Structure of past and present food webs from a semiarid wetland subjected to species invasion and environmental degradation

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Abstract words: 259

Main text words: 5599

Figures: 6

Tables: 2

References: 115

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Abstract

Species invasion and habitat degradation very often result in local species loss, which may result in a cascade of secondary extinctions that typically end up disrupting whole ecological netwroks. Herein, we used historical records and the natural abundance of stable isotopes (^{13}C and ^{15}N) of primary producers, aquatic animals and sediment/detritus to derive the past and present structure of food webs from the freshwater wetland “Las Tablas de Daimiel”, in central Spain. Before the green revolution and agricultural transformation of the area, this wetland was characterised by a high biodiversity of basal species, including primary consumers such as bivalves and gastropods, which are currently absent or very scarce. Our results demonstrate that the increase of anthropogenic disturbances, exotic species and changes in primary productivity of this wetland is affecting the biodiversity at all trophic levels (mainly herbivorous fish) but not the length of the food chain, which we estimated between 3.9 and 4.4 trophic levels. Using the mixing models, we showed that macrophytes represent an important contribution of matter and energy to higher trophic levels. Our model also suggested that a currently expanding, allochthonous halophytic tree (*Tamarix canariensis*) may be the main energy source for two species of commonly found butterflies (*Pieris rapae* and *Rhodometra sacraria*) as well as for invertebrates, while the macrophyte *Thypa dominguensis* was the main diet source for the exotic crayfish *Procambarus clarkii*, which occupies the niche left by the native crayfish. Our work demonstrates the importance of taking a whole-systems approach to characterize the magnitude of human impacts on the functioning of wetland ecosystems.

Keywords: Tablas de Daimiel; Food web; SISUS; *Procambarus clarkii*; *Tamarix canariensis;* Species invasion; Habitat degradation

Introduction

Species invasion and habitat degradation of wetlands, including water eutrophication, often cause an initial loss of local species that can result in a cascade of secondary extinctions, thus generating considerable challenges to conservation efforts (Sahasrabudhe and Motter, 2011). Although it is well established that nutrient enrichment and species invasion typicallly decrease diversity within and across trophic levels (Guy et al., 2012; Post, 2002; Sahasrabudhe and Motter, 2011; Schindler and Scheuerell, 2002; Vander Zanden et al., 1999), only a few studies have sought to assemble complete food webs in ecosystems that have historically been simultaneously subjected to multiple anthropogenic stressors and where both alien and native species currently coexist (Costanzo et al., 2005; Gartner et al., 2002; Spies et al., 1989). Studies related to the impacts of allochthonous species in wetlands usually report dramatic effects on the size, structure and species diversity of food webs, very often causing a loss of connectivity between native species (Demopoulos et al., 2007; Ings et al., 2009; Nilsson et al., 2012; Rennie et al., 2009; Woodward et al., 2008). However, other studies report no alteration in the length of the food chain under disturbance (Marks et al., 2000; McHugh et al., 2010; Pimm and Kitching, 1987; Walters and Post, 2008). In this context, natural abundance of stable isotopes from current living and non-living samples, coupled with information obtained from historical records, can be used to track changes in the structure of the food webs of ecosystems exposed to the simultaneous effects of eutrophication and invasion by allochthonous species (Brauns et al., 2011).

The National Park Tablas de Daimiel (NPTD) is one of the few relict floodplain wetland of the Iberian Peninsula. However, it has been subjected to numerous environmental pressures during the last 50 years, including drainage, sewerage, flooded area reduction, pollution (mainly organic) and the introduction of exotic species, many of them introduced on purpose for commercial reasons (Castaño et al., 2018). Although its biodiversity and the potential causes of its reduction in the NPTD have been the subject of numerous studies (Alvarez-Cobelas et al., 2001; Feilhauer et al., 2018), there is no previous study about the structure of food webs and the effects of environmental degradation, including those derived from exotic species and eutrophication, on this emblematic wetland. However, based on historical information, it has been suggested that fifty years ago the wetland must have had a very complex trophic structure that included, besides the European crayfish *Austropotamobius pallipes,* up to ten endemic species of fish, all disappeared today due to widespread degradation of the wetland (Alonso et al., 2000). Therefore, stablishing how the structure of the food web and the links within and between trophic levels have changed over time is key to the develpment of adequate conservation, mitigation and, eventually, restoration strategies.

There are many matter and energy sources that can potentially contribute to different consumers in the food web. The use of natural abundances of the stable isotopes ^{13}C and ^{15}N in organisms of a given ecosystem is useful to establish the range of possible contributions of each source (isotope mixing; (Galván et al., 2011). Since food webs are overly complex to solve due to multiple dietary sources (Layman et al., 2007), different mixing models have been designed for situations in which *n* isotopes can be used to determine the range of contribution of a mixture of *n* + 1 sources (Phillips and Gregg, 2003). These models are limited by isotopic mass balances (Phillips, 2001) and the absence of a unique solution for each case (Benstead et al., 2006). However, Bayesian mixing models have allowed the incorporation of $\delta^{13}C$- $\delta^{15}N$ signatures of multiple diets to generate potential solutions through probability distributions (Holtgrieve et al., 2010; Solomon et al., 2011). For example, SISUS (Stable Isotope Sourcing Using Sampling; Erhardt *et al.* 2014) is a mixing model based on Bayesian statistics that has been developed to estimate the proportional contributions from different sources to a mixture using stable isotope data (Erhardt and Bedrick, 2013), allowing many exact solutions, unlike other probabilistic models such as the deterministic mixture IsoSource (Phillips and Gregg, 2003), which yields only approximations.

In this study, we aimed to determine the past and present configuration of the food web in Las Tablas de Daimiel. For this, we used historical sources and stable isotopes (^{13}C and ^{15}N) of the main consumer species that are currently present in the ecosystem. We also assessed the most important structural and functional changes, including the trophic effects of introduced exotic species.

Material and methods

2.1. Study site

The study was conducted at the NPTD (39 ° 08'N, 3 ° 43'W), in the semiarid portion of central Spain. The representative species of the local flora restricted to coastal areas are emerging aquatic macrophytes such as the European sedge (*Cladium mariscus*), reed (*Phragmites australis*) and cattail (*Typha dominguensis*) (Alvarez-Cobelas and Cirujano, 1996), along with other species such as *Scirpus maritimus* and *Potamogeton pectinatus,* as well as submerged macrophytes such as charophytes (*Chara* sp.). In addition to the common carp (*Cyprinus carpio*), the local wildlife is represented exclusively by exotic species such as the American crayfish (*Procambarus clarkii*), bluegill (*Lepomis gibbosus*), mosquitofish (*Gambusia holbrookii*) and catfish (*Ameirus melas*).

2.2. Sampling

To examine the structure and composition of the food web of NPTD, we focused on the dominant taxa from all trophic levels. We considered all potential sources of energy in the wetland, including detritus/sediment, dissolved organic matter (DOC), particulate organic matter (POC), and primary producers (phytoplankton, epiphyton, filamentous algae, seaweed, aquatic and terrestrial vegetation). We also considered microbial mats (i.e., benthic communities composed of different layers of photosynthetic prokaryotes and eukaryotes, such as filamentous and unicellular cyanobacteria and diatoms). The microbial mats were included due to their key role in primary production and decomposition of organic matter (Goldsborough and Robinson, 1996). Benthic macroinvertebrates and zooplankton were considered as primary consumers, whereas crabs, fish and waterfowl were considered higher level consumers. Winged insects were also included to assess their contribution and importance in the food web. In the NPTD, no bivalve mollusks have been present since the mid-1970 (there are remains of shells of freshwater mussel *Unio* sp.), and during this study there were none observed in the substrate.

2.2.1. Collection of consumer organisms

The collection and preparation of samples of sediment/detritus, primary producers, zooplankton and benthic macroinvertebrates was performed as follows: different species of winged adult insects were collected in the vegetation, air (<2 m above the ground surface) and nests in twelve random points along the wetland during June 2011. The sampled insects were identified to genus level and, when possible, to species level. The collection of secondary consumers was conducted using different techniques. Fish and crabs were caught using gillnets (light path of 8 cm) and pots (1.5, 2.5, 4, 7 and 10 cm mouth opening) at five and ten locations in the wetland, respectively. Smaller fish (*G. holbrookii* and catfish) were caught using landing nets in wetland areas where they were present (Morenillo and Tablazo, mainly). For isotopic analysis, 5-10 specimens of each species from each sampling site were frozen until further preparation.

Samples of waterfowl were provided by the Toxicology Wildlife Research Institute of Hunting Resources (IREC-CSIC) and were collected post-mortem in the wetland and its surroundings during the spring and summer of 2011. Muscle tissue from the specimens were taken, packaged and frozen in liquid N immediately after collection in the field. Waterfowl were represented by stilt (*Himantopus himantopus*), black-headed gull (*Larus ridibundus*), yellow gull (*Larus* sp.), coot (*Fulica atra*), white-headed duck (*Oxyura leucocephala*), shoveler (*Anas clypeata*), mallard frieze (*Anas strepera*) and cattle egret (*Bubulcus ibis*). Since the specimens were exclusively collected post-mortem, we could not obtain samples from all species of waterfowl present in the wetland.

Filamentous algae were represented mainly by *Cladophora glomerata*, S*pirogyra* sp. and *Vaucheria dichotoma* (Aboal et al., 1996); charophytes consisted of the species *Chara hispida* var. *major*; microbial mats were mostly formed by the cyanobacteria *Schizothrix penicillata* (Barón, 2011); the epiphyton was represented mainly by *Pseudonabaena catenata, P. tenuis, Fragilaria acus, Nitzschia cf. capitelata* and *Navicula venta* and phytoplankton species *Cyclotella meneghiniana*, *Cryptomonas erosa*, *Rhodomonas bill* and *Monoraphidium griffithii* (Rojo and Rodrigo, 2010). The vegetation was represented by two species of vascular submerged macrophytes (*Potamogeton pectinatus* and *Ceratophyllum submersum*), four species of emergent macrophytes (*Cladium mariscus, Phragmites australis, Typha dominguensis* and *Scirpus maritimus*) and *Tamarix canariensis* as the dominant tree species.

Primary consumers consisted of zooplankton (rotifers and ciliates, mainly; Rojo & Rodrigo 2010), benthic macroinvertebrates (one Odonata [*Crocothemis erytrhaea*] larvae), one Heteroptera [*Naucoris maculatus* larvae] and one Diptera [*Chironomus* sp.] larvae), seven species of winged insects, including Lepidoptera (*Leptotes pirithous, Pieris rapae, Polyommatus icarus, Maniola jurtina, Pontia daplidice, Colias crocea, Rhodometra sacraria*), two species of Odonata (*Lestes* sp. and *Sympetrum* sp.), one crustacean decapod (*Procambarus clarkii*) and four species of fish (*Ameirus melas, Cyprinus carpio,* pumpkinseed and eastern mosquitofish). In some areas of the NPTD, we were also able to capture both juvenils and adults of *A. melas*. To perform isotopic analysis, *G. holbrookii* individuals were separated by size (1.5-3 mm, 3.1-4 mm and> 4.5 mm). Individuals of *P. clarkii* were separated between females and males. At each sampling location individuals of each species of fish and crabs were measured and weighed (Table 1).

2.3 Sample preparation and analysis

Prior to isotopic analysis, all samples were treated following the procedures cited in Lewis *et al.* (2001), Parkyn *et al.* (2001) and (Oreilly et al., 2002). We removed insect heads and extremities (Gratton and Denno, 2006; Gratton and E Forbes, 2006) Whenever there was enough biomass available, a composite sample was prepared combining 3-7 individuals of the same species for each capture site. Since the main limitation of the analysis was obtaining enough material for isotopic analysis (0.5 to 1 mg dry mass), those less abundant insect species whose presence was sporadic at any given sampling site had to be analysed together with a sample composed of several individuals, combining sites that were closer to one another. In samples of fish and crabs, muscle tissue was extracted by removing the viscera and digestive tract. Muscle tissue lipids were removed from all samples with a solution of chloroform: methanol (2: 1), following the procedures described in Hobson and Welch (1992), Murry et al., (2006) and Logan & Lutcavage (2008). Subsequently, all samples were washed with distilled water and oven-dried at 60 °C (insects) and 80 °C (crabs, fish and birds) for 24 to 48 hours. Once dried, samples were crushed with a grinder, pulverized in an agate mortar and finally sieved through a 1-mm mesh. Finally, all samples were stored in sealed plastic containers (ependorf type) for shipment to the stable isotope laboratory.

2.4 Stable isotope analysis

The analysis of stable isotopes ^{13}C and ^{15}N were carried out at the Environmental Isotope Laboratory of the University of Arizona, United States (http://www.geo.arizona.edu/node/153) using a continuous flow isotope ratio mass spectrometer (Delta Finnigan PlusXL) coupled to an elemental analyzer (Costech). Two standards, Peedee belemnite and atmospheric N_2, were used for C and N, respectively. The results are presented as parts per thousand (‰). Units used to express the ratios of stable isotopes in the notation (δ), a common form of isotopic measurement (Ben-David and Flaherty, 2012):

$\delta^{13}C$ or $\delta^{15}N$ (‰) = $[(R_{sample} / R_{standar}) - 1] \times 10^3$

Where $R = {}^{13}C / {}^{12}C$ and $^{15}N / {}^{14}N$. Analytical accuracy was greater than ± 0.2 ‰.

2.5 Determination of trophic levels of consumers in Daimiel

We used the equation developed by (Vander Zanden et al., 1997), and subsequently modified by Post (2002), to estimate the trophic levels of each of the organism: trophic level (TL) $TL = \lambda + (\delta^{15}N_C - \delta^{15}N_{base}) / \Delta_n$. Where λ ($\lambda = 1$ or 2) is the basis of the trophic level, whether primary producers or primary consumers, respectively. In this study the trophic levels with both λ (1 and 2) were determined to check whether there were differences. For the baseline $\lambda = 2$ (primary consumers), the average value of the $\delta^{15}N$ signature of zooplankton was used since bivalve mollusks were absent in this wetland. The use of primary consumers as a baseline ($\lambda = 2$) reduces the error in the estimation of trophic levels (Wolf et al., 2009). $\delta^{15}N$ represents the isotopic composition of the N consumers, and $\delta^{15}N_{base}$ is the isotopic composition of the base used. Finally, Δ_n is the trophic level enrichment, which was set at 2.54 as suggested in the meta-analysis by (Vanderklift and Ponsard, 2003).

2.6. Data analysis of present-day and past food webs

To explore the functional relationships between predators and potential prey, first, the eating habits of each species was investigated through literature search and, subsequently, using the Bayesian statistical model SISUS (vO.09 workbook template, (Erhardt et al., 2014) in R. This model uses $\delta^{13}C$ and $\delta^{15}N$ signatures to estimate the proportional contribution of each source to each consumer. To apply the SISUS model, and prior to resolving each consumer's diet, increases were made in $\delta^{13}C$ values and potential sources $\delta^{15}N$ according to the values suggested by previous studies ($\delta^{13}C$: 0-1 ‰ and $\delta^{15}N$: 2.54, 3.4 and 4 ‰; Minagawa & Wada, 1984; McCutchan Jr *et al.* 2003; Post 2002; Vanderklift & Ponsard 2003). Finally, we chose a value between 0 and 1 ‰ for $\delta^{13}C$ and 3 ‰ for $\delta^{15}N$ because we found that the average increase in $\delta^{15}N$ with increasing trophic level was around 3 ‰. In addition, this combination showed a greater number of relationships between consumers and sources studied in the ecosystem.

We complemented the isotopic analyses with literature search and records containing historical information that would allow us to reconstruct the foodweb of the wetland around 1950 (Alvarez-Cobelas and Cirujano, 1996). However, due to the lack of data and samples from that time, this part of the study was necessarily categorical rather than quantitative.

Results

3.1 Isotopic composition of the primary and secondary consumers of the wetland

^{13}C and ^{15}N signatures and ratios of C and N of all components of the NPTD are shown in Fig. 1. None of the species differed in terms of ^{13}C and ^{15}N depending on the sampling location, except in the case of *A. melas* and *P. clarkii* (Figs. 2 and 3). Catfish showed a significantly different isotopic signature depending on their size, stage of development and weight

(Fig. 2a and 2b). The crayfish also showed a different ^{15}N signature depending on its size but showed no difference regarding sex (Fig.3).

3.2 Food chain length and trophic levels

The food chain length in the NPTD was estimated at 3.9 trophic levels, where primary producers are considered as the base, and at 4.4 trophic levels (Fig. 4a and 4b), when the primary consumers are used as the base. Food chain length description, hereinafter, is based on the results obtained with primary consumers as the chain base.

Invertebrates, zooplankton, three species of birds (*B. ibis*, *Larus* sp. and *L. ridibundus*) and the most abundant winged insects (butterflies) and the damselfly (*Sympetrum* sp.), were placed in the primary consumers level. Secondary consumers were represented by two species of birds (*H. himantopus* and *F. atra*) and the winged insect *Lestes* sp. The fish species *C. carpio*, *G. holbrooki*, three species of birds (*Anas clypeata*, *A. strepera,* and *Aythya ferina*) and *P. clarkii* were integrated into the tertiary consumers group. Finally, *A. melas*, *L. gibbosus* and the white-headed duck (O. *leucocephala*) were at the top of the food web.

3.3 Organic source's contribution to consumers of the NPTD

The estimates of the SISUS model using two stable isotopes ^{13}C and ^{15}N-consumers are shown in Table 2 and Fig. 5. Concerning waterfowl, the diets of black-headed gull and stilt showed connection with microbial mats, perhaps a reflection of their opportunistic feeding behaviour in the mud. The laughing gull also revealed dependence on benthic macro invertebrates and sediment in their diet. The American crab and invertebrates were the main diet source of the shoveler. The diet of the coot reflected a varied eating habit with a similar distribution between zooplankton, macrophytes and crayfish. The main food source of Odonata class, represented by *Lestes* sp. And *Sympetrum* sp., was invertebrates (41 to 60.47% and from 23.05 to 60.25%, respectively).

Macrophytes were, in most cases, a primary or secondary energy source for organisms of higher trophic levels. For example, *P. clarkii* (trophic level = 3.2) based its diet intake on emergent macrophytes, representing up to 81.98% and 76.91% of its diet (*T. dominguensis* and *S. maritimus*, respectively). Another clear example is the secondary consumers *H. himantopus* and *F. atra* (trophic level= 2.0 and 2.9, respectively), which can base a significant percentage of their diet on submerged macrophytes. The isotopic signatures of *Tamarix canariensis* indicated that it was one of the main food sources for winged such as insects *Rhodometra sacraria* and *Pieris rapae*, and also for the cattle egret. The mixing model indicated that the contribution of macrophytes to the diet of the fish species collected is negligible.

3.4 Past and present foodweb in NPTD

In the mid-twentieth century, up to twelve native fish species in the wetland coexisted with carp and mosquito fish, including sea lamprey (*Petromyzon marinus*), eel (*Anguilla anguilla*), common catfish, comiza and cabecicorto (*Barbus bocagei*, *B. comiza*, *B. microcephalus*, respectively), boga (*Condrostoma willkommii polylepis*), chub (*Leuciscus pyrenaicus*) pardila (*Rutilus lemmingii*), tench (*Tinca tinca*), calandino (*Tropidophoxinellus alburnoides*), loach (*Cobitis malaria*) and monk (*Blennius fluviatilis*) (Elvira and Barrachina, 1996), along with other species of gastropods (*Lymnaea peregra, Pisidium* sp., *Bithynia tentaculata* and *Physella acuta*, among others; (Casado de Otaola, 1996). Nowadays, they have all disappeared. Based on historical information (Alvarez-Cobelas and Cirujano, 1996), we reconstructed the foodweb of the wetland around 1950 when, before the introduction of the mosquito fish, the NPTD was still a series of fish reserves (Fig. 6). The food web of NPTD for 1950 was composed of fish species, all autochthonous, and species of waterfowl supported by mollusks, macroinvertebrates, and zooplankton and accompanied by the autochthonous crab *Austropotamobius pallipes*. Filter-feeding mollusks fed on phytoplankton, clarifying the water and contributing to the development of submerged macrophytes. These should have been food for the native crayfish and ducks, who also consumed soft macroinvertebrates. The food chain length was probably similar to the one present today. In contrast, Fig. 5 shows the configuration of the current food web in 2011, strictly constructed with the results obtained with the results from the SISUS model. Here, we have included species of winged insects, although most butterflies do not show interactions with the species sampled. In today's food web, there are no shellfish, and the autochthonous crab has been completely replaced by the crayfish, which has a reduced number of interactions compared to those that *A. pallipes* could have potentially had in the food web of 1950. Higher trophic level organisms showed no trophic interactions with the rest of the organisms. Sediment/detritus is a food source for only egrets and gulls, while for benthic organisms like crayfish, its only of marginal importance. Some examples of food chains obtained through SISUS estimates are: *T. dominguensis* - *P. clarkii*- *A. clypeata; Tamarix canariensis* -invertebrates - *Lestes sp.*; or *C. submersum* - zooplankton - *F. atra.*

4. Discussion

Nowadays, the fish fauna of Las Tablas de Daimiel is only represented by opportunistic exotic species, such as in other heavily disturbed freshwater ecosystems (Copp et al., 2005). Four species, three of them exotic, make up the fish fauna of this wetland; three from North America: the bluegill (*Lepomis gibbosus*), the mosquitofish (*Gambusia holbrookii*), introduced for the control of malaria in 1950 (Alvarez-Cobelas and Cirujano, 1996) and catfish (*Ameirus melas*), emerging in the wetland (first sightings in 2010; National Park team, personal communication) and carp (*Cyprinus carpio*), which had already been mentioned in Las Tablas around 1910 (Elvira and Barrachina, 1996). NPTD has also been invaded by the American crayfish (*Procambarus clarkii*), native to the southeastern United States, replacing the European crayfish (*Austropotamobius pallipes*), also introduced in the late nineteenth century, and that was very abundant in these waters until the end of the 1960s, until it became extinct by the crayfish plague (Alvarez-Cobelas et al., 2010).

4.1 Food chain length of NPTD

The input of nutrients (including N, P and trace elements, etc.) and the invasion of exotic species greatly influence energy pathways, C balance, and nutrient budget of many habitats (Guy et al., 2012), but little is known about the effects on the food webs of these ecosystems. Las Tablas de Daimiel is a hypertrophic ecosystem (Alvarez-Cobelas et al., 2001), but the food chain may be considered moderate in length (from 3.9 to 4.4 trophic levels; Briand & Cohen, 1987; Beaudoin *et al.,* 2001). Several authors have cited that disturbances and changes in primary productivity resulting from nutrient enrichment do not necessarily exert direct impacts on the food chain length (McHugh et al., 2010; Walters and Post, 2008). However, there are clear indications that, among others, primary productivity (McHugh et al., 2010; Vander Zanden et al., 1999), the size of the ecosystem (Post, 2002; Schoener, 1989) and disruptions (Briand, 1983; Menge and Sutherland, 1987) are key in determining the configuration of food webs in aspects such as species diversity within trophic levels, the connection between species, and the influence of the trophic position of an organism.

The size of the ecosystem influences the food chain length, generating an increase or decrease in the number of trophic levels, allowing the coexistence of two predatory species in the same niche and even conditioning the abundance of a particular species (Takimoto et al., 2008). Disturbances, however, seem more crucial in controlling the abundance of species at higher trophic levels (Takimoto et al., 2008). In this situation it is expected that the inflow of nutrients in NPTD has helped to increase net primary productivity, changing the structure of the food web from the bottom-up (bottom-up effects; (Sahasrabudhe and Motter, 2011). However, changes in consumer species also alter the structure of the food chain via trophic cascades (i.e., top-down; (Scheffer, 1998). The nutrient enrichment registered in NPTD and the consequent deterioration of the habitat has contributed to the disappearance of many species of consumers, which has significantly modified the structure of the network (see Angeler *et al.* 2001; 2002)

The highest trophic level in Las Tablas is occupied by two species of exotic fish (*A. melas* and *L. gibbosus*) with a very irregular abundance over time (Sánchez-Carrillo and Angeler, 2010), probably conditioned by extreme changes suffered in the wetland from year to year (e.g., drought-flood cycles). In fact, *A. melas* has not been observed in recent surveys in the park, in contrast to what happens downstream (Llanos Gabaldón Lozano and Peña, 2009). Large fluctuations in the flooded area as well as disturbances have favored the invasion of the exotic species that are more resistant to the new hydrological conditions (Dudgeon et al., 2006). In fact, populations of *C. carpio* in the Upper Guadiana Basin are concentrated in Las Tablas. Apparently, the disruption suffered by the NPTD and the consequent reduction in both the size of the habitat and its quality is a key factor in the disappearance of populations of native fish species, which can still be seen in the upper reaches of the Záncara, Gigüela and Riansares rivers (seven-eight endemic species: (Elvira and Barrachina, 1996; Llanos Gabaldón Lozano and Peña, 2009). If the flooded area remained constant for a long-term cycle (over 10 years) and if the water quality improved, then it would be likely that some of these native species arrive to populate the NPTD again, which may eventually lead to a readjustment of the structure of trophic niches (Gathman et al., 2005; Wilcox et al., 2008).

Recently, the presence of comiza barbel (*Barbus comiza*) has been reported in the NPTD when the surface of the flooded area increased significantly after almost a decade without water. But its passage through the ecosystem has been ephemeral and it is unknown if this is related to its niche being occupied by other exotic species. Changes in the ecosystem can also alter the eating habits of certain species indirectly. For example, (Takimoto et al., 2008) showed that with the reduction of the ecosystem, the trophic cascade effect is magnified, and species of higher trophic levels seek food at lower trophic levels, consequently reducing their trophic position and length of the chain. This may be the reason why the specimens studied here of the genus *Larus,* which in other ecosystems reach a trophic level of 4 (e.g. on the Mediterranean coast; Navarro *et al.* 2011), are located at trophic level 1 (i.e., primary consumers) at NPTD.

4.2 Source contribution to the support of the food web of NPTD

There has been some controversy about the importance of macrophytes as the base of aquatic food webs because of their high biomass and production in these ecosystems (Hamilton et al., 1992; Hart and Lovvorn, 2002). Some studies using stable isotopes in aquatic food webs show that vascular plants are not as important in C flow for aquatic food webs (France, 1995). In wetlands, traditionally it is considered that food webs are supported by the decomposition of macrophytes as they are an important source of C for benthic macroinvertebrates (James et al., 2000; Kornijów, 1996). Numerous studies show that phytoplankton may become more important as secondary producers (Hamilton et al., 1992; Hart and Lovvorn, 2002). To our knowledge, only one study found vascular plants as major actors in wetland food webs through herbivores and detritivores (France R. L., 1995). Despite this, macrophytes are known to play a main role in trophic interactions in shallow lakes as they condition numerous chemical, behavioral and structural interactions (Burks et al., 2006).

Our isotopic results suggest that plants from NPTD contribute to the diet of some consumers that occupy the highest trophic levels. For example, *Tamarix canariensis* appeared as an important food source for benthic macroinvertebrates and for butterflies through its flowers (in fact, on the days of collection we noted that leaves were coated with pollen, indicating that it was their blooming time). In fact, the use of *Tamarix* sp. flowers by *Pieris rapae* has been described in the Mojave Desert (USA; Andersen & Nelson, 2013). Moreover, *T. dominguensis* and *S. maritimus* seem to be the main food sources of *P. clarkii* in the park. In support of this, other studies have reported that the diet of the American crab includes different macrophytes such as *Callitriche brutia*, *Chara connivens*, *Ranunculus peltatus* (Alcorlo et al., 2004) and *T. dominguensis* (Rosenthal et al., 2005).

Six of the nine species of waterfowl evaluated in this study showed isotopic signatures consistent with the sources studied (Carpenter and Lodge, 1986). *Fulica atra* bases its diet on submerged macrophytes, while the coot has a varied diet, feeding on macrophytes and other aquatic plants. However, its diet also includes invertebrates, filamentous algae, detritus, seeds, herbs, insects, snails, worms and even tadpoles (Perrow et al., 1997; Phillips et al., 1978). In contrast to waterfowl, the contribution of macrophytes to the fish fauna was negligible, something also noted by others (Bunn and Boon, 1993; Keough et al., 1996). This same pattern has been observed in Ruidera Laguna Complex (Cave Morenilla; Ruiz-Jimenez, 2015). Also, isotopic composition of fish does not reflect any consistency in the carbon sources evaluated in this study, as some authors also observed in other ecosystems (Matthews and Mazumder, 2003; Post, 2002). This may be because the fish community is subsidized by external habitats (Sierszen et al., 2019), all potential food sources of the wetland that were not captured, or because the park's isotopic variation of zooplankton is more heterogeneous than what was recorded in this study. Consistent with this, a study in several lakes in Finland showed that the ^{13}C-zooplankton can vary up to 19 delta ^{13}C (from -46.0 ‰ to -27.2 ‰;

(Jones et al., 1999), depending on the hydro-geomorphological conditions (E. Sierszen et al., 2006). The isotope fractionation accompanying the anaerobic decomposition of vascular plants in wetlands may result in that C fluxes between microbial populations and the remnants of macrophytes become decoupled, with the consequence that the isotopic signatures of the fish do not reflect the values of the vascular plants (Boon and Sorrell, 1991). Another factor may be the high concentration of sulphates in the wetland, mainly in the summer (Sánchez-Carrillo and Angeler, 2010), which makes $\delta^{13}C$-consumer values appear more depleted than their food source (Alongi, 1998; Brooks et al., 1987; Robinson and Cavanaugh, 1995).

The contribution of *P. australis*, the most abundant and productive macrophyte in the NPTD (Cirujano et al., 2010), to the food web of this wetland is almost insignificant, except for zooplankton and crawfish, for which it represented up to 54% and <20% of their diet. However, this species has a high range of isotopic variability (Hines et al., 2006; Treydte et al., 2009) and its consumption may remain masked by other sources (Chapin III et al., 1995; Hines et al., 2006). If this is confirmed, this would have major implications for ecosystem management, as the withdrawal of biomass would not critically affect trophic interactions, at least directly. To date, only in a marsh in the Delaware Bay, United States, there is knowledge of *P. australis* having a direct contribution to the diet of secondary producers (73% mixing models ^{13}C and ^{34}S; (Wainright et al., 2000). Moreover, most studies show adverse effects of proliferation of *P. australis*, including lower abundance of fish (Able and Hagan, 2003; Hunter et al., 2006) and lower abundance of some groups of algae, which indirectly influence higher trophic levels (Perez et al., 2013) and the reduction of birds and mammals due to the decrease in favourable areas (Hauber et al., 1991).

4.3 Las Tablas food web: past and present

More than five decades ago, the food web in Las Tablas was represented by a high diversity of organisms, including ten species of fish (Alvarez-Cobelas and Cirujano, 1996). Around the mid-twentieth century, it is likely that fish species coexisted at all trophic levels and with a trophic role of the crayfish *Austropotamobius pallipes* very similar to the American crayfish, based on the eating habits of this species (Reynolds and O'keeffe, 2005). Although it is likely that in the past the baseline wetland resources were much higher (with the presence of, for examples, bivalves and gastropods), a currently poorly connected trophic system suggests that ecosystem resources are currently not being fully exploited. Main causes may be related to the quality of the ecosystem or the presence of populations of certain opportunistic, versatile species that undermine potential competitors. The trophic configuration presented in Figure 5 shows a wide variety of fish species, mainly in the lower trophic levels, with high importance of benthic macroinvertebrates in sustaining the food web.

The water restrictions limit the diversity of intermediate predatory fish species (Hairston Nelson G. and Hairston Nelson G., 1993, 1997). It is more likely that in Las Tablas the lack of species populating the lower niches (trophic level 1 and 2), rather than resource constraints, may have allowed the expansion of opportunistic and resistant species such as *A. melas* and *P. clarkii*, as reported in other studies (Braig and Johnson, 2003; Novomeská and Kováč, 2009; Winemiller and Rose, 1992). This influences the low trophic complexity of the wetland present today, although the deterioration in water quality also has harmful effects on the diversity of fish species (e.g Serrano-Grijalva *et al.*, 2011). Likewise, the American crayfish may also be contributing indirectly to an important control of certain fish populations and may also be key for consumer interactions. It has been said that *P. clarkii* benefits limnephilus species, such as *A. melas*, that depends on specific substrate conditions to lay eggs and build nests (Braig and Johnson, 2003; Cucherousset et al., 2006). It has also been suggested that *P. clarkii* controls the abundance of certain species like *L. gibbosus* through the reduction of the macrophytes they depend on (Roth et al., 2007). The indirect influence of *P. clarkii* in the presence of amphibians for the removal of sediments has also been reported (Nyström et al., 1999). Opportunistic species such as *P. clarkii and A. melas* have a high trophic versatility and can occupy more than one trophic level throughout their life cycle (Matěna, 1995). The low abundance of carp, in decline since 1983 in the NPTD, may be allowing the entry of other exotic species to the ecosystem, altering the trophic structure of the wetland. The American crayfish can efficiently extract resources from niches left by other local and even exotic species that have disappeared from the ecosystem due to environmental degradation (Angeler et al., 2002; Leunda et al., 2008). The lack of success in capturing the diet of other species very adapted to the NPTD such as the mosquitofish may be due to its peculiar diet based on invertebrates captured in a wide range of microhabitats from the benthos (Diptera, Odonata and ostracods), surface water column (Hemiptera, Coleoptera, copepods, cladocerans), and even those that swarm on aquatic vegetation (Hymenoptera) (Rodríguez-Jímenez A. J., 1987). In our study, the mosquitofish occupies the 3.18-3.6 trophic level, demonstrating the plasticity of this species, whereas its previously described trophic level was lower (2.2; Capps *et al.*, 2009).

In summary, the food chain length of the NPTD wetland does not appear to have changed significantly during the last 50 years. In contrast, environmental variations sustained over time associated with the entry of nutrients from different sources and species invasion have reduced the diversity within trophic levels and also resulted in a loss of connectivity between species, which has allowed the success of versatile, opportunistic organisms at all trophic levels.

Acknowledgements

The DECAMERON project (2008/001) funded by the Agency for National Parks of the Spanish Ministry of Agriculture, Food and Environment, provided financial support for the wetland FACE facility. Thank you very much to Brian Fry for help me in the interpretation and Erick Heardt for allow me to use the SISUS model. Many thanks are due to TDNP staff, especially to the Principal, Carlos Ruiz de la Hermosa, and TRAGSA team for logistic and technical support. We also thank to José Luis Ayala, Carlos Menor, Jesus Iglesias, Adrian Carrero, María José Ortíz, Ana Meco and for their valuable support during FACE operation and field sampling. LSG and RSA were supported by JAE-PreDoc and JAE-Doc contracts respectively, co-funded by the European Social Funds.

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Table 1. Length and weight of the species of fish and crabs captured in NPTD n summer 2011. Small: <10.5 cm, Medium: 10.5-12 cm, large >12 cm.

				Length (cm)				Weight (g)			
Species	Common name	Stage	No.	Average	SD	MIN	MAX	Average	SD	MIN	MAX
Lepomis gibbosus	Pumpkinseed sunfish	Adult	1	11.5	11.5	11.5	11.5	14.8	14.8	14.8	14.8
Cyprinus carpio	Carp	Adult	61	35.3	1.7	32.0	39.0	648.1	91.0	500.0	800.0
Ameiurus melas	Cat fish	Adult	38	18.0	2.5	11.5	22.0	114.5	90.5	21.6	400.0
Ameiurus melas	Cat fish	Fry	40	2.4	0.3	1.8	3.0	0.2	0.1	0.1	0.4
Gambusia holbrookii	Gambusia	Fry	76	3.4	1.2	1.7	5.6	0.7	0.7	0.1	2.3
Procambarus clarkii	American crab	Small	106	9.3	1.0	6.5	10.5	24.1	8.7	4.3	41.8
Procambarus clarkii	American crab	Medium	54	11.4	0.4	11.0	12.0	47.0	7.3	30.2	64.5
Procambarus clarkii	American crab	Large	16	13.0	0.6	12.5	14.5	61.0	11.1	47.7	14.5

Table 2. Range of contribution (minimum and maximum) of the different food sources to consumers in the NPTD based on the mixing model SISUS through the use of stable isotopes (^{13}C and ^{15}N).

	Winged insects								Crustaceans		Birds											
	R. sacraria		*Pieris rapae*		*Lestes* sp.		*Sympetrum* sp.		*P. clarkii*		*L. ridibundus*		*H. himantopus*		*Fulica atra*		*A. clypeata*		*Larus* sp.		*B. ibis*	
	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX
Sediment					0.00	14.63	0.00	27.95	0.00	2.89	0.00	27.82	0.00	52.03					43.06	62.92	17.46	30.20
POC									0.00	23.12												
Phytoplankton					0.00	20.75	0.00	39.64			0.00	51.67	0.00	73.80					0.00	29.04	0.00	18.63
Periphyton					0.00	40.27	0.00	32.29			0.00	17.48	0.00	29.22					0.00	8.71	0.00	5.59
Filamentous algae					0.00	44.58	0.00	35.75			0.00	19.35	0.00	30.39					0.00	8.57	0.00	5.50
Microbial mats					0.00	26.73	0.00	51.07			16.90	83.51	0.00	73.53					0.00	20.84	0.00	13.37
C. hispida var. *major*	0.00	11.45	0.00	2.69					0.00	2.22			0.00	23.44	0.00	43.85	0.00	6.76				
C. submersum	0.00	5.15	0.00	6.35					0.00	8.59			0.00	72.74	0.00	21.33	0.00	25.33	0.00	12.68	0.00	8.14
P. pectinatus	0.00	5.76	0.00	3.79					0.00	3.13					0.00	61.45	0.00	9.37				
S. maritimus	0.00	1.97	0.00	2.43					0.00	76.91												
T. dominguensis	0.00	2.10	0.00	2.59					0.00	81.98									0.00	5.73	0.00	3.68
C. mariscus	0.00	80.64	0.00	18.94					0.00	15.65												
P. australis	0.00	2.39	0.00	2.95					0.00	16.14												
T. canariensis	18.68	86.88	78.80	95.08					3.02	21.53									25.66	33.76	62.47	67.67
Invertebrates					41.00	60.47	23.05	60.25			2.48	39.49	0.00	47.82	0.00	20.86	0.00	50.18	0.00	7.95	0.00	5.10
Zooplankton					0.00	32.27	0.00	25.88			0.00	14.01	0.00	20.36	0.00	76.06	0.00	16.88	0.00	6.31	0.00	4.05
P. clarkii small															0.00	27.89	0.00	67.08				
P. clarkii medium															0.00	45.77	0.00	83.35				
P. clarkii large															0.00	41.76	0.00	49.53				

Figure. 1. Scatterplot of δ13C and δ15N (mean and standard deviation) of the main sources, primary producers and consumers from the present food web in the NPTD. DIC = dissolved inorganic C. DOC = dissolved organic C. POC = paticulate organic C.

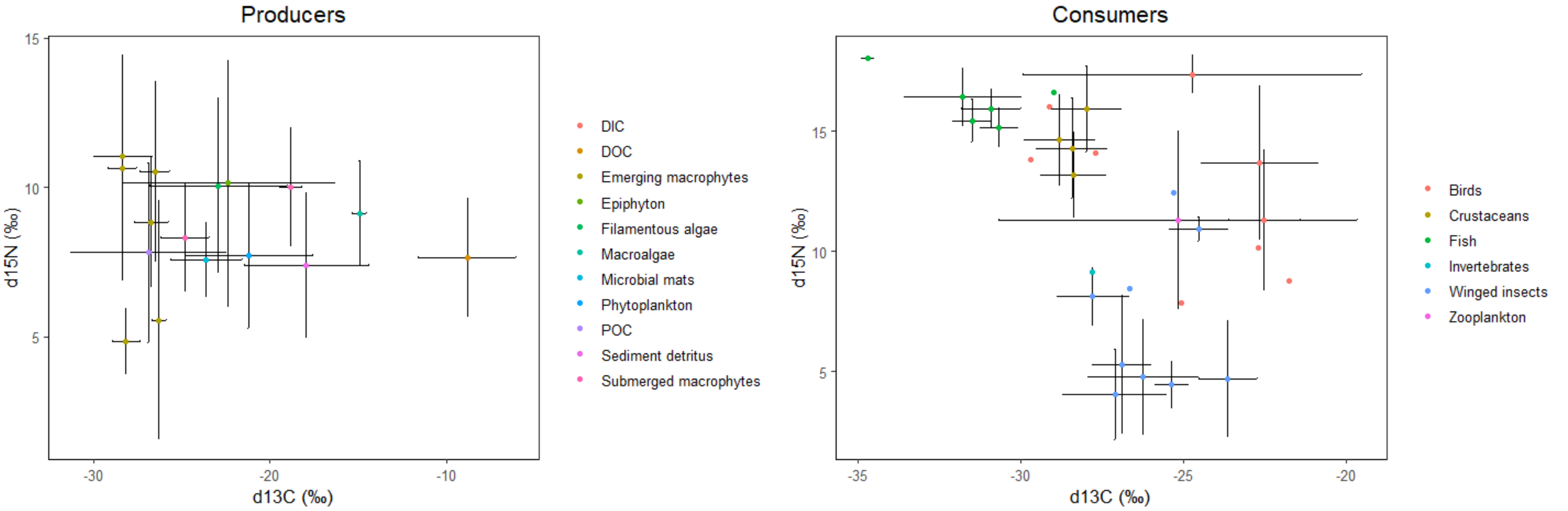

Figure 2. a) Mean ± SE of $\delta^{13}C$ y $\delta^{15}N$ of *Ameirus melas* in the four sites where it was collected. ANOVA: $\delta^{13}C$ p=0.003 and $\delta^{15}N$ p=0.036. b) Relationships between size and weight with isotopic signatures of *A. melas*.

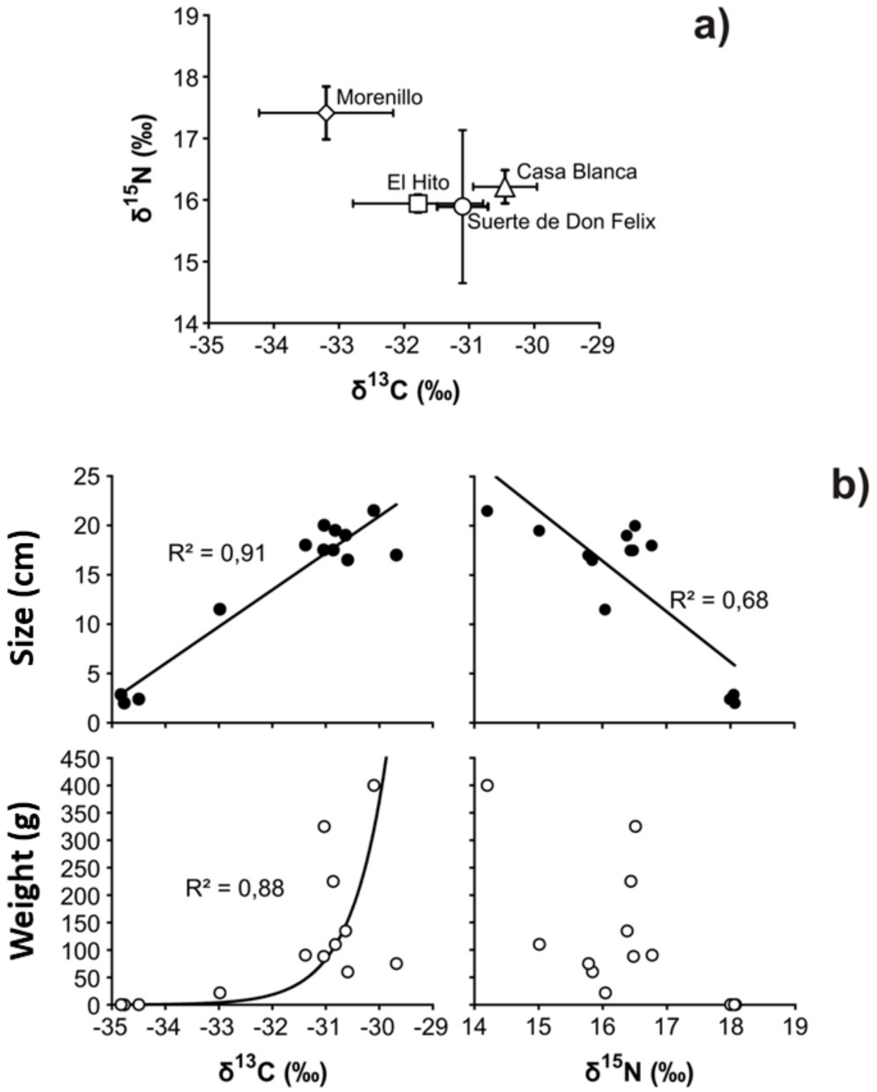

Figure 3. Scatterplot of δ^{13}C and δ^{15}N of *P. clarkii* based on (a) sampling location (b) and sex (ANOVA δ^{13}C $p > 0.05$ and δ^{15}N $p > 0.05$).

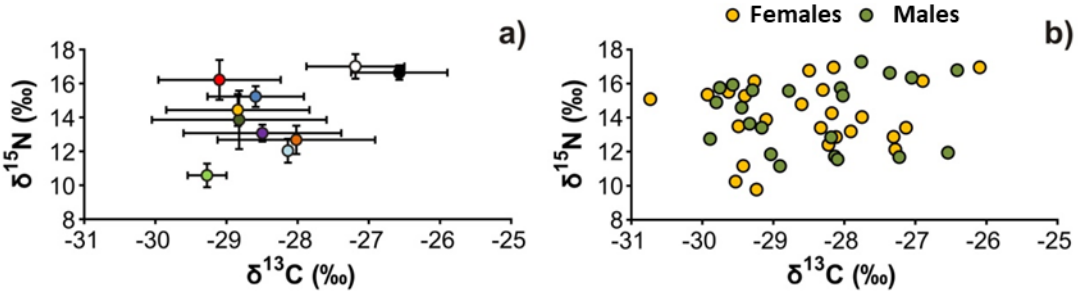

Figure 4. Trophic levels of the main consumers from NPTD when (a) producers or (b) primary consumers are considered as the base of the foodweb.

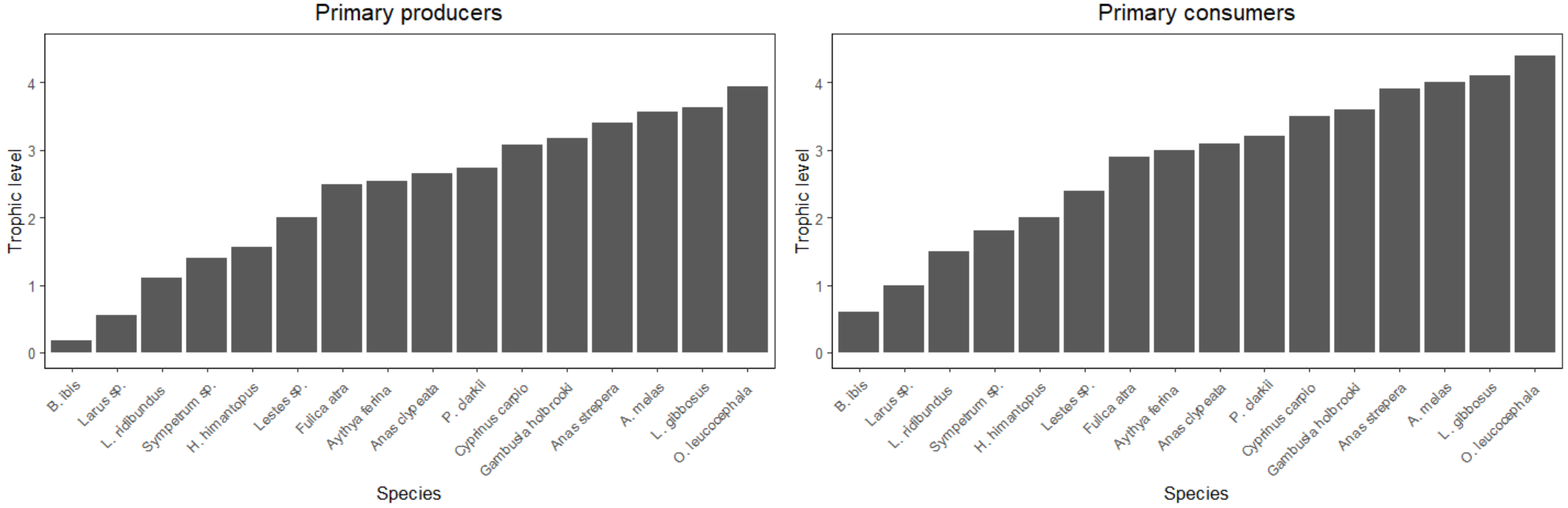

Figure 5. Representation of the present foodweb. The range of contribution (minimum and maximum) of the different food sources to consumers in the NPTD are based on the mixing model SISUS through the use of stable isotopes (^{13}C and ^{15}N).

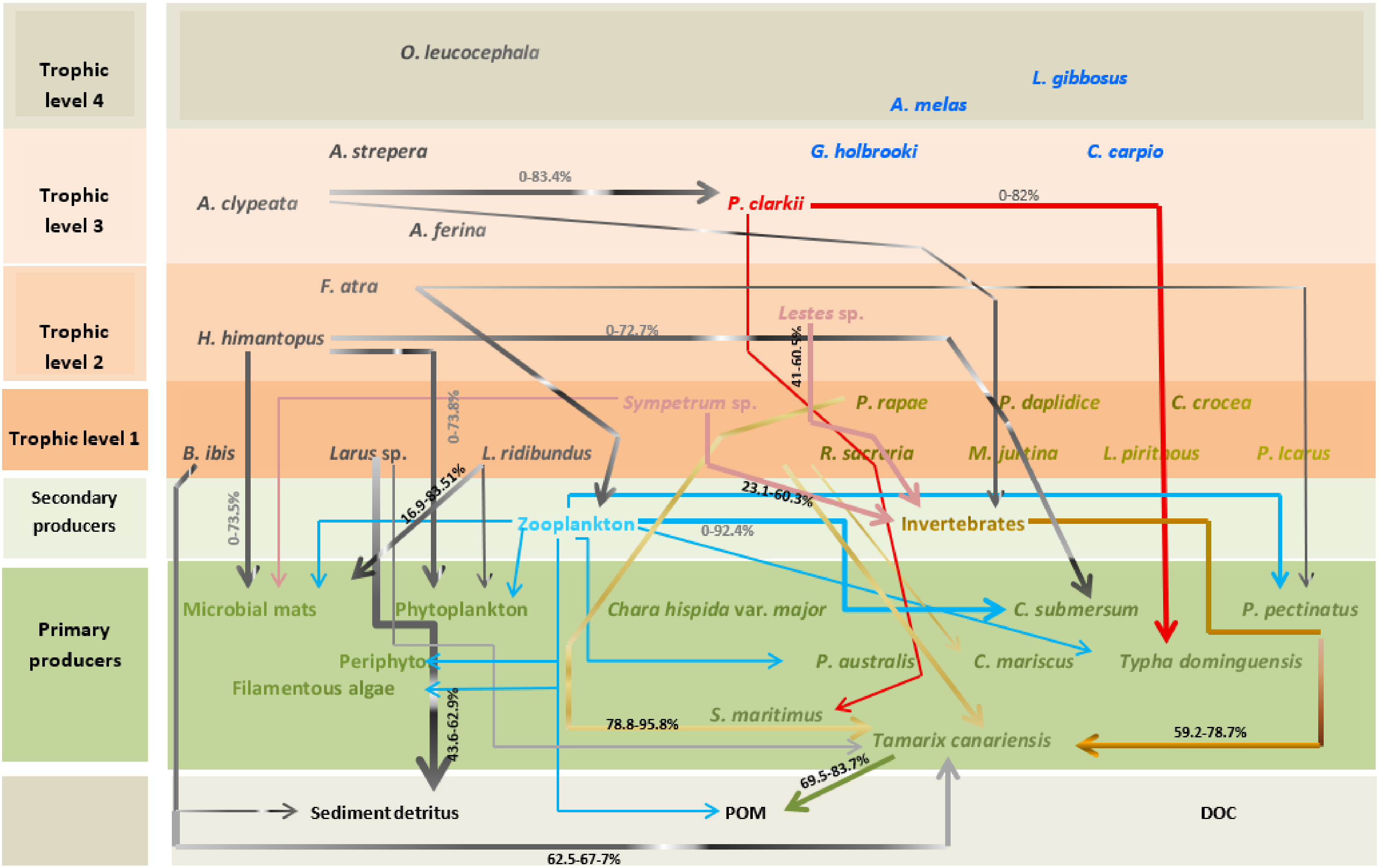

Figure 6. Schematic representation of the trophic network of NPTD around 1950 based on Alvarez-Cobelas and Cirujano (1996) and Cirujano Bracamonte et al. (1998).