performance of the function. As it was mentioned in section 3, the large variance in the values of c coefficient is not a serious problem since its bootstrap estimates fluctuate only in a positive range, while the parameter of Wt (which includes c according to Eq. 3b, 4b) shows small variance, which is also verified by Fig. 3.

Table 1. Statistics of LinBiExp parameters based on the 1,000 bootstrap values from Boot-NLR.

| Parameter | $c$ | $a_1$ | $L_t'$ | $a_2$ | $d$ | $a_1/a_2$ | $L_t$ (cm) | $W_t$ (g) |
|---|---|---|---|---|---|---|---|---|
| Mean | 0.0184 | 1.2182 | 1.0940 | 7.3222 | 1.1573 | 0.166 | 12.42 | 14.57 |
| Minimum | 0.0012 | 0.7434 | 1.0745 | 6.6148 | 1.1085 | 0.101 | 11.87 | 12.86 |
| Maximum | 0.0950 | 1.7869 | 1.1174 | 8.2024 | 1.2203 | 0.245 | 13.10 | 17.18 |
| 2.5% HPD interval | 0.0014 | 0.9209 | 1.0809 | 6.8973 | 1.1224 | 0.128 | 12.05 | 13.25 |
| 97.5% HPD interval | 0.0518 | 1.5721 | 1.1103 | 7.7591 | 1.2016 | 0.215 | 12.89 | 15.92 |
| Standard deviation | 0.0193 | 0.1691 | 0.0076 | 0.2233 | 0.0201 | 0.023 | 0.22 | 0.74 |
| Coef. Variation % | 104.5% | 13.9% | 0.7% | 3.0% | 1.7% | 13.6% | 1.7% | 5.1% |
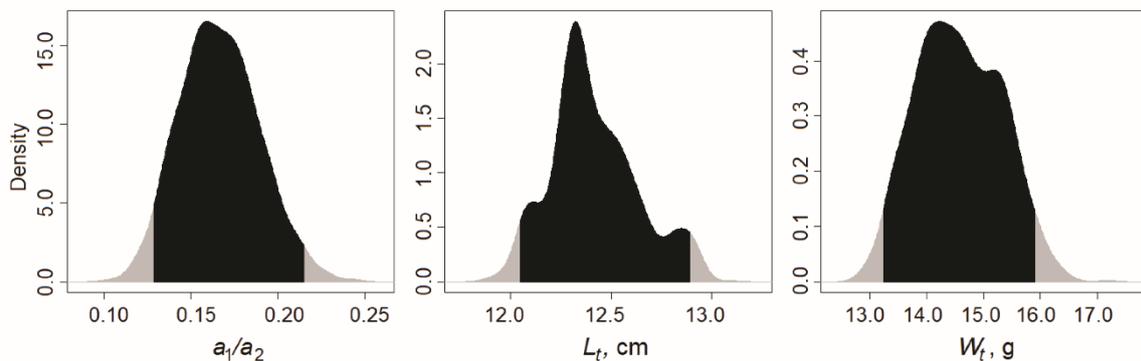


Fig. 3. HPD distribution graphs of the a1/a2, Lt, and Wt parameters (the central black portion of each graph describes the 95% central part of the distribution).

Step 3: Assessing the statistical significance of bilinearity through comparison of slopes and intercepts between the two linear segments

The third step examines the statistical robustness of the breakpoint and bilinearity by investigating if the slopes (b) and intercepts log(a) of Eq. 1b are statistically different between the two linear segments defined by the breakpoint of Eq. 2. Considering the above, the dataset was divided into two subsets using the breakpoint coordinates obtained from step 1 (Lt=12.39 cm, Wt=14.63 g). Thus, any observed pair of (W, L) with W>14.63 g and L>12.39 cm was included in subset "Large" (63 larger specimens) and the rest were included in subset "Small" (37 smaller specimens). Using a covariate that denotes the subset (i.e. "Large" or "Small"), a general linear regression analysis followed by ANCOVA was performed based on the following model:

$$\log(W) = \beta_0 + \beta_1 \log(L) + \beta_2 \, \text{COVLS} + \beta_3 \log(L) \, \text{COVLS} \qquad (5)$$

where the covariate term COVLS is equal to 1 for the subset "Small" and equal to 0 for the subset "Large". The analysis was performed in StatGraphics Centurion XV software (Statpoint Technologies, Warrenton, VA, USA). The results of the analysis showed that the coefficients and the model of Eq. 5 are all statistically significant at 0.001 level (Table 2a, b). The fitting performance of Eq. 5 showed R2 adj.df=0.922. Based on the estimated values of the coefficients of Table 2a, Eq. 5 forms two lines equivalent to Eq. 1b, which are given in Fig. 4 (the coefficients are symbolized as b and log(a) according to Eq. 1b) . The ANCOVA in Table 2c shows that the slopes and intercepts of these two lines are statistically different at 0.001 level, verifying the robustness of bilinearity.

Table 2. Results of the general linear regression analysis and ANCOVA for Eq. 5 using a covariate that separates the data based on the estimated breakpoint of LinBiExp.

| a) Coefficient | Estimate±SE | t-statistic | P-Value | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| $\beta_0$ | -2.154±0.186 | -11.5738 | <0.001 | | | |
| $\beta_1$ | 3.039±0.160 | 19.0521 | <0.001 | | | |
| $\beta_2$ | 2.770±0.204 | 13.6111 | <0.001 | | | |
| $\beta_3$ | -2.554±0.182 | -14.0558 | <0.001 | | | |
| | | | | | | |
| | | | | | | |
| b) Source | Sum of Squares | Df | Mean Square | F-Ratio | P-Value | |
| | | | | | | |
| Model | 3.4395 | 3 | 1.1465 | 388.19 | <0.001 | |
| Residual | 0.2806 | 95 | 0.0030 | | | |
| Total (Corr.) | 3.7201 | 98 | | | | |
| | | | | | | |
| | | | | | | |
| c) Source | Sum of Squares | Df | Mean Square | F-Ratio | P-Value | |
| | | | | | | |
| log(L) | 2.8155 | 1 | 2.8155 | 953.27 | <0.001 | |
| Intercepts | 0.0406 | 1 | 0.0406 | 13.74 | <0.001 | |
| Slopes | 0.5835 | 1 | 0.5835 | 197.56 | <0.001 | |
| Model | 3.4395 | 3 | | | | |
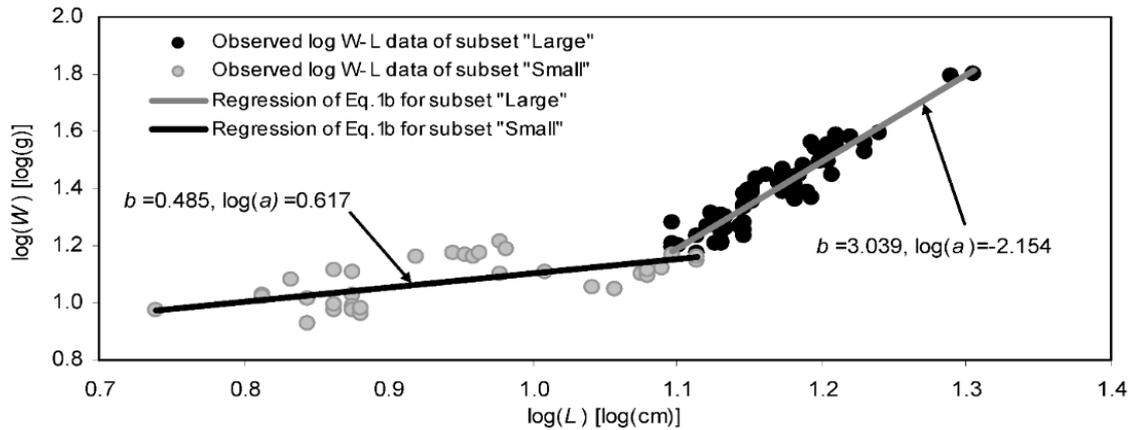
Fig. 4. Comparison of slopes b and intercepts log(a) of the regression lines (Eq. 1b) produced by the subsets "Large" and "Small".

*Comparative analysis based on log(a) vs. b graph using coefficients from other studies*

Finally, the four pairs of derived [b, log(a)] coefficients of Eq. 1 for Alburnus alburnus, which are given in Fig. 2a, 2c and Fig. 4, were compared with other fifteen pairs of respective coefficients obtained from other studies. Twelve of these pairs were derived from FishBase (last accessed in 31/6/2018) and concern studies conducted in Greece (Crivelli & Dupont 1987, Politou 1993, Kleanthidis et al. 1999, Koutrakis & Tsikliras 2003), Spain (Miranda et al. 2006, Leunda et al. 2006), Belgium (Verreycken et al. 2011) and UK (Mathews 1971). Another three pairs of the coefficients were also derived from studies conducted in Russia (Interesova & Chakimov 2015), Poland (Kompowski 2000) and Bulgaria (Raikova-Petrova et al. 2009) that are not included in FishBase. The comparison of [b, log(a)] pairs was performed using the graph of log(a) vs. b and it is given in Fig. 5. Based on Fig. 5, the derived coefficients for the subset "Large" (grey line in Fig. 4) are almost in the middle of the range values defined by the pairs from the other studies. On the other hand, the two pairs of coefficients that were obtained using all the data (Fig.2a and 2c), and especially the coefficients of subset "Small" (black line in Fig.4), are at the edge or completely out of the regular range defined by the other studies.
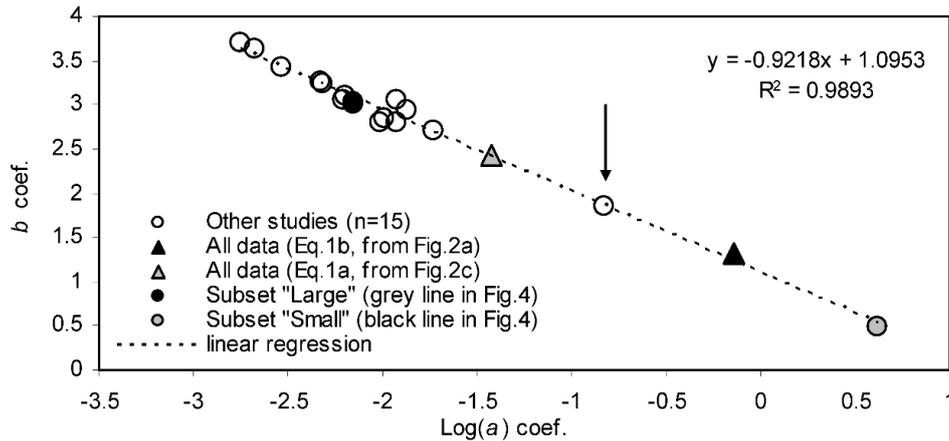
Fig. 5. Graph of log(a) vs. b using fifteen pairs of coefficients from other studies, the coefficients of Eq. 1b from Fig. 2a based on all data, the coefficients of Eq. 1a from Fig. 2c based on all data, the coefficients of subset "Large" (grey line in Fig. 4), and the coefficients of subset "Small" (black line in Fig.4) (the arrow corresponds to the coefficients given by Raikova-Petrova et al. 2009).

Discussion

*Origin of bilinearity and importance of the Linearized Biexponential model*

As it was mentioned in the introduction, the bilinear response of log-transformed W-L data might be associated with a shift in growth performance due to sexual maturation. Unfortunately, few studies have been carried out on size-at-maturity of Alburnus alburnus, but it has generally been reported that this species reaches maturity between 2 and 3 years of age (Koli 1990). However, it must be taken into account that Alburnus alburnus has been only recently introduced in Italy (Lanzoni et al. 2017) and thus it may reach maturity at a smaller size than what reported in its native range. Indeed, species in early stages of invasion tend to mature earlier and produce more offspring in order to offset colonization risks (Sakai et al. 2001). Moreover, Italy provides a more favorable environment due to the warmer climate than most countries where Alburnus alburnus is native. Politou (1993) reported that Alburnus alburnus matures at a length of ~8-10 cm in lake Koronia of Greece, which is close to the breakpoint highlighted in this analysis. Ultimately, future studies should focus on this species' biology in Italy in order to resolve whether the WLR breakpoint is a valid indication of size-at-maturity, and if there is any evidence of diet shift as it was observed in the case of *A. anguilla* from the same environment (Lanzoni et al. 2018). Gut content analysis for bleak has already been performed by Latorre et al. (2016) and Almeida et al. (2017), which showed that the species presents high plasticity in dietary traits at different habitats. Unfortunately, the observed differences in these traits were not further analyzed considering size or age classes. Thus, future research for justifying shifts in diet could be based on gut content analysis using specimens of different size/age classes.

Finally, regardless of the reasons of the breakpoint existence, its observation and identification through LinBiExp (Eq. 2) is very important since it can be used to improve the WLR analysis when it is performed using

Eq. 1. The coefficients of Eq. 1 are among the most important data of FishBase and it is unknown if any of the thousand coefficients of many fish species were biased due to the existence of a breakpoint in the initial data. For example, considering the pairs of coefficients from other studies for bleak, the pair of Raikova-Petrova et al. (2009) is indicated by an arrow in Fig. 5. This pair significantly deviates from the other published pairs. This deviation is probably due to more than 90% of specimens for assessing the coefficients of power function being shorter than the breakpoint identified in this study (Lt=12.39 cm) (see Table 1 in Raikova-Petrova et al. 2009). This probably indicates that the W-L dataset of Raikova-Petrova et al. (2009) corresponds mostly to smaller/younger specimens. The observations above, together with the fitting problems of the power function (Fig. 2), suggest that its coefficients highly depend on the range of length and the relative contribution of different length classes that participate in the fitting procedure. The LinBiExp can at least eliminate the problem of fitting differences between log-transformed and non-transformed W-L data (Fig. 2), while it can indicate the breakpoint existence in order to split the data for assessing more robust coefficients of the power function for each subset.

*General fitting performance of the Linearized Biexponential model*

Non-linear regression of Eq. 2 may lead to lack of convergence when the dataset is not large and not well distributed among different length classes because of the large number of fitting parameters. In the context of this study, preliminary fitting trials of Eq. 2 were performed on various realistic and artificial datasets using different software packages (StatGraphics, R) in order to evaluate the ability/flexibility of optimization algorithms (Levenberg-Marquardt, Gauss-Newton, Steepest Descent) to confront this problem. These trials showed that Levenberg-Marquardt performs better compared to the other two algorithms in smaller datasets. Additionally, all the software packages require initial values of the coefficients for the implementation of any non-linear regression. The successful implementation of non-linear regression using Eq. 2 strongly depends on the initial values of the coefficients given by the user and the number of iterations used by the software to converge (for example in R the "maxiter" argument was set larger than 100).

In a previous study, Aschonitis et al. (2015) made a detailed review about the most important non-linear equations and a thorough investigation about their general behavior. The authors identified that non-linear models that include the exponential function in internal calculations (as Eq. 2) may fail to fit any type of data because programming languages and software packages present numeric limitation to store values with more than 307 digits. For this reason, calculations inside exponentials that give values equal or greater than 710, cannot be calculated. This problem may also occur in the case of Eq. 2 when c coefficient is very close to 0. In this case, a solution is to use a larger metric unit of the independent variable (e.g. change of length unit from centimeters to decimeters or meters).

Conclusions

This study used W-L data of bleak *Alburnus alburnus* (Linnaeus, 1758) in order to test the applicability of LinBiExp to capture bimodal WLR responses. The use of LinBiExp model highlighted its flexibility to fit the data and to detect the absolute coordinates of a breakpoint in the log-log plot of WLR. The level of uncertainty in the breakpoint coordinates was analyzed based on bootstrap regression and confidence intervals analysis. The robustness of the breakpoint was verified through the comparison of slopes and intercepts of the two linear segments. The introduction of LinBiExp model for the analysis of WLR can be extremely valuable since it can improve the fitting performance of the typical power function, which is limited to describe a biphasic growth. Moreover, the existence and the precise determination of the breakpoint could assist future studies related to metabolic activity, sexual maturation and ontogenetic diet shifts.

References

- Acarli, D., Kara, A. & Bayhan B. 2014: Length–weight relations for 29 fish species from Homa Lagoon, Aegean Sea, Turkey. — *Acta Ichthyologica et Piscatoria* 44: 249–257.
- Almeida, D., Fletcher, D. H., Rangel, C., García-Berthou E. & da Silva, E. 2017: Dietary traits of invasive bleak *Alburnus alburnus* (Actinopterygii, Cyprinidae) between contrasting habitats in Iberian fresh waters. — *Hydrobiologia* 795: 23–33.
- Aschonitis, V. G., Castaldelli, G., Bartoli, M. & Fano, E. -A. 2015: A review and synthesis of bivariate non-linear models to describe the relative variation of ecological, biological and environmental parameters. — *Environmental Modeling and Assessment* 20: 169–182.
- Bernardo, J. M. 2005: Intrinsic credible regions: An objective Bayesian approach to interval estimation. — *TEST* 14: 317–384.
- Bevacqua, D., De Leo, G.A., Gatto, M. & Melià, P. 2009: Size selectivity of fyke nets for European eel Anguilla anguilla. — *Journal of Fish Biology* 74: 2178-2186.
- Bervian, G., Fontoura, N.F. & Haimovici, M. 2006: Statistical model of variable allometric growth: Otolith growth in Micropogonias furnieri (Actinopterygii, Sciaenidae. — *Journal of Fish Biology* 68: 196-208.
- Bolger, T. & Connolly, P. L. 1989: The selection of suitable indices for the measurement analysis of fish condition. — *Journal of Fish Biology* 34: 171–182.
- Buchwald, P. 2007: A general bilinear model to describe growth or decline time profiles. — *Mathematical Biosciences* 205: 108–136.
- Crivelli, A. J. & Dupont, F. 1987: Biometrical and biological features of *Alburnus alburnus×Rutilus rubilio* natural hybrids from Lake Mikri Prespa, northern Greece. — *Journal of Fish Biology* 31: 721–733.
- Efron, B. & Tibshirani, R. J. 1993: *An introduction to the bootstrap*. New York: Chapman and Hall/CRC.
- Elzhov, T. V., Mullen, K. M., Spiess, A. -N. & Bolker, B. 2016: *Package 'minpack.lm': R Interface to the Levenberg-Marquardt nonlinear least-squares algorithm found in MINPACK, Plus Support for Bounds*, Version 1.2-1.
- Froese, R. 2006: Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. — *Journal of Applied Ichthyology* 22: 241–253.
- Froese, R., Tsikliras, A. C. & Stergiou, K. I. 2011: Editorial note on weight-length relations of fishes. — *Acta Ichthyologica et Piscatoria* 41: 261–263.
- Gomiero, L. M. & Braga, F. M. S. 2005: The condition factor of fishes from two river basins in Sao Paulo state, Southeast of Brazil. — *Acta Scientiarum - Biological Sciences* 27: 73–78.
- Hashiguti, D.T., Soares, B.E., Wilson, K.L., Oliveira-Raiol, R.D. & Montag, L.F.D.A. 2018: Comparing three methods to estimate the average size at first maturity: A case study on a Curimatid exhibiting polyphasic growth. — *Ecology of Freshwater Fish*: Article in Press. DOI: 10.1111/eff.12451
- Interesova, E. A. & Chakimov, R. M. 2015: Bleak *Alburnus alburnus* (Cyprinidae) in the Inya River (southwestern Siberia). *Journal of Ichthyology* 55: 282-284.

- Kleanthidis, P. K., Sinis, A. I. & Stergiou, K. I. 1999: Length-weight relationships of freshwater fishes in Greece. *Naga* 22: 37–41.
- Koli, L. 1990: *Suomen kalat*. Werner Söderström Osakeyhtiö. Helsinki. [In Finnish]
- Kompowski, A. 2000: Growth rate of bleak, *alburnus alburnus* (L., 1758) in Międzyodrze waters. — *Acta Ichthyologica et Piscatoria* 30: 37–51.
- Kottelat, M. & Freyhof, J. 2007: *Handbook of European freshwater fishes*, Kottelat. Cornol, Switzerland, Freyhof. Berlin, Germany.
- Koutrakis, E. T. & Tsikliras, A. C. 2003: Length-weight relationships of fishes from three northern Aegean estuarine systems (Greece). — *Journal of Applied Ichthyology* 19: 258–260.
- Lanzoni, M., Aschonitis, V., Merighi, M., Bonnici, L., Alvisi, M. & Castaldelli, G. 2017: Rinvenimento di una specie alloctona nelle acque interne della Provincia di Ferrara, l'alborella europea *alburnus alburnus* (Linnaeus,1758). *Italian Journal of Freshwater Ichthyology* 1: 200–205.
- Lanzoni, M., Aschonitis, V., Milardi, M., Fano, E. -A. & Castaldelli, G. 2018: A method to identify bimodal weight-length relations: possible ontogenetic diet and/or metabolism shift effects in *Anguilla anguilla* (Actinopterygii: Anguilliformes: Anguillidae). — *Acta Ichthyologica et Piscatoria* 48: 163–171.
- Latorre, D., Masó, G., Hinckley, A., Rubio-Gracia, F., Vila-Gispert, A. & Almeida, D. 2016: Inter-population plasticity in dietary traits of invasive bleak *Alburnus alburnus* (Linnaeus, 1758) in Iberian fresh waters. — *Journal of Applied Ichthyology* 32: 1252–1255.
- Leunda, P. M., Oscoz, J. & Miranda, R. 2006: Length-weight relationships of fishes tributaries of the Ebro River, Spain. — *Journal of Applied Ichthyology* 22: 299–300.
- Lima-Junior, S. E., Cardone, I. B. & Goite, R. 2002: Determination of a method for calculation of allometric condition factor of fish. — *Acta Scientiarum - Biological and Health Sciences* 24: 397–400.
- Mathews, C. P.: 1971: Contribution of young fish to total production of fish in the River Thames near Reading. — *Journal of Fish Biology* 3: 157–180.
- Matthias, B.G., Ahrens, R.N.M., Allen, M.S., Tuten, T., Siders, Z.A. & Wilson, K.L. 2018. Understanding the effects of density and environmental variability on the process of fish growth. *Fisheries Research* 198: 209–219.
- Milardi, M, Lappalainen. J. L & Juntunen, T. 2015: Variation in length, girth and weight of large northern pikes (*Esox lucius*) in Finland. — *Annales Zoologici Fennici* 51: 335–339.
- Miranda, E., Oscoz, J., Leunda, P. M. & Escala, M. C., 2006: Weight-length relationships of cyprinid fishes of the Iberian Peninsula. — *Journal of Applied Ichthyology* 22: 297–298.
- Politou, C. -Y. 1993: *Biology and dynamics of the fish Alburnus alburnus (L. 1758) in lake Koronia*. Doctorate thesis, Aristotle University of Thessaloniki, Greece. [in Greek with English summary]
- Raikova-Petrova, G., Iliev, M. & Petrov, I. 2009: Growth rate and fecundity of bleak (Alburnus alburnus (L.) in the sand-pit lake chepintsi (Bulgaria). — *Biotechnology & Biotechnological Equipment* 23: 212–216.
- Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, D. M., Molofsky, J., With, K. A., Baughman, S., Cabin, R. J., Cohen, J. E., Ellstrand, N. C., McCauley, D. E., O'Neil, P., Parker, I. M., Thompson, J. N. & Weller, S. G. 2011: The population biology of invasive species. — *Annual Review of Ecology and Systematics* 32: 305–332.
- Simonović, P. D., Nikolić, V. P., Tošić, A. D. & Marić, S. P. 2011: Length-weight relationship in adult huchen *Hucho hucho* (L., 1758) from Drina River, Serbia. — *Biologia* 66: 156–159.
- Simonović, P., Marić, S. & Nikolić, V. 2000: Growth characteristics of huchen *Hucho hucho* (L.) from Rivers Drina, Una and Sana. — *Acta Biologica Iugoslavica - Ekologija Belgrade* 35: 123–126.
- Stergiou, K. I. & Fourouni, H. 1991: Food habits, ontogenetic diet shift and selectivity in *Zeus faber* Linnaeus, 1758. — *Journal of Fish Biology* 39: 589–603.
- Verreycken, H., Van Thuyne, G. & Belpaire, C. 2011: Length-weight relationships of 40 freshwater fish species from two decades of monitoring in Flanders (Belgium). — *Journal of Applied Ichthyology* 27: 1416–1421.
- Wilson, K.L., Matthias, B.G., Barbour, A.B., Ahrens, R.N.M., Tuten, T. & Allen, M.S. 2015: Combining samples from multiple gears helps to avoid fishy growth curves. North — American Journal of Fisheries Management 35: 1121–1131.
- Wilson, K.L., Honsey, A.E., Moe, B. & Venturelli, P. 2018: Growing the biphasic framework: Techniques and recommendations for fitting emerging growth models. — *Methods in Ecology and Evolution* 9: 822–833.

## 2.2.2. Case study 3. First evidence of bighead carp wild recruitment in Western Europe, and its relation to hydrology and temperature.

Abstract

Bighead carp (*Hypophthalmichthys nobilis*) have been introduced throughout Europe, mostly unintentionally, and little attention has been given to their potential for natural reproduction. We investigated the presence of young-of-the-year bighead carp in an irrigation canal network of Northern Italy and the environmental conditions associated with spawning in 2011–2015. The adult bighead carp population of the canal network was composed by large, likely mature, individuals with an average density of 45.2 kg/ha (over 10 fold more than in the main river). The 29 juvenile bighead carp found were 7.4–13.1 cm long (TL) and weighed 9.5–12.7 g. Using otolith-derived spawning dates we estimated that these juveniles were 94–100 days old, placing their fertilization and hatch dates in mid-to-end-June. Using this information in combination with thermal and hydraulic data, we examined the validity of existing models predicting the onset of spawning conditions and the viability of egg pathways to elucidate spawning location of the species. While evidence of reproduction was not found every year, we determined that potentially viable spawning conditions (annual degree-days and temperature thresholds) and pathways of egg drift suitable for hatching are present in short, slow-flowing canals.

Introduction

The bighead carp (*Hypophthalmichthys nobilis*) is a cyprinid fish native to South and Central China where it thrives in large rivers and associated floodplains. Bighead carps are primarily pelagic filter feeders, preferentially consuming zooplankton, but also phytoplankton and detritus [1–4]. Due to its rapid growth rates, it was the 7th most intensively cultured fish species worldwide in 2014 [5]. Because of its importance in aquaculture in China, bighead carp, along with silver carp (*H.molitrix*) grass carp (*Ctenopharyngodon idella*) and black carp (*Mylopharyngodon piceus*) are considered the "*four famous domestic fishes*". Herein, we follow many other authors in referring to this group of fishes as the "*Asian carps*", though there exist many species of carp in Asia. As with the other three Asian carps, bighead carp have been widely introduced outside their native range via unintentional escapes, biomanipulation programs, or for fishery enhancement [6]. The species is considered undesirable and a detrimental invasive species in many parts of its introduced range [7–10]. Probably the most notable of these invasions is occurring in North America. Bighead carp were initially imported to Arkansas in the early 1970s and were used in aquaculture, and sometimes sewage treatment, in many other states. Bighead carp have since escaped and become established throughout the central and

southern portions of the Mississippi River Basin, as well as having been reported in 24 states and in Lake Erie [11].

Bighead carp can outcompete native larval fishes and mussels for plankton resources [12], and potentially affect the entire ecosystem through a series of trophic cascades [13–15]. Bighead carp effects on the ecosystem are still largely unknown in Europe, where the species has also been introduced. Some sources claim that the species is established at least in the Danube River, e.g. [6,16,17], but others report they are only present in Europe through stocking and escapes [18]. In Western Europe, bighead carp was introduced at the beginning of the 1960s and is commonly considered unable to reproduce due to the limited length of its rivers, but there have been no studies on their population stability or their effects on the ecosystem. However, in many instances, bighead carp invasions likely are underreported in favor of silver carp (Hypophthalmichthys molitrix), which are often co-occurring and have a tendency to jump out of the water when boats pass [9], increasing the reporting frequency. These reasons all likely contribute to the lack of studies and management plans for bighead carp in Western Europe, over the more than 50 years since the first introduction.

Areas with high anthropogenic modification, where propagule pressure is strongest or where alien species introductions occurred first, are most likely to show signs of establishment [19–21]. One of the earliest introductions in West Europe was in Italy, where bighead carp were reported as early as 1975 [22]. Because there are no records of authorized stocking in open waters, the species probably was introduced through escapes from aquaculture or unintentional introductions when stocking other Asian carps, especially grass carp [23]. Unintentional introductions such as these are currently not investigated and are a likely vector for the spread of bighead carp in the Po River system, the largest river in Italy and potentially suitable for establishment of the species.

Many studies have focused on reproductive biology and early life history of Asian carps, either for sustainable management as foodfishes [24,25] or to identify the minimum suitable conditions for establishment and model their spread where they are considered undesirable [26–28]. The reproduction and early life history of Asian carps is thus well described, and is similar among the species. These rheophilic spawners typically migrate upstream to locations of high water velocity and turbulence for spawning [24,25,29]. Spawning events are usually closely associated with hydrograph peaks [25]. The eggs are very slightly demersal after water hardening [30] and the embryos develop while drifting in the current, kept from settling to the bottom by turbulence. Settling to the bottom is detrimental to the survival of the eggs [30] and sufficient river length and turbulence to keep the eggs from settling is considered a requisite for recruitment [27,31]. The early larvae also drift in the current, though they have the ability to swim vertically and avoid settling if turbulence is insufficient to keep them from settling [32]. Grass carp reproduction has been recently reported from a canal network in the lower floodplain of the Po River where nursery areas are present [33], suggesting

bighead carp could become established as well. However, the exact location of spawning remains unclear and no analysis of spawning condition viability has been performed.

Based on their similar spawning requirements, we hypothesized that adult bighead carp could also successfully recruit in the Po River or the associated canal system and that YOY bighead carp would most likely be found in nursery areas where juvenile grass carp had been previously reported. This is of great importance as early detection is paramount to manage potentially invasive species. To supplement the spawning hypothesis, we investigated data on thermal and hydraulic conditions to offer clues to discriminate among potential spawning locations. Finally, if natural reproduction of bighead carp is occurring, an analysis of pathways available within the canal network in relation to viability of egg transport would help ascertain potential locations suitable for recruitment.

Materials and methods

Field sampling permits have been granted by the Emilia Romagna regional administration and by the water management authority (ConsorziodiBonificaPianura diFerrara).

*Fish sampling and age analysis*

To verify the presence of potential spawners within the canal network, adult bighead carp were sampled during the fall of 2013 and 2014, at stretches of Canal Bianco, Canal Guarda, Canal Acque Alte and Andio, all located upstream of locations where juveniles were collected. Sampling of adult individuals was undertaken at locations where, due to minimum flow in the canals, fish were concentrated in an area of suitable depth for seining (0.6–1.3m) as described in [34]. Seine nets were 2m in height with a 25m mouth and 8mm mesh size, with a 3m long cod-end of 4mm mesh, and were dragged towards a blocking net of 4mm mesh spanning the canal width and depth. After measuring their weight and length, adult individuals were released in nearby canals which had higher water levels.

Density was estimated as number of individuals and their biomass per area of the canal, using the area of the canal before water level reduction. Density of adult individuals was also estimated in the Po River between August and October 2014, using a drifting trammel net 1.80 m high and 150 m long with an inner mesh size of 70 mm and an outer mesh size of 300 mm. In this case, density was estimated as number of individuals and their biomass per area swept by the net.

Investigations for the presence of young-of-the-year (YOY) bighead carp individuals were performed between 2011 and 2015 in similar habitats as used by juvenile grass carp [33]. YOY bighead carp sampling took place in rice field irrigation canals, which are characterized as short sections separated by flow control structures, often with low water velocity but abundant riparian and aquatic vegetation. These canals are situated at a lower elevation than the rice fields, requiring active pumping of the water for irrigation. Sampling was in 1–3 days of September and October of each year and was conducted with a 1m tall and 5m wide seine net (6mm mesh). All juvenile fish were measured (length and weight), transported to the laboratory, identified

by morphological discrimination [18] and frozen for further analysis. Silver carp are also present in the area, but extremely rare (~1% of adultHypophthalmichthys spp., Lanzoni unpublished data) and thus unlikely to be confused with bighead carp.

A total of 29 small bighead carp specimens were captured in October 2012 (n = 7), September 2013 (n = 4) and September 2014 (n = 18). No small specimens were captured in 2011 or in 2015. Unfortunately, 18 specimens were lost due to a refrigerator failure, including specimens from year 2012 (n = 1), 2013 (n = 1) and 2014 (n = 16).

To determine the age and the day-of-birth, lapillus otoliths were extracted, fixed with a resin medium on a glass plate and sanded to expose the nucleus. Daily growth rings were then counted under a stereomicroscope (10x or 20x magnification). Fertilization dates were estimated for each spawning year, based on date of capture and age of each individual and accounting for egg development time, related to temperature, according to [32]. Given that otolith formation occurs immediately before hatching, hatching dates were estimated to be the age of the fish subtracted from the collection date.

*Reproduction and hydraulic conditions*

Onset of Asian carp spawning is thought to be dependent on thermal and hydraulic cues (particularly spikes in the hydrograph; reviewed by [6]), therefore we collected hydraulic data (water temperatures and daily discharges) from both the main river and the canal network. Daily measures of water temperatures were taken by the public water treatment company (C.A.D.F.Ltd), in the Po River at the section of Berra municipality (Province of Ferrara) where both the studied siphoning plants of Contuga and Berra are located. Water from the river enters the canal network through these siphoning plants and flows through the system in a low gradient environment, usually with minimal change in temperature (Lanzoni, unpublished data). Temperature data were therefore considered representative of the canal network as well as the main river. Because specific information on bighead carp is lacking, we assumed available information on grass and silver carp would be reasonable surrogates, based on similarities in environments in both native and invaded habitats [27]. The total annual degree-days (hereafter ADD), were calculated to identify whether the thermal thresholds required for maturation (reported to be 2685 ADD during a calendar year; [35]) was reached. Also, the date on which the number of annual degree days above 15˚C (hereafter ADD15) reached the minimum for onset of spawning by silver carp (reported to be 655; [36]) was determined for each year. Grass carp has been reported to spawn at temperatures above 20˚C in the former Soviet Union [37]. We therefore assumed these three to be valid thresholds for the spawning of bighead carp.

Flooding events are known to trigger bighead carp spawning [6] but the hydrology of the canal network where putative YOY bighead carps were collected is complex. From the Po River, water enters the main irrigation canal, the Canal Bianco, through siphons at the siphoning plants of Contuga and Berra. The siphons are opened and closed according to irrigation needs. Secondarily, the Canal Bianco may receive drainage

water from areas upstream of the studied canal network, but only in case of heavy rains, usually in autumn and winter. Siphoning plants let little water through during winter (October-March), thus the water level in the canal network is low throughout these months. Siphons are progressively opened starting from the beginning of April (except in 2013, when they were opened in mid-March), which raises the water level in a flood-like event. Afterwards, the water level in the canal network is more or less constant until the next autumn. Thus, there are both natural (in the main river) and artificial (in the canal network) flood events that could trigger bighead carp spawning. Fish cannot move from the canal network to the Po River, but eggs, larvae and even juveniles drifting in the river could potentially pass through the siphons into the canal network. For a detailed description of migration pathways available to bighead carp see [33]. Po River water level was manually measured daily in Pontelagoscuro, roughly 40 km upstream of the siphoning plants, and was converted to discharge through a model developed by the water management authority (ConsorziodiBonificaPianura diFerrara, unpublished). Since no tributaries and water diversions are present between Pontelagoscuro and the plant of Contuga, this discharge was representative of the stretch of the main river where bighead carp spawning could potentially occur upstream of the siphons.

Data on discharge in the first section of the canal network were obtained from the water management authority (ConsorziodiBonificaPianura diFerrara) at the Contuga and Berra plants, the two main irrigation plants feeding water to Canal Bianco and the section where YOY were found. These data were measured daily at the siphons. Water velocity (m/s) was derived from discharge for each of the main canal derivations, based on canal sections, and data were verified with experimental measurements performed at main and secondary sections, using a current meter (Open Stream Current Velocity Meter, 2100, Swoffer Instruments Inc, Seattle, WA, USA). Water velocity was measured directly during peak flow in 2016 for each section of the putative egg pathways. The meter was mounted on a measuring pole with centimetric resolution, equipped with a modified propeller for low flow conditions.

Because adult fish in the canal network cannot migrate upstream of the siphons, we assumed that if spawning occurred within the canal system, it would occur in the turbulent area immediately downstream of the siphon site. We estimated all 5 potential egg pathways leading from the putative spawning location to the locations where YOY were captured (Fig 1). For each of these pathways, based on our hydrographic data, we calculated length and water transit times for every year when YOY were captured. To assess the viability of all pathways we then compared egg hatching times to water transit times. We also checked whether water velocity would be sufficient to keep eggs from sinking to the bottom, which can potentially prevent eggs from hatching.
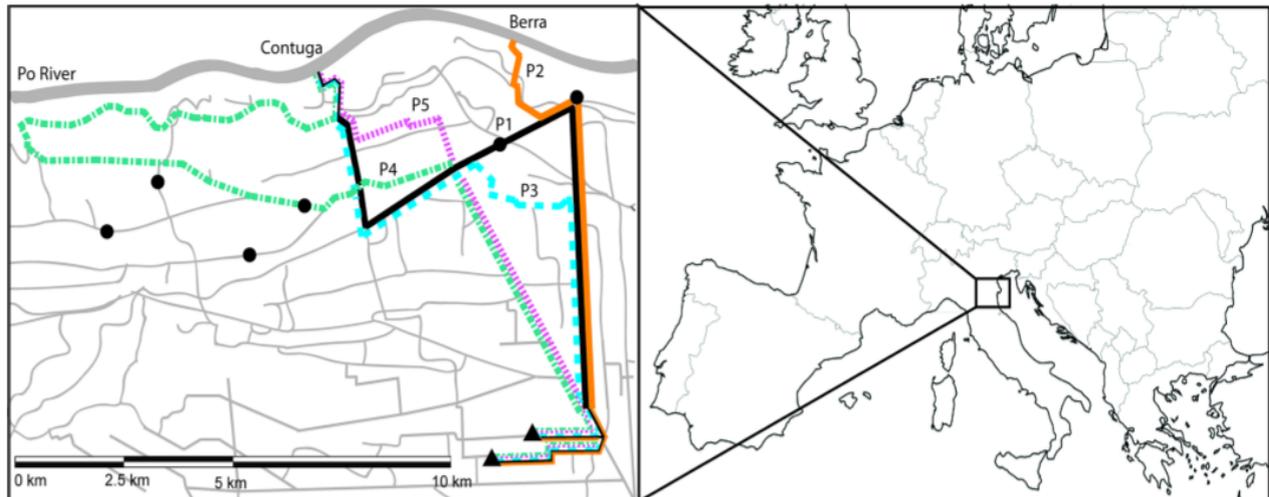
**Fig 1. Schematic representations of five potential egg pathways within the canal network.** Po River, at the top of the map, and canals of the network are presented with solid grey lines. Solid and dashed color lines represent available pathways from the Contuga (P1, in solid black, and P3–P5, in blue long dashes, green dash and dots and pink short dashes, respectively) and Berra (P2, in solid orange line) siphoning stations to the locations where YOY individuals were captured, represented with black triangles. Sampling locations of adult bighead carp are represented with black circles.

Results

A total of 350 adult bighead carp were sampled in the canal network between 2013 and 2014 (average weight 13.2 kg, maximum length 102.5 cm and maximum weight 21 kg). Average adult bighead carp density was 2.7 fish/ha and 45.2 kg/ha, but these values were also extremely variable among years and locations (1.0–7.0 fish/ha; 11.6–127.6 kg/ha). Density of bighead carp in the Po River was much lower than in the canal network (0.1 fish/ha; 1.8 kg/ha). A total of 29 YOY bighead carp were captured between 2012 and 2014 at two locations in the irrigation canals (Fig 2).
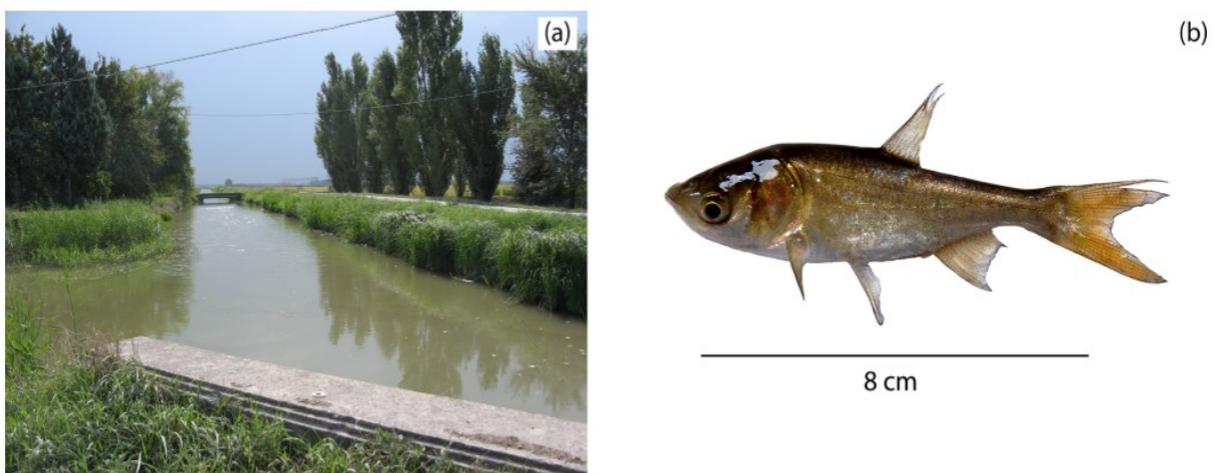


**Fig 2.** (a) the sampling location where all juvenile individuals were found and (b) one of the YOY bighead carp sampled during this study.

No YOY bighead carp were captured in 2011 or 2015. Young bighead carp were 7.4–13.1 cm long (TL) and weighed 9.5–12.7 g. The bighead carp in the subsample analyzed for age and DOB (n = 11) were 7.4–12.9 cm

(TL) and weighed 9.5–11.1 g. Age estimates from otoliths confirmed that all analyzed specimens were YOY. Daily growth ring analysis estimated that specimens from all years were 94–100 days old, placing their fertilization and hatch dates in mid-to-end-June, depending on the year (Table 1).

Table 1. Catch date, total length (TL), weight (g), age (in days) as well as hatch and fertilization dates for all specimens of YOY bighead carp examined in this study.

| Catch date | Total length (cm) | Weight (g) | Age (days) | Hatch date | Fertilization date |
|---|---|---|---|---|---|
| 02.10.2012 | 7.9 | 10.2 | 97 | 27.06.2012 | 26.06.2012 |
| 02.10.2012 | 7.9 | 9.5 | 96 | 28.06.2012 | 27.06.2012 |
| 02.10.2012 | 8.7 | 10.1 | 98 | 26.06.2012 | 25.06.2012 |
| 02.10.2012 | 11.5 | 11.1 | 100 | 24.06.2012 | 23.06.2012 |
| 02.10.2012 | 11.7 | 10.9 | 97 | 27.06.2012 | 26.06.2012 |
| 02.10.2012 | 11.4 | 10.8 | 100 | 24.06.2012 | 23.06.2012 |
| 16.09.2013 | 8.8 | 10.8 | 94 | 14.06.2013 | 13.06.2013 |
| 16.09.2013 | 12.1 | 10.4 | 97 | 11.06.2013 | 10.06.2013 |
| 16.09.2013 | 12.9 | 10.6 | 98 | 10.06.2013 | 9.06.2013 |
| 20.09.2014 | 7.4 | 9.6 | 94 | 18.06.2014 | 17.06.2014 |
| 20.09.2014 | 11.8 | 10.5 | 100 | 12.06.2014 | 11.06.2014 |

None of the fertilization dates corresponded with the mid-March to early-April flooding of the canal network, so we excluded those water level variations as cues for bighead carp spawning within the canal network.

The ADD threshold for maturation was always reached, both in non-spawning and spawning years, by first half of July. Overall, ADD were about 5000, meeting and exceeding annual thermal requirements for maturation. The ADD15 and 20˚C thresholds for onset spawning were always met, in that order, prior to estimated fertilization dates, in years when bighead carp YOY were captured (2012–2014) whereas in other years (2011 and 2015), the 20˚C threshold was reached before the ADD15 threshold (Fig 3).

In 2012, bighead carp spawning occurred in late June, during a moment of maximum flow from the Contuga plant but minimum and decreasing flow in the Po River and Berra plant. In 2013, bighead carp spawning also occurred during a peak in flow from the Contuga plant but concurring with a small peak in the main river flow. Similarly, in 2014 spawning occurred in mid-June, during a rising hydrograph in the main river.

Estimated egg hatching times were 20, 34.3 and 24.3 hours for 2012, 2013 and 2014, respectively. These hatching times were nearly all compatible with transit times within the different available egg pathways within the canal network and a viable pathway was always available in each year (Table 2).
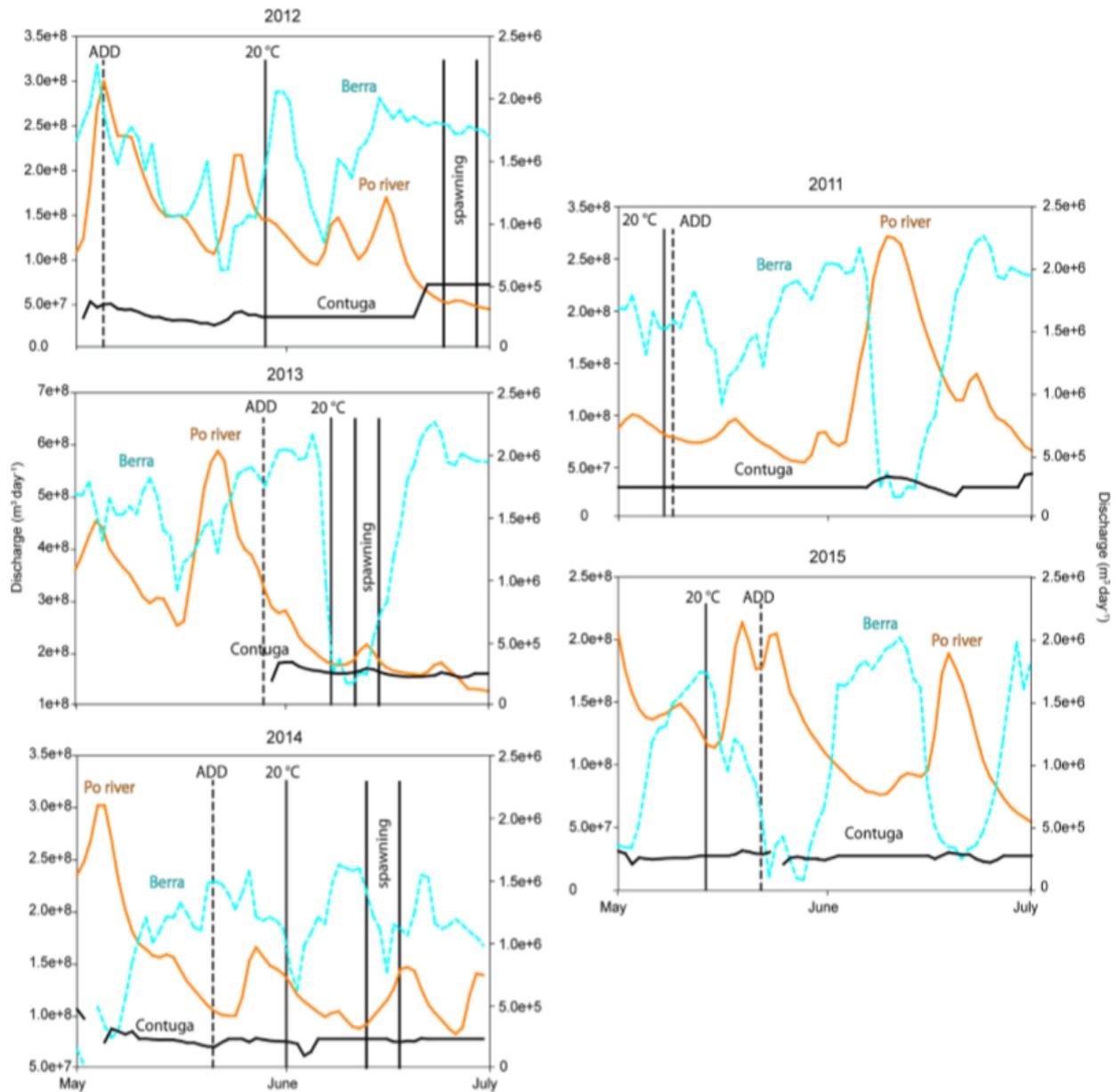
**Fig 3. Hydrographs of the discharge in the main river (in solid orange, left Y axis), the Contuga siphon (in solid black, right Y axis) and the Berra siphon (in dashed blue, right Y axis).** Vertical lines represent the ADD15 (dashed grey) threshold, first day of 20˚C (solid gray) and estimated fertilization dates (solid black).

**Table 2. Transit time difference (water transit time, minus egg hatching time) for each available egg pathway within the canal network, for every year when YOY were captured.** Bold values highlight incompatible times of transit (negative values indicate that eggs would hatch beyond the end of the pathway).

|  |  | 2012 | 2013 | 2014 |
|---|---|---|---|---|
|  | Total length (km) | Transit time difference (hours) | | |
| Pathway 1 | 18.75 | 20.64 | 6.38 | 16.71 |
| Pathway 2 | 11.41 | 3.97 | **-9.65** | 0.03 |
| Pathway 3 | 17.02 | 13.35 | **-0.91** | 9.43 |
| Pathway 4 | 31.63 | 201.13 | 186.84 | 196.95 |
| Pathway 5 | 13.97 | 16.78 | 2.50 | 12.68 |

All pathways included current velocity bottlenecks, with water velocity dropping as low as 0.03 m s-1. A common bottleneck was at the convergence of all Pathways, in Canale Leone, but this was limited to a short, terminal stretch for pathways 1–3. Conversely, Pathways 4 and 5 shared a greater length of the slow-flowing Canale Leone and Pathway 4 had low speed bottlenecks already in its initial stretch. Overall, Pathway 2 provided the closest matches with hatching times in all years.

Discussion

Large adult bighead carp and, in some years, YOY are present in the canal network, indicating successful recruitment. To our knowledge, this is the first account of bighead carp natural reproduction in West Europe. All thermal thresholds were met and seemed to corroborate the viability of spawning conditions and fertilization dates estimated from otoliths. Natural reproduction of grass carp, which has similar reproductive requirements, has been previously confirmed within the study area [33].

No inspection was conducted on the gonad development of the adult fish sampled in this study but bighead carp typically mature at lengths of 55–70 cm and weight around 3 kg [38] therefore the population in the canal network is composed in large part by mature fish that could be potential spawners. Bighead carp capture rates in the canal network were variable, likely reflecting a non-uniform spatial distribution of individuals, and marked differences in individual size. However, the highest density sampled in the canals (242.4 kg/ha, for 11.5 fish/ ha) indicates that large concentrations of spawning-size fish are possible and more likely to occur here than in the Po River, where densities were markedly lower. Higher densities could be reached within the canal network during the spawning season, if fish are grouped at impassable migration barriers such as the siphoning plants.

The question of whether the collected YOY bighead carp were spawned in the Po River or within the canal network itself is intriguing but cannot be fully resolved. In 2013, hydrograph peaks in both the Po River and in the canal coincided with the estimated egg fertilization dates, which is consistent with the preference of Asian carps for spawning during hydrograph peaks, but provides no clues as to whether spawning occurred in the Po River or in the canal. While in 2014 the rising hydrograph in the river could be the cue for spawning, the time of spawning in 2012, following an increased hydrograph from the Contuga plant, but not in the river, lends credit to a hypothesis that bighead carp spawned within the canal network. The system has very unusual conditions for spawning and recruitment by an Asian carp and if spawning did occur within the canal system it would constitute the only record of Asian carp spawning within a canal system other than the extremely large and river-like Karakum Canal in Turkmenistan (1375 km long and navigable for most of its length; [39]). However, it cannot be confirmed with certainty that the spawning event occurred within the canal network, because some Asian carp spawning (though reduced in intensity) does sometimes occur outside periods of hydrograph peaks [40,41]. Because of the intriguing potential that spawning may have occurred within the canal system itself, we investigated five pathways within the complex canal system and

their potential for egg transport that would support survival and recruitment (Fig 1). While some pathways (especially 2 and 3) had transport times close to hatching times in all years, some low velocity areas existed in the pathways where eggs may not have been adequately carried by the current. Pathway 4, in particular, was not likely to be viable, while Pathway 2 transit times were likely adequate in 2013 and 2014. Furthermore, hatching times in 2013 indicated that eggs in Pathway 2 and 3 could have hatched before they reached the potential low velocity bottleneck of Canale Leone, in the terminal stretch of the pathways. This estimation of drift distance is rather coarse but might work in these canal systems, with relatively simple hydraulics. However, a better understanding and modeling of these hydraulics would be needed to accurately estimate where spawning occurs. It has been reported that a minimum water velocity of 0.15 m s-1 is necessary to keep Asian carp eggs adrift even if higher turbulence resulting from shallow depth plays a relevant role in keeping eggs from settling [42]. The consequence of failing to meet this requirement is settlement to the sediment, which ultimately increases the chances of death for the embryo, especially if the egg is fully or partially buried [43].

In our study system, velocities below this threshold were detected, at least in Canale Leone, where all pathways ultimately converge. However, Asian carp eggs are large and mechanically resistant, and it may be that if the partially-hardened egg does not experience a complete stop, it could continue downstream, occasionally contacting the bottom without stopping its drift. This mechanism is possible in our study system where low sedimentation rates are accompanied by simplified canal morphology. Some of these canals have smooth hard bottoms and banks, either concrete or hard clay, free of macrophytes and large plant debris which decreases turbulence.

These characteristics also decrease the chance of eggs becoming lodged and ultimately buried. Furthermore, [43] found that mortality rates of settled eggs were much reduced if the eggs settled when they were near hatching, and that some eggs survived in some conditions even if they settled early in development. If eggs fertilized at the siphons reach the low velocity zone in Canale Leone, where all Pathways converge, they would have been very near the hatching (or even hatched, as in 2013) and thus they could potentially survive this bottleneck, unless they were buried by sediment in that short period. Oxygenation of the water column might still constitute a bottleneck, because some of the canals in the study area occasionally experience low-oxygen events during summer [34]. However, low oxygen concentrations typically occur later in the summer (July, August) and thus may not be relevant to the peak reproductive season of bighead carp. Thus, reproduction of bighead carp within the canal system cannot be ruled out. All thermal thresholds for maturation (ADD) and onset of spawning (ADD15 and 20˚C) were met in each year.

Notably, in 2011 and 2015, when no evidence of reproduction was found, the 20˚C threshold was reached first, contrary to years when reproduction was confirmed, when ADD15 was reached first. However, we do not know if this order is relevant to successful recruitment. There was no substantial difference between hydrographical and thermal regimes of years when recruitment was detected and years in which it was not

detected. It is possible that reproduction could have been successful in all years and that YOY were simply not detected, but YOY of grass carp were detected with similar sampling methods and locations [33] which suggests that sampling was at least appropriate. Bighead carp and grass carp have similar spawning requirements (e.g. [25]) so it is likely that if one species is able to reproduce, the other will also be able [44]. However, natural reproduction of grass carp in the area was reported to be fairly consistent from 2010–2015 [33], so there might be species-specific mechanisms yet unknown. It has been reported that, in China, grass carp are somewhat more plastic than the other Asian carps in terms of spawning requirements [45], which might explain the difference in recruitment consistency, but no literature is yet available to adequately evaluate species-specific differences [30,32,43]. Therefore, further studies would be needed to unravel the ultimate causes of differential reproduction success.

After flooding of the canals in spring time, because of artificial regulation of flow, there are low fluctuations of water level in the canal network until winter, unlike in natural rivers where increased flow is linked to rising water levels. While in the literature it is widely reported that one of the primary spawning cues for the species is a rising hydrograph [25], fish might only perceive variations in flow intensity and thus might not be able to gauge the height of the water level at any given time. Some spawning (usually with reduced intensity) has already been shown to occur during periods of stable or low flow in the introduced range in North America [40,41]. Thus, bighead carp could respond to artificial variations in flow and reproduce in systems previously considered unsuitable. Furthermore, bighead carp and grass carp spawning is generally thought to occur in rivers larger than these canals, but Coulter et al. [40] described substantial plasticity in bighead and silver carp spawning in its introduced range in the USA, including much smaller rivers than previously reported, and a spawning season protracted into early fall. Our results clearly support previous findings that the limitations to successful reproduction most commonly reported in the literature for this species are not rigid.

## References

1. Dong S, Li D (1994) Comparative studies on the feeding selectivity of silver carp Hypophthalmichthys molitrix and bighead carp Aristichthys nobilis. Journal of Fish Biology 44: 621–626.
2. Lazareva L, Omarov M, Lezina A (1977) Feeding and growth of the bighead, Aristichthys nobilis, in the waters of Dagestan. J Ichthyol 17: 65–71.
3. Opuszyński K (1981) Comparison of the usefulness of the silver carp and the bighead carp as additional fish in carp ponds. Aquaculture 25: 223–233.
4. Anderson KR, Chapman DC, Hayer C-A (2016) Assessment of dreissenid biodeposits as a potential food resource for invasive Asian carp. BioInvasions Records
5: 251–257. 5. FAO (2016) Fishery and Aquaculture Statistics yearbook. Rome 105 p.
6. Kolar C, DC C, Courtenay W Jr, Housel C, Williams J, et al. (2007) Bigheaded carps: a biological synopsis and risk assessment. Bethesda, Maryland: American Fisheries Society. 204 p.
7. Azevedo-Santos V, Magalhaes A, Rigolin-Sa O, Vitule J (2011) Registro de um grande exemplar de carpa-cabec¸ uda Hypophthalmichthys nobilis (Richardson, 1845) (Cypriniformes: Cyprinidae) no reservato ´rio de Furnas, Minas Gerais, Brasil. In: O R-S, editor. Bacia Hidrogra ´fica: Estudos do Rio Grande no Sudoeste de Minas Gerais. Brasil.

8. Boros G, Mozsa´r A, Vita´l Z, Nagy A, Speczia´r A (2014) Growth and condition factor of hybrid (Bighead Hypophthalmichthys nobilis Richardson, 1845×silver carp H. molitrix Valenciennes, 1844) Asian carps in the shallow, oligo-mesotrophic Lake Balaton. Journal of Applied Ichthyology 30: 546–548.

9. Conover G, Simmonds R, Whalen M (2007) Management and control plan for bighead, black, grass, and silver carps in the United States. Aquatic Nuisance Species Task Force, Asian Carp Working Group, Washington, DC Available: www.asiancarp org/Documents/Carps_Management_Plan.pdf (April 2010).

10. Xie P, Chen Y (2001) Invasive carp in China's plateau lakes. Science 294: 999–1000.

11. USGS (2016) Non-indigenous Aquatic Species Database.

12. Laird CA, Page LM (1996) Non-native fishes inhabiting the streams and lakes of Illinois. Illinois Natural History Survey Bulletin; v 035, no 01.

13. Cooke SL (2016) Anticipating the spread and ecological effects of invasive bigheaded carps (Hypophthalmichthys spp.) in North America: a review of modeling and other predictive studies. Biological invasions 18: 315–344.

14. Solomon LE, Pendleton RM, Chick JH, Casper AF (2016) Long-term changes in fish community structure in relation to the establishment of Asian carps in a large floodplain river. Biological Invasions 18: 2883–2895.

15. Zhao S-Y, Sun Y-P, Han B-P (2016) Top-down effects of bighead carp (Aristichthys nobilis) and Leptodora richardi (Haplopoda, Leptodoridae) in a subtropical reservoir during the winter–spring transition: a mesocosm experiment. Hydrobiologia 765: 43–54.

16. Ciolac A (2006) The Asian carps from Romanian Danube River. Annals of the University „Dunarea De Jos" of Galati 21: 91–95.

17. Jankovic D (1998) Natural reproduction by Asiatic herbivorous fishes in the Yugoslav section of the River Danube. Italian Journal of Zoology 65: 227–228.

18. Kottelat M, Freyhof J (2007) Handbook of European freshwater fishes. Cornol: Publications Kottelat.

19. Britton JR, Gozlan RE (2013) How many founders for a biological invasion? Predicting introduction outcomes from propagule pressure. Ecology 94: 2558–2566. PMID: 24400507

20. Jongejans E, Skarpaas O, Ferrari MJ, Long ES, Dauer JT, et al. (2015) A unifying gravity framework for dispersal. Theoretical Ecology 8: 207–223.

21. Leprieur F, Beauchard O, Blanchet S, Oberdorff T, Brosse S (2008) Fish Invasions in the World's River Systems: When Natural Processes Are Blurred by Human Activities. PLOS Biology 6: e28. https://doi. org/10.1371/journal.pbio.0060028 PMID: 18254661

22. Gandolfi G (1991) I pesci delle acque interne italiane: Istituto poligrafico e Zecca dello Stato, Libreria dello Stato.

23. Melotti P, Resta C, Cavallari A (1987) La carpa erbivora in Emilia-Romagna aspetti biologici e gestionali. Ferrara: Regione Emilia-Romagna e Amministrazione Provinciale di Ferrara. pp. 87.

24. Verigin B, Makeyeva A, Mokhamed MZ (1978) Natural spawning of the silver carp (Hypophthalmichthys nobilis), the bighead carp (Aristichthys nobilis), and the grass carp (Ctenopharyngodon idella) in the Syr-Dar'ya River. Journal of Ichthyology 18: 143–146.

25. Yi B, Yu Z, Liang Z, Shen S. Distribution, natural conditions and breeding production of the spawning grounds of four famous freshwater fishes on the main stream of the Changjiang River; 1991. pp. 181– 190.

26. Garcia T, Murphy EA, Jackson PR, Garcia MH (2015) Application of the FluEgg model to predict transport of Asian carp eggs in the Saint Joseph River (Great Lakes tributary). Journal of Great Lakes Research 41: 374–386.

27. Kocovsky PM, Chapman DC, McKenna JE (2012) Thermal and hydrologic suitability of Lake Erie and its major tributaries for spawning of Asian carps. Journal of Great Lakes Research 38: 159–166. 28. Long JM, Liang Y, Shoup DE, Dzialowski AR, Bidwell JR (2014) GIS-based rapid-assessment of bighead carp Hypophthalmichthys nobilis (Richardson, 1845) suitability in reservoirs. Management 5: 363–370.

29. Peters LM, Pegg MA, Reinhardt UG (2006) Movements of adult radio-tagged bighead carp in the Illinois River. Transactions of the American Fisheries Society 135: 1205–1212.

30. George A, Garcia T, Chapman D (2017) Comparison of Size, Terminal Fall Velocity, and Density of Bighead, Silver, and Grass Carp Eggs in Relation to Drift Modeling. Transactions of the American Fisheries Society Accepted.

31. Garcia T, Jackson PR, Murphy EA, Valocchi AJ, Garcia MH (2013) Development of a Fluvial Egg Drift Simulator to evaluate the transport and dispersion of Asian carp eggs in rivers. Ecological modelling 263: 211–222.

32. George AE, Chapman DC (2013) Aspects of embryonic and larval development in bighead carp Hypophthalmichthys nobilis and silver carp Hypophthalmichthys molitrix. PloS one 8: e73829. https://doi.org/10.1371/journal.pone.0073829 PMID: 23967350

33. Milardi M, Lanzoni M, Kiljunen M, Torniainen J, Castaldelli G (2015) Natural recruitment contributes to high densities of grass carp Ctenopharyngodon idella (Valenciennes, 1844) in Western Europe. Aquatic Invasions 10.

34. Castaldelli G, Pluchinotta A, Milardi M, Lanzoni M, Giari L, et al. (2013) Introduction of exotic fish species and decline of native species in the lower Po basin, north-eastern Italy. Aquatic Conservation: Marine and Freshwater Ecosystems 23: 405–417.

35. Abdusamadov A (1987) Biology of white amur (Ctenopharyngodon idella), silver carp (Hypophthalmichthys molitrix), and bighead (Aristichthys nobilis), acclimatized in the Terek Region of the Caspian Basin. Journal of Ichthyology 26: 41–49.

36. Gorbach E, Krykhtin M (1981) Maturation rate of the white amur Ctenopharyngodon idella and silver carp Hypophthalmichthys molitrix in the Amur River. Voprosy Ikhtiologii 21: 835–843.

37. Chilton EW II, Muoneke MI (1992) Biology and management of grass carp (Ctenopharyngodon idella, Cyprinidae) for vegetation control: a North American perspective. Reviews in fish biology and fisheries 2: 283–320.

38. Froese R, Pauly D (2017) FishBase.Version 03/2017. World Wide Web electronic publication, R Froese and D Pauly (eds) Available at: www.fishbase.org Accessed April.

39. Charyev R (1984) Some consequences of the introduction and acclimatization of grass carp, Ctenopharyngodon idella (Cyprinidae), in the Kara Kum Canal. J Ichthyol 24: 1–8.

40. Coulter AA, Keller D, Amberg JJ, Bailey EJ, Goforth RR (2013) Phenotypic plasticity in the spawning traits of bigheaded carp (Hypophthalmichthys spp.) in novel ecosystems. Freshwater Biology 58: 1029–1037.

41. Deters JE, Chapman DC, McElroy B (2013) Location and timing of Asian carp spawning in the Lower Missouri River. Environmental Biology of Fishes 96: 617–629.

42. Murphy EA, Jackson PR (2013) Hydraulic and water-quality data collection for the investigation of Great Lakes tributaries for Asian carp spawning and egg-transport suitability. Urbana 51: 61801–62347.

43. George AE, Chapman DC (2015) Embryonic and larval development and early behavior in grass carp, Ctenopharyngodon idella: implications for recruitment in rivers. PloS one 10: e0119023. https://doi.org/10.1371/journal.pone.0119023 PMID: 25822837

44. Chapman DC, Davis JJ, Jenkins JA, Kocovsky PM, Miner JG, et al. (2013) First evidence of grass carp recruitment in the Great Lakes Basin. Journal of Great Lakes Research 39: 547–554.

45. Liu J, He B (1992) Cultivation of the Chinese freshwater fishes. Science Press, Beijing.

## 2.3. Loss of native species and related effects on fisheries

### 2.3.1. Case study 4. A regional fish inventory of inland waters in Northern Italy reveals the presence of fully exotic fish communities

Abstract

The aim of the study is to present a complete and updated fish inventory of inland waters of the Emilia-Romagna region, Northern Italy, and to highlight the presence of fully exotic fish communities. Overall, based on 208 sampling locations, the observed fish fauna consisted of 45 species, 22 native and 23 exotics. A significant element of the inventory is the identification of xenodiversity hotspots (spatially clustered sites, one lowland and one upland region), where a complete substitution of native species by exotic species was observed (in total seven sites in the lowland and two sites in the upland with no native species presence). These xenodiversity hotspots were found to host specific combinations of exotic species, which may be able to constitute balanced exotic communities. The hotspots of the lowland region are located in the northeast lowland part of the territory, hosting exotic species combinations mainly composed by wels catfish (*Silurus glanis* Linnaeus, 1758, a large predator), common carp (*Cyprinus carpio* Linnaeus, 1758, a large benthivore), crucian carp (*Carassius spp.*, a small-bodied generalist) and other less dominant exotic species. The hotspots

in the upland region were located in the southwest part of the territory and were dominated by only one exotic species (rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792)). A difference between these xenodiversity hotspots is that in the lowland the introductions were mostly unintentional and are not continued, while in the upland the introduction of rainbow trout is intentional and currently carried out by local fishermen.

Introduction

The increase in exotic fishspecies invasions is alarming, creating an important threat for freshwater ecosystems (Leprieur *et al.*2008). Exotic species can promote habitat deterioration/alteration such as decline of aquatic vegetation, increase of turbidity and nutrients release due to sediment resuspension, increase of phytoplankton blooms and eutrophication, genetic alterations within populations, spreading of pathogens and parasites, competition with, and predation of, native species (Dibble & Kovalenko 2009; Leunda 2010; Ribeiro & Leunda 2012; Castaldelli et al. 2013). Among European nations, one of those most impacted by exotic species invasions is Italy (see e.g. Bianco & Ketmaier 2001; Bianco2014), with the Po River basin being one of the most invaded areas. The severity of the invasions has already reached critical limits, especially in the lowland areas near the estuary, where at least 10 native fish species faced local extinction while many exotic ones showed a population explosion during the period 1991–2009 (Castaldelli *et al*. 2013).

The first aim of this study is to present a complete and updated inland water fish inventory of the Emilia-Romagna region in Northern Italy. The Emilia-Romagna region belongs to the southern side of the Padanian-Venetian ichthyogeographic district (see Bianco 1995) and hosts a number of native fish species of great conservational interest (e.g. twaite shad *Alosa fallax* (Lacépède, 1803), Italian barbel *Barbus plebejus* Bonaparte, 1839, or Italian nase *Chondrostoma soetta* Bonaparte, 1840) according to the EU Habitat Directive (92/43/ EEC). Arising from the inventory, a significant element is the identification of xenodiversity hotspots, where there was a complete substitution of native species by exotic species (Castaldelli et al. 2013). These xenodiversity hotspots were found to host specific combinations of exotic species, which were able to constitute fully exotic communities. A discussion on the structure and attributes of these communities was the second aim of this study. A fish inventory including specific information on the distribution of exotic species could be relevant for managers, as it would be most useful to prioritize concrete conservation actions for native biodiversity.

Materials and methods

The study area is located in Northern Italy and it is defined by the administrative boundaries of the Emilia-Romagna Region with total coverage of 22,446 km2 (Figure 1). It is naturally bound north and south by the Po River and the Apennine Mountains, respectively. The study region has a Mediterranean continental climate. Altogether, data from 208 river monitoring sites were analyzed in this study (Figure 1), covering a

wide range of inland water habitats at different altitudinal zones. The samplings were performed in natural rivers (e.g. Po, Trebbia, Taro, Secchia, Panaro, Reno, Lamone, Fiumi Uniti, Bevano, Marecchia, etc.) and in large artificial irrigation canals which are mainly located in the lowlands (e.g. Po di Volano, Po di Primaro, Canal Bianco, Canale Circondariale, etc.).
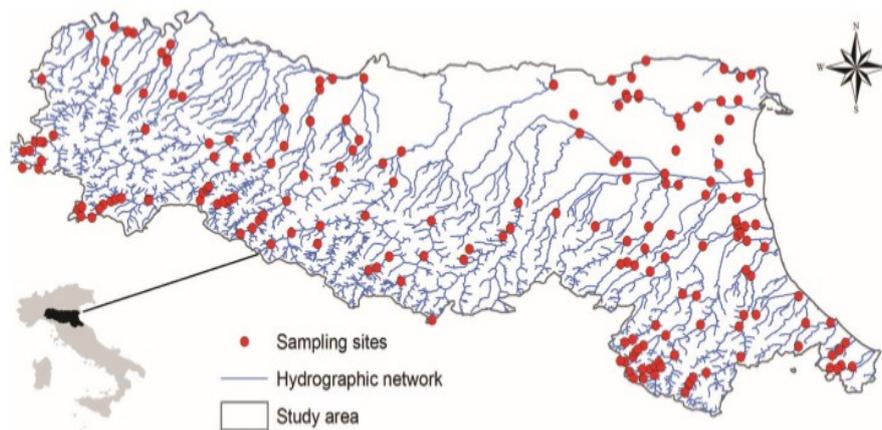


Figure 1. Study area (data source: http://gadm.org), hydrographic network of main rivers and streams (data source: http://www.eea.europa.eu/data-and-maps/data/european-river-catchments-1) and location of sampling sites.

Fish data were collected from sampling stations that were homogeneously positioned in 64 waterways of the region (Figure 1), away from recreationally managed sites, and with their section width ranging from 8 to 350 m (the maximum value corresponds to the Po River). The samplings were conducted during the warm season (from April to September) of the period 1998–2004 as part of the institutional regional monitoring program for the compilation of the official Fish Inventory of the Emilia-Romagna Region (Pascale et al. 2004, 2006; Castaldelli & Rossi 2008).

Fish sampling was performed by electrofishing, adapting the standard guidelines to the particular conditions of waterway typologies (Backiel & Welcomme 1980; Reynolds 1983). Electrofishing was performed thoroughly with a direct current at 400–600 V and 4–5 A (Reynolds 1983; Godinho & Ferreira 2000) once during daylight, in an upstream zigzag direction by wading, when depth was less than 1 m, and by boat in deeper waters. The transect lengths were equivalent to 10 times the river width, ensuring that the range of present macrohabitats of each site was fully surveyed (Hankin & Reeves 1988; Godinho & Ferreira 2000). The duration of sampling was therefore quite variable, ranging from half an hour to more than 2 hours, as in the case of the Po River. Electrofishing is considered the best quantitative method for fish sampling in shallow waters, up to a maximum of 1 m (Zalewski & Cowx 1990) but its efficacy may be low in deeper waters, with high conductivity, or in the presence of big and mobile specimens. Such special conditions occurred in almost all the lower stretches of rivers and in the large canals of the lowlands. For this reason, electrofishing in these sites was verified by sampling using a standard set of nets, composed and operated as follows: three sinking trammel nets (50 m long and 1.80 m high), composed of two external panels with knot-to-knot mesh size of 70 mm and an internal one of 300 mm, and three sinking gill nets (50 m long and 1.80 m high) with knot-to-

knot mesh sizes of 40, 20 and 10 mm, respectively. The presence of young-of-the-year specimens was assessed by using 2 × 2 m drop nets with a 5-mm mesh size in parallel with the other nets. Fishing with nets was performed immediately after electrofishing sessions, with the support of professional fishermen, and the duration was approximately 1 hour for the trammel nets and half an hour for the gill nets, in order to avoid mortality or damage of captured specimens. Fish species were identified according to Kottelat and Freyhof (2007) and Bianco (2014) and attributed either native or exotic status, relative to the Padanian-Venetian ichthyo-geographical district. Sampling sites were then grouped according to the presence/absence of native and exotic fish species.

Results

Overall, the observed fish fauna from the sampling sites consisted of 45 species, 22 native and 23 exotic, belonging to 12 families (Table I). Since a profound discussion on the Salmo trutta complex has been undertaken after the surveys, it was not possible to resolve whether the sampled individuals of this complex were native or exotic. Regarding Gobioninae, *Gobio gobio* (Linnaeus, 1758) should be added among the aliens due to its invasions in all waters of the Padano-Venetian district. *Gobio benacensis* (Pollini, 1816) is placed among the natives with a question mark as its presence was not certainly detected.

In a total of nine sampling sites, only exotic species were present. These xenodiversity hotspots were distributed in two main groups (one with seven sites in the lowlands, and one with two sites at higher elevation) which are shown in Figure 2. Table II summarizes the exotic species communities found in these sites.

Overall, it was possible to identify three different groups of sampling sites, based on the presence/ absence of native and exotic species:

- ✓ A group of nine sites populated solely with exotic species, mostly located in the lowlands. These xenodiversity hotspots showed different combinations of 14 out of the 23 exotic species detected in the region (the missing ones were European barbel *Barbus barbus* (Linnaeus, 1758), roach *Rutilus rutilus* (Linnaeus, 1758), asp *Leuciscus aspius* (Linnaeus,1758), channel catfish *Ictalurus punctatus* (Rafinesque, 1820), largemouth black bass *Micropterus salmoides* (Lacépède, 1803) and pond loach *Misgurnus anguillicaudatus* (Cantor,1842) (see Table II).
- ✓ A group of 92 sites hosting both native and exotic species, located from the lowlands to the foothills. These sites showed different combinations of native and exotic species, where all 23 exotic species were present, while from the 22 native species only three were missing (Italian nase, bullhead *Cottus gobio* Linnaeus, 1758, Eurasian minnow *Phoxinus phoxinus* (Linnaeus, 1758)).
- ✓ A group of 107 sites where only native species were present, located mostly at higher altitudes. These sites showed different combinations of 16 out of 22 native species (the missing ones were twaite shad, tench *Tinca tinca* (Linnaeus, 1758), Italian rudd *Scardinius hesperidicus* Bonaparte, 1845,

Southern pike *Esox cisalpinus* Bianco & Delmastro, 2011, thinlip grey mullet *Liza ramada* (Risso, 1827), flathead grey mullet *Mugil cephalus* Linnaeus, 1758, and European flounder *Platichthys flesus* (Linnaeus, 1758), the latter three typical of salt water but often found in inland waters.

Table I. Observed fish species in the freshwater systems of the Emilia Romagna region. The reported status of each species refers to the Emilia Romagna region (within the Padanian hydrographical district).

| Family | Species | Common name | S† | N‡ |
|---|---|---|---|---|
| Anguillidae | *Anguilla anguilla* (Linnaeus, 1758) | European eel | Ns | 17 |
| Clupeidae | *Alosa fallax* (Lacépède, 1803) | Twaite shad | Ns | 8 |
| Cyprinidae | *Sarmarutilus rubilio* (Bonaparte, 1837) | South European roach | Ns | 23 |
| | *Leucos aula* (Bonaparte, 1841) | Italian red-eye roach | Ns | 6 |
| | *Squalius squalus* (Bonaparte, 1837) | Cavedano chub | Ns | 85 |
| | *Squalius lucumonis* (Bianco, 1982) | Toscana stream chub | Es | 2 |
| | *Telestes muticellus* (Bonaparte, 1837) | Italian riffle dace | Ns | 55 |
| | *Phoxinus phoxinus* (Linnaeus, 1758) | Eurasian minnow | Ns | 4 |
| | *Tinca tinca* (Linnaeus, 1758) | Tench | Ns | 1 |
| | *Scardinius hesperidicus* Bonaparte, 1845 | Italian rudd | Ns | 30 |
| | *Alburnus arborella* (Bonaparte, 1841) | Italian bleak | Ns | 81 |
| | *Chondrostoma soetta* Bonaparte, 1840 | Italian nase | Ns | 2 |
| | *Protochondrostoma genei* (Bonaparte, 1839) | South European nase | Ns | 47 |
| | *Gobio benacensis* (Pollini, 1816) ? | Italian gudgeon | Ns | 20 |
| | *Barbus plebejus* Bonaparte, 1839 | Italian barbel | Ns | 76 |
| | *Barbus tyberinus* Bonaparte, 1839 | Tiber barbel | Es | 9 |
| | *Barbus barbus* Linnaeus, 1758 | European barbel | Es | 3 |
| | *Carassius* spp. | Crucian carp | Es | 71 |
| | *Cyprinus carpio* Linnaeus, 1758 | Common carp | Es | 82 |
| | *Abramis brama* (Linnaeus, 1758) | Common bream | Es | 31 |
| | *Blicca bjoerkna* (Linnaeus, 1758) | White bream | Es | 5 |
| | *Rutilus rutilus* Linnaeus, 1758 | Roach | Es | 1 |
| | *Rhodeus sericeus* (Pallas, 1776) | Bitterling | Es | 27 |
| | *Pseudorasbora parva* (Temminck & Schlegel, 1846) | Stone moroko | Es | 74 |
| | *Ctenopharyngodon idella* (Valenciennes, 1844) | Grass carp | Es | 3 |
| | *Leusciscus aspius* (Linnaeus, 1758) | Asp | Es | 6 |
| Cobitidae | *Misgurnus anguillicaudatus* (Cantor, 1842) | Pond loach | Es | 1 |
| | *Cobitis bilineata* Canestrini, 1865 | Italian spined loach | Ns | 27 |
| Siluridae | *Silurus glanis* Linnaeus, 1758 | Wels catfish | Es | 48 |
| Ictaluridae | *Ameiurus melas* (Rafinesque, 1820) | Black bullhead | Es | 20 |
| | *Ictalurus punctatus* (Rafinesque, 1820) | Channel catfish | Es | 4 |
| Esocidae | *Esox cisalpinus* Bianco & Delmastro, 2011 | Southern pike | Ns | 1 |
| Salmonidae | *Salmo trutta* complex | Brown trout | Ns/Es | 70 |
| | *Oncorhynchus mykiss* (Walbaum, 1792) | Rainbow trout | Es | 3 |
| Poeciliidae | *Gambusia holbrooki* Girard, 1859 | Eastern mosquitofish | Es | 6 |
| Cottidae | *Cottus gobio* Linnaeus, 1758 | Bullhead | Ns | 1 |
| Centrarchidae | *Micropterus salmoides* (Lacépède, 1803) | Largemouth black bass | Es | 9 |
| | *Lepomis gibbosus* (Linnaeus, 1758) | Pumpkinseed | Es | 26 |
| Percidae | *Perca fluviatilis* Linnaeus, 1758 | European perch | Es | 3 |
| | *Gymnocephalus cernua* (Linnaeus, 1758) | Ruffe | Es | 10 |
| | *Sander lucioperca* (Linnaeus, 1758) | Zander or pike-perch | Es | 29 |
| Mugilidae | *Mugil cephalus* Linnaeus, 1758 | Flathead grey mullet | Ns | 1 |
| | *Liza ramada* (Risso, 1827) | Thinlip grey mullet | Ns | 3 |
| Gobiidae | *Padogobius bonelli* (Bonaparte, 1846) | Padanian goby | Ns | 43 |
| Pleuronectidae | *Platichthys flesus* (Linnaeus, 1758) | European flounder | Ns | 1 |

†S: status; Ns is for native and Es is for exotic species.

‡N: number of sampling sites where the species is present.
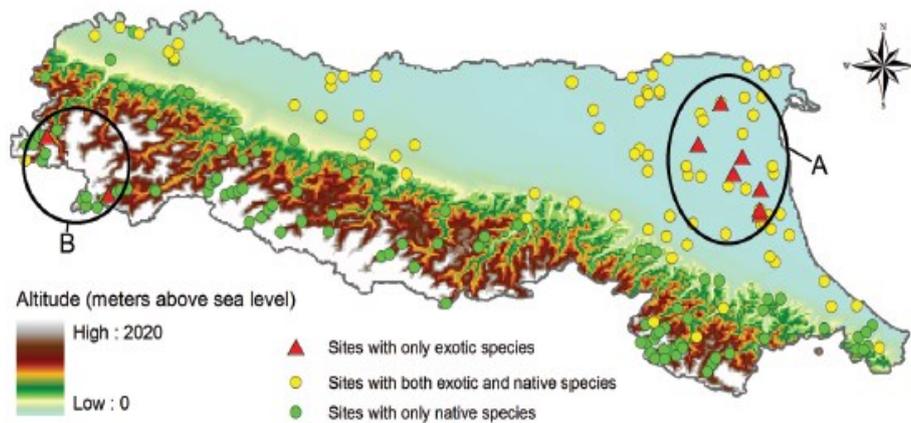
Figure 2. Altitude (data source: https://lta.cr.usgs.gov/GTOPO30) and separation of sampling sites based on the presence/absence of native and exotic species.

Table II. Exotic species present in the nine positions belonging to the xenodiversity hotspots where native species were absent (Figure 2).

| Site | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Crucian carp | x | | x | x | | x | | | | 4 |
| Common carp | x | x | x | x | | x | x | | | 6 |
| White bream | | | | | x | | | | | 1 |
| Common bream | x | | | | | | | | | 1 |
| Bitterling | x | | | | | | | | | 1 |
| Stone moroko | x | x | x | | | x | | | | 4 |
| Grass carp | | | | | x | | | | | 1 |
| Wels catfish | x | x | | x | x | | x | | | 5 |
| Black bullhead | x | | | x | | | | | | 2 |
| Eastern mosquitofish | | x | | | | | | | | 1 |
| Pumpkinseed | x | x | x | | | | | | | 3 |
| Pike-perch | x | | x | | | | | | | 2 |
| Ruffe | | | | | | | x | | | 1 |
| Rainbow trout | | | | | | | | x | x | 2 |
| **Total number of exotic species** | 9 | 5 | 5 | 4 | 3 | 3 | 3 | 1 | 1 | |

## Discussion

The existence of xenodiversity hotspots indicates that exotic fish species might be able to constitute fully exotic communities. These xenodiversity hotspots are surrounded by sites where the presence of native species is extremely low, at least in the lowlands. As fish surveys employed redundant sampling methods, it is unlikely that native species were not detected accurately. It is clear that other anthropogenic drivers (e.g. hydrologic alteration, habitat degradation) are also at play in the area and could favor exotic species, at least in the lowlands (Castaldelli *et al.* 2013). Therefore, it is likely that these xenodiversity hotspots could arise from species interactions occurring in altered environmental conditions. Unfortunately, because there are

no longterm records of the fish communities for all these sites, it is impossible to completely disentangle the exact mechanism of interaction with native fishes.

The presence of fish xenodiversity hotspots is particularly alarming, because there have been few documented cases of non-isolated freshwater systems with high interconnectivity where native fish communities have been completely substituted by exotics. It is well known that exotic species can cause the displacement of natives, by outcompeting natives for spatial and trophic niches (Mooney & Cleland 2001); however, reports that prove complete multiple local fish extinctions as a result of exotic fish species invasions are relatively scarce, and sometimes questionable for reasons explained in Gurevitch and Padilla (2004). The case of direct predation by brown trout (*Salmo trutta* Linnaeus, 1758) causing extinction of galaxiids fishes in New Zealand is one of the very few examples of these (Townsend 1996), while the case of Nile perch (*Lates niloticus* (Linnaeus, 1758)) in Lake Victoria is more controversial (e.g. Kitchell et al. 1997; Witte et al. 2000). Predatory interactions can only explain a limited part of the native species decline found in our study area, as the exotic fish communities comprise several species with a wide spectrum of eco-functional traits. The analysis of these traits, and how the combination of the specific exotic species may lead to fully exotic populations, should be the subject of future investigations.

The original native communities composed mostly by ciprinids such as Italian bleak Alburnus arborella (Bonaparte, 1841), Italian rudd, and exocids such as southern pike were locally extinct within the lowlands xenodiversity hotspots (Table II), where exotic communities were mainly composed by wels catfish *Silurus glanis* (a large predator), common carp *Cyprinus carpio* (a large benthivore) and crucian carp *Carassius sp*p. (a small-bodied generalist). These constitute the backbone of the fish communities in most xenodiversity hotspots. Biogeographical origin could partly explain this composition: the main exotic species in these sites (e.g. common bream *Abramis brama* (Linnaeus,1758) and wels catfish) come from the same area, the Danube River, where they likely co-evolved and developed mechanisms of niche partitioning and coexistence (Castaldelli et al. 2013). The large size attained by some of these species (e.g. common carp or grass carp *Ctenopharyngodon idella* (Valenciennes, 1844)) and the deep body of others (e.g. common bream, crucian carp) could also partly explain their coexistence with predators. A notable exception to Danube River species are the pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758), a North American centrarchid, and the black bullhead *Ameiurus melas* (Rafinesque, 1820), a North American ictalurid, which are both exotic species introduced over a century ago (Bianco 1998). These species seem able to constitute small populations within these sites, probably due to their well-known trophic and ecologic flexibility (Wainwright et al. 1991). The xenodiversity hotspots in the lowlands of our study area could provide a valuable example of invasional meltdown in fish communities.

Invasional meltdown is the mutual facilitation of invasion by different species (Simberloff & Von Holle 1999). Albeit not a new hypothesis, it still remains controversial (Simberloff 2006) and very few examples of it are known from fish communities (e.g. the opposite effect found in Britton et al.2010). The ecosystem

engineering capabilities of some of these exotic species could explain why these communities thrive. Common and crucian carp, for example, have been known to increase water turbidity and reduce macrophytes through their feeding actions (e.g. Richardson *et al.*1995; Bonneau & Scarnecchia 2015). While their invasion in Western Europe was completed long ago, they are capable of continued effects on the environment. Furthermore, grass carp have been shown to be established in the area (Milardi *et al.* 2015) and feed directly on macrophytes, increasing the positive feedback on turbidity.

Increased water turbidity can favor predators such as wels catfish or pike-perch, which are particularly adapted for predation in turbid waters, over native predators suchas southern pike, which largely rely on sight and do not have special adaptations. Moreover, the interactions between exotic and native species are likely magnified by hydrologic alteration, as already hypothesized by Castaldelli *et al.* (2013). Native fish are mostly riverine-adapted species, contrary to exotics which are more lacustrine in origin; therefore, the natives survive in streams or torrents on hills or sub-mountain zones, which should be considered sanctuaries for their survival, as previously discussed by Bianco and Ketmaier (2001). Ultimately, further studies are needed to confirm whether our study area shows clear signs of invasional meltdown. The xenodiversity hotspots at higher elevations (Table II) were located in the upper reaches of the Taro River and in a smaller stream (Rio Castello) feeding into the Trebbia River. These sites did not show significant habitat degradation or hydrologic alteration; however, the community was not composed by native trout and gobies or cottids, but rather solely composed by rainbow trout (*Oncorhynchus mykiss*). This species was previously reported to establish in different areas of Italy (Stanković et al. 2015), even if many more populations are known but not yet reported (Milardi, unpublished data), but its interactions with native fish and invertebrates are still largely un explored Candiotto *et al.* (2011) hypothesized that rainbow trout could colonize mainly river stretches where no other fish were present. Our data suggest that in the Taro River and the Trebbia River, a population of rainbow trout can exist well within systems where other natives are present both up- and downstream (suggesting also that it could occasionally effectively displace native species, atleastlocally). Eventhough surveyed sites are far from sites where recreational stocking occurs, it is probable that stocking of rainbow trout for recreational fisheries could strongly contribute to the distribution pattern of this species in sites at higher altitudes.

References

- Backiel T, Welcomme RL. 1980. Guidelines for sampling fish in inland waters. Rome, Italy: FAO.
- Bianco PG. 1995. Mediterranean endemic freshwater fishes of Italy. Biological Conservation 72:159–170. DOI:10.1016/ 0006-3207(94)00078-5.
- Bianco PG. 2014. An update on the status of native and exotic freshwater fishes of Italy. Journal of Applied Ichthyology 30:62–77. DOI:10.1111/jai.2014.30.issue-1.
- Bianco PG. 1998. Freshwater fish transfers in Italy: History, local changes in fish fauna and a prediction on the future of native populations. In: Cowx IG, editor. Stocking and introductions of fishes. Fishing New Book. Oxford: Blackwell Science. pp. 165–197.

- Bianco PG, Ketmaier V. 2001. Anthropogenic changes in the freshwater fish fauna of Italy, with reference to the central region and Barbus graellsii, a newly established alien species of Iberian origin. Journal of Fish Biology 59(Suppl. A):190– 208. DOI:10.1111/jfb.2001.59.issue-sa.
- Bonneau JL, Scarnecchia DL. 2015. Response of benthic macroinvertebrates to carp (Cyprinus carpio) biomanipulation in three tributaries of a eutrophic, GreatPlainsreservoir,USA.Transactions of the Kansas Academy of Science 118:13–26. DOI:10.1660/ 062.118.0103.
- Britton JR, Harper DM, Oyugi DO, Grey J. 2010. The introduced Micropterus salmoides in an equatorial lake: A paradoxical loser in an invasion meltdown scenario? Biological Invasions 12:3439–3448. DOI:10.1007/s10530-010-9742-7.
- Candiotto A, BoT, Fenoglio S. 2011. Biological andecological data on an established rainbo wtrout (Oncorhynchusmykiss) population in an Italian stream. Fundamental and Applied Limnology 179:67–76. DOI:10.1127/1863-9135/2011/0179-0067.
- Castaldelli G, Pluchinotta A, Milardi M, Lanzoni M, Giari L, Rossi R, Fano EA. 2013. Introduction of exotic fish species and decline of native species in the lower Po basin, northeastern Italy. Aquatic Conservation: Marine and Freshwater Ecosystems 23:405–417. DOI:10.1002/aqc.v23.3.
- Castaldelli G,Rossi R. 2008. Emilia-Romagna fishinventory, A and B zones. Emilia-Romagna region. Bologna, ITA: Greentime.
- Dibble ED, Kovalenko K. 2009. Ecological impact of grass carp: A review of the available data. Journal of Aquatic Plant Management 47:1–15.
- Godinho FN, Ferreira MT. 2000. Composition of endemic fish assemblages in relation to exotic species and river regulation in a temperate stream. Biological Invasions 2:231–244. DOI:10.1023/A:1010022123669. Gurevitch J, Padilla DK. 2004. Are invasive species a major cause of extinctions? Trends in Ecology & Evolution 19:470–474. DOI:10.1016/j.tree.2004.07.005.
- Hankin DG, Reeves GH. 1988. Estimating total fish abundance and total habitat area in small streams based on visual estimation methods. Canadian Journal of Fisheries and Aquatic Sciences 45:834–844. DOI:10.1139/f88-101.
- Kitchell JF, Schindler DE, Ogutu-Ohwayo R, Reinthal PN. 1997. The Nile perch in Lake Victoria: Interactions between predation and fisheries. Ecological Applications 7:653–664. DOI:10.1890/ 1051-0761(1997)007[0653:TNPILV]2.0.CO;2.
- Kottelat M, Freyhof J. 2007. Handbook of European freshwater fishes. Berlin: Kottelat, Cornol and Freyhof. pp. 646.
- Leprieur F, Beauchard O, Blanchet S, Oberdorff T, Brosse S. 2008. Fish invasions in the world's river systems: When natural processes are blurred by human activities. PloSBiology6:404–410.
- Leunda PM. 2010. Impacts of non-native fishes on Iberian freshwater ichthyofauna: Current knowledge and gaps. Aquatic Invasions 5:239–262. DOI:10.3391/ai.
- Milardi M, Lanzoni M, Kiljunen M, Torniainen J, Castaldelli G. 2015. Natural recruitment contributes to high densities of grass carp Ctenopharyngodon idella (Valenciennes, 1844) in Western Europe. Aquatic Invasions 10:439–448. DOI:10.3391/ai.
- Mooney HA, Cleland EE. 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences 98:5446–5451.
- Pascale M, Perosino G, Piccinini A. 2004. Carta Ittica dell'EmiliaRomagna Zona D. Regione Emilia-Romagna Ed., Crest, Torino. pp. 160.
- Pascale M, Perosino G, Piccinini A. 2006. Carta Ittica dell'Emilia Romagna Zona C. Regione Emilia-Romagna Ed., Crest, Torino. pp. 160. Reynolds J. 1983.
- Electrofishing. In: Nielsen L, Johnson D, editors. Fisheries techniques. Bethesda, MD: American Fisheries Society. pp. 147–163.
- Ribeiro F, Leunda PM. 2012. Non-nativefish impacts on Mediterranean freshwater ecosystems: Current knowledge and research needs. Fisheries Management and Ecology 19:142–156. DOI:10.1111/j.1365-2400.2011.00842.x.
- Richardson MJ, Whoriskey FG, Roy LH. 1995. Turbidity generation and biological impacts of an exotic fish Carassius auratus, introduced into shallow seasonally anoxic ponds. Journal of Fish Biology 47:576–585. Simberloff D. 2006. Invasional meltdown 6 years later: Important phenomenon, unfortunate metaphor, or both? Ecology Letters 9:912–919. DOI:10.1111/ele.2006.9.issue-8.
- Simberloff D, Von Holle B. 1999. Positive interactions of nonindigenous species: Invasional meltdown? Biological Invasions 1:21–32. DOI:10.1023/A:1010086329619.
- Stanković D, Crivelli AJ, Snoj A. 2015. Rainbow trout in Europe: Introduction, naturalization, and impacts. Reviews in Fisheries Science & Aquaculture 23:39–71. DOI:10.1080/ 23308249.2015.1024825.

- Townsend CR. 1996. Invasion biology and ecological impacts of brown trout Salmo trutta in New Zealand. Biological Conservation 78:13–22. DOI:10.1016/0006-3207(96) 00014-6.
- Wainwright PC, Osenberg CW, Mittelbach GG. 1991. Trophic polymorphism in the pumpkinseed sunfish (Lepomis gibbosus Linnaeus): Effects of environment on ontogeny. Functional Ecology 5:40–55. DOI:10.2307/2389554.
- Witte F, Msuku BS, Wanink JH, Seehausen O, Katunzi EFB, Goudswaard PC, Goldschmidt T. 2000. Recovery of cichlid species in Lake Victoria: An examination of factors leading to differential extinction. Reviews in Fish Biology and Fisheries 10:233–241. DOI:10.1023/A:1016677515930.
- Zalewski M, Cowx IG. 1990. Factors affecting the efficiency of electrofishing. In: Cowx IG, Lamarque P, editors. Fishing with electricity. London: Fishing News Books. pp. 89–111.

# 3. TRANSITIONAL FISH COMMUNITY OF PO RIVER DELTA

## 3.1. The characterization of Po Delta fish community: the case of the Valli di Comacchio

### 3.1.1. Case study 5. Meteorological factors influence marine and resident fish movements in a brackish lagoon

Abstract

Brackish lagoons are vulnerable aquatic environments that host specifically adapted fish species, as well as marine species on a temporary basis. We used long-term passive fishing gear data, to investigate how meteorological factors (i.e., wind and rain) affected resident and marine fish movement between inner and outer habitats in a Northern Mediterranean coastal lagoon. In particular, we used multivariate and threshold analyses to explore the relationship between fish catches in a transitional habitat and wind and rain variables, accounting for other major temporal and environmental variables. Our results indicated that meteorological factors had effects comparable to seasonal and annual variations, for wind and rain, respectively, and thus are potentially relevant drivers of the movement of fish species between coastal lagoons and marine habitats. Overall, prevalent wind direction and rain on the day before sampling were the most effective meteorological variables in enhancing fish movement. Furthermore, movement of lagoon resident species seemed to be enhanced by wind factors (both direction and wind speed), whereas marine species movement was enhanced by rain factors (but it was not possible to disentangle annual and seasonal effects for the latter). Among other factors, dissolved oxygen seemed to be linked to prevailing wind direction and showed significant thresholds around 7–8 mg/l for the increased movement of a number of marine species. Prevailing and gusty wind directions (around WSW) and speed ($2–6$ m s$^{-1}$) also showed significant thresholds for at least a resident species and a marine species that uses the lagoon as nursery.

Introduction

A solid literature body underlines the ecological importance of brackish lagoons for the functioning of coastal ecosystems (Newton et al. 2014). Lagoon ecosystems host a wide range of biotopes, which thrive on a delicate balance between multiple factors where salinity plays a key role (Blaber and Blaber 1980; Young et al. 1997; Gelin et al. 2001; Pe´rez-Ruzafa et al. 2011). Both marine and freshwater fish species cantemporarily in habit lagoons, when suitable salinity levels allow their presence (Franco et al. 2008; Potter et al. 2015). However, some species are specifically adapted to these environments and complete their whole life cycle in coastal lagoons [e.g., the sand goby Pomatoschistus minutus, Pallas 1770, Leita˜o et al. (2006)].

Given that lagoons are usually particularly productive areas in the coastal shelf, due to the input of landorigin nutrients, it is not surprising that marine fish often use lagoon as feeding grounds. Marine fish can enter periodically or occasionally the lagoon, mostly when salinity reaches higher levels (McLusky et al. 2004), for example, when tides bring in water from the open sea or when evaporation and scarce precipitation allow salinity levels to rise (Castro et al. 2009; Bruno et al. 2013; Bruno and Acha 2015). Some marine fish species are better adapted than others to withstand salinity transitions, and some have taken this so far that they can also use lagoons as spawning grounds or nurseries (Verdiell-Cubedo et al. 2013). In general, several coastal fish species use lagoons as nursery grounds, with juveniles growing in the sheltered lagoon environment and moving progressively out of it as they grow, but the factors regulating this behavior are scarcely investigated. On the contrary, some fish species take advantage of the lagoon habitats throughout their life and are adapted to live between different environments and salinity gradients (McLusky et al. 2004). Specialized physiological adaptations of the kidneys and gills allow them to withstand relatively rapid changes in salinity, which can occur at the monthly or seasonal scale, and thus exploit those transitional habitats usually inhospitable for salt- or freshwater fish (Vernberg 1982; McCormick et al. 2013). Furthermore, lagoon resident fish often possess behavioral and physical adaptations to live in areas with changing currents, turbidity and temperatures (Vernberg 1982). Among these is the ability to move from one habitat to another, following the even shifting conditions; however, not much is known about the exact factors that affect their movement between inner lagoon habitats. Among these factors, two likely drivers are wind and rain. Wind can increase water mixing by creating currents that affect oxygenation, temperature, and salinity, especially in shallow lagoons (Kjerfve and Magill 1989). Strong winds can also create waves and in general increase turbulence, thus potentially increasing turbidity through the resuspension of sediments and, in parallel, resuspending nutrients and algal films as well (Lawson et al. 2007). Particularly, in shallow environments, particle resuspension can induce large ecological effects (Chao et al. 2017). Besides wind intensity, also wind direction might dictate the strength of these effects, because of differences in fetch, which can be especially relevant in coastal lagoons. Furthermore, rain can dilute the salinity in the lagoon because of the freshwater input from rivers, and this in turn could drive some salinitysensitive species to move from their usual habitats, following salinity gradients. Rain can even alter the nutrient balance of the lagoon, by altering water residence time and affecting the connection with the sea (Mendoza-Salgado et al. 2005). Unfortunately, very little research has been undertaken on how these factors affect fish behavior. The Mediterranean Sea could be an ideal case study to test the effects of wind and rain on fish behavior, because of the lower tidal effects in its smaller basin compared to other seas, which potentially help reducing other confounding effects. To test this, we estimated fish movement rates in the Fattibello Lagoon, northern Adriatic Sea, using passive fishing gear operated in obligated pathways between the freshwater and marine environments. We used multivariate and threshold analyses to explore the relationship between fish catches and meteorological variables (wind and rain), with the goal of identifying major environmental drivers in these ecosystems. Based

on existing knowledge of the mechanisms of windandrainaction, wehypothesized thatmovements of lagoon resident species and marine species that use the lagoon as nursery would enhanced by wind factors, as turbulence, currents, and resuspension would affect their habitats most significantly. We also hypothesized that stronger winds, particularly those from directions close to south west, having the longest fetch, would have greater effects than weaker winds. Finally, we hypothesized thatmarinespeciesthatusethelagoonas feeding grounds would be more affected by rain factors, as they could be the most sensitive to changes in salinity within the lagoon. We expected that heavier rains would correspond to a decreased movement of marine species through the lagoon transitional habitats.

## Materials and methods

### *Study area*

This study used the Fattibello Lagoon, a small (ca. 700 ha) brackish (salinity ca. 13–31) lagoon in the northwestern shore of the Adriatic Sea (Fig. 1) as a model environment. It constitutes the northernmost part of the Comacchio Lagoons, an area renown worldwide for its long-standing tradition in extensive aquaculture of European eel (*Anguilla anguilla* L.). The region has a Mediterranean climate, with summer temperatures exceeding 30 °C and winter temperatures reaching below 0. The typical annual precipitations over the Lagoon are around 600–700 mm, with a peak during autumn. There are no definite trade winds, but wind storms are common during the autumn, winter and spring months.

The Fattibello Lagoon is connected to the sea through the Navigabile and Logonovo channels, two large waterways which are nowadays used mostly for recreational boat transit. The Navigabile Channel also extends inland and, together with the Fosse Foce Channel, can occasionally input freshwaters into the lagoon. Depth varies from a minimum of a few centimeters on the shallowest sandbars to a maximum of 3 m in the deepest channels (average depth 1 m). The lagoon substrate is also very variable, including muds and silts as well as sands and harder substrates (mussel beds, shell hash). Water currents reshape the softer sediments, sometimes creating temporally shifting patterns of sandbars. The high habitat complexity and variability make this lagoon an ideal habitat for several brackish species, such as gobies (e.g., the endemic Canestrini's goby, Pomatoschistus canestrinii, Ninni 1883), and a good foraging or nursery ground for marine species.
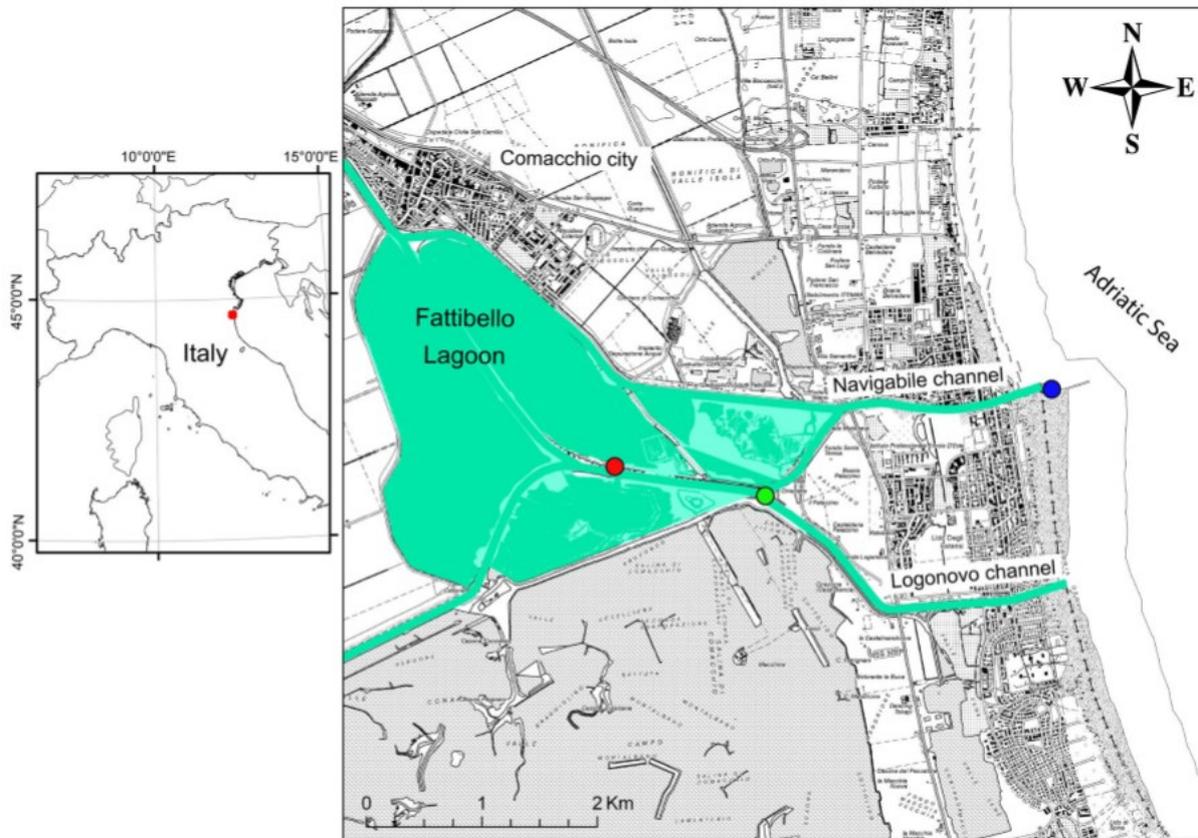
**Fig. 1** Map showing the location of the Fattibello Lagoon and its area, the fish sampling point (red dot, at the lagoon entrance of the Logonovo Channel) as well as the location of the water chemistry (green dot, in the Logonovo Channel) and weather (blue dot, at the seaward end of the Navigabile Channel) probes. (Color figure online)

*Fish sampling*

A total of 32 fish sampling events were carried out over the span of 6 years, between 2009 and 2014. Sampling events were not equally distributed in all years but, in each year, covered at least the relevant seasons when juvenile fishes are present in the lagoon. Most sampling was carried out during the spring and autumn months, when meteorological factors like wind and rain are most commonly at play. However, please note that, in 2011, at least one sampling event was carried out in each month, to ensure that the full year spectrum was also taken into account. Detailed information on the exact timing of each sampling event is given in Table 1 of Milardi et al. (2018b). Fish sampling was performed using stationary lift nets, a traditional fishing method in the area adapted to capture a wide range of fish sizes. Lift nets are 15 9 15 m square nets with an outer mesh of 12 mm, a second of 8 mm, and an inner one of 6 mm. Nets are operated from a sheltered housing through a series of beams and pulleys, which keep the net stretched and lower it into the water until the bottom; the net is subsequently lifted through the same system, capturing the fish in the water column and collecting them in the inner mesh. Sampling was undertaken at a fixed location on the banks of the Logonovo Channel, at the entrance of the Fattibello Lagoon and about 700 m inland from the sea (Fig. 1). The Logonovo Channel is a navigation pathway and an extremely simplified habitat; we considered this as a transitional habitat where no species reside permanently, but rather used for movement

between the lagoon and the open sea. Given this setup, the amount and composition of the catch in each sampling event depended on the number of net lifts. Each net lift captured a number of individuals of different species of fishes (each with their own catchability) moving through the channel and over the net area when it was being lifted, so that catch rates could be used to infer the magnitude of fish movement through the channel (i.e., between the lagoon and marine habitats). As our sampling gear was non-directional, we could only analyze the intensity, rather than the direction, of movement between these habitats. Sampling usually started in the afternoon (on average around 16:00) and lasted from a minimum of 57 min to a maximum of 9 h (from 12 to 32 net lifts in a sampling event). All catch data were converted in catch per unit of effort (CPUE, based on number of net lifts) to account for different sampling efforts. All captured fish specimens were identified to the species level, measured (to the nearest mm) and weighted with a resolution of 0.1 g [for further details on the catch composition see Table 2 in Milardi et al. (2018b)]. Fish species were classified taking into account recent taxonomical determinations and common names as listed in FishBase (Froese and Pauly 2017) and Kara and Quignard (2018). When samples contained a large amount of individuals of the same species, a subsample of 100 specimens was randomly selected to be measured in detail. Each species was assigned to a category, based on their prevalent functional use of the lagoon, following the concepts by Elliott and Hemingway (2008) and by Franco et al. (2008) (i.e., the prevalent use of the Fattibello Lagoon by a species, throughout their life cycle). We defined a total of four different categories, with the last one having three subcategories: Catadromous, Anadromous, Brackish (lagoon resident) and Marine (divided into feeding ground, nursery and stragglers subcategories). Marine species that enter the lagoon regularly to feed were categorized under ''feeding ground,'' those that live predominantly in the lagoon as juveniles were categorized under ''nursery'' and finally those that enter the lagoon only occasionally as ''stragglers.''

*Environmental parameters*

To account for seasonal and annual factors, both the month (Month) and the year (Year) of sampling were used as environmental parameters in our analysis. Wind speed and direction data were recorded by an automated weather station (OTT LOGOSENS), positioned at the entrance of the Navigabile Channel, roughly 700 m seaward from the sampling location, and operated by the Emilia-Romagna Region Environmental Protection Agency (ARPA Emilia Romagna). Four distinct wind variables were considered: the prevailing wind direction (PrevWindDir) and speed (PrevWindSpeed), and the strongest gust direction (GustWindDir) and speed (GustWindSpeed). Wind direction was measured with a tolerance of 22.5 degrees (a total of 16 different directions) and wind speed was measured in seven different classes (0–1, 1–2, 2–3, 3–5, 5–6, 6–10, 10–13.5 and 13–99 m s$^{-1}$).

Rain data were recorded using a rain gauge located at the same weather station, and it was considered as a proxy of meteorological events such as rainstorms. Three different rain variables were extracted: the total

precipitation (in mm) on the day prior to the sampling event (TotRain), on the sampling day (RainDay) and during the sampling itself (RainFish). Rain on the sampling day/momentcan affect surface salinity in the lagoon but, as the Fattibello Lagoon also receives freshwater input from a river source (the Fosse Foce channel), which can influence the salinity as well. Therefore, we also included the previous day precipitations, to account for the lag time between rainfall and drainage through the basin to the lagoon. Dissolved oxygen (DO), salinity (Salinity), and temperature (Temp) of the water were also measured by the Emilia-Romagna Region Environmental Protection Agency (ARPA Emilia Romagna) with a multiparametric probe (OTT DUOSENS) in the Logonovo Channel, in close proximity of the sampling site. The probe logs data with an hourly resolution, but an average daily value for each of the three parameters was considered as representative for each sampling event. These parameters had no significant spatial variations between the probe location and the fish sampling location, as verified by spot measures performed during sampling.

*Statistical analysis*

In order to explore the relationship between fish catches and meteorological as well as environmental parameters, multivariate statistics were employed. Table 1 contains a summary of the meteorological and environmental variables utilized in this study. A detrended correspondence analysis (DCA) was initially performed to select the most appropriate response model for gradient analysis (Leps and Smilauer 2003). The dominant gradient length in DCA was always lower than 3, so the redundancy analysis (RDA) was finally chosen (Leps and Smilauer 2003). RDA is a linear gradient analysis that allows to quantify the variation of a multivariate data set explained by independent variables (ter Braak and S ˇmilauer, 2002). The month and year of sampling (to account for seasonal and annual factors), as well as the meteorological and environmental data, were considered as independent variables, whereas the CPUEs of each fish species were considered as dependent. All environmental and fish CPUE data were $\log_{10}(X + 1)$ transformed. Rare species sampled in less than three sampling events were excluded from the analysis to avoid distortions (see, e.g., Godinho and Ferreira 2000), with the exception of rare marine species (*Belone belone, L., Scomber scombrus, L., Psetta maxima, L., Diplodus vulgaris*, Geoffroy Saint-Hilaire 1817, *Pagellus bogaraevo, Brunnich 1768 and Umbrina cirrosa, L.*) that were combined in a single variable called ''RareSpecies'' to test for effects common to all occasional marine stragglers. We also ran a similar analysis using meteorological and environmental data as independent variables, but using categories of lagoon functional use as independent variables. Collinearity of independent variables was investigated through the variance inflation factor (VIF) and collinear variables with VIF [8 were excluded from the analysis. A variance-partitioning scheme (Borcard et al. 1992; Liu 1997) was applied for each group of variables based on the overall variance explained by the partial RDAs. This procedure allowed the distinction between unique effects (i.e., the variance explained by a single group of variables), joint effects (i.e., the variance jointly explained by variables of two or three groups), and unexplained variance. Variance partitioning was also run with all variables to identify the marginal effects (k-

1) and the conditional effects (k-A) of each descriptor variable (see e.g., Milardi et al. 2018a). The marginal effect of a descriptor variable is equal to the eigenvalue of a partial RDA if the corresponding variable was the only environmental variable (additionally to the variance explained by covariables). The conditional effect of an environmental variable is equal to the additional amount of variance in species assemblages explained by the corresponding variable at the time it was included into the model during a selection procedure. Such effects were also examined to assess the relative contributions of environmental variables for predicting the catch composition (Ter Braak and Smilauer 2002). These analyses were performed using the CANOCO 4.5 for Windows software (Leps and Smilauer 2003). We used the Threshold Indicator Taxa ANalysis [TITAN, Baker and King (2010)] on all environmental parameters to identify the environmental threshold (the optimum value of a continuous variable) that partitions sampling units and distinguishes negative (= losses: z -) and positive (= gains: z +) taxon responses. Thus, TITAN helps to identify taxonspecific change points along an environmental gradient at which the decline/increase in a given taxon's frequency and abundance is most prominent. Bootstrapping (500 repetitions) is used to estimate two important diagnostic indices (reliability and purity) as well as uncertainty around the location of individual taxa and community change points (Baker and King 2010). TITAN analysis was performed with R Software (R Core Team, 2017), using the 'TITAN2' package (Matthew, Baker and King, 2015).

**Table 1** Mean annual values of the environmental variables examined in this study, based on daily averages recorded during sampling events. Each column heading also reports the number of sampling events during that year. Month and year were also used as temporal variables in the analysis

| Variable | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 |
|---|---|---|---|---|---|---|
| Total precipitation on the sampling day (mm) | 2.85 | 1.3 | 2.78 | 0.2 | 6 | 0.04 |
| Precipitation during the sampling event (mm) | 0 | 0 | 0.85 | 0.00 | 0.00 | 0.00 |
| Precipitation on the day prior to the sampling event (mm) | 0.85 | 4.4 | 1.35 | 0.44 | 15.70 | 0.08 |
| Prevailing wind direction on the sampling day | SSW | SSE | SE | WSW | N | SW |
| Prevailing wind speed on the sampling day (class) | 2.3 | 3.5 | 3.6 | 2.4 | 2.0 | 2.0 |
| Strongest gust direction on the sampling day | SE | SSE | SE | SSE | SSW | SSW |
| Strongest gust speed on the sampling day (class) | 4.5 | 4.5 | 4.67 | 4.6 | 3.5 | 3.8 |
| Dissolved oxygen (mg/l) | 8.2 | 8.5 | 6.2 | 6.9 | 6.8 | 8.5 |
| Salinity (ppm) | 19.4 | 19.3 | 25.5 | 27.8 | 16.8 | 20.3 |
| Water temperature (°C) | 15.6 | 15.7 | 17.8 | 18.2 | 23.2 | 14.8 |

## Results

Sampling collected a very variable amount of fish (0.49–38.2 kg per single event, 56.2–1378.7 g per net lift). A total of 36,336 individuals of 32 different fish species were captured, belonging to 19 families and with all four different lagoon functional use categories (Table 2). Sampling events were distributed rather equally between different wind directions and intensities. Prevailing winds came most often from NW (6 sampling events) and SE (5 sampling events), while no sampling events recorded winds from ENE, WNW and SSW (Fig.

2). Strongest gusty winds followed closely the pattern of prevailing winds, with NW and SE being the most common directions (5 sampling events each), and no gusts from N, E or ENE were recorded (Fig. 2). The average prevailing wind speed class was 2.87 ± 1.26, while the average gust wind class was 4.4 ± 1.18. Rainfall was completely absent during or around 12 sampling events (26.6% of the total), with only two instances of rainfall recorded, while the sampling took place (Fig. 3).

**Table 2** List of fish species captured in the Fattibello lagoon between 2009 and 2014, including the category of functional use of the lagoon for each species and their total CPUEs in the dataset

| Scientific name | Common name | Family | Functional use of the lagoon | Total CPUE |
|---|---|---|---|---|
| Alosa fallax | Twaite shad | Clupeidae | Anadromous | 20.34 |
| Atherina boyeri | Big scale sand smelt | Atherinidae | Brackish (lagoon resident) | 1621.84 |
| Salaria pavo | Peacock blenny | Blenniidae | Brackish (lagoon resident) | 0.77 |
| Aphanius fasciatus | Mediterranean banded killifish | Cyprinodontidae | Brackish (lagoon resident) | 2.16 |
| Zosterisessor ophiocephalus | Grass goby | Gobiidae | Brackish (lagoon resident) | 9.66 |
| Pomatoschistus canestrinii | Canestrini's goby | Gobiidae | Brackish (lagoon resident) | 54.81 |
| Knipowitschia panizzae | Adriatic dwarf goby | Gobiidae | Brackish (lagoon resident) | 13.72 |
| Pomatoschistus minutus | Sand goby | Gobiidae | Brackish (lagoon resident) | 7.55 |
| Gobius niger | Black goby | Gobiidae | Brackish (lagoon resident) | 9.84 |
| Gobius paganellus | Rock goby | Gobiidae | Brackish (lagoon resident) | 65.52 |
| Sygnatus acus | Greater pipefish | Syngnathidae | Brackish (lagoon resident) | 64.54 |
| Sygnathus abaster | Black striped pipefish | Syngnathidae | Brackish (lagoon resident) | 8.95 |
| Anguilla anguilla | European eel | Anguillidae | Catadromous | 119.55 |
| Sprattus sprattus | European sprat | Clupeidae | Marine (feeding grounds) | 8.28 |
| Engraulis encrasicolous | European anchovy | Engraulidae | Marine (feeding grounds) | 2957.27 |
| Dicentrarchus labrax | European seabass | Moronidae | Marine (nursery) | 5520.85 |
| Plathychtys flesus | European flounder | Pleuronectidae | Marine (nursery) | 3.63 |
| Chelidonichthys lucerna | Tub gurnard | Triglidae | Marine (nursery) | 0.68 |
| Liza ramada | Thinlip mullet | Mugilidae | Marine (nursery) | 0.40 |
| Liza aurata | Golden grey mullet | Mugilidae | Marine (nursery) | 0.27 |
| Liza saliens | Leaping mullet | Mugilidae | Marine (nursery) | 42.10 |
| Mugil cephalus | Flathead grey mullet | Mugilidae | Marine (nursery) | 407.75 |
| Chelon labrosus | Thicklip grey mullet | Mugilidae | Marine (nursery) | 102.18 |
| Belone belone | Garfish | Belonidae | Marine (straggler) | 39.87 |
| Scomber scombrus | Atlantic mackerel | Scombridae | Marine (straggler) | 6.21 |
| Psetta maxima | Turbot | Scophthalmidae | Marine (straggler) | 912.21 |
| Diplodus vulgaris | Common two-banded seabream | Sparidae | Marine (straggler) | 242.33 |
| Pagellus bogaraevo | Blackspot seabream | Sciaenidae | Marine (straggler) | 207.49 |
| Solea solea | Common sole | Soleidae | Marine (straggler) | 13.01 |
| Sparus aurata | Gilt-head bream | Sparidae | Marine (straggler) | 24.61 |
| Mullus barbatus | Red mullet | Mullidae | Marine (straggler) | 40.92 |
| Umbrina cirrosa | Shi drum | Sciaenidae | Marine (straggler) | 1.38 |
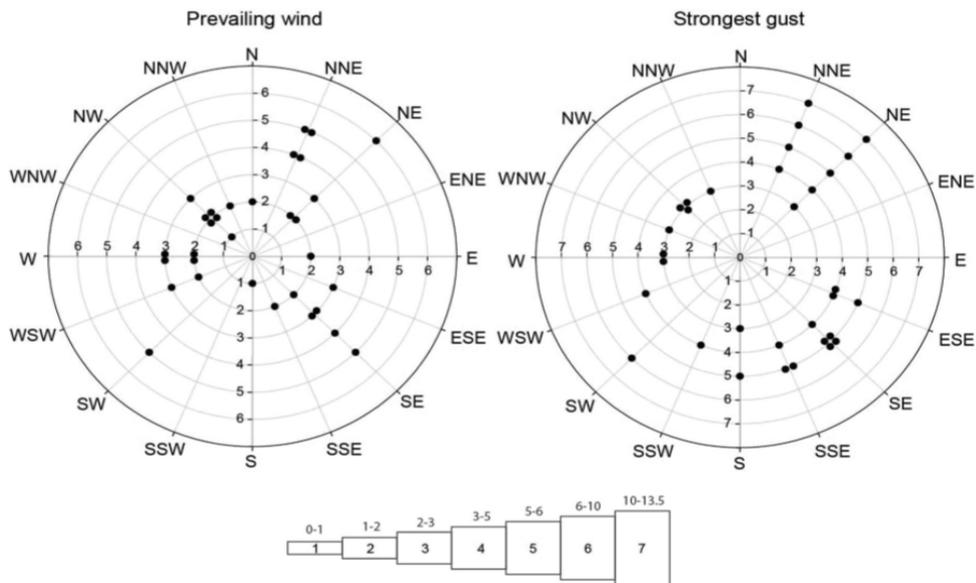
**Fig. 2** Prevailing (left) and gust (right) winds recorded at the Fattibello lagoon during sampling events. Each point position indicates wind direction (angular position) and intensity class (radial position, distance from center), and some points have been offset so as not to mask underlying ones. Below the radial plots, a legend is provided to interpret wind speed classes (velocities in m/s)

However, rainfall was overall rather frequent (63.4% of all events), especially during the day of sampling (11 events) and on the previous day (11 events). Only the peacock blenny (Salaria pavo, Risso 1810), sampled in two sampling events, and strongest gust direction had VIF [8 and were thus excluded from the RDA analysis. RDA analysis confirmed the relevance of wind and rain factors, which had effects comparable to seasonal and annual variations, respectively (Fig. 4a).

**Fig. 3** Rainfall potentially affecting sampling events. Each stacked bar represents the amount of rain in a 48 h period ending with the sampling events. Colors separate the rain on the day before sampling (black), on the sampling day (dark gray) and during the sampling itself (light gray). The horizontal dashed like marks the upper limit of what is commonly considered a light rainfall (30 mm in 12 h)
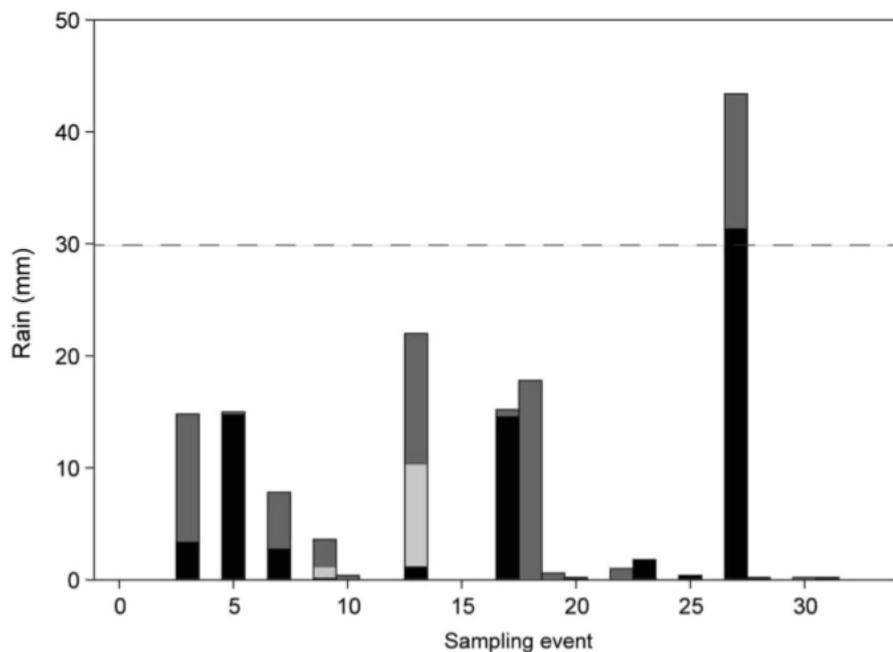


Overall, the RDA had a fair explanatory potential of the species variance (horizontal axis explained 29.2% of the data variance and vertical axis 22.1%, Fig. 4a), with the exception of rare marine species, which did not

show any significant pattern. Prevalent wind direction had one of the strongest effects detected, but also its speed and the speed of gusts had rather significant effects, especially on lagoon resident species (Fig. 4a). Prevalent wind direction effects showed a positive relationship with dissolved oxygen effects, while its speed effects showed a positive relationship with salinity (Fig. 4a). Rain during sampling had a negligible effect, but the amount of rain during the day and, even more, during the previous day had greater effects than most other variables examined and correlated with salinity changes (Fig. 4a). However, rain showed a weaker effect on lagoon resident species and influenced more marine species (Fig. 4a). A similar analysis on the categories of functional use of the lagoon had a higher explanatory power (horizontal axis explained 59.1% of the data variance and vertical axis 21.4%, Fig. 4c). Marine species that use the lagoon as feeding grounds were mostly influenced by precipitations and temperatures, as well as annual variables (Fig. 4c). Marine species that use the lagoon as nursery and stragglers were similarly affected by salinity, precipitation and gusty winds, as well as seasonal variables while lagoon residents where the least affected by these variables (Fig. 4c). Dissolved oxygen and temperature had relevant unique and marginal effects both for species and functional use groups (Fig. 4b, d). Wind gust speed, total rain, and wind on the previous day werethemeteorological factorswiththelargest effects on species (Fig. 4b). Conversely, rain on the fishing day, total rain, and wind gust speed were the meteorological factors with the largest effects on functional use groups (Fig. 4d).

Overall, dissolved oxygen, salinity, and temperature explained the largest proportion of the variance in our data for both species and functional groups (Fig. 5a, b). However, meteorological factors explained a proportion of variance comparable to that of dissolved oxygen, salinity and temperature and greater than seasonal/yearly factors (Fig. 5a, b). Dissolved oxygen was a significant factor for several species movements (Fig. 6a). Gust direction and intensity showed significant thresholds for thinlip mullet (*Liza ramada*, Risso 1827, Fig. 6b, c). Similarly, Canestrini's goby movement showed a threshold for prevailing wind direction (Fig. 6d).
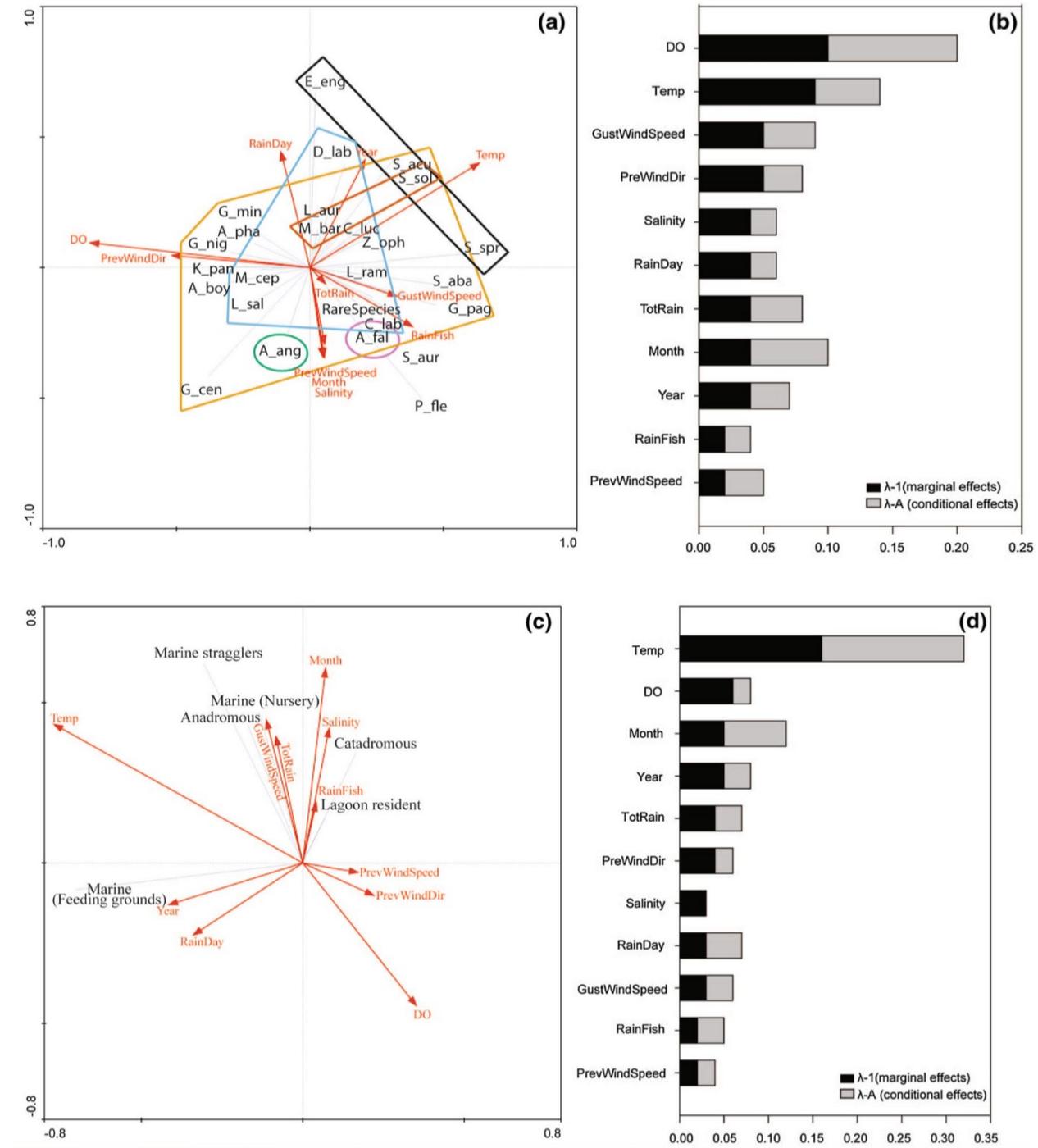
**Fig. 4 a** Redundancy analysis (RDA) triplot showing the relationships between fish species (black labels) and environmental parameters (red arrows and labels) and **b** marginal (λ-1) and conditional (λ-A) effects of variables that affect fish species. Fish species labels consist of abbreviations where the first letter is the initial of the genus, and the three following are the first letters of the species name, environmental variables abbreviations are given in the Materials and Methods section. Polygons and ellipses of different colors group species into categories of functional use of the lagoon: anadromous (reddish purple), catadromous (green), lagoon resident (orange), marine nursery (blue), marine feeding ground (black) and marine stragglers (vermillion). **c** Redundancy analysis (RDA) triplot showing the relationships between different categories of functional use of the lagoon (black labels) and environmental parameters (red arrows and labels) and **d** marginal (λ-1) and conditional (λ-A) effects of variables that affect functional use groups. (Color figure online).
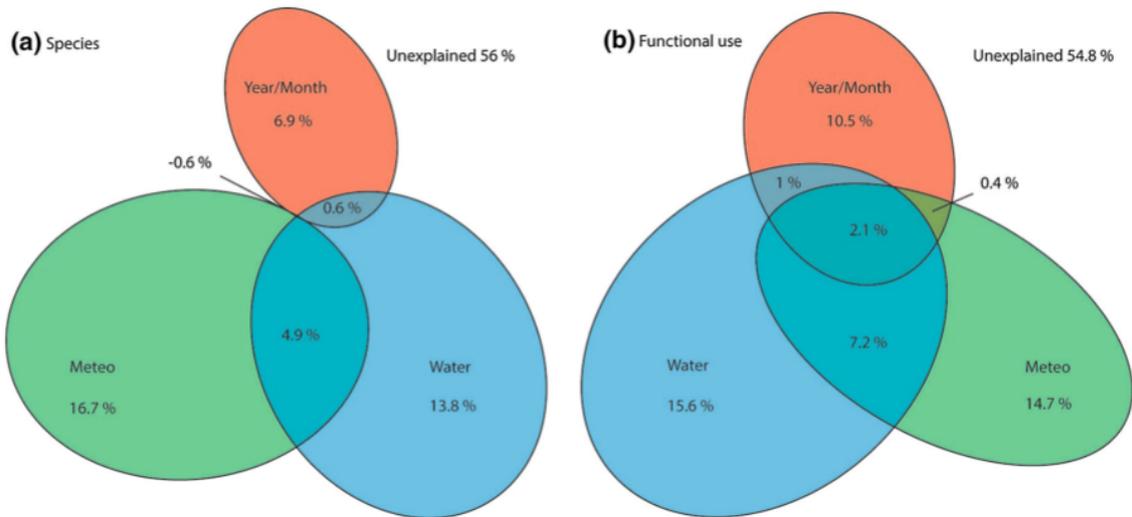
**Fig. 5** Euler–Venn diagram of unique and joint effects of time (Year/Month), meteorological (Meteo) and dissolved oxygen, salinity, and temperature (Water) factors on fish species movement (**a**) and functional use groups movement (**b**). Numbers indicate the variance explained by each component.

Occasionally, a shared variance fraction might have a negative value, indicating that the joint explanatory effect of the groups of variables is stronger than a sum of their marginal effects. (Color figure online)
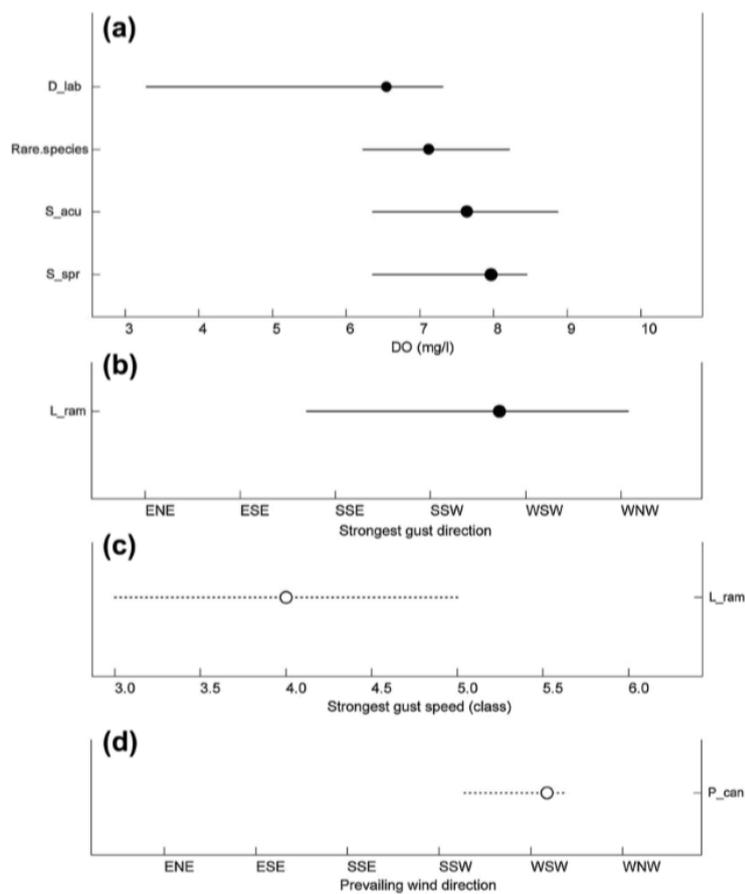


**Fig. 6**. Significant thresholds for different taxa along dissolved oxygen (a), strongest gust direction (b), strongest gust speed (c) and prevailing wind direction (d) gradients, according to the TITAN analysis. Lines represent the 95% confidence interval of the bootstrapped distribution across the gradient, with circles identifying the species change points across the gradient. Solid lines and circles represent species that decline along the gradient (listed on the left axis), while dashed lines and empty circles represent species which distribution increases along the gradient (listed on the right axis). Species not shown in these figures have a distribution not predictable according to the environmental gradients examined.

Discussion

Our results indicated that meteorological factors had effects on fish movement comparable to dissolved oxygen, salinity, and temperature and larger than seasonal and annual variations, and thus could be relevant drivers of the movement of fish species between coastal lagoons and the open sea.

Overall, prevalent wind direction and rain on the day before sampling had the greatest effects on fish movement. Furthermore, the movement of lagoon residents, and marine species that use the lagoon as nursery, seemed to be most affected by wind factors (both direction and wind speed, especially gusty ones), whereas marine species that use the lagoon as feeding grounds were most affected by rain factors (but it was not possible to disentangle annual and seasonal effects for the latter), which confirmed our initial hypotheses. Lagoon resident species movement was the least affected by meteorological factors, perhaps due to their specific adaptation to the shifting conditions of this habitat. Among other factors, dissolved oxygen seemed to be connected with prevailing wind direction and showed significant thre sholds for the movement of a numberof marine species that use the lagoon as nursery grounds or are lagoon residents, but also of some rare marine species that occasionally enter the lagoon. Prevailing wind directions and speed as well as strongest gust speed also showed significant thresholds for at least a resident species (Canestrini's goby) and a marine species that uses the lagoon as nursery (thinlip mullet). Stronger effects could be hypothesized for wind directions from the sea and perpendicular to the coast (i.e., around NW), which should be most effective in pushing the sea water toward the lagoon. However, fetch could also play a role in the significance of wind effects, as the longest fetch is reached for a different direction (i.e., SEwinds). The relevance of a particular wind direction was not overly clear in our multivariate analysis, but our gradient analysis (Fig. 6) highlighted the relevance of offshore winds (with SW winds being particularly significant at least for one species, Fig. 6). While it is true that lagoon resident species responded the least to meteorological factors (Fig. 4c), different species within this group showed marked behavioral responses to specific environmental variables (Fig. 4a). Gobiidae, in particular, seemed to be affected by wind, but even within this group radically opposite behavioral responses were found, e.g., for rock goby compared to other species. As was also confirmed by the TITAN analysis, both thinlip mullet juveniles (a marine species that uses the lagoon as nursery), and Canestrini's goby, a lagoon resident species, seemed to be the most clearly affected by wind direction and intensity. Indeed, our analyses highlighted wind as one of the most important factors affecting movement of species living in the lagoon (either brackish species or marine species at their juvenile stage), but also other parameters such as dissolved oxygen. Wind-generated currents increase the water mixing in the lagoon, providing an increased oxygenation and decreasing potential anoxia caused by eutrophication which could be detrimental to several organisms in the lagoon (Powers et al. 2005).

Generally speaking, stronger average wind speeds (i.e., sustained speeds) and stronger gusts should also have stronger effects (e.g., on water mixing through wave induction). Our data showed a positive linkage

between increased oxygen and movement behavior of some lagoon resident species and at least some marine species (stragglers but also juveniles of European seabass). Similarly, these currents could also increase sediments and nutrient resuspension, thus ultimately increasing turbidity, but we did not have any direct data to support conclusions based on specific or secondary effects.

Winds can also potentially enhance the action of tides, pushing seawater into the lagoon and thus magnifying the amplitude of tides (see, e.g., Brunoand Acha 2015, with an example for juvenile fish). In our dataset, prevailing wind directions from NW would have likely prevented marine water from entering the lagoon (offshore winds), while those from SE would have pushed seawater into the lagoon (onshore winds with the longest fetch). Indeed, a tidal effect on fish activity was found in a parallel study on the Fattibello lagoon (Milardi et al. 2018b), but wind direction and speed were not correlated with tide amplitude in this dataset (Milardi, unpublished data) and prevailing wind directions that theoretically should have had the greatest effects were not singled out by our analysis (see, e.g., Figure 6).

Moreover, tides had greater effects on different species than those affected by wind (e.g., the European anchovy, *Engraulis encrasicolous*, L., that was more affected by tides than by winds), perhaps suggesting that there could be different mechanisms regulatingthe influence of these variables on fish behavior. Indeed, both wind and rain could be partly related toseasonal factors, asthe summer period is usually characterized by stable high pressure and no meteorological events are common (Lanzoni, unpublished data). During autumn, winter, and spring, the weather is more variable, leading to a higher frequency of low-pressure events. These ultimately can also cause rainfall, and its amount was also a relevant factor in our analysis, especially for marine species that use the Fattibello Lagoon as a feeding ground (even though rain was not clearly correlated with salinity changes, Milardi unpublished data). Our data clearly indicated that the highest catches of both sprat, *Sprattus sprattus*, L., and European anchovy occurred when no rain diluted the salinity of the Fattibello Lagoon, confirming our initial hypothesis that these species would not tolerate lower salinities (McCormick et al. 2013). This is particularly significant, as most catches of sprat and European anchovy occurred during the autumn–winter–spring period, when rainfall is usually more frequent than in the summertime. A similar mechanism, limiting marine species presence in a coastal lagoon, was found in a Brazilian lagoon (Garcia et al. 2001), where salinity changes were caused by marked seasonal rainfall variations existed. Intense rainfall and increased freshwater load could occasionally cause salinity in the Fattibello lagoon to drop below usual brackish levels, transporting some typical freshwater species which can be exceptionally found there (the topmouth gudgeon, *Pseudorasbora parva*, Temminck &Schlegels 1825, and the freshwater bream, *Abramis brama, L*., Lanzoni, unpublished data); which is again similar to what published for the Patos Lagoon (Garcia et al. 2003). However, notably there is little published information available on Mediterranean coastal lagoons, and most literature focuses on broadly different areas and usually higher salinities (see, e.g., Vega-Cendejas and de Santillana 2004). It is entirely possible that more complex mechanisms and subtler influences could be at play here (see Pauly 1975, for a counterintuitive

example of salinity mechanisms) therefore our work can only becom paredata general level tothe published literature, and we encourage further studies dealing with this aspect in similar environments. Perhaps the combined action of wind and rain events could not be fully disentangled from other factors affecting fish in the Fattibello Lagoon; in particular, it was evident that seasonal and annual factors could not be completely discounted in this analysis. Furthermore, temporal variability in the lagoon habitat can also constitute an additional confounding factor (Pe ´rez-Ruzafa et al. 2006, 2007) which was not considered in our study but should be included in future ones. It is very likely that all factors can be at play at any single given time, so that, to fully resolve the contribution of each, longer-term monitoring specifically designed for this purpose would be needed as suggested also by Pe ´rez-Ruzafa et al. (2019). However, we believe our data constitute a first valuable insight into the mechanisms underlying broad fish behavior dynamics in a shallow lagoon, which could be useful not only to better understand the ecology of fish but also to better conserve and manage these critical coastal environments.

## References

- Baker ME, King RS (2010) A new method for detecting and interpreting biodiversity and ecological community thresholds. Methods Ecol Evol 1:25–37
- Blaber S, Blaber T (1980) Factors affecting the distribution of juvenile estuarine and inshore fish. J Fish Biol 17:143–162
- Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. Ecology 73:1045–1055
- Bruno DO, Acha EM (2015) Winds vs. tides: factors ruling the recruitment of larval and juvenile fishes into a micro-tidal and shallow choked lagoon (Argentina). Environ Biol Fishes 98:1449–1458
- Bruno DO, Barbini SA, Dı ´az de Astarloa JM, Martos P (2013) Fish abundance and distribution patterns related to environmental factors in a choked temperate coastal lagoon (Argentina). Braz J Oceanogr 61:43–53
- Castro MG, de Astarloa JD, Cousseau M, Figueroa D, Delpiani S, Bruno D, Guzzoni J, Blasina G, Antoni MD (2009) Fish composition in a south-western Atlantic temperate coastal lagoon: spatial–temporal variation and relationships with environmental variables. JMar Biol Assoc UK 89:593–604
- Chao J-Y, Zhang Y-M, Kong M, Zhuang W, Wang L-M, Shao K-Q, Gao G (2017) Long-term moderate wind induced sediment resuspension meeting phosphorus demand of phytoplankton in the large shallow eutrophic Lake Taihu. PLoS ONE 12:e0173477
- Elliott M, Hemingway KL (2008) Fishes in estuaries. Wiley, Hoboken
- Franco A, Elliott M, Franzoi P, Torricelli P (2008) Life strategies of fishes in European estuaries: the functional guild approach. Mar Ecol Prog Ser 354:219–228
- Froese R, Pauly D (2017) FishBase. Version 03/2017. World Wide Web electronic publication, R Froese and D Pauly (eds) Available at: www fishbase org Accessed April 2017
- Garcia A, Vieira J, Winemiller K (2001) Dynamics of the shallow-water fish assemblage of the Patos Lagoon estuary (Brazil) during cold and warm ENSO episodes. J Fish Biol 59:1218–1238
- Garcia A, Raseira M, Vieira J, Winemiller K, Grimm A (2003) Spatiotemporal variation in shallow-water freshwater fish distribution and abundance in a large subtropical coastal lagoon. Environ Biol Fishes 68:215–228
- Gelin A, Crivelli AJ, Rosecchi E ´, Kerambrun P (2001) The effect of salinity changes on the population structure and reproductive traits of Crangon crangon L. populations in the Camargue (Rho ˆne Delta, France). Ecoscience 8:8–17
- Godinho FN, Ferreira MT (2000) Composition of endemic fish assemblages in relation to exotic species and river regulation in a temperate stream. Biol Invasions 2:231–244

- Kara MH, Quignard J-P (2018) Fishes in lagoons and estuaries in the mediterranean: diversity, bioecology and exploitation. Wiley, Hoboken
- Kjerfve B, Magill KE (1989) Geographic and hydrodynamic characteristics of shallow coastal lagoons. Mar Geol 88:187–199
- Lawson S, Wiberg P, McGlathery K, Fugate D (2007) Winddriven sediment suspension controls light availability in a shallow coastal lagoon. Estuaries Coasts 30:102–112
- Leita ̃o R, Martinho F, Neto J, Cabral H, Marques J, Pardal M (2006) Feeding ecology, population structure and distribution of Pomatoschistus microps (Krøyer, 1838) and Pomatoschistus minutus (Pallas, 1770) in a temperate estuary, Portugal. Estuar Coast Shelf Sci 66:231–239
- Leps ̌ J, S ̌milauer P (2003) Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge Liu Q (1997) Variation partitioning by partial redundancy analysis (RDA). Environmetrics 8:75–85
- McCormick SD, Farrell AP, BraunerCJ(2013)Fishphysiology: euryhaline fishes. Academic Press, Cambridge
- McLusky DS, Elliott M, Elliott M (2004) The estuarine ecosystem: ecology, threats and management. Oxford University Press, Oxford
- Mendoza-Salgado RA, Lechuga-Deve ́ze CH, Ortega-Rubio A (2005) Identifying rainfall effects in an arid Gulf of California coastal lagoon. J Environ Manage 75:183–187
- Milardi M, Aschonitis V, Gavioli A, Lanzoni M, Fano EA, Castaldelli G (2018a) Run to the hills: exotic fish invasions and water quality degradation drive native fish to higher altitudes. Sci Total Environ 624:1325–1335. https://doi. org/10.1016/j.scitotenv.2017.12.237
- Milardi M, Lanzoni M, Gavioli A, Fano EA, Castaldelli G (2018b) Tides and moon drive fish movements in a brackish lagoon. Estuar Coast Shelf Sci 215:207–214
- Newton A, Icely J, Cristina S, Brito A, Cardoso AC, Colijn F, Dalla Riva S, Gertz F, Hansen JW, Holmer M (2014) An overview of ecological status, vulnerability and future perspectives of European large shallow, semi-enclosed coastal systems, lagoons and transitional waters. Estuar Coast Shelf Sci 140:95–122
- Pauly D (1975) On the ecology of a small West African lagoon. Ber Deutsch Wiss Kom Meer 24:46–62
- Perez-Ruzafa A, Garcıa-Charton J, Barcala E, Marcos C (2006) Changes in benthic fish assemblages as a consequence of coastal works in a coastal lagoon: the Mar Menor (Spain, Western Mediterranean). Mar Pollut Bull 53:107–120
- Pe rez-Ruzafa A, Marcos C, Pe ́rez-Ruzafa I, Barcala E, Hegazi M, Quispe J (2007) Detecting changes resulting from human pressure in a naturally quick-changing and heterogeneous environment: spatial and temporal scales of variability in coastal lagoons. Estuar Coast Shelf Sci 75:175–188
- Perez-Ruzafa A, Marcos C, Pe ́rez-Ruzafa IM, Pe ́rez-Marcos M (2011) Coastal lagoons:''transitional ecosystems'' between transitional and coastal waters. J Coast Conservation 15:369–392
- Perez-Ruzafa A, Pe ́rez-Ruzafa IM, Newton A, Marcos C (2019) Coastal lagoons: environmental variability, ecosystem complexity, and goods and services uniformity coasts and estuaries. Elsevier, Amsterdam, pp 253–276
- Potter IC, Tweedley JR, Elliott M, Whitfield AK (2015) The ways in which fish use estuaries: a refinement and expansion of the guild approach. Fish Fish 16:230–239
- Powers SP, Peterson CH, Christian RR, Sullivan E, Powers MJ, Bishop MJ, Buzzelli CP (2005) Effects of eutrophication on bottom habitat and prey resources of demersal fishes. Mar Ecol Prog Ser 302:233–243
- Ter Braak CJ, Smilauer P (2002) CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5). www. canoco. com
- Vega-Cendejas ME, de Santillana MH (2004) Fish community structure and dynamics in a coastal hypersaline lagoon: rio Lagartos, Yucatan, Mexico. Estuar Coast Shelf Sci 60:285–299
- Verdiell-Cubedo D, Oliva-Paterna FJ, Ruiz-Navarro A, Torralva M (2013) Assessing the nursery role for marine fish species in a hypersaline coastal lagoon (Mar Menor, Mediterranean Sea). Mar Biol Res 9:739–748
- Vernberg F (1982) Environmental adaptation to lagoon systems. Oceanol Acta (special issue)
- Young G, Potter I, Hyndes G, De Lestang S (1997) The ichthyofauna of an intermittently open estuary: implications of bar breaching and low salinities on faunal composition. Estuar Coast Shelf Sci 45:53–68

### 3.1.2. Case study 6. Tides and moon drive fish movements in a brackish lagoon

Abstrac

Brackish lagoons, on the edge between marine and freshwater ecosystems, are vulnerable aquatic environments that act as nursery grounds for several of the most commercially exploited fish families. We used long-term passive gear data, to investigate whether the moon and tides affected fish movement between inner and outer habitats in a Northern Mediterranean coastal lagoon. In particular, we used multivariate, threshold and nonlinear correlation analyses to explore the relationship between fish catches and moon and tide variables in transitional habitats, accounting for th epresence of potential prey and other major temporal and environmental variables. Fish movements between habitats were influenced by moon and tide factors, which had effects comparable to annual and seasonal variations, respectively. Overall, the magnitude e of effects related to the moon parameters were smaller than most environmental parameters examined, but still larger than e.g.the presence of invertebrate prey (lagoon shrimp) or someof the tidefactors. European flounder catches were positively correlated with disk illumination, while sand and black goby were influenced by the moon phase. Other benthic and pelagic species showed no significant correlation. Tide direction affected negatively the movement of boxlip mullet and tide amplitude prior to sampling had far more effect than tide amplitude during sampling. Water temperature, salinity and the presence ofi nvertebrate prey (greyshrimp) had significant but contrasting effects on some, but not all, of the species examined. Ultimately, this information could improve the und erstanding of the drivers in the see cosystem as well as potentially provide use fulin sights for improved fisheries management.

Introduction

Brackish lagoons are some of the most important and vulnerable aquatic environments worldwide, sitting on the edge between marine and freshwater ecosystems (Newton et al., 2014). Their biotopes thrive on a delicate balance between multiple factors where salinity plays a key role (Blaber & Blaber, 1980; Gelin, Crivelli, Rosecchi, & Kerambrun, 2001; Young, Potter, Hyndes, & De Lestang, 1997). Some species have adapted specifically to this balance and complete their whole life cycle in coastal lagoons (e.g. the sand goby Potamoschistus minutus, Leitão et al., 2006). However, both marine and freshwater fish species can temporarily inhabit lagoons, when the environmental conditions allow their presence (Franco, Elliott, Franzoi, & Torricelli, 2008). Furthermore, brackish lagoons constitute nursery grounds for several of the most commercially exploited families of marine fish such as Pleuronectidae, Sparidae or Clupeidae (Tournois et al., 2017). Juvenile fishes might reside in coastal lagoons for a variable amount of time, since some of the lagoon

habitats can provide abundant prey and refuge from predators (Beck et al., 2001). Yet, despite their reduced movement capabilities, juveniles can and will move from one habitat to the other within the lagoon to capitalize on the available resources (Verdiell-Cubedo, Oliva-Paterna, Ruiz-Navarro, & Torralva, 2013), often transitioning to coastal waters. However, while much effort has been devoted to investigate the role of these habitats in juvenile fish growth (e.g. Tournois et al., 2017), much less is known about the factors that affect fish movement between inner and outer lagoon habitats.

Fish movement has been traditionally investigated in the field using tracking systems, which can identify the position of single individuals equipped with passive or active transmitters to a certain degree of precision (Abecasis & Erzini, 2008; Gonzalez & Gerlotto, 1998; Hussey et al., 2015). Alternatively, some insights could also be derived through the capture of individuals with passive fishing gear, provided that the gear is operated in a suitable position (i.e. on movement routes, e.g. channels between different habitats). This is very well known to fishermen, who traditionally set their gears in these positions in an attempt to maximize their catches. Some insights could be derived from catches and traditional knowledge but fishermen's knowledge has not yet been fully recognized as relevant for the management of marine fish resources (Johannes & Hviding, 2000). Among this traditional knowledge, there is a wealth of contrasting theories about the effects of the moon and tides, which are yet to be thoroughly investigated. Perhaps the most famous is the "Solunar" theory, which is at the origin of tables claiming to predict periods of increased fish activity based on lunar, tidal and solar information (Knight, 1942). Moon cycles are supposed to affect catches through an influence on the behavior and movements of fishes (Banks, 1969; Stoner, 2004), but neither their effect nor mechanisms seem to be fully understood.

There are multiple ways in which the moon can affect fish movement, but these could be simplified in two categories: primary (i.e. gravitational) and secondary (i.e. illumination and tide influence) effects (Kuparinen, O'hara, & Merilä, 2009). Primary effects would assumedly be regulated by the presence/absence of the moon in the sky, while secondary effects would also be modulated by the moon phase (waxing or waning) and the disk illumination (Battaglia et al., 2017; Griffiths, 1999; Kuparinen, Klefoth, & Arlinghaus, 2010). The moon cycle and its position in the sky can also affect tides: tides move water in and out of the lagoon, therefore potentially exerting a strong influence on juvenile fishes, which are poor swimmers and might be dragged by tidal currents but could also be adapted to exploit these currents to move between different foraging grounds (Bennett, Cowley, Childs, & Næsje, 2015; Childs et al., 2008; Næsje et al., 2012). Some of these effects have been recognized as relevant factors in fisheries catches (Pulver, 2017), and it has been suggested that they could potentially affect our estimates of population size (Stoner, 2004).

The Mediterranean Sea could be a prime ground to test whether fishermen knowledge on moon and tides reflects actual patterns in juvenile fish movement between marine and brackish habitats. In this area, coastal lagoons are prime nursery areas for juvenile fish and have also been the focus of extensive ecological research and environmental monitoring (e.g. Aschonitis et al., 2017; Franco et al., 2006; Zucchetta, Franco, Torricelli,

& Franzoi, 2010), creating ideal conditions for further research. The Fattibello Lagoon, in the Northern Adriatic Sea, hosts a long-standing fishing tradition with lift nets along channels, which could act as fish pathways both within the lagoon and between the freshwater and marine environments. We thus used traditional passive fishing gear operated in channels to gauge fish presence in transitional habitats of a coastal lagoon in northern Italy. Based on local fishermen knowledge, we hypothesized that both moon and tides would have a significant effect on the movement of fish between marine and brackish habitats, with new moon and rising tide constituting the best conditions for movement. Specifically, we hypothesized that new moon phases would favor the movement of fish during the day and that marine fishes would enter the lagoon on flooding tides. We used multivariate, threshold and non-linear correlation analyses to explore the relationship between fish catches and moon and tide variables, taking also into account the presence of potential prey and other major temporal and environmental variables. Ultimately, this information would not only test whether there is a basis for traditional knowledge, but also improve the understanding of the drivers in these ecosystem as well as potentially provide useful insights for improved fisheries management.

## Materials and Methods

### Study area

This study focused on the Fattibello Lagoon, a small (ca. 700 ha) brackish (salinity ca. 13-31) lagoon in the north-western shore of the Adriatic Sea (Figure 1). It constitutes the northernmost part of the Comacchio Lagoons, an area renown worldwide for its long-standing tradition in extensive aquaculture of European eel (Anguilla anguilla L.). The Fattibello Lagoon is connected to the sea through the Navigabile and Logonovo channels, two large waterways which are nowadays used mostly for recreational boat transit. The Navigabile Channel also extends inland and, together with the Fosse Foce Channel, can occasionally input freshwaters into the lagoon. Both channels are transitional habitats, used by marine and brackish fishes to move between sea and lagoon environments, constituting the only passage between the two.
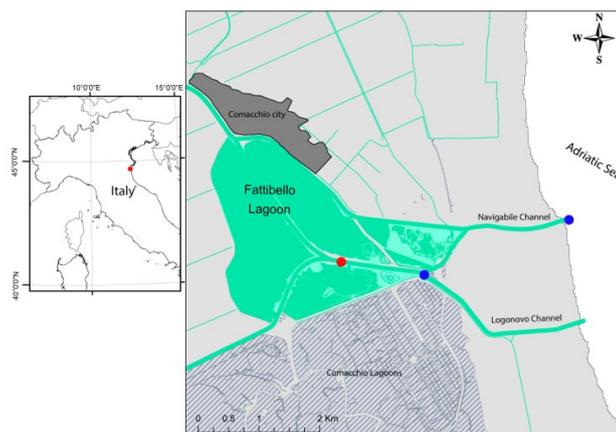


Figure 1 – Map showing the location of the Fattibello Lagoon and its area, as well as the sampling point (red dot) and location of the water chemistry and tide probes (blue dots)

Depth varies from a minimum of a few centimeters on the shallowest sandbars to a maximum of 3 meters in the deepest channels (average depth 1 meter). The lagoon substrate is also very variable, including muds and silts as well as sands and harder substrates (mussel beds, shell hash). Water currents reshape the softer sediments, sometimes creating temporally shifting patterns of sandbars. The average tidal event in this area has a magnitude of approximately 40cm, but tidal range is rather variable (5–90 cm tidal amplitude)

The high habitat complexity and variability, as well as the connection to the sea, make this lagoon an ideal nursery for several fish species of commercial interest, including European eel, European flounder, European seabass and gilthead seabream.

*Fish sampling*

A total of 32 fish sampling events were carried out over the span of 6 years, between 2009 and 2014 (Table 1). Sampling events were not equally distributed in all years but, in each year, covered at least the relevant seasons when juveniles of marine species are present in the lagoon. In 2011, at least one sampling event was carried out in each month, to ensure that the full year spectrum was taken into account.

**Table 1**
Sampling event schedule detailing for each event its effort as well as its duration and timing.

| Fishing event | Date | Start Time | End Time | Total duration (hours) | N of net lifts |
|---|---|---|---|---|---|
| 1 | 3/25/2009 | 19:25 | 20:22 | 0:57 | 12 |
| 2 | 4/14/2009 | 20:35 | 21:42 | 1:07 | 19 |
| 3 | 4/24/2009 | 19:45 | 21:25 | 1:40 | 18 |
| 4 | 5/7/2009 | 17:50 | 20:25 | 2:35 | 12 |
| 5 | 3/4/2010 | 13:52 | 18:50 | 4:58 | 29 |
| 6 | 3/25/2010 | 16:18 | 20:57 | 4:39 | 30 |
| 7 | 5/11/2010 | 18:20 | 22:30 | 4:10 | 32 |
| 8 | 9/23/2010 | 17:30 | 21:38 | 4:08 | 30 |
| 9 | 1/20/2011 | 10:20 | 19:20 | 9:00 | 32 |
| 10 | 2/9/2011 | 13:20 | 22:00 | 8:40 | 27 |
| 11 | 4/18/2011 | 18:10 | 23:30 | 5:20 | 15 |
| 12 | 5/10/2011 | 17:40 | 23:00 | 5:20 | 15 |
| 13 | 6/7/2011 | 16:20 | 21:40 | 5:20 | 17 |
| 14 | 6/28/2011 | 17:00 | 22:40 | 5:40 | 17 |
| 15 | 7/14/2011 | 16:30 | 22:30 | 6:00 | 18 |
| 16 | 8/8/2011 | 13:00 | 19:10 | 6:10 | 19 |
| 17 | 9/9/2011 | 16:20 | 21:40 | 5:20 | 15 |
| 18 | 10/20/2011 | 15:45 | 21:45 | 6:00 | 18 |
| 19 | 11/22/2011 | 12:50 | 19:15 | 6:25 | 19 |
| 20 | 12/2/2011 | 13:00 | 18:00 | 5:00 | 17 |
| 21 | 3/28/2012 | 17:30 | 23:00 | 5:30 | 15 |
| 22 | 5/16/2012 | 17:10 | 22:40 | 5:30 | 15 |
| 23 | 6/13/2012 | 16:40 | 22:20 | 5:40 | 17 |
| 24 | 9/15/2012 | 16:20 | 18:00 | 1:40 | 14 |
| 25 | 10/20/2012 | 15:50 | 19:30 | 3:40 | 14 |
| 26 | 5/22/2013 | 16:20 | 21:40 | 5:20 | 17 |
| 27 | 7/14/2013 | 15:00 | 16:10 | 1:10 | 12 |
| 28 | 2/24/2014 | 15:50 | 21:00 | 5:10 | 15 |
| 29 | 3/12/2014 | 16:20 | 19:35 | 3:15 | 14 |
| 30 | 3/20/2014 | 14:30 | 19:30 | 5:00 | 16 |
| 31 | 4/14/2014 | 15:30 | 20:10 | 4:40 | 16 |
| 32 | 4/22/2014 | 14:10 | 20:20 | 6:10 | 17 |

Fish sampling was performed using stationary lift nets, a traditional fishing method in the area adapted to capture also juvenile individuals. Lift nets are 15 x 15 m square nets with an outer mesh of 12 mm, a second of 8 mm, and an inner one of 6 mm. Nets are operated from a sheltered housing through a series of beams and pulleys, which keep the net stretched and lower it into the water until the bottom; the net is subsequently lifted through the same system, capturing the fish in the water column and collecting them in the inner mesh. Sampling was undertaken at a fixed location on the banks of the Logonovo Channel, at the

entrance of the Fattibello Lagoon and about 700 m inland from the sea (Figure 1). Given this setup, the amount and composition of the catch in each sampling event depended on the number of net lifts. Each net lift captured a number of individuals of different species of fishes (each with their own catchability) moving through the channel and over the net area when it was being lifted. All catch data was converted in catch per unit of effort (CPUE, based on number of net lifts) to account for different sampling efforts.

All captured fish specimens were identified to the species level, measured (total length, TL, to the nearest mm) and weighted with a resolution of 0.1 g. When samples contained a large amount of individuals of the same species, a subsample of 100 specimens was randomly selected to be measured in detail. Freshwater species were occasionally sampled but were not retained in this analysis.


*Environmental parameters*

To account for seasonal and annual factors, both the month (Month) and the year (Year) of sampling were used as environmental parameters in our analysis.

Moon data were derived through an online application of the United States Naval Observatory (http://aa.usno.navy.mil/data/docs/RS_OneDay.php) using the location and date of fishing as input parameters. Both the moon phase itself (waxing or waning, MoonPhas) and the percentage of illuminated moon disk (%Disk) were used in the analysis, as these two parameters are able to describe accurately the aspect of the moon as seen from earth. Additionally, we used moon rise and set times to estimate the share of fishing time that was carried out while the moon was in the sky (MoonPres), under the assumption that moon presence in the sky could have an additional effect on fishes.

Tide data were recorded by an automated tide station (OTT LOGOSENS), equipped with both radar and floater measuring systems. The station is positioned at the outlet of the Navigabile Channel, roughly 700 m seaward from the sampling location, and operated by the Emilia-Romagna Region Environmental Protection Agency (ARPA Emilia Romagna). A time delay of 30 minutes was applied to the data, to account for the different timing of tides between the tide station and the Fattibello Lagoon (derived from an empirical correlation between tidal times at the tide station and the fish sampling location). Both prevalent tide direction (i.e. the most represented tide direction during sampling, TideDir) and tide amplitude (i.e. the maximum recorded amplitude during sampling, TideFish) were used in the analysis, as well as the direction (TideDirPrev) and total amplitude (TideMax) of the strongest tide event prior to sampling. The latter was used in order to account for factors that could potentially influence fish presence before sampling began.

Dissolved oxygen (DO), salinity (Salinity) and temperature (Temp) of the water were also measured by the Emilia-Romagna Region Environmental Protection Agency (ARPA Emilia Romagna) with a multiparametric probe (OTT DUOSENS) in the Logonovo Channel, in close proximity of the sampling site. The probe logs data with an hourly resolution, but an average daily value for each of the three parameters was considered as

representative for each sampling event. These parameters had no significant spatial variations between the probe location and the fish sampling location, as verified by spot measures performed during sampling.

As crustaceans prey has been suggested as a relevant factor for the distribution of some species by previous studies (Maree et al., 2016), small-sized crustaceans captured while sampling fishes were also tested as environmental descriptors (LagoonShrimp). Crustacean catches comprised two relevant species: the grey shrimp (Crangon crangon L.) and the lagoon shrimp (Palaemon spp.), but these were caught only occasionally (13 out of 32 events), and their total biomass in the catch never exceeded a total of 1.2 kg per sampling event. These species constitute a relevant food resource for several fish species in the Fattibello Lagoon (e.g. for European eel, Lanzoni, Aschonitis, Milardi, Fano, & Castaldelli, 2018; and for other species, Lanzoni, unpublished data) and in other Mediterranean lagoons (Rogdakis, Ramfos, Koukou, Dimitriou, & Katselis, 2010; Sá, Bexiga, Veiga, Vieira, & Erzini, 2006)

*Statistical analysis*

In order to explore the relationship between fish catches and environmental factors (seasonal, annual, moon, tidal, dissolved oxygen levels, salinity, temperature and the presence of crustacean prey) multivariate statistics were employed. A Detrended Correspondence Analysis (DCA) was initially performed to select the most appropriate response model for gradient analysis (Lepš & Šmilauer, 2003). The dominant gradient length in DCA was always lower than 3 so the Redundancy Analysis (RDA) was finally chosen (Lepš and Šmilauer, 2003). RDA is a linear gradient analysis that allows to quantify the variation of a multivariate data set explained by independent variables (Ter Braak & Smilauer, 2002). The month and year of sampling (to account for seasonal and annual factors), as well as the moon, tides, environmental and crustacean prey variables were considered as independent parameters, whereas the CPUEs of each fish species were considered as dependent. All environmental and fish CPUE data were log10(X+1) transformed, except moon disk illumination which was arcsin transformed. Variables were investigated through Variance Inflation Factor to exclude collinearity problems, however no variable was found to be collinear. These analyses were performed using the CANOCO 4.5 for Windows software (Lepš & Šmilauer, 2003).

We used the Spearman rank test to investigate the presence of correlations between single fish species and moon and tide factors. We also used the Threshold Indicator Taxa ANalysis (TITAN, Baker & King, 2010) on all environmental parameters to identify the environmental threshold (the optimum value of a continuous variable) that partitions sampling units and distinguishes negative (= losses: z−) and positive (= gains: z+) taxon responses. Thus, TITAN helps to identify taxon-specific change points along an environmental gradient at which the decline/increase in a given taxon's frequency and abundance is most prominent. Bootstrapping (500 repetitions) was used to estimate two important diagnostic indices (reliability and purity) as well as uncertainty around the location of individual taxa and community change points (Baker & King, 2010). Both Spearman correlations and TITAN analysis were performed using R software (R Core Team, 2017).
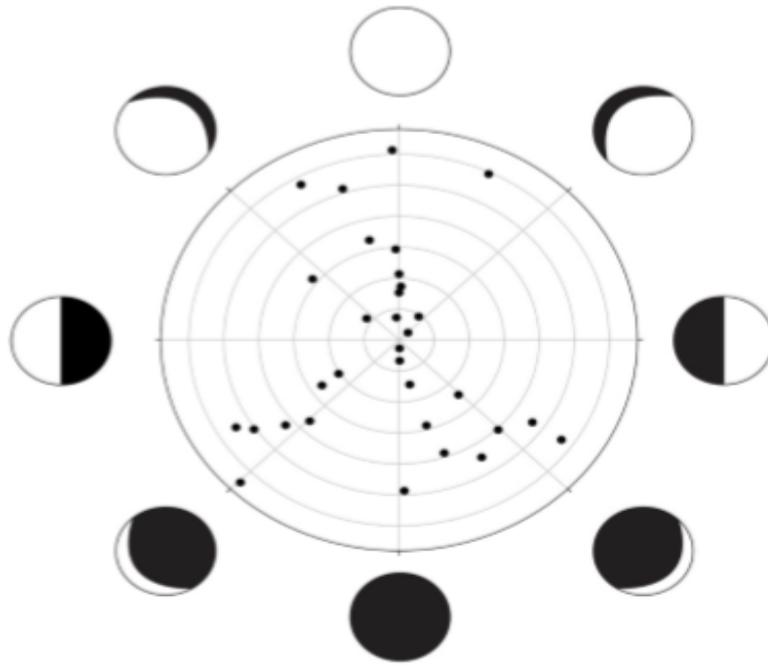
**Fig. 2.** Moon disk illumination and its phase. Each point represents a fishing event, with the angular position indicating the exact moon phase (waxing or waning and degree of illumination). The distance of each point from the center represents the progressive number of sampling events.

## Results

Sampling collected a very variable amount of fish: from 0.49 to 38.2 kg captured in a single event. CPUE also varied widely, from a minimum of 56.2 g to a maximum of 1378.7 g of fish per net lift. A total of 36,336 individuals of 32 different fish species were captured, belonging to 19 families (Table 2).

**Table 2**
List of fish species captured in the Logonovo Channel between 2009 and 2014, detailing the number of individuals per each species, their average size (in cm) and its standard deviation.

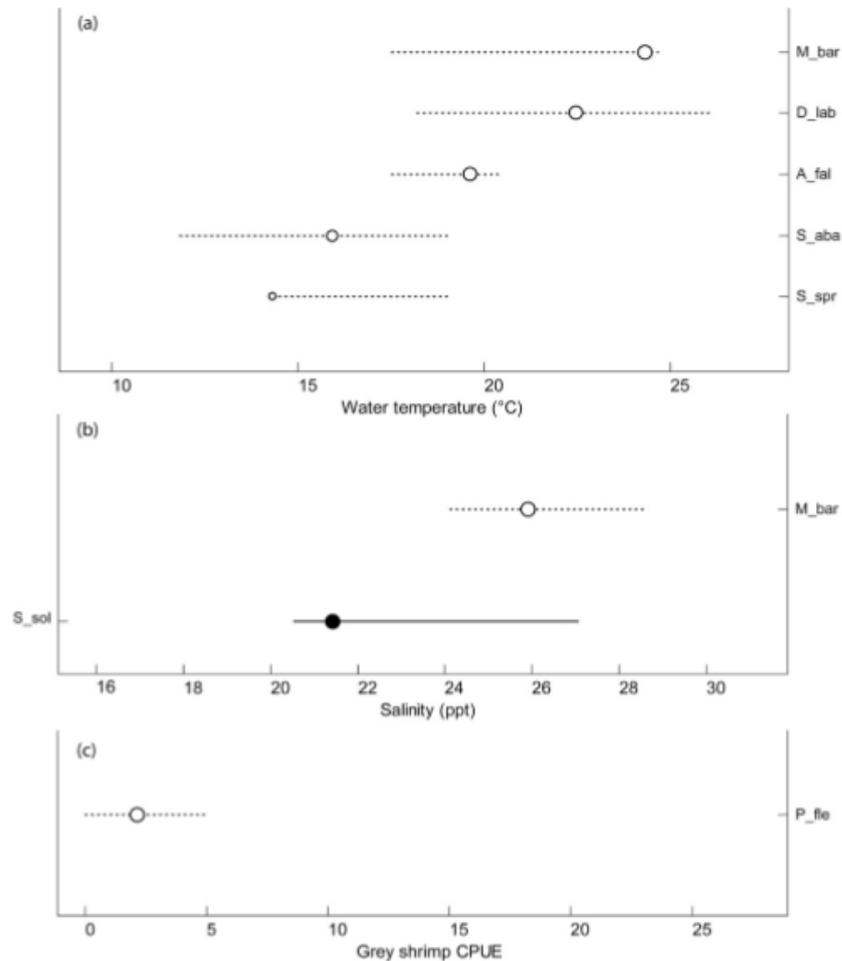| Family | Scientific name | Common name | Number of individuals | Average size (cm) | Standard Deviation |
|---|---|---|---|---|---|
| Anguillidae | Anguilla anguilla | European eel | 78 | 24.3 | 12.0 |
| Atherinidae | Atherina boyeri | Big scale sand smelt | 5050 | 6.7 | 2.0 |
| Belonidae | Belone belone | Garfish | 3 | 38.0 | 3.8 |
| Blenniidae | Salaria pavo | Peacock blenny | 2 | 8.9 | 1.2 |
| Clupeidae | Alosa fallax | Twaite shad | 26 | 12.4 | 5.4 |
| | Sprattus sprattus | European sprat | 8484 | 5.8 | 1.2 |
| Cyprinodontidae | Aphanius fasciatus | Mediterranean banded killifish | 9 | 3.7 | 0.2 |
| Engraulidae | Engraulis encrasicolous | European anchovy | 12398 | 7.4 | 2.4 |
| Gobiidae | Zosterisessor ophiocephalus | Grass goby | 15 | 11.2 | 3.0 |
| | Pomatoschistus canestrinii | Canestrini's goby | 191 | 4.8 | 1.2 |
| | Knipowitschia panizzae | Adriatic dwarf goby | 50 | 3.5 | 1.0 |
| | Pomatoschistus minutus | Sand goby | 55 | 4.2 | 0.8 |
| | Gobius niger | Black goby | 27 | 8.0 | 1.3 |
| | Gobius paganellus | Rock goby | 133 | 8.0 | 2.1 |
| Moronidae | Dicentrarchus labrax | European seabass | 17 | 10.9 | 3.5 |
| Mugilidae | Liza ramada | Thinlip mullet | 1463 | 11.5 | 6.9 |
| | Liza aurata | Golden grey mullet | 320 | 13.4 | 6.7 |
| | Liza saliens | Leaping mullet | 368 | 10.2 | 5.1 |
| | Mugil cephalus | Flathead grey mullet | 24 | 10.5 | 9.1 |
| | Chelon labrosus | Thicklip grey mullet | 18 | 11.9 | 7.0 |
| Mullidae | Mullus barbatus | Red mullet | 225 | 8.0 | 1.7 |
| Pleuronectidae | Plathychtys flesus | European flounder | 986 | 7.2 | 3.3 |
| Sciaenidae | Pagellus bogaraevo | Blackspot seabream | 1 | 7.8 | – |
| | Umbrina cirrosa | Shi drum | 2 | 14.2 | 5.4 |
| Scombridae | Scomber scombrus | Atlantic mackerel | 11 | 7.5 | 4.2 |
| Scophthalmidae | Scophthalmus maximus | Turbot | 2 | 7.3 | 1.1 |
| Soleidae | Solea solea | Common sole | 267 | 5.6 | 3.0 |
| Sparidae | Sparus aurata | Gilt-head bream | 84 | 7.2 | 3.4 |
| | Diplodus vulgaris | Common two-banded seabream | 1 | 7.5 | – |
| Syngnathidae | Sygnatus acus | Greater pipefish | 76 | 12.3 | 3.6 |
| | Sygnathus abaster | Black striped pipefish | 15 | 9.4 | 3.3 |
| Triglidae | Chelidonichthys lucerna | Tub gurnard | 14 | 7.3 | 3.8 |

Sampling events were rather equally distributed between waning (17 events) and waxing (15 events) moon phases, with two events occurring when the moon was full (Figure 2). However, a smaller amount of fishing events occurred between the first quarter and the full moon (Figure 2). The vast majority of fishing events occurred while the moon was either always in the sky (15) or not in the sky at all (11), with only few events (a total of 6) between these two extremes.

Tide direction during fish sampling was unevenly distributed: most fishing events (19) occurred with flooding tide, a smaller part (8) occurred with ebbing tide and only few (5) with dead tide. Also tide magnitude during sampling events showed greater variations with a minimum of 0.003 m and a maximum of 0.807 m level variation. Tide direction in the period before sampling events was also relatively evenly distributed, with only one period of dead tide and the other periods distributed among flooding (13 events) and ebbing (18 events). Tide magnitude in the period before sampling varied from 0.066 to 0.889 m water level variation.

RDA analysis confirmed the relevance of moon and tide factors, which had effects comparable to annual and seasonal variations, respectively (Figure 4). Overall, the RDA had a fair explanatory potential (the horizontal axis explained 35.2% of the fish data variance, the vertical axis 20.2%). Moon phase (waxing or waning) and duration of moon presence in the sky during sampling had greater effects than the actual illumination of the moon disk. Overall the magnitude of effects related to the moon parameters were smaller than most environmental parameters examined, but still larger than e.g. the presence of lagoon shrimp and some of the tide factors. Tide direction (ebb or flood) during sampling had a greater effect than tide direction prior to sampling. Conversely, tide amplitude prior to sampling had far more effect than tide amplitude during sampling. Disk illumination had an overall weak effect on fish (Figure 4). Some of the highest CPUE of European flounder were sampled during periods of high moon illumination (Spearman rank $P = 0.05$, correlation coefficient 0.34, Figure 4), but there was no significant correlation with other moon parameters. Other benthic species (i.e. gobies) showed different degrees of correlation with moon variables: the Spearman Rank $P$ was $> 0.12$ for most goby species and all moon parameters considered, with the exception of sand goby (Spearman Rank $P = 0.06$, correlation coefficient -0.33) and black goby (Spearman Rank $P < 0.05$, correlation coefficient -0.4) which negatively correlated with moon phase. However, moon variables were also poorly correlated with pelagic species (European anchovy and sprat, Spearman Rank $P > 0.09$ for all moon parameters and both species). Tide direction negatively affected boxlip mullet and garfish (Spearman Rank $P < 0.05$, correlation coefficients of -0.47 and -0.38, respectively) but the latter was only caught once. On the other hand, maximum tide amplitude positively affected European anchovy (Spearman Rank $P = 0.05$, correlation coefficient 0.35). However, the other fish species seem not to be affected by tides (Spearman Rank $P > 0.09$ for all species and tide parameters).

Water temperature was a significant factor for several species movements (Figure 5a). Common sole and red mullet had significant but opposite threshold for salinity (Figure 5b). European flounder presence in the catch was correlated with the catch of grey shrimp (Figure 5c).

**Fig. 3.** Tide amplitude during (a) and right before (b) each sampling event. Bar colors indicate whther sampling occurred during prevalent flowing tides (white), dead tides (grey) and ebbing tides (black).



**Fig. 4.** Redundancy analysis (RDA) triplot showing the relationships between fish species (black labels) and environmental parameters (red arrows and labels). Fish species labels consist of abbreviations where the first letter is the initial of the genus and the three following are the first letters of the species name. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Fig. 5.** Significant thresholds for different taxa along temperature (a), salinity (b) and presence of grey shrimp in the catch (c), according to the TITAN analysis. Lines represent the 95% Confidence Interval of the bootstrapped distribution across the gradient, with circles identifying the species change points across the gradient. Solid lines and circles represent species that decline along the gradient (listed on the left axis), while dashed lines and empty circles represent species which distribution increases along the gradient (listed on the right axis). Species not shown in these figures have a distribution not predictable according to the environmental gradients examined.

Discussion

Our results confirmed that juvenile fish movements in the Fattibello Lagoon were indeed influenced by moon and tide factors, which had effects comparable to annual and seasonal variations, respectively. Overall, the magnitude of effects related to the moon parameters were smaller than most environmental parameters examined, but still larger than e.g. the presence of invertebrate prey (lagoon shrimp) or some of the tide factors. Contrarily to the fishermen knowledge and our initial hypothesis, European flounder movement was positively correlated with disk illumination, while sand and black goby were influenced by the moon phase. Other benthic and pelagic species showed no significant correlation. On the other hand, tide direction affected negatively the movement of boxlip mullet and tide amplitude prior to sampling had far more effect than tide amplitude during sampling, positively affecting European anchovy presence and somewhat

conforming to traditional knowledge. Among other factors, water temperature, salinity and the presence of invertebrate prey (grey shrimp) had significant but contrasting effects on some, but not all, of the species examined. Ultimately, our study represents a significant albeit preliminary look at the effect of these variables, but could not completely disentangle or quantify the direct and indirect effects of moon and tide variations.

It is not yet clear if and how the direct gravitational effect of the moon could be sensed by fish or other animals, given that the sensitivity of the known body structures devoted to sense gravity (i.e. the otoliths in fish, or similar structures in other animals) do not seem to be sufficient to detect such small changes. While traditional fisheries knowledge often refers to direct effects of the moon on the catches of fish it is not easy to disentangle such effects from secondary effects. Exceptionally, Kuparinen et al. (2009) were able to investigate the moon direct influence on Atlantic salmon (Salmo salar L.) spawning runs in a place where secondary effects were negligible, but did not find a significant effect. In our case, moon direct effects could not be fully disentangled, as the moon creates tides even in the northern Adriatic Sea. However, strongest tides should be created during full and new moon phases because during these phases the gravitational effects of the moon and the sun are combined, creating so-called spring tides. This suggests that we should be able to detect an additional direct effect, should this effect be added to indirect effects (Stoner, 2004). Conversely, in our dataset, full and new moon phases did not correspond to higher catches of all species, suggesting that there is probably a relatively weak direct effect on the juvenile fishes of the Fattibello Lagoon. On the other hand, among indirect effects, the illumination provided by the moon is commonly believed to provide increased light during the night, allowing fishes to effectively move and forage during this period and, as a consequence, less during daytime. An artificial supply of light has been previously investigated as a factor for fish aggregation (Becker, Whitfield, Cowley, Järnegren, & Næsje, 2013), and the moon illumination could act in a similar way. If present, this effect should be all the more evident in shallow lagoons, especially where water transparency allows for a tighter coupling between moon illumination and light penetration in the water. However, this effect could be masked by other factors; the presence of a thick cloud cover or high turbidity should negate the effects of a strong moonlight. Cloud cover and water turbidity could act as regulators of the moonlight penetration in the water column, thus influencing which species could be most affected. In our results, European flounder, a benthic invertebrate predator, was mostly caught when the moon illumination was high, perhaps as a result of the increased movement during the night. However, we did not fully resolve whether there was a direct link between fishing effort distribution between day/night times and European flounder catches. That a benthic species was affected would suggest that moonlight can penetrate all the way to the bottom and therefore other fish, living in higher layers of the water column, should be even more influenced by moonlight. Yet, this was not seen in our data, since pelagic species (e.g. European anchovy) movement was not significantly correlated with moon illumination. This could partly depend on sampling timing, which often included both daylight and moonlight conditions in the same

sampling event and thus mix contrasting effects. Moreover, turbidity is highly variable in the lagoon and was not recorded in our data, adding a potentially confounding factor to the interpretation of our results on moon.

Among its indirect effects, the moon influences heavily the amplitude and timing of tides, so that moon and tides cannot be analyzed separately. In our data, tide amplitude, particularly of the event before sampling rather than during the sampling, had an effect on at least some species. European anchovy and boxlip mullet, two marine species, were found to enter the lagoon with flooding tides, conforming to traditional knowledge. In this area, tides can be further enhanced by winds if the wind strength and direction push water towards the shore, which could in turn affect the presence, abundance and movement of marine fish in the lagoon (Bruno & Acha, 2015). However, this effect was not evident in a parallel study conducted on the same Fattibello Lagoon dataset (Milardi et al. 2018, under review). Flooding tides can also contribute to increase salinity in the lagoon, by bringing in saltier water from the open sea. However, in the Fattibello Lagoon, only common sole and red mullet had significant (but opposite) thresholds for salinity. Both common sole and red mullet do not spawn in brackish waters and come into the lagoon only to feed, moving in and out with the tides and following salinity gradients. It is not easy to relate our results to previously published research (Bennett et al., 2015; Næsje et al., 2012), as species-specific and local factors might come into play in these interactions. Ultimately, the lack of stronger effects of tides on all species could perhaps be attributed to the complexity of some of the habitats within the lagoon (which can shelter fish from currents) and to the wide range of species, from benthic to pelagic ones.

Some of these species (e.g. European anchovy) have a rather relevant seasonality in the commercial catches of the Fattibello Lagoon (Lanzoni, unpublished data), suggesting that temporal factors might also play a role in regulating the presence of these species in the lagoon, more than just their movements. Annual fluctuations in the population of engraulids are very well known (see e.g. Kawasaki, 1983) and this was reflected also in our data. Seasonal and annual factors were indeed some of the strongest in our multivariate analysis, but their magnitude was still comparable to moon and tide factors. However, seasonal factors could have also been at the root of the temperature thresholds found for several species, which seemed to prefer warmer waters during summer and fall rather than cold winter and spring waters. Similarly, the presence of grey shrimp in the lagoon is known to increase during the winter and spring (Lanzoni, unpublished data, but see also e.g. Spaargaren (2000)), when also European flounder enters the lagoon to spawn (Franzoi, Franco, & Torricelli, 2010; Zucchetta et al., 2010). This could partly explain the linkage underlined by our analyses, but it must be noted that the catch was comprised mostly of juveniles, whereas spawners were relatively rare. Perhaps specifically-aimed studies could help to resolve prey-predator linkages in the lagoon, similarly to what was undertaken in other areas by Maree et al. (2016).

Ultimately, our data did not have the necessary resolution to fully disentangle and quantify the primary and secondary effects of moon and tidal factors. However, it was sufficiently detailed to assess that their

magnitudes were at least comparable. Furthermore, our results underlined that overall moon and tides factors are potentially strong drivers for fish movement, confirming at least in part the local fishermen traditional knowledge. The Fattibello Lagoon is a good example of brackish lagoon in the Mediterranean with long standing fisheries traditions, which had not been previously investigated from the fish ecology perspective. Our results underline that this area is a spawning and feeding ground for a variety of species, as well as a vital nursery area used by juvenile individuals of several species of commercial interest. As such, it would be vital to preserve its ecological balance and its ability to provide a primary ecosystem service (i.e. healthy fish stocks). While our results might not directly translate into clear recommendations for fisheries management, they shed some further light on previously unknown ecology and movement of fishes in the lagoon and should at least be taken into account when trying to regulate fishing with passive gear.

## References

- Abecasis, D., & Erzini, K. (2008). Site fidelity and movements of gilthead sea bream (Sparus aurata) in a coastal lagoon (Ria Formosa, Portugal). Estuarine, Coastal and Shelf Science, 79(4), 758-763.
- Aschonitis, V., Castaldelli, G., Lanzoni, M., Rossi, R., Kennedy, C., & Fano, E. A. (2017). Long-term records (1781–2013) of European eel (Anguilla anguilla L.) production in the Comacchio Lagoon (Italy): evaluation of local and global factors as causes of the population collapse. Aquatic Conservation: Marine and Freshwater Ecosystems, 27(2), 502-520.
- Baker, M. E., & King, R. S. (2010). A new method for detecting and interpreting biodiversity and ecological community thresholds. Methods in Ecology and Evolution, 1(1), 25-37.
- Banks, J. (1969). A review of the literature on the upstream migration of adult salmonids. Journal of Fish Biology, 1(2), 85-136.
- Battaglia, P., Ammendolia, G., Cavallaro, M., Consoli, P., Esposito, V., Malara, D., . . . Andaloro, F. (2017). Influence of lunar phases, winds and seasonality on the stranding of mesopelagic fish in the Strait of Messina (Central Mediterranean Sea). Marine Ecology, 38(5).
- Beck, M. W., Heck Jr, K. L., Able, K. W., Childers, D. L., Eggleston, D. B., Gillanders, B. M., . . . Minello, T. J. (2001). The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates: a better understanding of the habitats that serve as nurseries for marine species and the factors that create site-specific variability in nursery quality will improve conservation and management of these areas. BioScience, 51(8), 633-641.
- Becker, A., Whitfield, A. K., Cowley, P. D., Järnegren, J., & Næsje, T. F. (2013). Potential effects of artificial light associated with anthropogenic infrastructure on the abundance and foraging behaviour of estuary-associated fishes. Journal of Applied Ecology, 50(1), 43-50.
- Bennett, R. H., Cowley, P. D., Childs, A.-R., & Næsje, T. F. (2015). Movements and residency of juvenile white steenbras Lithognathus lithognathus in a range of contrasting estuaries. Estuarine, Coastal and Shelf Science, 152, 100-108.
- Blaber, S., & Blaber, T. (1980). Factors affecting the distribution of juvenile estuarine and inshore fish. Journal of Fish Biology, 17(2), 143-162.
- Bruno, D. O., & Acha, E. M. (2015). Winds vs. tides: factors ruling the recruitment of larval and juvenile fishes into a micro-tidal and shallow choked lagoon (Argentina). Environmental Biology of Fishes, 98(5), 1449-1458.
- Childs, A.-R., Cowley, P. D., Næsje, T., Booth, A. J., Potts, W., Thorstad, E., & Økland, F. (2008). Do environmental factors influence the movement of estuarine fish? A case study using acoustic telemetry. Estuarine, Coastal and Shelf Science, 78(1), 227-236.
- Franco, A., Elliott, M., Franzoi, P., & Torricelli, P. (2008). Life strategies of fishes in European estuaries: the functional guild approach. Marine Ecology Progress Series, 354, 219-228.
- Franco, A., Franzoi, P., Malavasi, S., Riccato, F., Torricelli, P., & Mainardi, D. (2006). Use of shallow water habitats by fish assemblages in a Mediterranean coastal lagoon. Estuarine, Coastal and Shelf Science, 66(1-2), 67-83.

- Franzoi, P., Franco, A., & Torricelli, P. (2010). Fish assemblage diversity and dynamics in the Venice lagoon. Rendiconti Lincei, 21(3), 269-281.
- Gelin, A., Crivelli, A. J., Rosecchi, É., & Kerambrun, P. (2001). The effect of salinity changes on the population structure and reproductive traits of Crangon crangon L. populations in the Camargue (Rhône Delta, France). Ecoscience, 8(1), 8-17.
- Gonzalez, L., & Gerlotto, F. (1998). Observation of fish migration between the sea and a mediterranean lagoon (Etang de l'Or, France) using multibeam sonar and split beam echo sounder. Fisheries research, 35(1-2), 15-22.
- Griffiths, S. P. (1999). Effects of lunar periodicity on catches of Penaeus plebejus (Hess) in an Australian coastal lagoon. Fisheries research, 42(1-2), 195-199.
- Hussey, N. E., Kessel, S. T., Aarestrup, K., Cooke, S. J., Cowley, P. D., Fisk, A. T., . . . Kocik, J. F. (2015). Aquatic animal telemetry: a panoramic window into the underwater world. Science, 348(6240), 1255642.
- Johannes, R. E., & Hviding, E. (2000). Traditional knowledge possessed by the fishers of Marovo Lagoon, Solomon Islands, concerning fish aggregating behaviour. Current Anthropology, 39, 223-252.
- Kawasaki, T. (1983). Why do some pelagic fishes have wide fluctuations in their numbers? Biological basis of fluctuation from the viewpoint of evolutionary ecology. FAO Fish. Rep, 291(3), 1065-1080.
- Knight, J. A. (1942). Moon Up, Moon Down: New York: C. Scribner's Sons.
- Kuparinen, A., Klefoth, T., & Arlinghaus, R. (2010). Abiotic and fishing-related correlates of angling catch rates in pike (Esox lucius). Fisheries research, 105(2), 111-117.
- Kuparinen, A., O'hara, R., & Merilä, J. (2009). Lunar periodicity and the timing of river entry in Atlantic salmon Salmo salar. Journal of Fish Biology, 74(10), 2401-2408.
- Lanzoni, M., Aschonitis, V., Milardi, M., Fano, E. A., & Castaldelli, G. (2018). A method to identify bimodal weight-length relations: possible ontogenetic diet and/or metabolism shift effects in anguilla anguilla (Actinopterygii: Anguilliformes: Anguillidae). Acta Ichthyologica et Piscatoria, 48(2).
- Leitão, R., Martinho, F., Neto, J., Cabral, H., Marques, J., & Pardal, M. (2006). Feeding ecology, population structure and distribution of Pomatoschistus microps (Krøyer, 1838) and Pomatoschistus minutus (Pallas, 1770) in a temperate estuary, Portugal. Estuarine, Coastal and Shelf Science, 66(1-2), 231-239.
- Lepš, J., & Šmilauer, P. (2003). Multivariate analysis of ecological data using CANOCO: Cambridge university press.
- Maree, B., Cowley, P., Næsje, T., Childs, A., Terörde, A., & Thorstad, E. (2016). Influence of prey abundance and abiotic factors on the long-term home-range and movement dynamics of spotted grunter Pomadasys commersonnii in an intermittently open estuary. African Journal of Marine Science, 38(2), 171-180.
- Næsje, T. F., Cowley, P. D., Diserud, O. H., Childs, A.-R., Kerwath, S. E., & Thorstad, E. B. (2012). Riding the tide: estuarine movements of a sciaenid fish, Argyrosomus japonicus. Marine Ecology Progress Series, 460, 221-232.
- Newton, A., Icely, J., Cristina, S., Brito, A., Cardoso, A. C., Colijn, F., . . . Holmer, M. (2014). An overview of ecological status, vulnerability and future perspectives of European large shallow, semi-enclosed coastal systems, lagoons and transitional waters. Estuarine, Coastal and Shelf Science, 140, 95-122.
- Pulver, J. (2017). Does the Lunar Cycle Affect Reef Fish Catch Rates? North American Journal of Fisheries Management, 37(3), 536-549.
- R Core Team. (2017). R: A language and environment for statistical computing. Vienna, Austria; 2014.
- Rogdakis, Y., Ramfos, A., Koukou, K., Dimitriou, E., & Katselis, G. (2010). Feeding habits and trophic level of sea bass (Dicentrarchus labrax) in the Messolonghi-Etoliko lagoons complex (Western Greece). Journal of Biological Research, 13, 13.
- Sá, R., Bexiga, C., Veiga, P., Vieira, L., & Erzini, K. (2006). Feeding ecology and trophic relationships of fish species in the lower Guadiana River Estuary and Castro Marim e Vila Real de Santo António Salt Marsh. Estuarine, Coastal and Shelf Science, 70(1-2), 19-26.
- Spaargaren, D. (2000). Seasonal and annual variations in the catches of Crangon crangon,(L., 1758)(Decapoda, Natantia) near the coast of Texel, The Netherlands. Crustaceana, 73(5), 547-563.
- Stoner, A. (2004). Effects of environmental variables on fish feeding ecology: implications for the performance of baited fishing gear and stock assessment. Journal of Fish Biology, 65(6), 1445-1471.
- Ter Braak, C. J., & Smilauer, P. (2002). CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5): www. canoco. com.
- Tournois, J., Darnaude, A. M., Ferraton, F., Aliaume, C., Mercier, L., & McKenzie, D. J. (2017). Lagoon nurseries make a major contribution to adult populations of a highly prized coastal fish. Limnology and Oceanography, 62(3), 1219-1233.
- Verdiell-Cubedo, D., Oliva-Paterna, F. J., Ruiz-Navarro, A., & Torralva, M. (2013). Assessing the nursery role for marine fish species in a hypersaline coastal lagoon (Mar Menor, Mediterranean Sea). Marine Biology Research, 9(8), 739-748.

- Young, G., Potter, I., Hyndes, G., & De Lestang, S. (1997). The ichthyofauna of an intermittently open estuary: implications of bar breaching and low salinities on faunal composition. Estuarine, Coastal and Shelf Science, 45(1), 53-68.
- Zucchetta, M., Franco, A., Torricelli, P., & Franzoi, P. (2010). Habitat distribution model for European flounder juveniles in the Venice lagoon. Journal of Sea Research, 64(1-2), 133-144.

## 3.2. The decline of the hystorical eel fishery and the developement of new ones

### 3.2.1. Case study 7. A method to identify bimodal weight–length relations: Possible ontogenetic diet and/or metabolism shift effects in Anguilla anguilla (Actinopterygii: Anguilliformes: Anguillidae).

<u>Abstract</u>

The power function W = a Lb is commonly used to describe the weight–length (W–L) relation (WLR) of fish. Smaller/younger specimens may present different WLR from larger/older ones, introducing errors in the derivation of WLR of the total population. This difference appears through a breakpoint in the log–log plot of W–L data and can be justified due to biological factors or due to errors in the sampling procedure. The aim of the study is to propose a bilinear model (LinBiExp) that identifies the specific coordinates of the breakpoint in the log-transformed W–L measurements. The analysis was performed using 2627 W–L measurements of European eel, *Anguilla anguilla* (Linnaeus, 1758), from the Comacchio Lagoon (Italy). The bilinearity produced by LinBiExp model was verified through comparison of slopes and intercepts (ANOVA) of the two linear segments and through the 95% intervals of the highest posterior density (HPD) distribution of breakpoint coordinates estimated by bootstrap regression of LinBiExp. Additionally, gut content analysis was performed in order to detect any diet shift in order to justify the existence of the breakpoint. The LinBiExp function identified the breakpoint coordinates (Lt, Wt) = (28.9 cm, 35.9 g). The ANOVA showed that there was a statistically significant difference between the slopes and between the intercepts of the two linear segments at 99.9% confidence level. The 95% HPD intervals of Lt and Wt were 28.4–29.4 cm and 34.5–38.0 g, respectively, based on 10 000 bootstrap estimates. The gut content analysis showed inclusion of other fish preys in the diet of eels when their weight and length exceeded the coordinates of the breakpoint in W–L data. The estimated breakpoint for the specific dataset was justified by the possible interrelation of ontogenetic diet shift with other metabolic processes (e.g., beginning of sexual maturation). The study showed that the LinBiExp function can be a valuable tool for detecting the absolute coordinates of a

breakpoint in logtransformed W–L data, while the presented methodology can increase the robustness of weight–length analysis of fishes using the typical power function.

Introduction

The knowledge of fish weight-length (W–L) relation (WLR) is one of the most important elements for the description of body proportions of fish species. The typical power function (W = a Lb) is the most widely used function for the description of WLRs, facilitating weight predictions from length measurements (Froese 2006). Furthermore, an important contribution of the typical power function is that its coefficients (a, b) for various fish species are stored in FishBase (Froese and Pauly 2018). These coefficients have also been used to analyse growth allometry and growth condition factors, which show the degree of well-being of a species in its habitat (Knights 1982, Bolger and Connolly 1989, Lima-Junior et al. 2002, Gomiero and de Souza Braga 2005, Acarli et al. 2014). The most extensive review about different types and methods for obtaining WLRs was provided by Froese (2006) while additional guidelines were proposed in a more recent editorial note by Froese et al. (2011). Froese (2006) noted that the derivation of WLRs for fish species may seem a simple technical procedure but there are many issues associated with their derivation, which need special attention. One of the most important issues was that small specimens from very young age classes might present different WLR from the older ones introducing errors in the final WLR of the total population. For this reason, a recommendation was the exclusion of the young specimens for improving fitting performance. The different WLR of the young specimens can be explained by justifications such as (Stergiou and Fourtouni 1991, Froese 2006, Simonović et al. 2011, Milardi et al. 2014):

• The young specimens present different WLR from the larger/older ones due to ontogenetic shifts in diet while this difference can also be regulated by the availability of the different food types required at different growth stages.

• Usually the amount of data from very young age classes is small and not representative due to the efficacy reduction of fishing techniques for capturing smaller specimens (gear/net selectivity.

• Shifts in growth performance due to metabolic changes associated to sexual maturation.

Taking into account the above, the safer procedures to obtain the WLR are either to use the typical power function after the removal of young specimens or to use a more complex model to describe the complete data. The method for removing the data of younger specimens is either empirical (e.g., from visual inspection) or it is based on statistical outliers' identification procedures. If indeed, the young specimens have a different WLR, outliers' identification methods do not usually remove all the observations of young specimens, which present different WLR response, but may indicate as outliers, specimens of the larger classes. Thus, a robust method to identify a specific threshold of length or weight in order to remove the "problematic" young

specimens is still missing. Such a method would allow the users to derive a more robust WLR for the older/larger specimens using the typical power function. The aim of this study was:

• To propose a bilinear model that can describe the complete dataset of weight–length measurements identifying the specific coordinates of any existing breakpoint in the log-log plot of weight–length data.

• To use the specific breakpoint for dividing the data in parts, which would improve the fitting efficacy of the typical power function.

The proposed methodology is applied using as an example a large dataset of weight–length data of the specimens of the European eel, *Anguilla anguilla* (Linnaeus, 1758), captured in the Comacchio Lagoon (Italy). Additional gut content data from the specimens were used to evaluate the hypothesis that the observed breakpoint in the log-log plot of weight–length data is related to an ontogenetic diet shift while additional justifications were given in order to justify the interrelation of diet shift with other shifts in metabolic processes.

## Matirial and methos

*Data*

A total of 2627 yellow and silver European eels (with a proportion of 73% being yellow and 27% being silver) were sampled during 2011 from the Comacchio Lagoon (44°37′ 12.6′′N, 12°09′35.2′′E) in northern Italy.

Silver eels were sampled at fishing screens called "lavorieri" during the period of seaward migration (November–December, while silver eel migration has not been observed in the period January–October). The screens permit the entry or escape of elvers in the lagoon but entrap all silver eels when they begin their migration, thus, the eel catch in the screens represents ~100% of the migrating silver eel population of the Comacchio Lagoon. The total yield of silver eels of 2011 (for the fishing area of 8470 ha) was 3811.5 kg with a total silver eel abundance equal to 0.45 kg ha$^{-1}$ (Aschonitis et al. 2017a). The value of silver eel abundance of 2011 was among the 1% lower annual records starting from 1781. The last thirty years the silver eel abundance in Comacchio showed a continuous decline, and after 2010, has been reduced more than 96% compared to the mean value (~14.5 kg ha$^{-1}$) of 1781–1980 (Aschonitis et al. 2017b). Yellow eels were caught by a set of 20 trap nets evenly distributed over the entire lagoon area. The fishing gear was a modified fyke net, locally called "cogollo", which is typically used for eel fishing in the shallow lagoons of the northern Adriatic. It consists of a leader, 50 × 1.5 m, that directs the fish toward two conical trap nets positioned at its distal ends. The structure consists of 8 × 8 mm mesh, large enough to prevent blocking by macroalgae, periphyton and detritus, but small enough to prevent loss of small age class specimens. The nets were monitored every two days in September and October, a period when metamorphosis to silver eels is considered complete, preventing overlap between yellow and silver eel population groups (van Ginneken et al. 2007). Yellow and silver eels were counted and anaesthetized with ice in order to measure length and weight. Subsamples of 573 yellow and 366 silver eels (939 in total) were randomly selected for age and sex

determination (Castaldelli et al. 2014, Aschonitis et al. 2017a) while all the rest eels were released. The age of specimens was determined by double reading after grinding and polishing the otoliths (Anonymous 2009). Sex was determined by macroscopic examination of the gonads and when specimens were smaller than 35 cm, then microscopic examination of the gonads was performed (Colombo and Grandi 1996, Tesch 2007). The females represented 97.5% of the collected specimens, the males represented 1.4%, and the share of sexually undifferentiated specimens was 1.1% (Aschonitis et al. 2017a). The extremely high percentage of females is attributed to the extremely low abundance of the population in the lagoon (Aschonitis et al. 2017a, 2017b) (it is already known the strong negative correlation of European eel feminization rate with the abundance of the population) (Roncarati et al. 1997, Krueger and Oliveira 1999, Han and Tzeng 2006, Aschonitis et al. 2017a, 2017b). Male and female eels present different WLR and so the error, which might be introduced by mixing males and females in WLR analysis, is negligible in this dataset. Thus, the analysis that will follow, mainly describes the characteristics of a mixed yellow and silver female European eel population. The sub-dataset of mixed yellow and silver female individuals (925 in total) of known age and length have already been used by Castaldelli et al. (2014) for the derivation of the length–age relation using von Bertalanffy function:

$$L = L_\infty \cdot \left(1 - e^{-k \cdot X}\right) + L_0 \cdot e^{-k \cdot X} \qquad (1)$$

where L is length [cm], X is age [years], with $L_\infty$ = 155.94, $L_0$ = 7.5, k = 0.087 and R2 = 0.97. The total observed age classes of specimens subjected to age analysis using otoliths were 11 (from 0+ to 10+ years, the + accounts for ~0.5). The small number of sexually undifferentiated European eels was restricted to the 0+ and 1+ age classes (Aschonitis et al. 2017a). Since ontogenetic diet shifts have been documented as one of the reasons for the existence of such breakpoints (Stergiou and Fourtouni 1991, Froese 2006), additional observations about the gut content of eels were also obtained. Observations of the gut content were performed based on 62 specimens selected from the initial dataset, with length ranging between 11 and 83 cm. Identification of prey type was performed using a stereo-microscope. The initial number of analysed specimens was much larger, but their majority was excluded because their gut content did not allow robust identification of prey due to almost complete digestion. Prey was identified to the species level and gut content of the selected specimens was expressed as a percentage of biomass per food/prey type.

*Weight–length relations*

The analysis of WLR was performed using the typical power function and the linearized biexponential function (LinBiExp) proposed by Buchwald (2007). The typical power function and its respective logtransformed form are the following:

$$W = a \cdot L^b \qquad\qquad (2a)$$

$$\log(W) = \log(a) + b \cdot \log(L) \qquad\qquad (2b)$$

where W is the weight [g], L is the length [cm] and *a* and *b* are regression coefficients. The common procedure for analysing WLRs using Eq. 2 requires the initial use of simple regression for Eq. 2b using the log-transformed W and L variables for identification and removal of outliers (Froese 2006). Then, the fitting procedure is repeated without outliers using either non-linear regression with Eq. 2a or linear regression with Eq. 2b. The LinBiExp provides smooth and fully parametrizable transitions between two linear segments maintaining a clear connection between them. The LinBiExp is fitted on the log-transformed W, L variables and is given by the following function (Buchwald 2007):

$$W' = f(L') = c \cdot \log\left\{\exp\left[a_1 \cdot (L' - L'_t) \cdot c^{-1}\right] + \right.$$
$$\left. + \exp\left[a_2 \cdot (L' - L'_t) \cdot c^{-1}\right]\right\} + d \quad \text{for } c \neq 0 \quad (3)$$

where W' and L' are the log-transformed weight [log(g)] and length [log(cm)], $a_1$ and $a_2$ are coefficients that regulate the slopes of the two linear segments, *c* is a parameter for adjusting the smoothness/abruptness of the transition and the form of angle between the two linear segments, d is a constant for shifting the curve along the vertical axis (log-W axis), and $L_t'$ is a constant that defines the break point between the two linear segments at horizontal axis (log-L axis). The transition between the two linear segments does not require a sharp break-point, it can take place along a smooth, continuously differentiable, curved portion of adjustable width (with the deviation from linearity having an exponential character). Nevertheless, a model should be considered bilinear only if it shows linearity at both ends of its considered range (Buchwald 2007). Positive values of c coefficient indicate that the angle above the two linear segments is < 180o while negative c values indicate that the respective angle is > 180°. The larger the absolute value of c is, the smoother is the transition between the two linear segments. The c coefficient is generally sensitive and a large variation of its values only in a positive or a negative range just affects the steepness in the transition between the two linear segments. For this reason, a high statistical significance for the case of c coefficient is not required. The angle is regulated by the $a_1 \div a_2$ ratio and when it is equal or close to 1 indicates that bilinearity hardly exists. Thus, the smaller the ratio is, the higher is the degree of bilinearity. It has also to be mentioned that the only difference between the two exponent factors inside Eq. 3 is the value of $a_1$ and $a_2$ coefficient and thus exchanging their values does not change the curve (e.g., for $a_1 = 1$ and $a_2 = 4$ or $a_1 = 4$ and $a_2 = 1$, the curve is the same). The coordinates of the breakpoint in the log(W)− log(L) plot are provided by the following functions:

for log-*L* axis:

$$x' = L'_t \qquad\qquad (4a)$$

and for log-*W* axis:

$$y' = W'_t = c \cdot \log(2) + d \qquad\qquad (4b)$$

The respective coordinates of the breakpoint in regular weight–length curves after removing the logarithmic transformation are provided by:

$$x = L_t = 10^{x'} \qquad\qquad (5a)$$

$$y = W_t = 10^{y'} \qquad\qquad (5b)$$

*Steps of analysis*

The first step of the analysis included simple non-linear regression (Simple-NLR) of LinBiExp (Eq. 3) using the full dataset for detecting outliers. The fitting analysis together with outliers' detection was performed using StatGraphics Centurion XV software (Statpoint Technologies, Warrenton, VA, USA). Those observations with studentized residuals greater than 2 in absolute value were considered outliers (standard procedure of the aforementioned software). The new dataset without outliers was used again to perform SimpleNLR of Eq. 3 for the final calculation of its coefficients and the respective coordinates of the breakpoint.

The second step of the analysis was performed for assessing the robustness of bilinear response. This step included two procedures that complement each other. In the first procedure, the analysis aimed to investigate the robustness of $a_1 \div a_2$ ratio and the robustness of breakpoint coordinates ($L_t$, $W_t$) provided by LinBiExp (Eq. 3) based on their observed variation when Eq. 3 is subjected to bootstrap non-linear regression (Boot-NLR). Boot-NLR is based on the generation of a large number of new datasets by randomly sampling data with replacement (Efron and Tibshirani 1994) and it is considered among the most robust methods for assessing the variability of regression coefficients. The Boot-NLR was performed by applying the "nls.lm" function of the {minpack.lm} package (Elzhov et al. 2016) in R software. The "nls.lm" function uses the Levenberg-Marquardt non-linear leastsquares algorithm. The Boot-NLR procedure was applied for 10 000 iterations that led to a respective number of ($a_1$, $a_2$, $c$, $d$, $L_t'$) solutions. The 10 000 bootstrap sets of coefficients were then used to assess the respective values of $a1 \div a2$ ratio and the coordinates of the breakpoint ($L_t$, $W_t$) based on Eq. 4 and Eq. 5 for each bootstrap set of variables. The range of bootstrap estimations of the regression coefficients but also of $a1 \div a2$, $L_t$, and $W_t$, was defined by the 95% confidence interval, which was estimated based on the probability distribution of their 10 000 estimations. This method was applied in order to estimate the values of the highest posterior density (HPD) distribution that indicates the 2.5% and 97.5% thresholds (HPD thresholds), which contain the central 95% of the HPDD distribution.

The probability interval was computed using the "*p. interval*" of {LaplacesDemon} package (Bernardo 2005) in R software.

In the second procedure, the analysis aimed to investigate if the slopes (*b*) and intercepts $\log_{(a)}$ of Eq. 2b are statistically different between the two linear segments defined by the breakpoint of Eq. 3. For this reason, the clean dataset of observations, after the removal of outliers from step 1, was divided in two subsets using the breakpoint coordinates that were also obtained from step 1. Thus, any observed pair of (*W, L*) with $W < W_t$ and $L < Lt$ was included in subset 1 (smaller specimens) and the rest were included in subset 2 (larger specimens). Comparison of the linear regressions based on the log-transformed variables was performed for both subsets using Eq. 2b, while ANOVA was used to compare the statistical difference of their slopes (b) and intercepts $\log_{(a)}$. The third step included the examination of possible hypotheses related to the existence of breakpoint in the log-WLRs. The very small size of nets used for capturing eels (8 mm) was chosen in order to minimize the possible errors of nets selectivity for young specimens. Thus, any possible occurrence of a breakpoint in the logWLR of this dataset could be attributed to other reasons (e.g., environmental or biological factors). Three possible hypotheses of biological background for the existence of the breakpoint in the specific dataset were examined:

• A shift of metabolic activity to achieve higher elongation rates at the young age classes.

• A shift of metabolic activity to achieve sexual maturation.

• An ontogenetic diet shifts.

Results

*Step 1: Simple-NLR of LinBiExp for removing outliers and determination of the breakpoint.*

The minimum and maximum observed weights in the dataset of 2627 specimens were 8.4 and 2371.5 g, respectively, and the minimum and maximum observed lengths were 11.0 and 105.0 cm, respectively. Simple-NLR of Eq. 3 was performed based on the full dataset (n = 2627) of log-transformed variables for detecting outliers (Fig. 1A). This procedure identified 143 outliers (Fig. 1A), which were removed from the dataset. Simple NLR of Eq. 3 was applied again based on the new clean dataset (n = 2484) and the results are given in Fig. 1B. The log-transformed coordinates of the breakpoint in the log(L) vs. log(W) plot (Fig. 1b) were equal to $(L_t', W_t') = (1.461, 1.555)$ (Eqs. 4a, 4b), while their values without the log-transformation were equal to $(L_t, W_t) = (28.9 \text{ cm}, 35.9 \text{ g})$ (Eqs. 5a, 5b). Step 2: Robustness of bilinear response. The general statistics together with the 2.5% and 97.5% HPD thresholds for $a_1, a_2, c, d, L_t'$ but also for $a_1 \div a_2, L_t$, and $W_t$ using the 10 000 bootstrap estimates of Boot-NLR of Eq. 3 are given in Table 1. For $a_1 \div a_2, L_t$, and $W_t$ are also given the HPDD graphs (Fig. 2). Taking into account the results of Table 1, it is observed that almost all the parameters present a restricted range of variation according to HPD thresholds, which suggests a robust performance of

the function. The only parameter that presents large variance is the c coefficient, which is very sensitive because it regulates the smoothness/abruptness of the transition between the two linear segments. This is not a serious problem since its bootstrap estimates fluctuate only in a positive range, while the parameters of $a_1 \div a_2$, $L_t$, and especially $W_t$ (which includes c according to Eq. 4b) show small variance, which is also verified by Fig. 2. The clean dataset (n = 2484) was further divided into two subsets based on the breakpoint of Eq. 3 obtained from step 1. Eq. 2b was then fitted on the two subsets (Fig. 3) and ANOVA was used to compare their slopes (b) and intercepts log(a). The ANOVA showed that there was a statistically significant difference between the slopes (b) but also between the intercepts $\log_{(a)}$ of the regression lines of the two subsets at 99.9% confidence level, respectively, verifying the robustness of the bilinear response. The individual regressions of the two lines showed R2 = 0.58 (P < 0.001) and R2 = 0.98 (P < 0.001) for subset 1 and 2, respectively. Step 3: Possible hypotheses that justify the breakpoint of log-WLR. Regarding the first hypothesis about the regulation of metabolic activity to achieve higher elongation rates at the young age classes, it was found that when L is equal to $L_t \approx 28.9$ cm, eels have already reached approximately the 28% of their maximum observed length in comparison to the weight, which is still less than 2% of their maximum observed value. This performance may be related to a regulation of metabolic activity to achieve higher elongation rates at the young age classes, which presents a shift after reaching $L_t$. The elongation rates of subset 1 and 2 can also be considered statistically different since the slopes b of Eq. 2b (Fig. 3) were also statistically different.

The fact that the elongation rate of subset 2 was smaller than the one of subset 1 may also be associated to a metabolic shift for achieving sexual maturation. This hypothesis can be justified based on the length–age relation (Eq. 1). According to Eq. 1, the range between the 2.5% and 97.5% HPD values of $L_t$ (28.4–29.4 cm, Table 1) corresponds to eels age of 1.7–1.9 years.
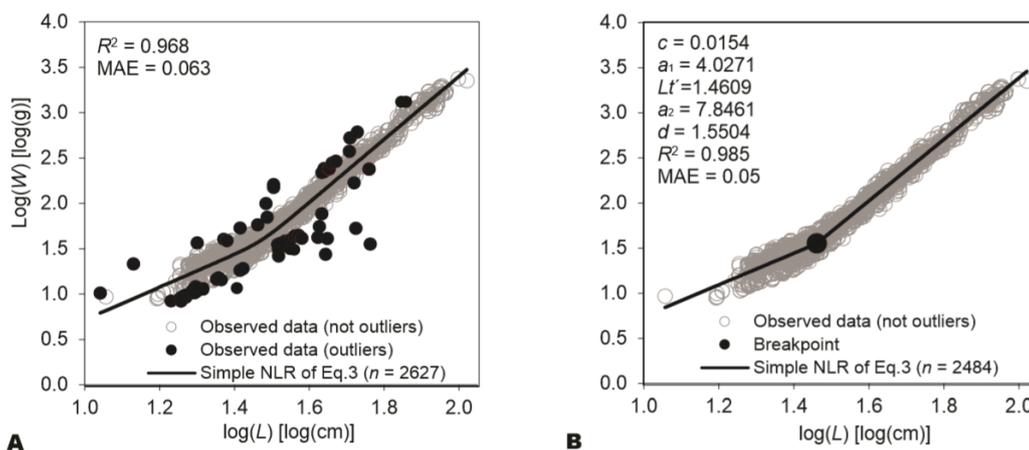


**Fig. 1. (A)** Simple-NLR of Eq. 3 using the complete dataset ($n = 2627$) and identification of outliers (red dots), **(B)** Simple-NLR of Eq. 3 after removing 143 outliers ($n = 2484$) and identification of breakpoint coordinates (black dot) for the European eel, *Anguilla anguilla*, of the Comacchio Lagoon

General statistics together with 2.5% and 97.5% HPD intervals for $a_1$, $a_2$, $c$, $d$, $L_t'$, $a_1 \div a_2$, $L_t$ and $W_t$ using the 10 000 bootstrap estimates obtained from Boot-NLR of Eq. 3

| Parameter | $c$ | $a_1$ | $L_t'$ | $a_2$ | $d$ | $a_1 \div a_2$ | $L_t$ [cm] | $W_t$ [g] |
|---|---|---|---|---|---|---|---|---|
| Mean | 0.0260 | 4.0252 | 1.4609 | 7.8469 | 1.5503 | 0.5130 | 28.90 | 36.17 |
| Standard deviation | 0.0220 | 0.1367 | 0.0038 | 0.0292 | 0.0125 | 0.0177 | 0.25 | 0.91 |
| Coefficient of variation | 84.50% | 3.40% | 0.26% | 0.37% | 0.81% | 3.45% | 0.88% | 2.52% |
| Minimum | 0.0054 | 3.4976 | 1.4476 | 7.7276 | 1.5007 | 0.4468 | 28.03 | 33.16 |
| Maximum | 0.1210 | 4.4825 | 1.4745 | 7.9696 | 1.5928 | 0.5737 | 29.82 | 39.86 |
| 2.5% HPD interval | 0.0055 | 3.7713 | 1.4532 | 7.7917 | 1.5246 | 0.4787 | 28.41 | 34.48 |
| 97.5% HPD interval | 0.0643 | 4.3021 | 1.4678 | 7.9074 | 1.5731 | 0.5478 | 29.37 | 38.04 |

The fact that the observed sexually undifferentiated eels of this population were all < 2 years old (Aschonitis et al. 2017a) may suggest that the high metabolic performance for achieving high elongation rates is reduced after Lt on behalf of metabolic processes associated to sexual maturation.
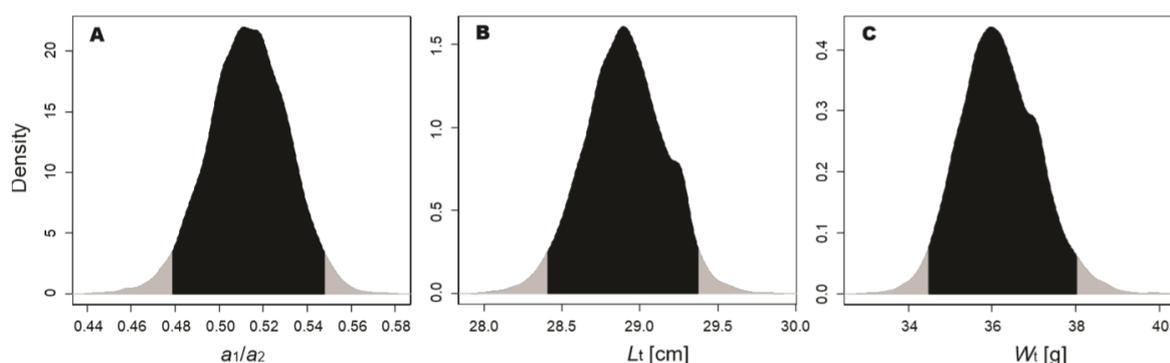


**Fig. 2.** HPDD graphs of the $a_1 \div a_2$, $L_t$, and $W_t$ parameters (the central black portion of the graph describes the 95% central part of the distributions) for the European eel, *Anguilla anguilla*, of the Comacchio Lagoon

The third hypothesis of ontogenetic diet shift was investigated based on the gut content data. The gut content analysis is presented using five length classes (Fig. 4). The threshold value between the second and the third length class is equal to $L_t$ = 28.9 cm. The gut observations for the specimens with L < $L_t$, showed that eels diet primarily consisted of small amphipods (*Gammarus spp.*) and secondly by small shrimps (*Palaemon spp.*). On the other hand, the analysis of specimens with L > L*t*, showed a diet shift by showing higher shrimps content in comparison to amphipods and inclusion of small fish species like anchovies, *Engraulis encrasicolus* (Linnaeus, 1758). The percentage of fish species in their diet was gradually increased with the length increase, including also other species like grass goby, *Zosterisessor ophiocephalus* (Pallas, 1814), or larger shrimps and crabs (*Carcinus aestuarii*). The existence of brackish/saltwater fish species like anchovy and grass goby in their diet is due to the saltwater conditions of the Comacchio Lagoon, which is connected to the Adriatic Sea.
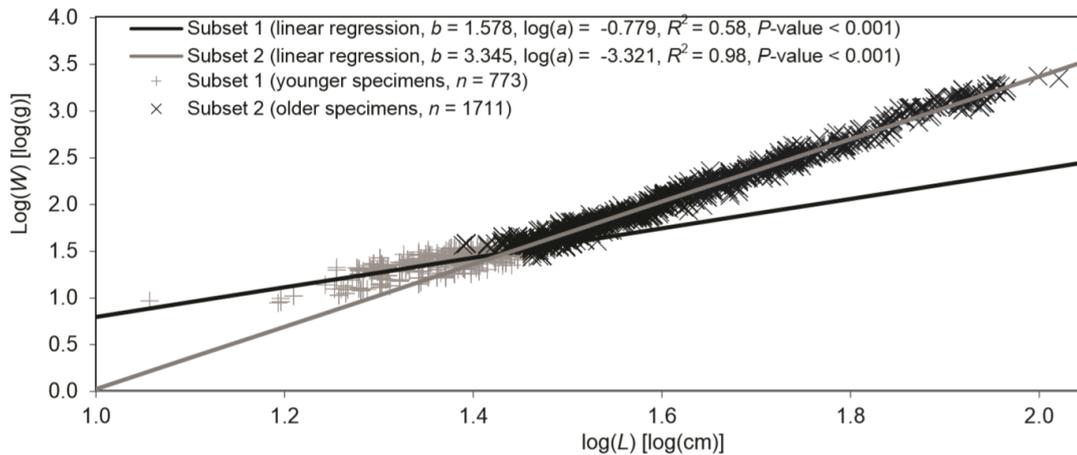
**Fig. 3.** Comparison of slopes $b$ and intercepts $\log(a)$ of the regression lines (Eq. 2b) produced by the subset 1 (smaller specimens with $W < 35.9$ g and $L < 28.9$ cm) and subset 2 (the rest larger specimens) for the European eel, *Anguilla anguilla*, of the Comacchio Lagoon
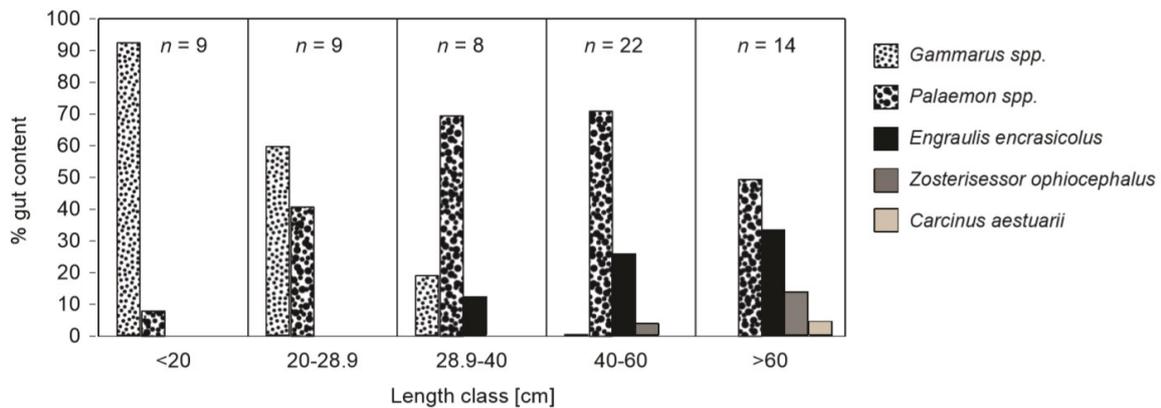


**Fig. 4.** Percent of gut content in different length classes of *Anguilla anguilla* of the Comacchio Lagoon ($n$ is the number of specimens used for each length class)

Discussion

The results of this study could not exclude any of the initial three hypotheses as causes of the breakpoint existence. On the other hand, it is likely that an interrelation and synergy among them exists as a result of both biological (metabolic processes) and environmental (prey type and availability) factors.

The combined explanation about the specific breakpoint occurrence could be that the specimens are trying to optimize the exploitation of energy, which is derived by various prey types of different size, in this specific environment. At the initial stages of growth, individuals invest energy on somatic growth in order to perform faster the following two strategies:

• Their protective mechanisms (swimming performance). A justification for this behaviour is that it probably increases the defensive ability of the species from predators since the longer elvers swim faster and since they are still thin (small trunk section), they can easily hide in small holes of the bottom or inside the stones.

• Their ability to capture larger preys of higher energetic content that will allow them to cover the higher energy demand of metabolic processes associated with sexual maturation. When their size reaches a specific threshold (breakpoint), which allows them to consume enough energy for boosting sexual maturation, then their metabolic processes change towards this latter purpose.

Fish consumption (especially anchovy), which started to appear in the gut content analysis when L became larger than L$t$ (Fig. 4), probably plays a key role in European eel maturation. Anchovy belongs to the oily fish group, providing high amounts of oil and fatty acids (Üstün et al. 1996) that have extremely high nutritional value for eels, which also belong to the same fish group (Pike and Jackson 2010). The fact that young European eels invest more energy to increase their length in comparison to their transect diameter probably leads to a faster elongation of their stomach, which also has an elongated shape (see fig. 1 in Peters 1982). This may also help them to consume and digest more efficiently anchovies, which have an elongated body shape in spite of their small size (Whitehead et al. 1985). The process of elongation is, of course, a genetic attribute of eel species associated with their particular snakelike morphology but it could be hypothesized that this rate might be enhanced at the initial growth stages in order for a specific population to adapt itself to better exploiting the fish preys present in the environment. If this hypothesis is true, then it would indicate high plasticity of elongation rates associated to specific food types. European eels have been shown to be capable to adjust their morphology for similar reasons. For example, in a study by De Meyer et al. (2016), it was found that a controlled population of eels presented phenotypic difference in their head when they were fed either hard or soft diets. The authors found that hard feeders developed a broader head and a larger abductor mandibulae region that made them capable of stronger bites. On the other hand, soft feeders developed a sharper and narrower head, which could reduce hydrodynamic drag, allowing more rapid strikes towards their prey.

Furthermore, it should be emphasized that this is the first time that a breakpoint in the log-WLR of European eel is documented. This can be attributed either to the fact that other eel datasets were not adequately expanded in the small age classes for identifying the existence of a breakpoint or to the fact that the existence of the breakpoint in this dataset is a rare case based on the local preys' preferences of eels (Fig. 4). It has to be noted that dietary shifts of European eels with the gradual inclusion of fish preys may not be observed in other areas due to differences in the availability of different prey types and the high competition for fish preys with other predators. For example, Dörner et al. (2009) analysed the dietary habits of European eel in two lakes—one in Germany (Großer Vätersee) and the other one in Denmark (Vallum Sø). Despite the fact that Vallum Sø had significantly higher amount of small prey fishes and macrozoobenthic preys compared to Großer Vätersee, the eels of Vallum Sø were mainly fed with macrozoobenthic preys (e.g., chironomid larvae), while the eels of Großer Vätersee used fishes as the main food component. The above-cited authors concluded that the density of macrozoobenthos generally controlled the degree of piscivory. Another element that should also be considered is the possible seasonal variability in eel diet. The samplings of this

study were performed during September–December, and thus seasonal effects could not be identified. On the other hand, Bouchereau et al. (2009) found seasonal variations in the diet of eels sampled in Languedocian Mauguio Lagoon (Gulf of Lion, France) but they did not consider the diet differences among different eel size classes. Taking into account the aforementioned findings and comments, it is observed that there is a large knowledge gap regarding:

• The feeding preferences of European eel which can vary between different size classes, different seasons, or different habitats.

• The effects of dietary habits on its growth and morphometric characteristics in natural environments. Future research should focus on the aforementioned issues giving also special attention to the sampling procedures for improving the capture of smaller specimens in order to verify the breakpoint existence in the log-WLR of other eel populations. If other breakpoints can be found in other extensive eel datasets, excluding the possibility that they are related to the selectivity of fishing technique, then the breakpoints could be used as a biological and environmental indicator. Furthermore, the aforementioned analysis should be expanded to other fish species, since the breakpoint point was also observed in log-transformed weight–length data of other species (Simonović et al. 2000, 2011). Additionally, it would be of special interest the investigation of breakpoint existence in log-transformed WLRs of fish species, which are considered to have isometric or slight allometric growth.

Finally, regardless of the reasons of the breakpoint existence, its observation and identification through LinBiExp (Eq. 3) are very important since it can be used to improve the WLR when it is described by Eq. 2. The coefficients of Eq. 2 are among the most important data of FishBase (Froese and Pauly 2018) and it is unknown if any of the thousand coefficients of many fish species were biased due to the existence of a breakpoint in the initial data.

Bibliografy

- Acarli D., Kara A., Bayhan B. 2014. Length–weight relations for 29 fish species from Homa Lagoon, Aegean Sea, Turkey. Acta Ichthyologica et Piscatoria 44 (3): 249–257. DOI: 10.3750/AIP2014.44.3.09
- Anonymous 2009. Workshop on age reading of European and American Eel (WKAREA), 20–24 April 2009, Bordeaux, France. ICES CM 2009\ACOM, 48.
- Aschonitis V.G., Castaldelli G., Lanzoni M., Merighi M., Gelli F., Giari L., Rossi R., Fano E.A. 2017a. A size-age model based on bootstrapping and Bayesian approaches to assess population dynamics of Anguilla anguilla L. in semi-closed lagoons. Ecology of Freshwater Fish 26 (2): 217–232. DOI: 10.1111/eff.12269
- Aschonitis V.G., Castaldelli G., Lanzoni M., Rossi R., Kennedy C., Fano E.A. 2017b. Long-term records (1781–2013) of European eel (Anguilla anguilla L.) production in the Comacchio Lagoon (Italy): Evaluation of local and global factors as causes of the population collapse. Aquatic Conservation Marine and Freshwater Ecosystems 27 (2): 502–520. DOI: 10.1002/aqc.2701
- Bernardo J.M. 2005. Intrinsic credible regions: An objective Bayesian approach to interval estimation. TEST 14 (2): 317–384. DOI: 10.1007/BF02595408
- Bolger T., Connolly P.L. 1989. The selection of suitable indices for the measurement analysis of fish condition. Journal of Fish Biology 34 (2): 171–182. DOI: 10.1111/j.1095-8649.1989.tb03300.x

- Bouchereau J.-L., Marques C., Pereira P., Guélorget O., Vergne Y. 2009. Food of the European eel Anguilla anguilla in the Mauguio Lagoon (Mediterranean, France). Acta Adriatica 50 (2): 159–170.
- Buchwald P. 2007. A general bilinear model to describe growth or decline time profiles. Mathematical Biosciences 205 (1): 108–136. DOI: 10.1016/j. mbs.2006.08.013
- Castaldelli G., Aschonitis V., Lanzoni M., Gelli F., Rossi R., Fano E.A. 2014. An update of the length–weight and length–age relationships of the European eel (Anguilla anguilla, Linnaeus 1758) in the Comacchio Lagoon, northeast Adriatic Sea, Italy. Journal of Applied Ichthyology 30 (3): 558–559. DOI: 10.1111/jai.12392
- Colombo G., Grandi G. 1996. Histological study of the development and sex differentiation of the gonad in the European eel. Journal of Fish Biology 48 (3): 493– 512. DOI: 10.1111/j.1095-8649.1996.tb01443.
- De Meyer J., Christiaens J., Adriaens D. 2016. Dietinduced phenotypic plasticity in European eel (Anguilla anguilla). Journal of Experimental Biology 219: 354–363. DOI: 10.1242/jeb.131714
- Dörner H., Skov C., Berg S., Schulze T., Beare D.J., Van der Velde G. 2009. Piscivory and trophic position of Anguilla anguilla in two lakes: Importance of macrozoobenthos density. Journal of Fish Biology 74 (9): 2115–2131. DOI: 10.1111/j.10958649. 2009. 02289.
- Efron B., Tibshirani R.J. 1994. An introduction to the bootstrap. Chapman and Hall/CRC. Monographs on Statistics and Applied Probability No. 57. Chapman and Hall, New York, NY, USA.
- Elzhov T.V., Mullen K.M., Spiess A.N., Bolker B. 2016. Package 'minpack.lm': R Interface to the Levenberg Marquardt nonlinear least-squares algorithm found in MINPACK, Plus Support for Bounds. Version 1.2-1.
- Froese R. 2006. Cube law, condition factor and weight– length relationships: History, meta-analysis and recommendations. Journal of Applied Ichthyology 22 (4): 241–253. DOI: 10.1111/j.1439-0426.2006.00805.
- Froese R., Pauly D. (eds.) 2018. FishBase. [Version 02/2018] www.fishbase.org Froese R., Tsikliras A.C., Stergiou K.I. 2011. Editorial note on weight–length relations of fishes. Acta Ichthyologica et Piscatoria 41 (4): 261–263. DOI: 10.3750/AIP2011.41.4.01
- Gomiero L.M., de Souza Braga F.M. 2005. The condition factor of fishes from two river basins in São Paulo state, southeast of Brazil. Acta Scientiarum, Biological Sciences 27 (1): 73–78. DOI: 10.4025/actascibiolsci. v27i1.1368
- Han Y.S., Tzeng W.N. 2006. Use of the sex ratio as a means of resource assessment for the Japanese eel Anguilla japonica: A case study in the Kaoping River, Taiwan. Zoological Studies 45 (2): 255–263.
- Knights B. 1982. Body dimensions of farmed eels (Anguilla anguilla L.) in relation to condition factor, grading, sex and feeding. Aquacultural Engineering 1 (4): 297–310. DOI: 10.1016/0144-8609(82)90037-1
- Krueger W., Oliveira K. 1999. Evidence for environmental sex determination in the American eel, Anguilla rostrata. Environmental Biology of Fishes 55 (4): 381–389. DOI: 10.1023/A:1007575600789
- Lima-Junior S.E., Cardone I.B., Goite R. 2002. Determination of a method for calculation of allometric condition factor of fish. Acta Scientiarum, Biological and Health Sciences 24 (2): 397–400.
- Milardi M., Lappalainen J.L., Juntunen T. 2014. Variation in length, girth and weight of large northern pikes (Esox lucius) in Finland. Annales Zoologici Fennici 51 (3): 335–339. DOI: 10.5735/086.051.0305
- Peters G. 1982. The effect of stress on the stomach of the European eel, Anguilla anguilla L. Journal of Fish Biology 21 (5): 497–512. DOI: 10.1111/j.10958649.1982. tb02855.
- Pike I.H., Jackson A. 2010. Fish oil: Production and use now and in the future. Lipid Technology 22 (3): 59–61. DOI: 10.1002/lite.201000003
- Roncarati A., Melotti P., Mordenti O., Gennari L. 1997. Influence of stocking density of European eel (Anguilla anguilla, L.) elvers on sex differentiation and zootechnical performances. Journal of Applied Ichthyology 13 (3): 131–136. DOI: 10.1111/j.14390426.1997.tb00112.
- Simonović P., Marić S., Nikolić V. 2000. Growth characteristics of huchen Hucho hucho (L.) from rivers Drina, Una and Sana. Ekologija 35 (2): 123–126.
- Simonović P.D., Nikolić V.P., Tošić A.D., Marić S.P. 2011. Length–weight relationship in adult huchen Hucho hucho (L., 1758) from Drina River, Serbia. Biologia 66 (1): 156–159. DOI: 10.2478/s11756-0100135-2
- Stergiou K.I., Fourtouni H. 1991. Food habits, ontogenetic diet shift and selectivity in Zeus faber Linnaeus, 1758. Journal of Fish Biology 39 (4): 589– 603. DOI: 10.1111/j.1095-8649.1991.tb04389.
- Tesch F.W. 2007. The eel. 3rd edn. Blackwell Science, Oxford, UK. DOI: 10.1002/9780470995389
- Üstün G., Akova A., Dandik L. 1996. Oil content and fatty acid composition of commercially important Turkish fish species. Journal of the American Oil Chemists' Society 73 (3): 389–391. DOI: 10.1007/BF02523436

- van Ginneken V., Durif C., Balm S.P., Boot R., Verstegen M.W.A., Antonissen E., van Den Thillart G. 2007. Silvering of European eel (Anguilla anguilla L.): Seasonal changes of morphological and metabolic parameters. Animal Biology 57 (1): 63–77. DOI: 10.1163/157075607780002014
- Whitehead P.J.P. 1985. FAO Species Catalogue. Vol. 7. Clupeoid fishes of the world (suborder Clupeoidei). An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads, anchovies and wolf-herrings. Part I - Chirocentridae, Clupeidae and Pristigasteridae. FAO Fisheries Synopsis 125. FAO, Rome

### 3.2.2. Case study 8: Qualitative and quantitative analysis of the Valli di Comacchio fish Fauna (years 2010-18) for drafting the first district's management fishing plan, guidelines for increasing fish production, integrated with the regulation for species protection, and the drafting of a hypothesis for the fishery regulation in the Comacchio District

(Published as: **Mattia Lanzoni**, Anna Gavioli, Marco Milardi, Giuseppe Castaldelli 2018. Qualitative and quantitative analysis of the Valli di Comacchio fish Fauna (years 2010-18) for drafting the first district's management fish plan. Projest Co-management Final Report, 2018)

Introduction

The main purpose of the project was the formulation of innovative guidelines for the integrated management of fish fauna in the Valli di Comacchio, the formulation of a model for exploiting fishery resources which, by recovering the practices of fish farming, does not result in conflict with the other ecosystem services of the valleys and the primary obligations of the Park Authority for the conservation of habitats and protected species. In accordance with the job proposal, the following actions, each of which was dealt with in a dedicated paragraph, were considered in the following order:

- Action 1: collection and organization of the database of previous data related to the research in question and in the period of interest;
- Action 2: sampling of species of interest and collection of field data related to fishing in the area under study, useful for updating the data series 2010-2018 and/or for verifying and validating aspects of particular interest;
- Action 3: data analysis including those gradually collected through sampling during the project period to verify aspects relating to species of particular interest;
- Action 4: writing of the Fishing Charter of the Comacchio Valleys and the adductor channels;
- Action 5: data analysis and writing of the final report, including guidelines for management.

Study area

Comacchio Lagoon comprises three main basins: Valle Campo, Valle Magnavacca, and Valle Fossa di Porto, which cover an area of ~104 ha. The lagoon is connected to the Adriatic Sea by two canals (Bellocchio and

Foce) that are hydraulically regulated by gateways where where V-shaped colled the "lavorieri" are placed and used to capture silver eels (figure 1a and 1b). The study site is recognized as one of the most important coastal wetlands in Europe for biodiversity conservation, and since 1988 has been protected by the institution of the Regional Park of the Po Delta of the EmiliaRomagna (Regional Law 27/88).
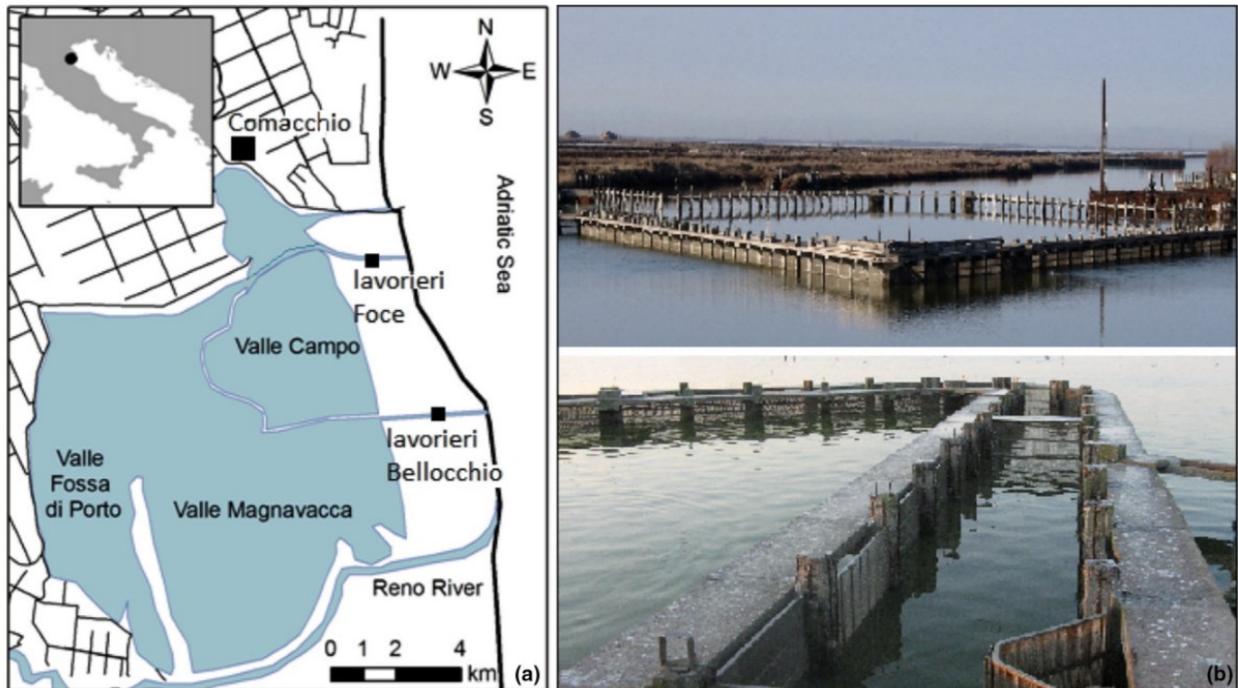


Fig. 1. (a) Study site – Comacchio Lagoon and (b) V-shaped screens 'lavorieri' of selective size used to capture silver eels.

Action 1: collection and organization of the database of previous data
Action 2: sampling of species of interest and collection of field data related to fishing in the area under study

The results of both actions were collected and organized in a single database, in order to be able to analyze and compare them. It contains all the data relating to the fish productions of the Comacchio valleys and all those of sampling carried out for scientific purposes and falling in the same period, using the fky nets "*cogollo*". The monitoring of the fish fauna with fky nets has led to the qualitative and quantitative evaluation of the catch relative to the totality of the fky nets daily put into fishing, with particular attention to the species of commercial interest. The choice of positioning the fky nets, both during the sampling carried out during this research and in previous years, has from time to time represented a compromise between obtaining information, in one or more portions of the valleys, and the availability of personnel at two fishing stations Foce and Bellocchio. The characteristics of the used fky nets and the fishing and data collection methods have been standardized and kept constant throughout the entire period, so as to be able to compare the fishing yield of the different areas in the different periods. To this end, the results are expressed as daily catch per unit of effort, the single head (CPUE, Catch per Unit of Effort), this for each species caught. The following evaluations concern three species: the eel (*Anguilla anguilla*), the shrimp (*Palemon sp*.) And the gray shrimp or schilla (*Crangon crangon*). These three species alone, in fact, represented more than 98% of the total

annual catch with fky nets and are the only ones to be addressed to the commercial chain. The other species of commercial interest fished with fky nets have shown CPUE completely insignificant for the purposes of commercial fishing and with sizes generally below those legal for commercialization.

Eel

The yield of "*cogollo*" for this species was considered in the years between 2011 and 2016. In this period the annual average of CPUE was equal to 0.27 kg / cogollo / month ± 0.43 ds, in the interval between a maximum of 2.86 kg / cogollo / month and a minimum of 0.00 kg / cogollo / month. The year with the highest average CPUE was 2013 with 0.69 kg / cogollo / month ± 0.48 ds, while 2012 was the year with the lowest average CPUE (0.09 kg / cogollo / month 0.12 ± ds). Given the different environmental factors that can act within the valley, a comparison was made between the fishing values in the two sectors within the Valle Magnavacca basin, placed respectively at the two fishing stations of Foce and Bellocchio. These two stations were called by analogy with the two respective "l*avorieri*" at Stazione Foce (Lat 44.6614 ° Long 12.1890 °) and Stazione Bellocchio (Lat 44.6386 ° Long 12.2316 °). In relation to a reduced availability of personnel, although both stations have been monitored throughout the entire period, the CPUE relating to the Bellocchio Station is available until 2014.

The Bellocchio station has recorded an average CPUE of 0.22 kg / cogollo / month ± 12:39 D.S. and a maximum of 1.72 in October 2013 (Figure 1). As for the Foce station, the average CPUE was 0.32 kg / cogollo / month ± 0.47 ds, with a maximum of 2.86 kg / cogollo / month reached in 2014, in July (Figure 1). Overall, the average size of the specimens fished was 40.16 cm ± 6.54 d.s. and 234.2 ± 132.01 g D.S. Out of the total of the specimens fished, 93.5% was composed exclusively of specimens at the "yellow" stage, of which 16.5% still at the juvenile "ragano" stage, with lengths between 11 and 14.8 cm of length. In particular, according to what was expected, during the spring between the months of March and June, the smaller average monthly sizes were recorded, between 30.3 and 37.4 cm, while the larger medium sized specimens were fished in the months of November and December.
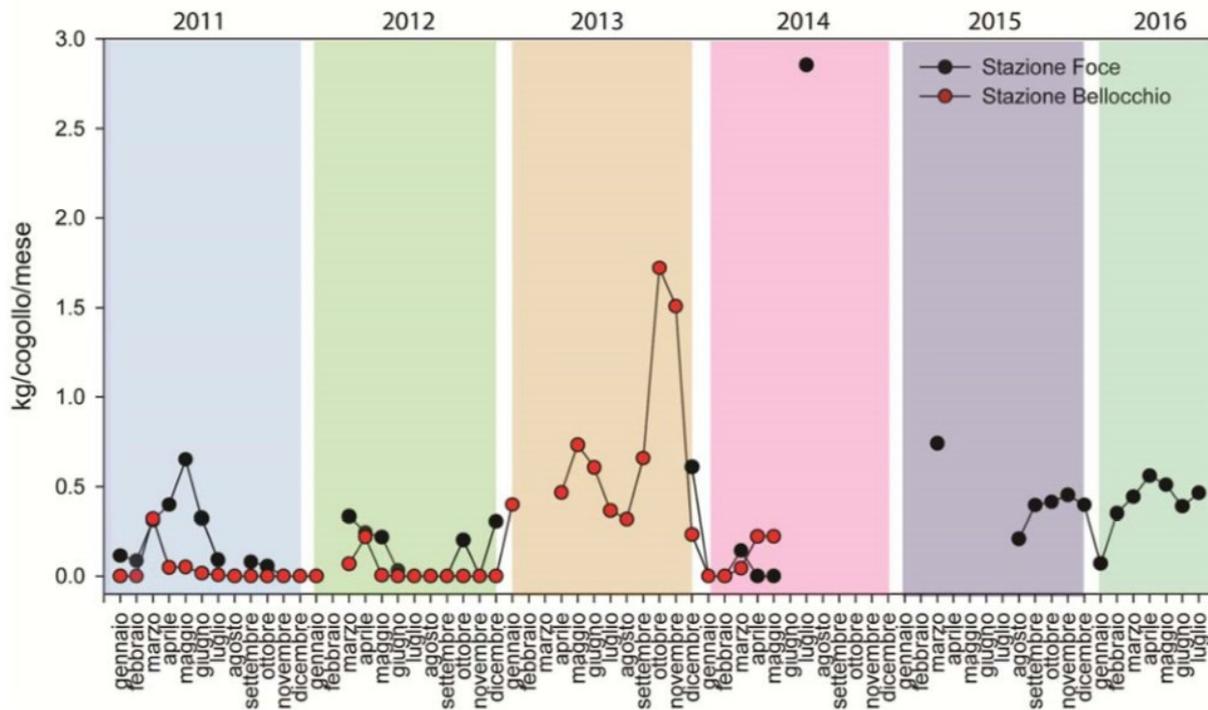
Figure 1. CPUE (kg / cogollo) monthly average for the eel from 2011 to 2016 for the Foce station (black circles) and the Bellocchio station (red circles).

Shrimp and gray shrimp (schilla)

The analysis period extends from 2011 and 2016 for both portions of Valle Magnavacca, placed in front of the lavorieri of Foce and Bellocchio, with an interruption in the years 2014 and 2015. The shrimp (*Palemon sp*.) and the gray shrimp or "*schilla"* (*Crangon crangon*) have a well-defined temporal distribution: the shrimp is dominant in the summer and autumn months, making up almost the totality of the catch, particularly between June and September. At the end of October, as the season progresses and in relation to the lowering of the temperature (indicatively below 12-13 ° C), the appearance of the gray shrimp appears. This species is more active in the late autumn and winter periods, so much so as to constitute almost all of the catch with maximum values between 80 and 90% in January and February. The average CPUE for the Foce station is equal to 3.47 kg / cogollo / month ± 1.97 D.S. with a maximum of 8.78 kg / scroll / month recorded in 2012 in September (Figure 2). For Bellocchio station, a slightly higher average CPUE (4.06 kg / cogollo / month ± 1.78 d.s.) was found, with a maximum of 7.78 kg / cogollo / month, reached in October 2011 (Figure 2).
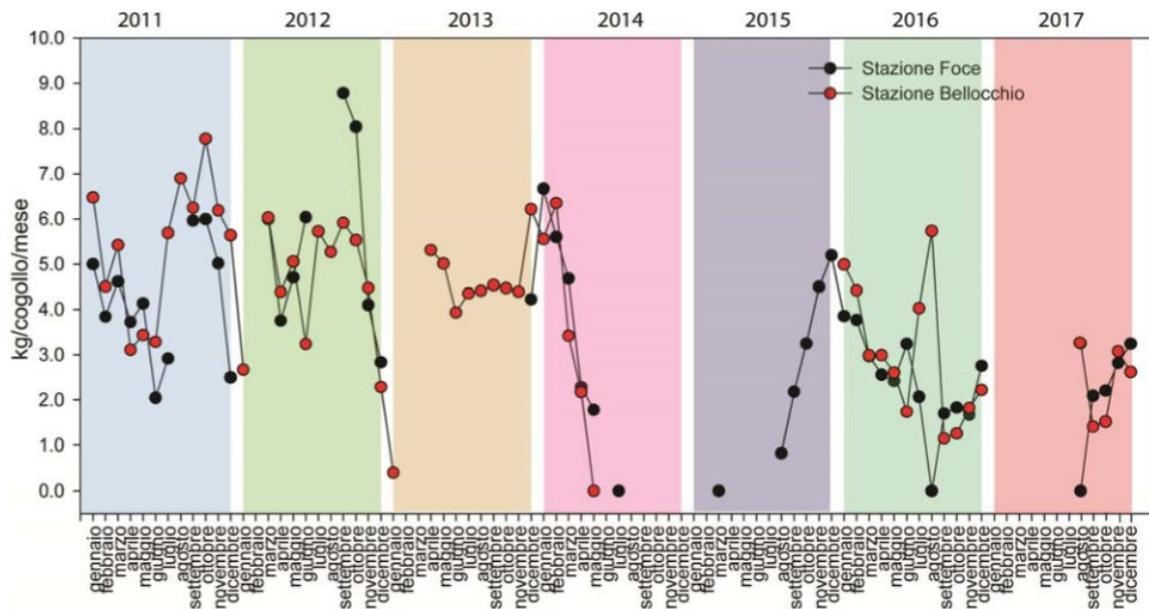
Figure 2. CPUE (kg / cogollo) monthly average for the shrimp from 2011 to 2017 for the Foce station (black circles) and the Bellocchio station (red circles).

Other species of commercial interest underrepresented in the catch (2010-2013)

From 2010 to 2013 it was carried out an analysis of other species which with cogollo. A total of 15 species of potential commercial interest have been surveyed: anchovy (*Engraulis encrasicolous*), gurnard or capon (*Chelidonichthys lucerna*), goby Gò (*Zosterisessor ophiocephalus*), dairy (*Atherina boyeri*), mullet (*Liza ramada*), golden mullet ( *Liza aurata*), mullet (*Liza saliens*), mullet mullet (*Mugil cephalus*), mullet labbrone (*Chelon labrosus*), sea bream (*Sparus aurata*), plaice (*Plathychtys flesus*), sole (*Solea solea*), sea bass (*Dicentrarchus labrax*), sprat (*Sprattus sprattus*) and soaso or smooth turbot (*Scophthalmus rhombus*). The data are reported separately for the Bellocchio station (Table 1) and Foce (Table 2), in order to highlight the possible differences in terms of capture. The data shows that for all the 15 species the fishing with cogollo has recorded catches for extremely low effort units and average sizes constantly lower than the minimum marketing size. This evidence confirms the non-applicability of this method of fishing to these species within the Comacchio valleys.

Table 1:

| Anno | Mese | Acciuga | Capone/Galinella | Ghiozzo Go | Latterino | Muggine Calamita | Muggine cefalo | Muggine dorato | Muggine labbrone | Muggine musino | Orata | Passera | Sogliola | Spigola | Spratto | Soaso (Rombo liscio) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo |
| 2010 | novembre | 0.58333 | 0 | 0.02631 | 0.00150 | 0.13274 | 0 | 0 | 0.22473 | 0.09900 | 0.09198 | 0 | 0 | 0 | 0 | 0 |
| | dicembre | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2011 | marzo | 0.01400 | 0 | 0.00437 | 0.00286 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | aprile | 0.01188 | 0 | 0.02312 | 0.01244 | 0.00820 | 0 | 0 | 0 | 0 | 0 | 0.00001 | 0.00006 | 0 | 0 | 0 |
| | maggio | 0.06914 | 0.00006 | 0.01463 | 0.02290 | 0.00495 | 0.00054 | 0 | 0 | 0.00730 | 0.00062 | 0.00070 | 0.00524 | 0 | 0.00013 | 0.00040 |
| | giugno | 0.34821 | 0.00009 | 0.02634 | 0.02682 | 0.02653 | 0 | 0.00141 | 0 | 0.01733 | 0.00222 | 0.01961 | 0.07107 | 0.00022 | 0.03213 | 0.00160 |
| | luglio | 0.37548 | 0 | 0.03425 | 0 | 0 | 0 | 0 | 0 | 0.00891 | 0.00970 | 0.01528 | 0.08520 | 0 | 0 | 0 |
| 2012 | gennaio | 0 | 0 | 0 | 0 | 0 | 0 | 0.00020 | 0 | 0.05462 | 0 | 0 | 0 | 0 | 0 | 0 |
| | febbraio | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | marzo | 0.05952 | 0 | 0.13680 | 0.02194 | 0.08104 | 0 | 0.00481 | 0 | 0.01102 | 0 | 0 | 0 | 0 | 0 | 0 |
| | aprile | 0.55000 | 0 | 0.01768 | 0.00400 | 0.33564 | 0 | 0 | 0 | 0 | 0.00817 | 0 | 0.00015 | 0 | 0 | 0.00010 |
| | maggio | 0.06250 | 0 | 0.00889 | 0.00064 | 0.06560 | 0 | 0 | 0 | 0.01679 | 0.00993 | 0 | 0.00088 | 0 | 0 | 0 |
| | giugno | 0 | 0 | 0.01189 | 0.00398 | 0.14909 | 0 | 0 | 0.07229 | 0.05735 | 0.02671 | 0 | 0 | 0 | 0 | 0 |
| | luglio | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 1. CPUE (kg / cogollo / month) for the 15 fish species of interest, for the Bellocchio station, Valli di Comacchio

Table 2:

| Anno | Mese | Acciuga | Capone/Galinella | Ghiozzo Go | Latterino | Muggine Calamita | Muggine cefalo | Muggine dorato | Muggine labbrone | Muggine musino | Orata | Passera | Sogliola | Spigola | Spratto | Soaso (Rombo liscio) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo | kg/cogollo |
| 2010 | novembre | 0.07011 | 0.00001 | 0.03465 | 0.00758 | 0.00079 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | dicembre | 0 | 0 | 0.00826 | 0.00140 | 0.00000 | 0 | 0 | 0 | 0.00000 | 0 | 0.00004 | 0.00003 | 0 | 0 | 0 |
| 2011 | marzo | 0.07620 | 0 | 0.03607 | 0.03426 | 0.00549 | 0 | 0 | 0 | 0.00573 | 0.00017 | 0.00302 | 0.00887 | 0 | 0 | 0 |
| | aprile | 0.41379 | 0 | 0.03114 | 0.06122 | 0.01717 | 0 | 0 | 0 | 0.01696 | 0.00060 | 0.00986 | 0.03411 | 0.00001 | 0.01114 | 0.00090 |
| | maggio | 0.25019 | 0 | 0.00721 | 0.02067 | 0.00824 | 0 | 0.00263 | 0 | 0.02854 | 0.00194 | 0.00181 | 0.02801 | 0 | 0 | 0 |
| | giugno | 0.01765 | 0 | 0 | 0.01533 | 0.03610 | 0 | 0.01467 | 0 | 0.01828 | 0.00067 | 0 | 0 | 0 | 0 | 0 |
| | luglio | 0 | 0 | 0.00440 | 0.00260 | 0.01393 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2012 | gennaio | 0.09049 | 0 | 0.02161 | 0.09238 | 0.00333 | 0 | 0.05544 | 0 | 0.00832 | 0 | 0 | 0 | 0 | 0 | 0 |
| | febbraio | 0.00023 | 0 | 0.05762 | 0 | 0 | 0 | 0 | 0 | 0.01647 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Marzo | 0.07855 | 0 | 0.19584 | 0.07053 | 0.02025 | 0 | 0.05531 | 0 | 0.06347 | 0.03002 | 0 | 0.01555 | 0 | 0 | 0 |
| | Aprile | 0.19333 | 0 | 0.18564 | 0.22167 | 0.28243 | 0 | 0 | 0 | 0.06117 | 0.00375 | 0.01397 | 0.04220 | 0 | 0 | 0 |
| | Maggio | 0.58125 | 0 | 0.36999 | 0.06881 | 0.29749 | 0 | 0.01688 | 0 | 0.01819 | 0.02688 | 0.04016 | 0 | 0.00383 | 0 | 0 |
| | Giugno | 0.06372 | 0 | 0.21838 | 0.02115 | 0.02840 | 0 | 0.00243 | 0 | 0.01092 | 0.00000 | 0 | 0 | 0 | 0.01175 | 0 |
| 2013 | settembre | 0.02190 | 0 | 0.02940 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.46140 | 0 |
| | Ottobre | 0.52160 | 0 | 0.37600 | 0.03755 | 0.07512 | 0 | 0.00335 | 0 | 0.03078 | 0 | 0 | 0 | 0 | 0.22505 | 0 |

Table 2. CPUE (kg / cogollo / month) for the 15 fish species of interest, for the Foce station, Valli di Comacchio.

## Fishing with "*lavoriero*" (2008-2017)

Worker monitoring activities were carried out at the two fishing stations of Foce and Bellocchio, during the autumn and spring and summer months, and therefore concerned the main species targeted at the commercial chain. The quantitative data of the catch are cumulative of both the "lavorieri" of Foce and Bellocchio, obtained from the daily sales data kindly granted by the Park Management Body and the Biodiversity-Delta of the Po, for the period between 2008 and 2017. The species that can be caught are the following: anchovy, eel, dairy, mugilidae (data including the 5 species that can be caught in the valley), sea bream, flounder, sole and sea bass. The data relating to the eel are representative of the maximum quantity feasible in the period, while for anchovy and mugillidii, they are indicative of the order of magnitude of the

quantities fished in a given year. For these species, which are easily perishable once captured, the work of the workers was subordinated to the demands of the market which did not always correspond to the quantities available in the valley for fishing, on some major occasions. From 2008 to 2017, the average production of fish was 26,571.5 kg / year ± 15,452.9 ds (Table 3). The largest quantities refer to anchovy with an average of 17.929.1 kg / year ± 12.420,0 d.s. and an average yield per unit area of 2.13 kg / ha / year. The eel had an average production of 4,224.4 kg / year with a yield of 0.50 kg / ha / year. The trends of these two species, the most important in terms of quantity and marketing, are shown in Figure 3, where, in addition to different temporal fluctuations in the amount of anchovy, an increase in eel catch is particularly evident from 2014 until 2017, in which production reached 8393 kg, with an average increase of 100% compared to the years 2008 to 2015, including (Figure 3).
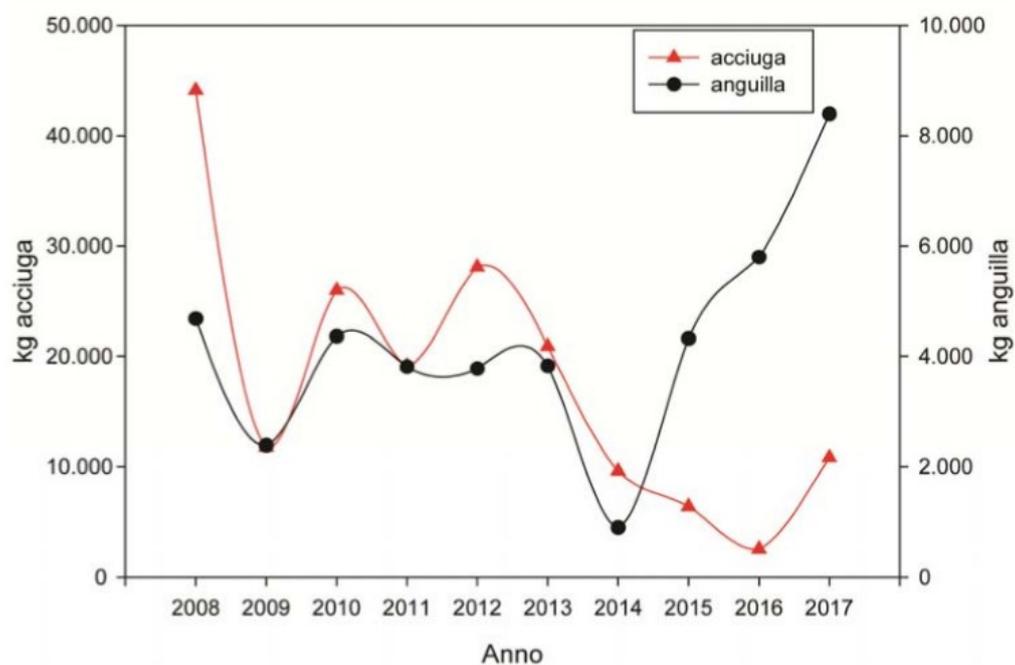


Figure 3. Annual productions analysis of anchovy and eel 2008-2017.

The mugilidis reach values equal to 4.453.5 kg / year ± 4.309,6 d.s. and unit yield of 0.53 kg / ha / year and the sea bream, 84.4 kg / year ± 92.0 d.s. and yield of 0.01 kg / ha / year (Table 3). From the annual production data for each species, it is clear that the species of commercial interest such as S*. solea, D. labrax and A. boyeri* in the present are completely marginal for commercial fishing, both for the small quantities fished and for the discontinuity of the catches (Table 3).

| PRODUZIONE VALLIVA PER SPECIE (kg), 2008-2017. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ANNO | acciuga | anguilla | latterino | mugilidi | orata | passera | sogliola | spigola |
| 2008 | 44074 | 4676 | 2521 | 8907 | | 104 | | 2 |
| 2009 | 11779 | 2383 | 536 | 5379 | | 243 | | |
| 2010 | 25965 | 4359 | 6 | 792 | 1 | | | |
| 2011 | 19109 | 3812 | | 13657 | 87 | | 26 | |
| 2012 | 28081 | 3778 | | 4019 | 38 | | | |
| 2013 | 20894 | 3827 | 2 | 2748 | 212 | | 14 | 26 |
| 2014 | 9608 | 901 | | 1422 | | | | |
| 2015 | 6402 | 4321 | 4 | 2275 | | | | |
| 2016 | 2546 | 5795 | 10 | 883 | | | | |
| 2017 | 10833 | 8393 | | | | | | |
| Produzz. media annua (kg) | 17929.1 | 4224.4 | 513.0 | 4453.5 | 84.4 | 173.5 | 20.0 | 14.0 |
| Resa unitaria media annua (kg/ha) | 2.134 | 0.503 | 0.061 | 0.530 | 0.010 | 0.021 | 0.002 | 0.002 |

Table 3. Lagoon production values (kg) for each species analyzed in the period 2008-2017.

Trends of all the species show a strong seasonality, according to the expectations regarding the fishing in the lagoon, due to the particular biological cycles of the single species and to the relative behavioral modalities of exploitation of the lagoon environment (Figure 4). The anchovy reaches the maximum quantities of fish caught during the late spring and summer season, with very high seasonal quantities, on average between 180 and 220 q / season. In contrast to species such as eel, and "white" fish, or mugilidis + sea bream, the catch is concentrated almost exclusively during the autumn season, taking full advantage of the migration of adult specimens returning from the valley to the sea (Figure 4).
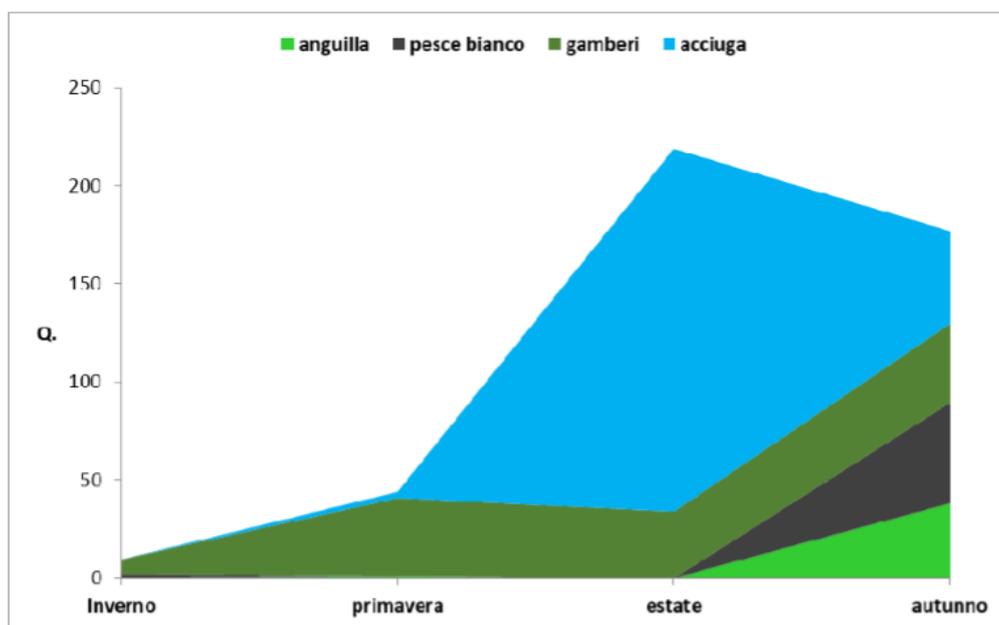


Figure 4. Average annual trend of the lagoon production (q), in the period 2008-2017, for eel, anchovy, shrimp and white fish (mugilidis + sea bream)

Action 3: data analysis including those gradually collected through sampling during the project period to verify aspects relating to species of particular interest

In 2007, at the XIV CITES Conference (Convention on International Trade in Threatened Animal and Plant Species), created on the progressive decline of the eel population, recorded globally in recent decades, the European Union has proposed and obtained that the eel was recognized as an endangered species, asking the international community for an effort to preserve it. Site of Community Importance (SCI) and Special Protection Area (SPA, IT4060002), Comacchio, with its "Valleys", is inextricably linked to this species and could only be in the front row for its preservation.

The Po Delta Park of Emilia-Romagna has activated numerous actions, including a Life Nature project entitled "*Conservation of habitats and species in the Natura 2000 sites of the Po delta*". In this project, the monitoring of the fauna, carried out by the writing group of the Department of Biology and Evolution of the University of Ferrara, was aimed at studying some of the most important characteristics of the eel population of the valleys. In his life, in fact, an eel can "inhabit" all types of aquatic environments, from the depths to mountain streams, from the Scandinavian peninsula to the Sargasso Sea. Of this life cycle, the most touching aspect is the return of the small eels that cross the Atlantic from the Sargasso, still at the post-larval stage of leptocephalus and, transported by the currents, reach the mouths of the rivers as "glass eels", small eels of few centimeters, now equal to the adult form, but still transparent. Numerous on the Tyrrhenian coast, the glass eels have been fished for decades at the mouth of the Tuscan, Lazio and Campania rivers, to be resold on farms and / or eaten as a local specialty.

In Comacchio, in fact, until 2011 there were no scientific reports to document the rise of glass eels in the delta area. However, the lagoons report, in the past, when the lagoons produced thousands of quintals per year, it was common practice to follow their movements, from the coast to the canals leading to the lagoons and then inside the lagoons themselves, with visual reliefs or with the method of little-woods-nets.

On January 19, 2011, on the Gobbino, an adductor channel of the Comacchio lagoons, at the fishing station called Bellocchio, with a specially designed juveniles fishing nets, built by personnel of the Park, and university researchers, they were caught glass eels at various times during the study period, which extended from January to April. The monitoring was repeated in 2012. The analysis of the results is reported in the annual reports of the activities carried out by the research group of the University of Ferrara, and are the object of analysis for the publication of a scientific article related to the factors that control the dynamics of ascent of the glass eels. Other aspects of the ecology of the eel stock of the Magnavacca and Fossa di Porto Valleys have been the subject of recent publications by Prof. Castaldelli's group, both in the form of technical reports and, subsequently, of scientific publications in international journals. These publications have brought the international scientific interest to Comacchio lagoons, the most known environment abroad in

reference to eel fishing. For the sake of completeness, the references of these international publications are reported below, also regarding other fish species of the Comacchio lagoons, marginally affected by fishing but subject to protection under the Habitats Directive. Historically, fishing in the Comacchio lagoons has always had eel as its main target species, while mullet, flounder and other minor lagoon species, have always been considered a sort of marginal yield, of little economic importance and mainly used for the local consumption and not for sale. The capture of these species took place simultaneously with that of the eel but in a non-priority way. Therefore, to maximize the yield of eel capture at the "silver" stadium, a dogma has historically been confirmed in the Comacchio wetlands: the only fishing gear that can be used is the "lavoriero". On the contrary, all the other techniques currently used in the lagoon, and based on the use of nets of various shapes, as well as primed hooks and spears of various types, were prohibited in an absolute sense in the valleys, as their use went to interfere with the eel maturation cycle, allowing it to be captured at the "yellow" stage and therefore decreasing its probability of maturation, and therefore yields, at the silver stage. It is worth dwelling on this fact because its importance in understanding the inviolable rules of traditional fish farming is fundamental. The prohibition of all fishing techniques outside the lavoriero in the lagoons is a sort of truth of faith on which, in Comacchio more than in any other place of the Po delta and the north-western Adriatic, it is not discussed. It has always represented the watershed between what is good practice and what is illegal. Today, however, for a critical and constructive analysis, free from prejudices and free from easy exploitation, it is important to underline that the lagoon fisheries, in Comacchio as in the entire Po delta, are no longer those in which the practice of fish farming is It has evolved.

The drainage of wetlands, eutrophication and subsidence onwards, have profoundly changed. In addition, the reduction of eel populations at European level and with it the recruitment of glass eels and "ragani", especially in the north-western Adriatic, did the rest, bringing the productions of silver eel to very low values compared to only forty years. Although these productions can be increased, as demonstrated by the first results of the application of the Valli di Comacchio Management Plan, for all the reasons listed they will not be able to reach the values of the first post-war period. At this point it should be made a reflection on fish productivity of the Comacchio. It has evidently changed in the last half century, due to changes in the lagoons environment, but it is not necessarily decremented. Rather, from almost monospecific to the eel alone, it now consists of other fish species, including anchovy, and shellfish. To confirm, consider that just the anchovy was completely absent in the catch until the end of the seventies, and now it is the dominant fish species, with much greater quantities involved. In fact, if the catch of this species is on the average of the order of two hundred quintals per year, it is very likely that the production may actually be much greater, given that, together with the mullets, it is the prey of election of miles of ichthyophagous birds, moreover almost entirely absent in the late seventies. Another very interesting case is represented by the shrimps, both golden shrimp and grey shrimp, which most likely were present in extremely lower quantities before the trophic change of the lagoons due to eutrophication. Also in this case, a punctual comparison between the beginning of the

seventies and the present day is not possible, as we do not have any previous estimates, given that the shrimp cannot be caught by the lavorieri, the only type of fishing practiced then in the Magnavacca, Fossa Porto and Campo lagoons. Regardless, if we look at the chart of eel productions, extracted from the article by Aschonitis and collaborators (2016) and shown below (Figure 5), where the productions of silver eel from the Comacchio lagoons are indicated from the end of 1700 to 2013, we note that at the end of the seventies the values stood at around one thousand quintals per year.
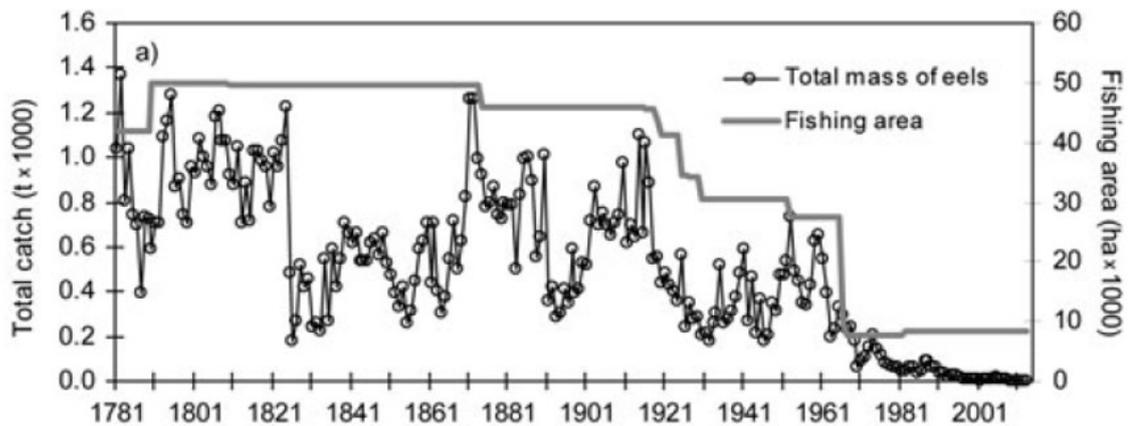


Figure 5. Chart taken from Aschonitis and collaborators (2016) showing the fishing area of the eel (fishing area) in hectares, and the annual variation of the silver eel catch (total mass of eels) expressed in tonnage × 1000, for the period 1781-2013.

This value of production of eel alone is equivalent to the overall secondary production of the Comacchio lagoons, deriving from a valley surface that is, in fact, the current one, since the Mezzano drainaged was already it has been completed. This value is probably not substantially different from the current one but only different for the species involved, in the early seventies mainly eel and now mainly anchovy and shrimp, while eel has become a minor species. This hypothesis is also supported by numerous scientific articles, produced by various authors and independent institutions, which unitarily document the trophic change of the ecosystem of the lagoons, occurring between the two periods considered, the early seventies and the present. This type of discussion would weigh down the text and the subject in question and therefore should be referred to a dedicated in-depth study, if requested. The meaning of this dissertation, supported by fishing data, is to bring attention to the change in the Comacchio Valleys in order to highlight the new opportunities for exploiting fishery resources. In order to do this, for the purpose of an overall revaluation of the district, it is compulsory to succeed in defining new operating methods, sustainable from the environmental and conservation point of view and such as not to interfere with the eel production cycle that for historical reasons and of application of the European Regulation, remains however a priority.

To this end, in the case of the Comacchio lagoons anchovy, locally called "sardoncino", there are no critical issues relating to its capture, which is easily carried out with the lavoriero, in a period that is not superimposed with that of the eel. In fact, fishing begins around July 15, when the anchovy reaches its minimum commercial size and continues approximately until early October, thus ending before the eel fishing season opens. Regarding the exploitation of the shrimp, some elements and technical aspects must be clarified; in particular, how much biomass can be captured in the various periods, without jeopardizing the stock and, a subject of more difficult resolution, which tool to authorize for its capture, without negatively interfering with the eel and other species development cycle object of protection present in the lagoons. Currently in the Comacchio lagoons there are two species of shrimp, both belonging to the genus Palaemon and the gray shrimp or schilla (Crangon crangon). The abundance of catches of all three species, verified in numerous experimental campaigns, is proof that these species find in the current conditions of the Comacchio lagoons a favourable environment for reproduction and growth. In the seven years of study there has never been a decline in their presence, except as a result of the exceptional proliferation event within the valleys of a ctenophore, *Mnemiopsis leidyi*, similar to a jellyfish. The species appeared in the summer of 2016 along the entire north-western Adriatic coast, according to development dynamics remained unknown. It is probable, however, that once he entered the Comacchio lagoons he interacted with the shrimps reproductive cycle, of which a marked drop in catches was recorded in the period to follow. Certainly, in the period in which the ctenophore was present in the lagoons, it prevented the use of the " cogollo" nets for monitoring, as it ended up on the crosspieces and in the same deceptions, clogging them making them unusable. Apart from this unpredictable and quite exceptional phenomenon, the field data collected over many years and locations within the valleys indicate that the resource is substantial and that the overall annual yield can be of the order of several hundred quintals. It is evident that the problematic nature of the exploitation of this resource lies more in the fishing technique, the cogollo, than in the available quantities. As mentioned above, the use of the cogollo has always been forbidden in the lagoons, as it is an elective technique also for eel fishing and therefore can interfere with its development and maturation cycle.

An alternative technique for shrimp fishing is the use of the seine, locally called "treats", of suitable mesh and size and operated without the use of boats. However, this technique is effective only in the autumn and winter season, when the reduced motility of the crayfish allows an easier capture, while in the spring and summer season it is generally not very effective. The following figure (Figure 6) shows the sites within the lagoons where, during the course of this agreement, a trial was conducted on the possibility of using this tool during the summer season with the management staff. The map also shows the overall length of the transects along which the route was pulled. The network used was 1.30 m high, 7 m long (5 meters in fishing), with 7 mm mesh.
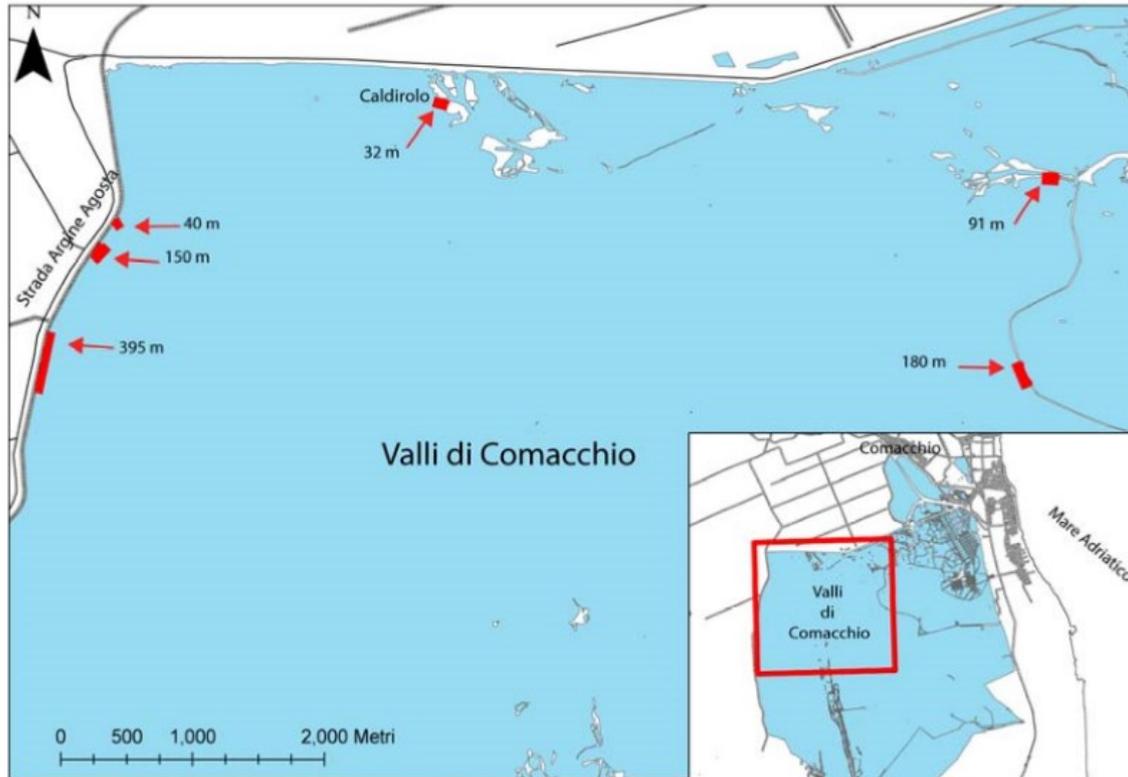
Figure 6. Map of the Comacchio Valleys with indicated sites and the overall length of the transepts where the use of treats for shrimp fishing was tested.

In validation the monitorings with cogolli, carried out in previous years, the results indicated a homogeneous distribution of the shrimp in all the considered stations. However, due to the great mobility of the animals, typical of the warm months, the amount of catches was very poor, and such as to exclude this technique for fishing in the spring and summer period. Eventually, after experimentation carried out in the period of interest, fishing with seine can be considered in autumn-winter. However, there remains a heavy spatial limitation related to its use, in fact bound only to areas with low depth and sandy and sandy bottom silty, with the exclusion of large areas of the valley basin, where the depth in the cold period exceeds one meter and the seabed is muddy and incoherent. To investigate the issue and try to highlight a possible solution to minimize the disturbance on the eel that would derive from the use of the cogollo, some results of the monitoring carried out in previous years have been considered. In particular, during a recent monitoring campaign with the cogolli, carried out by Lanzoni 2016 "Monitoring of the fish fauna of the Comacchio Valleys with particular reference to the eel stock, the comeback of juveniles and species of commercial interest - August 2015 - July 2016 " interesting information emerged that was confirmed in the previous monitoring period (2010-13).

The seasonal analysis of the percentage composition in biomass of the catch showed in all the seasons a clear prevalence of prawns (shrimp and dill) with biomass values always higher than 60% of the total. The other species always present in the seasonal composition of the catch is eel, with values ranging between 15.4% of

the catch in the summer and 19.4% in the autumn. Concerning the shrimps, shrimp and shells considered cumulatively, the average capture per unit of effort (a single cogollo, with only one deception and a 50 m traverse) was equal to 4.9 kg ± 2.9 d.s. per day for cogollo, from a minimum of 0.5 (kg / g) to a maximum of 15 (kg / g), (Figure 7). The analysis of average monthly catches per unit of effort found in the winter months of January and February the highest average values equal to 8.84 ± 3.8 d.s. and 8.18 ± 1.9 D.S. (kg / g) respectively, while in the months of August and September the lowest values of 1.37 ± 0.36 d.s. and 2.18 ± 1.09 D.S. (Kg / g), (Figure 7).
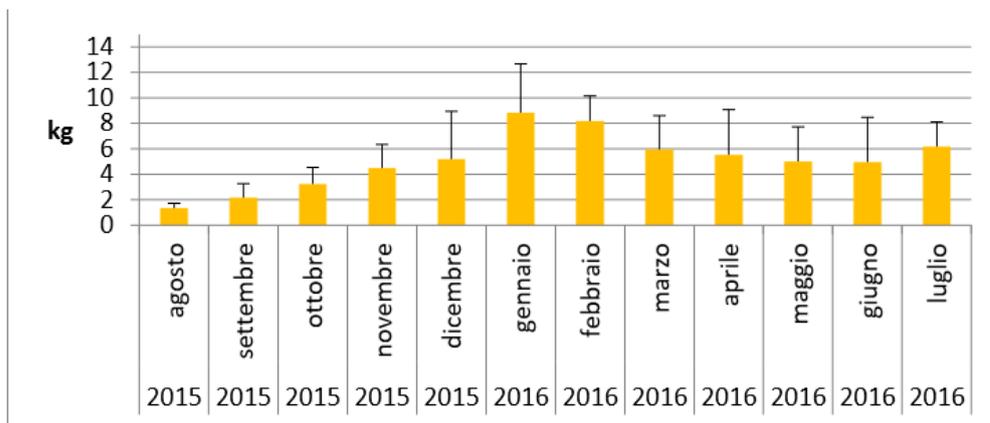


Figure 7. Monthly trend of average daily catches per unit of effort (CPUE) (kg / day / cogollo) of shrimp and grey shrip, considered cumulatively, from August 2015 to July 2016.

For the qualitative analysis, the shrimp was dominant in the summer and autumn months with values equal to 100% of the catch between June and September (Figure 8). As the season continues and in relation to the lowering of the temperature (indicatively below 12-13 ° C), the percentage of the "schilla" increases, a more active species in the late autumn and winter periods, which with the progress of the cold season almost replaces completely the shrimp. The presence of schilla in the catch started from the end of October, with a proportion equal to 10% of the total shrimps, to then reach the maximum values between 80 and 90% in January and February.
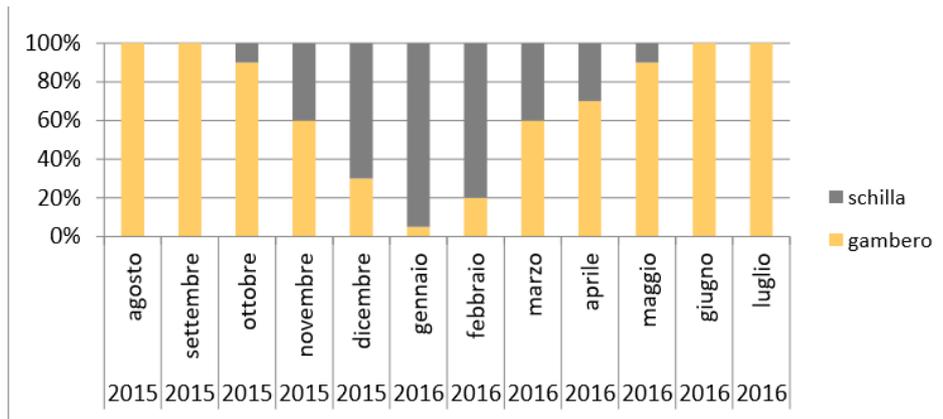
Figure 8. Monthly trend in the percentage distribution of the shrimp and the shilla compared to their total catch with cogollo, from August 2015 to July 2016.

For the eel, the average capture per unit of effort, or the quantity captured by a single cogollo, armed with a single deception and endowed with a barrier length of 50 m, was equal to 0.19 kg ± 0,7 ds in a single day of fishing, varying from a minimum of 0.10 kg ± 0.7 ds, in August, to a maximum of 0.28 kg ± 0.2 ds, in April, therefore with higher monthly average values in spring (Figure 9). The average size of the captured specimens was 40.16 cm ± 6.54 d.s. and 234.2 ± 132.01 g D.S. 93.5% of the fished specimens were constituted by "yellow" stage, of which 16.5% still at the juvenile "ragano" stage, between 11 and 14.8 cm long. In particular, according to what was expected, during the spring between the months of March and June the smaller average monthly sizes were recorded, between 30.3 and 37.4 cm, while the larger medium sized specimens were caught in November and December (Figure 9). In April, and in general during the spring, the highest eel capture values were recorded for each cogollo.

At the same time, the average size of the captured specimens was the smallest compared to the other periods of the year. These indications draw attention to the possible negative effects of the fishing activity with the cogollo during this time of the year, in which the greater mobility of young individuals makes them more easily captured with this fishing technique, exposing them to a catch stress and to the risk of possible injury in the release phase. Furthermore, in the same period the damages on the stock are maximum from possible illegal fishing activities that can take away a large number of individuals from growth and development, placing serious limits on the general productivity of the Magnavacca and Fossa di Porto complex, understood. Obviously, as the production of mature, silver eels, captured at the lavorieris' site, to conclusion the growth cycle, as foreseen by the good practices of traditional aquaculture.
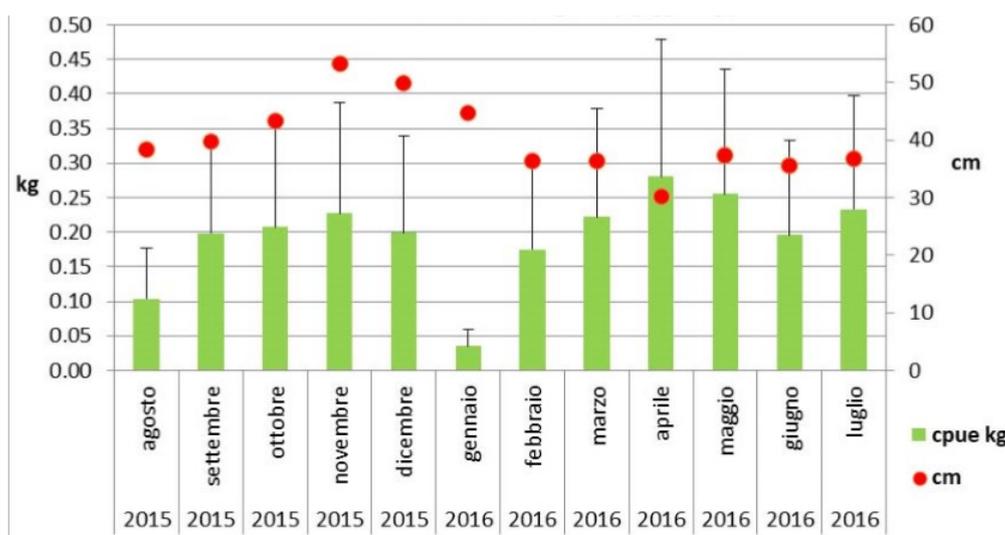
Figure 9. Monthly trend of average daily catches per unit of effort (CPUE) (kg / day / cogollo) of fishing with eels and its medium size (cm), in red, from August 2015 to July 2016.

## Considerations and guidelines

In all months the crayfish catches overlap with those of the eel. Therefore, the extensive use of the cogollo for catching shrimps interferes with the eel development cycle throughout most of the year, posing risks to its correct completion, according to the good practices of the hives. The only partial "windows", in which the catchability of the eel with the cogollo is reduced, due to the reduced mobility of the species, it turned out to be January, in which the catches of the srimps, mainly gray shrimps, are at annual maximums, and August, when, also shrimp fishing has produced poor catches, due to high temperatures and low oxygen saturations. The use of the seine "trezza", as catching method for the srimps, has so far been evaluated only in June and July of 2018, when it has not given satisfactory results, as proof of its difficulty if not impossiblity of use in Spring and Summer months, due to the high mobility of the animals.

Therefore, alternative solutions must be identified. A hypothesis of a useful solution for shrimps fishing without causing damage to the eel stock is represented by the use of a modified cogollo, in order to minimize eel catching, while maintaining high that of shrimps. Experimental tests are in progress to verify this possibility. A first modification of the cogollo, useful to prevent the capture of the size eels (length greater than 60 cm), is obtainable modifying the tool through the application of a front screen net on the mouth of the fyke net part of the cogollo. A further modification, more difficult to define and subject to experimentation, is aimed at allowing eels less than 60 cm to exit, by creating an escape route from the last chamber of the cogollo deception, usable by eels but not by shrimps. The sizing and positioning of this exit for eels is being studied.

If this method of selective fishing with the cogollo will be quantitatively effective and repeatable, it will open to its use without temporal and spatial limitation, except in the areas forbidden to navigation due to the presence of sand bars occupied by colonies of birds and used as nesting sites.

In the case of an effective definition of the selective fishing method with the cogollo, the only limitation to consider would concern the maximum fishing effort, i.e. the total number of cogolli. The evaluation of these numbers is still ongoing. It is however possible to anticipate, that given the natural interannual fluctuation of the fishery resources in an environment such as the Comacchio lagoon, the correct management of the basin should forsee the annual authorization of a precise fishing effort, as maximum number of cogolli and linear meters of blocking net. This evaluation should be based on catches in the previous years and parallel field estimates and put into practice by the Po Delta Park.

### Action 4: Fish Plan of Comacchio lagoons, Magnavacca and Fossa di Porto, Fattibello lagoons and adducted canals and Comacchio salt-work

The fish species surveyed by the means of monthly or bimonthly monitoring in six years, from 2009 to 2014 in the two main districts of Comacchio (the lagoon system of Magnavacca and Fossa di Porto and the lagoon system of Valle Fattibello - Spavola and related channels). To complete analysis, the data relating to the dismissed saltwork of Comacchio has also been reported, whose sampling was carried out in an earlier period (2001-2006), as part of the Life Project LIFE00NAT / IT / 7215 - Ecological restoration and conservation of habitat in the Saline of the SIC Valli di Comacchio (AA.VV., 2006. Management Plan of the Salina di Comacchio portion of the Sic "Valli Di Comacchio" - IT4060002).

Fattibello-Spavola lagoon and channels

A total of 34 species reported in Table 4 were counted in the Fattibello-Spavola and canals of connection to the sea.

| Famiglia | Nome scientifico | Nome comune inglese | Nome comune italiano |
|---|---|---|---|
| Anguillidae | *Anguilla anguilla* | European eel | Anguilla |
| Atherinidae | *Atherina boyeri* | Big scale sand smelt | Latterino |
| Belonidae | *Belone belone* | Garfish | Aguglia |
| Blenniidae | *Salaria pavo* | Peacock blenny | Bavosa |
| Clupeidae | *Alosa fallax* | Twaite shad | Cheppia |
| | *Sprattus sprattus* | European sprat | Spratto |
| Cyprinidae | *Abramis brama* | Common bream | Abramide |
| | *Pseudorasbora parva* | Stone moroko | Pseudorasbora |
| Cyprinodontidae | *Aphanius fasciatus* | Mediterranean banded killifish | Nono |
| Engraulidae | *Engraulis encrasicolous* | European anchovy | Acciuga |
| Gobiidae | *Zosterisessor ophiocephalus* | Grass goby | Ghiozzo Gò |
| | *Pomatoschistus canestrinii* | Canestrini's goby | Ghiozzo cenerino |
| | *Knipowitschia panizzae* | Adriatic dwarf goby | Ghiozzo laguna |
| | *Pomatoschistus minutus* | Sand goby | Ghiozzo minuto |
| | *Gobius niger* | Black goby | Ghiozzo nero |
| | *Gobius paganellus* | Rock goby | Ghiozzo paganello |
| Moronidae | *Dicentrarchus labrax* | European seabass | Spigola |
| Mugilidae | *Liza ramada* | Thinlip mullet | Muggine calamita |
| | *Liza aurata* | Golden grey mullet | Muggine dorato |
| | *Liza saliens* | Leaping mullet | Muggine musino |
| | *Mugil cephalus* | Flathead grey mullet | Muggine cefalo |
| | *Chelon labrosus* | Thicklip grey mullet | Muggine labbrone |
| Mullidae | *Mullus barbatus* | Red mullet | Triglia |
| Pleuronectidae | *Plathychtys flesus* | European flounder | Passera |
| Sciaenidae | *Pagellus bogaraveo* | Blackspot seabream | Occhione |
| | *Ombrina cirrosa* | Shi drum | Ombrina |
| Scombridae | *Scomber scombrus* | Atlantic mackerel | Sgombro |
| Scophthalmidae | *Scophthalmus maximus* | Turbot | Rombo |
| Soleidae | *Solea solea* | Common sole | Sogliola |
| Sparidae | *Sparus aurata* | Gilt-head bream | Orata |
| | *Diplodus vulgaris* | Common two-banded seabream | Sarago |
| Syngnathinae | *Sygnatus acus* | Greater pipefish | Pesce ago |
| | *Sygnathus abaster* | Black striped pipefish | Pesce ago di rio |
| Triglidae | *Chelidonichthys lucerna* | Tub gurnard | Capone/Gallinella |

Table 4. Fish species present in Valle Fattibello-Spavola and related channels

## Magnavacca and Fossa Porto lagoons

In Magnavacca and Fossa di Porto lagoonsit was caught a total of 28 species (Table 5).

| Famiglia | Nome scientifico | Nome comune inglese | Nome comune italiano |
|---|---|---|---|
| Anguillidae | *Anguilla anguilla* | European eel | Anguilla |
| Atherinidae | *Atherina boyeri* | Big scale sand smelt | Latterino |
| Blennidae | *Salaria pavo* | Peacock blenny | Bavosa |
| Clupeidae | *Alosa fallax* | Twaite shad | Cheppia |
| | *Sprattus sprattus* | European sprat | Spratto |
| Cyprinidae | *Pseudorasbora parva* | Stone moroko | Pseudorasbora |
| Cyprinodontidae | *Aphanius fasciatus* | Mediterranean banded killifish | Nono |
| Congridae | *Conger conger* | European conger | Grongo |
| Engraulidae | *Engraulis encrasicolous* | European anchovy | Acciuga |
| Gasterosteidae | *Gasterosteus aculeatus* | Three-spined stickleback | Spinarello |
| Gobiidae | *Zosterisessor ophiocephalus* | Grass goby | Ghiozzo Gò |
| | *Pomatoschistus canestrinii* | Canestrini's goby | Ghiozzo cenerino |
| | *Knipowitschia panizzae* | Adriatic dwarf goby | Ghiozzo laguna |
| | *Gobius paganellus* | Rock goby | Ghiozzo paganello |
| Moronidae | *Dicentrarchus labrax* | European seabass | Spigola |
| Mugilidae | *Liza ramada* | Thinlip mullet | Muggine calamita |
| | *Liza aurata* | Golden grey mullet | Muggine dorato |
| | *Liza saliens* | Leaping mullet | Muggine musino |
| | *Mugil cephalus* | Flathead grey mullet | Muggine cefalo |
| | *Chelon labrosus* | Thicklip grey mullet | Muggine labbrone |
| Pleuronectidae | *Plathychtys flesus* | European flounder | Passera |
| Scophthalmidae | *Scophthalmus maximus* | Turbot | Rombo |
| | *Scophthalmus rhombus* | Brill | Soaso (rombo liscio) |
| Soleidae | *Solea solea* | Common sole | Sogliola |
| Sparidae | *Sparus aurata* | Gilt-head bream | Orata |
| Syngnathinae | *Sygnatus acus* | Greater pipefish | Pesce ago |
| | *Sygnathus abaster* | Black striped pipefish | Pesce ago di rio |
| Triglidae | *Chelidonichthys lucerna* | Tub gurnard | Capone/Galinella |

Table 5. Fish species present in Magnavacca and Fossa Porto lagoons

## Comacchio Salt-work

In the area of the Salina of Comacchio salt-work were sampled 8 species (Table 6).

| Famiglia | Nome scientifico | Nome comune inglese | Nome comune italiano |
|---|---|---|---|
| Anguillidae | *Anguilla anguilla* | European eel | Anguilla |
| Cyprinodontidae | *Aphanius fasciatus* | Mediterranean banded killifish | Nono |
| Gobiidae | *Pomatoschistus canestrinii* | Canestrini's goby | Ghiozzo cenerino |
| | *Knipowitschia panizzae* | Adriatic dwarf goby | Ghiozzo laguna |
| Mugilidae | *Liza ramada* | Thinlip mullet | Muggine calamita |
| | *Liza aurata* | Golden grey mullet | Muggine dorato |
| | *Liza saliens* | Leaping mullet | Muggine musino |
| Syngnathinae | *Sygnatus acus* | Greater pipefish | Pesce ago |

Table 6. Species of fish present in Comacchio salt-work.

Action 5: data analysis, final report and management guide lines


Based on fishing and monitoring data, covering the last decade and collected in this relationship, as well as on long term ell catches, and other evidences of ecological shifts occurred in the basin which took place from the second half of the seventies to the present, the following concluding ramarks are formulated. The most striking evidence is that the resource historically prevalent, the eel, at present it is very scarce while the anchovy that was completely absent in the lagoon before the eighties, and the goden shimp, once much less abundant, now are the dominant catches. This evidence opens up to the exploitation of new fisheries.

Another evidence of the results collected, important for the management, is the reduction of the overall fishing yields, especially if compared to those of the past. However, lower yields do not correspond to a real shortage of secondary production but are the resultant of a management that has not yet been updated to the changes occurred. In order to reverse the current trend, guidelines are summarized below:

a) the recruitment of the main target species, i.e. eel, is scarce and is due to global and not local factors. The causes are therefore independent by the management of the Comacchio lagoon as extensively debated in the article by Aschonitis et al., "Long-term records (1781–2013) of European eel (*Anguilla anguilla* L.) production in the Comacchio Lagoon (Italy): evaluation of local and global factors as well as causes of the population collapse", recently published in Aquatic Conservation: Marine and Freshwater Ecosystems in 2016. Actions to increase the number of juveniles available have already been undertaken as part of the Comacchio lagoons Management Plan and the first positive results have already been acheived. However, other actions can be forseen, like a better exploitation of the wild juveniles present in the neighboring basins, as Valle Fattibello, and / or by the restocking with juveniles from the Camargue;

b) need to develop and promote innovative fishing techniques dedicated to the exploitation of individual resources in different periods of the year. An experiment in this sense is still in progress to define a shrimp fishing technique that does not interfere with the eel development cycle; it is also needed to extend shimp fishing in the southern and western portions of the lagoon, presently unexploited;

d) neet to promote the local commercialization of anchovy and goldel shrimp that, unlike eel and grey shrimp, are available in conjunction with the peak of the touristic season, and represent a model of short supply chain; this would also increase the promotion of the Valli di Comacchio brand to a wider audience;

e) the need to define a new management model that solves the problems reported in the previous points, for which the current direct management by the Park is not fully effective. By fact, the park has different primary institutional goals, that are habitat conservation, species protection, and the promotion of cultural and historical heritage and not the quantitative enhancement of productions;

f) the need to carry out an overall reorganization of the fishing regulation, both for the public part of the lagoon (Magnavacca, Fossa di Porto and Campo, Fattibello and related canals) and for the basins of private property.

# References

- Lanzoni M., Aschonitis V., Milardi M., Fano E. A. and Castaldelli G. A method to identify bimodal weight-length relations: possible ontogenetic diet and/or metabolism shift effects in Anguilla
- anguilla (Actinopterygii: Anguilliformes: Anguillidae) (2018) Acta Ichthyologica et Piscatoria, 48 (2), pp. 163–171.
- Aschonitis, V., Castaldelli, G., Lanzoni, M., Rossi, R., Kennedy, C., Fano, E.A. Long-term records (1781–2013) of European eel (Anguilla anguilla L.) production in the Comacchio Lagoon (Italy): evaluation of local and global factors as causes of the population collapse (2017) Aquatic Conservation: Marine and Freshwater Ecosystems, 27 (2), pp. 502-520.
- Aschonitis, V.G., Castaldelli, G., Lanzoni, M., Merighi, M., Gelli, F., Giari, L., Rossi, R., Fano, E.A. A size-age model based on bootstrapping and Bayesian approaches to assess population dynamics of Anguilla anguilla L. in semi-closed lagoons (2017) Ecology of Freshwater Fish, 26 (2), pp. 217-232.
- Lanzoni, M., Gavioli, A., Aschonitis, V., Merighi, M., Fano, E.A., Castaldelli, G. Length–weight relationships of three estuarine species in the Comacchio Lagoon, Po River delta, Italy (2016)
- Journal of Applied Ichthyology, 32 (6), pp. 1284-1285. Dezfuli, B.S., Manera, M., Bosi, G., DePasquale, J.A., D'Amelio, S., Castaldelli, G., Giari, L. Anguilla anguilla intestinal immune response to natural infection with Contracaecum rudolphii A larvae (2016) Journal of fish diseases, 39 (10), pp. 1187-1200.
- Giari, L., Guerranti, C., Perra, G., Lanzoni, M., Fano, E.A., Castaldelli, G. Occurrence of perfluorooctanesulfonate and perfluorooctanoic acid and histopathology in eels from north Italian waters (2015) Chemosphere, 118 (1), pp. 117-123.
- Castaldelli, G., Aschonitis, V., Lanzoni, M., Gelli, F., Rossi, R., Fano, E.A. An update of the length-weight and length-age relationships of the European eel (Anguilla anguilla, Linnaeus 1758) in the Comacchio Lagoon, northeast Adriatic Sea, Italy (2014) Journal of Applied Ichthyology, 30 (3), pp. 558-559.
- Dezfuli, B.S., Giari, L., Castaldelli, G., Lanzoni, M., Rossi, R., Lorenzoni, M., Kennedy, C.R. Temporal and spatial changes in the composition and structure of helminth component communities in
- European eels Anguilla anguilla in an Adriatic coastal lagoon and some freshwaters in Italy (2014) Parasitology Research, 113 (1), pp. 113-120.