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Research Article

Natural recruitment contributes to high densities of grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) in Western Europe

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Abstract

Introductions of grass carp, well known for their potentially negative ecosystem effects, have been performed in several countries around the world. As the species was considered unable to reproduce naturally under non-native environmental conditions, little attention was initially given to its invasive potential. We studied an area in northern-Italy where, contrary to expectations, introductions that were performed in the early 80s still exert a considerable pressure on aquatic macrophytes. In order to reveal whether the observed population dynamics are the result of natural events or stocking we analysed the density, age- and size-structure of the grass carp population and the migration pathways available to it. Telephone surveys were also used to check for fish transport from national and international suppliers. We also sampled potential spawning and nursery areas for young individuals and, when some were captured, we applied stable isotope analyses to discriminate their origin. We found that the population of large individuals likely originated solely from early stocking. We also documented the first analytical evidence of grass carp recruitment in the study area and, to our knowledge, in Western Europe. Therefore the species has the potential to become invasive in these areas and more detailed studies are needed to assess this potential. Further management should account at least for natural recruitment and potential negative environmental effects, controlling the species where needed.

Key words: Italy; natural reproduction; spawning; stable isotopes

Introduction

It is often assumed that species introductions are a consequence of unintentional transport (Hulme 2009). However, this is not entirely true for fish, where a high number of introductions are the result of intentional fisheries management activities (García-Berthou et al. 2005). Grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) is an Asian fish species and prime example of such management, as it has been voluntarily introduced worldwide.

Native to eastern Asia (Berg 1964), grass carp has been stocked, as a means for biological weed control or with the purpose of establishing new fisheries for the species, in 139 countries worldwide (Welcomme 1988). Grass carp live in large rivers, lakes, and reservoirs with abundant vegetation and relatively shallow waters in sub-tropical and temperate climates (Cudmore and Mandrak 2004). Due to the restrictive spawning conditions required by this species (Shireman and Smith 1983), locations where the species could establish were believed to be limited. Indeed, introductions of grass carp in numerous countries worldwide were not actually successful, e.g. in Finland (Urho and Lehtonen 2008), and in several cases required continued stocking. Although unable to reproduce, such populations have still been shown to cause severe adverse consequences on the ecosystem, related to overgrazing of aquatic vegetation (e.g. Dibble and Kovalenko 2009). Natural recruitment has been detected in several countries: Mexico, Japan, the Philippines, Taiwan and the United States

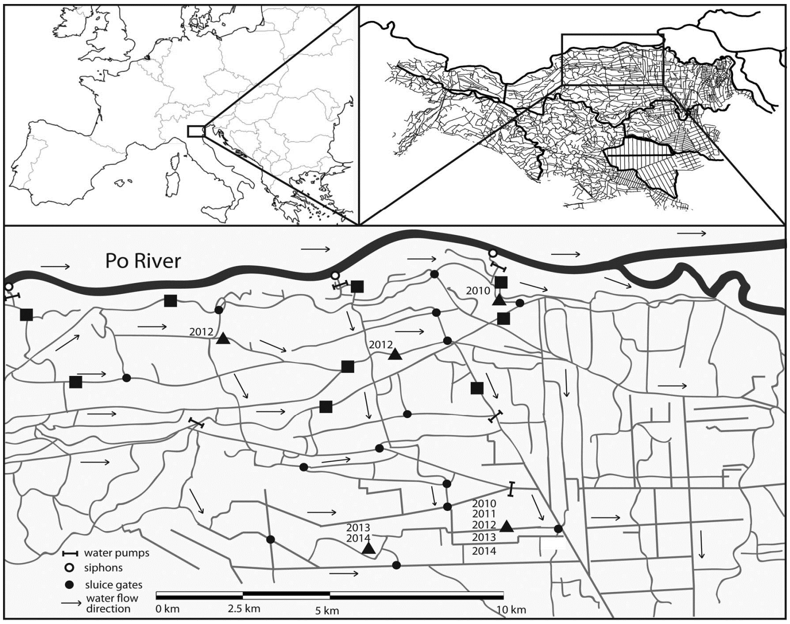


Figure 1. Map of the Po River (thick lines) and study area canal framework (thin lines). Arrows show the water flow direction and potential migration barriers are represented with different symbols summarized in the legend. The map also includes locations where young specimens (▲) and large specimens of grass carp (■) were sampled and caught during the study period (numbers next to markers indicate the sampling year for each small specimen location).

(e.g. Tsuchiya 1979; Chapman et al. 2013). Although introduced into all European countries (Welcomme 1988), natural recruitment of grass carp has been reported solely for eastern European countries (Kottelat and Freyhof 2007), in particular in the Danube River drainage basin (Opuszyński 1972), where it was introduced first, probably in historical times. However, introductions in western European countries were performed at later times, from the late 1950's onwards (Welcomme 1988), possibly delaying the onset of natural reproduction and its detection.

While fry and juveniles consume planktonic crustaceans, rotifers, and insect larvae, adults are almost completely vegetarian (Shireman and Smith 1983). Adult grass carp feed on a wide range of aquatic vegetation, and can consume 40–150% of their body mass per day depending on size and temperature (Cudmore and Mandrak 2004). Thus, a dense-enough population of grass carp can over-consume the aquatic vegetation, leading to a complete elimination (Allen and Wattendorf 1987; Bain 1993). At the end of the 1960's eutrophication became a major problem in Italian waters, mainly due to the increased nutrient load from agriculture. This was particularly true in the Po plain, where weed growth limited the flow in water canals used for agriculture (i.e. rice, sugar beet). Between 1975 and 1985, the Emilia-Romagna region ran the first experimental introductions of grass carp to biologically control weed growth (Melotti et al. 1989). As trials proved to be successful, 20300 kg of young grass carp (20–30 cm length) were stocked in 1987–1988 by the Consorzio di Bonifica (Water Management Authority) (Melotti et al. 1989). The effectiveness of grass carp as a weed-control mean was immediately evident, with a 50% reduction in mechanical weed removal efforts by the following year (Melotti et al. 1989). Stocking continued thereafter and other authorized introductions were carried out yearly until 2000.

Typically, grass carp management is done under the assumption that water management authorities are able to control their density through stocking and fishing regulations, as they were believed to be unable to recruit under the local environmental conditions (Melotti et al. 1987). After almost 15 years since the last introduction, populations of grass carp were found to be still relatively abundant in the area in 2009 (Castaldelli et al. 2013). This could be one of the causes of relatively low macrophyte abundance, as observed in several sites of the channel network (Lanzoni, personal observation). The low density of aquatic macrophytes cannot be explained by a change in environmental conditions (e.g. pollution) because the system has been rather stable over a long period of time (Castaldelli et al. 2013).

However, grass carp is targeted by illegal fishing, which is believed to have considerably reduced the biomass of all cyprinids in the area, especially in the last five years (Lanzoni, unpubl.

data). Moreover, natural mortality should have resulted in a decrease in grass carp foraging capacity, even accounting for the growth of surviving individuals. The continued low density of macrophytes in 2014 can thus hardly be attributed to the effects of a small population of grass carp, which by now should exert only a moderate grazing pressure. Moreover, in 2007 small specimens of grass carp were captured for the first time during fish surveys in the drought period, and by recreational anglers.

Thus, we hypothesized that grass carp densities are higher than previously believed, at least on a local scale. Higher densities could be due to either human-driven introductions and/or to natural movement through migration pathways within the canal network. We also hypothesized that young individuals are either the result of unauthorized introductions or of natural reproduction.

Materials and methods

Density, age and size-structure

The density of large-sized grass carp had been specifically monitored between 2005–2013 using seine nets in larger sections of canals of the Po plain drainage system, in the province of Ferrara (Northern Italy) (Figure 1). These sampling sites are characterized by large sections, moderate flow, relatively low aquatic vegetation abundance and the presence of riparian vegetation. All sampling was undertaken during autumn-winter, when the water level is at its lowest, using a seine net 2m in height with a 25m mouth and a knot-to-knot mesh size of 25mm. The cod-end was 3m long and a knot-to-knot mesh size of 15mm. In each canal, the fish community was surveyed by fixing a blocking net (25mm knot-to-knot mesh size) spanning the canal width and depth across one end and dragging the seine towards it.

Over the eight year monitoring period (2005–2013), a total of 138 large grass carp specimens were captured, counted and their weight, length and age were estimated through a subsample of 62 randomly selected specimens. Total fish biomass was estimated based on the subsample and the density was estimated taking into account the area swept by the trawl. Afterwards, the rest of the catch was released alive far from the sampling site, beyond unsurpassable migration barriers. The population size distribution was derived from all captured specimens. Age was determined through scale analysis, using a stereoscope. Year class distribution was then derived from all aged specimens.

Specific sampling for smaller individuals was performed from 2010 to 2014, using a seine net 1m in height with a 5m mouth and a knot-to-knot mesh size of 6mm. The area investigated was extended to include smaller waterways, where young individuals were thought to occur most likely. Netting was repeated typically over the course of 1–3 days, until at least some specimens were caught. All young specimens in this study were captured during such surveys, in rice irrigation canals, belonging to the same hydrological network (Figure 1). Such areas are characterized by smaller sections, lower flows and riparian and aquatic vegetation. Rice irrigation canals are separated and on a lower level than the rice fields themselves, water needs to be pumped from these canals to irrigate the rice fields. Specimen taxonomic determination was performed by morphological discrimination (Kottelat and Freyhof 2007).

A total of 67 small grass carp specimens were captured in October 2007 (n=6), March 2010 (n=5), September–October 2011 (n=17), October 2012 (n=16), September 2013 (n=8) and September 2014 (n=15).

Fish captured in October 2007 and March 2010 were photographed, measured and released on site. All fishes captured 2011–onwards were kept alive in a tank at the University of Ferrara and measured under MS-222 sedation or euthanized (fish from 2014). Scale samples were collected from each specimen for age determination.

Migration and introduction pathways

Presence and type of migration barriers were derived from maps and with the cooperation of the Water Management Authority. Opening of migration pathways and water flow direction were reported by the Water Management Authority and verified *in situ*.

A review of the regional archives was performed to check if stocking authorizations were issued for these species in the period 2001–2014. Moreover, in order to check for accidental aquaculture releases, a telephone survey was conducted in 2014 including all fish farms within a radius of 100 km from the sampling sites. Face-to-face interviews were performed for each fish farm after the first phone survey. A fish importer, the only one operating in the study area, was also involved in the study in 2014 through several face-to-face interviews to check for possible importation from other countries. These surveys also allowed for collection of information on grass carp aquaculture production in Italy.

Stable isotope analyses

In order to discriminate whether grass carp sampled in the wild originated from natural reproduction or unauthorized introduction, stable isotope analyses (SIA) were conducted on a subsample of grass carp individuals captured in the study area during autumn 2014 and on a batch of hatchery reared fish. Reared fish were imported from a farm in Hungary (close to the city of Sarvar), through the only available trade channel to Italy.

The scales of 30 grass carp individuals (Italy n = 15, Hungary n = 15) were analysed for carbon, nitrogen and hydrogen isotopes. As a pre-treatment for SIA, scales were soaked in deionized water and cleaned with non-lint paper tissue to remove tissue other than scale material (e.g. mucus, pigment, adhered paper) (Perga and Gerdeaux 2003; Torniainen et al. 2013). In order to separate early and late life phases, scales of older individuals captured in Italy (n = 5, aged 2+) were cut to separate inner growth rings (corresponding to 0–1 years) from outer growth rings (corresponding to 1–2+ years), which were then analysed separately. The outer growth region was determined using a microfilm reader and was cut using a scalpel against a glass edge to sample a minimum of 0.2 mg of scale material.

All scale material was prepared and analysed for stable isotopes at the University of Jyväskylä, Finland.To prevent error in stable isotope values arising from extraneous carbonates, scale material analysed for carbon and nitrogen isotope ratios were acidified for 2 min in 1.2 N HCl, rinsed five times in deionized water and dried overnight at 60°C (Perga and Gerdeaux 2003). SIA for carbon and nitrogen was performed using a Thermo Finnigan DELTA^{plus} Advantage continuos-flow stable isotope-ratio mass spectrometer (CF-SIRMS) coupled with a FlashEA 1112 elemental analyser (Thermo Electron Corporation, Waltham, MA, USA). Powdered pike (*Esox lucius* L.) white muscle tissue was used as a laboratory reference material for carbon and nitrogen. Scale material for hydrogen SIA was cleaned of oils in a 2:1 chloroform/methanol solvent rinse. Hydrogen stable isotope ratios were measured using Isoprime 100 CF-SIRMS (Isoprime UK), coupled with an Elementar Pyrocube elemental analyser (Elementar, Germany). Hydrogen SIA was conducted using the equilibration method described by Wassenaar and Hobson (2003). Two keratin laboratory reference materials (CBS, KHS) obtained from Environment Canada were used to normalize the results, using the previously assigned values (Wassenaar and Hobson 2010). Water hydrogen isotope analysis was also performed, at the Dating Lab of the University of Helsinki, Finland, on water samples collected in 2014 both at the young individuals sampling sites (Italy, n = 3) and at the farming site (Hungary, n = 3) to test for differences in water hydrogen isotope composition. The samples were first equilibrated with He+H_2 at 25°C for 24 hours in the presence of a platinum catalyst, then analysed using a ThermoQuest Finnigan Delta^{plus} XL mass-spectrometer coupled to a GasBench. Results are expressed using the standard δ notation ($\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$) as parts per thousand (‰) differences from the international standard. The reference materials used were International Atomic Energy Agency (IAEA) standards of known relation to the international standards of Vienna Pee Dee Belemnite (for carbon), atmospheric N_2 (for nitrogen) and Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale (for hydrogen). Precision for each run was always better than 0.13‰ for C, 0.17‰ for N and 3‰ for H based on the standard deviation of replicates of the laboratory reference materials.

Statistical analyses

Student's t-tests were performed to test differences between distributions of stable isotope ratios of Hungarian and Italian samples. Discriminant analysis was used to evaluate the isotopic differences of the two assumed origins (Hungary, Italy) of grass carp. A stepwise method was used with variables of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ (leave-one-out method for cross validation) (Efron 1983). The criterion for including a variable was Wilks'λ ($F \geq 3.84$). The stepwise approach minimizes the multicollinearity occurrence and between variable correlations; tolerances of the variables entered at each step were checked (included if tolerance > 0.4) for confirmation. All statistical analyses were performed using PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA).

Results

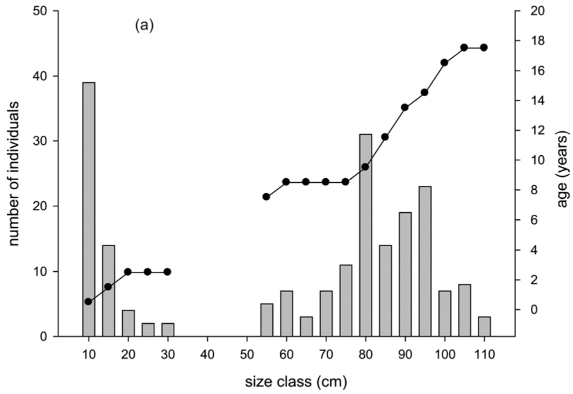
Density, age and size-structure

Grass carp densities varied greatly between different sampling points (8–107 kg/ha), but individuals were generally large.

On average, large specimens were 81.73 cm (±19.23 cm S.D.) and 10752.7 g (± 4288.6 g S.D.). Length and age distributions were relatively broad, spanning from 55 to 110 cm and 7+ to 17+ years without evident gaps (Figure 2a). Year classes spanned from 1988 through 2000 and no large specimens belonging to more recent year classes were found (Figure 2b).

Small-bodied specimens (mean length 20 cm, mean weight 180 g) were found during the surveys in late October 2007 at Canal Bianco, Canale Andio and Canale Seminiato, three relatively large canals of the network. Grass carp sampled in March 2010 were found in smaller canals and could have been 1+, given their size range (116–203 mm) and comparing them to other individuals that were aged in our study. Unfortunately, the capture of these individuals was initially overlooked and no scale samples were available to perform age analysis.

Fish caught in 2011 were all 0+ (size range 54–100 mm) whereas fish from 2012 (size range 81–255 mm) were 0+, 1+ and 2+. In 2013, only 0+ and 1+ fish were caught (size range 78–97 mm) (Figure 3). In 2014, young grass carp (size range 90–210 mm) belonged to 3 age groups (0+, 1+ and 2+). Overall, the year classes of 2011 and 2012 were the most represented, both containing 19 specimens each (Figure 2b). On average, samples of young grass carp spanned a rather large size interval (54–255 mm total length; 1.4–246.35 g body weight) (Figure 2a).



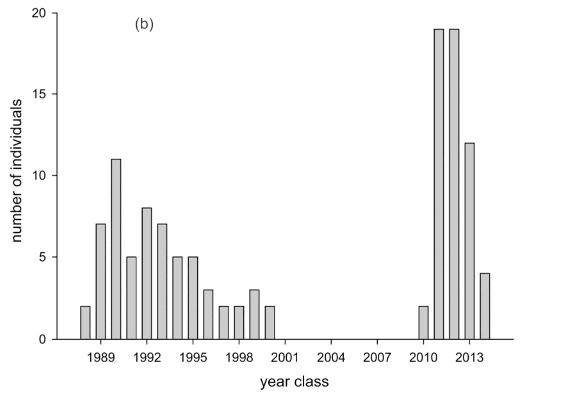


Figure 2. Size and average age distribution (a) and year class distribution (b) of all the grass carp specimens sampled and aged in this study. Grey bars represent size- and year-class frequencies and are scaled on the left vertical axis, while black line and dots represent average age distributions and are scaled on the right vertical axis.

Migration and introduction pathways

Four types of migration barriers exist between the main river and the sampling sites. From the main course of the Po River, siphons are open for 60-120 days a year between March and September (according to irrigation needs). While open, these allow passage of fish without significant restrictions. Water from the Po River moves into forebays, and pumping plants feed water into the main irrigation canals. Passage through these pumps is extremely unlikely for fish > 30 cm, because of the limited space between pumping blades. From the main irrigation canals water is distributed through a network of canals of progressively decreasing cross-section where it flows with gravity alone. Flow is regulated through sluice gates, which are typically open year round and do not limit fish passage. Within the secondary canal network there are 3 pumping stations, which are activated on an as needed basis and form a very effective barrier to fish migration, given the small space between pump blades (< 10 cm).

No authorization was given to stock grass carp in the period 2001–2014. No hatchery spawning of grass carp is carried out in Italy. A total of three fish farms fit the criteria of relevance and all were therefore contacted during this study, until a response was given. Fish farms and private ponds in the area are occasionally supplied with smaller size grass carp (minimum 200–300 g) that are grown and sold for both human

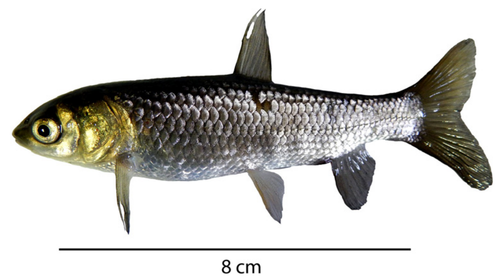


Figure 3. One of the young-of-the-year specimens of grass carp, captured in 2013. Photograph by Mattia Lanzoni.

consumption and for stocking in private ponds. Fish farmers reported that no accidental escapes have occurred during 2007–2014. Grass carp are supplied by only one fish importer for the whole area. Only one trade channel has been open in the period 2007–2014 to import young individuals of grass carp to the study area: a fish farm site in Hungary. Grass carp of sizes similar to those sampled in the wild (around 15 cm) were rarely imported in the past from east-Europe by the fish importer, and always from the same source. According to the importer, the last trade of this kind was performed in 2011, but its destination was not disclosed.

Stable isotope analysis

Carbon stable isotope ratios of Hungarian carp were densely grouped (mean $\delta^{13}C$ -21.77‰ ± 0.98 S.D.) as were nitrogen stable isotope ratios (mean $\delta^{15}N$ 8.80‰ ± 0.36 S.D.). In Italian fish, $\delta^{13}C$ and $\delta^{15}N$ ratios were more variable (mean $\delta^{13}C$ -22.75‰ ± 4.30 S.D., mean $\delta^{15}N$ 8.81‰ ± 1.09 S.D.) but still close to values from Hungarian fish (Figure 4). No significant differences were found between Hungarian and Italian $\delta^{13}C$ or $\delta^{15}N$ ratios (t-test: P-value > 0.1).

Measurements on inner (mean $\delta^{13}C$ -19.73‰ ± 0.43 S.D, mean $\delta^{15}N$ 8.31‰ ± 0.25 S.D, mean $\delta^{2}H$ –133.66‰ ± 1.8 S.D.) and outer parts (mean $\delta^{13}C$ -18.74‰ ± 0.74 S.D, mean $\delta^{15}N$ 9.04‰ ± 0.24 S.D, mean $\delta^{2}H$ -139.76‰ ± 2.09 S.D.) of the scale for 2+ specimens captured in Italy yielded similar values to other age classes sampled in the same locations.

An average difference of 20‰ was found between hydrogen isotope ratios of water samples from Hungary (mean $\delta^{2}H$ -39‰ ± 1.45 S.D.) and from Italy (mean $\delta^{2}H$ -59‰ ± 1.05 S.D.). Similarly, scale samples from the Hungarian hatchery (mean $\delta^{2}H$ -66.62‰ ± 6.08 S.D.) and from the Italian fish caught in the wild (mean $\delta^{2}H$ -123.72‰ ± 15.1 S.D.) had an average difference of 57‰ in $\delta^{2}H$ ratios (Figure 5).

Water samples (t-test: P-value < 0.005) and fish samples (t-test: P-value < 0.005) showed a significant difference in $\delta^{2}H$ signatures between Italian and Hungarian sites. The discriminant analysis excluded $\delta^{13}C$ and $\delta^{15}N$, with tolerances <0.4. However, $\delta^{2}H$ results alone indicated there was 100% site discrimination for each individual fish. In other words, the results of discriminant analysis strongly suggested that all fish captured in Italy were not of hatchery origin but were rather born and grown *in situ*.

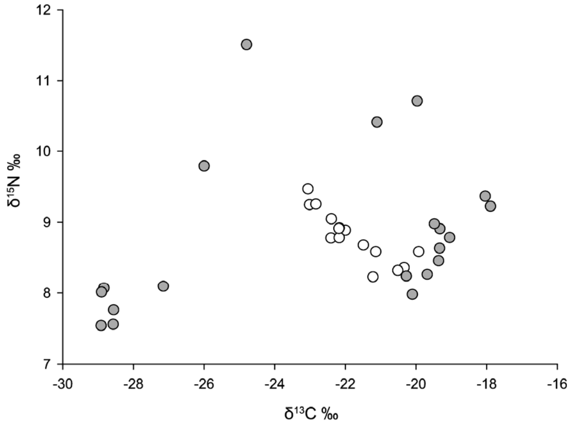


Figure 4. Carbon and nitrogen stable isotopes ratios of fish scale samples showing the distribution of values for individuals with different origins. White circles represent isotopic ratios of Hungarian individuals and grey circles represent isotopic ratios of Italian individuals.

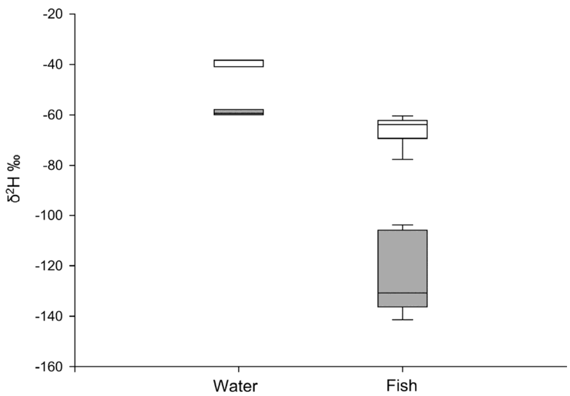


Figure 5. Hydrogen stable isotopes ratios of water and grass carp fish scale samples showing the separation between waters and individuals of different origin. Mid-line: median, outer edges: 50%, whiskers: 95%. White plots represent isotopic ratios of Hungarian water and grass carp individuals whereas grey plots represent isotopic ratios of Italian water and grass carp individuals.

Discussion

All examined evidence strongly suggested that no stocking has occurred in recent years and that natural recruitment of grass carp is currently occurring in the study area.

Size and age distributions of the sampled specimens suggested that the population was mainly composed of relatively old individuals. The size frequency of large individuals showed a distribution compatible with a population resulting from previous stocking. Year classes of large individuals were all connected to legal stockings performed between 1988 and 2000 and no fish were either born or stocked between 2000 and 2007. We cannot fully exclude illegal stocking of already large sized fish, but this is not very likely as it is not an economically viable option.

Densities varied greatly, and without an apparent pattern, between sampling locations and sampling events. This could be due to fish movement, but no information is currently available on grass carp movement in this system. Within the canal network, migration pathways are practically always open for large fish to move and reach any of the sampling locations. However, no fish of large size can reach the canal network from the Po River, due to impassable migration barriers. Thus, high concentrations of large grass carp could only be explained by immigration of fish from other areas within the canal network and are most likely the results of natural distribution patterns within a semi-artificial area. However, the possibility still exists that some individuals of large size might originate from older (i.e. before 2007 and most likely prior to 2001) accidental escapes from fish farms in the area or that the escapes have been misreported. Locally high densities are equal, or higher, than densities used in the first experimental trials of the mid 1980s, therefore suggesting that, at current densities, grass carp might be still be able to overgraze aquatic macrophytes, at least locally.

Small grass carp specimens sampled in 2007 were initially thought to be the result of illegal introductions, but the continuity of catches (2010–2014) seems to discredit such a hypothesis. The financial and logistic resources needed to import and introduce illegally large numbers of grass carp, without being discovered, would be beyond the capabilities of most entities. As no grass carp of similar size are farmed in the area, it is very unlikely that the small fish sampled in the wild originate from accidental or intentional releases, as they are not farmed or used as baitfish. Although we cannot completely exclude the possibility of an accidental, or even illegal, introduction of grass carp in 2011, young-of-the-year specimens were caught also in later years. Moreover, in 2008, adult specimens of grass carp were found to be mature (Grandi et al. 2014), lending credit to the hypothesis that natural reproduction can at least potentially occur.

Young specimens were found in rice-field irrigation canals, which are natural places of occurrence for early life stages in the native range (Kottelat and Freyhof 2007; Shireman and Smith 1983). Because young grass carp are similar in appearance to other elongated cyprinids, it might be possible that their presence is overlooked during surveys that process a large number of fish of many species. Similarly, recreational fishermen might fail to identify a fish species when in uncommonly small sizes (for grass carp). Thus, it is possible that grass carp recruitment has already occurred in other areas of west-Europe as well, but has been under-detected.

Age-at-maturity of grass carp in the native range is 2–4 years (e.g. Li et al. 1990), but fish can mature at older ages in colder climates (e.g. in the Danube River 6–7 years, Opuszyński 1972). Because most large specimens captured in this study were older than those minimum ages, we assume that factors other than age/size might play a role on the actual spawning dynamics of this species in the study area. Temperature, length of the waterways and water flow appear to be more important factors and, in the study area (an artificial irrigation canal system), the man-made water regime probably mimics natural spawning conditions.

For example, Stanley et al. (1978) have estimated a minimum length of the river that grass carp need for spawning as 50–180 km, based on water flow. However, Chapman et al. (2013) found that grass carp recruited successfully in only 26 km of river. Furthermore, Murphy and Jackson (2013) and Garcia et al. (2013) successfully predicted that Asian carp could recruit in as little as 14 km of river. A system of siphons, pumps and sluice gates such as in the study area could create localized discharge conditions ideal to trigger spawning, while maintaining low water velocity in subsidiary canals, thus reducing the continuous river length actually needed for egg and larval development.

In the study area, spawning might occur between the end of May and early July, according to the model developed by Kocovsky et al. (2012) and temperatures recorded at permanent monitoring stations within the canal network (Lanzoni, unpubl.). This would be supported by the growth observed in the fall, the temperature regime in the canal network, the productivity of the canals and the timing of hydraulic regime shifts in agriculture. However, when the siphons are open, migration pathways exist for larvae or young specimens up to 10 cm to reach any place within the canal network. Therefore it cannot be excluded that some specimens could be the result of transport/migration from other areas of the canal network or even upstream reaches of the main river.

Grass carps are covered with cycloid scales mainly composed of proteins (collagen and ichthylepidin) and some minerals like magnesium carbonate and calcium carbonate (Hutchinson and Trueman 2006; Ehrlich 2010). Ideally, $\delta^{13}C$ and $\delta^{15}N$ in animal tissues reflects dietary traits (e.g. Fry 2008; Layman et al. 2012) over a variable span of time depending on the tissue analysed (Dalerum and Angerbjörn 2005). Aquaculture rearing typically utilizes formulated industrial feeds based on marine fish oil which can be traced by stable isotopes (Dempson and Power 2004; Schröder and de Leaniz 2011). However, in our study grass carp scales $\delta^{13}C$ and $\delta^{15}N$ ratios could not discriminate between sites of origin. This might be due to the type of farming typically used to rear grass carp (extensive, rather than intensive), which uses semi-natural feed and rearing conditions. The densely distributed $\delta^{13}C$ and $\delta^{15}N$ values from the Hungarian specimens could be attributed to a relatively homogenous diet available in aquaculture conditions, which is reflected in uniform stable isotopes values of different age classes (1+ and 2+). The $\delta^{13}C$ and $\delta^{15}N$ values of Italian specimens were more dispersed, even within the same age classes (0+, 1+, 2+), suggesting a wider dietary niche, compatible with conditions in the wild, where more food sources would be available. Furthermore, age classes of Italian fish differed in $\delta^{13}C$ and $\delta^{15}N$, possibly indicating ontogenetic dietary shifts during the early growth stages in the wild.

It is known that fish stable isotope composition also reflects that of their environment (Whitledge et al. 2006). Hydrogen isotopes have been previously used to discriminate geographical origins of various animals (e.g. Hobson 1999; Hobson et al. 2004, Cerling et al. 2006) or as a conservation forensics tool (Bowen et al. 2005). Fish scale δ^2H is partly influenced by diet (Birchall et al. 2005), but the ambient water contribution to fish tissue δ^2H has been estimated to be up to 50% (Soto et al. 2013). Especially on a large geographical scale, differences in tissue δ^2H are largely driven by the differences in the ambient water (Hobson et al. 1999). The observed δ^2H values were sufficient different to clearly separate Italian and Hungarian waters, and to separate Italian from Hungarian fish. This observation offers strong evidence that small-sized fish caught from Italy are of natural origin instead of being hatched and transported from the Hungarian farm. Despite the fact that the hydrogen fractionation between water and fish scales is currently unknown, the distinct values of water and fish scales still offer convincing support for the hypothesis that Italian fish were born *in situ*. Furthermore, through the analysis of inner and outer rings in the scale, it was also possible to exclude that 2+ fish were the result of past stockings, because no Hungarian water hydrogen signal was detected in the inner scale.

In general, the study area has low habitat heterogeneity and nutrient rich waters which lead to algal blooms and oxygen depleted conditions (Castaldelli et al. 2013). These factors have been hypothesized to be among the causes responsible for the observed decline of other fish species (Rose 1972; Bain 1993) which has also occurred in the study area (Castaldelli et al. 2013). The presence of young year-classes of grass carp could further increase nutrient cycling and habitat modification, because young individuals feed at higher rates than adults (Vietmeyer 1976) and natural recruitment could ultimately lead to a self-sustaining population with higher numbers of individuals and total biomass.

While fishing of grass carp was initially restricted after introduction, currently no restriction or other management actions are in place for this species (Castaldelli et al. 2011). Anglers focus on large sized grass carp, but mostly apply catch and release techniques (Lanzoni, pers. observation). Recently, the study area has been under illegal fishing pressure. The extent of such pressure is not precisely known, but is assessed to be high (Lanzoni, pers. observation). Illegal fishing targets grass carps among other cyprinid species, using gear with low species- or size-selectivity (e.g. nets or electric stunning).

Currently, the potential for grass carp to become an invasive species in the study area or in Western Europe is unknown. It also is unknown how often conditions in the study area will support recruitment or whether conditions for recruitment might be present in other areas or not. In any case, the management of this species should take into account the possibility of natural recruitment and thus of establishment. The potential for natural recruitment should be accounted for, in particular, when new introductions of these species are proposed and stocking should be allowed only with sterile individuals. Furthermore, surveys are needed to verify if natural recruitment occurs in other areas of Western Europe as well.

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