University of Sassari
Ph.D. School in Natural Sciences
Via Muroni 25
I-07100 Sassari (SS) Italy

Dissertation for the Degree of Doctor of Philosophy in:
Environmental Biology presented at Sassari University in 2012

XXIV cycle

Trophic webs in Mediterranean freshwater environments through Stable Isotope Analysis (S.I.A.) of carbon and nitrogen:
Lake Sos Canales (Sardinia, Italy)

PH.D. CANDIDATE: ***Dr. Amedeo Fadda*** DIRECTOR OF THE SCHOOL: ***Prof. Marco Curini Galletti***

SUPERVISOR: ***Prof. Antonella Lugliè***

CO-SUPERVISORS: ***Dr. Bachisio Padedda***

Dr.ssa Marina Manca

University of Sassari
Ph.D. School in
Natural Sciences Via
Muroni 25, I-07100
Sassari, Italy

INDEX

SUMMARY

ABSTRACT

(ENGLISH)

Reservoirs are complex man-made ecosystems, with environmental traits intermediate between rivers and lakes. Previous studies highlighted that eutrophication is the most important issue of Sardinian reservoirs. Studies on zooplankton were, in this context, addressed to understand whether and how peculiar traits of these environments, also related to their trophic status, might influence transfer of matter and energy through the food web. A preliminary study on 15 Sardinian reservoirs led to the decision of choosing Lake Sos Canales to apply carbon and nitrogen Stable Isotope Analysis (S.I.A) for investigating the role and seasonality of different sources sustaining pelagic food web. Between October 2010 and October 2011 suspended particulate matter (SPM), zooplankton and fish were collected. SPM was strongly affected by hydrological seasonal dynamics of the reservoir. Results of SPM isotopic signature were used to quantify contribution of different sources to zooplankton consumers. Results on crustacean zooplankton taxa highlighted common seasonal patterns and taxa-specific roles in matter and energy transfer to upper levels of the trophic web. The fish community was constituted by two allochthonous species, only one relying upon pelagic food sources. Data showed that paths of transfer of matter and energy and trophic interactions between fish species were peculiar of these environments, intensely affected by hydrological stressors.

Key words: aquatic food web, stable isotope analysis, suspended particulate matter, zooplankton, allochthonous fish, reservoir

(ITALIANO)

I laghi artificiali sono dei complessi ecosistemi costruiti dall’uomo, con caratteristiche ecologiche intermedie tra i fiumi e i laghi. Studi precedenti hanno evidenziato come l’eutrofizzazione sia il maggiore problema dei bacini artificiali della Sardegna. Gli studi sullo zooplankton, in questo contesto, sono stati indirizzati alla compresione di come e se le caratteristiche peculiari di questi ambienti, anche legate al loro stato trofico, influnenzino il trasferimento di materia e di energia attraverso la rete trofica. Uno studio preliminare su 15 laghi artificiali della Sardegna ha portato alla scelta del Lago Sos Canales per applicare l’analisi degli isotopi stabili (S.I.A.) di carbonio ed azoto per investigare il ruolo e la stagionalità delle differenti fonti che supportano la rete trofica pelagica. Tra l’ottobre del 2010 e l’ottobre del 2011 sono stati campionati il materiale particellato sospeso (SPM), lo zooplankton e i pesci. La SPM è risultata essere fortemente influenzata dalle dinamiche idrologiche stagionali dell’invaso. I risultati isotopici della SPM, sono stati utilizzati per quantificare il contributo delle differenti fonti per i consumatori zooplanctonici. I crostacei planctonici hanno utilizzato differenti fonti alimentari nel corso dell’anno, mostrando di concorrere al trasferimento di materia ed energia dai più bassi a i più alti livelli della rete trofica. Solamente una delle due specie alloctone di pesci ritrovate nel lago è risultata alimentarsi su fonti pelagiche. Inoltre, i dati mostrano che le vie di trasferimento di materia ed energia e le interazioni trofiche tra le specie ittiche è peculiare in questi ambienti, intensamente influenzati dagli stress idrologici.

Parole chiave: reti trofiche acquatiche, analisi degli isotopi stabili, materiale sospeso particellato, zooplancton, pesci alloctoni, laghi artificiali

PREFACE

The present thesis, entitled "Trophic webs in Mediterranean freshwater environments through Stable Isotope Analysis (S.I.A.) of carbon and nitrogen: Lake Sos Canales (Sardinia, Italy)" - Ph.D degree in Enviromental Biology of the Ph.D School in Natural Sciences of the University of Sassari (Italy), summaries the activities and the results of the Ph.D project of Dr. Amedeo Fadda, planned and supported with the collaboration of the Department of Sciences for Nature and Environmental Resources (DipNET) of the University of Sassari (Italy), the Institute of Ecosystem Study of the National Council of Research (CNR-ISE), Verbania (Italy) and the Ente Acque della Sardegna (ENAS), Cagliari (Italy).

The study was performed under the supervision of Prof. Antonella Lugliè and the co-supervision of Dr. Bachisio Padedda of the (DipNET) and the co-supervision of the senior research scientist Dr. Marina Manca (CNR-ISE).

Other researchers involved significantly in the study were:

Dr. Silvia Markovà of Department of Vertebrate Evolutionary Biology and Genetics, Academy of Science (Czech Republic) (Chapter I);

Dr. Piero Olla of the Institute for the Study of Atmosferic Science and Climate of the National Council of Research (CNR-ISAC), Cagliari (Italy) (Chapter II);

Prof. Andrea Sabatini of the Department of Animal Biology of University of Cagliari (Italy) (Chapter V)

Dr. Federica Camin and Dr. Luca Ziller of the Istituto Agrario San Michele all'Adige (IASMA) (Italy).

In particular, field activities and part of the analysis were carried out in close collaboration with ENAS. Fish caught was planned and performed with the collaboration of the Department of Animal Biology of University of Cagliari (Italy). The analyses of stable isotope samples were performed at the Istituto Agrario San Michele all'Adige (IASMA) (Italy) at the Stable Isotope Platform. The genetic analysis of *Daphnia pulex* was performed at the Department of Vertebrate Evolutionary Biology and Genetic, Academy of Sciences of Czech Republic.

The thesis consists of a general introduction, the aims, five chapters (scientific papers) in published and manuscript form, general conclusions and attachments.

The first part of the Introduction summarizes a brief overview of the main characteristics of reservoirs as man-made ecosystems and their aquatic communities, in particular focusing on Sardinian reservoirs. The second part summarizes the general knowledge of stable isotope analysis and its application in food web studies.

Scientifics results were organized in the following chapters:

Chapter 1 - Zooplankton in Sardinian reservoirs:

"First record of planktonic crustaceans in Sardinian reservoirs"

Chapter 2 - C and N stable isotope analysis of suspended particulate matter (SPM) in Lake Sos Canales (North-Sardinia):

"Do hydrological stressors affect stable isotopic signature of carbon and nitrogen in Mediterranean reservoirs?"

Chapter 3 - C and N stable isotope analysis of zooplankton consumers in Lake Sos Canales:

"Spatio-temporal dynamics of C and N isotopic signature of zooplankton: an annual study on a man- made reservoir in the Mediterranean Region"

Chapter 4 - C and N stable isotope analysis of fish fauna in Lake Sos Canales:

"Allochthonous fish impact on zooplankton community in upland man-made lake through Stable Isotope Analysis"

Chapter 5 - C and N stable isotope analysis of planktivorous fish in a large, deep subalpine lake (Lake Maggiore):

"Littoral vs. pelagic sources for planktivorous fish: applying a Dynamic Mixing Model to C, N Stable Isotope Analyses in deep subalpine lake" (Submitted to Freshwater Biology)

The conclusion gives an overall picture of the obtained results.

In the final part were attached scientific papers published in national language:

1- "Verso un approccio funzionale allo studio della diversità: risultati di una prima indagine sullo zooplancton e la rete trofica pelagica del Lago Maggiore attraverso analisi d'isotopi stabili di carbonio ed azoto".

2- "Indagini sulla rete trofica pelagica del Lago Maggiore mediante analisi d'isotopi stabili di carbonio e azoto (SIA): risultati della campagna 2009".

INTRODUCTION

RESERVOIRS: MAN-MADE ENVIROMENTS

Reservoirs represent peculiar freshwater ecosystems, whose existence is always connected to specific, often multiple, uses (Straškraba and Tundisi, 1999).They are in the middle between rivers and lakes (Straškraba and Tundisi, 1999) and show mainly unidirectional changes of limnological variables, with environmental gradients along their longitudinal axis (Straškraba et al., 1993; Straškraba, 1997; Caputo et al., 2008). Thornton (1990) proposed a heuristic model to describe them as a continuum from river inflow to dam, with the establishment of three distinct zones: riverine, transition and lacustrine zones. The amplitude of each zone mainly depends on the size and the shape of reservoir.

Compared to natural lakes, artificial lakes have higher watersheds area/water body area, shorter and varying retentions times at both intra- and inter-annual scale, a rapid ageing process in relation to watershed uses, high capability to retain organic and inorganic matter (Straškraba, 1998; Straškraba and Tundisi, 1999; Tundisi and Matsumura-Tundisi, 2003). External inputs from watershed are relevant during wet season, or filling phase, while internal recycling processes support biological community during the drought season (Zoary et al., 2011). Moreover, they have a littoral zone relatively wide, only periodically submerged, due to the strong water level fluctuation (WLF) along the seasons, especially when artificial lakes are devoted to human water exploitation (e.g., agriculture, hydropower, drink water production). Strong WLF limits the development of well-structured shoreline macrophyte community and limits the number of species able to survive in these conditions. Thus, phytoplankton develops mainly and results the most important primary producer at the base of the aquatic food web. The reason of reservoir existence, and mainly its water destination use, changes its management, hence hydropower reservoirs differing by those for drinking water or agricultural supply (Barbanti et al., 1980). Thus, reservoirs are strongly influenced by management practices, making the study of reservoirs much more difficult than that of natural lakes (Wetzel, 2001). Consequently, reservoirs need a different study approaches than natural lakes (Straskraba, 1973).

In the Mediterranean basin, reservoirs represent strategic water resources to support different human needs. It is especially in the southern regions and islands, where the semiarid character of the climate, the strong seasonality of wet and arid periods and the scarcity of other sources of surface waters are more intense. In these conditions, the water demand increase during the dry months, causing heavy and abrupt reduction of water level in reservoirs. In Sardinia, the second larger island in the Mediterranean, since early of last century, several rivers have been dammed to support increased water demand for different purposes (Sechi and Lugliè 1992, 1996). With the exception of Lake Baratz, an oligo-aline waterbody in the northwestern area, all Sardinian lakes are reservoirs, most of them eutrophic (Sechi and Cossu 1979). Actually, there are about forty reservoirs with a volume > 0.5 x 10^6 m^3, for a total water stored of about 1.9 x 10^9 m^3.Ente Acque of Sardinia (ENAS) is the Water Authority and the instrumental entity of Autonomous Region of Sardinia that manages the multi-sector regional water system. The ENAS was established in 2006 and currently 32 artificial lakes of Sardinia are under its direct management. Waters are withdrawn in order to produce hydropower or for agricultural, industrial and other human supply, such is drinking water. All the Sardinian reservoirs have multiple uses of the waters even if some of them were created for only one use.

Reservoirs initially devoted to drinking water production were commonly small and located in the upland (e.g., Lake Sos Canales and Torrei). The small size of these water bodies enhances the effects of management on the hydrological dynamics. In order to reduce the cost of water drinkable treatment, some reservoirs have an intake mobile water structures (e.g., Lake Sos Canales). Such mobile structure allow to chose the depths from which withdraw the water of best available quality along the water column. Commonly, the water intake structure is located slightly below to the photic zone, to avoid the depths where phytoplankton is usually more abundant, and above to the hypolimnetic layers, where the oxygen depletion triggers remobilization processes from the bottom. The middle position of the water withdrawn can create a barrier between surface and bottom waters. Furthermore, at a general level, the continuous flow reduces the possibility for the organism movement along the water column (Straskraba and Tundisi, 1999).

AQUATIC COMMUNITIES OF SARDINIAN RESERVOIRS

Autotrophic primary producers: phytoplankton

Sardinian reservoirs are typically and only dominated by phytoplankton due to the wide amplitude of WLF throughout the year. So far, they have been studied mainly in relation to their trophic status, hydrochemistry and changes over time, especially for the identification of best management strategies and a better exploitation for a precious resource for the Island, particularly for drinking water supply (Sechi and Cossu, 1979). Studies highlighted eutrophication as the major issue of Sardinian reservoirs (Sechi and Lugliè, 1992, 1996; Marchetto et al., 2009). The excessive amount of nutrient inputs was considered the main cause of their generally high trophy. Eutrophication process has been ascertained since the middle of seventies, with high chlorophyll *a*, phytoplankton biomass and dominance of Cyanobacteria in about 2/3 of the reservoirs. In particular, phytoplankton of the eutrophic and hypertrophic reservoirs appeared dominated by Cyanobacteria, whereas those of mesotrophic and oligotrophic reservoirs by Chlorophyceae and Bacillariophyceae. In eutrophic and hypertrophic reservoirs the typical species were *Microcystis aeruginosa* (Kützing), *Dolichospermum flosaquae* (Brébisson ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek, *Aphanizomenon flos-aquae* (Linnaeus), *Dolichospermum planctonicum* (Brunnth.) Wacklin, L.Hoffm. & Komárek, *Closterium aciculare* (T. West) and *Aulacoseira distans* (Ehrenberg) (Sechi and Lugliè, 1996). Toxic cyanobacteria are an important issue in Sardinian reservoirs (Messineo et al., 2009). In reservoirs between eutrophic and mesotrophic, species of genus *Planktothrix* (i.e. Lake Mulargia) and dinoflagellates, like *Gymnodinium uberrimum* (Kofoid and Swezy) dominated (e.g., in Lake Sos Canales; Sechi and Lugliè, 1996).

Planktonic consumers: zooplankton

Untill the study carried out with this PhD thesis, the main studies on freshwater planktonic fauna in Sardinia had considered zooplankton in pond communities (Stella, 1970, 1982; Margaritora, 1983). Zooplanktonic communities of Sardinian reservoirs were poor investigated and only sporadic information exists in literature

(Cioglia, 1969). Consequently, the first step of this thesis was to obtain basic information on planktonic crustacean populations in Sardinian reservoirs to choose the artificial lake where carry out the subsequent phases of the research (1st Chapter of the thesis). In fact, this work has built the basis of knowledge on zooplanktonic communities in Sardinian reservoirs. The screening activity in 15 Sardinian reservoirs highlighted that they are “banks” of diversity, hosting both Mediterranean endemisms (i.e. Copidodiaptomus numidicus, Gurney) and invasive species, such is the hybrid clone of Daphnia pulex (Leydig). Overall, 18 crustacean zooplankton taxa were identified, ten belonging to the Cladocera, eight to the Copepoda. Within the latter, seven were of Cyclopoids, only one belonged to Calanoids (C. numidicus Gurney). The latter was the only non-cosmopolitan species, known for a geographical distribution restricted to the Mediterranean area and North Africa (Armengol, 1980; Cherbì 1984). This species was found in all of the investigated reservoirs, at different levels of relative abundance. Cyclopoids included taxa of a large to very large body size (1.9-2.5 mm; Einsle 1993), namely Macrocyclops albidus (Jurine), Megacyclops gigas (Claus) and Megacyclops viridis (Jurine), as well as Acanthocyclops vernalis-robustus (Sars). Species of the Cyclops abyssorum group (Sars) were also found (Sos Canales and Torrei lakes). Among smaller-sized taxa, Mesocyclops leuckarti (Claus) was found in Lake Monte Lerno at low abundance, and Thermocyclops dybowski (Lande) in lakes Cantoniera, Is Barrocus, Bidighinzu and Medio Flumendosa. Among small sized Cladocerans, Bosmina longirostris (O. F. Müller) was found in most reservoirs (11), in one case (Lake Sos Canales) representing a large proportion of total zooplankton abundance in 2009. Ceriodaphnia pulchella (Sars) and Ceriodaphnia reticulata (Jurine) were ubiquitous; in some cases the former made up the largest proportion of total zooplankton abundance. Chydorus sphaericus (Müller) was detected in hypertrophic and eutrophic reservoirs (such as lakes Cixerri, Cucchinadorza and Cantoniera). Two species of the genus Diaphanosoma, D. lacustris (Korinek) and Diaphanosoma sp., were found in 9 of the 15 reservoirs. Moina brachiata (Jurine) was found in lakes with a relatively high conductivity. The Daphnia genus was well represented in the sampled reservoirs (in 9 out of 15), with species of the “pulex”, namely, Daphnia pulex (Leydig); (after Margaritora, 1983) as well as of the “longispina” group namely, D. longispina (Müller).

So far, D. pulex was reported only from fishless temporary ponds; therefore, this is the first record for the presence of this species in Sardinian reservoirs. In eight reservoirs only species of the “longispina” group were detected, whereas in one case (Lake Sos Canales), they coexisted with D. pulex.

Because of the highest diversity of zooplankton found in Lake Sos Canales, it was chosen for the further research activities of this thesis. Moreover, the overall results obtained with the preliminary screening have suggested the importance of surveys on zooplankton community in all reservoirs managed by ENAS, during the following two years (2010-2012). This activity increased the list of zooplankton taxa inhabit Sardinian reservoirs, including rotifers, and showed the presence of new taxa for the island. In particular, the presence of the invasive freshwater jellyfish, Craspedacusta sowerbyi (Lankester) in the southern-western part of the island and the North-American origin Daphnia parvula (Fordyce) in Lake Bidighinzu (personal observations) were recorded.

Fish

The majority of freshwater fish species inhabiting Sardinia reservoirs are introduced (Orrù et al., 2010). Introduction of allochthonous fishes in Sardinian reservoirs occurred for different purposes (aquaculture, stock enhancement, angling and biological control). The origin areas of introduced fish species are North-America, Asia and Europe and most of them have already established self-sustaining populations elsewhere in Mediterranean-type eco-regions (Orrù et al., 2010; Massidda et al., 2008). Species found in Sardinian reservoirs mainly belong to Cyprinidae, Centrarchidae, Salmonidae, Cobitidae, Ictaluridae, Percidae and Poecilidae. Moreover, anadromus fishes have already established self-maintaining population into the new built environments, i.e *Alosa fallax nilotica* (Fatio) in Lake Medio Flumendosa and Lake Cantoniera. Among Cyprinidae, the presence of carp (*Cyprinus carpio*, L.) is well known in almost all of the Sardinian reservoirs. Further presences are those of tench (*Tinca tinca*, L.), goldfish (*Carassius auratus*, L.), rudd (*Scardiunus erythrophthalmus*, L.), bleak (*Albunus alburnus alborella*, De Filippi), and topmouth gudgeon (*Pseudorasbora parva*, Temminck and Schlegel). Among Centrachidae, the largemouth bass (*Micropterus salmoides*, Lacépède) and pumplinseed (*Lepomis gibbosus*, L.) were assessed. Salmonidae were introduced both in reservoirs and streams, in particular the North-Atlantic brown trout origin (*Salmo trutta*, L.) and the rainbow trout (*Oncorhynchus mykiss*, Walbaum). Sardinian rivers host the endemic brown trout which belong to the evolutionary lineage of Mediterranean haplotype (Sabatini et al., 2011 and references therein). The release of North-Atlantic brown trout origin could have modified the distribution of endemic salmonid populations and caused genetic “pollution” effects (Gandolfi et al., 1991). Other introduced fishes species frequently occurred in Sardinian reservoirs are spined loach (*Cobitis taenia*, L.), black bullhead (*Ameiurus melas*, Rafinesque), perch (European perch, *Perca fluviatilis*, L.) and mosquitofish (*Gambusia holbrooki*, Girard).

SOME ASPECTS OF STABLE ISOTOPE ANALYSIS (S.I.A.) IN FRESHWATER ECOLOGY

Preface

Isotopes are atoms with the same number of protons and electrons but differing numbers of neutrons. Stable isotopes are defined as those that are energetically stable and do not decay, thus, they are not radioactive. An isotope tends to be stable when the number of neutrons (N) and the number of protons (Z) are quite similar. There are roughly 300 stable isotopes, over 1200 radioactive isotopes, and only 21 elements that are known to have only one isotope (Hoefs, 1997). Carbon, Nitrogen and Sulfur are commonly the stable (heavy) isotopes used in food web studies.

The isotopic differences between various materials (e.g., leaves, scales, organisms) are commonly small, so isotopic composition is reported relative to an internationally accepted standard and expressed in parts per thousand deviations from that standard by:

$$\delta(‰) = \left\{\frac{\text{R sample}}{\text{R standard}} - 1\right\} x1000$$

where R is the ratio of heavy-to-light (typically, but not always, rare-to abundant) isotope, R_{sample} is that ratio in the sample, and R_{standard} is that in the standard.

A positive δ ("delta") indicates that the sample has more of the heavy isotope than does the standard, whereas a negative delta value indicates the sample has less of the heavy isotope than the standard (i.e. is "depleted" in heavy isotope). There are several common means to compare isotopic composition of two materials, including "heavy" vs. "light", high vs. low values (the latter can be confusing as we often deal with negative values), more/less positive, and "enriched" vs. "depleted". The last should only be used with clarification as to which sample is enriched or depleted in the isotope, the lighter isotope, or the heavier one. The standard approach when using this terminology is to refer in terms of the heavy isotope (which is often, but not always, more rare); for example, "sample X is depleted in ^{15}N whereas sample Y is enriched in ^{15}N" (Sulzman, 2007).

Differences between source and product isotopic composition of chemical reaction is called fractionation. Three mechanisms lead to isotopic fractionation: equilibrium (also called thermodynamic or exchange), kinetic, and nuclear spin. Equilibrium fractionation reactions are those in which the distribution of isotopes differs between chemical substances (reactant vs. product) or phases (e.g., vapour vs. liquid) when a reaction is in equilibrium. Kinetic fractionation reactions are normally associated with processes such as evaporation, diffusion, dissociation reactions, and enzymatic effects. Kinetic fractionations are often quite large, usually much larger than equilibrium fractionations, and result in the lighter isotope accumulating in the product (lighter goes faster). Nuclear spin isotope effects are not mass dependent; rather, they arise because of differences in the nuclear structure among isotopes and lead to differences in nuclear spin. It is not clear how important nuclear spin fractionation is in most circumstances, but it does allow for coupling tracers (e.g., a ^{13}C-labeled substrate) with nuclear magnetic resonance (NMR) techniques.

Every isotope is unique, thus several characteristics are needed to use stable isotopes in ecological research, and in particular:

- large mass difference between the rare-stable and abundant-light-isotope;
- low atomic mass;
- elements with more than one oxidation state.

During a chemical reaction, the different isotopic forms of an element behave at slightly different rates: the lighter isotope tends to react more quickly than the heavier, slower one. Thus, the isotope ratio of reactant molecules differs from that of product molecules (“isotopic fractionation”) giving different, if minimally so, values (0.11% for C, 0.04% for N) between products and reactants (cfr. “Carbon and Nitrogen”).

Stable isotope ratios are typically measured through Isotope Ratio Mass Spectrometry (IRMS), invented by J.J. Thompson in 1910. A mass spectrometer separates charged atoms or molecules on the basis of their mass-to-charge-ratio, m/z. There are two basic types of IRMS, dual-inlet (DI-IRMS) and continuous flow (CF-IRMS). A continuous flow system allows to introduce multiple component samples (e.g., atmospheric air, soil, leaves) and obtain isotopic information for individual elements or compounds (Sulzman, 2007). The instrument that can calculate small differences in gaseous samples determines the chemical composition of a sample in terms of its molecular structure, splitting sample into different fractions along a mass gradient. Different components of the analysed sample (e.g. different isotopes of a molecule) are split depending on their atomic mass, and their mass/charge ratio measured. In addition, the C:N ratio of every sample analysed is calculated (cfr. “C:N ratio”). Samples for IRMS must be prepared according to international protocols and the internal directive of the laboratory (cfr. “Sampling spatio-temporal variability”).

Stable Isotope in food web ecology

Stable isotope analysis (SIA) is commonly used in ecological studies to answer questions related to energy pathways in aquatic ecosystems, trophic structure, plant and animal ecophysiology.

In aquatic environments it is a useful tool because direct observations are limited, spatial complexity is high, and the potential sources of nutrients, organic matter and prey are multiple (Finlay and Kendall, 2007). In particular, carbon and nitrogen stable isotope analysis is a powerful tool to estimate trophic position of organisms and carbon flow through food web (Post, 2002). Stable isotope analysis potentially captures the complex interactions, including omnivory, and traces energy and mass flow through ecological communities (Peterson and Fry 1987, Cabana and

Rasmussen 1996). In food webs, i.e in prey-predator relationships, the ratio of carbon isotopes ($\delta^{13}C$) changes little. Therefore, carbon isotopic signature can be used to evaluate the ultimate sources of carbon for an organism when the isotopic signatures of the sources are different (France, 1985). In lakes, $\delta^{13}C$ is useful e.g. for differentiating between littoral and pelagic sources (France, 1985).

Instead, the ratio of nitrogen ($\delta^{15}N$) isotopes of an organism is typically enriched by 2.3-3.2‰ relative to its diet, thus can be used to estimate trophic position (Minagawa and Wada ,1984; Peterson and Fry, 1987). Isotopic signature of a consumer alone is not sufficient to infer trophic position or carbon sources without an appropriate isotopic baseline (Post, 2002).

An important step in the study of aquatic food webs through stable isotope analysis is to define a starting point, called isotopic baseline. The simplified model of pelagic aquatic trophic web involves primary producers (phytoplankton), primary consumers (zooplanktonic grazers), secondary consumers (invertebrate predators and zooplanktivours fish) and ichthyophagous fishes. The first studies of lake pelagic food webs (focused on energy fluxes and nutrient turnover), assumed pelagic phytoplankton as primary carbon source sustaining zooplankton primary consumers (Gu et al., 1994). Later on, however, it was demonstrated that contribution to pelagic food web of allochthonous organic material, both of littoral (terrestrial vegetation) and allochthonous (terrestrial sources and soil) origin, may be equal to that of phytoplankton (Hecky and Hesslein, 1995; Jones et al., 1998; Kiyashko et al., 1998). Moreover, 40-55% of Particulate Organic Carbon (POC) and 22-50% of carbon taken up by primary consumers may be of terrestrial origin, and fish may be able to feed on secondary benthic production to about 63% (Pace et al., 2004). The dependence of zooplankton on allochthonous sources tends to decrease with increasing lake trophy (Grey and Jones, 1999; Grey et al., 2000); thus zooplankton of eutrophic lakes may feed primarily on autochthonous sources, whereas relying more on external sources in oligotrophic ones. Furthermore, different lake zones (i.e. pelagic and littoral) are characterized by different isotopic signatures, which make possible to identify the origin of analyzed material (Jones et al., 1998). Isotopic signature of allochthonous carbon is less negative (ranging between -21 and -27‰) than that of autochthonous material (mean $\delta^{13}C$ around -30‰: phytoplankton $\delta^{13}C$ -31.2‰ and detritus -27.8 ‰)

(Jones et al., 1998; Grey et al., 2000; Grey and Jones, 2001). Therefore, pelagic phytoplankton is characterized by more negative δ^{13}C signature than littoral producers (Jones et al., 1998; Grey et al., 2000; Grey and Jones, 2001). On the other hand, nitrogen is affected by anthropogenic activity in the watershed (Cabana and Rasmussen, 1996; Anderson and Cabana, 2006). δ^{15}N baseline can therefore be used as eutrophication proxy (Wada, 2009). Trophic status of a water body influences isotopic signatures of organisms it hosts (Post et al., 2000; Cabana and Rasmussen, 1996; Cattaneo et al., 2004).

Variability among and within (spatial and temporal) aquatic ecosystems makes necessary to define site and time specific baselines to investigate trophic relationships of organisms and their food sources.

Signatures of primary producers are characterized by wide temporal fluctuations depending on seasonal Dissolved Inorganic Carbon (DIC) variations, Particulate Organic Matter (POM) composition, algal productivity, species composition, metabolic processes (Matthews and Madzumder, 2005; Vuorio et al., 2006; Gu et al., 2006), temperature and inorganic nutrient recycling (Perga and Gerdeaux, 2006). Different sources contribute to particulate matter; the signature of which, therefore, integrate isotopic signatures of single components, which can hardly be separated. Contribution of primary producer to suspended matter may prevail in deep, large natural lakes (Cabana and Rasmussen, 1996; Vander Zanden and Rasmussen, 1999; Post, 2002; Matthews and Madzumder, 2003; Vuorio et al., 2006, Visconti et. al, 2011). High turnover rates and seasonal changes in phytoplankton species composition result in seasonal changes in isotopic signatures. Generally in natural lakes of temperate regions, carbon isotopic signature oscillates between summer maxima (^{13}C-enriched) and winter minima (^{13}C depleted) (Perga and Gerdeax, 2006; Visconti and Manca, 2011). It may be not so in reservoirs of the Mediterranean region, as a consequence of the above mentioned peculiarities.

Carbon isotopic signature of a consumer integrates its food isotopic composition over a proper time-scale, raging between weeks for zooplankton and benthos, months for fish (Grey, 2000; Heisslein et al., 1991; Hesslein et al., 1993; Perga and Gerdeaux, 2005; Woodland et al., 2012). Therefore, integration of temporal variations of diet signature is a necessary step for estimating consumer’s role in food web. Primary consumer carbon isotopic signature integrates different carbon sources exploited (pelagic/littoral, allochthonous/autochthonus), leading to spatial differences (Vander Zanden and Rasmussen, 1999 and 2006; Post et al., 2000; Jeppesen et al., 2002). Usually, $\delta^{13}C$ and a $\delta^{15}N$ signatures of primary consumers decrease and increase respectively from littoral to pelagic zone, therefore allowing for tracing origin of carbon and nitrogen sources they use. SIA allowed for tracing opportunistic feeding behavior of zooplankton primary consumers, previously regarded as strict herbivores (e.g., Calanoid copepods and planktonic cladocerans, able to feed upon both, photosynthetic and non-photosynthetic suspended particulate matter; Matthews and Madzumder, 2003). Feeding behavior and diet chosen were influenced both by food availability and by chemical-physical lake conditions (Matthews and Madzumder, 2003). The $\delta^{13}C$ isotopic signatures of individuals of the same species feeding on the same prey may vary as much as 2‰. Differences between isotopic signatures of different species may result from taxa-specific feeding behavior (choice of food and predation depth) and physiology (e.g., body lipid content in different taxa) (Grey and Jones, 1999; Matthews and Madzumder, 2003). Signature of large organisms such as fish, is tissue-specific, depending on tissue turnover rate (De Niro and Epstein, 1978), faster in blood, plasma and hepatic tissue than in muscle and bones (Tieszne et al., 1983; Phillips and Eldrige, 2006).

When stable isotope analysis is applied to study trophic webs, baselines are chosen with particular care, as this is the “starting point” to which all the values obtained will be referred (cfr. “Baseline choice and calculation of trophic levels”). Sampling methods vary according to the different organisms analysed, which must be collected using specific techniques and instruments. Once in the laboratory, samples for SIA analysis are prepared, and for some compartments of organisms of particular interest (phytoplankton and zooplankton, and fish in this case), population density and biomass of taxa are determined to estimate their contribution to matter and energy flow (cfr. “Sampling spatio-temporal variability”). The data obtained are statistically analysed and visualized graphically to produce the final results and define trophic relationships between organisms.

How to use stable isotope signatures

The common elements used in food web studies are carbon (C) and nitrogen (N). C isotopic composition of animals is similar to that of their diet, little varying along the food chain (Peterson and Fry, 1987; Gu et al., 1994; Post, 2002) as $\delta^{13}C$ fractionation during assimilation and respiration is low. Predator-prey difference in $\delta^{13}C$ signature (fractionation: F= δ^{13}Cpredator - δ^{13}Cprey) is usually ≤ 0.8 (SD = ±1.1‰; DeNiro and Eipstein, 1978). C isotopic signature allows identifying energy fluxes from primary producers to consumers of upper levels of the food chain (Gu et al., 1994; Vander Zanden and Rasmussen, 1999, Xu et al., 2005; Gerdeaux and Perga, 2006). Allochthonous matter, e.g. plant detritus, have carbon signature similar to that commonly detected in C3 terrestrial plants (Jones et al., 1998; Grey et al., 2000; Grey and Jones, 2001; Pace et al., 2004). Suspended particulate matter in lakes and reservoirs is made up by detritus carried by streams/rivers as well as by plankton organisms living within (Pace et al., 2004; Xu et al., 2005). Temporal intra-specific variations in $\delta^{13}C$ signature of zooplankton and its food are non-linear, because of changes in lipid content (Matthews and Madzumder, 2005). Lipids have a lower $\delta^{13}C$ signature than other compounds, thus, the relationship between $\delta^{13}C$ and lipids will always be negative. The lipid content in organic tissues is directly proportional to C:N ratio and hence, the relationship between $\delta^{13}C$ and C:N ratio is usually negative (Madzumder, 2005).

Conversely, N isotopic signatures of different organisms are useful for assessing their trophic relationship. ^{15}N enrichment in predator-prey relationship usually ranges between 3-5‰ (SD = ±1.3‰) (Peterson and Fry, 1987; Gu et al., 1994; France and Peters, 1997; Vander Zanden and Rasmussen, 1999; Grey and Jones, 2001; Jeppesen et al., 2002). Values of 3-5‰ are quite approximate, however, representing a mean of the statistical distribution of the $\delta^{15}N$ differences along different trophic chains (Adams et al., 2000). The most used values to estimate source-consumer enrichment is 3.4‰ (S.D. = ±1.0‰) suggested by Post (2002). Moreover, difference in nitrogen stable isotopes (ΔN= Enrichment (E) =δ^{15}N taxon - δ^{15}N source) between consumer and diet depends on availability of environmental nitrogen (Adams et al., 2000). The increase in $\delta^{15}N$ between trophic levels, resulting from ^{15}N enrichment during metabolism, is mainly due to retention of the heavy ^{15}N isotope during biosynthesis of aminoacids and to the excretion of the lighter ^{14}N isotope (Gu et al., 1994; Adams et al., 2000).

Other organisms can be used as a proxy for suspended particulate matter or other primary food sources to trace the isotopic baseline. Filter-feeding bivalves like zebra mussel (*Dreissena polymorpha*, Pallas 1771) can be used as proxy for seston material, because they are good temporal integrators of seasonal isotopic variability. Instead, gastropod grazers, such as river limpet (*Ancylus fluviatilis*, O.F. Müller 1774), as well as other freshwater snails (Post et al*.,* 2000; Post, 2002), can be used as proxy for integrating littoral sources. Zooplankton primary consumers were used to trace seasonal changes in pelagic baseline, as they have shorter life and metabolic rates (Gu et al*.,* 1994; Cabana and Rasmussen, 1996; Perga and Gerdeaux, 2006).

When multiple sources are exploited by consumers, contribution of each source can be estimated by applying a linear mixing model (Phillips, 2001; Phillips and Koch, 2002). Carbon signature of a consumer tissue is assumed to be the linear mass balance of elemental mass from each food source. Mass balance must equal 1; therefore, when two sources are used, the mass balance is given by:

$$\delta^{13}C\text{predator} = \alpha x \ x \delta^{13}Cx + \alpha y \ x \ \delta^{13}Cy \quad [2]$$

Where: $\alpha x + \alpha y = 1$. x and y are the two food sources (e.g.: x = pelagic, y = littoral) and α is the fractional carbon contribution to each sources to the predator diet. When three or more food sources are available, the mass balance becomes $1 = \alpha x + \alpha y + \alpha z \ldots + \alpha$ and so on (Phillips, 2001).

Accordingly, when three food sources are available, they can be partitioned using isotopic ratios of two elements (e.g., $\delta^{13}C$ and $\delta^{15}N$), and the mass balance equation becomes a system of three equations with three unknowns:

$$\delta^{13}C \text{ predator} = \alpha x * \delta^{13}Cx + \alpha y * \delta^{13}Cy + \alpha z * \delta^{13}Cz$$
$$\delta^{15}N \text{ predator} = \alpha x * \delta^{15}Nx + \alpha y * \delta^{15}Ny + \alpha z * \delta^{15}Nz$$
$$1 = \alpha x + \alpha y + \alpha z$$

Where: x, y and z are the three food sources and α is the fractional carbon contribution to each source to the predator diet.

For organisms with turnover rates longer than their sources, such as fish, a linear dynamic mixing model can be applied (Woodland et al., 2011). Isotopic signature of a consumer is the result of both, diet signature and specific growth rate, which is also tissue-specific. Isotopic consumer signature measured at any given time t (δ^{13}Ccons (t)) is therefore expressed as:

$$\delta^{13}Xcons(t)= \delta^{13}Xn + (\delta^{13}X0 - \delta^{13}Xn) e-(k+m)t$$

Where δ^{13}X0 is the initial isotope value of a consumer in equilibrium with its food at time t = 0, in natural populations assumed as <2‰ richer than the food source signature (Hesslein et al., 1993); δ^{13}Xn is the isotope signature of a consumer in equilibrium with its diet at time t (in the case of fish t = 60 days); k is the growth rate (per day) of the consumer and m is the metabolic tissue turnover constant (per day).

AIMS

The main purpose of this thesis was to contribute to knowledge of artificial lakes of Mediterranean area, carrying out a research to understand the functioning of food webs in artificial lakes of Sardinia, as examples of the Mediterranean typology.

With an initial screening on several Sardinian artificial lakes, the Lake Sos Canales was identified as case-study to achieve the following milestones by the use of stable isotopes analysis of carbon and nitrogen to:

1) suspended particulate matter, composition of phytoplankton and seasonal dynamics, relationships with environmental variables and their isotopic signatures of carbon and nitrogen,

2) composition of zooplankton and seasonal dynamics, its relationships with environmental variables and isotopic signatures of carbon and nitrogen,

4) composition of fish community and its seasonal dynamics, its relationships with environmental variables and isotopic signatures of carbon and nitrogen.

VERSITA

Biologia **66**/5: 856—865, 2011
Section Zoology
DOI: 10.2478/s11756-011-0092-4

First record of planktonic crustaceans in Sardinian reservoirs

Amedeo Fadda[1,2], Silvia Marková[3], Petr Kotlík[3], Antonella Lugliè[1], Bachisio Padedda[1], Paola Buscarinu[2], Nicola Sechi[1] & Marina Manca[4]

[1] *Dipartimento di Scienze Botaniche, Ecologiche e Geologiche dell'Università degli Studi di Sassari Via Piandanna 4, 07100 Sassari (SS), Italy; e-mail: amedeo.fadda@enas.sardegna.it*
[2] *Ente acque della Sardegna (ENAS), Via Mameli 88, 09123 Cagliari (CA), Italy*
[3] *Department of Vertebrate Evolutionary Biology and Genetics, Academy of Sciences of the Czech Republic, CZ-27721 Liběchov, Czech Republic*
[4] *CNR Institute for Ecosystem Studies (CNR ISE), Largo Tonolli 50, 28922 Verbania (VB), Italy*

Abstract: Sardinian man-made lakes are reservoirs of species richness, hosting zooplankton taxa from the Mediterranean region and North Africa. To provide a first record of the taxa composition and diversity of zooplankton communities, we sampled 15 reservoirs during 2008–2009, from the north of the island to the south, representative of a range of size, depth, renewal time, and trophy. The survey was complemented by seasonal sampling in one of the largest lakes studied. Water samples collected from surface to bottom provided data on hydrochemistry and trophy. Crustacean dormant stages were inspected from sediments of the richest, and most diverse, Lake Sos Canales. RDA suggested that productivity, water depth, renewal time and altitude were the main variables related to taxa composition. The ubiquitous *Copidodiaptomus numidicus*, and its persistence in the water column, resulted from the production of subitaneous eggs throughout the year, an adaptive strategy in perennial water bodies. Genetic analyses of DNA sequences of the diagnostic gene ND5 placed the Sardinian *Daphnia pulex* in the North American group. Moreover, the ND5 sequence found in Sardinia was identical with that of an asexual hybrid clone between the American *D. pulex* and American *D. pulicaria* that replaced native *D. pulex* throughout Africa. The presence of this ND5 haplotype in Sardinia shows that this invasive clone also poses an invasive threat to native populations in Europe.

Key words: zooplankton; *Copidodiaptomus*; *Daphnia*; endemisms; reservoirs; Sardinia

Introduction

Freshwater zooplankton taxa composition, richness and diversity are mainly influenced by geographical and morphoedaphic characteristics (Hobæk et al. 2002). The Mediterranean Region represents an important biodiversity spot, in which freshwater environments host endemic as well as cosmopolitan taxa (Marrone et al. 2006a). Located in the central part of the Mediterranean Basin, Sardinia is of great significance for the biodiversity of aquatic species, in both temporary ponds and perennial water bodies. So far, zooplankton studies in Sardinia have mainly focused on ponds, with reservoirs only occasionally being investigated. The latter are the only perennial water bodies in Sardinia, as in most Mediterranean Region. Because they are often used as a drinking water supply, they have mainly been monitored for hydrochemistry and primary productivity, with a view to promoting sustainable exploitation (Sechi & Cossu 1979; Sechi & Lugliè 1992).

In a first attempt to provide a record of crustacean zooplankton taxa richness and diversity, we sampled zooplankton from 15 reservoirs from northern to southern Sardinia, representative of the main river basins, and of different size, depth, renewal time, and productivity. As a general rule, lake size is positively correlated with zooplankton species richness (Dodson 1992). Shape, in particular how elongated a reservoir is, may also be important: longitudinal gradients in canyon-shaped reservoirs provide opportunities for spatial segregation of species and hybrid zones (Seďa et al. 2007; Petrusek et al. 2008a). Lake depth is also important, since deeper lakes should allow more room for vertical niche segregation (Størm 1946). In reservoirs, in addition to these factors, zooplankton taxa richness and diversity are also related to water renewal time (Tundisi et al. 1998), which is affected by both natural and human-driven impacts. Trophy is also known to influence planktonic crustacean diversity, with a decline of diversity and richness at high productivity levels (Mittelbach et al. 2001).

Sampling at a single time is not sufficient to cover the local biodiversity but, given the constraints, we sampled a number of sites representative of different characteristics, in a "space per time approach", successfully applied for other sites in Europe (e.g., for alpine lakes as part of the EMERGE Project; Tolotti et al. 2006), which was complemented with additional information on the seasonal dynamics of the second largest reservoir. Seasonality is much more pronounced

Springer

in Mediterranean reservoirs than in natural deep lakes of other regions, as a result of human activities and the climate.

In highly variable aquatic environments, zooplankton often produces resting stages that represent a pool from which active specimens are recruited over the seasons. Analysis of resting egg bank may provide additional data on local species richness, which cannot be covered by one-time sampling. To account for this aspect, the resting stages preserved in the sediments in one of the more diverse reservoirs were analyzed, in an attempt to characterize potential biodiversity and estimate vulnerability to biodiversity loss.

Crustaceans were determined using identification keys based on morphological characters (see Material and methods). The exception was the cladoceran *Daphnia pulex* (L., 1758) complex where the species identity was checked by sequencing a diagnostic mitochondrial DNA gene. Although the common perception now is that genetically different species of *D. pulex* and *D. pulicaria* (Forbes, 1893) inhabit temperate Europe than America (e.g., Colbourne et al. 1998; Marková et al. 2007), recent studies showed the presence in Europe of the North American *D. pulicaria* (Marková et al. 2007) and of its hybrids with the North American *D. pulex* (Mergeay et al. 2006).

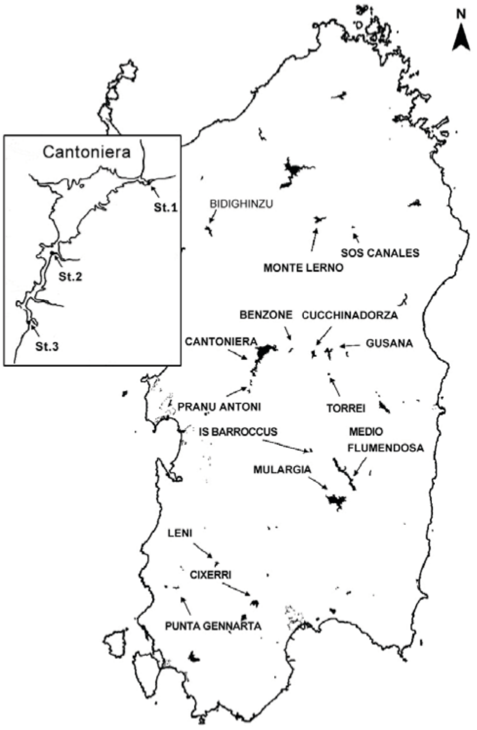


Fig. 1. Location of the 15 Sardinian reservoirs selected for our survey on zooplankton in 2008–2009. Sampling stations in Lake Cantoniera are also shown (for explanation see text).

Material and methods

Between late summer and early autumn 2008 (August 27 and October 15), zooplankton samples were collected from 15 Sardinian reservoirs belonging to the main river systems of the island (Fig. 1 and Table 1). Samples were collected at a station located near the dam, in the deepest part of each reservoir, where the depth was always >20 m. In Lake Cantoniera (previously known as Omodeo), the largest reservoir in Sardinia, two other stations in the main basin were also included (in a central area and at the inflow of the Taloro River; Fig. 1). All reservoirs were visited once, with the exception of Sos Canales, which was sampled approximately bi-monthly between May 2008 and August 2009.

In all lakes but Benzone, Cucchinadorza, Gusana and Pranu Antoni, the Secchi depth (SD) water transparency, *pH* and temperature vertical profiles, as well as conductivity, dissolved oxygen (DO), total nitrogen (TN) and reactive phosphorus (RP) were measured on the same date of our sampling (data kindly provided by Ente Acque della Sardegna, ENAS; Table 1). Conductivity, DO, TN and RP were analyzed following Strickland & Parsons (1972) (Table 1). Data on the morphology, renewal time and trophy of all reservoirs were taken from previous studies (Sechi & Lugliè 1992, 1996; Marchetto et al. 2009). Qualitative samples were collected via vertical hauls with an Apstein zooplankton net (mesh size 200 μm), from 20 m depth to the surface, and preserved in 90% ethanol.

In Lake Sos Canales, in addition to zooplankton, sediment samples were collected at the same station with an Ekman grab, to inspect dormant stages of crustacean zooplankton. The samples were kept for 6 months at 4℃ in the dark, before being treated according to Onbè (1978) for extraction of cladoceran ephippia and calanoid resting eggs. Both were identified, counted and sorted into hatched and un-hatched specimens. The contribution of each taxon to total abundance was estimated. We ran hatching experiments on 50 eggs/taxa. Each egg was transferred into 5 ml of oligomineral water filtered through 0.4 μm GF/C glass fibre filters, and incubated in a thermostatic cell at a constant temperature of 20℃, a light intensity of 2.8–3.3×10^3 Lux and a photoperiod of 16 h light : 8 h dark for hatching. The culture medium was renewed every other day, and hatchlings recorded daily for one month.

Copepods and cladocerans were identified at least to genus, and in most cases to species level. Identification keys by Einsle (1993, 1996), Dussart (1967) and Stella (1982) were used for copepods, while Cladocera (except *D. pulex*, see below) were identified following Margaritora (1983) and Alonso (1996). Each sample was counted on at least half of the total volume, to estimate the contribution of the various taxa to total abundance. We calculated the Shannon-Wiener diversity index (*H*, base 2-logarithm; Shannon & Weaver 1949), as well as the Evenness index (*e*) according to Pielou (1966).

To compare with data on reservoirs of the same biogeographic region, and hypothesizing that differences might be environmental-sound, the body length (prosome + urosome, furcal rami excluded; Einsle 1993) of adults of the ubiquitous *Copidodiaptomus numidicus* (Gurney, 1909) (50 females and 50 males or, when fewer, all individuals) was

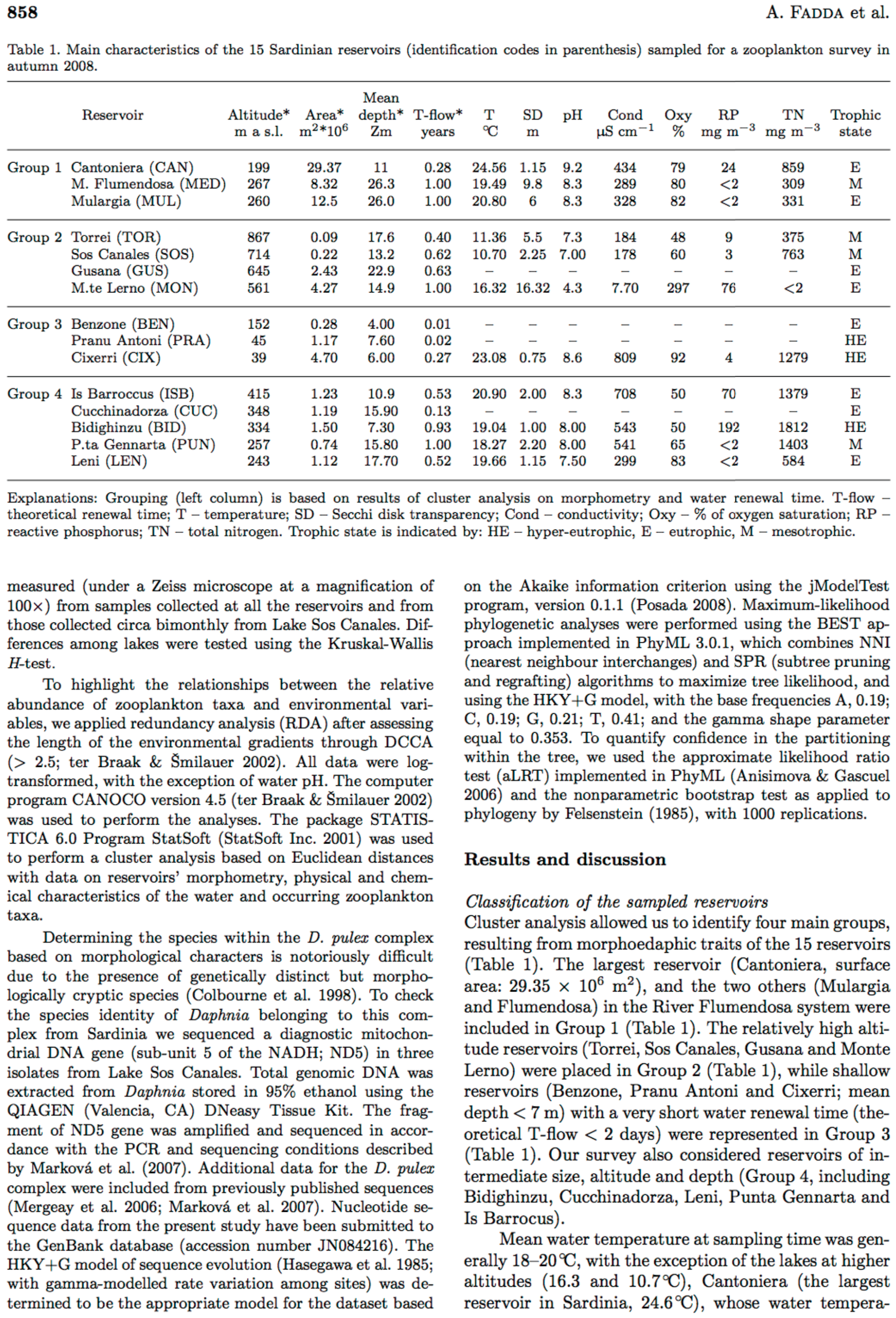


Table 1. Main characteristics of the 15 Sardinian reservoirs (identification codes in parenthesis) sampled for a zooplankton survey in autumn 2008.

	Reservoir	Altitude* m a s.l.	Area* m^2*10^6	Mean depth* Zm	T-flow* years	T °C	SD m	pH	Cond µS cm^{-1}	Oxy %	RP mg m^{-3}	TN mg m^{-3}	Trophic state
Group 1	Cantoniera (CAN)	199	29.37	11	0.28	24.56	1.15	9.2	434	79	24	859	E
	M. Flumendosa (MED)	267	8.32	26.3	1.00	19.49	9.8	8.3	289	80	<2	309	M
	Mulargia (MUL)	260	12.5	26.0	1.00	20.80	6	8.3	328	82	<2	331	E
Group 2	Torrei (TOR)	867	0.09	17.6	0.40	11.36	5.5	7.3	184	48	9	375	M
	Sos Canales (SOS)	714	0.22	13.2	0.62	10.70	2.25	7.00	178	60	3	763	M
	Gusana (GUS)	645	2.43	22.9	0.63	–	–	–	–	–	–	–	E
	M.te Lerno (MON)	561	4.27	14.9	1.00	16.32	16.32	4.3	7.70	297	76	<2	E
Group 3	Benzone (BEN)	152	0.28	4.00	0.01	–	–	–	–	–	–	–	E
	Pranu Antoni (PRA)	45	1.17	7.60	0.02	–	–	–	–	–	–	–	HE
	Cixerri (CIX)	39	4.70	6.00	0.27	23.08	0.75	8.6	809	92	4	1279	HE
Group 4	Is Barroccus (ISB)	415	1.23	10.9	0.53	20.90	2.00	8.3	708	50	70	1379	E
	Cucchinadorza (CUC)	348	1.19	15.90	0.13	–	–	–	–	–	–	–	E
	Bidighinzu (BID)	334	1.50	7.30	0.93	19.04	1.00	8.00	543	50	192	1812	HE
	P.ta Gennarta (PUN)	257	0.74	15.80	1.00	18.27	2.20	8.00	541	65	<2	1403	M
	Leni (LEN)	243	1.12	17.70	0.52	19.66	1.15	7.50	299	83	<2	584	E

Explanations: Grouping (left column) is based on results of cluster analysis on morphometry and water renewal time. T-flow – theoretical renewal time; T – temperature; SD – Secchi disk transparency; Cond – conductivity; Oxy – % of oxygen saturation; RP – reactive phosphorus; TN – total nitrogen. Trophic state is indicated by: HE – hyper-eutrophic, E – eutrophic, M – mesotrophic.

measured (under a Zeiss microscope at a magnification of 100×) from samples collected at all the reservoirs and from those collected circa bimonthly from Lake Sos Canales. Differences among lakes were tested using the Kruskal-Wallis *H*-test.

To highlight the relationships between the relative abundance of zooplankton taxa and environmental variables, we applied redundancy analysis (RDA) after assessing the length of the environmental gradients through DCCA (> 2.5; ter Braak & Šmilauer 2002). All data were log-transformed, with the exception of water pH. The computer program CANOCO version 4.5 (ter Braak & Šmilauer 2002) was used to perform the analyses. The package STATISTICA 6.0 Program StatSoft (StatSoft Inc. 2001) was used to perform a cluster analysis based on Euclidean distances with data on reservoirs' morphometry, physical and chemical characteristics of the water and occurring zooplankton taxa.

Determining the species within the *D. pulex* complex based on morphological characters is notoriously difficult due to the presence of genetically distinct but morphologically cryptic species (Colbourne et al. 1998). To check the species identity of *Daphnia* belonging to this complex from Sardinia we sequenced a diagnostic mitochondrial DNA gene (sub-unit 5 of the NADH; ND5) in three isolates from Lake Sos Canales. Total genomic DNA was extracted from *Daphnia* stored in 95% ethanol using the QIAGEN (Valencia, CA) DNeasy Tissue Kit. The fragment of ND5 gene was amplified and sequenced in accordance with the PCR and sequencing conditions described by Marková et al. (2007). Additional data for the *D. pulex* complex were included from previously published sequences (Mergeay et al. 2006; Marková et al. 2007). Nucleotide sequence data from the present study have been submitted to the GenBank database (accession number JN084216). The HKY+G model of sequence evolution (Hasegawa et al. 1985; with gamma-modelled rate variation among sites) was determined to be the appropriate model for the dataset based on the Akaike information criterion using the jModelTest program, version 0.1.1 (Posada 2008). Maximum-likelihood phylogenetic analyses were performed using the BEST approach implemented in PhyML 3.0.1, which combines NNI (nearest neighbour interchanges) and SPR (subtree pruning and regrafting) algorithms to maximize tree likelihood, and using the HKY+G model, with the base frequencies A, 0.19; C, 0.19; G, 0.21; T, 0.41; and the gamma shape parameter equal to 0.353. To quantify confidence in the partitioning within the tree, we used the approximate likelihood ratio test (aLRT) implemented in PhyML (Anisimova & Gascuel 2006) and the nonparametric bootstrap test as applied to phylogeny by Felsenstein (1985), with 1000 replications.

Results and discussion

Classification of the sampled reservoirs

Cluster analysis allowed us to identify four main groups, resulting from morphoedaphic traits of the 15 reservoirs (Table 1). The largest reservoir (Cantoniera, surface area: 29.35×10^6 m^2), and the two others (Mulargia and Flumendosa) in the River Flumendosa system were included in Group 1 (Table 1). The relatively high altitude reservoirs (Torrei, Sos Canales, Gusana and Monte Lerno) were placed in Group 2 (Table 1), while shallow reservoirs (Benzone, Pranu Antoni and Cixerri; mean depth < 7 m) with a very short water renewal time (theoretical T-flow < 2 days) were represented in Group 3 (Table 1). Our survey also considered reservoirs of intermediate size, altitude and depth (Group 4, including Bidighinzu, Cucchinadorza, Leni, Punta Gennarta and Is Barrocus).

Mean water temperature at sampling time was generally 18–20 °C, with the exception of the lakes at higher altitudes (16.3 and 10.7 °C), Cantoniera (the largest reservoir in Sardinia, 24.6 °C), whose water tempera-

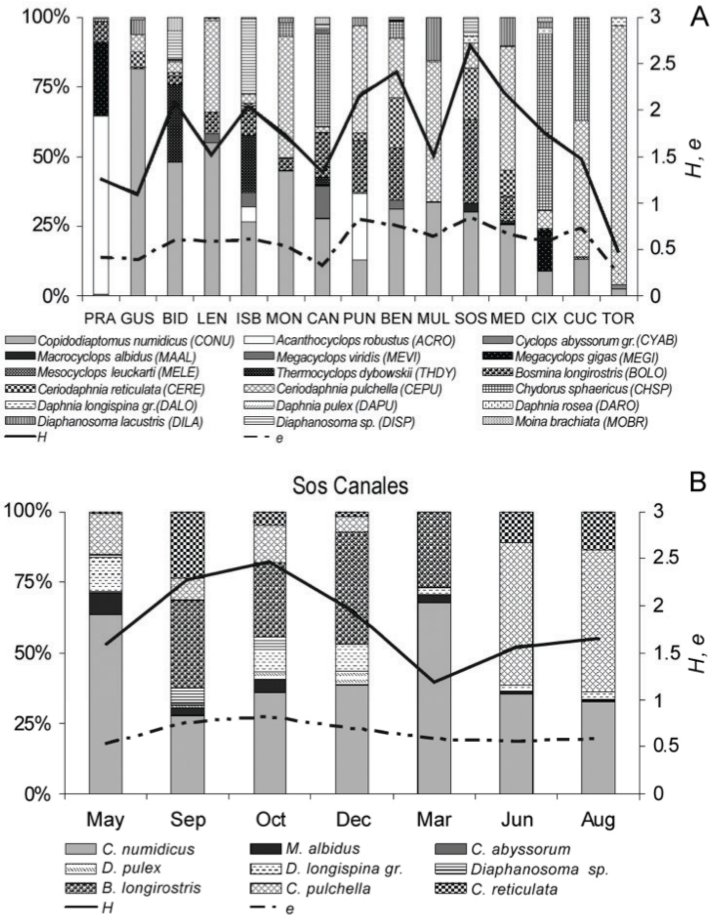


Fig. 2. A: Relative abundance (left axis), Shannon-Wiener diversity (*H*) and evenness (*e*) of crustacean zooplankton taxa from 15 Sardinian reservoirs in autumn 2008. The lake ordination from left to right is based on results of a cluster analysis of taxa relative abundances (only those with ≥2% of the total shown). DARO refers to the presence of a "rosea-like" (Alonso 1996) morph within *D. longispina* populations.
B: Relative abundance (left axis), Shannon-Wiener diversity (*H*) and evenness (*e*) of crustacean zooplankton taxa from Lake Sos Canales in 2008–2009.

ture was measured at the end of August, and Cixerri (23.1℃; Table 1).

Secchi depth (water transparency) ranged from 0.75 m in the shallow Lake Cixerri to 9.8 m in Lake Medio Flumendosa (the deepest reservoir in Sardinia). Water pH varied between values close to 7, measured in higher altitude reservoirs (Group 2), and values around 8 in large to medium sized ones (Group 4). The highest pH value (9.2 units) was measured in Lake Cantoniera (the only lake sampled in late August, Table 1). Water conductivity ranged from 178 µS cm^{-1} (Sos Canales, Group 2) to 809 µS cm^{-1} (Cixerri, the lake at the lowest altitude (39 m a.s.l., Group 3).

Oxygen saturation level varied from a minimum of 48% (Torrei, Group 2) to a maximum of 92% (Cixerri, Group 3).

TN and *RP* concentrations ranged from respective values of < 2 mg P m^{-3} and 309 mg N m^{-3} in Medio Flumendosa reservoir (Group 1) to 192 mg P m^{-3} and 1812 mg N m^{-3} in the most productive lake (Bidighinzu; Group 4, Table 1). The reservoirs were classified as eutrophic (Cantonieria, Mulargia, Gusana, Monte Lerno, Benzone, Is Barrocus, Leni and Cucchinadorza), mesotrophic (Medio Fludendosa, Torrei, Sos Canales, Punta Gennarta) and hyper-eutrophic (Bidighinzu, Cixerri, Pranu Antoni) (Sechi & Lugliè 1992; Marchetto et al. 2009) according to the OECD (1982).

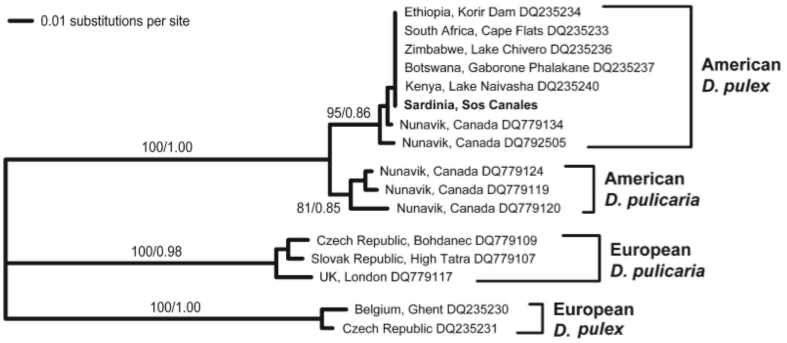


Fig. 3. Phylogenetic tree of *ND5* sequences showing the relationships of the haplotype carried by the Sardinian isolates with other members of the *Daphnia pulex* complex. Statistical support for the major clades is expressed as the percentage bootstrap proportions of 1000 bootstrap replicates and as the SH-like aLRT probabilities.

Crustacean zooplankton taxa composition

Overall, we identified 21 crustacean zooplankton taxa, thirteen belonging to the Cladocera and eight to the Copepoda (Fig. 2A). Species richness was only slightly smaller than that reported by Calvo et al. (1993) and Alfonso et al. (2010) for permanent lakes in Sicily, although in the latter case, samplings were repeated for the four seasons and in double number of lakes than was ours (30, 25 of which artificial). In our study, the number of taxa/lake varied between a minimum of 4 (in Torrei and Cucchinadorza) to a maximum of 12 (in Cantoniera); 53% of the lakes had a number of taxa > 7 at the time of sampling. Within the copepods, seven were cyclopoids, and only one was a calanoid (*Copidodiaptomus numidicus* Gurney, 1909). The latter was the only species with a geographical distribution restricted to the Mediterranean area and North Africa (Armengol 1980; Cherbí 1984). This species was found in all reservoirs, at different levels of relative abundance. When largely dominant, as in the case of Lake Gusana, it was the only copepod species present. Cyclopoids included taxa of a large to very large body size (1.9–2.5 mm; Einsle 1993), namely *Macrocyclops albidus* (Jurine, 1820), *Megacyclops gigas* (Claus, 1857), *M. viridis* (Jurine, 1820), and of the *Acanthocyclops vernalis-robustus* species complex (Sars, 1863) (Blaha et al. 2010). Species in the *Cyclops abyssorum* group (Sars, 1863) were also found (in Sos Canales and Torrei; Figs 2A, B). As for the smaller-sized taxa, *Mesocyclops leuckarti* (Claus, 1857) was found in Lake Monte Lerno at a low abundance, and *Thermocyclops dybowski* (Lande, 1890) was observed in Cantoniera, Is Barrocus, Bidighinzu and Medio Flumendosa.

Of the small-sized cladocerans, *Bosmina longirostris* (O.F. Müller, 1776) was found in most reservoirs (11), in one case (Lake Sos Canales) representing a large proportion of total zooplankton abundance (Figs 2A, B). *Ceriodaphnia pulchella* (G.O. Sars, 1862), and *C. reticulata* (Jurine, 1820) were almost ubiquitous; in some cases (particularly in Torrei, Mulargia, Medio Flumendosa, Cucchinadorza and Punta Gennarta), the former made up the largest proportion of total zooplankton abundance. *Chydorus sphaericus* was detected in hyper-eutrophic and eutrophic reservoirs (such as Cixerri, Cucchinadorza and Cantoniera). In addition, *Leydigia leydigi* (Schödeler, 1862), *Chydorus* sp. and *Alona affinis* (Leydig, 1860) were found in lakes Mulargia, Monte Lerno and Pranu Antoni, respectively, although at very low (< 2% of the total) abundance. Two species of the genus *Diaphanosoma*, *D. lacustris* (Kořínek, 1981) and *Diaphanosoma* sp., were found in 9 of the 15 reservoirs, and with a remarkable abundance in Lake Is Barrocus. *Moina brachiata* (Jurine, 1820) was found in lakes with a relatively high conductivity (Table 1 and Fig. 2A).

The *Daphnia* genus was well represented in the sampled reservoirs (in 9 out of 15). We observed species of the *D. pulex* group (putative *D. pulex* Leydig, 1860; after Margaritora 1983, see below) and of the *D. longispina* group (as defined in Petrusek et al. 2008b; Thielsch et al. 2009) with parental species and hybrids. Previously, *D. pulex* had only been reported from fishless temporary ponds in Sardinia; this is the first record of the presence of this species in lakes with fish like the Sardinian reservoirs. In eight reservoirs, we only detected species of the *D. longispina* group, while in one case (Sos Canales), they coexisted with *D. pulex*.

Genetic analyses of Daphnia pulex

The phylogenetic analysis of the ND5 gene sequences unequivocally placed the putative *D. pulex* isolates from Lake Sos Canales within the American *D. pulex* clade, which is clearly distinct from the European *D. pulex* (Fig. 3). Moreover, the haplotype carried by the Sardinian isolates was identical to the homologous part of the haplotype carried by an asexual clone described by Mergeay et al. (2006) as being widely distributed throughout eastern and southern Africa. Mergeay et al. (2006) inferred that this clone was actually a hybrid between the American *D. pulex* and American *D. pulicaria* that recently invaded Africa and has spread and replaced native *D. pulex* throughout its African range (Mergeay et al. 2006). Although Mergeay et al. (2006) did not find the invasive clone outside Africa, they refer to a previously published 12S rRNA mitochondrial gene sequence from Spain as being identical to the 12S sequence of the invasive African clone (Mergeay et al. 2006). Our results for the isolates from Lake Sos Canales in Sardinia unequivocally demonstrate that *Daphnia* carrying the ND5 sequence of the invasive clone occur in the Mediterranean region of Europe (Fig. 3). Although it remains to be demonstrated whether *Daphnia* in Sardinia with this mtDNA clone actually belongs to the invasive hybrid lineage, the significance of this finding should not be underestimated. This clone replaced native *D. pulex* throughout Africa within 60 years of its introduction (Mergeay et al. 2006), and although it is presently known to occur in Europe only in the Mediterranean region, it presents a real threat to *Daphnia* populations elsewhere in Europe.

Diversity and seasonality

The Shannon-Wiener diversity index (H) and evenness (e; Fig. 2A) did not appear to be influenced by lake size and depth: their maximum values were observed in Lake Sos Canales, one of the smaller lakes at a relatively high elevation. Eight zooplankton taxa were detected in this reservoir, including *D. pulex*, *D. longispina*, the ubiquitous *C. pulchella*, the rarer *C. reticulata*, *B. longirostris* and *Diaphanosoma* sp. The copepod taxa observed were *C. numidicus*, *M. albidus* and a species of the *C. abyssorum* group, present at a lower proportion. Diversity and evenness were also relatively high in Lake Benzone, which is also in the group of shallow lakes (Group 3; Table 1). All but *D. pulex* were common, widely distributed cladoceran taxa, already included in the inventory from inland, mainly temporary, waters of Sicily (Marrone et al. 2006b), and from temporary ponds of Sardinia and Corsica (Margaritora 1975). Up to eight copepod species were found, seven of which being small to large-sized cyclopoids, and only one of calanoids, namely, the ubiquitous *C. numidicus*. The latter was one of the nine calanoid taxa reported from temporary ponds of Sicily (Marrone et al. 2006a).

The lowest diversity and evenness were observed in Torrei (Fig. 2A), where a single species (*C. pulchella*) accounted for $> 80\%$ of the total abundance.

Both H and e can vary widely throughout the seasons. An analysis of seasonal changes based on bimonthly samples collected from 2008 to 2009 in one of the studied reservoirs (Sos Canales) indicates that H and e were highest in September – October (Fig. 2B). This suggests that, in agreement with data in literature, the time of the year selected for our survey of crustacean zooplankton taxa was appropriate (Manca & Armiraglio 2002; Tolotti et al. 2006) for the detection of adult stages of copepods and of most cladoceran taxa in the various reservoirs. The Cladocera were especially well represented, with *Bosmina* and *Ceriodaphnia* coexisting with both the large *D. pulex* and the smaller *D. longispina* gr. As further suggested by the seasonal data on Lake Sos Canales, *Ceriodaphnia*'s ability to grow in all seasons may explain its ubiquitous presence in reservoirs (Figs 2A, B). In Lake Sos Canales, *Ceriodaphnia* became dominant in June-August, replacing *Bosmina longirostris*, which tended to dominate between September and March (Fig. 2B).

Bosmina longirostris disappeared from the water column in summer, probably after the production of ephippial eggs, which were found in significant numbers in the surface sediments of the lake. *Bosmina* ephippia represented the largest fraction of total ephippia recovered from the sediments (550 out of 794, corresponding to 69% of the total ephippia found). They resulted viable in non-negligible numbers (15% of total eggs), suggesting that hatching of ephippial eggs may be important for yearly recruitment of *Bosmina* specimens into the water column (Hairston 1996).

Similarly, the persistence of *C. numidicus* throughout the year in Lake Sos Canales may be related to its ubiquitous presence in all sampled reservoirs (Figs 2A, B). Such persistence suggests that, unlike the situation observed in temporary ponds, this species does not rely upon the production of resting stages in reservoirs. Our results seem to confirm those previously reported by Stella (1970), who found that only subitaneous eggs were produced in perennial water bodies, while resting eggs' production was confined to temporary water bodies.

Body length of Copidodiaptomus numidicus

Adult body length of *C. numidicus* from the 15 sampled reservoirs ranged from a minimum of 0.90 mm (in Sos Canales and Monte Lerno lakes) to a maximum of 1.62 mm (in Lake Punta Gennarta; Table 2A). Differences in the size of adults from different lakes (Kruskal-Wallis H-test: 14, $n = 1401$) resulted highly statistically significant ($P < 0.001$). The differences were also statistically significant when data on the various segments of the adult population (i.e., males, females and ovigerous females) were compared for the different sites. Males' body length ranged between 0.90 (in Sos Canales and Monte Lerno lakes) and 1.37 mm (in Lake Punta Gennarta). Ovigerous females measured between 1.44 (in Lake Punta Gennarta) and 1.07 mm (in Lake Monte Lerno). Overall, specimens from the 15 reservoirs tend to be smaller than those reported by Stella from temporary ponds in north-eastern Sardinia. They are also generally smaller than those reported from reservoirs in Portugal (Parra et al. 2009), except for three sites (lakes Punta Gennarta, Torrei, Is Barroccus) where body length was fully comparable to data in the literature.

Smaller body sizes are often observed in permanent reservoirs compared to temporary ponds. This fact may be explained by factors such as predation, as predation by invertebrates encourages zooplankton to grow larger in ponds, while fish predation in lakes favours smaller body sizes (e.g., Brooks & Dodson 1965). Clutch size (n. eggs clutch^{-1}) was correlated with the body length (BL, expressed as logBL) of ovigerous females at highly

Table 2. Body length of adult (n = number of specimens) *C. numidicus* from 15 sampled reservoirs in Sardinia. Data are compared with those from Sardinian temporary ponds and from reservoirs in Portugal. The presence of *Mesostoma* is also reported.

Site	Adults			Ovigerous females			Clutch size			Females			Males		
	Mean (n)	Min	Max	Mean (n)	Min	Max	Mean (n)	Min	Max	Mean (n)	Min	Max	Mean (n)	Min	Max
PRA*	1.01 (6)	0.95	1.08	–	–	–	–	–	–	1.07 (3)	1.05	1.08	0.96 (3)	0.95	0.98
GUS*	1.10 (100)	0.96	1.25	1.17 (77)	1.15	1.20	4.57 (7)	4	6	1.15 (43)	1.10	1.62	1.02 (50)	0.96	1.10
BID*	1.13 (100)	0.96	1.25	1.18 (39)	1.12	1.25	8.87 (39)	6	13	1.19 (1)	1.15	1.20	1.07 (50)	0.96	1.15
LEN	1.14 (100)	0.99	1.20	1.18 (21)	1.15	1.20	5.00 (21)	4	6	1.18 (29)	1.12	1.20	1.10 (50)	0.99	1.15
ISB	1.27 (100)	1.13	1.45	1.30 (39)	1.25	1.45	9.89 (39)	2	16	1.34 (11)	1.13	1.41	1.20 (50)	1.13	1.27
MON	1.02 (100)	0.90	1.15	1.07 (11)	1.02	1.09	2.73 (11)	2	6	1.08 (39)	1.05	1.15	0.96 (50)	0.90	0.99
CAN	1.10 (100)	0.98	1.25	1.16 (17)	1.09	1.25	3.35 (17)	1	5	1.15 (32)	1.06	1.25	1.04 (50)	0.98	1.11
PUN	1.36 (100)	1.02	1.62	1.44 (14)	1.42	1.47	7.86 (14)	2	12	1.44 (36)	1.32	1.62	1.28 (50)	1.20	1.37
BEN	1.19 (100)	1.06	1.35	1.24 (15)	1.12	1.35	5.86 (15)	4	8	1.24 (35)	1.11	1.32	1.13 (50)	1.06	1.22
MUL*	1.08 (100)	0.98	1.18	1.12 (14)	1.10	1.13	3.69 (16)	2	8	1.12 (34)	1.08	1.18	1.04 (50)	0.98	1.12
SOS*	1.07 (100)	0.90	1.38	1.19 (25)	1.09	1.38	4.24 (25)	2	6	1.24 (25)	1.13	1.33	0.93 (50)	0.90	0.98
MED*	1.08 (100)	1.02	1.18	1.14 (5)	1.13	1.16	4.00 (5)	4	4	1.21 (45)	1.05	1.18	1.05 (50)	1.02	1.08
CIX*	1.17 (100)	1.06	1.28	1.23 (44)	1.16	1.28	7.93 (44)	6	12	1.25 (6)	1.18	1.23	1.12 (50)	1.06	1.17
CUC*	1.12 (100)	1.06	1.23	1.11 (27)	1.06	1.16	3.18 (27)	1	6	1.15 (23)	1.07	1.23	1.11 (50)	1.09	1.15
TOR*	1.29 (100)	1.12	1.51	1.42 (34)	1.31	1.51	9.41 (34)	5	12	1.40 (16)	1.30	1.50	1.17 (50)	1.12	1.22
Temporary ponds										1.70**			1.65**		
S. Luzia, Portugal				1.41***			6.86***								
Maranhao, Portugal				1.52***			9.84***								

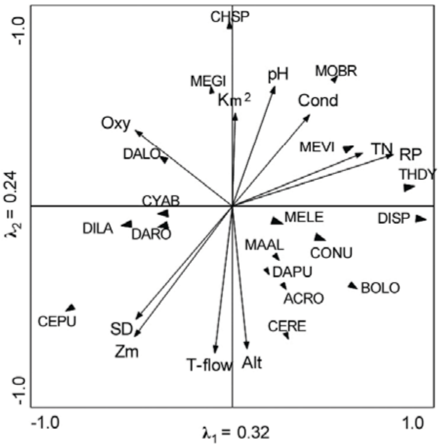


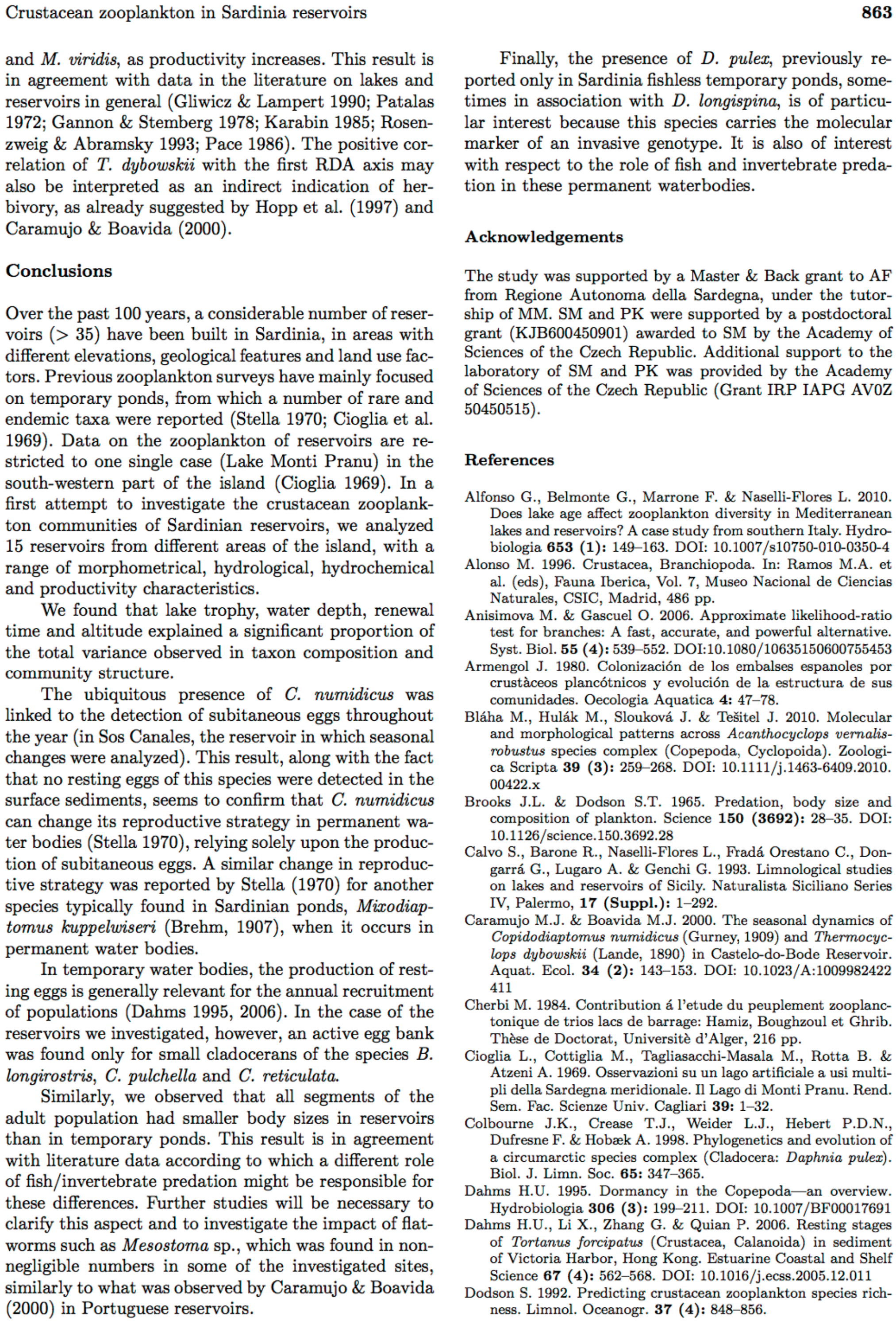
Fig. 4. RDA biplot of zooplankton taxa (acronyms refer to the first two letters of the genus and the first two of the species) and of environmental variables (indicated by arrows) measured in Sardinian reservoirs during our survey in autumn 2008. For further explanations, see Table 1, Fig. 2 and the Material and methods section.

statistical level (Pearson product moment $r = 0.65$; $P < 0.005$; $n = 314$). It increased with algal nutrients' concentration, and was inversely related to water transparency, thus suggesting dependence from food availability (e.g., Maly 1973).

Community structure and environmental variables

Relationships between zooplankton community composition and environmental variables were highlighted by means of an RDA biplot (Fig. 4). We used lake altitude as a substitute for water temperature, to which it was significantly correlated (Pearson product moment correlation coefficient $r = 0.782$; $P < 0.01$; $n = 11$). Overall, the first four RDA axes accounted for 77.4% of the total observed variance, of which 56.4% was explained by the first two RDA axes, shown in the biplot ($\lambda_1 = 0.318$; $\lambda_2 = 0.24$). We interpreted the first RDA axis as being correlated to lake productivity, with lakes of increasing total nitrogen and reactive phosphorus concentrations on the right side of the graph. Species such as *Thermocyclops dybowskii* Lande, 1890 and *Diaphanosoma* sp. were positively correlated with this first axis. The second RDA axis was interpreted as being more related to morphological and hydrological variables, allowing us to separate reservoirs with longer theoretical water renewal times (T-flow), greater mean depth, higher altitudes and higher water transparency. This second axis was associated with species such as *C. reticulata*, positively correlated with altitude and T-flow, and *C. pulchella*, positively correlated with water depth (Zm) and transparency (SD). The latter observation is consistent with report of Alonso (1996) of *C. pulchella* in clear water bodies, with a relatively low pH and conductivity and relatively low productivity (based on RP and TN). Lakes with lower T-flow rates (represented on the upper half of the biplot graph; Fig. 4) seem to be more characterized by the species *Chydorus sphaericus* O.F. Mueller, 1785. The latter is reported to prefer littoral or shallow water bodies. In deeper water bodies, it is associated with the occurrence of Cyanobacteria (Armengol 1980; Alonso 1996). We also observed that *Moina brachiata* was more significant at higher conductivity and pH levels and lower water transparencies; this observation was in agreement with finding of Alonso (1996) that the species prefers turbid waters, with a relatively high pH.

The RDA biplot also points out the increasing importance of cycloploid copepods, namely *T. dybowskii*



and *M. viridis*, as productivity increases. This result is in agreement with data in the literature on lakes and reservoirs in general (Gliwicz & Lampert 1990; Patalas 1972; Gannon & Stemberg 1978; Karabin 1985; Rosenzweig & Abramsky 1993; Pace 1986). The positive correlation of *T. dybowskii* with the first RDA axis may also be interpreted as an indirect indication of herbivory, as already suggested by Hopp et al. (1997) and Caramujo & Boavida (2000).

Conclusions

Over the past 100 years, a considerable number of reservoirs (> 35) have been built in Sardinia, in areas with different elevations, geological features and land use factors. Previous zooplankton surveys have mainly focused on temporary ponds, from which a number of rare and endemic taxa were reported (Stella 1970; Cioglia et al. 1969). Data on the zooplankton of reservoirs are restricted to one single case (Lake Monti Pranu) in the south-western part of the island (Cioglia 1969). In a first attempt to investigate the crustacean zooplankton communities of Sardinian reservoirs, we analyzed 15 reservoirs from different areas of the island, with a range of morphometrical, hydrological, hydrochemical and productivity characteristics.

We found that lake trophy, water depth, renewal time and altitude explained a significant proportion of the total variance observed in taxon composition and community structure.

The ubiquitous presence of *C. numidicus* was linked to the detection of subitaneous eggs throughout the year (in Sos Canales, the reservoir in which seasonal changes were analyzed). This result, along with the fact that no resting eggs of this species were detected in the surface sediments, seems to confirm that *C. numidicus* can change its reproductive strategy in permanent water bodies (Stella 1970), relying solely upon the production of subitaneous eggs. A similar change in reproductive strategy was reported by Stella (1970) for another species typically found in Sardinian ponds, *Mixodiaptomus kuppelwiseri* (Brehm, 1907), when it occurs in permanent water bodies.

In temporary water bodies, the production of resting eggs is generally relevant for the annual recruitment of populations (Dahms 1995, 2006). In the case of the reservoirs we investigated, however, an active egg bank was found only for small cladocerans of the species *B. longirostris*, *C. pulchella* and *C. reticulata*.

Similarly, we observed that all segments of the adult population had smaller body sizes in reservoirs than in temporary ponds. This result is in agreement with literature data according to which a different role of fish/invertebrate predation might be responsible for these differences. Further studies will be necessary to clarify this aspect and to investigate the impact of flatworms such as *Mesostoma* sp., which was found in non-negligible numbers in some of the investigated sites, similarly to what was observed by Caramujo & Boavida (2000) in Portuguese reservoirs.

Finally, the presence of *D. pulex*, previously reported only in Sardinia fishless temporary ponds, sometimes in association with *D. longispina*, is of particular interest because this species carries the molecular marker of an invasive genotype. It is also of interest with respect to the role of fish and invertebrate predation in these permanent waterbodies.

Acknowledgements

The study was supported by a Master & Back grant to AF from Regione Autonoma della Sardegna, under the tutorship of MM. SM and PK were supported by a postdoctoral grant (KJB600450901) awarded to SM by the Academy of Sciences of the Czech Republic. Additional support to the laboratory of SM and PK was provided by the Academy of Sciences of the Czech Republic (Grant IRP IAPG AV0Z 50450515).

References

Alfonso G., Belmonte G., Marrone F. & Naselli-Flores L. 2010. Does lake age affect zooplankton diversity in Mediterranean lakes and reservoirs? A case study from southern Italy. Hydrobiologia **653 (1):** 149–163. DOI: 10.1007/s10750-010-0350-4

Alonso M. 1996. Crustacea, Branchiopoda. In: Ramos M.A. et al. (eds), Fauna Iberica, Vol. 7, Museo Nacional de Ciencias Naturales, CSIC, Madrid, 486 pp.

Anisimova M. & Gascuel O. 2006. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. Syst. Biol. **55 (4):** 539–552. DOI:10.1080/10635150600755453

Armengol J. 1980. Colonización de los embalses espanoles por crustàceos plancótnicos y evolución de la estructura de sus comunidades. Oecologia Aquatica **4:** 47–78.

Bláha M., Hulák M., Slouková J. & Tešitel J. 2010. Molecular and morphological patterns across *Acanthocyclops vernalis-robustus* species complex (Copepoda, Cyclopoida). Zoologica Scripta **39 (3):** 259–268. DOI: 10.1111/j.1463-6409.2010.00422.x

Brooks J.L. & Dodson S.T. 1965. Predation, body size and composition of plankton. Science **150 (3692):** 28–35. DOI: 10.1126/science.150.3692.28

Calvo S., Barone R., Naselli-Flores L., Fradá Orestano C., Dongarrá G., Lugaro A. & Genchi G. 1993. Limnological studies on lakes and reservoirs of Sicily. Naturalista Siciliano Series IV, Palermo, **17 (Suppl.):** 1–292.

Caramujo M.J. & Boavida M.J. 2000. The seasonal dynamics of *Copidodiaptomus numidicus* (Gurney, 1909) and *Thermocyclops dybowskii* (Lande, 1890) in Castelo-do-Bode Reservoir. Aquat. Ecol. **34 (2):** 143–153. DOI: 10.1023/A:1009982422411

Cherbi M. 1984. Contribution á l'etude du peuplement zooplanctonique de trios lacs de barrage: Hamiz, Boughzoul et Ghrib. Thèse de Doctorat, Universitè d'Alger, 216 pp.

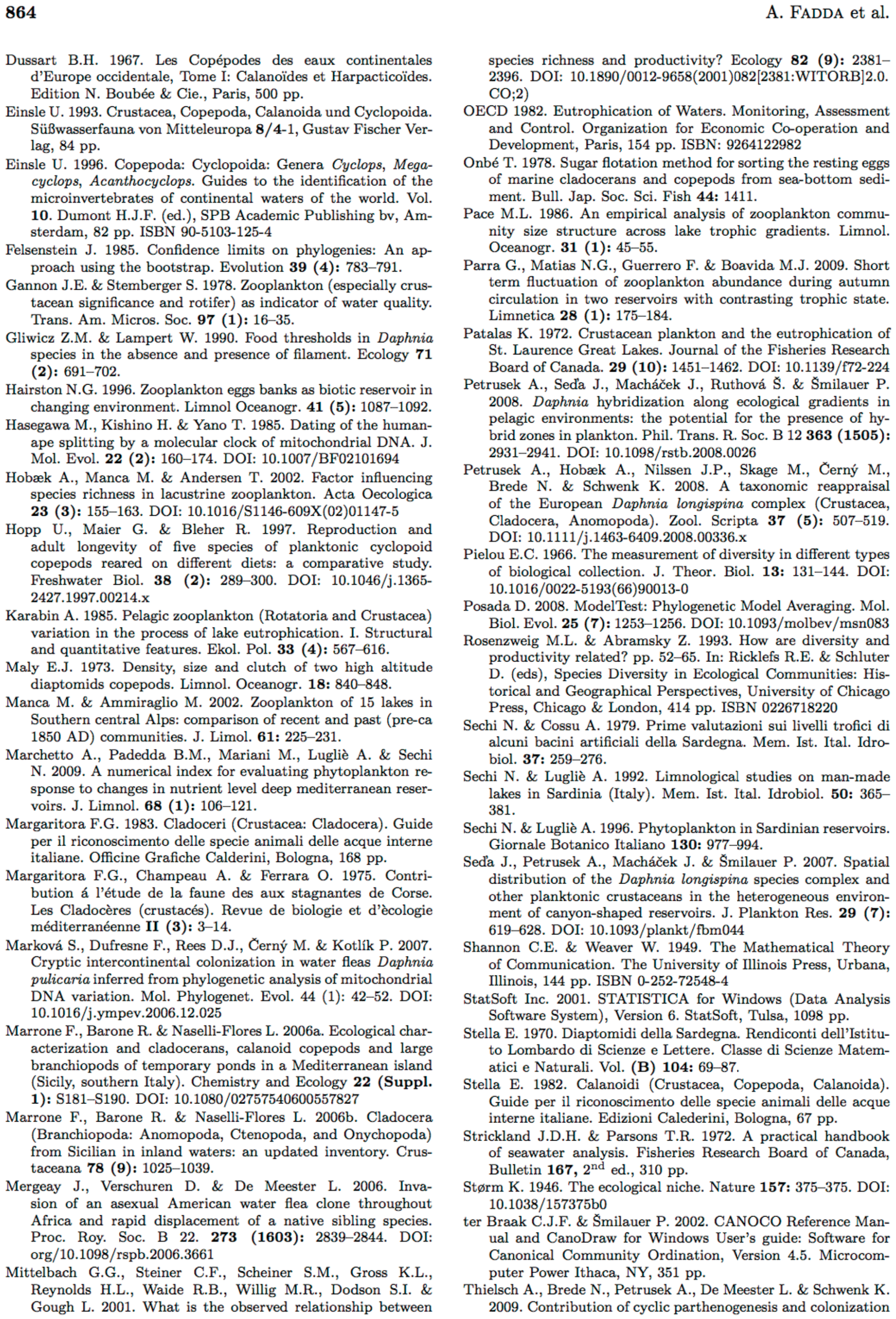
Cioglia L., Cottiglia M., Tagliasacchi-Masala M., Rotta B. & Atzeni A. 1969. Osservazioni su un lago artificiale a usi multipli della Sardegna meridionale. Il Lago di Monti Pranu. Rend. Sem. Fac. Scienze Univ. Cagliari **39:** 1–32.

Colbourne J.K., Crease T.J., Weider L.J., Hebert P.D.N., Dufresne F. & Hobæk A. 1998. Phylogenetics and evolution of a circumarctic species complex (Cladocera: *Daphnia pulex*). Biol. J. Limn. Soc. **65:** 347–365.

Dahms H.U. 1995. Dormancy in the Copepoda—an overview. Hydrobiologia **306 (3):** 199–211. DOI: 10.1007/BF00017691

Dahms H.U., Li X., Zhang G. & Quian P. 2006. Resting stages of *Tortanus forcipatus* (Crustacea, Calanoida) in sediment of Victoria Harbor, Hong Kong. Estuarine Coastal and Shelf Science **67 (4):** 562–568. DOI: 10.1016/j.ecss.2005.12.011

Dodson S. 1992. Predicting crustacean zooplankton species richness. Limnol. Oceanogr. **37 (4):** 848–856.



Dussart B.H. 1967. Les Copépodes des eaux continentales d'Europe occidentale, Tome I: Calanoïdes et Harpacticoïdes. Edition N. Boubée & Cie., Paris, 500 pp.

Einsle U. 1993. Crustacea, Copepoda, Calanoida und Cyclopoida. Süßwasserfauna von Mitteleuropa **8/4**-1, Gustav Fischer Verlag, 84 pp.

Einsle U. 1996. Copepoda: Cyclopoida: Genera *Cyclops*, *Megacyclops*, *Acanthocyclops*. Guides to the identification of the microinvertebrates of continental waters of the world. Vol. **10**. Dumont H.J.F. (ed.), SPB Academic Publishing bv, Amsterdam, 82 pp. ISBN 90-5103-125-4

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution **39 (4):** 783–791.

Gannon J.E. & Stemberger S. 1978. Zooplankton (especially crustacean significance and rotifer) as indicator of water quality. Trans. Am. Micros. Soc. **97 (1):** 16–35.

Gliwicz Z.M. & Lampert W. 1990. Food thresholds in *Daphnia* species in the absence and presence of filament. Ecology **71 (2):** 691–702.

Hairston N.G. 1996. Zooplankton eggs banks as biotic reservoir in changing environment. Limnol Oceanogr. **41 (5):** 1087–1092.

Hasegawa M., Kishino H. & Yano T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. **22 (2):** 160–174. DOI: 10.1007/BF02101694

Hobæk A., Manca M. & Andersen T. 2002. Factor influencing species richness in lacustrine zooplankton. Acta Oecologica **23 (3):** 155–163. DOI: 10.1016/S1146-609X(02)01147-5

Hopp U., Maier G. & Bleher R. 1997. Reproduction and adult longevity of five species of planktonic cyclopoid copepods reared on different diets: a comparative study. Freshwater Biol. **38 (2):** 289–300. DOI: 10.1046/j.1365-2427.1997.00214.x

Karabin A. 1985. Pelagic zooplankton (Rotatoria and Crustacea) variation in the process of lake eutrophication. I. Structural and quantitative features. Ekol. Pol. **33 (4):** 567–616.

Maly E.J. 1973. Density, size and clutch of two high altitude diaptomids copepods. Limnol. Oceanogr. **18:** 840–848.

Manca M. & Ammiraglio M. 2002. Zooplankton of 15 lakes in Southern central Alps: comparison of recent and past (pre-ca 1850 AD) communities. J. Limol. **61:** 225–231.

Marchetto A., Padedda B.M., Mariani M., Luglié A. & Sechi N. 2009. A numerical index for evaluating phytoplankton response to changes in nutrient level deep mediterranean reservoirs. J. Limnol. **68 (1):** 106–121.

Margaritora F.G. 1983. Cladoceri (Crustacea: Cladocera). Guide per il riconoscimento delle specie animali delle acque interne italiane. Officine Grafiche Calderini, Bologna, 168 pp.

Margaritora F.G., Champeau A. & Ferrara O. 1975. Contribution á l'étude de la faune des aux stagnantes de Corse. Les Cladocères (crustacés). Revue de biologie et d'ècologie méditerranéenne **II (3):** 3–14.

Marková S., Dufresne F., Rees D.J., Černý M. & Kotlík P. 2007. Cryptic intercontinental colonization in water fleas *Daphnia pulicaria* inferred from phylogenetic analysis of mitochondrial DNA variation. Mol. Phylogenet. Evol. 44 (1): 42–52. DOI: 10.1016/j.ympev.2006.12.025

Marrone F., Barone R. & Naselli-Flores L. 2006a. Ecological characterization and cladocerans, calanoid copepods and large branchiopods of temporary ponds in a Mediterranean island (Sicily, southern Italy). Chemistry and Ecology **22 (Suppl. 1):** S181–S190. DOI: 10.1080/02757540600557827

Marrone F., Barone R. & Naselli-Flores L. 2006b. Cladocera (Branchiopoda: Anomopoda, Ctenopoda, and Onychopoda) from Sicilian in inland waters: an updated inventory. Crustaceana **78 (9):** 1025–1039.

Mergeay J., Verschuren D. & De Meester L. 2006. Invasion of an asexual American water flea clone throughout Africa and rapid displacement of a native sibling species. Proc. Roy. Soc. B 22. **273 (1603):** 2839–2844. DOI: org/10.1098/rspb.2006.3661

Mittelbach G.G., Steiner C.F., Scheiner S.M., Gross K.L., Reynolds H.L., Waide R.B., Willig M.R., Dodson S.I. & Gough L. 2001. What is the observed relationship between species richness and productivity? Ecology **82 (9):** 2381–2396. DOI: 10.1890/0012-9658(2001)082[2381:WITORB]2.0.CO;2)

OECD 1982. Eutrophication of Waters. Monitoring, Assessment and Control. Organization for Economic Co-operation and Development, Paris, 154 pp. ISBN: 9264122982

Onbé T. 1978. Sugar flotation method for sorting the resting eggs of marine cladocerans and copepods from sea-bottom sediment. Bull. Jap. Soc. Sci. Fish **44:** 1411.

Pace M.L. 1986. An empirical analysis of zooplankton community size structure across lake trophic gradients. Limnol. Oceanogr. **31 (1):** 45–55.

Parra G., Matias N.G., Guerrero F. & Boavida M.J. 2009. Short term fluctuation of zooplankton abundance during autumn circulation in two reservoirs with contrasting trophic state. Limnetica **28 (1):** 175–184.

Patalas K. 1972. Crustacean plankton and the eutrophication of St. Laurence Great Lakes. Journal of the Fisheries Research Board of Canada. **29 (10):** 1451–1462. DOI: 10.1139/f72-224

Petrusek A., Seďa J., Macháček J., Ruthová Š. & Šmilauer P. 2008. *Daphnia* hybridization along ecological gradients in pelagic environments: the potential for the presence of hybrid zones in plankton. Phil. Trans. R. Soc. B 12 **363 (1505):** 2931–2941. DOI: 10.1098/rstb.2008.0026

Petrusek A., Hobæk A., Nilssen J.P., Skage M., Černý M., Brede N. & Schwenk K. 2008. A taxonomic reappraisal of the European *Daphnia longispina* complex (Crustacea, Cladocera, Anomopoda). Zool. Scripta **37 (5):** 507–519. DOI: 10.1111/j.1463-6409.2008.00336.x

Pielou E.C. 1966. The measurement of diversity in different types of biological collection. J. Theor. Biol. **13:** 131–144. DOI: 10.1016/0022-5193(66)90013-0

Posada D. 2008. ModelTest: Phylogenetic Model Averaging. Mol. Biol. Evol. **25 (7):** 1253–1256. DOI: 10.1093/molbev/msn083

Rosenzweig M.L. & Abramsky Z. 1993. How are diversity and productivity related? pp. 52–65. In: Ricklefs R.E. & Schluter D. (eds), Species Diversity in Ecological Communities: Historical and Geographical Perspectives, University of Chicago Press, Chicago & London, 414 pp. ISBN 0226718220

Sechi N. & Cossu A. 1979. Prime valutazioni sui livelli trofici di alcuni bacini artificiali della Sardegna. Mem. Ist. Ital. Idrobiol. **37:** 259–276.

Sechi N. & Luglië A. 1992. Limnological studies on man-made lakes in Sardinia (Italy). Mem. Ist. Ital. Idrobiol. **50:** 365–381.

Sechi N. & Luglië A. 1996. Phytoplankton in Sardinian reservoirs. Giornale Botanico Italiano **130:** 977–994.

Seďa J., Petrusek A., Macháček J. & Šmilauer P. 2007. Spatial distribution of the *Daphnia longispina* species complex and other planktonic crustaceans in the heterogeneous environment of canyon-shaped reservoirs. J. Plankton Res. **29 (7):** 619–628. DOI: 10.1093/plankt/fbm044

Shannon C.E. & Weaver W. 1949. The Mathematical Theory of Communication. The University of Illinois Press, Urbana, Illinois, 144 pp. ISBN 0-252-72548-4

StatSoft Inc. 2001. STATISTICA for Windows (Data Analysis Software System), Version 6. StatSoft, Tulsa, 1098 pp.

Stella E. 1970. Diaptomidi della Sardegna. Rendiconti dell'Istituto Lombardo di Scienze e Lettere. Classe di Scienze Matematici e Naturali. Vol. **(B) 104:** 69–87.

Stella E. 1982. Calanoidi (Crustacea, Copepoda, Calanoida). Guide per il riconoscimento delle specie animali delle acque interne italiane. Edizioni Calederini, Bologna, 67 pp.

Strickland J.D.H. & Parsons T.R. 1972. A practical handbook of seawater analysis. Fisheries Research Board of Canada, Bulletin **167,** 2nd ed., 310 pp.

Størm K. 1946. The ecological niche. Nature **157:** 375–375. DOI: 10.1038/157375b0

ter Braak C.J.F. & Šmilauer P. 2002. CANOCO Reference Manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination, Version 4.5. Microcomputer Power Ithaca, NY, 351 pp.

Thielsch A., Brede N., Petrusek A., De Meester L. & Schwenk K. 2009. Contribution of cyclic parthenogenesis and colonization history to population structure in *Daphnia*. Mol. Ecol. **18** **(8):** 1616–1628. DOI: 10.1111/j.1365-294X.2009.04130.x

Tolotti M., Manca M., Angeli N., Morabito G., Thaler B., Rott E. & Stuchlik E. 2006. Phytoplankton and zooplankton association in a set of Alpine high altitude lakes: geographic distribution and ecology. Hydrobiologia **562** **(1):** 99–122. DOI: 10.1007/s10750-005-1807-8

Tundisi J.G., Rocha O., Matsumura-Tundisi T. & Braga B. 1998. Reservoir management in South America. Int. J. Water Resourc. Develop. **14** **(2):** 141–155. DOI:10.1080/07900629849367

Received March 2, 2011
Accepted June 20, 2011

CHAPTER 2

Do hydrological stressors affect stable isotopic signature of carbon and nitrogen in Mediterranean reservoirs?

Amedeo FADDA[ae], Marina MANCA[b], Federica CAMIN[c], Luca ZILLER[c], Piero OLLA[d], Paola BUSCARINU[e], Maria Antonietta MARIANI[a], *,Bachisio Mario PADEDDA[a], Nicola SECHI[a], Tomasa VIRDIS[e] and Antonella LUGLIÈ[a]

(a) University of Sassari, Department of Sciences for Nature and Environmental Resources, via Piandanna, 4 07100 Sassari (SS) Italy

(b) CNR Institute of Ecosystem Study (CNR-ISE), Largo Tonolli 50 28922 Verbania

(c) IASMA Fondazione Edmund Mach, San Michele all Adige TN, Italy

(d) Institute for the Study of Atmosferic Science and Climate of the National Council of Research (CNR-ISAC), Cagliari (Italy)

(e) Ente Acque della Sardegna, via Mameli 88 Cagliari (Italy)

Abstract

Artificial lakes are complex freshwater environments, with ecological characters in the middle between rivers and lakes. In the Medietrranean basin, reservoirs strongly varied their water level throughout the year mainly related by annual hydrological dynamics as well as management activity. Lake Sos Canales is a small reservoir in a quite pristine upland area of Sardinia (Italy), devoted to drinkable water production. Despite low anthropogenic activity in the watershed, Lake Sos Canales was classified as meso-eutrophic reservoir. Lake is characterized by a low water-level phase since summer to late fall and a “re-filling” phase in winter and spring. The nature and origin of suspended particulate matter (SPM) was study to quantify how annual hydrological dynamics influenced its composition. The composition of this matter can be autochthonous, from an internal production, mainly phytoplankton, bacteria and zooplankton, as well as allochthonous, from external origin, mainly transported by river inputs. Stable Isotope Analysis (SIA) of carbon and nitrogen is an important useful tool to study the origin of SPM able to trace nutrient fluxes between ecosystems, trophic relationships and pathways of energy flow. SPM $\delta^{13}C$ and $\delta^{15}N$ isotopic signature were analyzed ca. monthly along an annual hydrological cycle (October 2010-October 2011) both in the shallow area, near to the river inflow, and in the deepest one, close the dam (horizontal axis) in Lake Sos Canales. Furthermore SPM carbon and nitrogen signatures were studied in surface (0-7.5m) and deep water (>10m) in the deep area (vertical axis), united to the physical, chemical and phytoplankton analysis. Our results highlighted an overlapping of isotopic signature during a low-water phase and a differentiation during the re-filling of reservoir. In particular was observed a negative relationship between $\delta^{15}N$ and water level, with an enrichment in low-water phase, whereas $\delta^{13}C$ of surface water increase in hottest months . Moreover a peculiar distribution of $\delta^{13}C$ signatures was recorded along the water column during the re-filling phase, with depleted signature in surface. While the origin of SPM from shallow and deeper water layer appeared most heterogeneous, SPM of surface water was related to phytoplankton biomass.

Phytoplankton community in Lake Sos Canales was dominated (in terms of biomass) by a mixotrophic dinoflagellate (*G. uberrimum*), that can responsible for this anomalous δ^{13}C distribution.

Key word: water level fluctuation, stable isotope analysis, reservoir, dinoflagellate, suspended particulate matter.

Introduction

Water bodies of geographical areas characterized by climate with strong variability throughout the year, often show great water-level fluctuations (WLF). WLF is one of the most important stressor in lentic environments (Leira and Cantonati, 2012; and references therein) and has greatest effects in shallow lakes and in the littoral zone. In thermally-stratified lakes, WLF can cause unexpected water mixing, with strong effects on biota and ecosystem functioning (Bond et al., 2008), including both top-down and bottom-up effects (Zoary et al., 2011). Furthermore, loss of biodiversity and degradation of littoral community as well as presence of alien species, are other effects linked to extreme variability of water level (Zoary et al., 2011). The rapid climate change, that is concerning our planet, is expected further aggravate these effects, with wider WLF due to increase in mean annual temperature and summer evaporation, modification of rainfall seasonal patterns, intensity and frequency of extreme rainfall events and very drought periods (Mooij et al., 2005; Mulholland et al., 1997; Barone et al., 2010).

Compared to natural lakes, artificial lakes are more subject to this important stress factors (Naselli-Flores, 2003). Artificial lakes are in the middle between rivers and lakes (Straškraba & Tundisi, 1999) and they show mainly unidirectional changes of limnological variables with environmental gradients along their longitudinal axis (Straškraba et al., 1993; Straškraba, 1997; Caputo et al., 2008). Thornton (1990) proposed a heuristic model to describe them as a continuum from river inflow to dam, with the establishment of three distinct zones: riverine, transition and lacustrine zones. Compared to natural lakes, artificial lakes have higher watersheds area/water body area, shorter and varying retention times at both intra- and inter-annual scale, a rapid ageing process in relation to watershed uses, high capability to retain organic and inorganic matter (Straškraba, 1998; Straškraba & Tundisi, 1999; Tundisi & Matsumura-Tundisi, 2003). External inputs from watershed are relevant during wet season, or filling phase, while internal recycling processes support biological community during the drought season (Zoary et al., 2011). Moreover, they have a littoral zone relatively wide, only periodically submerged, due to the strong WLF along the seasons, especially when artificial lakes are devoted to human water exploitation (e.g., agriculture, hydropower, drink water production). Thus, phytoplankton develops mainly and results the most important primary producer at the base of the aquatic food web. In addition, it influences water quality and can interfere with the subsequent steps in the water purifying plants for drinking waters due to both of its particulate nature and its "living" character (Niesel et al., 2007).

Phytoplankton is one of the main components of the suspended particulate matter (SPM) of water ecosystems. The composition of this matter can be autochthonous, from an internal production, mainly phytoplankton, bacteria and zooplankton, as well as allochthonous, from external origin, mainly transported by river inputs. Stable Isotope Analysis (SIA) of carbon and nitrogen is an important useful tool to study the origin of SPM. SIA is a relative recent methodology to study food webs, tracing nutrient fluxes among ecosystems, trophic relationships and pathways of energy flow, in particular in aquatic environments (Grey, 2006). One of the most difficult methodological issues in the application of SIA to food web studies is the assignment of an appropriate isotopic baseline (Post, 2002; Grey, 2006). At a general level, in a same habitat, C isotope composition ($^{13}C/^{12}C$; $\delta^{13}C$) is expected to remain constant, whereas N ($^{15}N/^{14}N$; $\delta^{15}N$) tends to be enriched (referred to the heavy isotope; Sulzman, 2007). Consequently, C isotope composition can be used to determine the food source for different organisms, whereas N composition allows to estimate their relative position in the trophic chain (Cattaneo et al., 2004). In fact, $\delta^{13}C$ has helped to gain important insights into sources of organic matter, primary productivity, and CO_2 concentration in the surface water (Lehman et al., 2004). Instead, $\delta^{15}N$ of SPM has been used also to understand various N cycling processes (Teranes and Bernasconi, 2000; Lehman et al., 2004).

Marked differences exist between terrestrial isotopic signature of SPM and aquatic origin matter (Kendall, 2006). In lacustrine systems, $\delta^{13}C$ and $\delta^{15}N$ signatures vary over time (Zohary et al., 1994; Lehman et al., 2004) and these variability is related to external loadings, phytoplankton species composition, primary productivity, as well as sources and concentrations of dissolved inorganic C and N (Caroni et al., 2012; Grey et al., 2001). Several variables affect phytoplankton $\delta^{13}C$ signature in lentic ecosystems, primary the uptake of inorganic C and its fractionation, which is the differences between source and product isotopic composition of chemical reaction defined as the isotopic differences between the source and product compounds (Hayes, 2004). Every species fractionate δ^{13}C in different way, thus SPM main constitute by phytoplankton reflects its taxonomic composition (Vuorio et al., 2006, Caroni et al., 2012). δ^{15}N signature is also influenced by N uptake (Teranes and Bernasconi, 2000), nitrification (Mariotti et al., 1981), denitrification (Brandes et al., 1998), and organic matter degradation processes (Lehmann, 2002).

In the Mediterranean region, since the last century, the increasing water needs for socio-economical development of local populations have been answered, in many cases, by damming rivers or streams and the construction of artificial lakes. Even if of strategic importance, they are subject to very strong stresses, of natural (e.g., climate of the region) as well as anthropogenic origins (e.g., eutrophication, water management). For this, limnological studies are necessary to better understand the functioning of artificial lakes, evaluate the effects of these stresses in deep and elaborate site-specific adequate managing plans recovery and, if necessary, adequate recovery projects. In any case, studies should be in tightly relationship with managers of artificial lakes and able to formulate useful suggestions and develop adequate tools to a better utilization of water resources (Tundisi and& Matsumura-Tundisi, 2003).

In this contest, the determination of SPM origin is of extreme importance to understand the “weight” of autochthonous primary trophic level in respect to allochthonous input in the lacustrine food web. At a management level, for example, it gives basic information to value if a top-down approach (e.g., grazing) may be a useful strategy to control phytoplankton growth (Søndegard et al., 1999).

The aims of this study were to evaluate the origin and the annual dynamics of SPM through SIA of C and N in a Mediterranean artificial lake (Sos Canales Lake, Sardinia, Italy) devoted to drinking-water production and affected by strong annual WLF.

In small artificial lakes (*sensu* Straškraba and Tundusi, 1999), a large contribution by external inputs is expected in wet season (filling phase), when a relative lower isotopic signal of C is expected (terrestrial signal), whereas by phytoplankton in dry months (empty phase), with a relative increase in the isotopic signature (aquatic signal).

However, hydrological stressors, such as WLF and the relative morphometric characters of the lake, can affect the composition of SPM. In addition, the variability of phytoplankton further increases the complexity of the signals.

Material and methods

Study area

Sos Canales Lake (SC) was built in 1956 damming the upper stream of River Tirso, the most important river of Sardinia (Italy;

Fig. 1). It is located in a small quite pristine watershed, surrounded by cork-oaks woods. The water-catchment is small and granitic (Tab. 1), with little anthropogenic activities, mainly related to the practices of cork production and of wild breeding, difficult to be quantified. Despite a low impact of human activities and a relative low theoretic phosphorus load (Sechi, 1989), the lake is classified as meso-eutrophic according to OECD criterions (Sechi, 1989; Sechi & Lugliè, 1992; Lugliè et al., 1996; Marchetto et al., 2009). SC belongs to the Italian Network of Long Term Ecological Research (LTER-Italy; www.lteritalia.it). Long term studies have highlighted that phytoplankton, the main primary producer of SC, is co-dominated by Cyanobacteria, Dinophyceae, Chlorophyceae and Bacillariophyceae (Sechi and Lugliè, 1996). Fadda et al. (2011) highlighted that it showed the richest and most diverse zooplankton community among those of the 15 Sardinian artificial lakes studied on a space per time approach (Tolotti et al., 2006).

Sos Canales Lake falls in the Mediterranean climate area, characterized by warm dry summers, and mild to cool and wet winters. Rainfall events are concentrated since late autumn till early-late spring. Consequently, as in many others upland Mediterranean cases, the first rainfall wash the riverbed and the watershed, carrying a great amount of detritus and nutrients. Water overflow occurs occasionally in SC. Therefore, most of the conveyed water is retained by the dam, for future supply. For this, even if SC is affected by large WLF, as many others Mediterranean artificial lakes, it remains enough deep to show stable thermal stratification from late spring to autumn, as the majority of the Sardinian artificial lakes (Marchetto et al., 2009).

The lake provides drinking water supply for resident population (15.000 inhabitants) and its outflow, only due to human intake, is quite constant during the year, never exceeding 265 m^3 $month^{-1}$. Furthermore, in order to reduce the costs of treatments for the drinkable waters, a mobile device for choose waters with the best quality into the water column was installed in the 2006. Water quality is monitored by a Remote System Monitoring in Real Time, managed by the Ente Acque della Sardegna (ENAS), the regional manager of dams and lakes. The system allows obtain data along the vertical profile for different basic variables (temperature, pH, conductivity, dissolved oxygen and fluorimetric chlorophyll *a*).

Suspended particulate matter and stable isotope determination

Sampling

Suspended particulate matter (SPM; 1.2≥µm≤76) was collected from October 2010 to September 2011 at two stations: near to the river inflow (40°33’16” N 9°19’13”E; St. 2, riverine area) and close to the dam (40°33’17”N, 9°18’49”E; St. 1, lacustrine area), where the maximum lake depth is reached (max 40 m). The sampling time was restricted to the early afternoon (between 13:00 to 14:00 h p.m.).

Two integrated water samples were obtained on each date at St. 1: one between surface and -7.5 m depth and the other from -10 m depth up to the deeper waters. These two water layers were called in the text SUR the former, and DEP the latter. SUR samples integrated waters collected with a Niskin bottle (5 l) at 0, -1, -2.5, -5, -7.5 m, and DEP samples at -10, -15, -20 and -30 m (when possible) but never below 2 m from the bottom.

On the same sampling dates, 0-7.5 m depth integrated water samples were collected at the riverine area (St. 2; RIV), where water depth ranged between 8 and 10 m. RIV samples integrated waters collected with a Niskin bottle (5 l) at 0, -1, -2.5, -5, -7.5 and 10 m (when possible).

To evaluate the effect of surrounding soil, earthworms were collected along the seasons into the artificial lake catchment.

To assess the influences from the bottom sediments, benthic oligochaetes were collected through an Ekman grab in February 2011.

Determination

Water samples, pre-filtered on a 76 µm sieve, to remove zooplankton and large detritus material, were filtered on WATMAN GF/C glass-fiber filters (4.5 cm diameter, 1.2 µm pore size) and oven dried at 60 °C for 24 h. The dry-weight of SPM (µg l^{-1}) was evaluated calculating the net weight between the pre-weighted empty and full filters, taking in account the water litters filtered. Filters portions were transferred into tin capsules (6X4 mm) for $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis.

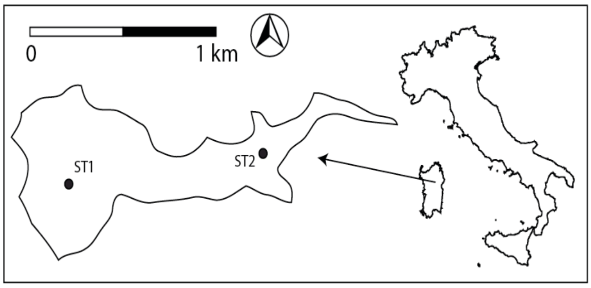


Fig. 1 – Sos Canales Lake and sampling stations localization (St. 1 = lacustrine station; St. 2 = riverine station).

Tab. 1 – Main morphometric characteristics of

Max volume	4.3 x 10^6
Max surface area	0.33 x 10^6 m^3
Max volume	4.3 x 10^6 m^2
Mean depth	13.2 m
Watershed area	15.9 x 10^6 m^2
Max altitude	714 m a.s.l.

Worms, from surrounding soil and bottom sediments, were washed from sediment residuals and oven dried at 60 °C for 24 h. Dried organisms were powdered and transferred in tin capsules (6X4 mm). Two replicates/sample were performed. The samples were analyzed by Continuous Flow-Isotope Ratio Mass Spectrometer (CF-IRMS; Delta plus XP ThermoFinnigann, Bremen, Germany) at the Istituto Agrario San Michele all'Adige (I.A.S.M.A, Trento, Italy), after total combustion (≈ 0.5 mg) in elemental analyzer (EA Flash 1112 ThermoFinnigan) (Camin et al., 2004 and 2008).

Values were expressed in δ‰ against international standards (Vienna-Pee Dee Belemnite for $\delta^{13}C$, air for $\delta^{15}N$) and computed against acetanilide, which were calibrated against international reference materials (MRI-ET-ISO 64, MRI-ET-ISO 63; Camin et al., 2008). Uncertainty of measurements was <0.3‰ for $\delta^{13}C$ and $\delta^{15}N$.

Environmental variables, nutrients and phytoplankton

Sampling

Samplings were conducted ca. monthly, at the same dates of SPM, at the station in the lacustrine zone (St. 1) for chemical-physical, nutrients and phytoplankton analyses and also at the riverine station (St. 2) for only phytoplankton analysis. For nutrients and chemical-physical variables, water samples were collected along the vertical profile, from the surface downward, at fixed depths using a Niskin bottle (0, 1, 2.5, 5, 7.5, 10, 15 m) and below at intervals of 10 m till the bottom but never below 2 m from the bottom. Phytoplankton samples (100 ml) were collected from the first six depths (0, 1, 2.5, 5, 7.5, 10 m) and immediately fixed in the field with Lugol's solution.

Measures, determinations and statistical data treatment

Secchi disk transparency (SD) and temperature (T), pH, conductivity (Cond) and dissolved oxygen (DO) were measured *in situ* by a multiparametric probe (multiprobe Hydrolab DS5). Values registered in the field were replicated in laboratory for pH (Orion Research Model 960) and conductivity (Mod Analytical Control 120).

Water samples were preserved in cold and dark conditions for the laboratory analysis of alkalinity (IRSA, 1994), ammonium (N-NH4) (Fresenius et al., 1988), nitrite (N-NO2), nitrate (N-NO3), total nitrogen (TN), reactive silica (RSi), reactive (RP), and total phosphorus (TP), iron (Fe) and manganese (Mn) according to Strickland and Parsons (1972), and chlorophyll *a* (Chl a; Golterman et al., 1978). Dissolved inorganic nitrogen (DIN) was obtained as the sum of ammonia, nitrate and nitrite. The environmental and nutrient data were averaged in the SUR and DEP layers.

Phytoplankton was analyzed with Utermöhl's technique (1958). Cell density was evaluated in subsamples of 5-10 ml of the fixed samples with an inverted microscope (Zeiss, Axiovert 10) at 200x and 400x magnifications in an adequate number of fields. The species were determined on alive and fixed samples according to Bourrelly (1966-1981), Germain (1981), Huber-Pestalozzi (1938, 1941, 1942, 1955, 1961, 1968, 1982, 1983), Hustedt (1985), Komárek and Anagnostidis, (1999, 2005), Komárek and Komárková (2002), Komárek and Zapomělová (2007, 2008), Krammer and Lange-Bertalot (1986-1991), Suda et al. (2002). Biovolume was obtained by multiplying the cell density of each taxon with the unitary cell biovolume of the same taxon, which was obtained by geometrical approximations, after measurement of at least 30 specimens according to Sun and Liu (2003). Biovolume were converted to biomass assuming 1 mm^3 = 1 mg of fresh-weight biomass (Liu et al., 2011). Phytoplankton carbon and nitrogen contents were calculated applying the Menden-Deuer and& Lessard equations (2000). The phytoplankton data were averaged in the SUR and DEP layers of the lacustrine station and in the RIV. Moreover, weighted means in the photic zone (PZ = 2.5 SD) were calculated.

Monthly rainfall values in the studied period and those of the latest six-year period (2006-2011) - recorded by the meteo-climatic station located close to the dam - were analyzed to evaluate the rainfall trends.

Correlations among $\delta^{13}C$ signature and temperature, SPM dry-weight vs phytoplanktonic biomass and Chl-*a*, $\delta^{15}N$ vs nitric-nitrate and N-content of phytoplankton biomass, phytoplankton density and biomass vs photic-zone, were tested applying parametric or non-parametric tests for correlation, depending on normality and homoscedasticity using the program Statistica for Windows (StatSoft 1994).

Results

Environmental variables and nutrients

The lake volume and, consequently the water level, increased slowly from November to January and abruptly from February to March due to heavy rainfall in the autumn-winter months (maximum of 180 mm in November; Fig. 2). Water level remained relatively stable until June. It decreased of about 5 m in July and the same trend continued until September, due to the low or absent rainfall in summer months.

The observed rainfall dynamic was similar to those of the period 2006-2011 even if rainfall between autumn and spring was greater than the pluriannual average. As expected, wide seasonal variations were highlighted.

Coherently, Secchi disk transparency was lower in February-March (1.2 and 1.5 m), after the rainiest period and the consequent intense water input from the catchment (Fig. 3a). Water transparency gradually increased in spring, remaining stable on ca. 3.5 m throughout summer.

Water temperature was homogeneous along the vertical profile and relatively low in winter (5.5-7.3°C) when the lake was in a mixing condition (Fig. 3a and Fig. 4). Thermal stratification started in April, lasting until September. Temperature maximum was recorded in SUR in July (24.8 °C as peak).

pH was around 7 units, highlighting slightly higher values during summer months in SUR layer. Contemporaneously, more acid, values, around 6 units, were observed in the DEP layer. During mixing, the values overlapped (Fig. 3b).

Dissolved oxygen percentage varied

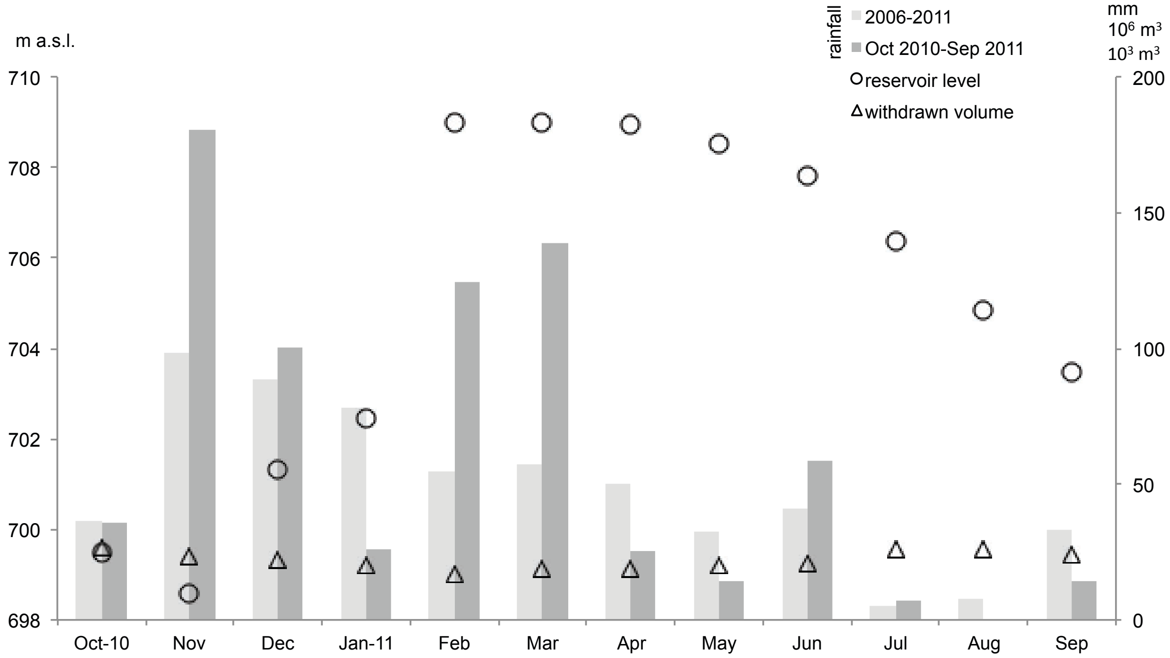


Fig.2 - Lake Sos Canales seasonal water level, lacustrine volume, withdrawn volume and rainfall.

accordingly, gradually declining towards the end of summer and beginning of fall, especially in DEP (Fig. 3b). Values were never more than 100% in SUR and less than 38% in DEP.

Total nitrogen ranged between a minimum of ca. 600 mg N m^{-3} in SUR (September) and a maximum of ca. 1600 mg N m^{-3} in DEP (October) (Fig. 4a). Organic nitrogen contributed most to TN all the year round. Nitrate was the main component of the dissolved inorganic nitrogen (DIN) both in SUR and DEP samples (at least 75%). Nitrate were of ca. 350 mg N m^{-3} during mixing; with the onset of thermal stratification, nitrate decreased in the epilimnion while increased lightly and were more constant in the hypolimnion (Fig. 5).

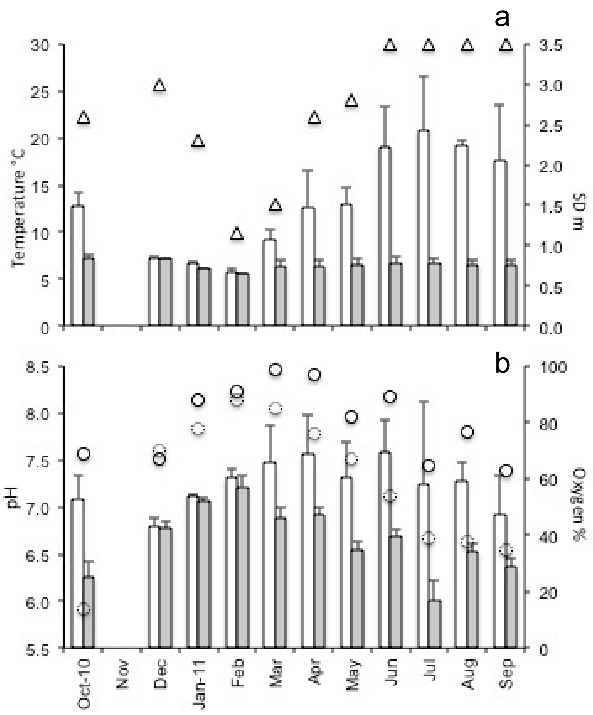


Fig. 3 – Dynamics of a) temperature (column) and SD (triangles) and b) pH (column) and oxygen (circles) at St. 1. (SUR = white column and solid circles; DEP = grey column and dotted circles; bars = standard error).

maximum of ca. 1600 mg N m^{-3} in DEP (October) (Fig. 4a).Organic nitrogen contributed most to TN all the year round. Nitrate was the main component of the dissolved inorganic nitrogen (DIN) both in SUR and DEP samples (at least 75%). Nitrate were of ca. 350 mg N m^{-3} during

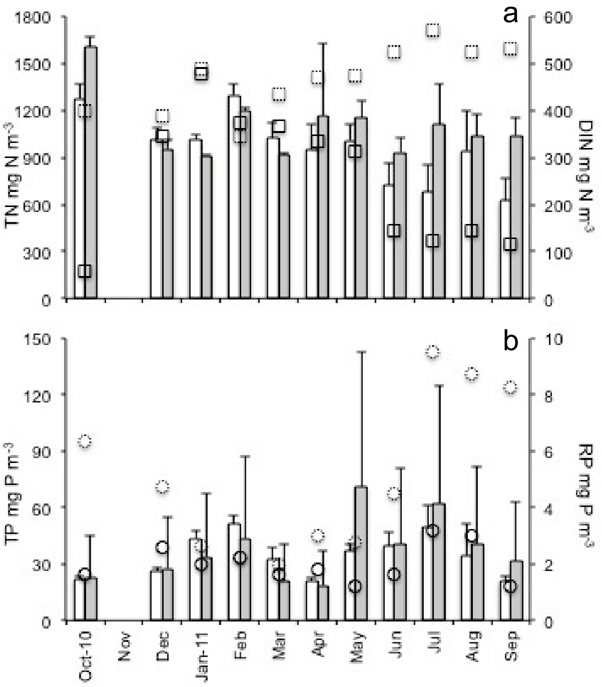


Fig. 4 – Observed dynamics of a) total nitrogen (column) and dissolved inorganic nitrogen (DIN) (squares) and b) total (column) and reactive phosphorous (circles) at St. 1. (SUR = white column and solid circles and squares; DEP = grey column and dotted circles and squares; bars = standard error).

mixing; with the onset of thermal stratification, nitrate decreased in the epilimnion while increased lightly and were more constant in the hypolimnion (Fig. 5).

Total phosphorus ranged between ca. 20 mg P m^{-3} in April to ca. 70 mg P m^{-3} in May in DEP (Fig. 4b). Both TP and RP increased strongly in DEP from June to September, during stratification, even if RP never exceeded 10 mg P m^{-3}.

Phytoplankton

Chlorophyll *a* was always higher in SUR than in DEP where, exceptionally, surpassed 2 mg m^{-3} (Fig. 6a). SUR values increased from October to winter months, up to the maxima of 21.1 mg m^{-3} in February and 20.4 mg m^{-3} in March. Contemporaneously, the photic zone (PZ = 2.5 SD) decreased, achieving the minimum of 2.9 m and 3.8 m, respectively in February and March (Fig. 5a). Chl *a* showed lower values in the spring months (ca. 7 mg m^{-3}), showing further peaks in summer (ca. 11 mg m^{-3}), when PZ was highest (8.8 m from June to September). Vertical distribution of Chl *a* along water column showed an evident upper layer preference in the colder months (Fig. 5). From April, the higher values were recorded below 2.5 m and, in particular from June to August, at the depth of -5 m.

Phytoplankton cell density and biomass were higher in SUR than in DEP, except than in October and December for density and only in December for biomass, when values were similar in the two layers (Fig. 6b). SUR values overlapped with the weighted means in the photic zone (R^2 = 0.99745 for density; R^2 = 0.96664 for biomass; n = 11), which ranged between 0.5 x 10^6 cells l^{-1} in April and 14 x 10^6 cells l^{-1} in September for cell density, and from 1.64 mg l^{-1} in September to 32.74 mg l^{-1} in March for biomass (Fig. 6b).

Class composition of density and biomass differed largely. In both the stations (St. 1 and St. 2) and in all the samplings, density was clearly more diversified than biomass. Density was dominated by Chlorophyceae (mainly *Chlorella* sp.) in October-December, Cryptophyceae (mainly *Plagioselmis lacustris* (Pascher & Ruttner) P.Javornick) and Dinophyceae (due to *Gymnodinium*

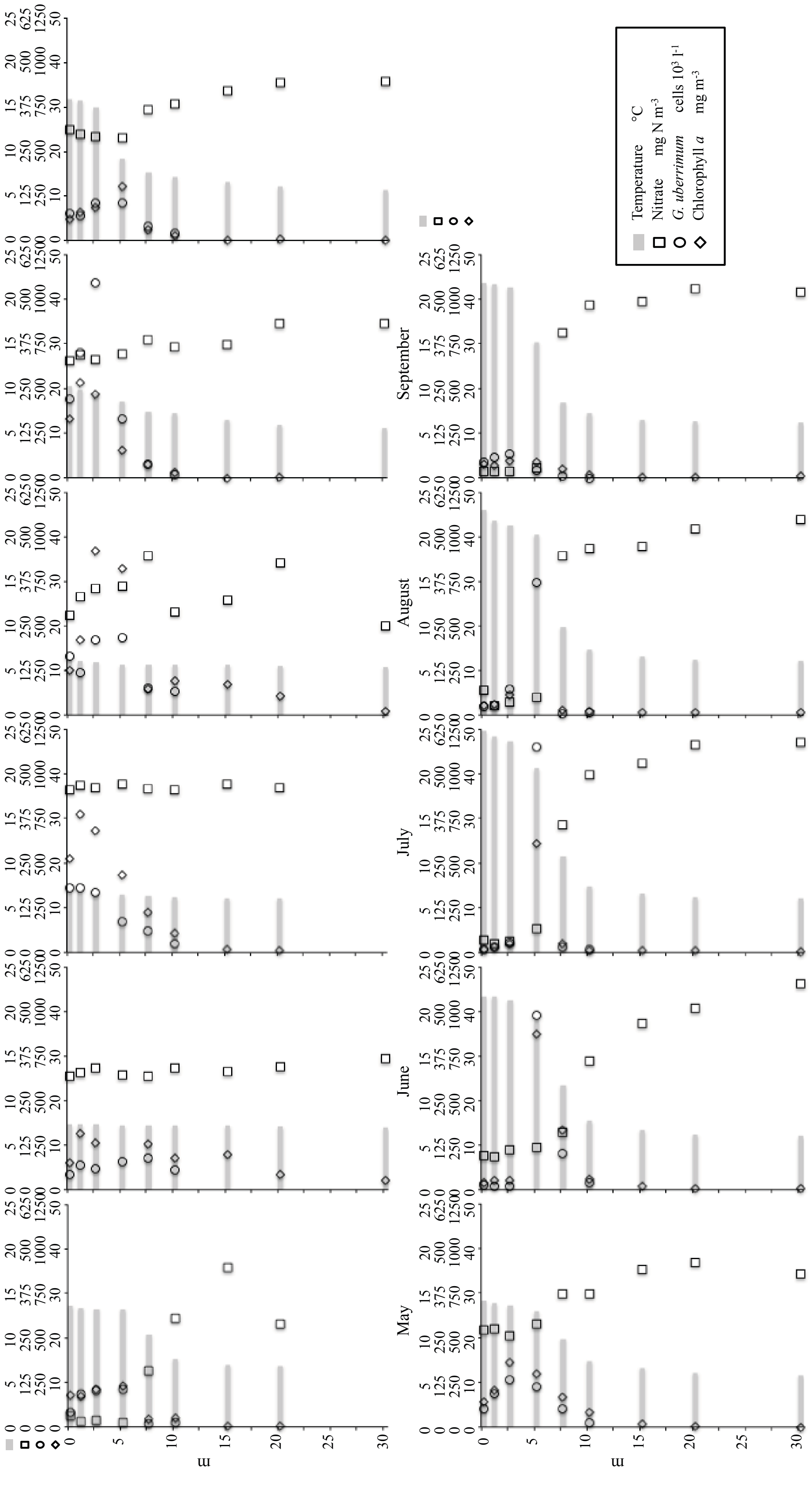


Fig. 5 – Monthly vertical dynamics of temperature, nitrate, *G. uberrimum* abundance and chlorophyll *a* at St. 1 in the Lake Sos Canales.

uberrimum (G.J.Allman) Kofoid & Swezy) from January to May, and Cyanobacteria (mainly *Aphanothece minutissima* (W. West) Komárková-Legnerová et Cronbergmainly) in August and September, when cell density value was highest. On the other hand, biomass resulted strongly dominated only by Dinophyceae (*G. uberrimum*). The vertical distribution of *G. uberrimum* along the water column showed a strong seasonal dynamic, preferring superficial depths (0-2.5 m) in winter-spring months and deepest epilimnetic depths (5-7.5 m) in summer months (Fig. 5). Phytoplankton biomass, expressed as carbon and nitrogen contents, was constituted largely by Dinophyceae (namely, *G. uberrimum*), both at St. 1 and St. 2. Phytoplankton biomass was lower in RIV than in SUR samples. The highest values were observed in the colder winter months, from January till March, with annual peaks of 5 mg C l^{-1} and 3.8 mg N l^{-1}. Since April, when water temperature increased, biomass decreased.

Suspended particulate matter and isotopic signatures

Suspended particulate matter in SUR layer ranged between 1.74 µg dw l^{-1} in July and 5.25 µg dw l^{-1} in March and in DEP layer between 1.13 µg dw l^{-1} in May and 5.42 µg dw l^{-1} in February, at St. 1 (Fig. 7a). At St. 2, the values ranged from 1.93 µg l^{-1} in June and 6.67 µg dw l^{-1} in July, highlighting a different seasonality. When the lake level was lower, at the beginning and ending of the study period (from October to January and September), the amounts of SPM were closer in SUR and DEP layers, as well as in RIV samples. In February, when lake level was at the maximum and mixing occurred, SPM values increased both at St.1 and St. 2. At St. 1, SUR and DEP values overlapped and were twice than in RIV samples. From March, the dynamics were more differentiated. In fact, a progressive decrement of SPM was revealed in RIV samples, coherently with the rainfall dynamic, till the unexpected and intense peak in July. On this occasion, the maximum of the all dataset was recorded and it might be due to shoreline erosion. At St. 1, SPM dynamic was less regular. Therefore, the peaks were observed in the wet season, in February and March, in correspondence of the maximum level of the lake, and contemporaneously with the maxima of Chl a and of *G. uberrimum* in upper depths (0-2.5 m; Fig. 6). In March, an abrupt decrement was recorded in DEP while SPM was highest in SUR. In April, SUR value diminished at about the same level of DEP. In May and June, SPM in DEP layer showed lower values than in SUR and RIV.

$\delta^{13}C$ signatures of SPM at the different stations and lake water layers largely varied along the season (Fig. 7b). The ranges were very close for SUR and DEP values at St. 1, respectively of -34.89‰ (December) and -26.72‰ (May) in SUR and -35.26‰ (December) and -26.48‰ (May-June) in DEP, and also for RIV (-35.42‰ in

December and -26.28‰ in June).

Indeed, the most negative values were measured in winter (December), less negative ones in summer (June and July) and the signatures fully overlapped at the beginning (from October to December) and ending of the study period (September), in the fall-winter months.

The dynamics were more differentiated in winter-spring months. In fact, δ^{13}C signatures of SPM in SUR increased from December to February and then remained quite constant and more negative than δ^{13}C in DEP and RIV samples till May. DEP and RIV values were very similar in February, April and May, while differed in March, when DEP value approached more to SUR.

All the δ^{13}C signatures became less negative (-26±0.005‰) in June but maintaining remarkable differences and less negative values in DEP and RIV than in SUR. Finally, SUR values further increased becoming less negative than DEP and RIV in July and September.

δ^{15}N of SPM of SUR (range: 4.56‰ in June and 10.60‰ in October) and DEP (range: 2.97‰ in June May and 8.82‰ in October-December) and RIV (range: 4.37‰ in June and 10.05‰ in December) were close in October and December, with values of ca. 10‰ (Fig. 7c), similarly to δ^{13}C dynamic. The values sharply decreased, becoming less enriched in DEP from February to May and in SUR and RIV until June. In this period, DEP values were lower (less enriched) than in SUR and RIV values from October to May, becoming higher from June and to July-September. Instead, SPM signatures in samples from SUR and RIV were very similar and tended to decrease from December till June (ca. 4‰), increasing then from July to September.

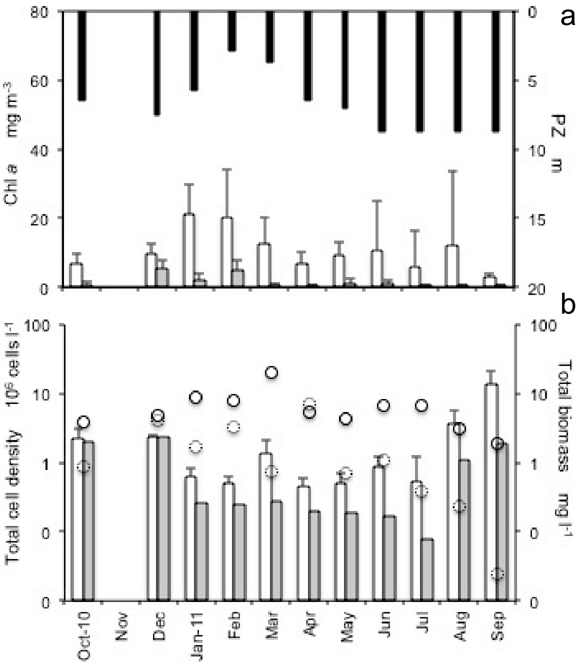


Fig. 6 - Observed dynamics of a) chlorophyll *a* and photic zone (black column), b) total cell density (column) and total phytoplankton biomass (circles). (SUR = white column and solid circles; DEP = grey column and dotted

Oligochaetes collected from the sediments, showed light δ^{13}C signatures (-34±0.19‰), close to the values recorded in SMP in December, when SUR, DEP and RIV signatures overlapped. Values of earthworms were less negative (-22.9±1.68‰, n=10). δ^{15}N values were of 9.20±0.15 (n=4) for oligachaetes and of 4.67±1.18 (n=10) for terrestrial worms, respectively similar to the values at St. 1 and St. 2 in fall and June (SUR and RIV samples).

Discussion

Hydrological, environmental conditions, suspended particulate matter and isotopic signatures

Lake Sos Canales (SC) was strongly affected by water level fluctuation (WLF) throughout the year, with a clear seasonality as usually reported for reservoirs in the Mediterranean area (Naselli-Flores, 2003; Naselli-Flores and Barone, 2005; Carmujo and Boavida, 2005). The lowest water level was reached in fall months (October and December), at the end of the driest period, and because of the all-year constant water withdrawal (Fig. 2), to produce drinkable water. Filling phase occurred in winter, and it was mainly due to rainfall conveyed into the tributary by the watershed.

The unpredictable climatic variability and the prolongation of drought time in Mediterranean basin often cause the completely dry of the upland tributary, as in the upper stream of River Tirso (personal observation). In the dry season (late spring-summer-early autumn) a large amount of plant detritus may accumulate into the dry river-bed, which adding to the runoff of watershed, can be transported into the reservoir after the first rainfall. Consequently, a relevant input of allochthonous material from the watershed can be hypothesized and, in turn, signatures close to terrestrial origin, usually more enriched than that of aquatic origin (Finlay and Kendall, 2006), expected in the lake. In the case of SC, during the filling phase in February (WLF of +10 m than in December), transparency decreased, reaching the minimum of 1.2 m SD, and suspended particulate matter (SPM) increased. Contemporaneously, $\delta^{13}C$ values were less negative than in fall, when SUR, DEP and RIV $\delta^{13}C$ and $\delta^{15}N$ overlapped, showing values of more lacustrine origin. In particular, in February, $\delta^{13}C$ SUR value was more negative than DEP and RIV values, indicating a more conservative behavior than DEP and RIV and also the presence of autochthonous origin SMP. On the contrary, $\delta^{13}C$ DEP overlapped with RIV value, probably tracing the water inflow from watershed into the reservoir. In fact, because inflow density usually differs from that of the water already present in a reservoir (e.g., due to temperature, total dissolved solid and SPM), inflows enter and move through reservoirs as density current (Ford, 1990 and references therein). $\delta^{13}C$ of bottom-benthic oligochaetes (-34±0.24‰) collected from the sediments of SC was closer to $\delta^{13}C$ in SUR than in DEP and RIV during the filling phase, supporting this hypothesis. On the other hand, the SPM origins during the filling phase traced by $\delta^{13}C$ were also supported by the lower $\delta^{15}N$ values during the filling phase, when especially DEP values were close to $\delta^{15}N$ of earthworms (4.67 ± 1.18, n=10). Signature of terrestrial material origin is in fact, usually, less enriched than that of aquatic origin (Finlay and Kendall, 2006). Overall values of $\delta^{15}N$ were negatively correlated with lake water levels (Spearman-Rank, R =

-0.72, n = 54; P = 0.0001).

More than hydrological variability, others physical variables, such as temperature, are important determinants of $\delta^{13}C$ seasonal changes (Rau et al., 1989). In relation to the seasonal thermal dynamic, SC confirmed its warm-monomictic character. In monomitic lakes a positive correlation between $\delta^{13}C$ and temperature is often observed (Caroni et al., 2012; Perga and Gerdeax, 2006; Zoary, 1994). When temperature increase, atmospheric dissolution decrease, hence changes in internal CO_2 sources can led to a positive correlation with temperature. Temperature controls the solubility of CO_2 and in turn influences the fractionation of carbon isotopes during phytoplankton uptake (Rau et al., 1989 and 1992). In our study, $\delta^{13}C$ of SPM increased from more to less negative values, from May to July, when the reservoir was thermally stratified and surface temperature reached maximum. Anyway, a positive correlation was assessed only between SUR $\delta^{13}C$ and the relative temperature (Sperman-Rank, R = 0.883, n = 9; p = 0.007). A different uptake of Dissolved Inorganic Carbon (DIC) by phytoplankton may have been responsible for this relationship.

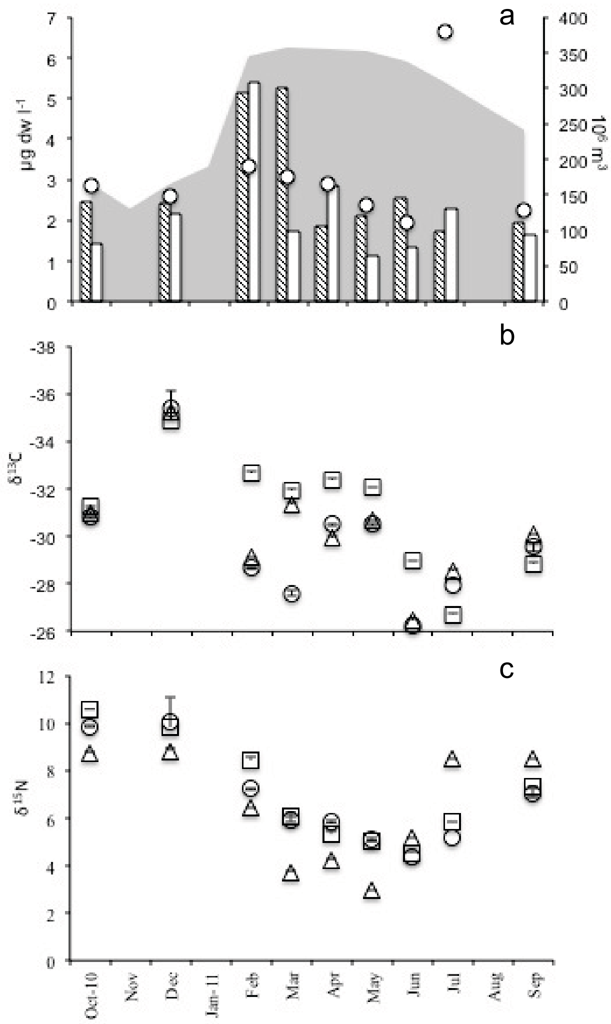


Fig. 7 - Observed dynamics of a) SPM dry weights and mean monthly reservoir volume, (b) $\delta^{13}C$ and and (c) $\delta^{15}N$ isotopic signatures of SPM. (SUR = striped column and squares; DEP = white column and triangles; RIV =

On the other hand, in stratified lakes and reservoirs, biological activities affect also hypolimnetic waters, decreasing the dissolved oxygen, pH and redox potential at the water-sediment interface (Nowalin et al., 2005). Moreover the prolongation of dry season and the stagnation of waters, with consequent lower redox potential, can enhance the remobilization of nutrients from the sediments (Zoary, 1994; Boström et al., 1998). In SC, DEP pH and oxygen reduction occurred in summer, even if anoxia was not recorded. Consequently, process of nitrification may be occurred, contributing to the increment of the dissolved inorganic nitrogen (DIN) in DEP in summer months (in June and especially in July). Indeed, $\delta^{15}N$ in DEP may have reflected degradation processes. On the contrary, from April to June, DIN decrement in SUR may have been due to phytoplankton uptake, and, in agreement, $\delta^{15}N$ signature decreased. In fact, a conceptual model developed in the Great Lakes (USA) describes that the $\delta^{15}N$ of seston is controlled by the balance between NH_4^+ uptake and degradation processes that increase $\delta^{15}N$, and NO_3^- uptake that decrease the $\delta^{15}N$ of seston (McCusker et al., 1999). Furthermore, in SC also phosphorous tended to increase in DEP during stratification and when external input was negligible, suggesting the importance of an internal recycle in deeper waters.

Finally, considering carbon signatures, hypolimnetic intense respiratory activity can lead to the decrease in the isotopic signals: organisms which rely on deep water sources, where respiration activity increase, are more negative than those living closer to the surface and/or littoral zone (Vander Zanden and Rasmussen, 1999; Grey et al. 2000; Cattaneo et al., 2004).

Phytoplankton, suspended particulate matter and isotopic signatures.

The important contribution of phytoplankton to surface SPM in SUR samples was indicated by the significant correlations between SPM weight and Chl *a* (Spearman-Rank R = 0.883, n = 9, p = 0.001) and total phytoplankton biomass (Spearman-Rank R = 0.636, n = 11, p = 0.03). Correlations were not found between phytoplankton and SPM weight in DEP and RIV, whose dynamics may have been mainly related to hydrological processes, as already highlighted in the above paragraph. Also the anomalous high value of SPM recorded in July in RIV may have been related with the shoreline erosion after the decrease of water level. On these considerations, in the following, the discussion is focused only on data relative to SUR.

Phytoplankton biomass was mainly due to *Gymnodinium uberrimum* (Spearman-Rank R = 0.636, n = 11, p = 0.03), which is a mixotrophic naked dinoflagellate. In SC, it found more favorable life conditions in the colder winter months (rom December till March), when the lake was in mixing condition, in the upper depths (-2.5 m), and in summer months, when temperature increased (June and July) and the lake was stratified, in deeper layers (-5 m). The capability to move daily along the water column is well known for this species (Tiezen, 1973; Queimaliños et al., 2002; Niesel et al. 2007), as well as to settle down seasonally at specific water layer (Niesel et al. 2007 and reference therein). Although stratified condition was supposed to help motile species, recent studies reported a high competitiveness of *G. uberrimum* in mixed water, when these motile cells cannot utilize their ability to move (Niesel et al., 2007). Moreover, a positive phototaxis was reported for G*. uberrimum* in high altitude lake whereas a negative phototaxis in mid-range altitude (Rott, 1988), in according to the observed behavior in SC: during winter, when photic zone was lower, *G. uberrimum* was mainly recorded in upper water layer (-2.5 m), whereas, when photic zone increased, swam in epilimnetic deeper layers. *G. uberrimum* is considered a oligo to mesotrophic species with a positive benefit from low phosphorus concentrations (Tielzer, 1973; Niesel et al., 2007). It is also reported as a species of good environmental quality in Mediterranean reservoirs (Marchetto et al., 2009). In SC, a relationship between *G. uberrimum* seasonal distribution and the concentration of nitrate along the water column was also observed, as reported for other gymnodiales (Cullen and Horrigan, 1981). Moreover, N-dinoflagellate biomass content was strongly correlated with nitrate (Spearman-Rank R : 0.7, n = 11, p = 0.001). When SC was thermally stratified, NO_3^- tended to decrease in epilimnetic layers, as already discussed, with decrease in $\delta^{15}N$. In September, when *G. uberrimum* decreased, the concentration of nitrate in epilimnetic was strongly reduced.

Conclusions

Lake Sos Canales is a small monomitic reservoir where annual hydrological cycles affected the carbon and nitrogen signature of suspended particulate matter (SPM). Been a Mediterranean reservoir, SC was characterized by two phases, one of stagnant low waters, from the late summer to winter beginning, and the other, more dynamic, from winter to spring, of “re-filling”. SPM and its composition were influenced by these annual dynamics. In fact, hydrological dynamics, mirrored by water level fluctuation, led the $\delta^{15}N$ seasonality related to annual nitrogen cycle whereas $\delta^{13}C$ allowed tracing the water inflow from watershed into the reservoir. Further, isotopic signatures of SPM were influenced by phytoplankton composition and its uptake activity (Zoary, 1994; Gu and Schelke 1996; Lehmann et al., 2004). The apparent anomalous $\delta^{13}C$ signatures distribution along the water column during filling phase suggests a conservative behavior of the peculiar phytoplanktonic inhabitants, which retain partially the signatures of previous months. Phytoplankton community and SPM in surface waters were dominated, in terms of biomass, by *Gymnodinium uberrimum*, a mixotrophic dinoflagellate, which prefers oligo-mesotrophic conditions. *G. uberrimum* found advantageous condition in winter mixed water, when preferred superficial layers. When summer thermal stratification occurred, settle down at specific water layers during the daylight, as reported for other environments (Tiezen, 1973; Queimaliños et al., 2002; Niesel et al. 2007).

Our study confirms the high ecological complexity of reservoirs and highlights that stable isotope analysis is a useful tool in the interpretation of their dynamics related both to hydrologic and biotic processes.

References

Bond N.R., Lake P.S., Arthington A.H., The impacts of frought on freshwater ecosystems: an Australian perspective. Hydrobiologia, 600: 3-6, 2008.

Boström B., Andersen J., Fleischer S. & Jansson M., Exchange of phosphorus across the sediment–water interface. Hydrobiologia, 170, 229–244, 1988.

Boström., Jansson M. & Forsberg G., Phosphorus release from lake sediments. Archiv für Hydrobiologie, 18, 5–59, 1982.

Camin F., Perini M., Colombari G., Bontempo L., Versini G., 2008. Influence of dietary composition on the carbon, nitrogen, oxygen and hydrogen stable isotope rations of milk. Rapid. Comm. Mass. Spectrom., 22: 1690-1696.

Camin F., Wietzerbin K, Blanch Cortes A., Haberhauer G., Less M., Versini G.J., 2004. Application of multielement stable isotope ratio analysis to the characterization of French, Italian, and Spanish cheeses. J. Agric. Food Chem., 52: 6592-6606.

Caroni R., G. Free, A. Visconti and M. Manca. Phytoplankton funnctional traits and seston stable isotopes signature: a funcitional-based approach in a deep, subalpine lake, Lake Maggiore (N. Italy). Journal of Limnology, 71(1), 84-94, 2012.

Cattaneo A., M. Manca and J.B. Rasmussen. 2004. Peculiarities in the stable isotope composition of organisms from an alpine lake. Aquat. Sci., 66: 440–445.

Cullen J.J. and Horrigan S.G., Effects of Nitrate on the Diurnal Vertical Migration, Carbon to Nitrogen Ratio, and the Photosynthetic Capacity of the Dinoflagellate *Gymnodinium splendens*. Marine Biology 62: 81-89, 1981.

Fadda A., S. Marková, P. Kotlík, A. Lugliè, B. Padedda, P. Buscarinu, Ni. Sechi and M. Manca. First record of planktonic crustaceans in Sardinian reservoirs. Biologia 66(5): 856-865.

Finlay and Kendall, 2008. In “ Stable Isotopes in Ecology and Environmental Science, second edition,. Finlay J.C. and Kendall C.- Stable Isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems, pp. 283-324.

Geraldes A.M. and Boavida M.J., Seasonal water level fluctuations: implications for reservoir limnology and management. Lakes Reserv. Res. Manage. 10: 59-69, 2005.

Gosselain V., Hamilton P.B. and Descy J., Estimation phytoplankton carbon from microscopic counts: an application for riverine system. Hydrobiologia, 438: 75-90, 2000.

Grey J. 2000. Trophic fractionation and the effects of diet switch on the carbon stable isotopic “signatures” of pelagic consumers. Verh. Internat. Verein. Limnol. 27:3187-3191.

Grey J. and R.I. Jones 2001. Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. Limnol. Oceanogr., 46(3): 505–513.

Grey J. Trophic fractionation and the effects of diet switch on the carbon stable isotopic “signatures” of pelagic consumer. Verh. Internat.Verein. Limnolol. 27: 3187-3191, 2000.

Grey J., R.I. Jones, D. Sleep. 2000. Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state. Oecologia, 123: 232–240.

Gu B., A.D. Chapman, C.L. Schelske. 2006. Factors controlling seasonal variations in stable isotope composition of particulate organic matter in a soft water eutrophic lake. Limnol. Oceanogr., 51(6): 2837-2848.

Gu B., D.M. Schell and V. Alexander. 1994. Stable Carbon and Nitrogen Isotopic Analysis of the Plankton Food web in a Subarctic Lake. Can. J. Fish. Aquat., 51: 1338-1344.

Lehman M.F., Bernasconi S.M., and McKenzie J.A., Seasonal variation of the δ13C and δ15N of particulate and dissolved carbon and nitrogen in Lake Lugano: Constraints on biogeochemical cycling in a eutrophic lake. Limnol. Oceanogr. 49 (2): 415-429, 2004.

Lehman M.F., Reichert P., Barbieri A. and McKenzie J.A., Modelling nitrogen and oxygen isotope fractionation during denitrification in a lacustrine redox-transition zone. Geochim. Comochi. Acta. 67: 2529-2543, 2002.

Leira M. Cantonati M., Effects of water-level fluctuations on lakes: an annotated bibliography. Hydrobiologia 612: 171-184, 2008.

Marchetto, B.M Padedda, M. Mariani, A. Lugliè, N. Sechi, A numerical index for evaluating phytoplankton response to changes in nutrient level deep mediterranean reservoirs, J.Limnol., 68-1 (2009), pp. 106-121.

Menden-Deuer S. and Lessard E.J., Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol. Oceanogr., 45(3): 569-579, 2000.

Mulholland P.J., Marzolf E.R., Webster J.R., Hart D.R. & Hendricks S.P. 1997. Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams. Limnology and Oceanography, 42: 443-451.

Naselli-Flores L., Barone R., Water-level fluctuations in Mediterranean reservoirs:setting a deatering threshold as a management tool to improve water quality. Hydrobiologia, 548: 85-95, 2005.

Naselli-Flores L., Man-made lakes in Mediterranean semi-arid climate:the strange case of Dr Deep Lake and Mr Shallow Lake. Hydrobiologia, 506 (1-3): 13-21, 2003.

Naselli-Flores. Man-made lakes in Mediterranean semi-arid climate: the strange case of Dr. Deep an Mr Shallow Lake- Hydrobilogia, 506 (1-3): 13-21.

Niesel V., Hehn E., Sudbrack R., Willmitzer H., and Chorus I., 2007. The occurrence of the Dynophyte species *Gymnodinium uberrimum* and PeridiniumWillei in German reservoirs. J. Plank. Res., 29(4): 347-357.

Nowlin W., Evarts J.L. and Vanni J., Release rates and potential fates of nitrogen and phosphorous from sediments in a eutrophic reservoir. Fresh. Biol., vol. 50: 301-322, 2005.

OECD 1982. Eutrophication of waters. Monitoring, assessment and control. Organization for Economic Co-operation and Development. Paris, France.

Perga, M.E. & D. Gerdeaux. 2006. Seasonal variability in the $\delta^{13}C$ and $\delta^{15}N$ values of the zooplankton taxa in two alpine lakes. Acta Oecologica, 30: 69-77.

Post. D. M. Using stable isotopes to estimate trophic position: models, methods, and assumption. Ecology 83: 703-718, 2000.

Queimaliños C., Perez G. and Modenutti B., Summer population development and diurnal vertical distribution of dinoflagellates in an ultraoligotrophic Andean lake (Patagonia, Argentina). Alogological Studies, 107 :117-129, 2002.

Rau, G.H., T. Takahashi & D.J. des Marais. 1989. Latitudinal variations in plankton ^{13}C: implications for CO2 and productivity in past oceans. Nature, 341: 516-518.

Rau, G.H., T. Takahashi, D.J. des Marais, D.J. Repeta & J.H. Martin. 1992. The relationship between 13C of organic matter and [CO2 (aq)] in ocean surface water: data from a JGOFS site in the northeast Atlantic Ocean and a model. Geochim. Cosmochim. Acta, 56: 1413-1419.

Rau, G.H., U. Riebesell & D. Wolf-Cladrow. 1996. A model of photosynthetic fractionation by marine phytoplankton based on diffusive molecular CO2 uptake. Mar. Ecol. Prog. Ser., 133: 275-285.

Rott, E. 1981. Some results from phytoplankton counting intercalibrations. Schweizerische Zeitschrift für Hydrologie, 43: 34-63.

Sechi N. and A. Lugliè, Limnlogical studies on man-made lakes in Sardinia (Italy). Mem. Ist. Ital. Idrobiol., 50 (1992), pp. 365-381.

Sechi N. and A. Cossu, Prime valutazioni sui livelli trofici di alcuni bacini artificiali della Sardegna, Mem. Ist. Ital. Idrobiol., 37 (1979), pp. 259-276.

Sechi N. and A. Lugliè, Limnlogical studies on man-made lakes in Sardinia (Italy). Mem. Ist. Ital. Idrobiol., 50 (1992), pp. 365-381.

Sechi N., and A. Luglié, Phytoplankton in Sardinian reservoirs.Giornale Botanico Italiano, 130 (1996), pp. 977-994.

Sechi N., and A. Luglié, Phytoplankton in Sardinianreservoirs.Giornale Botanico Italiano, 130 (1996), pp. 977-994.

Straskraba, M., Tundisi, J.G., Duncan, A. 1993. Comparative Reservoir Limnology and

Water Quality Management. Developments in Hydrobiology. Volume DH 77, Kluwer Academic Publishers, Boston.

Straškraba, M., Tundisi, J. G. 1999. Reservoir water quality management. Guidelines of lake management, volume 9. Ed. International Lake Environment Committee, Kusatsu. 1999.

Strickland J.D.H., and T.R. Parsons A practical handbook of water analysis. J. Fish. Res. Bd. Canada. 167 (1972).

Sulzman E., 2008. In " Stable Isotopes in Ecology and Environmental Science, second edition,. Finlay J.C. and Kendall C.- Stable Isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems, pp. 283-324.

Syväranta J., Tiirola M. and Jones R. I., 2008. Seasonality in lake pelagic $\delta^{15}N$ values: patterns, possible explanations, and implications for food web baseline. Fun. App. Limnol. Arch. Hydr., 172(3) 255-262. (Fundamental Applied Limnology Archiv fur Hydrobiologie).

Thornton K. W., Kimmel B.L., Payne F.E. 1990. Reservoir limnology: Ecological perspectives. John Wiley & Sons, Inc., Somerset, NewJersey.

Tilzer M.M., Diurnal periodicity in the phytoplankton assemblage of a high mountain lake. Vol. 18: 15-30, 1973.

Tranes J. and Bernasconi S.M., The record of nitrate utilization and productivity imitation provided by $\delta^{15}N$ values in lake organic matter-a study of sediment trap and core sediments from Baleggersee, Switzerlandk. Limnol. Oceanogr. 45: 801-813.

Tundisi G. and Matzumura-Tundisi J., 2003. Integration of research and management in optimizing multiple uses of reservoirs: the experience in South America and Brazilian case studies. Hydrobiologia. Vol. 500, no. 1-3, pp. 231-242.

Tundisi J. G., O. Rocha, T. Matsumura-Tundisi and B. Braga, Reservoir management in South America, Water Resources Development, 14-2 (1999), pp. 141-155.

Vander Zanden, M.J. & J.B. Rasmussen. 1999. Primary consumer 13C and 15N and the trophic position of aquatic consumers. Ecology, 80: 1395-1404.

Vuorio, K., M. Meili & J. Sarvala. 2006. Taxon-specific variation in the stable isotopic signatures (13C and 15N) of lake phytoplankton. Freshwat. Biol., 51: 807-822.

Zohary T. an Ostrovsky I., Ecologica impacts of excessive water level fluctuations in stratified freshwater lakes. Inland Water 1,: 47-59, 2011.

Zohary, T., J. Erez, M. Gophen, I. Bermanfrank & M. Stiller. 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. Limnol. Oceanogr., 39: 1030-1043.

CHAPTER 3

Spatio-temporal dynamics of C and N isotopic signature of zooplankton: an annual study on a man- made reservoir in the Mediterranean Region

Amedeo Fadda [1], Bachisio Padedda[1], Antonella Lugliè[1] , Marina Manca[2]
(1) Department of Sciences for Nature and Environmental Resources (DipNET) of the University of Sassari (Italy), 4 07100 Sassari (SS) Italy
(2) CNR Institute of Ecosystem Study (CNR-ISE), Largo Tonolli 50 28922 Verbania.

Abstract

Seasonal changes in carbon and nitrogen stable isotope signatures of crustacean zooplankton and Suspended Particulate Matter (SPM) were studied in a Mediterranean small reservoir. Reservoirs are complex environments affected by several stressors from climatic and management processes. Seasonal water level fluctuation is the most visible stress in semi-arid countries, characterized by a “low-water” and a “re-filling phase” in each hydrological year cycle. Stable isotope analysis (SIA) of C and N is a useful tool to trace energy and matter flow between SPM and pelagic primary consumers. In Sos Canales Reservoir (SCR), SPM and zooplankton were collected monthly in deep, close the dam, and in the shallow, near to the river inflow, zones throughout an annual hydrological cycle. SPM was also collected in surface and deep water layers of deepest zone, as well as from littoral, deep-benthic, and surrounding soil organisms to evaluate origin of available sources for primary consumers. A seasonal trend related to hydrological annual cycle highlighted an overlapping of C and N signature among SPM collected in different zones and in deep water during the low-water phase, and a differentiation during the re-filling phase between surface and deep samples (winter-spring). Seasonal changes observed in consumer taxa were consistent with patterns observed in natural lakes, with more δ^{15}N-enriched and δ^{13}C depleted isotopic signatures in fall and winter and less δ^{13}C depleted in summer. Zooplankton taxa isotopic signatures of deep and shallow stations, following SPM, overlapped in fall and winter, while differing in spring. Impact of meteo-climatic events, such as heavy rainfall and water level increase, clearly detected in SPM isotopic signatures of both shallow and deep stations, were not detected in zooplankton taxa. Between-station differences in spring were attributed to exploitation of different food sources (δ^{13}C signature) and/or differences in contribution of early/late (small/large body size) developmental stages (degree of ^{15}N enrichment). δ^{15}N signature of copepods was exceptionally high, particularly in late fall and winter. In December and February, copepod δ^{13}C signature was independent from that measured in SPM: we provided possible explanations for this apparent paradox, based on analyses of population structure and /or of possible exploitation of alternative food sources (benthic). Overall, our results provide evidence of importance of copepods for tracing changes in matter and energy transfer along the pelagic food web of reservoirs. Peculiarities observed in some taxa along the year, e.g. high ^{15}N-enrichment during the re-filling phase of reservoir, were also identified and discussed. Overall, our results provide evidence of importance of copepods for tracing changes in matter and energy transfer along the pelagic food web of reservoirs.

Keyword: trophic web, zooplankton, copepods, suspended particulate matter, stable isotope

Introduction

Ecosystem functioning and the study of biological processes through which energy and matter are transferred through the food web are of crucial importance for both theoretical and practical aspects of aquatic ecology (Finlay and Kendall, 2008). Studies focused on functioning and processes highly contribute to definition of operational diversity by identifying ecological roles of species within ecosystems (Pace et al., 1999, Davic, 2003; Moss et al. 2009). In turn, these studies provide a basis for addressing impact of perturbations and how they are processed through the food web.

Pathways by which energy and matter are transferred through ecosystems form the basis of trophic dynamics (Lindeman 1942, Karlsson 2003). Carbon and nitrogen are basal elements of organic matter, commonly used to track energy and matter flow through food web, and among-taxa trophic relationships. Analysis of their stable isotopes (^{13}C and ^{15}N) is increasingly used to investigate freshwater ecosystem functioning (Finlay and Kendall, 2007). Carbon and nitrogen stable isotope ratios can provide time-integrated information about energy flow and feeding relationships in a food web (Post 2002). Stable isotopes potentially capture complex interactions, including omnivory and they trace energy through ecological communities (Peterson and Fry 1987, Cabana and Rasmussen 1996).

Lake food webs were, in this context, relatively well investigated, while being attempts to study small reservoirs never,especially on those in West-Mediterranean Region. Reservoirs are more impacted by human activities than natural lakes: they were made to meet water demand in areas by their nature subject to a high degree of within- and between-year variability. In an attempt to preserve water quality, reservoirs devoted to drinkable water supply are often small and located in pristine areas (Tundisi and Straskraba, 1999). They are often exploited to the highest possible degree, particularly in seasons during which water availability is naturally low. As a consequence, they are markedly affected by hydrological dynamics. Human management tend to further emphasize natural, climate-driven variability.

In reservoirs, high temporal variability is over-imposed on spatial heterogeneity, which is in turn consequent to their being somewhat intermediate between lakes and rivers (Straškraba & Tundisi, 1999). External inputs from the watershed are relevant during the wet season, or filling phase, while internal recycling processes support biological communities similar to those of natural lakes during the drought season (Zohary et al., 2011).

Because of large water level fluctuations, reservoirs are stressed environments (Leira and Cantonati, 2008 and references therein; Naselli-Flores, 2003; Heinry et al., 2010; Perbiche-Neves, 2010). Water level fluctuations may cause unexpected water mixing during thermal stratification, affecting top-down and bottom-up control mechanisms (Bond et al., 2008; Zohary et al., 2011).

Reservoirs in the Mediterranean Region are, among the others, most sensitive to climate change, for both water and biodiversity conservation purposes (Moss et al. 2009).

Crustacean zooplankton is of key importance in transferring matter and energy along the pelagic food web. Different taxa and developmental stages may play different ecological roles along the season (Smyentek et al., 2012; Manca and Comoli, 1997), because of different abilities in exploiting different food sources in a spatially and temporally variable environment. They also differ in vulnerability to fish predation (Lampert and Sommer, 1997; Mauchline, 1998; de Bernardi et al. 1987, Sprules and Bowerman, 1988; Einsle, 1996).

In the present study, we investigated seasonal changes in carbon and nitrogen isotopic signatures of crustacean zooplankton of a small reservoir in Sardinia. Our main aim was to highlight peculiar traits which might be attributed to natural and man-driven impacts; in particular we wanted to see whether and how changes in food sources resulting from water level and in hydrological regime were transferred through the food web by zooplankton consumers, in a spatially and temporally heterogeneous man-made environment. By analyzing taxa-specific isotopic signatures we were also interested in defining trophic relationships and different abilities to exploit available resources.

CHAPTER 3

Material and methods

Study area

Sos Canales (SCR) reservoir was built in 1956 damming the upper stream of River Tirso, the most important river in Sardinia (Italy) in a Mediterranean climate Region (Fig. 1). It is a small reservoir (0.33 km2) lying in a granite bedrock surrounded by Cork-Oaks woods in the North-Eastern part of the Island at 714 m a.s.l. Reservoir watershed is small (16 km^2), with little anthropogenic activity, mainly related to production of cork and wild breeding practices.

The lake provides drinking water supply for resident population (15.000 inhabitants). Its outflow, for human consumption only, is quite constant along the year. It never exceeds 265 m^3 $month^{-1}$. Mediterranean climate is characterized by warm and dry summers, and mild to cool, and wet, winters. Rainfall events are concentrated since late fall till early-late spring, often causing floods, while streams may almost completely dry up in summer. As typical for Mediterranean man-made lakes, SCR is subject to large water level fluctuations (for this reason, water conveyed into the lake is in large part retained by the dam for future supply (Naselli Flores, 2003; Zohary & Ostrovsky, 2011).

Sampling and analyses

Since October 2010 till September 2011, water and zooplankton samples were collected monthly at two sampling stations: station S (“Shallow”), near the river inflow (where water depth is <8 m throughout the year); station D (“Deep”) in the deepest area, near the dam (Fig. 1). Integrated water samples were collected with a 5 L Niskin bottle for analyzing Suspended Particulate Matter (SPM), from surface to 7.5 m depth (at 0, 1, 2.5, 5 and 7.5 m depth in the two stations; SPM-S and SPM D-S for stations S and D, respectively) and from 10 m depth to 2 m above bottom (at depths 10, 15, 20, 30 m and at 2 m above bottom; SPM-D-D) at the D station. All samples were pre-filtered in the field on a 76 μm nylon sieve, to remove zooplankton and large-size detritus. They were then filtered in the laboratory on pre-weighted GFC glass-fiber filters (1.2 μm pore size) for SPM-SIA (size fraction 1.2≤μm≥0.76). Surface water samples were collected at the same depths as for the lake monitoring since the late 70s at the D station. Integrated zooplankton samples from 2 m above bottom to surface were collected with a 76 μm mesh size Apstein’s zooplankton net, and fixed in pure (99%) ethanol. Copepods, cladocerans and rotifers were identified and counted to species. Population density and SSB (Standing Stock Biomass, from length/weight regression equations, LWRE; (McCauley 1984, Manca et al., 1997) were estimated. Specimens of the main taxa composing zooplankton samples on each date were sorted from zooplankton samples which were collected on each date with a 100 μm mesh size large mouth plankton net. Copepods were sorted by Cyclopoids and Calanoids. Cladocerans by genus (*Daphnia* and *Ceriodaphnia*). On two dates also the colonial rotifer *Conochilus unicornis-hippocrepis* gr. (Ruttner-Kolisko, 1974) was analyzed.

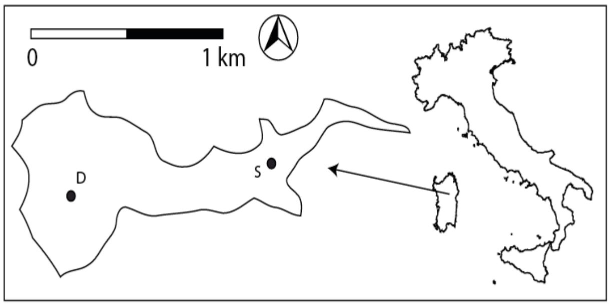


Fig. 1- Sampling stations in Lake Sos Canales, D as a deep and S as a shallow.

On each sampling date, water temperature vertical profile (Hydrolab multiprobe HdS5) and water level were measured at the two stations.

In addition, littoral and deep benthic samples were collected. Seasonal changes in $\delta^{13}C$, $\delta^{15}N$ isotopic signatures were investigated using soft tissues of a shredder decapod (*Atyaephyra desmaresti*, Millei) and of an algal-grazer gastropod (*Ancylus fluviatilis*, Müller). Oligochaetes collected from sediments of the deep station with an Ekman grab in February provided estimate of sotopic signature of profundal macrozoobenthos used as a proxy of deep lacustrine origin sources. Earthworms were also collected monthly in surrounded soil of SCR and they were used as a proxy of terrestrial origin sources.

Duplicates of each sample for SIA were oven dried for 24 h at 60°C and transferred into 6x4 mm tin-capsules. Analyses were run on a Continuous Flow-Isotope Ratio Mass Spectrometer (CF-IRMS; Delta plus XP ThermoFinnigan, Bremen, Germany), after total combustion (≈ 0.5 mg) in elemental analyzer (EA Flash 1112 ThermoFinnigan), (Camin et al. 2004, 2008). Values were expressed in δ‰ against international standards (Vienna-Pee Dee Belemnite for $\delta^{13}C$, air for $\delta^{15}N$) and computed against acetanilide, which was calibrated against international reference materials (MRI-ET-1SO 64, MRI-ET-ISO 63; Camin et al. 2008). Uncertainty of measurements was < 0.3 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. Statistical analyses were performed with Statistica for Windows (StatSoft 1994); they included between-station and among-season differences as well as potential correlation between SMP and zooplankton signatures.

Results

Zooplankton taxonomical composition

Copepods community were constituted by one calanoid species, *Copidodiaptomus numidicus* (Gurney, 1909), and two cyclopoids, *Macrocyclops albidus* (Jurine, 1820) and *Cyclops abyssorum* gr. (Sars, 1863) Cladocerans by two Daphnia species, *D. pulex* (Leydig, 1860) and *D. longispina* gr.(as defined in Petrusek et al. 2008b; Tielsch et al. 2009), two *Ceriodaphnia* species, *C. pulchella* (G.O. Sars, 1862) and *C. reticulata* (Jurine, 1820), and by *Bosmina longirostris* (O.F. Müller, 1776). Rotifers species were *Poyarthra vulgaris-dolychoptera* gr.(Ruttner-Kolisko, 1974), *Synchaeta tremula-oblonga* gr. (Ruttner-Kolisko, 1974), *Filinia longiseta* (Ehrenberg, 1834) and *Conochilus unicornis-hippocrepis* (Ruttner-Kolisko, 1974).

Water temperature was homogeneous along the water column and relatively low (7.3-5.5 °C in December 2010 and February 2011). Thermal stratification started in March, lasting until October 2011. Annual maximum temperature was recorded in July (20°C).

Water level strongly varied throughout the year, due to a continuous withdraw for human supply of c.ca 265 m^{-3}/month. At the end of 2010 the smallest water level was recorded (27 and 28 m in October and December 2010). Water level rapidly increased in February 2011 due to heavy rainfall, reaching 37 m. It weakly decreased till June 2011. Since July to September 2011, water level constantly decreased, highlighting thar low water input from the watershed was low.

Seasonal changes in isotopic signature of SPM from different stations and water depths

Isotopic signatures of SPM baselines changed with the season, ($\delta^{13}C$ Kruskal-Wallis test: H (n= 26) =18.05 p =.0208; $\delta^{15}N$ Kruskal-Wallis test: H (n= 26) =20.67 p =. 0081) with relatively more ^{13}C-depleted values between December and May, with the exception of February, less depleted values in full summer, and a reverse trend, towards increasingly 13C-depleted signatures in autumn (FIG. 2). Nitrogen isotopic signatures varied between higher values in October-September to lower values in spring and summer. Variations were much more pronounced in SPM-D-S than in SPM-D-D samples (Fig. 2). Overall, range of variation was much more marked in SPM-D-S than in SPM-D-D samples. On each date, SPM-DS and SPM-SS were quite similar, with the exception of February, when they significantly differed

Seasonal changes in isotopic signatures of zooplankton taxa

Zooplankton $\delta^{13}C$ and $\delta^{15}N$ signatures varied seasonally ($\delta^{13}C$ Kruskal-Wallis test,H:63, n=100, p=0.037; $\delta^{15}N$ Kruskal-Wallis H (63, n=100, p=0.005).In October and December, carbon isotopic signature of *Copidodiaptomus* from the two sampling sites (S and D) and of *Ceriodaphnia* and the Cyclopoids from D station were consistent with those of SPM on the same date. The latter did not differ among stations/depths. Carbon signature of *Ceriodaphnia* from S station, however, did not fit into any SPM signature in October. In the same month, $\delta^{15}N$ from D station seemed to suggest that *Copidodiaptomus* might be able to feed on Ceriodaphnia, while in the S station it might be able to feed on *Daphnia*. Nitrogen isotopic signatures of *Copidodiaptomus* and the Cyclopoids further suggest that they might have the same trophic position (Fig. 3b).

Isotopic signatures of *Copidodiaptomus* and of the Cyclopoids from the two sampling sites were quite similar in December, as were those of *Daphnia* and *Ceriodaphnia*. Based on $\delta^{13}C$, we might suggest that

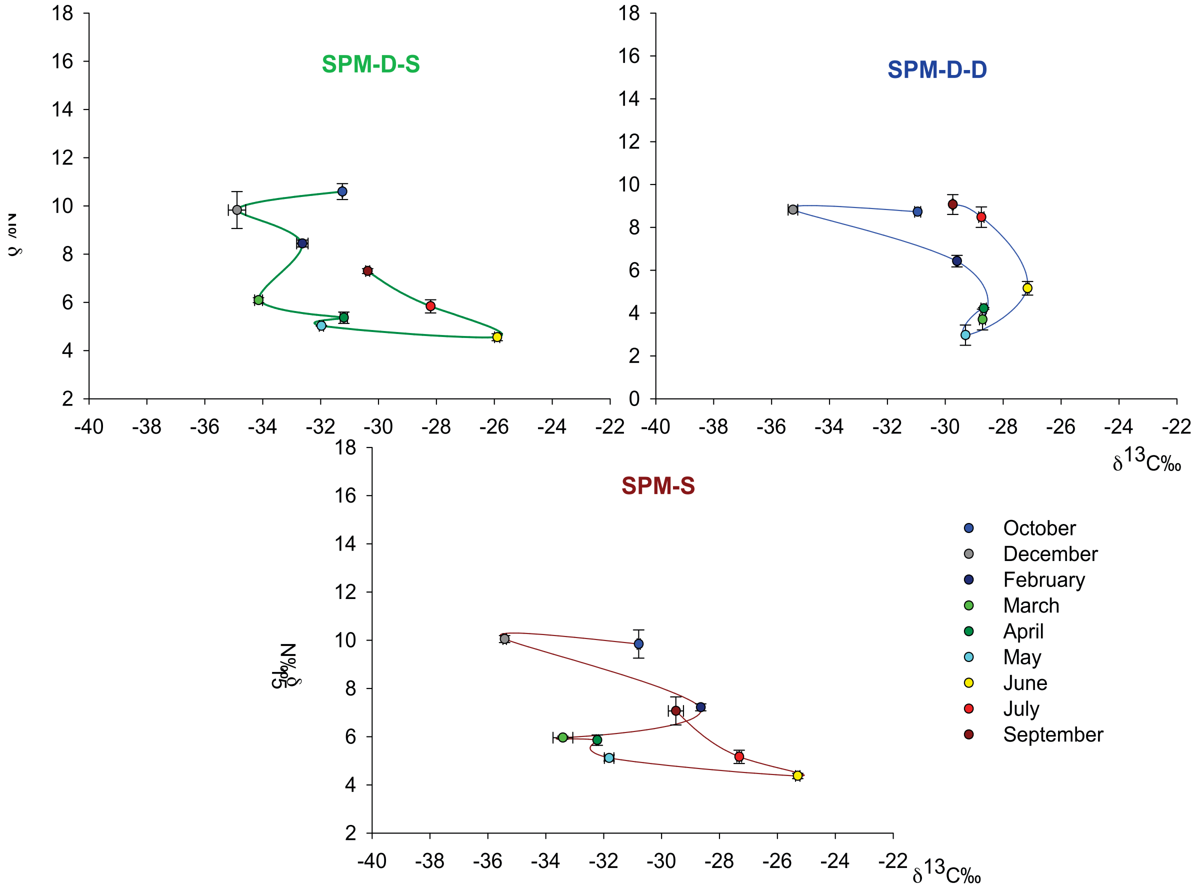


Fig. 2. Seasonal changes in carbon and nitrogen isotopic signatures of SPM from different stations and water depths of Lake Sos Canales in 2010-2011. For further explanation of symbols see text.

Copidodiaptomus and the cyclopids could be able to consume *Daphnia*, while being isotopic signatures of *Copidodiaptomus* from D station consistent with a consumption of *Ceriodaphnia* from both stations (D and S). *Copidodiaptomus,* however, likely did not feed on Daphnia from D station (Fig.3a).

Copidodiaptomus and the cyclopoids isotopic signatures were in February the same as those measured in December; they did not fit with SPM signatures measured from different sites and depths on the same date. Signatures of the latter were in fact quite different among stations, with SPM-D-S much more depleted in ^{13}C (ca. -32.5‰ δ^{13}C) than in the shallow station (SPM-S; ca. -28‰ δ^{13}C (Fig.3a). Isotopic signatures measured in March suggest a clear separation between an S and a D zone of the lake, with similar SPM-S-S and SPM-D-S signatures, both differing from SPM-D-D. *Copidodiaptomus* and the cyclopoids from both stations had isotopic signatures consistent with those of SPM-D-S. The same similarity was found for Daphnia collected at S station . *Daphnia* and *Ceriodaphnia* signatures from station D were, instead, similar to their corresponding baselines (FIG. 3b).

A distinction between Surface and deep

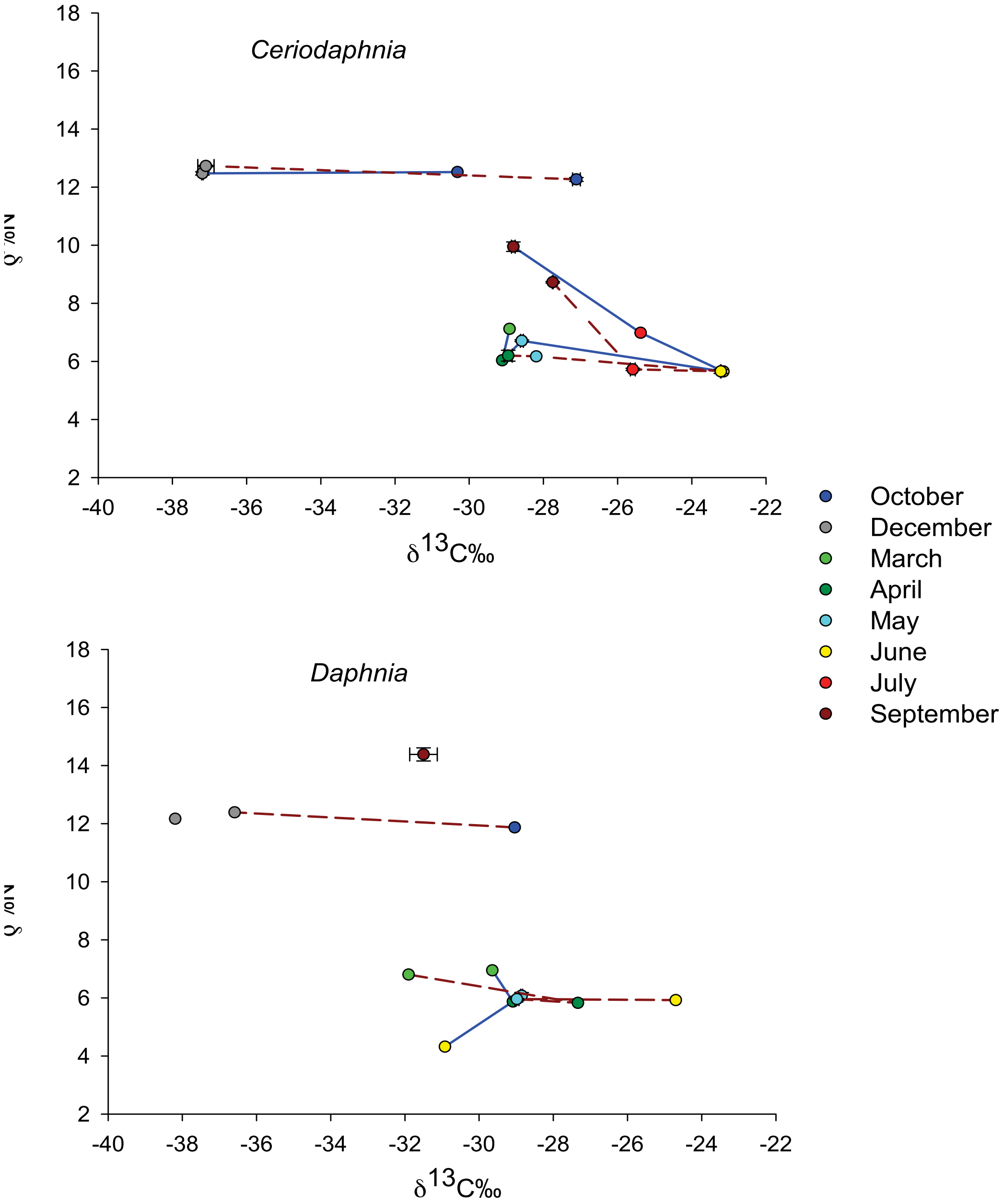


Fig.- 3a Seasonal changes in carbon and nitrogen isotopic signatures of zooplankton cladocera taxa from shallow (brown dashed line) and deep sampling stations of Lake Sos Canales, 2010-2011. For further explanation see text.

isotopic signatures was also evident in April; δ^{13}C of *Copidodiaptomus* and of the cyclopoids was close to surface SPM signature at both sampling stations Daphnia and *Ceriodaphnia*, signatures were, instead, close to SPM-D-D.

δ^{15}N of *Copidodiaptomus*, however, differred consistently between stations. Notably, when isotopic signatures of SPM-D-D differred from those of SPM-D-S they were less ^{13}C-depleted that the latter (Fig. 3c).

In May, SPM isotopic signature of SPM-D-D and SPM-S/D-S samples was still well distinguishable; *Daphnia* and *Ceriodaphnia* from both S and D station were more closely related to SPM-D-D than to SPM-DS/S

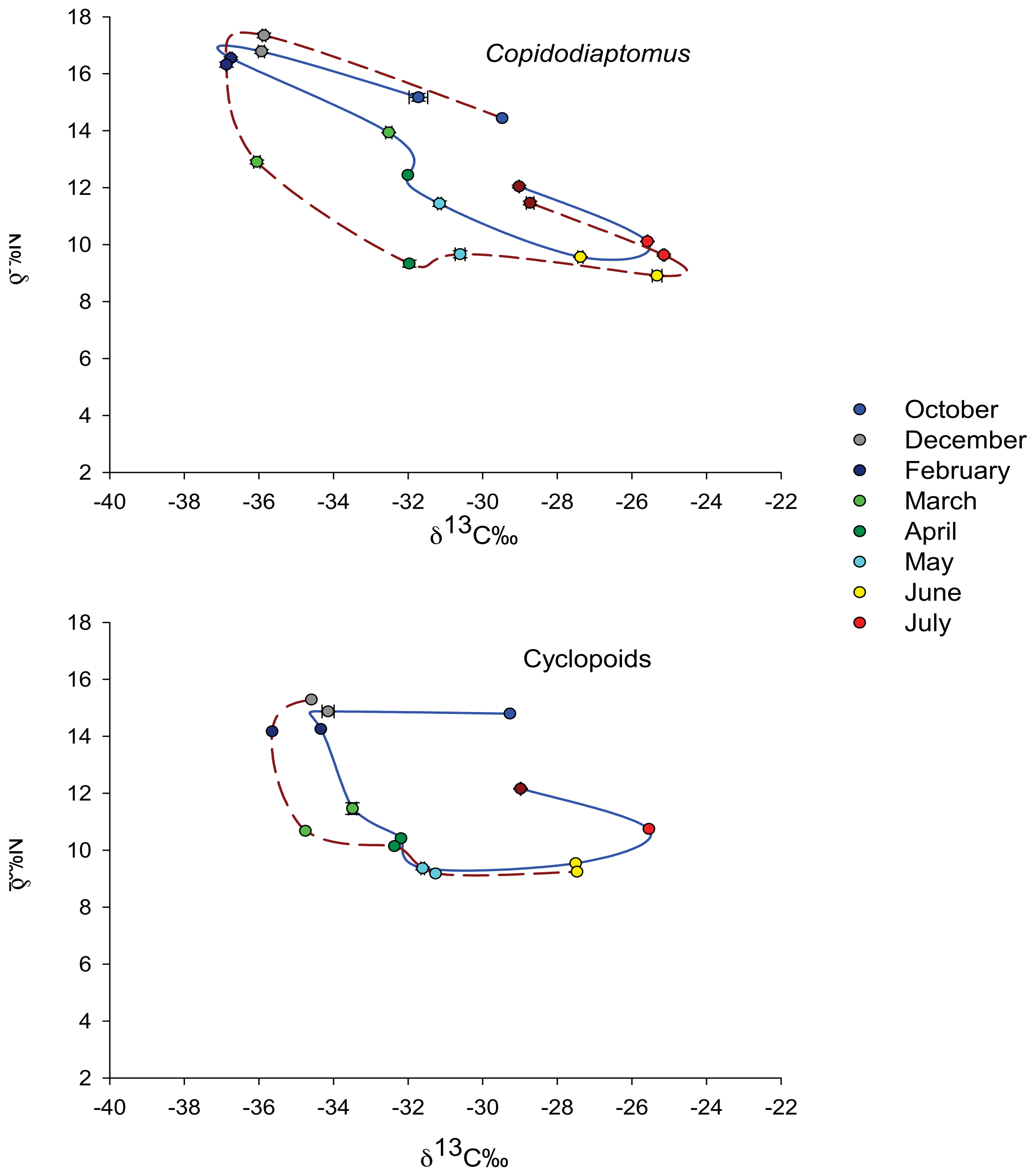


Fig.- 3b Seasonal changes in carbon and nitrogen isotopic signatures of zooplankton copepod taxa from shallow (brown dashed line) and deep sampling stations of Lake Sos Canales, 2010-2011. For further explanation see text.

carbon sources. On the other hand, *Copidodiaptomus* and the cyclopoids from both sampling stations were close to SPM-D-S/S isotopic signature. Also in this case, *Copidodiaptomus* from the D station resulted much more $\delta^{15}N$-enriched than specimens collected at S station (Fig. 3c) In June, SPM-D-S/S carbon signature were least $\delta^{13}C$-depleted, with values of $\delta^{13}C$ around ca.-26‰. *Copidodiaptomus,* and *Conochilus* colonies from D station, but also Cyclopoids from station S, were close to SPM-D-D $\delta^{13}C$. Much more $\delta^{13}C$ -depleted, and different from that of the other zooplankton taxa, was the $\delta^{13}C$ Daphnia isotopic signature. Carbon istotopic signatures of *Copidodiaptomus* and

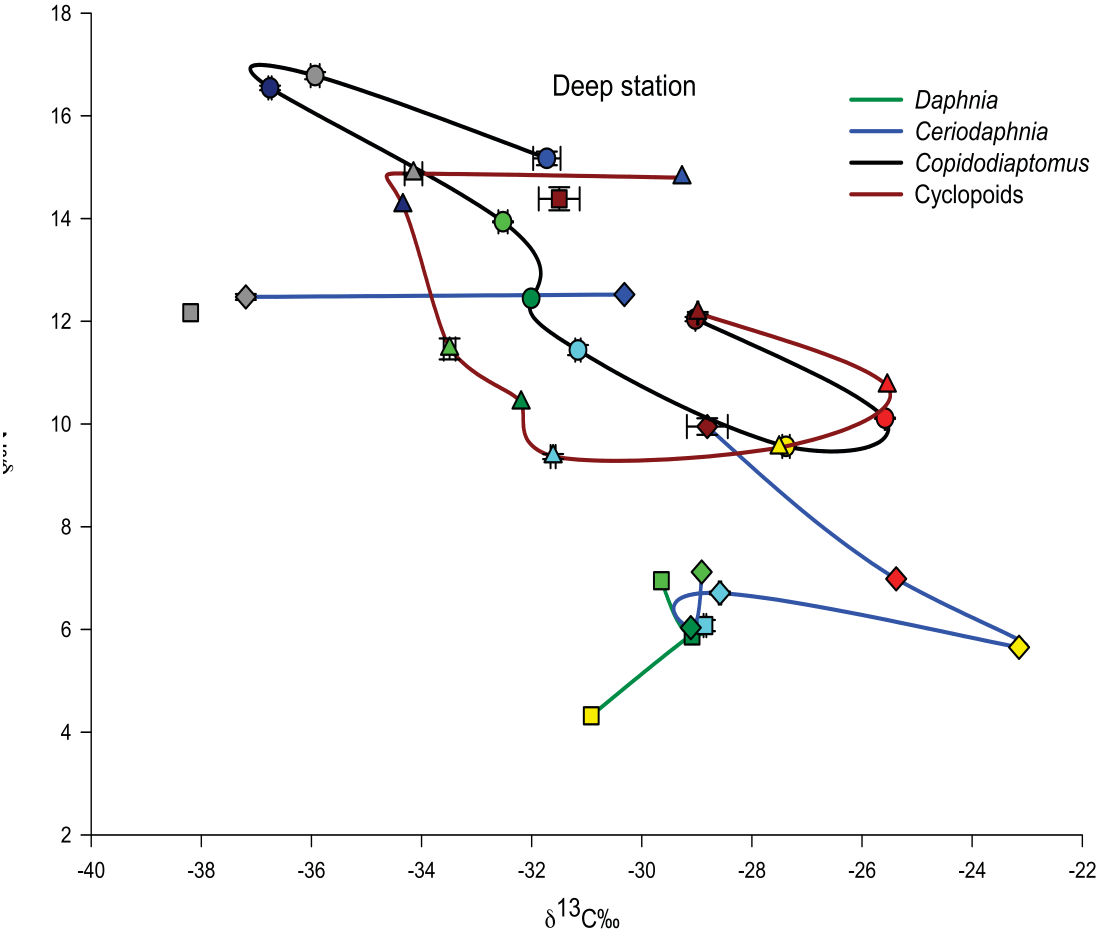


Fig. 4a- Seasonal changes in carbon and nitrogen isotopic signatures of zooplankton taxa from the deep sampling station of Lake Sos Canales, 2010-2011. Symbol colours refer to months as shown in figure 3a/b.

Daphnia from S station and of *Ceriodaphnia* from both S and D stations were more closely related to SPM-D-S/S baselines. The latter did not differ substantially between the two sampling stations (Fig. 3d). In July, SPM–D-D was much more 15N-enriched than SPM-DS and SPM-S. The latter did not differ between the two sampling stations. Isotopic signatures of zooplankton taxa, however, were substantially different from those of SPM baselines measured in this month. ^{15}N-enrichment of *Copidodiaptomus* and cyclopoid copepods with respect to *Ceriodaphnia* might suggest that the latter was the main prey for both copepods (FIG. 3d).

Samples of September were very similar to those of October, with carbon signatures of *Copidodiaptomus* and of *Cyclopoids* from both sampling stations, and of *Ceriodaphnia* from D station consistent with those of SPM, not differing substantially among sampling sites and depths. δ^{15}N signatures of *Copidodiaptomus* from the two stations (D and S) and for cyclopoids from station D were very close to each other. Cyclopoids from S station were quite different, less ^{15}N-enriched, probably because of differences in contribution of developmental stages (Fig 3b). *Daphnia* was particularly enriched in ^{15}N, with an isotopic signature far from those of the other zooplankton taxa.

Comparing isotopic signatures of different zooplankton taxa throughout the year with those of SPM baselines we found that *Copidodiaptomus* from D station were mainly related to SPM-D-D carbon baseline in December, June and September (Fig. 4a). In April, May and July *Copidodiaptomus* carbon signatures were similar to those of the S station. *Copidodiaptomus* from the S station was closely related to carbon SPM isotopic baseline from the same station in October, December, April, June and September. Substantially similar isotopic SPM signatures from S and D water layers and in the two stations corresponded to a close similarity in isotopic signatures of *Copidodiaptomus* from S and D sampling stations.

On each sampling date, isotopic signatures of the Cyclopoids from D station were very close to those of *Copidodiaptomus*. Isotopic signatures of the latter were related to SPM-D-D δ13C baseline in June and Septemper, while being closer to the SPM baseline of the S station in December, March and April. Cyclopoids from the S station were similar to SPM carbon baseline of samples from the same site only in April and May. Comparing isotopic signature of *Copidodiaptomus* from both D and S sampling site, we may observe that they differ for carbon signature in October, March and in June (Fig. 5). In October and June, carbon signatures of specimens from S station were less ^{13}C-depleted, as expected. In March, however, the reverse was found, and specimens from the deeper sampling station were less 13C-depleted than those from the shallow station. Such result may at least partially reflect the fact that in March, SPM-D-S was more δ^{13}C-enriched than SPM-DS/SPM-S. Between-station differences were also observed in δ^{15}N signature in April and, to a less extent, May; specimens from the D station were more 15N-enriched than those from S station (by ca. 2‰).

Isotopic signatures of cyclopoids were quite comparable in the two sampling sites, with the exception of carbon isotopic signature of February and March (Fig. 4). As for *Copidodiaptomus*, cyclopoids from S station were more depleted in ^{13}C than those from station D. They retained, however, the same δ^{15}N signature.

Discussion

As commonly observed in natural lakes, SPM carbon signature was least ^{13}C-depleted in summer; in SCR, the least ^{13}C-depleted value (of ca. -26--25‰) was detected in June in all samples. More than hydrology, physical variables, such as temperature, might be responsible for such pattern (Rau et al., 1989). SCR is a warm-monomictic lake. In monomictic lakes, a positive correlation between δ^{13}C and temperature is often observed (Caroni et al., 2012; Perga and Gerdeax, 2006; Zoary, 1994, Visconti et al., 2010). When temperature increases, atmospheric C

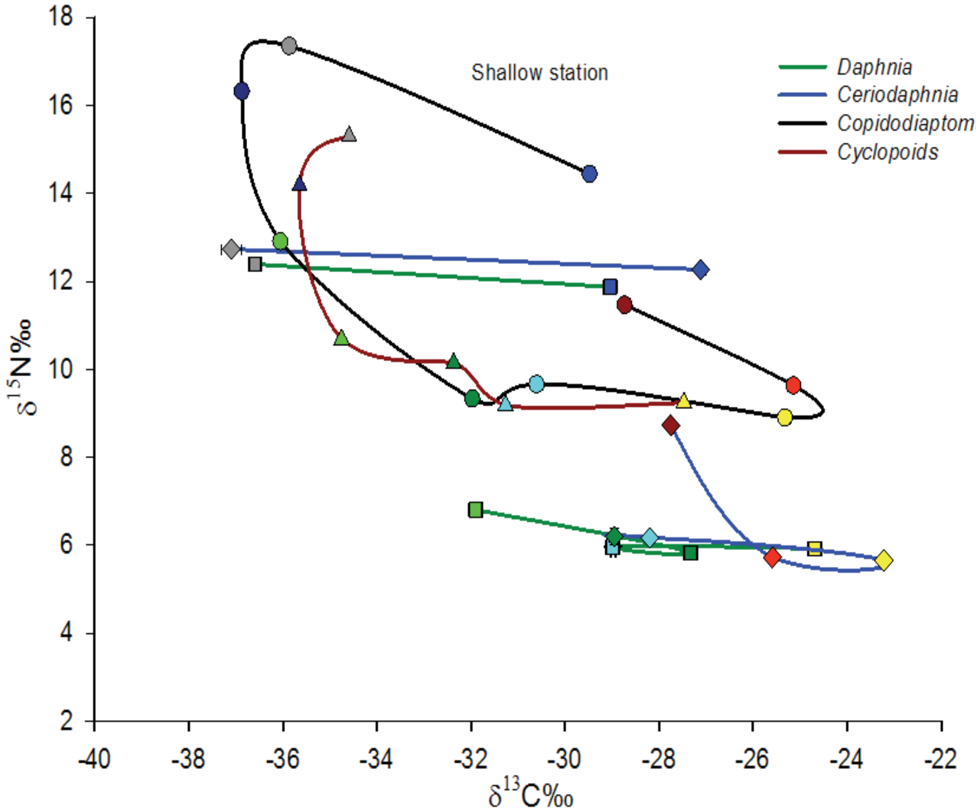


Fig. 4b- Seasonal changes in carbon and nitrogen isotopic signatures of zooplankton taxa from the shallow sampling station of Lake Sos Canales, 2010-2011. Symbol colours refer to months as shown in figure 3a/b.

dissolution decreases, hence changes in internal CO_2 sources may lead to a positive correlation with temperature. Temperature controls the solubility of CO_2 and in turn influences the fractionation of carbon isotopes during phytoplankton uptake (Rau et al., 1989 and 1992). In our study, $\delta^{13}C$ increased from more to less negative values between May and July, when SCR was thermally stratified and surface temperature reached the year maximum. Correlation between SPM $\delta^{13}C$ and water temperature was positive and statistically highly significant for values measured at D station in surface water (Sperman-Rank, R=0.883, n=9; p=0.007). Important contribution of phytoplankton to SPM-D-S was, in fact, suggested by the significant correlation between SPM and Chl a concentration (Spearman R=0.883, n=9, p=0.001) and total phytoplankton biomass (Sperman R=0.636, n=11, p=0.03) (data not showed).

As expected, SPM-D-D was more ^{13}C-depleted than SPM-DS/-S. Such depletion is consequent to an increase in respiration activity in deep waters (Cattaneo et al., 2004). In deep lakes (sensu Straskraba, 1999), carbon isotopic signature is strongly influenced by depth; organisms relying on deep water carbon sources, where respiration activity increases, are more ^{13}C-depleted than those living closer to the surface and/or in the littoral zone (Vander Zanden and Rasmussen, 1999; Grey et al. 2000; Cattaneo et al., 2004). Seasonal changes in SPM isotopic signature were in turn detected in zooplankton of S and D stations. $\delta^{15}N$ signature of *Ceriodaphnia* in October and December was quite constant between the two stations, enriched by ca. 2‰ with respect to SPM, as expected for primary consumers. $\delta^{13}C$ signature, however, while being more depleted and completely overlapped between the two stations in December, differed between stations in October: the value was much more depleted for *Ceriodaphnia* from D than from S station. Such difference was apparently more evident from SPM-D-D signature than from SPM- D-S/-S. It might be attributed to the fact that SPM was not analyzed along the whole water column, being SPM-DS collected from the upper 7.5 meters and SPM-D-D collected below 10 m water depth.

In July, while SPM $\delta^{13}C$ signature did not differ among stations/depths, SPM $\delta^{15}N$ signature was more ^{15}N -enriched in SPM-D-D than in the other two samples. Such enrichment was also observed in September. In stratified lakes and reservoirs, biological activity in sediments leads to a decrease in hypolimnetic dissolved oxygen concentration and in pH, lowering the redox potential at the sediments' interface (Nowlin et al., 2005). Moreover the prolongation of drought season and the stagnation of water can help the remobilization of nutrients kept in the sediments (Zohary, 1994). In hottest months, and particularly in July, $\delta^{15}N$ increased in deep waters, with an increase in nitric-nitrogen and reactive phosphorus (data not shown). The enrichment might be consequent to internal nutrient remobilization by nitrification processes. In particular, $\delta^{15}N$ signature of SPM depends on dissolved inorganic nitrogen origin (Hadas et al., 2009). Nitrate deriving from nitrification of ammonia is less ^{15}N-enriched than residual ammonium (Lehman et al., 2006). In SCR, oxygen decreased in hottest months; although anoxia was not detected, nitrification processes likely occurred. Concentration of dissolved inorganic nitrogen affects ^{15}N signature (Rau et al, 1989, Grey et al., 2001).

In February, isotopic $\delta^{13}C$ and $\delta^{15}N$ signature of *C. numidicus* from the two stations were, not only overlapped between stations, but also very close to those of the previous months. They were also completely independent from SPM isotopic signature. Such result might suggest that specimens analyzed were not actively feeding, most likely on diapause. Copepods far from SPM signatures and ^{15}N enriched suggest an increase in body lipid content (El-Sabaawi et al., 2009). Storage lipids provide energy for reproduction, overcoming periods of low food supply, to escape predation and for vertical and horizontal migrations (Lee et al., 2006). In heterogeneous or temporary habitats, copepods evolved adaptive mechanisms that guarantee synchronization in time and space between growth, reproduction and favorable environmental conditions (Santer, 1998). The state of dormancy is the main strategy adopted by copepods. Dormancy in copepods occurs in several forms: true diapausing eggs, resting or dormant eggs, dormant copepodites, or dormant and diapausing adults (Uye, 1986; Watson, 1986; Elgmork and Eie, 1989; Hairston and Van Brunt, 1994).

If for cyclopoids only the dormant copepodites and dormant and diapausing adults were known, calanoids mainly produce resting or diapausing eggs. Analysis of resting stages in SCR sediments, however, highlighted that *C. numidicus* resting eggs were lacking (Fadda et al., 2011). Structure of the population, mainly composed by copepodites and adults, support our hypothesis of being *C. numidicus* in diapause in February 2011. During diapause, copepods tend to migrate in deep cool water layers (Vanderploeg, 1998). Upward of hypolimnetic water may have transported diapausing copepods which carried bottom sediment signatures. This interpretation was supported by data on carbon signatures of the oligochaetes collected from bottom sediments (-34.07 ±0.19‰), substantially similar to those of C. numidicus and of the cyclopoids in February 2011. Whereas for large cyclopoid species, such as *C. abyssoroum* gr. and *M. albidus*, predation on cladocerans was well documented (Dussart and DeFaye, 2001; Fernando, 1998), little is known on feeding behavior of *C. numidicus*. Adults and large copepodites of calanoid copepods are often referred as selective feeders, able of a taste selection on fresh, good quality detritus (Demott, 1995; Demott, 1996) . A recent study on Portuguese estuaries (Gonçalves et al. 2012), however, reported *C. numidicus* as omnivorous.

Between March and May between-station differences became evident: in March, *Copidodiaptomus* of the shallow station was more 13C-depleted than at the deep station, while being characterized by similar $\delta^{15}N$ signatures. Such result suggests that at the D station *Copidodiaptomus* might prefer feeding in deeper (>7.5 m) waters.

Notably, April and May samples, no ^{15}N-enrichment with respect on SPM was observed. *Daphnia* of D station was in April more ^{13}C -depleted than in S station. Such pattern, which, among the others, does not correspond to differences between S and deep D signature (S- being more depleted than SPM-D-D), might suggest horizontal migration (Hamza et al., 1993), or complementary feeding on other food sources. Zooplankton is known to migrate vertically and horizontally. Diurnal horizontal migration (DHM) has been observed in shallow lakes, where zooplankton settle down near to the littoral zone during the day and migrate into pelagic open waters during the night (Wickulum 1999, Masson et al. 2001, Burks et al. 2002, and Romare and Hansson 2003). Studies have shown that zooplankton would hide during the day from predation in macrophytes in the littoral zone and then at night, when zooplankton are less visible to vertebrate predators (fish), move into the open water to forage for food (Burks et al 2001, Romare and Hansson 2003, Lewin et al., 2004). Such result might be explained by hypothesizing that larger/older specimen/developmental stages contributed more to *Copidodiaptomus* population in D than in S station. The difference between the two stations was still evident, although to a lower extent, in May.

Following changes in SPM, *Copidodiaptomus* isotopic signature from the two stations was, in June, more ^{13}C -depleted in D than in S, while being overlapping for δ^{15}N. Quite similar between-station values were measured in July and September, the latter being more ^{13}C-depleted than the former, as observed in SPM. Seasonal changes and between-station differences/similarities were found also on cyclopoid copepods. Notably, however, despite being contributed by two different taxa, cyclopoids seemed not affected by differences in population structure/contribution of different developmental stages. They were, at any time of the year, quite comparable between stations for δ^{15}N signature. They were also less ^{15}N -enriched than *C. numidicus* in December. Substantially similar between-station values of SPM-D-S/-S, and the same pattern of change between October and December (from less depleted to more depleted values), were identifiable in *Copidodiaptomus* isotopic signature of these two months. Also in this case, however, δ^{13}C signature of specimens of the D station was, as expected, and differently from SPM, more depleted than the signature of specimens collected from the S station. While SPM δ^{15}Nsignature did not change between October and December, however, δ^{15}N signature of *Copidodiaptomus* increased from 14.5 to 17.5 δ^{15}N ‰ between October and December. The enrichment was steeper in S than D station. Such a difference might suggest that the two months were, indeed, characterized by a different structure of the population, namely, being in December composed by larger/older individuals/developmental stages than in October

Between-station differences were, in April, evident for nitrogen, with a more ^{15}N-enriched signature in *Copidodiaptomus* from D than from S station (12 with respect to 9). Such difference cannot be explained by between-station differences in SPM signature, according to which D samples were, on the contrary, less enriched than SPM-D-S/-S. A clear separation between D and S Daphnia δ^{13}C signature was detected in June: as expected, *Daphnia* from D station was more 13C -depleted than Daphnia from S station. The latter was also much more 15N -enriched than the former. Unusually high δ15N *Daphnia* signature in September can hardly be explained. Other sequential events over the lifetime could lead to an increase in δ15N with age in *Daphnia*, including reproduction (juveniles have lower δ^{15}N than their mothers), and ephippia have a low δ^{15}N (Matthews and Mazumer, 2008). Increase in δ^{15}N has been found related to age and/or decreasing food quality (Adam and Sterner, 2000).

A general trend towards δ^{13}C-depleted values and no δ^{15}N enrichment with respect to SPM isotopic signature was detected between March-May and June, with substantially overlapping values in samples from D and S stations. A slight difference between the two, not in δ^{13}C but in δ^{15}N isotopic signature, could be traced in July, with slightly 15N -enriched specimens from the D sampling station. Such enrichment, however, was by far less evident than between SPM-D-S and SPM- D-D. Such result might suggest that *Ceriodaphnia* was able to integrate between the two (i.e. between epilimnetic and hypolimnetic food sources).

Similarly, δ^{13}C and δ^{15}N isotopic signature of S and D stations could be distinguished in October. This result may be explained by *Ceriodaphnia* feeding behavior, this small caldoceran being one of the most efficient bacteria consumers of all zooplankton (Porter et al. 1983, Gophen et al. 1974).

Daphnia δ^{15}N signature,remained quite constant in October and December, showing the same ^{15}N-enrichment as *Ceriodaphnia*. Differently from the latter, however, in December *Daphnia* was more δ^{13}C - depleted in D than in S station. Such a difference, however, cannot be explained by a difference in SPM δ^{13}C signature, being the latter substantially the same in SPM samples from different stations/depths. In a relatively uniform aquatic environment, such as SCR during the “still water phase”, food niche overlapping may be commonly observed (Hessen 1990 and references therein). *C. pulchella* was the dominant taxa among cladocerans in both shallow and deep areas. *Daphnia* was generally less abundant than *Ceriodaphnia* all year round. The latter exponentially increased in warmer months. Seasonal increase in *Ceriodaphnia* in SCR (Fadda et al. 2011), as well as in other Mediterranean meso-eutrophic reservoirs, is related to an increase in water temperature (Geraldes and Boavida, 2004). Such a difference in abundance, however, is likely not a result of competition between young *Daphnia* and adult *Ceriodaphnia*, as commonly observed in ponds (Lynch, 1978). Other environmental conditions, such as increasing water temperatures, leading to increasing *Ceriodaphnia* feeding rates (Gophen, 1976), food quality (Porter et al. 1983) and size-selective predation may be responsible for the low abundance of *Daphnia* in both stations (Lauridsen et al., 1999) in summer. *Daphnia* from S station was more ^{13}C-depleted than *Daphnia* from D station in March. Such a difference might also result from the large difference between δ^{13}C signatures of surface vs. that deep SPM. Unexpectedly, SPM-D-D was, in fact, less ^{13}C-depleted than the former. Such between-station differences in carbon isotopic signature might be indicative of differences between populations of the two stations in: 1) feeding (the two were feeding in different water layers); 2) body size. In deep lakes niche segregation is well known (Størm, 1946) as well as that isotopic signatures are related to age and size of individuals (Power et al., 2003; Matthews and Mazumer, 2008).

Only in July, however was δ^{13}C and δ^{15}N signature of copepods consistent with a possibility of using zooplankton primary consumers, namely *Ceriodaphnia*, as food. During this month, *Ceriodaphnia* was in turn likely feeding on SPM-D-S, thus allowing carbon from upper water layers to be incorporated into the food web. The same ability of copepods to exploit *Ceriodaphnia* was also traced through isotopic signatures of September. Data measured in June, however, did not confirm this hypothesis: during this month, carbon signature of *Ceriodaphnia* was closer to values of shallow (S) and littoral sources, which were less ^{13}C-depleted (*Atyephyra desmaresti*, -23.66±1.43 δ13C) than those measured at D sampling station. Specimens sampled from D station might be able to retain isotopic signature of the place where they migrated, or were passively transported, for feeding. In Summer in SCR mosquitofish became easily visible along the shoreline (AF pers. observation), thus being likely stimulating inverse DHM. A study on mountain fish- and fishless lakes showed that fish predation caused DHM: during the day, 7.9% of zooplankton population was distributed near the shore in fish- lakes, while in fishless lakes upo to 61.9% of zooplankton population were distributed near the shore (Wicklum, 1999).

Wicklum (1999) suggested that fish predation caused the zooplankton to behave in reverse DHM (movement away from the shore during the day), because most of the fish lived in shallow water. In Spring (March-May), *C. numidicus* differed from the cyclopoids for trophic position.

Comparing signatures of copepods with those of *Daphnia* and *Ceriodaphnia*, we may hypothesize that they relied upon the same food source. The latter, however, was certainly not constituted by *Daphnia* and/or *Ceriodaphnia*. According to this result, during spring, carbon from surface (epilimnetic) water layers (SPM-DS/S) is incorporated into copepod tissues via other-than-*Daphnia*/-*Ceriodaphnia* prey. The latter fully overlapped for both carbon sources and trophic level during this season. Cyclopoid copepods might prey on *Ceriodaphnia* in October, when SPM δ^{13}C signatures fully overlapped. Copepods from S station, occupied the same trophic position, higher than that of *Daphnia* and *Ceriodaphnia*, in Summer (June-September). Differences in their carbon isotopic signatures, however, suggest that they might be feeding at different depths and/or in different zones of the lake, with *C. numidicus* closer than the cyclopoids to shallow/ littoral carbon sources. Particularly in June, and in September, *C. numidicus* might incorporate shallow/littoral carbon through *Daphnia* (June) or *Ceriodaphnia* (September). In April-May copepods full overlapped, exploiting the same food sources, and relying upon SPM-D-S carbon. They do not seem, however to incorporate carbon from *Daphnia*. The latter seem to be feeding in deeper water layers during this months, as both an effect of vertical migration/transport from the D station.

Conclusions

- Seasonal changes of zooplankton consumer taxa of Lake Sos Canales were consistent with patterns observed in natural lakes. Zooplankton taxa isotopic signatures were overall less ^{13}C-depleted in spring and summer. Such enrichment might be interpreted as consequent to changes in carbon sources available for primary producers, related to increased water temperatures and thermal stratification.

- Impact of meteo-climatic events, such as heavy rainfall and water level increase, clearly detected in SPM isotopic signatures of both shallow and deep stations, were not detected in zooplankton taxa. Therefore, suspended solids carried into the lake, responsible for abrupt changes in SPM isotopic signature, were not carried into the food web through the zooplankton. Zooplankton taxa present during periods of heavy rainfall, increase in water level and increased input of suspended solids (copepods, e.g. in February) were either, not actively feeding or, exploiting other food sources (zoobenthos).
- Zooplankton taxa isotopic signatures of deep/shallow sampling stations overlapped during late summer and winter, while differing in spring. Such differences resulted from differences in vertical distributions, with copepods feeding in more superficial waters than cladocerans.
- Between-station differences in spring were attributed to exploitation of different food sources ($\delta^{13}C$ signature) and/or differences in contribution of early/late (small/large body size) developmental stages (degree of ^{15}N enrichment). The latter was likely more important for *Copidodiaptomus* than for cyclopoid population.
- $\delta^{15}N$ signature of copepods was exceptionally high, particularly in late fall and winter. In December and February, copepod $\delta^{13}C$ signature was independent from that measured in SPM. The un-coupling of $\delta^{13}C$ signature of zooplankton and SPM was interpreted as indicative of being copepods not actively feeding because of entering diapause. Indeed. An alternative explanation was that, copepods might be able to select other-than-SPM food sources (e.g. zoobenthos) during high input of suspended solids, and low quality food sources from the river.
- Unexpectedly, no or very little ^{15}N enrichment was detected in zooplankton cladocera with respect to SPM during spring-summer. We speculated that such pattern might result from intensive, size-specific, fish predation: selectively removing the largest, more visible fraction, zooplanktivorous fish might deplete cladocera primary consumer populations of the component likely more ^{15}N-enriched.
- Overall, our results provide evidence of importance of copepods for tracing changes in matter and energy transfer along the pelagic food web of reservoirs.
- Despite being ephemeral, cladoceran primary consumers were important prey for copepods and fish in spring and summer.

References

Adams T.S. & Sterner R.W. (2000) The effect of dietary nitrogen content on the trophic level 15N enrichment.Limnology and Oceanography, 45, 601–607.

Alonso M., Crustacea Branchiopoda. Fauna Iberica vol. 7, Museo nacional de Ciencias

Naturales Consejo Superior de Investigaciones Cientificas, Madrid, 1996.

Armengol J., Colonizaciòn de los embalses espanoles por crustàceos planctònicos y evoluciòn de la estructura de sus comunidades, Oecologia acquatica, 4 (1980), pp. 45-70.

Armengol J., Zooplankton crustaceans in Spanish reservoir, Verh. Internat. Verein. Limnol., 20 (1978), pp. 1652-1656.

Armengol X., Boronat L., Camacho A.and Wurtsbaugh W. A., Grazing by a dominant rotifer Conochilus unicornis Rousselet in a mountain lake: in situ measurements with synthetic microspheres. Hydrobiologia 446-447 (1): 107-114.

Bockstahler K. and D. Coats, Grazing of the mixotrophic dinoflagellate *Gymnodinium sanguineum* on ciliate populations of Chesapeake Bay. Marine Biology , Volume 116 (3), 1993;

Bond W. J. What Limits Trees in C4 Grasslands and Savannas? Annual Review of Ecology, Evolution, and Systematics, 39: 641-659, 2008

Burkholder J. M., 1990. Phytoplankton and episodic suspended sediment loading: Phosphate partitioning and mechanisms for survival. Limnol. Oceanogr., 37: 974-988.

Cabana G. and J.B. Rasmussen, Comparision of aquatic food chains using nitrogen isotopes. Proceeding of the National Academy of Sciences of the United States of America, 93: 10844-10847, 1996.

Calieri C., Pugnetti A. and Manca M., Carbon partitioning in the trophic web of a high mountain lake: from bacteria to zooplankton. In: Straškrabovâ V., C. Calieri an J. Fott (eds.), Pelagic food webs in mountain lakes. Mountain Lakes Reserch Program. Journal ofn Limnology. 58: 144-151.

Camin F., Perini M., Colombari G., Bontempo L., Versini G., 2008. Influence of dietary composition on the carbon, nitrogen, oxygen and hydrogen stable isotope rations of milk. Rapid. Comm. Mass. Spectrom., 22: 1690-1696.

Camin F., Wietzerbin K, Blanch Cortes A., Haberhauer G., Less M., Versini G.J., 2004. Application of multielement stable isotope ratio analysis to the characterization of French, Italian, and Spanish cheeses. J. Agric. Food Chem., 52: 6592-6606.

Caramujo M.J. and M.J. Boavida, The seasonal dynamics of *Copidodiaptomus numidicus* (Gurney, 1909) and *Thermocyclops dybowskii* (Lande, 1890) in Castelo-do-Bode Reservoir. Aquatic Ecology 34 (2000), pp. 143-153.

Caroni R., G. Free, A. Visconti and M. Manca. Phytoplankton funnctional traits and seston stable isotopes signature: a funcitional-based approach in a deep, subalpine lake, Lake Maggiore (N. Italy). Journal of Limnology, 71(1), 84-94, 2012.

Cherbi M., Contribution à l'etude du peuplement zooplanctonique de trios lacs de barrage: Hamiz, Boughzoul et Ghrib. Thèse de Doctorat, Universitè d'Alger, pp. 216, 1984.

Davic L., Linking Keystone Species and Functional groups. A new operational definition of Keystone Species concept. Conservation Ecology vol. 7, 2003.

de Bernardi R., G. Giussani and M. Manca. Cladocera: Predators and prey. Hydrobiologia 145(1): 225-243, 1987.

de Niro M.J. and S. Epstein. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimca Acta. 42: 495-506, 1978.

DeMott W. R. (1986) The role of taste in food selection by freshwater zooplankton.

Oecologia, 69, 334-340.

DeMott W. R. (1995) Optimal foraging by a suspension-feeding copepod: responses to short-term and seasonal variation in food resources. Oecologia, 103, 230-240

DeMott W. R., R. D. Gulati and E. Van Donk, *Daphnia* Food Limitation in Three Hypereutrophic Dutch Lakes: Evidence for Exclusion of Large-Bodied Species by Interfering Filaments of Cyanobacteria. Limnology and Oceanography, Vol. 46 (8): pp. 2054-2060, 2001.

DeMott W.R. Food selection by calanoids in response to between-lake variation in food abundance. Fresh. Biol. Vol. 33 (2): 171-180 (1995).

DeMott W.R.. The role of taste in food selection by freshwater zooplankton. Oecologia vol. 69: 334-340, 1986.

Dussart B. H. & Defaye D. (2001) Introduction to Copepoda. Backhuys Publishers, Leiden

Dussart, B. H. and Fernando, C. H. (1988) Sur quelques Mesocyclops (Crustacea; Copepoda). Hydrobiologia, 157, 241–264

Einsle U., Copepoda: Cyclopoida: Genera Cyclops, Megacyclops, Acanthocyclops. Guides to the identification of the microinvertebrates of continental waters of the world (H.J.F. Dumont, Ed.) vol. 10, SPB Publisching, Amsterdam, 1996.

Fadda A., S. Marková, P. Kotlík, A. Lugliè, B. Padedda, P. Buscarinu, Ni. Sechi and M. Manca. First record of planktonic crustaceans in Sardinian reservoirs. Biologia 66(5): 856-865.

Finlay and Kendall, 2008. In “ Stable Isotopes in Ecology and Environmental Science, second edition,. Finlay J.C. and Kendall C.- Stable Isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems, pp. 283-324.

France R.L. (1995) Carbon-13 enrichment in benthic compared to pelagic algae: food web implications. Marine Ecology Progress Series, 124, 307–312.

Geller W. & Müller H. (1981) The filtration apparatus of cladocera: Filter mesh-sizes and their implications on food selectivity. Oecologia, 49, 316-321

Geraldes A.M. and Boavida M.J., What factors affect the pelagic cladocerans of the meso-eutrophic Azibo Reservoirs?. Ann. Limnol. Int. J. Lim., 40(2): 101-111.

Gliwicz Z. M. and W. Lampert, Food thresholds in Daphnia species in the absence and presence of filament, Ecology 71 (1990), pp. 691-702.

Gliwicz ZM, Lampert W (1994) Clutch-size variability in *Daphnia*: body size related effects of egg predation by cyclopoid copepods. Limnol Oceanogr 39:479–485

Gonçalves A. M.M., M. Â. Pardal, S. C. Marques, S. Mendes, M. J. Fernández-Gómez, M. P. Galindo-Villardón, and U. M. Azeiteiro. Responses of Copepoda Life-History Stages to Climatic Variability in a Southern-European Temperate Estuary. Zoological Studies 51(3): 321-335 (2012).

Gophen M. & Geller W. (1984) Filter mesh size and food particle uptake by Daphnia. Oecologia, 64, 408-412

Gophen M. 1976, Temperature Dependance of Food Intake, Ammonia Excretion and Respiration in *Ceriodaphnia reticulata* (Jurine), Lake Kinneret, Israel. Freshwater Biology 6(5): 451-455.

Grey J.. Trophic fractionation and the effects of diet switch on the carbon stable isotopic “signatures” of pelagic consumer. Verh. Internat. Verein. Limnolol. 27: 3187-3191, 2000.

Hamza, W., Ruggiu D., Manca M. Diel

Zooplankton Migrations and their effect on the grazing impact in lake Candia (Italy). Ergebnisse der Limnologie ERLIA6, vol. 39: 175-185, 1993.

Henry R., E. A. Panarelli, S. M. Caglierani Casanova, D.C. Plankton richness and abundance in several differnt hydrologica situation in lakes later to a river: case a study in the mouth zone of a tributary into a tropica reservoir. Oecologia Australis15(3): 537-558, 2011.

Hessen D. O. & Lyche A. (1991) Inter- and intraspecific variations in zooplankton element composition. Archiv für Hydrobiologie, 121, 343-353

Hessen D.O., Niche overlap between herbivorous cladocerans; the role of food quality and habitat homogeneity. Hydrobiologia 190: 61-78, 1990.

Karlsson J., Jonsson A., Meili M. & Jansson M. 2003. Control of zooplankton dependence on allochthonous organic carbon in humic clear water lakes in Northern Sweden. Limnol. Oceanog. 48: 269–276.

Karlsson J., Jonsson A., Meili M. & Jansson M. 2003. Control of zooplankton dependence on allochthonous organic carbon in humic clear water lakes in Northern Sweden. Limnol. Oceanog. 48: 269–276.

Lampert W. & Sommer U. (1997) Limnoecology. Oxford University Press.

Lauridsen, T. L., E. Jeppesen, S. F. Mitchell, D. M. Lodge, and R. L. Burks. 1999. Diel Variation in Horizontal Distribution of *Daphnia* and *Ceriodaphnia* in Oligotrophic and Mesotrophic Lakes with Contrasting Fish Densities. Hydrobiologia 408/409(0): 241-250.

Lawton, J.H. 1987. Water fleas as population model. Nature (London), 316: 577–578.

Leira M. Cantonati M., Effects of water-level fluctuations on lakes: an annotated bibliography. Hydrobiologia 612: 171-184, 2008.

Lindeman R.L. 1942. The trophic-dynamic aspect of ecology. Ecology 23: 399–417.

Lynch M., Complex Interactions between Natural Coexploiters-*Daphnia* and *Ceriodaphnia*. Ecology, Vol. 59(3): 552-564. 1978

Maier G. (1993) An example of niche partitioning in three coexisting freshwater cyclopoid copepods. Journal of Plankton Research, 15, 1097-1102

Marchetto A., B.M Padedda, M. Mariani, A. Lugliè, N. Sechi, A numerical index for evaluating phytoplankton response to changes in nutrient level deep mediterranean reservoirs, J.Limnol., 68-1 (2009), pp. 106-121.

Marten G. G., Bordes E. S. and & Nguyen M. Use of cyclopoid copepods for mosquito control Hydrobiologia 292/293: 491-496, 1994

Matthewes B. and A. Mazumder. Compositional and interlake variability of zooplankton affect baseline stable isotope signatures. Limnology and Oceanography, 48(5), 1977-1987; 2003.

Matthews B. and Mazumer A., Detecting trophic-level variation in consumer assemblages. Fresh. Biol., doi: doi:10.1111/j.1365-2427.2008.02018.x, 2008.

Mauchline J. (1998) The biology of calanoid copepods. Academic Press, London

May, R.M. 1981. Models for single populations.In. Theoretical ecology: principles and applications. 2nd ed. Edited by R.M. May. Blackwell Scientific, Oxford, U.K. pp. 4–25

McCauley E.. The estimation of abundance and biomass of zooplankton in samples. In: A manual on methods for the assessment of secondary productivity in fresh water (Eds

Dowinig J.A & F.H. Rigler), pp. 228-265. Blackewell Scientific Publications.

McCauley, E., and Murdoch, W.W. 1987. Cyclic and stable populations:plankton as paradigm. Am. Nat. 129: 97121

Minagawa M. & Wada E. (1984) Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ15N and animal age. Geochimica et Cosmochimica Acta, 48, 1135-1140

Moss, B., D. Hering, A.J. Green, A. Aidoud, E. Becares, M. Beklioglu, H. Bennion, D. Boix, S. Brucet, L. Carvalho, B. Clement, T. Davidson, S. Declerck, M. Dobson, E. van Donk, B. Dudley, H. Feuchtmayr, N. Friberg, G. Grenouillet, H. Hillebran, A. Hobaek, K. Irvine, E. Jeppesen, R. Johnson, I. Jones, M. Kernan, T.L. Lauridsen, M. Manca, M. Meerhoff, J. Olafsson, S. Ormerod, E. Papastergiadou, W.E. Penning, R. Ptacnik, X. Quintana, L. Sandin, M. Seferlis, G. Simpson, C. Trigal, P. Verdonschot, A.M. Verschoor & G.A. Weyhenmeyer. 2009. Climate change and the future of freshwater biodiversity in Europe: a primer for policy-makers. Freshwat. Rev.: 2: 103-130.

Naselli-Flores. Man-made lakes in Mediterranean semi-arid climate: the strange case of Dr. Deep an Mr Shallow Lake- Hydrobilogia, 506 (1-3): 13-21.

Niesel V., Hehn E., Sudbrack R., Willmitzer H., and Chorus I., 2007. The occurrence of the Dynophyte species Gymnodinium uberrimum and Peridinium Willei in German reservoirs. J. Plank. Res., 29(4): 347-357.

Pace M.L., Cole J.L., Carpenter S.R., Kitchell J.F.. Trophic cascades revealed in diverse ecosystem. Trend in Ecology and Evolution vol. 14(2): 483-488.

Perbiche-Neves G.R., R.. Romero Ferreira and M. Gomes Nogueira. Phytoplankton structure in two contrasting cascade reservoirs (Paranapanema River, Southeast Brazil). Biologia 66 (6): 967-976, 2011.

Perga M. E. and Gerdeaux D. Seasonal variability in the δ13C and δ15N values of the zooplankton taxa in two alpine lakes. Acta Ecologica 30, 69-77; 2006.

Peterson B. J. & Fry B. (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics, 18, 293-320

Petrusek, A.Hobæk, J. P. Nilssen, M. Skage, M. ČErný, N. Brede, K. Schwenk. A taxonomic reappraisal of the European Daphnia longispina complex (Crustacea, Cladocera, Anomopoda) Zoologica Scripta, 37 (5): 507–519, 2008

Porter, K. G., Y. S. Feig, and E. F. Vetter. 1983. Morphology, Flow Regimes, and Filtering Rates of *Daphnia*, *Ceriodaphnia*, and *Bosmina* Fed Natural Bacteria. Oecologia. 58(2): 56-163.

Post D. M.. Using stable isotopes to estimate trophic position: models, methods, and assumption. Ecology 83: 703-718.

Power M., Guiguer K. & Barton D.R. (2003) Effects of temperature on isotopic enrichment in *Daphnia magna*: implications for aquatic food-web studies. Rapid Communications in Mass Spectrometry, 17, 1619–1625.

Ringelberg J., Flik B. J. G., Lindenaar D. & Royackers K. (1991) Diel vertical migration of *Daphnia hyalina* (sensu latiori) in Lake Maarsseveen: Part 1. Aspects of seasonal and daily timing. Archiv für Hydrobiologie, 121, 129-145

Roman M. R., Holliday D.V., and Sanford L.P., Temporal and spatial patterns of zooplankton in the Chesapeake Bay turbidity maximum. Mar. Ecol. Prog. Ser., 213: 215-227, 2001.

Sechi N. and A. Lugliè, Limnlogical studies on man-made lakes in Sardinia (Italy). Mem. Ist. Ital. Idrobiol., 50 (1992), pp. 365-381.

Sechi N., and A. Luglié, Phytoplankton in Sardinian reservoirs.Giornale Botanico Italiano, 130 (1996), pp. 977-994.

Smyntek P.M., Teece M.A., Kimberly L.S. Storch A.J. Taxonomic differences in the essential fatty acid composition of groups of freshwater zooplankton relate to reproductive demands and generation time. Fresh. Biol. Vol. 53 (9): 1768–1782.

Smyntek P.M., Teece M.A., Schulz K.L. & Storch A.J. (2008) Taxonomic differences in the essential fatty acid composition of groups of freshwater zooplankton relate to reproductive demands and generation time. Freshwater Biology, 53, 1768–1782.

Sprules, W. G., and J. E. Bowerman.. Omnivory and Food Chain Length in Zooplankton Food Webs. Ecology 69:418–426, 1988

Syväranta J. & Rautio M. (2010) Zooplankton, lipids and stable isotopes: importance of seasonal, latitudinal and taxonomic differences. Canadian Journal of Fisheries and Aquatic Sciences, 67, 1721–1729.

Syväranta J., Tiirola M. and Jones R. I., 2008. Seasonality in lake pelagic δ15N values: patterns, possible explanations, and implications for food web baseline. Fun. App. Limnol. Arch. Hydr., 172(3) 255-262. (Fundamental Applied Limnology Archiv fur Hydrobiologie).

Taipale S.J., Kankaala P., Tiirola M. & Jones R.I. 2008. Whole-lake dissolved inorganic 13C additions reveal seasonal shifts in zooplankton diet. Ecology 89: 463–474.

Thielsh A., Brede N., Petrusek A., De Meesteer L., Schewnk K. Contribution of cyclic parthenogenesis and colonization history to population structure in *Daphnia*. Molecular Ecology 18 (8): 1616–1628, 2009

Tundisi J. G. et al. Theoretical basis for reservoir management1999.In Tundisi J.G and Straskraba M, Theoretical Reservoir Ecology and Its Applications s.l.: IIE. BAS, Backhuys Publishers, pp. 505.

Visconti A. and Manca M., Seasonal changes in the δ13C and δ15N signatures of the Lago Maggiore pelagic food web. Journal of Limnology, 67(2), 87-92.

Wetzel R.G. 2001. Limnology: Lake and river ecosystems. 2nd edition. Academic Press, London (Pages 1006).

Zoary T. and Ostrovsky I., Ecologica impacts of excessive water level fluctuations in stratified freshwater lakes. Inland Water 1,: 47-59, 2011.

Zoary T., Erez J., M. Gophen, I. Berman-Frank, M. Stiller. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret,. Limnology and Oceanography, 39: 1030-1043, 1994.

CHAPTER 4

Allochthonous fish impact on zooplankton community in a Mediterranean man-made lake through Stable Isotope Analysis (Lake Sos canales, Sardinia, Italy)

Amedeo Fadda[1], Francesco Palmas[2], Federica Camin[3], Bachisio Padedda[1], Antonella Lugliè[1], Marina Manca[3], Andrea Sabatini[2]

(1) Department of Sciences for Nature and Environmental Resources (DipNET) of the University of Sassari 4, Sassari, Italy

(2) Department of Animal Biology and Ecology, University of Cagliari, Cagliari, Italy

(3) Istituto Agrario San Michele all'Adige IASMA, Fondazione Edmund Mach, Trento, Italy

(4) Institute of Ecosystem Study (CNR-ISE), Verbania, Italy

Abstract

In relatively young environments such as reservoirs non indigenous fish were released for different purposes. In Sardinia mainly since the early 20ies of the last century 14 allochthonous fish species were introduced in rivers and reservoirs. In particular, North-Atlantic brown trout and mosquitofish were introduced, among the others, in Sos Canales Reservoir (SCR), a small reservoir located in North-Eastern Sardinia, devoted to drinkable water supply. Despite a low impact of human activities in the watershed, the lake is classified as meso-eutrophic, according to OECD guidelines criterions. The lake is among the richest for planktonic crustacean diversity, hosting both Mediterranean endemism as well as invasive hybrid taxa. To quantify ecological effects of non-native fish on plankton community structure and functioning, we analyzed seasonal changes in carbon and nitrogen stable isotopes of fish and of pelagic/littoral food sources in SCR during 2010-2011. We found that brown trout main relied on littoral sources, particularly on a common shredder decapod (*Atyeaphyra desmaresti*). Mosquitofish changed feeding behavior along the year, relying on littoral sources from winter to early summer, and on pelagic sources in late summer and early fall. Brown trout population was manly composed by adults which had clearly visible problems in reproductive trail. Such problems may lead to a decrease in abundance of this effective predatory fish. Such a decrease, and occurrence of a prolonged drought season may result in an exponentially increase of the mosquitofish population. The latter might decimate zooplankton, ultimately causing a deterioration of Sos Canales water quality.

Key words: stable isotope analysis, zooplankton, brown trout, mosquitofish, trophic web, non indigenous species

Introduction

Fish are frequently introduced in freshwater ecosystems because are strongly associated to human activities (Coppe et al., 2005; Garcia-Berthou et al., 2005). Introduction and release of allochthonous fish species occur for angling/sport, aquaculture, fisheries and ornamental purposes (Gozlan, 2008). Although some introduced fish species may fail to establish self-sustained population or may have few effects on native community (Gozlan, 2008), several studies show strong ecological impacts (review in Cucherousset and Olden 2011), Ecological impacts of non-native fishes acts at different levels of biological organization, biodiversity loss and biotic homogenization (e.g. Allendorf et al. 2001; Olden, 2006, Cucherousset et al., 2012), and hybridization (Cucherousset et al. 2008; Sabatini et al., 2006). Furthermore, fish can alter trophic status of water bodies reducing the pressure of grazers on phytoplankton community (Søndergaard et al., 1990). Fish predation-pressure can change zooplanktonic communities in terms of size (Manca et al. 2008; de Bernardi et al. 1987) and species (Lynch, 1979) composition. New interaction arising from direct (e.g. predation) and indirect (e.g. trophic cascade) allochthonous fish impacts can destabilize food webs (e.g. Baxter et al. 2004; Lookwood et al. 2007).

Stable isotope analysis (SIA) of carbon and nitrogen is a usefull tool to quantify ecological effects of non-native fishes on plankton community (Cucherousset et al., 2012, Visconti et al. 2012). SIA is based on the relationship between isotopic composition of consumer and its prey, allowing for the investigation of diet composition and energy flow in food webs (Fry, 2006). Fish feeding behavior by means of analysis of gut content provided an instantaneous snapshot of diet composition, unable to provide a real comprehension of assimilated sources and direct prey-predator relationships (Gu, Schell and Alexander, 1994; Vander Zanden and Rasmussen, 1999). SIA, provides an integrated value covering a wide time span (Visconti et al. 2011). Moreover provides a basic knowledge on trophic relationship among taxa, providing an useful tool in management approaches, and a better prediction of impacts of external and internal perturbations (Visconti et al. 2012).

Since early 1900s in Sardinian streams and reservoirs, 14 allochthonous fish species have been introduced, by local governments and privates (Orrù et al., 2010). Mediterranean reservoirs are complex man-made systems markedly affected by hydrological dynamics due to human management and climate. Small size euriecious fishes, with high reproductive rates, able to protect new generations (ovoviviparous) are favored in stressed environments such as reservoirs. Lake Sos Canales (SC) is a small reservoir devoted to produce drinkable water in the North-Eastern Sardinia (Italy). Water devoted to human supply should derive from reservoirs of good environmental quality to assure both the quality of the product and reduction of the treatment costs to obtain it. Despite a low impact of human activities in the watershed, SC is classified as meso-eutrophic, according to OECD guidelines criterions (Sechi and Lugliè, 1992; Lugliè et al., 1996; Marchetto et al., 2009).

Changes in hydrodynamics, water level fluctuations and the presence of a mixotrophic dinoflagellate species as dominant in the phytoplankton community, affected the C, N SIA seasonality of suspended particulate matter (SPM) (Chapter 2 of this thesis).

Furthermore, SC is a “reservoir” of planktonic crustaceans, hosting Mediterranean endemism (i.e., *Copidodiaptomus numidicus* Gurney, 1909) as well as invasive hybrids of *Daphnia pulex* complex (Fadda et al., 2011; Chapter 1 of this thesis). C, N SIA of crustacean zooplankton taxa suggested a ability to feed on different food sources, influenced by hydrological dynamics (Chapter 3 of this thesis).

In the present work we aimed to evaluate fish species composition and their trophic relationships in SC, SC was especially suitable for studying effects of non indigenous species (NIS) on pelagic crustacean community because its fish community was poor (only two species living in), and mainly two sources (pelagic vs littoral) were available for fish feeding, particularly during the dry season. To evaluate feeding behavior and effects of fishes on pelagic mesozooplankton, C, N SIA of both pelagic and littoral organisms and fishes of SC were seasonally analyzed.

Methods

Study site

Lake Sos Canales (SC) is a small reservoir (0.33 km^2) lying on granite bedrocks, surrounded by a cork-oaks woods, in the North-Eastern part of Sardinia (Italy), at 714 m a.s.l. Reservoir watershed is small (16 km^2) with little anthropogenic activity, mainly related to production of cork and wild breeding practices. SC was built in 1956 by damming the upper stream of the River Tirso, the most important river in the Island.

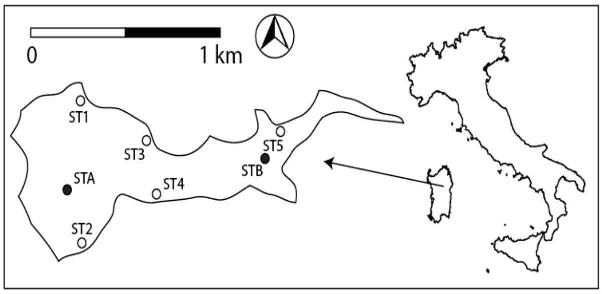


Fig. 1 Sampling station**s** in **Lake** Sos Canales. Underlined number (A, B) indicate SPM and zooplankton sampling stations. Fishes and *A. desmaresti* sampling station (i.e. St. 1) around the reservoir shoreline used to evaluate isotopic differences among areas and *G. holbrooki* abundances in October 2011.

The lake provides drinking water supply for a resident population of 15.000 inhabitants. Its outflow, only due to human intake, is quite constant during the year, never exceeding 265 m^3 month^{-1}. Mediterranean area are characterized by warm dry summers, and mild to cool and wet winters. Rainfall events are concentrated since late fall till early-late spring, often causing floods. Consequently, as in many others upland Mediterranean streams, the SC’s tributary has a temporary regimes, thus water overflow in SC occurring occasionally. Therefore, most of the waters conveyed are retained by the dam for future supplies.

Sample collection and laboratory analysis

Suspended particulate matter (SPM) and zooplankton were collected ca. monthly since February 2011 till September 2011 in the deeper area (St. 1), close to the dam, and near to the river inflow (St. 2) (Fig.1). All samples were collected during the same days. Integrated SPM were collected in surface (0, 1, 2.5, 5 and 7.5 m) and deep (10, 15, 20 and 30 m) water layers with a 5L Niskin bottle. In the field, lake water was pre-filtered by a 76 µm mesh size sieve to remove zooplankton organisms and large detritus. SPM size fraction (1.2≥µm≤0.76) included detritus, phytoplankton and smaller organisms. SPM was collected by filtration on pre-weighted GFC glass fiber-filters and dried at 60 °C for 24 h and transferred into tin capsules for SIA.

For phytoplankton analyses (biomass and taxa composition and abundance), subsamples from surface depths (0, 1, 2.5, 5 and 7.5 m) were fixed with Lugol's solution and analyzed with Utermöhl's technique (1958) and according to Sun and Liu (2003) and Liu et al. (2011). Chlorophyll *a* concentrations (Chl-*a*) were measured following Golterman et al., (1978).

Zooplankton species composition and abundance and Standing Stock Biomass (SSB) were estimated from samples collected through a Apstein plankton nylon net of 0.76 µm mesh size. The samples were fixed in pure ethanol (99%). We calculated zooplankton biomass from length-weight regression equations of the different taxa (McCauley, 1984; Manca and Comoli, 2000). Zooplankton samples for SIA were collected with a large-mouth plankton net of 100 µm and than fixed with ethanol (99%) and sorted within the next two weeks. Zooplankton were carefully washed on tap water, then sorted by species: *Copidodiaptomus numidicus*, genera (*Daphnia*, *Ceriodaphnia* and *Conochilus*) and order (cyclopoids).

Littoral organisms were gathered ca. monthly on the shoreline, through a sieve of 1 mm mesh size, then sorted by shredder decapods (*Atyaephyra desmaresti* Millet), grazer gastropods (*Ancylus fluviatilis* Müll), and amphibian earthworms. Furthermore, when founded, dragonfly larvae, bivalves (*Sphaerium* spp.) and terrestrial insects trapped on surface waters were collected.

Macroinvertebrates were kept overnight in clear water in glass Petri dishes. Only soft tissue of decapods and mollusks were used for SIA.

Since March to October 2011 ca. every three months, relatively large size fishes were sampled with a drifting nets of 3.5 cm mesh size and a 13 m Seine net (Dragnet) of 1 mm mesh size to evaluate the species composition and abundance, and for SIA. Fish nets were put along the shoreline and near to tributary input, at 5 sampling stations around the reservoir shoreline. Five individuals were collected on each sampling station to estimate potential differences among areas. Small-sized fish for SIA were captured on the shoreline with a hand net of 0.5 mm of mesh size.

Individual size (total body length, BL, mm) and total body mass (weight, BW, mg) of fishes were measured.

On each sampling date, five individuals per species, when possible, were used for SIA, as well as to evaluate among-station differences for small-sized fishes and stomach contents.

Stomachs were removed from the fishes and preserved in 4% buffered formaldehyde for further diet analysis. Fishes were stored deep frozen before subsequent samples treatments.

The stomach contents were identified, categorized and counted, for large fishes, to decapods, terrestrial and aquatic insects, aquatic larvae (e.g., dragonfly larvae) and oligochaetes, for small fishes to cladocera genera (i.e. *Daphnia*, *Ceriodaphnia*) and in calanoids, cyclopoids, ostracoda, terrestrial and aquatic insetcs, *Atyeaphyra desmaresti*, and into other prey (e.g., aquatic mites, chironomid). Gut contents of small fishes were stained with crystal violet for taxonomic identification.

For SIA a small portion the dorsal muscle (between head and dorsal fin and above the lateral line) was dissected from each large species. Small fishes were skinned and a body muscle tissue were taken. Muscle samples were oven dried at 60 °C for at least two days and then grounded, in order to obtain a fine powder. As for samples, SIA was run on two replicates per individual per sampling.

All SIA samples were transferred into tin-capsules of 6X4 mm. The samples were analyzed by a Continuous Flow-Isotope Ratio Mass Spectrometer (CF-IRMS; Delta plus XP ThermoFinnigan, Bremen, Germany), after total combustion (≈ 0.5 mg) in elemental analyzer (EA Flash 1112 ThermoFinnigan), (Camin et al., 2004, 2008), for $\delta^{13}C$, $\delta^{15}N$ and C:N ratio. Values were expressed in δ‰ against international standards (Vienna-Pee Belemnite for $\delta\ ^{13}C$, air for $\delta\ ^{15}N$) and computed against acetanilide, which was calibrated against international reference materials (MRI-ET-1SO 64, MRI-ET-ISO 63; Camin et al., 2008). Uncertainty of measurements was < 0.3‰ for $\delta\ ^{13}C$ and $\delta\ ^{15}N$.

Isotopic baseline

To evaluate relative contribution of pelagic vs. littoral carbon sources to fish diet, a linear Dinamic Baselin Mixing Model (DBMM; Woodland et al., 2012) was applied. SPM and *A. desmaresti* isotopic values were used as proxies for pelagic and littoral carbon isotopic baselines, respectively. Isotopic signatures of potential food sources were integrated over two-month intervals, a time consistent with turnover metabolic rates of fish dorsal muscle tissue (Hesslein et al., 1991 and 1993; Grey, 2000; Perga and Gerdeaux, 2005; Phillips and Eldrige, 2006; Visconti et al., 2012). We assumed that monthly carbon signature of fish tissues was a linear mass balance average of elemental mass from each food sources (linear mixing model; Phillips, 2001; Phillips and Gregg, 2001; Phillips and Koch, 2002) within the 60-day time interval necessary to reach equilibrium (Woodland et al., 2012). Sos Canales fish community was constitute by a fast growing species, *Gambusia holbrooki* (G.), and a slow growing rate population of brown trout, *Salmo trutta* (L.). To apply the DBMM, growth rates (*k*) of *G. holbrooki* (Cabral and Marques, 1999) and brown trout (Borroni et al, 2003) recorded in other Mediterranean climate sites were applied. For fish dorsal muscular tissue, *m* was assumed as 0.0018 d^{-1} both for $\delta^{13}C$ and $\delta^{15}N$ (Hesslein, Hallard and Ramlal, 1993). We applied DBMM equation reported in Visconti et al. (2012).

Trophic position and $\delta^{15}N$ enrichment of fishes were calculated from results of DBMM, when fishes relied on littoral and pelagic carbon sources (see Visconti et al., 2012), or by the trophic equation suggested by De Niro and Epstein (1981). Bimonthly average of SPM and zooplankton taxon isotopic signatures were used to evaluate trophic linkage between fishes and invertebrates.

Among-station and among-season differences of isotopic signatures were tested for statistical significance by applying parametric or non-parametric tests for comparison, depending on normality and homoscedasticity using the program Statistica for Windows (StatSoft 1994).

RESULTS

Fish community of Sos Canales Reservoir

In Sos Canales only two introduced fish species were caught; the North-Atlantic brown trout (*Salmo trutta*, L.) and a mosquitofish (*Gambusia holbrooki*, G.). Brown trout population abundance as extremely low, only one individual on each sampling date was collected. All individuals were mature adults. One had a deformity in the caudal region, likely due of breeding farm origin (Mehrdad et al., 2011). The female captured in July carried mature gonads with mature eggs. Total body length ranged between 16 and 38 cm, while gutted weights between 105 to 705 gr in all captured individuals. Mosquitofish reaced abundance of 56 ind m^{-2}, total body length ranged between a maximum of 2.4 cm to a minimum of 0.7 cm in October 2011. Mosquitofish abundance did not differ among the 5 sampling stations in October 2011 (Kruskal-Wallis test H=4, n=5, p=0.406). Among station differences were not significantly different for BL (Kruskall-Wallis: H:4, n=60, p=0.06) and guttered weight (Kruskall-Wallis, H=4, n=60, p=0.180). The maximum individual length (3.4 cm), was recorded in July in female with embryos; and the minimum in October on juvenile.

Brown trout gut contents (Fig. 2.a) showed a larger spectrum of prey in March than were recorded in July, the latter being mainly constituted by aquatic insects (90 %, in particular by *Ditiscus* spp.). In October, *A. desmaresti* as the main prey (70%). Mosquitofish gut contents, as expected was mainly constituted by zooplankton, particularly in March, 50% of cladocerans (*Daphnia* and *Ceriodaphnia*) and 50 % of copepods (*C. numidicus* and the cyclopoids). In July, more than 90% of gut contents were constituted by

Tab.1 - Averages of isotopic values and C:N ratio of mosquitofish and *A. desmaresti*, measured among the 5 stations in October, and statistical significances (Friedman-Anova test).

	Mosquitofish		*Atyaephyra desmaresti*	
n=5	Mean	p	Mean	p
$\delta^{13}C$	-27.35 ± 0.36	0.28	-23.71 ± 0.25	0.27
$\delta^{15}N$	13.80 ± 0.18	0.21	8.89 ± 0.24	0.27
C:N	4.05 ± 0.17	0.2	4.15 ± 0.15	0.17

Ceriodaphnia. In fall, *G. holbrooki* also on insects and juvenile *A. desmaresti*, in September, prey were mainly cyclopoids, while diet was more heterogeneous in October (Fig. 2.b). Gut content of *G. holbrooki* in *May* was lost.

Among-stations differences in carbon and nitrogen isotopic signatures and C:N ratios, of both *G. holbrooki* and*A. desmaresti*, were not observed (Tab.1) in individuals collected around the reservoir shoreline in October.

Littoral organisms – carbon and nitrogen isotopic signature

Carbon isotopic signatures varied seasonaly with depleted values in cool months (February-March) and a slight increase till summer. *A. desmaresti* was used as a proxy of the littoral baseline. δ^{13}C depleted values were recorded in March (-25.5 ±0.82 ‰ δ^{13}C). River limpet (*Ancylus fluviatilis*), used as a proxy of periphyton signatures, showed an inverse trend, with more enriched values in February (-15.71 ±0.17 δ^{13}C) and more depleted in September (-23.9±3.24 δ^{13}C). *Sphaerium* spp., collected in October (-19.34 ±0.19 δ^{13}C) carried more δ^{13}C enriched values than on SPM (see below), which exclude it for a annual integrative proxy of seston (as SPM). The same was suggested for other filter-feeder bivalves, such as zebra-mussel (*Dreissena polymorpha*). Dragonfly larvae (ODON) recorded only in May (-25.30±0.09 ‰ δ^{13}C) overlapped with mosquito fish signature (-25.13±0.01 ‰ δ^{13}C). δ^{13}C signatures of adults brown trout were similar among individuals caught in April, July and October (ca. -24.5±0.5 ‰ δ^{13}C). Mosquitofish δ^{13}C was enriched during the hottest months (-21.4±1.06 ‰ δ^{13}C), while being close between March and September (-25.13±0.05 ‰ δ^{13}C, -25.72±1.00 ‰ δ^{13}C , respectively). Signature was in October (-27.3 ±0.36 δ^{13}C).

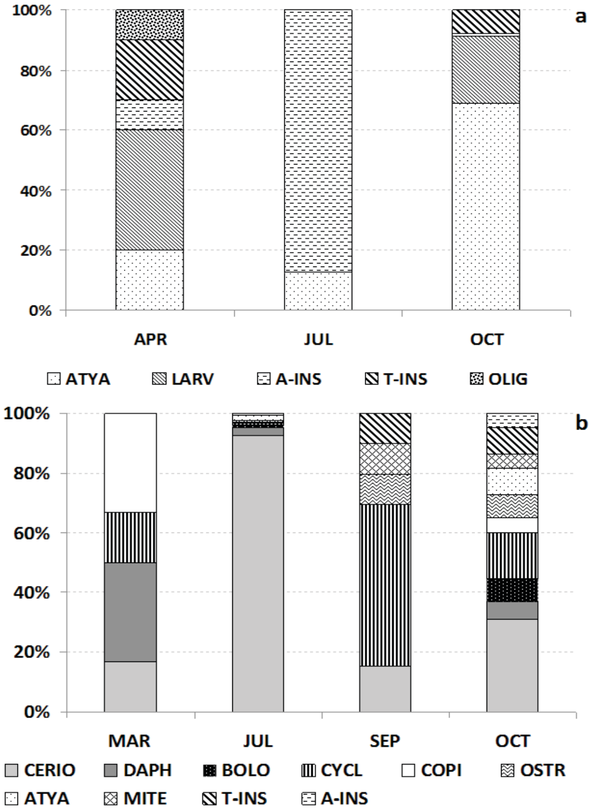


Fig. 2 Analysis of gut contents in brown trout (a), and mosquitofish (b), the first 4 letters (ATYA as *Atyaephyra*) indicate prey recorded in gut, A and T (indicate Aquatic or Terrestrial insects).

Nitrogen signature of *A. desmaresti* highlighted an inverse trend with respect to isotopic carbon signatures (Fig. 3.b). From March (12 ± 0.02 $\delta^{15}N$) to September (8.18±0.24 $\delta^{15}N$) it constantly decreased (Fig. 3.b). Oligochaetes showed closer values since April to June (ca 3.8 ±0.2 $\delta^{15}N$) and slightly increased in July (5.51±0.01 $\delta^{15}N$) and September (6.31±0.03 $\delta^{15}N$). $\delta^{15}N$ signatures of *A. fluviatilis* were similar (8.58±0.74 $\delta^{15}N$) in March, June and September. Adults brown trout $\delta^{15}N$ signatures, as just seen for carbon, were close all over the time (ca 14.43± 0.93 $\delta^{15}N$). Mosquitofish signatures in March, May and October were of ca 14 $\delta^{15}N$ ‰ (Fig. 3.b). Less enriched values were measured in July (10.03±0.41 $\delta^{15}N$).

C:N ratio

C:N ration of *A. desmaresti* never exceeded 4 (mean value 3.39±0.25), also observed for littoral oligochaetes (mean value 4.08±0.19‰ $\delta^{15}N$). Mollusks were more enriched in nitrogen (5.93±0.55 $\delta^{15}N$ *A. fluviatilis* and 8.89±0.18 $\delta^{15}N$ *Sphaerium* spp.) than dragonfly larvae (3.9±0.3) were closer to shredders decapods *A.desmaresti*. C:N ratio for both fish species were close among sampling dates (3.82±0.24 $\delta^{15}N$ for adults brown trout as well as for mosquitofish 4.00±0.46 $\delta^{15}N$).

Pelagic- Isotopic signatures

SPM carbon isotopic signature in Sos Canales showed a marked seasonality, with more $\delta^{13}C$ depleted values in Fall and Winter and more enriched $\delta^{13}C$ in late Spring and Summer . SPM of surface water of the deep station, showed more negative $\delta^{13}C$ signatures than in deep water layers and in shallow station river area. Surface SPM dry weight was correlated with phytoplankton C biomass (Sperman R=0.636, n=11, p= 0.03), and surface Chl-*a* (Sperman R=0.883, n=9, p=0.001) mainly constituted by dinoflagellates (Fadda et al. Chapter 2). SPM from deep water and from the shallow station were more enriched and had closer values (Wilcoxon-test Z=1.40, n=16, p=0.16). In Sos Canales, mezooplankton primary consumer taxa relied on a mixture of photosynthetic and non-photosynthetic carbon sources, highlighting a high tendency to an omnivorous diet (Fadda et al. Chapter 3). Two main affected seasonal changes in planktonic communities of Sos Canales, water temperature and water level fluctuations. Temperature , with values increasing (less $\delta^{13}C$ depleted) affected carbon isotopic signature than increase in hot months, as observed in temperate large deep temperate lakes (i.e. Perga and Gerdeaux, 2006; Visconti and Manca, 2011). Water temperature in Sos Canales was homogeneous along the water column and relatively low in winter (5.5 °C in February 2011). Thermal stratification started in April, lasting until October 2011. Annual maximum was recorded in July (20°C). In Sos Canales, temperature plays an important role during the thermal stratification, both for isotopic signatures as well as for zooplankton community development. Copepods, *Ceriodaphnias* and rotifers showed a marked thermophily, increasing in biomass in late spring and summer. Water level also affected indirectly isotopic signatures of both SPM, in

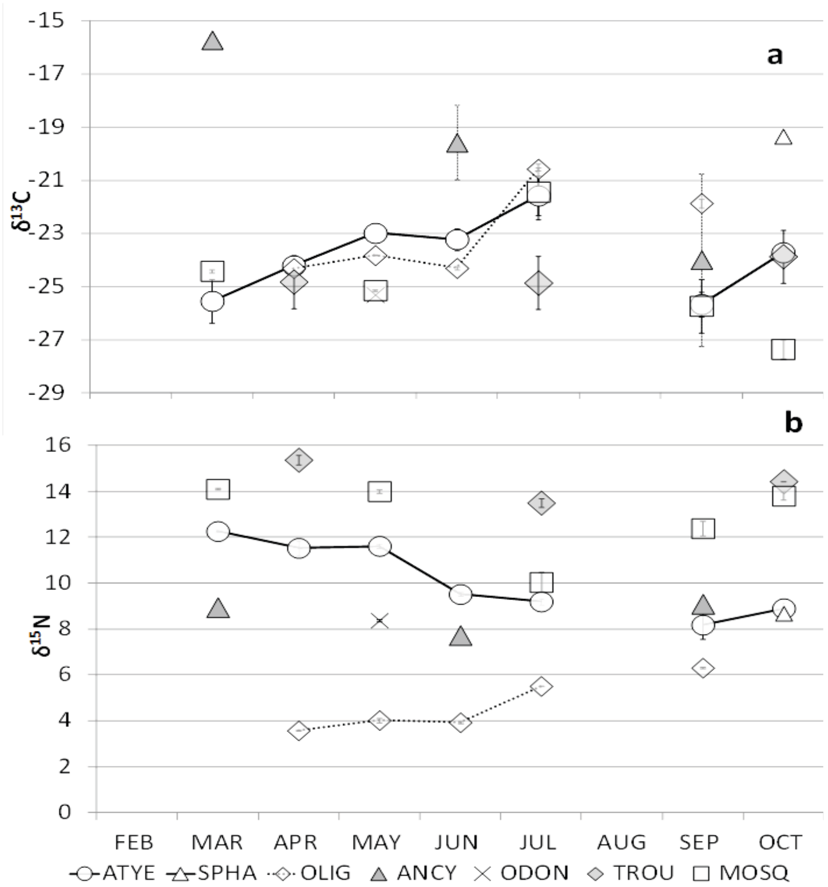


Figure 3. Carbon (a) and nitrogen (b) stable isotope results on littoral organism and fish collected in Sos Canales reservoir, the first 4 letter indicate organisms (i.e. TROU as a trout).

Tab. 2 - Bimonthly average values of carbon and nitrogen isotopic signatures of suspended particulate matter (SPM) and main mesozooplankton crustaceans in Sos Canales, *Daphnia* spp. (DAPH), *Ceriodaphnia* spp. (CERI), *C.numidiucus* (COPI), and cyclopoids (CYCL). Months are identified by their initial letters.

	SPM		DAPH		CERI		COPI		CYCL	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
F-M	-30.78±0.12	6.59±2.44	-32.41±2.32	9.14±3.21	-30.21±1.84	9.27±3.03	-34.63±2.99	14.92±2.13	-33.91±0.60	12.64±2.22
A-M	-31.31±0.01	4.47±0.63	-28.98±0.13	5.93±0.11	-28.84±0.37	6.27±0.22	-30.73±0.59	9.91±0.89	-31.90±0.41	10.53±1.78
J-J	-27.57±0.12	5.89±1.53	-27.80	5.12	-24.26±1.57	6.00±0.49	-26.48±1.27	9.55±0.45	-26.52±1.38	10.06±0.95
S-O	-29.32±0.85	7.82±0.22	-31±0.26	14.38±0.22	-28.81±0.37	9.33±0.095	-29.02±0.10	11.75±0.05	-28.98±0.05	12.15±0.02

particular $\delta^{15}N$ increased when water level decreased, and for the available carbon sources for the phytoplankton community. Water level strongly varied throughout the year due to continuous withdraw for human supply of ca. 265 m^{-3}/month. Water level rapidly increased in February 2011, as a consequence of heavy rainfall, reaching 37 m (respect to 27 m recorded in October 2010). It weakly decreased till June 2011. Since July to September 2011 water level constantly decrease highlighting decreased water input from the watershed.

To evaluate the feeding behavior of fish in Sos

Canales, we used SPM average values (surface, deep and river inflow area SPM isotopic signatures). Fish do not use directly SPM (1.2≥μm≤0.76) but next primary consumers. Fish caught started in March; therfore we considered SPM and zooplankton isotopic signatures sinceFebruary, assuming that muscles fish tissues carried a two months integrate signatures respects to its diet (Woodland et al., 2012). Instead we used a bimonthly averages carbon and nitrogen isotopic signatures of SPM and zooplankton taxa as a proxies of season in Mediterranean area. Thus February-March (F-M) as a winter, April-May (A-M) spring, June-July (J-J) summer and September-October (S-O) as a fall. SPM $\delta^{13}C$ was more depleted in winter (-30.78±0.12 $\delta^{13}C$, F-M), spring (-31.31±0.01 $\delta^{13}C$ A-M) and fall (-29.32±0.85, $\delta^{13}C$, S-O) than in summer (-27.57±0.12 $\delta^{13}C$, J-J). SPM $\delta^{15}N$ were more enriched in winter (6.59±2.44 $\delta^{15}N$, F-M) and fall (7.82±0.22, $\delta^{15}N$ S-O) than in spring (4.46±0.63 $\delta^{15}N$, A-M) and summer (5.89±1.53 $\delta^{15}N$, J-J). Mesozooplankton pelagic community was main composed both by large and small sized cladocerans (*Daphnia* spp. and *Ceriodaphnia* spp.) as well as by calanoid (*C. numidicus*) and cyclopoid copepods (*M. albidus* and *C. abyssorum* gr.). As was recorded on SPM, mesozooplankton crustaceans carbon and nitrogen isotopic signatures were more depleted in $\delta^{13}C$ in winter and spring than in summer and fall (Tab. 2). $\delta^{15}N$ mesozooplankton signatures were more enriched in winter (F-M) and fall (S-O) than in spring and summer months (Tab. 2).

DBMM

To evaluate the contribution of the two main food sources in Lake Sos Canales a DBMM was applied. Isotopic signatures recorded on fish dorsal muscles tissues are the results of assimilated carbon and nitrogen in an time of ca. two months. DBMM of adults brown trout highlighted that they rely mainly on littoral organisms, only 40% on pelagic carbon source origin exploited in July (Fig. 4a). *G. holbrooki* relied upon littoral food (85%) in late winter (F-M) and summer (J –J) (76%). In spring (M-A) fed on both sources, while in late summer and early fall relied mainly on pelagic carbon sources (Fig. 4b).

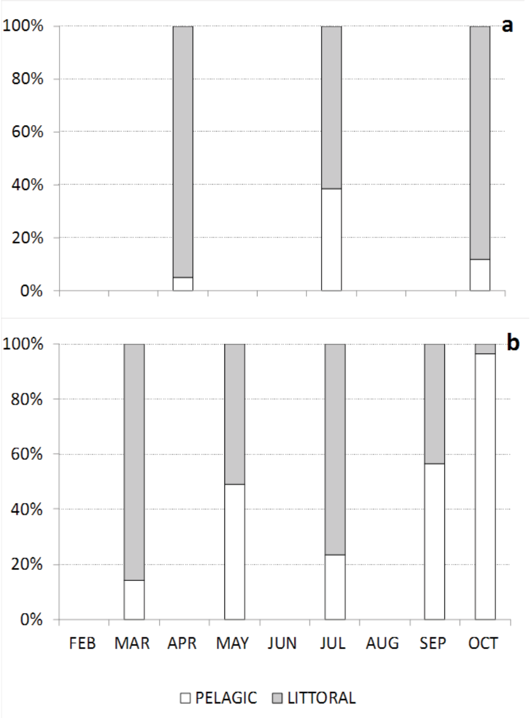


Fig. 4 - DBMM results indicate proportion of littoral (grey) and pelagic (white) carbon sources exploited by brown trout

Trophic position

Considering changes in fish feeding behavior as suggested by results on SIA, we suggest

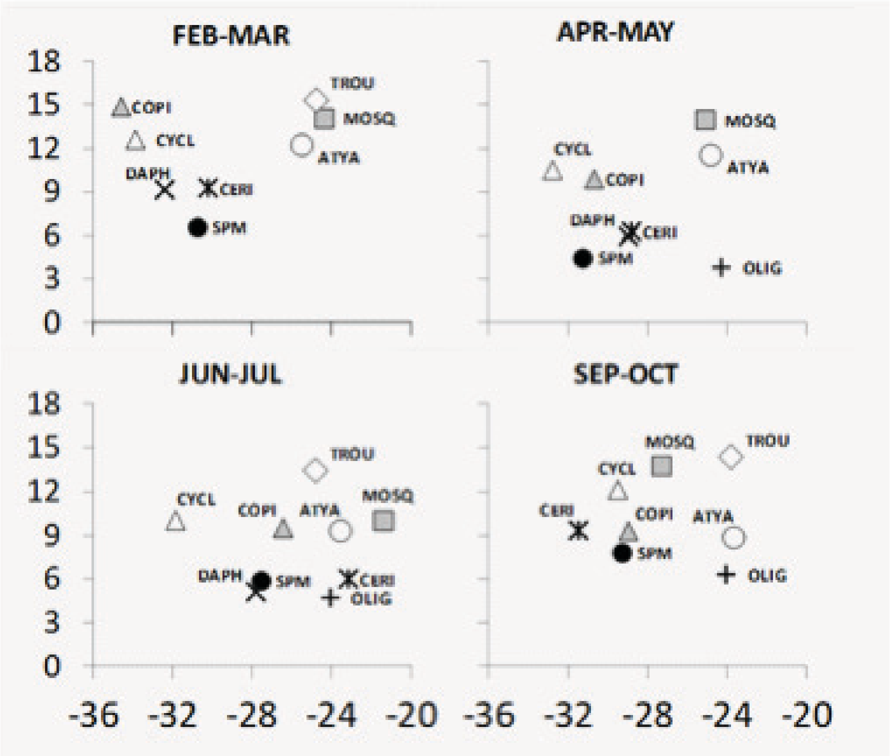


Fig. 5 Bimonthly results and trophic relationship among pelagic and littoral community in Sos Canales. Months as indicate with the first letter (i.e. June-July as J-J), organisms were indicated with the first 4 letters of organism (i.e. MOSQ as mosquitofish), expet SPM than indicate Suspended Particulate Matter.

that littoral sources were important for brown trout throughout the year, while mosquitofish changed their diet since summer to fall. In winter and spring Fish exploited mainly littoral carbon sources, while during thermal stratification, and a decrease in external water inputs small sized fish started to rely on pelagic food sources. Trophic position equation highlighted that adults brown trout relied on littoral carbon sources and occupied level 3 in littoral food web (3.2 on F-M, 3.5 in J-J and 3.9 in S-O). Nitrogen enrichment of brown trout ranged between 4.1 $\delta^{15}N$ in F-M and 6.5 $\delta^{15}N$ in S-O. *A. desmaresti* was the primary consumer in littoral food chain throughout the study period. *G. holbrooki* in cool months (F-M) occupied the same trophic position as brown trout (3.2) highlighting close values of nitrogen enrichment (4.31 $\delta^{15}N$). In spring (A-M) was a primary consumer, while in summer fed on a mixture of the littoral and pelagic sources (T=2.8 whit a E=2.7 δ15N). In fall, relied on pelagic carbon sources of a pelagic food web (6.8 $\delta^{15}N$ of nitrogen enrichment).

DISCUSSION

In Sos Canales reservoir two allochthonous fish species were introduced likely to recreational fishing purpose, the non endemic brown trout and mosquitofish (*G. holbrooki*). Sardinian streams hosted the endemic and introduced North Atlantic origin brown trout (Massidda, 1995; Sabatini et al. 2006).

Massive stocking of North Atlantic origin brown trout was conducted in several streams (Sabatini, 2011) as well as in reservoirs (Orrù et al. 2010). The release of these invasive fish into Sardinian rivers could have modified the distribution of endemic salmonids populations and caused genetic pollution effects (Gandolfi et al. 1991). *Gambusia holbrooki* were introduced in Sardinia in 1920, mainly close to the coastal malaria areas. Mosquitofish in Sardinia is very abundant in reservoirs, streams, wetland and ponds (Orrù et al. 2010). In Sos Canales only brown trout have a fishing interest, the *G. holbrooki*, was likely introduced as a forage fish. Sos Canales reservoir is devoted to drinkable water production, in a small quite pristine upland area of North-East Sardinia. Little catchment area and absence of important tributary can exclude a natural spread of inhabitant fish. Furthermore Sos Canales, as well as, reservoir in general rule, are subject to several stress, enhancing the spread capability of non indigenous species (NIS). The main stressors affecting aquatic community in this reservoir was the water level fluctuation, that varied among 10 m during the year (Fadda et al. submitted). Instead strongly water level fluctuation stopped the development of structured macrophytic littoral community, thus only few submerged plants comparing in summer (pers. obs.). Aquatic primary producer, in Sos Canales, was principle constitute by phytoplankton. Phytoplankton community were dominate by mixotrophic dinoflagellate, in particular by *Gimnodinium uberrimum*. *G. uberrimum* founded suitable condition in this slightly acid waters (Fadda et. al). Carbon and nitrogen stable isotope analysis of SPM collected both near to the river inflow and in the deepest area close the dam, showed a strong contribution by external carbon sources during the cool months. Water bodies in Mediterranean climate area, are subject to marked annual hydrological cycle, rainfall events were restricted to winter and early spring, followed by several drought months. The absence of water input during warm period, and constant human intake, causing a visible drop of water level. The first relevant rainfall episode, often are a flood, transport a large amount of detritus from watershed that rapidly sank close the dam. Furthermore the new water inputs re-suspended a large amount of bottom sediments. Instead SPM composition varied widely throughout the year and were constitute both by photosynthetic and no-photosynthetic fractions (Fadda et. al. submitted). Sos Canales mesozooplankton crustacean community highlighted an higher diversity, hosting endemic species, *C. numidicus*, as well as invasive hybrid clone of *Daphnia pulex* complex (Fadda et al. 2011). Cladocera community were composed by small (*Bosmina longirostris*, *C. pulchella*, *C. reticulala*) and large size (*D. pulex*, *D. longispina* gr.) filter-feeders. Copepods by calanoids and predaceous cyclopoids (*M. albidus*, *C. abyssorum* gr.). Stable isotope analysis in Sos Canales mesozoplankton crustacean, highlighted a prevalence of omnivorous behavior by cladocerans and *C. numidicus*, cyclopoids, represented by large size species, were for almost of the time the secondary consumer rely on phytoplankton carbon origin (Fadda in preparation). Furthermore, dial horizontal migration (DHM) on zooplankton community were recorded in spring months (Fadda et al., in preparation).

Fish population were constituted by few adults of brown trout likely affected by reproductive trail problems. At each sampling date, only one adults individual of brown trout were caught, and one with a skeletal deformity in a caudal area, suggesting a farming origin (Mehrdad, 2011) and another one was a female with a mature eggs in July. The long spawning period in Mediterranean climate, is a an advantage for survival in unpredictable habitats (Gortàzar, 2007). Stress during part or all of vitellogenesis may compromise reproduction by diverting energy away from this process towards coping with stressor (Contreras-Sànchez, 1998). Eggs trout deposition in Mediterranean area were reported from December until April, mature eggs during summer can indicate inability to founded a suitable area for spawning or interruption of vitellogenesis. Analysis of brown trout gut contents highlighted the prevalence of littoral invertebrate prey, in particular aquatic insects and the common *A. desmaresti*. Mosquitofish population was relatively abundant in littoral area, 56 ind. m^{-2}, analysis of gut contents, as was expected, main composed by zooplankton. In July more the 90% of prey were *Ceriodaphnia*, this latter reached their annual peak in biomass in July (Fadda et. in preparation).

Carbon and nitrogen isotopic signatures collected on mosquitofish and *A. desmaresti* in 5 station around the reservoir shoreline were closer (Tab. 1), highlighting similar suitable carbon sources for littoral community. Carbon isotopic signature of littoral invertebrate, as was observed on SPM and mesozooplankton showing a marked seasonality (Fig. 3.a). Relative depleted $\delta^{13}C$ were recorded in cool months (March, October), and more enriched in summer (July). $\delta^{15}N$ followed an inverse trend, was more enriched in late winter and spring (since March till May) and decreased in summer (June-July). If analysis of gut contents was like a picture of the last meal of caught fish, stable isotope analysis is an integrate tool, able to detect the assimilate carbon sources. Assuming that the monthly carbon signature of fish tissue was a linear mass balance average of elemental mass from each food sources (Phillips and Gregg, 2001), within 60-day time interval to reach equilibrium (Woodland et al. 2012). Thus isotopic values integrated over two-months intervals, a time consistent with turnover metabolic rates of fish dorsal muscle tissue (Hesslein et al., 1991). To clarify the main exploited available sources for fish in Sos Canales, DBMM was applied. DBMM results confirms adults brown trout a littoral feeder, while mosquitofish changed their died during the year, main rely on pelagic sources in Fall. If we consider a bimonthly as a proxy of season we founded that littoral and pelagic organism had well a defined food web pattern relationships in winter (F-M) and spring (A-M), while since summer (J-J) till fall (S-O) became closer (Fig. 5). Oligochaetes showed the lower level of littoral trophic web while shredder decapods *A. desmaresti* was the primary consumer. In winter both brown trout and mosquitofihs rely on littoral sources at the 3th trophic position (T = 3.3 and 3.2, respectively), with closer nitrogen enrichment value (δ^{15}N E=4.31‰, and E= 4.1‰). In spring mosquito fish at the same level of the primary consumer *A. desmaresti* (T=2; δ^{15}N E=4.89‰). In summer a prey-predator relationship were observed between large fish (T=3.5, E=5.2) and the small one (T=2.8;, E=2.71). In fall fishes were at the 4th trophic position, but brown trout rely on littoral, and mosquitofish on pelagic sources. In S-O mosquitofish rely on pelagic copepods and 3 rings of pelagic food chain were observed, mosquitofish (T=4.4; E=6.8), cyclopoids (T=3.2; E=4.09), *C. numidicus* (T=2.4; E=1.51).

The feeding behavior of introduced fish species highlighted that littoral carbon sources was a main exploited sources by inhabitant fish. Hydrology dynamics also can influenced fish behavior in particular small fish. Littoral area of Sos Canales is rich in invertebrate fauna, in particular *A. desmaresti* were more abundant, as well as insects larvae (i.e. dragonfly), thus represented a quite palatable and easily sources for fish community. Reservoir is surrounded by a Cork-Oak wood than supply a large amount of food for shredder decapods. During the filling phase, since February to May (Fadda et al. submitted), *G. holbrooki* mainly exploited on littoral carbon sources. After the drought summer (J-J), when external water inputs not occurred, reservoir was in a standing water phase, and *G. holbrooki* exploit on pelagic carbon sources. The effects of mosquitofish on invertebrate community, is well documented (George et al. 2008 references therein) causing a shift by large size filter-feeders to small cladocerans and rotifers in planktonic community (Margaritora et al. 2001).

CONCLUSION

Mosquitofish is a voracious predator, introduced in Europe to control mosquito larvae, and in
Sardinia since 1920s (Orrù et al., 2010), which significantly alters aquatic community structure (Hulber et la., 1972). In Mediterranean area, mosquitofish population strongly decrease in winter, and few survivors reforms population in early spring (Fernandez-Delgado, 1989; Fernandez-Delgado et al., 1997; Hughes, 1985; Haynes, 1993). One fertilized female are able to replace a new mosquitofish population with little or no damaging founder effects (Chesser et al,, 1984; Alemadi and Jenkins, 2008). Furthermore, this species is resistant to a wide range of environmental conditions, e.g. low oxygen concentrations and temperature (Peterson and Peterson, 1990, Rupp, 1997). Our results highlighted a prey-pressure by G. holbrooki on pelagic zooplankton only in fall months. The shift from littoral to pelagic sources may be led by hydrological condition in SC at the end of drought season. This species mainly inhabits slow flow or still

waters in Sardinia (Orrù et al., 2010), than suggesting a favorable hydrological condition since summer to early fall months, when external water inputs were absent. Although a predator fish species was also introduced, the Atlantic brown trout, its population was low in abundance and evidenced reproductive trail problems. The concurrent absence of a effective predator and the enlargement of "standing-water" phase, can enhance the mosquitofish population. Furthermore, SC is a reservoir of planktonic crustancean diversity (Fadda et al., 2011). An increase in abundance of SC's mosquitofish population can affect planktonic crustacean community reducing diversity of inhabitant mesozooplanktonic crustaceans as well as indirectly the water quality.

References

Alemadi, S. D.; Jenkins, D. G., 2008: Behavioral constraints for thespread of the eastern mosquitofish, Gambusia holbrooki (Poeciliidae).Biol. Invasions 10, 59–66.

Allendorf F.W., Leary R.F., Spruell P., Weinburg J.K., The problems with hybrids: setting conservation guideline. Trends in Ecology and Evolution, 16:613-622, 2001.

Baxter C.V., Faush K.D., Murakami M., Chapman P.L., Fish invasion restructures stream and forest by interrupting reciprocal prey subsidies. Ecology 85:2656-2663, 2004.

Bence J. R., Indirect effects and biological control of mosquitoes by mosquitofish. Journal of Applied Ecology 25: 505-521, 1988.

Borroni I., Trasforini S., Bardazzi M., Gentili G., Caratterizzazione Fenotipica e Genetica della popolazione di trota de Rio Baracca (Bacino dell'Orba). Carta ittica 1999-2003.

Cabral J. A., Marques J.C., Life history, population dynamics and production of eastern mosquitofish, Gambusia holbrooki (Pisces, Poeciliidae), in rice fields the lower Mondego River Valley western Portugal. Acta Oecologica 20(6): 607-620, 1999.

Camin F., Perini M., Colombari G., Bontempo L., Versini G., 2008. Influence of dietary composition on the carbon, nitrogen, oxygen and hydrogen stable isotope rations of milk. Rapid. Comm. Mass. Spectrom., 22: 1690-1696.

Camin F., Wietzerbin K, Blanch Cortes A., Haberhauer G., Less M., Versini G.J., 2004. Application of multielement stable isotope ratio analysis to the characterization of French, Italian, and Spanish cheeses. J. Agric. Food Chem., 52: 6592-6606.

Chesser, R. K.; Smith, M. W.; Smith, M. H., 1984: Biochemical genetics of mosquitofish. III. Incidence and significance of multiple insemination. Genetica 64, 77–81.

Contreras-Sànchez W.M., Schreck C.B., Fitzpatrick M.S., Pereira C.B., Effects of Stress on Reproductive Performance of Rainbow Trout (Oncorhynchus mykiss). Biology of Reproduction 58: 439-447, 1998.

Coop G.H., Bianco P.G., Bogutskaya N.G., Eros T., Falka I., Ferreira M.T.. To be, or not to be, a non-native freshwater fish? Journal of Applied Ichthyology, 21: 242-262, 2005.

Cottiglia, M., 1968: La distribuzione dellittiofauna dulciacquicola in Sardegna. Riv. Idrobiol. 7, 64–116.

Cucherousset J., Bouletreau S., Martino A., Roussel J. M., Santoul F., Using stable isotope analysis to determine the ecological effects on non-native fishes. Fish. Manag. Ecol., 19: 111-119, 2012.

Cucherousset J. and Olden J.D., The ecological impacts of nonnative freshwater fishes. Fisheries, 36: 215-230, 2011.

Cucherousset J., Aymes J.C., Poulet N., Santoul F. and Cèrèghino R., Do native brown trout and non-native brook trout interact reproductively? Nuturwissenchaften 95: 647-654, 2008.

De Bernardi R., Giussani G., Manca M., Cladocera predator and prey. Hydrobiologia, 145: 225-243, 1987.

De Niro M.J. and Epstein S., Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta. 42: 495-506, 1978.

Fadda A., S. Marková, P. Kotlík, A. Lugliè, B.Padedda, P. Buscarinu, Ni. Sechi and M. Manca. First record of planktonic crustaceans in Sardinian reservoirs. Biologia

66(5): 856-865.

Fernandez-Delgado, C. Rossomanno S.: Reproductive Biology of the Mosquitofish in a Permanent Natural Lagoon in South-West Spain: Two Tactics for One Species. J. Fish Biol. 1997, 51: 80-92.

Fernandez-Delgado, C: Life-history Patterns of the Mosquitofish, Gambusia affinis, in the Estuary the Guadalquivir River of South-West Spain. Freshwater Biol. 1989; 22: 395-404.

Fry B., Stable Isotope Ecology. New York, USA: Springer, 308 pp., 2006.

Garcìa-Berthou E., Alcaraz C., Pou-Rovira Q., Zamora L., Coenders G. and Feo C., Introducion pathways and establishment rates of invasive aquatic species in Europe. Canadian Journal of Fisheries and Aquatic Sciences, 65: 453-463, 2005.

Gozlan R.E., Introduction of non-native freshwaterfish: is it all bad? Fish and Fisheries 9: 106-115, 2008.

Gu B., Schell D.M., Alexander V., Stable carbon and nitrogen isotope analysis of plankton food web in subartic lake. Can. J. Fish. Aquat. Sci. 51: 1338-1344.

Haynes J.L.: Annual reestablishment of mosquitofish populations in Nebraska. Copeia 1993, 11(1): 232-235.

Hesslein R.H., Capel M.J., Fox D.E., Hallard K.A. Stable Isotopes of Sulfur, Carbon, and Nitrogen as Indicators of Trophic Level and Fish Migration in the Lower Mackenzie River Basin, Canada. Canadian Journal of Fisheries and Aquatic Sciences, 48 (11): 2258-2265, 1991.

Hughes A.L., Seasonal Changes in Fecundity and Size at First Reproduction in an Indiana population of the Mosquitofish Gambusia affinis. Am. Midl. Nat. 1985,114: 30-36

Lookwood J.L., Hoopes M.F, and Marchetti M.P., Invasion Ecology. Oxford, UK: Blackwell Publishing, 312 pp., 2007.

Lynch M., Predation completion, and zooplankton community structure: An experimental study. Limnol. Oceanogr., 24(2): 253-272, 1979.

M.J. de Niro and S. Epstein. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimca Acta. 42: 495-506, 1978.

M.J. de Niro and S. Epstein. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimca Acta. 42: 495-506, 1978.

Manca M., Vijverberg, Polishchuk V.L. and Voronov D.A., Daphnia body size and population dynamics under predation by invertebrate and fish in Lago Maggiore: an approach based on contribution analysis. J. Limnol. 67(1): 15-21, 2008.

Marchetto A., Padedda B.M, Mariani M., Lugliè A., Sechi N., A numerical index for evaluating phytoplankton response to changes in nutrient level deep mediterranean reservoirs, J.Limnol., 68-1 (2009), pp. 106-121.

Margaritora G., Ferrara O., Vagaggini D., Predatory impact of the mosquitofish (Gambusia holbrooki, Girard) on zooplanktonic populations in a pond a Tenuta di Castelporziano (Rome, Central Italy). J. Limnol., 60 (2): 189-193, 2001

Massidda, P., 1995: Salmo (trutta) macrostigma in Sardegna. Biol. Ambient. 5, 40–43.

Massidda, P.; Conti, G.; Loddo, A.; Cau, A., 2008: Pesci dacqua dolce della Sardegna. Aı`sara, Cagliari, Italia, pp. 93.

Massidda, P.; Orru` , F.; Davini, M. A.; Pirastu, A.; Cau, A., 2006: Introductions of non-native fishes in Sardinia: first occurrence of Carassius auratus (L. 1758) in Lake Coghinas (Sardinia, Italy). J. Freshw. Biol. (Quaderni ETP) 34, 299–303.

McCauley, E., and Murdoch, W.W. 1987. Cyclic and stable populations: plankton as paradigm. Am. Nat. 129: 97–121

Mehrdad Y., Mehdi R., Mahsa A., A radiographical Study on Skeletal Deformities in Cultured Rainbow Trout (Oncorhynchus mykiss) in Iran. Global Veterinaria, 7 (6): 601-604, 2011.

Sechi N. and A. Lugliè, Limnlogical studies on man-made lakes in Sardinia (Italy). Mem. Ist. Ital. Idrobiol., 50 (1992), pp. 365-381.

Sechi N., and A. Luglié, Phytoplankton in Sardinian reservoirs.Giornale Botanico Italiano, 130 (1996), pp. 977-994.

Nesbit, D. H., Meffe, G.K.: Cannibalism Frequencies in Wild Populations of the Eastern Mosquitofish (G. holbrooki: Poecilidae) in South Carolina, Copeia 1993: Aug 18(3): 867-870

Olden J.D., Biotic homogenization: a new research agenda for conservation biogeography. Journal of Biogeography 33: 2027-2039, 2006.

Orrù F., Deiana A.M., Cau A., Introduction and distribution of alien freshwater fishes on the island of Sardinia (Italy): assessment on the basis of existing data sources. J. App. Ichthyol. 26 (2): 46-52, 2010.

Peck G. W. and Walton W.E. Effects of mosquitofish (Gambusia affinis) and sestonic food abundance on the invertebrate community within a constructed treatment wetland. Freshwater Biology, doi:10.111/j.. 1365-2427.2008.

Philips D.L, Gregg J.W. , Uncertainty in source portioning using stable isotope mixing models. Oecologia, 130: 114-125, 2001.

Rupp, H.R. Mosquito Control with Gambusia affinis J. Am. Mosqu. Contr. Assoc. 1997: 296.

Sabatini A., Cannas R., Follesa M.C., Palmas F., Manunza A., Matta G., Pendugiu A.A., Serra P., Cau A., Genetic characterization and artificial re production attempt of endemic Sardinia trout Salmo trutta L., 1758 (Osteichthyes, Salmonidae): Experience in captivity. Ital. Journal of Zoology, 78(1): 20-26, 2011.

Sabatini, A.; Orru` , F.; Cannas, R.; Serra, P.; Cau, A., 2006: Conservation and management of Salmo (trutta) macrostigma. in Sardinian freshwaters: first results of genetic characterization. J. Freshw. Biol. (Quaderni ETP) 34, 335–340.

Şükrü Ö., Some biological properties of mosquitofish populations (Gambusia affinis) living in inland waters of western Mediterranean region of Turkey. Turk. J. Vet. Anim. Sci., 28: 355-361, 2004.

T. Zoary an Ostrovsky I., Ecologica impacts of excessive water level fluctuations in stratified freshwater lakes. Inland Water 1,: 47-59, 2011.

Visconti A., Fadda A., Volta P., Di Guardo A., Manca M., Littoral vs. pelagic sources for planktivorous fish: applying a Dynamic Mixing Model to C, N Stable Isotope Analyses in deep subalpine lake. Submitted 24-06-2012.

Woodland R.J., Rodrìguez M.A., Magnan P., Glèmet H. and Cabana G.. Incorporating temporally dynamic baselines in isotopic mixing models. Ecology, 93, 131-144, 2012.

CHAPTER 5

Freshwater Biology

Freshwater Biology

Littoral vs. pelagic sources for planktivorous fish: applying a Dynamic Mixing Model to C, N Stable Isotope Analyses in a deep subalpine lake

Journal:	*Freshwater Biology*
Manuscript ID:	FWB-A-Jun-12-0368
Manuscript Type:	Applied Issue
Date Submitted by the Author:	24-Jun-2012
Complete List of Authors:	Visconti, Anna; CNR Intitute of Ecosystems Studies, Fadda, Amedeo; CNR Intitute of Ecosystems Studies, Volta, Pietro; CNR Intitute of Ecosystems Studies, Di Guardo, Antonio; University of Insubria, Department of Science and High Technology Manca, Marina; CNR Intitute of Ecosystems Studies,
Keywords:	Fisheries < Applied Issues, Lakes < Habitat, Ecosystem < Level of Organisation, Food webs < Process / Approach / Methods, Stable isotopes < Process / Approach / Methods, Fish < Taxonomic Group / Assemblage, Zooplankton < Taxonomic Group / Assemblage

CHAPTER 5

Littoral vs. pelagic sources for planktivorous fish: applying a Dynamic Mixing Model to C, N Stable Isotope Analyses in a deep subalpine lake

VISCONTI A.(1,2)*, VOLTA P.(1), FADDA A.(1), DI GUARDO A.(2) AND MANCA M.(1)

(1) CNR ISE, Largo Tonolli 50, 28048 Verbania VB Italy

(2) University of Insubria, Department of Science and High Technology, Via Valleggio, 11, 22100 Como, Italy

*Author for correspondence

e-mail address: a.visconti@ise.cnr.it

Keywords: stable isotopes, planktivorous fish, trophic web, seasonality, deep lakes

TO BE SUBMITTED TO: FWB, APPLIED ISSUES

Running head: Dynamic Mixing Models and SIA: contribution of littoral/pelagic sources for planktivorous fish

CHAPTER 5

SUMMARY

1. Stable isotope analysis (SIA) is increasingly being used for quantitative estimates of trophic relations among organisms. Nitrogen and carbon isotopes are used respectively to investigate trophic web interactions and carbon sources fuelling the food web. Identifying and quantifying contribution of different sources (littoral and pelagic) and the steps through which they are conveyed along the food web is essential also in view of reliable predictions of the impact of different stressors acting on different spatial and time scales.
2. During 2008, we detected seasonal changes of carbon and nitrogen isotopic signature to the main components of the pelagic food web of a deep subalpine lake, Lake Maggiore (Northern Italy), from putative food sources (primary producers) to zooplankton primary and secondary consumers (in 2008 and 2009) and three planktivorous fish species.
3. Reconstructing seasonal fluctuations in the isotopic baselines (from -35.8 to -25.7 for pelagic and from -26.7 to -18.3 for littoral baselines) and how they are attenuated by organisms with different turnover rates and trophic position (7.2 time less in fish compared to baselines) is essential also for understanding mechanisms through which matter, energy, and pollutants, are transferred through aquatic food webs, and to predict impacts and patterns consequent to human impacts, either direct or indirect.
4. We investigated seasonal changes in δ13C and δ15N of three fish species (European whitefish, shad and roach) and of their potential preys in Lake Maggiore, by applying a recently developed linear Dynamic Baseline Mixing Model (DBMM) in which temporal changes in littoral and pelagic baselines, as well as metabolic turnover (m) and growth rate (k) of the fish (0-3+) were included. We used fish dorsal muscle and integrated prey signatures over a 60 day time span. We found that seasonal shifts between pelagic and littoral food sources, and the other way round, were far from negligible for all three fish species.
5. We provided quantitative estimates of seasonal changes in trophic position of primary (λ=2) and secondary (λ=3) consumers within the zooplankton. Predatory zooplankters, particularly *Bythotrephes longimanus*, seem to occupy the same trophic position of fish (T=4) in pelagic food web in winter, when the latter were exploiting littoral food sources. Such a role might be crucial to elucidate seasonal variations of persistent organic pollutant (POPs) accumulation along the food web.

CHAPTER 5

1. INTRODUCTION

Nitrogen and carbon stable isotopes analysis (SIA) is increasingly used to investigate trophic web interactions and carbon sources fueling the food web. While $\delta^{15}N$ signatures allow us to calculate the relative trophic position of the organisms belonging to the same trophic web, the analysis of carbon isotope ratio allows us to identify and quantify contributions of different sources (e.g. littoral and pelagic) and the steps through which they are conveyed along the food web.

Reconstructing sources sustaining secondary production and paths of transfer of matter and energy through trophic webs is of crucial importance. These analyses provide basic knowledge on how ecological systems operate, which allow for sounder ecosystem management, and better prediction of the impacts of external perturbations. External perturbations are mediated through the food web, their exact impacts depending on how organisms interact both with the environment and each others. In aquatic environments and in lakes in particular, seasonal changes may be crucial controlling these impacts. Deep lakes are optimal systems for investigating the importance of seasonality for transferring carbon and nitrogen stable isotopes signatures to planktivorous fish because of their relatively high homeostasis, well defined pelagic environment, and relatively low between-year variations in plankton seasonal dynamics (Woodland *et al.*, 2012).

In the present work, we aimed at quantifying seasonal changes in relative contribution of pelagic/littoral food sources and the pattern by which they were transferred to fish through the food web. For this purpose, C and N Stable Isotopes Analysis (C, N SIA) was performed on pelagic and littoral primary producers, primary and secondary consumers and three planktivorous fish species: the European whitefish *Coregonus lavaretus* (Linnaeus, 1758), the shad *Alosa agone* (Scopoli, 1786) and the roach *Rutilus rutilus* (Linnaeus, 1758) which recently invaded Lake Maggiore (Volta & Jepsen, 2008).

Gut analysis and laboratory experiments are usually used to investigate prey preferences and feeding rates of fish. These approaches, however, suffer from being an instantaneous snapshot in time and these provide only a partial comprehension of real energetic sources and direct predator-prey relations (Gu *et al.*, 1994; Vander Zanden & Rasmussen, 1999). SIA , instead, provides the integrated values of the assimilated food over a long term period, i.e. what the animal realistically uses for development and growth.

Recently, Woodland *et al.* (2012) proposed a linear “dynamic baseline mixing model” (DBMM) to estimate diet composition, which incorporates temporal gradients in isotopic baselines available for consumers, as well as estimatiscy consumer tissue growth (k), and metabolic turnover (m) rates.

Food webs delineated by stable isotope (SI) also provide a better model for potential after its well-known, and recent, history of contamination by DDTs (Di Guardo *et al.*, 2006; Volta *et al.* 2009. Bettinetti *et al.*, 2012). Here we demonstrate that, depending on the season, shifts in littoral vs. pelagic food sources may be of crucial importance also for quantifying the role of zooplankton secondary consumers in transferring matter, energy and e.g. bioaccumulative pollutants through the pelagic food web.

2. MATERIALS AND METHODS

2.1 Study site

Lake Maggiore (45°58'30"N; 8°39'09"E) lying at an altitude of 194 m a.s.l., is the second deepest (mean depth, 177 m; maximum, 370 m) and largest (area, 212.5 km^2; volume, 37.5 km^3) subalpine lake in Italy. Phosphorus limited, the lake was driven back to oligotrophy by the middle 1980s and 1990s (total phosphorus concentration at winter turnover ca 10 µg L^{-1}), after eutrophication in the 1960s and late 1970s. The impact of multiple stressors (i.e. changes in algal nutrients, introduction of exotic fish, climate warming and meteo-climatic inter-annual variability) on Lake Maggiore is discussed in several papers (e.g., Manca & Ruggiu, 1998; Manca *et al.*, 2004; Ambrosetti & Barbanti, 1999; Manca & DeMott, 2009; Visconti *et al.*, 2008; Guilizzoni *et al.*, 2011).

2.2 Sample collection and laboratory analyses

Seston and zooplankton were collected monthly during 2008 from three stations: an open-water station at the maximum depth of the lake (Ghiffa, G: 45°58'30"N; 8°39'09"E); one station influenced by the inflow of the Toce River (Baveno, B: 45°54'28"N; 8°31'44"E) (Ambrosetti *et al.*, 1980); and a third station influenced by littoral conditions (Lesa, L: 45°49'70"N; 8°34'70"E; Ambrosetti et al. *ibidem*) (Fig. 1). All stations were sampled on the same day. Integrated water samples were collected from the epilimnion (0-20 m) and from the hypolimnion (20-50 m) with Niskin bottles.Water was filtered through a 76-µm nylon filter to obtain the sestonic 1.2 ≤ 76 µm portion which included detritus, phytoplankton and smaller organisms. From each sample, a subsample was fixed in Lugol acetic solution, for phytoplankton microscopic analysis (biomass and abundance of different taxa, analysis of functional traits, i.e. size and cell geometry). Chlorophyll *a* concentration (Chl*a*) was also measured following Lorenzen (1967).

Zooplankton population density and biomass were estimated from samples collected with two Clarke-Bumpus plankton samplers (75-µm and 125-µm mesh size nets) towed along a sinusoidal trajectory (from a boat moving at a low and constant speed of 0.6 m s^{-1}) to integrate the first 50 m of water column. The samples were fixed with ethanol (99%). We calculated biomass from length–weight regression equations of the different taxa (McCauley, 1984; Manca & Comoli, 2000). Live zooplankton samples for isotopic analysis were collected with large (58-cm opening mouth diameter), 450-µm and 1000-µm mesh nets. Our use of larger net mesh sizes facilitated high filtration efficiency, while ensuring that large phytoplankton colonies were avoided; moreover, taxa of small body size, such as rotifers, and early developmental stages of copepods were excluded. These components are of marginal importance for the purpose of our study which is focused on the relative contribution of primary and secondary zooplankton consumers to zooplanktivorous fish. Zooplanktivorous fish preferentially capture large-bodied and well visible zooplankters are largely preferred by zooplanktivorous fish (Zaret, 1980; de Bernardi *et al*., 1988).

Organisms were kept overnight in filtered lake water for gut clearance and than sorted and isolated in a proper amount (from 50 to 700, based on species body size) for each taxon (*Daphnia galeata*, *Bythotrephes longimanus*, *Leptodora kindtii* among cladocerans*;*, *Mesocyclops leuckarti* and *Cyclops abyssorum* among copepods).

Samples of benthic littoral organisms (chironomids and amphipods) were collected with benthic nets in four different littoral stations of the shore along the major axis of the lake, from Cannero, to Meina (three stations: “Cannero, Baveno, Meina”) and in a station (“Toce”) near the mouth of the Toce River (Fig.1, crossed symbols). Organisms were kept overnight in filtered lake water for gut clearance and than sorted and isolated in a proper amount (approximately 15 organisms from each group).

All samples were oven dried at 60°C for 48 hours and finely powdered; subsamples of ca 1 mg dry weight each were transferred into 5x9 mm tin capsules for Carbon, Hydrogen, Nitrogen (CHN) and Continuous Flow-Isotope-Ratio Mass Spectrometry (CF-IRMS) Stable Isotope Analyses. Three replicates were performed on each sample.

In the same year, zooplanktivorous fish (European whitefish, shad and roach) were sampled monthly in the central zone of the lake. Whitefish and shad were sampled approximately in the same station where zooplankton was collected, roach was sampled nearest to the shore. A set of drifting nets of different mesh size (32-34-40-50 mm knot to knot) was used in pelagic waters for the whitefish and shad whilst benthic multimesh survey gillnets were used in littoral and sublittoral for the roach. They were set at dusk and taken out on the following morning.

Individual size (total body length, BL, cm) and total body mass (weight, BW, g) of fish were measured. Age was estimated by scale reading (Volta & Jepsen 2008, Volta & Giussani 2010). On each sampling date, five individuals per species of homogeneous size were collected. A small section of dorsal muscle between head and dorsal fin and above the lateral line was taken from each individual and then frozen at -20 °C.

Muscle samples were oven dried at 60 °C for at least two days and then ground in order to obtain a fine powder. As for the other samples, SIA were run on three replicates per individual per date. Atotal, of85 fish specimens (26 of shad, 30 of whitefish and 29 of roach) were analyzed.

Samples were sent to the G.G. Hatch Stable Isotope Laboratory (University of Ottawa, Canada), where the isotopic composition of organic carbon and nitrogen was determined by the analysis of CO_2 and N_2, produced by combustion on a Carlo Erba 1110 Elemental Analyser followed by gas chromatograph separation and on-line analysis by continuous-flow with a DeltaPlus Advantage isotope ratio mass spectrometer coupled with a ConFlo III. The analytical precision of the analyses (S.D.), based on the laboratory internal standards (C-55) was usually better than 0.2 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. Isotope ratios are expressed as parts per thousand (‰) differences from a standard reference, which is PeeDee Belemnite for carbon and atmospheric N_2 for nitrogen:

$$\delta^{13}C = [(^{13}C \text{ sample } / ^{12}C \text{ sample}) / (^{13}C \text{ standard } / ^{12}C \text{ standard}) - 1]; [1]$$

$$\delta^{15}N = [(^{15}N \text{ sample } / ^{14}N \text{ sample}) / (^{15}N \text{ standard } / ^{14}N \text{ standard}) - 1]; [2]$$

Statistical analysis (ANOVA and Pearson’s product moment correlation coefficient, *r*) was performed for assessing independence of fish isotopic signatures for BL, BW and age, using Statistica software version 6 (STATSOFT, Tulsa, OK, USA).

2.3 Integration of baseline fluctuations into a dynamic linear mixing model

At any given time, isotopic signature of a consumer is a result of a time-integration of diet signatures and a (tissue-) specific growth rate. Moreover, different food sources may be exploited simultaneously.

We applied a linear Dynamic Baseline Mixing Model (DBMM; Woodland et al., 2012), to take into account the seasonally shifting baseline signatures common to the food webs and the underlying physiology and growth rates of the fish.

Isotopic signatures of potential food sources were integrated over two-month intervals, a time consistent with turnover metabolic rates of fish dorsal muscle tissues (Hesslein *et al*., 1991 and 1993; Grey, 2000; Perga & Gerdeaux, 2005; Phillips & Eldridge, 2006). Fast (ca. weekly), homogeneous, turnover rates within the zooplankton and potential food (i.e. littoral and pelagic) sources allowed for a time-specific direct estimate of their trophic relationships. Missing data were estimated by applying linear regression equations or by linear interpolation.

We assumed that the monthly carbon signature of fish tissues was a linear mass balance average of elemental mass from each food source (linear mixing models; Phillips, 2001; Phillips & Gregg 2001; Phillips & Koch, 2002) within the 60-day time interval necessary to reach equilibrium (Woodland *et al*., 2012). The following equation was applied:

$$\delta^{13}C_{fish(t)} = \delta^{13}C_{fish(0)}\, e^{-(k+m)t} + p\,(h_{p(t)} - h_{p(0)}\, e^{-(k+m)t}) + (1\text{-}p)\,(h_{l(t)} - h_{l(0)}\, e^{-(k+m)t}); \quad [3]$$

where: δC_0 is the initial isotopic value of a consumer (fish) in equilibrium with its diet at time t=0; $\delta^{13}C_t$ is the isotopic signature of a consumer (fish) in equilibrium with its diet at time t (where t=60 days); k and m are the consumer's growth rate (d^{-1}) and tissue turnover metabolic constant (d^{-1}), respectively. p and (1- p) are the percentage contributions of the two sources (pelagic and littoral, respectively) useful for the trophic level calculation (equation [5]). For fish dorsal muscular tissue, m was assumed as $0.0018 d^{-1}$ both for $\delta^{13}C$ and $\delta^{15}N$ (Hesslein *et al.*, 1993). The growth rates k assigned to each fish species, were calculated according to Hesslein *et al.* (1993): $k_{shad}=0.01\ d^{-1}$; $k_{whitefish}=0.03\ d^{-1}$; $kroach=0.03\ d^{-1}$.

Prevalence of a single food source was estimated at carbon isotopic maximum fractionation ($F = \delta^{13}C_{cosnumer}-\delta^{13}Cdiet \leq 0.8$ ‰ ±1.1 SD; de Niro & Epstein, 1978) between consumers and their diet over time.

Trophic position (T) of fish (and zooplankton) with respect to baselines (littoral or pelagic) were calculated as:

$$T= (\delta^{15}N_{fish} - \delta^{15}N_{baseline})/\lambda + 2; \quad [4]$$

Where: λ = trophic fractionation ≈ 3.4 ‰; and 2 is a constant value usually assigned to primary consumers (e.g. *Daphnia* for pelagic and chironomids/amphipods for littoral). When both food sources were exploited, the following equation was applied:

$$T= (\delta^{15}N_{fish} - (p * \delta^{15}N_{pelagic} + (1\text{-}\ p)* \delta^{15}N_{littoral})/\lambda) + 2; \quad [5]$$

where: p and (1- p) contributions (pelagic and littoral, respectively) are taken from equation [3].

3. RESULTS

3.1 *Fish*

In whitefish and shad carbon and nitrogen isotopic signatures were not correlated with BL and BW. However, they were significantly correlated: r= 0.44 p=0.018 for $\delta^{15}N$ vs. BW; r= 0.42 p=0.024 for $\delta^{15}N$ vs. BL and r=0.61, p<0.001, n= for $\delta^{13}C$ vs. BL and r=0.68, p<0.001, n= for $\delta^{13}C$ vs. BW. Correlations became statistically non-significant when older specimens (>4 y) were excluded. Thus, we did not include the oldest fish as we aimed at testing homogeneous within-taxa samples.

Our result suggest that whitefish and shad did not change their food preferences seasonally, while roach tended to be more opportunistic and omnivorous. Indeed, from December to June, $\delta^{13}C$

isotopic signature of roach ranged between -24.10 and -26.28‰ being close (i.e. F≤ 0.8±1.1) to values detected in the littoral (chironomids and amphipods) (Figs. 2 and 3). In summer, however, the roach seemed to change feeding preference, approaching pelagic carbon isotopic signature. From August to October the fractionation F of $\delta^{13}C$ of roach were ≤ 0.8±1.1 with respect to *Daphnia* indicating that the majority of the diet was pelagic. Maximum contribution of pelagic food sources was detected in August (96.4%) (Fig. 3). Intermediate values between pelagic and littoral $\delta^{13}C$ signatures were found in July and in November suggesting a contemporaneous exploitation of the two carbon sources during this period of the year. We estimated a 64% contribution of pelagic sources in July and a 54% contribution of littoral sources in November. Between March and May, carbon isotopic signatures of roach were also close to those of Diaptomids (F≤ 0.8±1.1; data not shown). The latter carry a littoral-like carbon signature which has been interpreted as consequent to their feeding habits and/or the extent to which these organisms may be infested by epibionts and parasites. Depending on the time of the year and or the above mentioned traits, these zooplankters may act as carriers of littoral carbon into the lake pelagia. A mixed diet based on chironomids, amphipods and diaptomids might be inferred from carbon isotopic signature of roach during this time of the year.

Carbon isotopic signature of shad and whitefish were very close, and quite constant, through the year. For shad, values ranged between -27.76 and -29.47 ‰ (mean=-28.68 ‰ ± 0.62SD), and for whitefish between -26.94 and -28.94 ‰ (mean = -28.26 ‰ ± 0.58SD). The two species signatures were closely related to the littoral signature in winter and early spring (from January-February to April; Fig. 3). During this time of the year, both pelagic and littoral carbon baselines reached their most negative values (Fig. 2). Isotopic signatures intermediate between pelagic and littoral carbon sources were measured for both fish species in January and May.

Dependence of fish on both carbon sources during some seasons is further highlighted in Fig. 4A, in which seasonal changes in $\delta^{15}N$ are also reported. Roach signature is clearly on a lower right side of the graph; annual range variation in $\delta^{15}N$ was low (ΔN= 1.82), with values around 8.59 ‰ in November and a maximum of 10.41 ‰ in September. Whitefish tended to be higher in $\delta^{15}N$; range of variation is higher (ΔN= 2.13) with values ranging between 9.65 ‰ in June to 11.79 ‰ in September. Range of variation was also high for the shad (ΔN= 3.24) with nitrogen isotopic signature ranging between a minimum of 8.56 ‰ in August and a maximum of 11.74 ‰ in December. Mean $\delta^{15}N$ annual values were of 9.77 ‰ ± 0.74, 10.97 ‰ ± 0.64 and of 9.14 ‰ ± 0.60 (mean ± SD) were calculated for nitrogen signatures of shad, whitefish and roach, respectively.

3.2 *Zooplankton*

seston (r = 0.86; p <0.01; N = 13), thus confirming that in deep lakes such as Maggiore *Daphnia* is an appropriate proxy for the pelagic baseline against which the carbon isotopic signals of other zooplankton taxa can be compared.

Among stations differences in both $\delta^{13}C$ and $\delta^{15}N$ signatures of the different components of the pelagic food web, from seston to predatory zooplankton, were statistically non significant ($p < 0.05$; Friedman ANOVA test), thus confirming that allochthonous input may become important only after exceptional rainfall events which did not occur during 2008 (see Visconti & Manca, 2009 for further details).

The variance about the stable carbon and nitrogen signatures decreased up the food web (Fig 4, A and B).

3.3 *Baselines*

Seasonal changes in carbon pelagic baseline (either expressed by *Daphnia* or seston) were large, ranging between more negative values in winter to less negative values in summer (-35.78‰ in March and – 25.72‰ in July) (Fig. 2). They were consistent with seasonal changes in seston carbon baseline; the latter was consequent to changes in availability of carbon sources and differences in isotopic fractionation and the rate at which phytoplankton may incorporate carbon, in turn depending on morphological and functional traits of taxonomic groups (Caroni *et al.*, 2012).

Seasonal variations were also detected in littoral carbon signature; the latter were more negative in winter and less negative in summer (from -26.7‰ in March to -18.3‰ in September). Values measured in February-March (-25.28‰ and -26.67‰ respectively) were quite similar to those measured in the pelagic baseline in July-August (-25.72‰ and -27.30‰)

Such a clear seasonality further emphasizes the importance of applying dynamic models, when contribution of pelagic littoral vs. carbon sources for secondary and primary consumers are investigated.

With respect to seston, *Daphnia* enrichment in $\delta^{15}N$ was quite constant over the year (mean annual nitrogen signature of 5.7‰1 ± 0.74) and an average enrichment of 3.5 with respect to seston.

In winter and early spring, cyclopoid copepods and *Bythotrephes* were close to *Daphnia* and the pelagic carbon signature, occupying in the pelagia a trophic position fully comparable to that of planktivorous fish, and likely, both feeding on *Daphnia*. A slightly higher $\delta^{15}N$ signature of *Bythotrephes* with respect to *Cyclops* in March-May might also suggest that the former could also feed on Cyclopoid copepods. Large *C. abyssorum* gr. and small *Mesocyclops leuckartii* co-occur in Lake Maggiore during these months; the two species also differ in ability to escape predators; we hypothesize that *Bythotrephes* signature might reflect its ability to capturing the latter, while being the former, larger and faster swimmer, top predators on *Daphnia* during this period of the year. As did not discriminate between the two in preparing samples for SIA, however, we cannot discriminate between their isotopic signatures. Occurring all year round, *Bythotrephes* experience a relatively large range of variation in both carbon and nitrogen isotopic signatures, although less pronounced that in *Daphnia*. Carbon signature tends to be less negative and nitrogen tends to decrease during summer and autumn, reflecting, although in a lower range of variation, changes in pelagic baseline. When present (i.e. between May and November, *Leptodora* tends to overlap with *Bythotrephes* for carbon isotopic signature and for nitrogen until October, being in November lower in δ^{15}N, as to suggest that it may become a prey for large *Bythotrephes* in late autumn ($T_{Bythotrephes}$ = 3.4 and $T_{Leptodora}$ = 2.6).

An overview of seasonal changes in isotopic signatures of fish and of their putative food resources (preys) is provided in figure 5. Littoral-like carbon isotopic fingerprints of all three fish species are quite evident in March; here *Bythotrephes* basically plays in open waters the same trophic role as whitefish and shad. This predatory cladoceran is tightly related to lake pelagia all year round, and when fish rely upon pelagic food sources, it may act as intermediate between *Daphnia* (in November through *Leptodora*) and whitefish (and shad). Equally evident is a fully pelagic carbon signature of roach in August, and of a mixed contribution by littoral and pelagic sources in July.

In terms of trophic position (T), predatory zooplankton (*Bythotrephes* and *Leptodora* and Cyclopoids) tend to occupy levels of ca. 3 (mean annual values: $T_{Bythotrephes}$ = 3.08; $T_{Leptodora}$ = 2.79; $T_{Cyclopoids}$= 3.13), i.e. of secondary consumers, directly feeding on *Daphnia*. In March, however, for *Bythotrephes* T = 3.5, which suggests that it may feed on Cyclopoids (T = 3.07).

When dependent on pelagic food sources (i.e. from July to October), roach had T = 3 from August to October, while in July the T=4 is probably influenced from the previous littoral signature. Shad occupied level 3 from June to November, directly feeding on *Daphnia* from July to November (F < 0.8±1.1). In December*,* it might have exploited intermediate, pelagic, prey (T = 3.9), which we could not detect as we missed samples on zooplankton predators. Whitefish used *Daphnia* as carbon source (T = 3) in June and July. From August to December and in January, however, they belonged to an upper consumer's level (T = 4), probably feeding on predatory zooplankters, such as *Bythotrephes* and *Leptodora.* In August, whitefish δ^{13}C signature was indeed different from that of *Daphnia* (F > 0.8±1.1), intermediate between those of *Bythotrephes* and *Leptodora* suggesting a close link with these two zooplankton predators. During this month, the former is fully pelagic (with F < 0.8±1.1 with respect to *Daphnia*), while the latter carries non-pelagic carbon signature (F > 0.8±1.1 with respect to *Daphnia*); by feeding on both, whitefish incorporates a mixed carbon signature. In May, T levels ≥4 of these two fish were probably influenced from previous littoral signatures integrated in their muscle tissues. When related to littoral food sources (Fig. 3) all three fish species have T values≥ 4, suggesting that one (at T=4) or two (at T=5) intermediate food items were missed between primary consumers we used to trace littoral baseline and the fish.

4. DISCUSSION AND CONCLUDING REMARKS

Our study has contributed not only to a better knowledge of the food web dynamics in Lake Maggiore, but has shed light on ecological displacement amongst competitors (whitefish, shad and roach) common to many of the larger lakes, provided insight into pathways of exchange between the littoral and pelagic subsystems with potential importance in these larger lakes, and provided refinement for trophic models of organic biomagnification.

Methods

Isotopic signatures of different fish tissues integrate prey signatures over different time periods (Woodland *et al*., 2012). Fast turnover tissues (such as fat, blood and liver) are used when SIA are compared with analysis of gut content (Perga & Gerdeaux, 2005). By choosing to analyze fish dorsal muscular tissues (Mac Avoy *et al*., 2001) we were aware that the latter integrated signatures of prey over a *ca.* two months time span (Tieszen *et al*., 1983). The diet signature integration in muscular tissue also depends on the somatic growth period: when growth is faster (i.e. in spring-summer) fish muscles more readily integrate food isotopic signatures than in not-growth periods. On the other hand, we were also aware that dorsal muscle's isotopic signature has almost no carbon fractionation with respect to consumed food (de Niro & Epstein, 1978). By incorporating in our calculations taxa-specific growth rate coefficients (Heisslein *et al*., 1993; Volta & Jepsen, 2008, Volta & Giussani, 2010) and specimens of homogenous age and size class we could rule out variations consequent to fast-growing and slow-growing species (as defined by Heisslein et al., 1993) and age. By applying SIA to fish tissues we were able to detect, instead of "what the animal was eating" at the sampling date (detectable with the classic methodologies, e.g. gut content analysis), what was absorbed by the fish, i.e. of the only part useful for fish growth (Maruyama *et al*., 2001). By applying a linear Dynamic Baseline Mixing Model (Woodland *et al*., 2012) we were able to link temporal fluctuations in food preferences of the three most important planktivorous fish in Lake Maggiore with those of littoral and pelagic baselines.

Movement of fish and nutrients

The European whitefish (*Coregonus lavaretus*) and the shad (*Alosa agone*) are key fish species in the large, deep southern Alpine lakes (Volta *et al*., 2011). Due to their pelagic lifestyle, they have been often used as indicators to monitor the transfer of pollutants through the pelagic food webs (Broman *et al*., 1999, Bettinetti *et al*., 2006, Volta *et al*., 2009) and the impact of climate change (Jeppesen *et al*., in press). Our study demonstrated however, that the contribution of littoral sources to European whitefish diet is far from negligible, especially in winter and spring. During this time of the year, whitefish approach the littoral before spawning (Berg & Grimaldi, 1965; Perga & Gerdeaux, 2005) where they may feed on benthic food sources such as larvae of chironomids and amphipods (Berg & Grimaldi, 1965). Such interpretation is consistent with results obtained in similar environments (i.e. Lake Geneva, Perga & Gerdeaux, 2005), where benthic prey were consumed by whitefish between February and April. Similar results were obtained for shad, suggesting that the two strictly zooplanktivorous fish species (Berg & Grimaldi, 1966a; Volta, 2010) may incorporate carbon littoral signatures over relatively long time periods (4-months in shad and 3-months in whitefish) each year.

Yet these fish species were caught in the offshore through out the year. Are there other possible sources of littoral carbon signature? Between March and May, carbon isotopic signatures of offshore diaptomids were close to those of roach (F≤ 0.8±1.1; data not shown), and thus had a strong littoral signal. They could provide an early spring signal to fish. Other sources might include emergent benthos or surface insects.

Instead, a tight dependence of roach (*Rutilus rutilus*) on littoral food sources was detected during most part of the year, although the contribution of pelagic sources was far from being negligible during late summer- early autumn. Indeed we found a sudden shift from littoral to pelagic $\delta^{13}C$ signature suggesting a shift in roach feeding behaviour towards *Daphnia*. This is not surprising as roach are omnivorous and opportunist, able to modify the diet in relation to the food availability. For instance, Townsend *et al*. (1986) found that roach switched to copepods when the density of small cladocerans was lower than 40 ind L^{-1}. Between March and late May, roach approach littoral shallow areas to spawn (Volta & Jepsen, 2008), and in fact we found that carbon isotopic signature was highly related to that of the littoral in these months. Our results also suggest that the feeding niche of the roach does not overlap with that of the two other species. Indeed, whilst roach spawn in March-May, the spawning season of the shad is in June-July and that of whitefish is in winter. Accordingly they tend to approach the littoral in different times of the year thus feeding on different food sources.

Thus, within the lake there is a natural rhythm of movement of fish species between the inshore and offshore regions minimizing their competitive overlap and maintaining a transfer of nutrients (and pollutants) between the two systems.

Foodweb Anomoly

In the spring, *Bythotrephes longimanus* and cyclopoid $\delta^{15}N$ SI signatures were similar to those of the fish (Fig. 5A-C, F). Only in the summer did it appear that the zooplanktivorous fish could be consuming predatory zooplankton, namely, *B. longimanus* and *Leptodora kindti* .(Fig. 5 E). Although at this time (Visconti & Manca, 2011). The lack of predation allowed the population to develop and attain high enough abundances that it was not readily controlled by the planktivorous fish. Hence we still see large populations present in the fall (Visconti & Manca ibidem)

Biomagnification Estimation

Biomagnification (biomagnification factor, BMF) along food chains (or within food webs) are largely estimated from nitrogen stable isotope signatures (Broman *et al*., 1999; Fisk *et al*., 2001; Villa *et al.*, 2006). Relationships between pollutant concentration and trophic structure can also be analyzed using specific characteristics of SIA, such as, trophic level (T) (Fisk *et al*., 2001; Hoekstra *et al*., 2003), δ^{15}N signature (Broman *et al*., 1999; Kidd *et al.*, 1995), predator-prey N rates (Fisk *et al*., 2001; Hoekstra *et al*., 2003); and enrichment values, E (Borgå *et al*., 2004). All these analyses assume linear movement of pollutants up the food chain.

The European whitefish (*Coregonus lavaretus*) and the shad (*Alosa agone*) are key fish species in large, deep southern Alpine lakes (Volta *et al*., 2011) and due to their pelagic lifestyle, have often been used as indicators to monitor the transfer of pollutants through the pelagic food webs (Broman *et al*., 1999, Bettinetti *et al*., 2006, Volta *et al*., 2009). Our study results caution against using this approach blindly. Even these strictly zooplantivorous fish contained signals of littoral feeding at some times of the year. Weighted pollutant concentrations from both food webs would need to be incorporated into trophic bioaccumulation models to accurately capture bioaccumulation.

Bioconcentration and biomagnification of three different POPs (DDT, DDD, DDE) were estimated in Lake Maggiore whitefish in 1998 (Broman *et al*., 1999). Our novel contribution, highlighting and quantifying seasonal changes in both food sources and trophic position of fish and of their potential prey might be also useful for exploring relations and mechanisms responsible, for example, of the relationship between fish biomagnification potential (B, Broman *et al*., 1999) and concentration detected in the water column (Borgå *et al*., 2004; Borgå & Di Guardo, 2005).

Acknowledgements

The study was part of a project funded (to MM, who also partly supported contribution by PV) by the CIPAIS (International Commission for the Protection of Italian-Swiss Waters on Lake Maggiore; 2008-2013 Special Project on “Use of C and N stable isotopes analyses to quantify the role of non pelagic carbon sources for zooplankton production”). Financial support to AV was given by the “Emilia Stella” fellowship to young, post-graduate students and to AF by a post-graduate training program of Ente Autonomo Acque della Sardegna (ENAS). PV was also funded by the WISER Project. We are all indebted to I. Cerutti, CNR ISE, for his valuable help in fish sampling and laboratory work. We are extremely grateful to Dr. O. E. Johannsson for helping to revise the MS.

CHAPTER 5

CHAPTER 5

References:

Ambrosetti, W., Barbanti L., de Bernardi R. & Libera V. (1980) Alcune risposte limnologiche ad un evento meteorologico eccezionale: il nubifragio del 7 Agosto 1978 nel bacino del Lago Maggiore. *Atti 3° Congresso A.I.O.L.*, 389-406.

Ambrosetti, W. & Barbanti L. (1999). Deep water warming in lakes: An indicator of climatic change. *Journal of Limnology,* 58, 1–9.

Berg A. & Grimaldi E. (1965) Biologia delle due forme di Coregone (*Coregonus* sp.) del Lago Maggiore. *Memorie dell'Istituto Italiano di Idrobiologia*, 18, 25-196.

Berg A. & Grimaldi E. (1966a) Biologia dell'Agone (*Alosa ficta lacustris*) del lago Maggiore. *Memorie dell'Istituto Italiano di Idrobiologia*, 20, 41-83.

Berg A. & Grimaldi E. (1966b) Ecological relationships between planktophagic fish species in the Lago Maggiore. *Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 16, 1065-1073.

Bettinetti R., Galassi S., Guzzella L., Quadroni S. & Volta P. (2010) The role of zooplankton in DDT biomagnification in a pelagic food web of Lake Maggiore (Northern Italy). *Environmental Science and Pollution Research*, 17 (9), 1508-1518.

Bettinetti R., Quadroni S., Manca M., Pisciahttp://www.sciencedirect.com/science/article/pii/S0045653512003232 - aff3 R., Volta P., Guzzellahttp://www.sciencedirect.com/science/article/pii/S0045653512003232 - aff4 L., Rosciolid C. & Galassi S. (2012) Seasonal fluctuations of DDTs and PCBs in zooplankton and fish of Lake Maggiore (Northern Italy). *Chemosphere,* 88 (3), 344–351.

Borgå K., Fisk A. T., Hoekstra P. F. & Muir D. C. G. (2004) Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. *Environmental Toxicology and Chemistry*, 23 (10), 2367–2385.

Borgå K. & Di Guardo A. (2005) Comparing measured and predicted PCB concentrations in Arctic seawater and marine biota. *Science of The Total Environment*, 342 (1–3), 281–300.

Broman D., Di Guardo A. & Calamari D. (1999) Food chain accumulation of DDT and some of its metabolites in fish from Lake Maggiore (Italy) after an accidental relase-evaluated by the use of stable isotopes and other biotic parameters. Organohalogen Compounds,, 43, 45-48.

Cabana G. & Rasmussen J. B. (1996) Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences of the United States of America*, 93: 10844-10847.

Caroni R., Free G., Visconti A. & Manca M. (2012) Phytoplankton functional traits and seston stable isotopes signature: a functional-based approach in a deep, subalpine lake, Lake Maggiore (N. Italy) *Journal of Limnology*, 71(1), 84-94.

de Bernardi R. (1984) Methods for the estimation of zooplankton abundance. In: *A manual of methods for the assesmen of secondary productivity in fresh water* (Eds Downing J.A. & F.H. Rigler), pp. 59- 86 Blackwell Scientific Publications.

de Niro M.J. & Epstein S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta.* 42: 495-506.

Di Guardo A., Ferrari F. & Infantino A. (2006). Development of a dynamic aquatic model (DynA model): estimating temporal emissions of DDT to Lake Maggiore (N. Italy). Environtal Science & Pollution Research, 13: 50-58.

Fisk A. T., K. Hobson & R. J. Norstrom (2001) Influence of Chemical and Biological Factors on Trophic Transfer of Persistent Organic Pollutants in the Northwater Polynya Marine Food Web. *Environmenthal Science and Technology*, 35, 732-738.

Grey J. 2000. Trophic fractionation and the effects of diet switch on the carbon stable isotopic "signatures" of pelagic consumers. *Verh. Internat. Verein. Limnol*. 27:3187-3191.

Gu B., Schell D. M. & Alexander V. (1994) Stable Carbon and Nitrogen Isotopic Analysis of the Plankton Food web in a Subarctic Lake. *Canadian Journal of Fisheries Aquatic Sciences*, 51, Guilizzoni P., Marchetto A., Lami A., Gerli S. & Musazzi S. (2011) Use of sedimentary pigments to infer past phosphorus concentration in lakes. *Journal of Paleolimnology*, 45(4), 433-445.

Hesslein R. H., Capel M. J., Fox D. E. & Hallard K. A. (1991) Stable Isotopes of Sulfur, Carbon, and Nitrogen as Indicators of Trophic Level and Fish Migration in the Lower Mackenzie River Basin, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 48(11), 2258-2265.

Hesslein R. H., Hallard K. A., Ramlal P. (1993) Replacement of Sulfur, Carbon, and Nitrogen in Tissue of Growing Broad Whitefish (*Coregonus nasus*) in Response to a Change in Diet Traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. *Canadian Journal of Fisheries and Aquatic Sciences*, 1993, 50(10), 2071-2076.

Hoekstra P. F., O'Hara T. M., Fisk A. T., Borgå K., Solomon K. R. & Muir D. C. G. (2003) Trophic transfer of persistent organochlorine contaminants (OCs) within an Arctic marine food web from the southern Beaufort–Chukchi Seas. *Environmental Pollution*, 124, 509–522.

Jones R.I., Grey J., Sleep D., Quarmby C. (1998) An assessment, using stable isotopes, of the importance of allochtonous organic carbon sources to the pelagic food web in Loch Ness. *Proceedings of the Royal Society of London B Biological Sciences*, 265, 105-111.

Kidd K. A., Schindler D. W., Hesslein R. H. & Muir D. C. G. (1995) Correlation between stable nitrogen isotope ratios and concentrations of organochlorines in biota from a freshwater food web. Science of The Total Environment, 160/161, 381-390.

Lorenzen, C.J. (1967) Determination of chlorophyll and pheopigments: spectrophotometric equations. *Limnology and Oceanography*,12, 343-346.

Mac Avoy S. E., Macko S. A. & Garman G.C. (2001). Isotopic turnover in aquatic predators: quantifying the exploitation of migratory prey. *Canadian Journal of Fisheries and Aquatic Sciences,* 58 (5), 923-932.

Manca, M. & Ruggiu D. (1998) Consequences of pelagic food web changes during a long-term lake oligotrophication process. *Limnology and Oceanography*, 43, 1368-1373.

Manca, M. & Comoli P. (2000) Biomass estimates of freshwater zooplankton from length-carbon regression equations. Journal of Limnology, 59 (1), 15-18.

Manca, M. & DeMott W.R. (2009) Response of the invertebrate predator *Bythotrephes* to a climate-linked increase in the duration of a refuge from fish predation. *Limnology and Oceanography*, 6, 2506-2512.

Manca M. (2011) Invasions and re-emergences: an analysis of the success of *Bythotrephes* in Lago Maggiore (Italy). *Journal of Limnology*, 70(1), 76-82.

Maruyama A., Yamada Y., Rusuwa B. & Yuma M. (2001) Change in stable nitrogen isotope ratio in the muscle tissue of a migratory goby, Rhinogobius sp., in a natural setting. Canadian Journal of Fisheries and Aquatic Sciences, 58(11), 2125-2128.

Matthews B. & Mazumder A. (2003) Compositional and interlake variability of zooplankton affect baseline stable isotope signatures. *Limnology and Oceanography*, 48(5), 1977–1987.

McCauley E. (1984) The estimation of abundance and biomass of zooplankton in samples. In: *A manual on methods for the assessment of secondary productivity in fresh waters (Eds Dowing* J.A. & F.H. Rigler), pp. 228-265. Blackewell Scientific Publications.

Pangle, K.L, Peacor S.D. & Johannsson O.E. (2007) Large non lethal effects of an invasive invertebrate predator on zooplankton population growth rate, *Ecology*, 88, 402-412.

Perga M. E. & Gerdeaux D. (2005). ''Are fish what they eat'' all year round? *Oecologia*, 144, 598–606.

Phillips D. L. (2001) Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia*, 127,166–170.

Phillips D.L. & Gregg J. W. (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia*, 127,171–179.

Phillips D.L. & Koch P. Ll (2002) Incorporating concentration dependence in stable isotope mixing models. *Oecologia*, 130,114–125

Phillips D. L. &and Eldridge P. M. (2006) Estimating the timing of diet shifts using stable isotopes. *Oecologia*, 147, 195-203.

Post D. M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83(3), 703–718.

Tieszen L.L., T.W. Boutton, K.G. Tesdahl, N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for d13C analysis of diet. *Oecologia*. 57, 32–37.

Townsend C. R., Winfield I. J., Peirson G. & Cryer M. (1986) The Response of Young Roach Rutilus rutilus to Seasonal Changes in Abundance of Microcrustacean Prey: A Field Demonstration of Switching. *Oikos*. 46 (3), 372-378

Vander Zanden M. J. & Rasmussen J. B. (1999) Primary consumer δ13C and δ15N and the trophic position of aquatic consumers. Ecology, 80(4), 1395-1404.

Villa S., Bizzotto E. C. & Vighi M. (2011) Persistent organic pollutant in a fish community of a sub-alpine lake. *Environmental Pollution*, 159(4), 932–939.

Visconti A., Manca M. & de Bernardi R. (2008) Eutrophication-like response to climate warming: an analysis of Lago Maggiore (N. Italy) zooplankton in contrasting years. *Journal of Limnology,* 67 (2), 87-92.

Visconti A. & Manca M. (2011) Seasonal changes in the δ^{13}C and δ^{15}N signatures of the Lago Maggiore pelagic food web. *Journal of Limnology*, 70(2), 263-271.

Volta, 2010. Diet of the shad (Alosa falla lacustris) in Lake Maggiore (in Italian). Studi Trentini di Scienze naturali. Acta Biologica.

Volta P. & Jepsen N. (2008) The recent invasion of *Rutilus rutilus* (L.) (Pisces: Cyprinidae) in a large South Alpine lake: Lago Maggiore. *Journal of Limnology*, 67, 163-170.

Volta, P., P. Tremolada, M. C. Neri, G. Giussani & S. Galassi, 2009. Age-dependent bioaccumulation of organochlorine compounds in fish and their selective biotransformation in top predators from Lake Maggiore (Italy). Water Air & Soil Pollution 197: 193–209

Volta, P. & Giussani G. (2010) Weight–length relationships (WLRs) and von Bertalanffy growth function (VBGF) parameters of *Alosa agone*, *Rutilus pigus* and the anadromous *Salmo trutta* from a deep south-alpine lake: Lago Maggiore (Northern Italy). *Journal of Applied Ichthyology*, 26, 474–476.

Volta P., Oggioni A., Bettinetti R & E. Jeppesen. 2011. Assessing lake typologies and indicator fish species for Italian natural lakes using past fish richness and assemblages. Hydrobiologia 671: 227-240

Wada E. (2009) Stable δ15N and δ13C isotope ratios in aquatic ecosystems. *Proceedings of Japan Academy* (B) 85: 98-107.

Woodland R. J., Rodríguez M.A., Magnan P., Glémet H. & Cabana G. (2012) Incorporating temporally dynamic baselines in isotopic mixing models. *Ecology*, 93, 131–144.

Zaret T.M. (1980) Predation and freshwater communities. New Haven: Yale University Press: 187pp.

CHAPTER 5

Captions of figures

Fig. 1. Lake Maggiore bathymetric map showing littoral and pelagic sampling stations (for explanation of symbols see text).

Fig. 2. Seasonal changes in fish carbon isotopic signatures and in pelagic and littoral baselines in Lake Maggiore, 2008.

Fig. 3. Seasonal changes in contribution of pelagic (black) and littoral (grey) food sources to fish carbon isotopic signature. For further explanation see text. .

Fig. 4. Carbon and nitrogen isotopic signatures of (A) fish; (B) zooplankton, littoral and pelagic stable isotopes baselines (values integrated over a two-month time interval; letters refer to the initials of months) measured monthly in 2008.

Fig. 5. Carbon and nitrogen stable isotope signatures measured in fish and zooplankton species in littoral and pelagic baselines during different months, representative of the most relevant seasonal changes. Letters refers to organism names, seston (ses) and littoral isotopic baselines (lit).

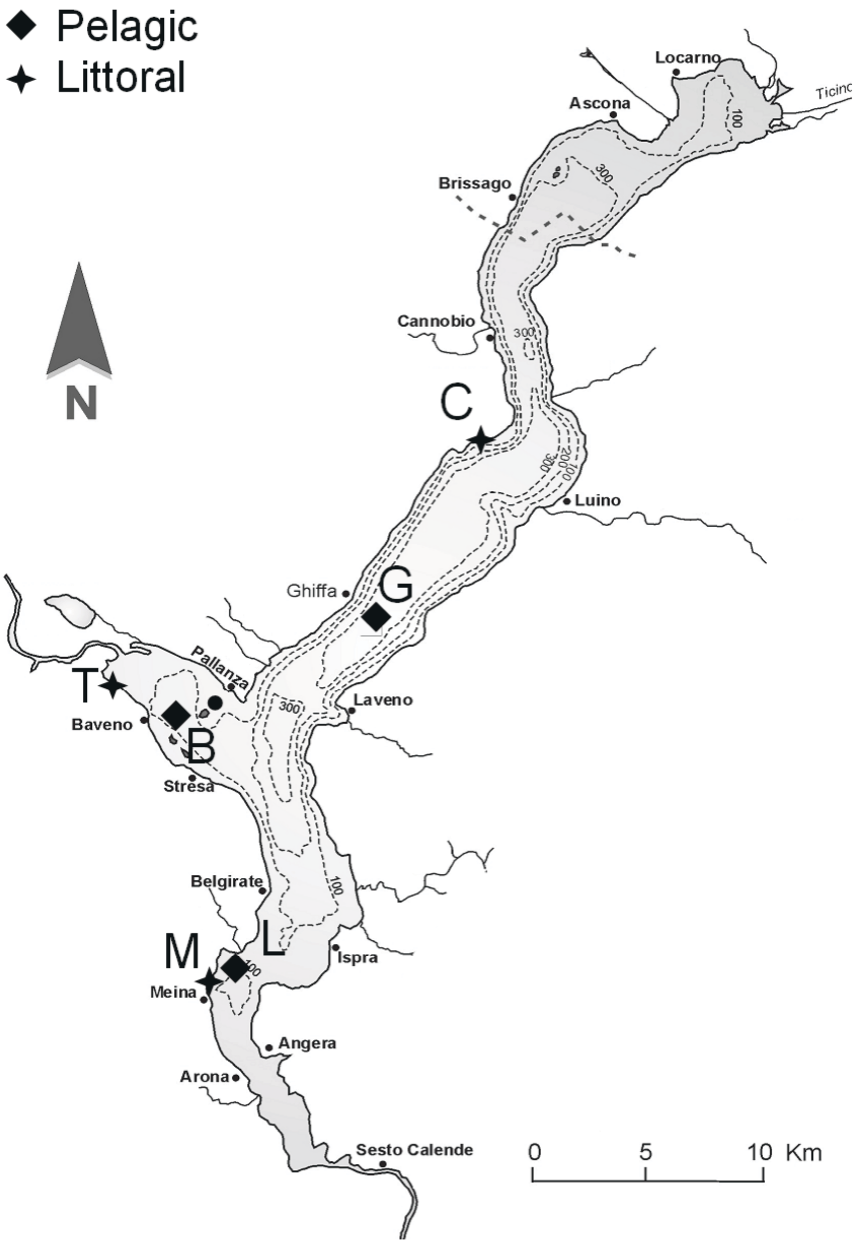

◆ Pelagic
✦ Littoral
N
Locarno
Ascona
Ticin
Brissago
100
300
Cannobio
300
C
300
200
100
Luino
Ghiffa
G
Pallanza
T
Baveno
B
Stresa
300
Laveno
Belgirate
100
M
L
Ispra
Meina
100
Angera
Arona
Sesto Calende
0
5
10 Km

Fig. 1.

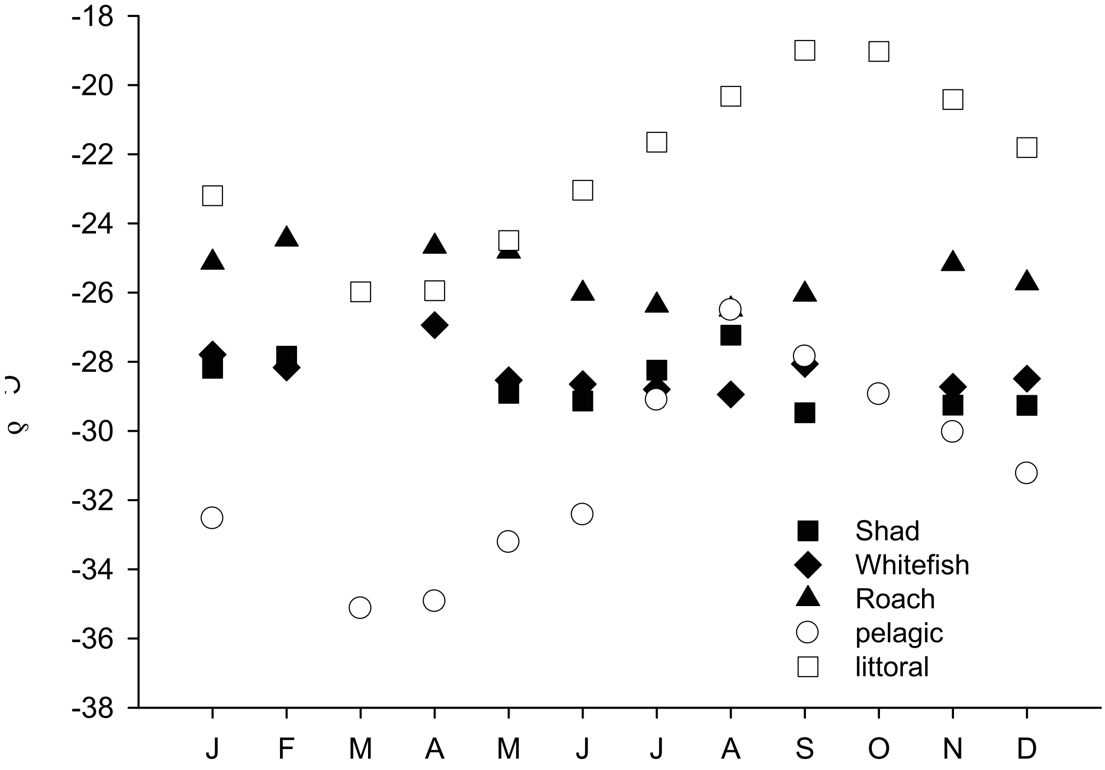

-18
-20
-22
-24
-26
-28
-30
-32
-34
-36
-38
δ C
J
F
M
A
M
J
J
A
S
O
N
D
Shad
Whitefish
Roach
pelagic
littoral

Fig. 2.

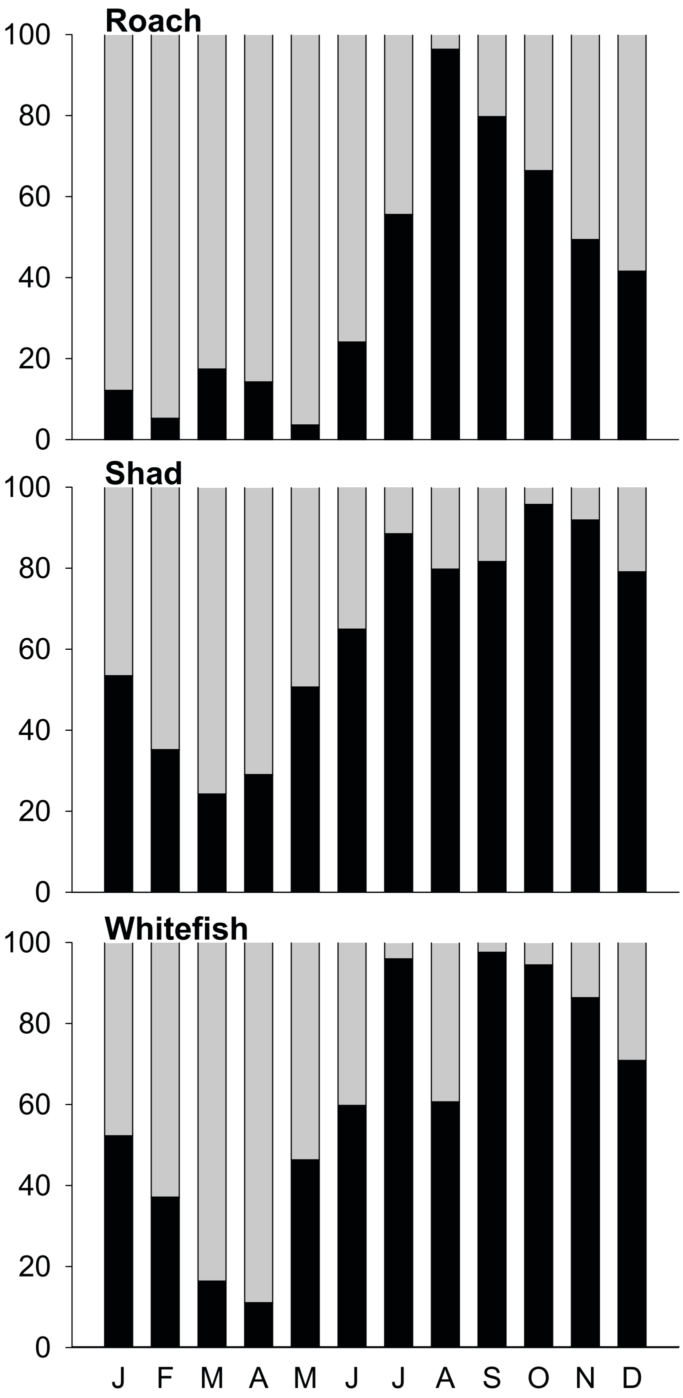

Roach
100
80
60
40
20
0
Shad
100
80
60
40
20
0
Whitefish
100
80
60
40
20
0
J
F
M
A
M
J
J
A
S
O
N
D

Fig. 3.

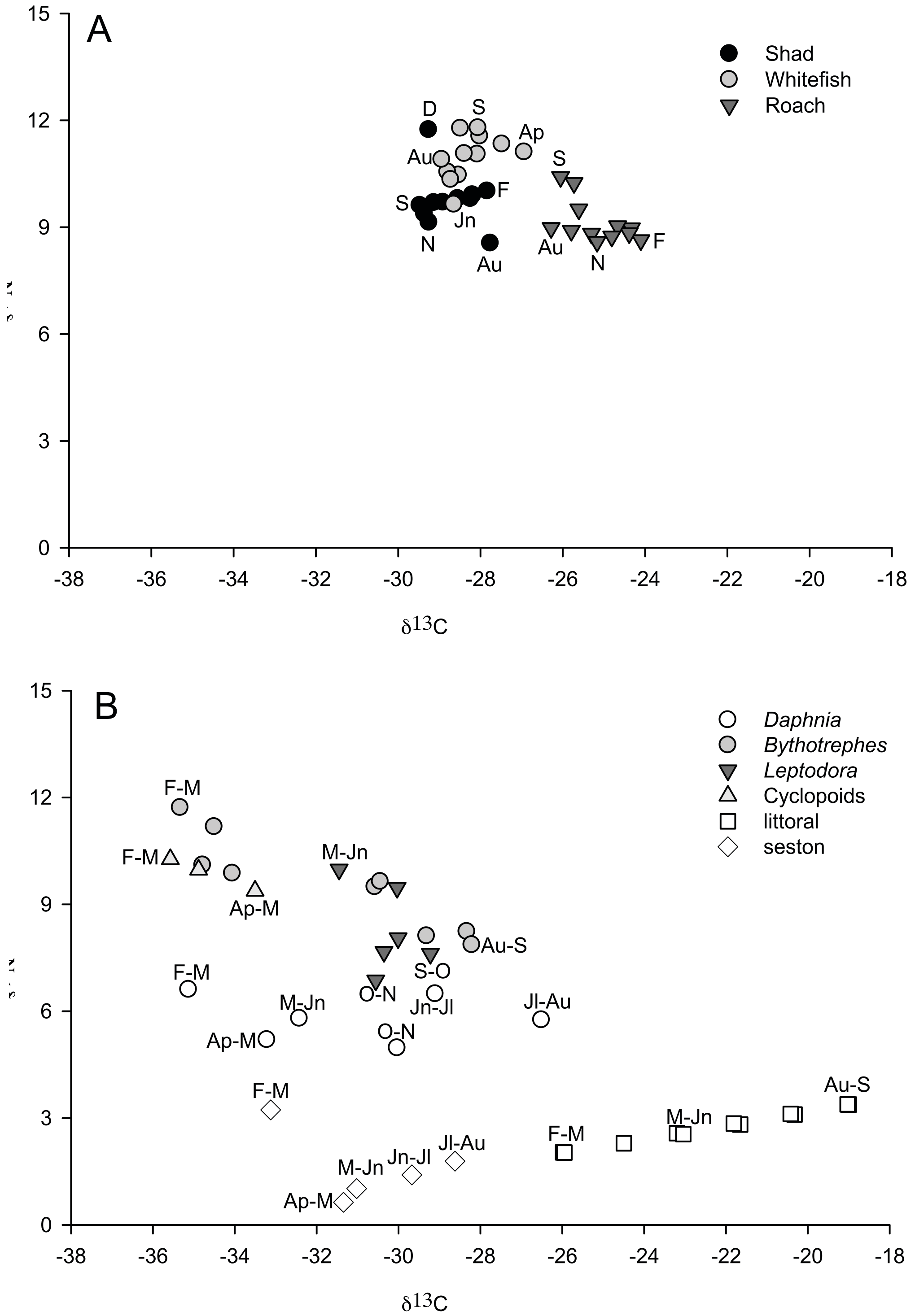
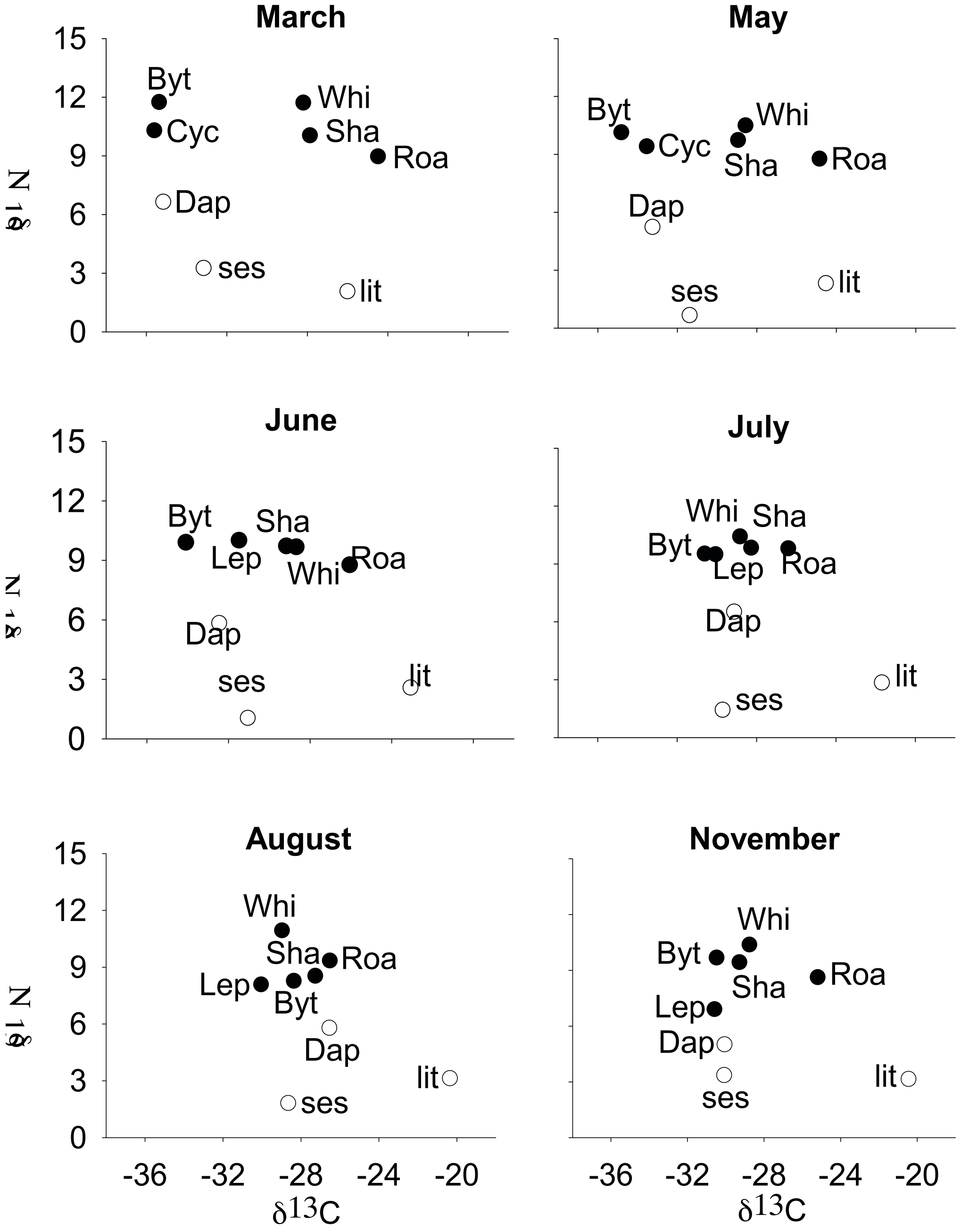

A
Shad
Whitefish
Roach
D
S
Ap
Au
S
F
S
Jn
N
Au
Au
N
F
15
12
9
6
3
0
-38
-36
-34
-32
-30
-28
-26
-24
-22
-20
-18
δ13C
B
Daphnia
Bythotrephes
Leptodora
Cyclopoids
littoral
seston
F-M
F-M
M-Jn
Ap-M
Au-S
S-O
F-M
O-N
Jn-Jl
M-Jn
Jl-Au
Ap-M
O-N
F-M
Au-S
M-Jn
F-M
Jl-Au
Jn-Jl
M-Jn
Ap-M
δ13C

Fig. 4.



March

May

June

July

August

November

Fig.5.

CONCLUSION

The present PhD thesis is a first attempt to study food webs of Sardinian perennial freshwater lakes through Carbon and Nitrogen Stable Isotope Analysis. In Sardinia, permanent freshwater bodies are reservoirs, built to answer the increased water demand during the last century. Reservoirs are peculiar man-made ecosystems, in-between rivers and lakes, strongly affected by human management activities as well as by climate interannual variability.

Reservoirs devoted to human-supply (i.e. drinkable water supply and agriculture) in semi-arid countries, like those in the Mediterranean Region, are heavily affected by year water level fluctuations.

Almost all Sardinian reservoirs are affected by eutrophication: while studies aimed at quantifying trophic level, primary productivity and hydrochemistry have been carried out since the late-1970s information on zooplankton and fish is very scanty.

Indeed, the first step of this study was to investigate crustacean planktonic communities of a large part of Sardinian reservoirs. Previous zooplankton surveys have mainly focused on temporary ponds, from which a number of rare and endemic taxa were recorded (Stella, 1970, Margaritora, 1983). In a first attempt to investigate the crustacean zooplankton communities, 15 reservoirs from different regions of the island were analyzed. Reservoirs selected for this analyses covered a wide range of morphometrical, hydrological, hydrochemical and productivity characteristics.

Results highlighted that lake trophy, water depth, renewal time and altitude explained a significant proportion of the total variance observed in taxa composition and community structure. Overall, 21 crustacean zooplankton taxa were identified,thirteen belonging to the Cladocera an eight to the Copepoda. In particular, the ubiquitous presence of a Mediterranean endemic calanoid was observed as well as the presence of an invasive hybrid *Daphnia pulex* clone . Phylogenetic analysis of ND5 gene sequence unequivocally placed the putative *D. pulex* isolated from Lake Sos Canales, within the American *D. pulex* clade. Moreover, the haplotype carried by Sardinia isolates was identical to the homologous part of haplotype carried by an asexual clone widely distributed throughout eastern and southern Africa (Mergeay, 2006). Among the investigated lakes, Sos Canales Reservoir (SCR) resulted the most diverse and rich in zooplankton taxa. Analyses of resting stages in surface sediments of this lake, carried out in 2009, highlighted that the largest amount of viable resting eggs collected was of small-sized cladocerans. Unexpectedly, resting eggs of *C. numidicus*, were not found. We interpreted such result as an adaptive strategy of the species perennial water bodies, being the production of resting eggs by this species well-known in temporary pond populations. The peculiar traits above mentioned of SCR, along with a little watershed in a quite pristine area, and availability of long-term limnological data stimulated interest to investigate potential sources supporting primary and secondary production, and patterns and trophic links through which matter an energy is transferred along the pelagic trophic web. To meet these goals, C and N SIA was applied.

Samples were collected from October 2010 till September 2011. Lake Sos Canales, as all reservoirs devoted to human supply, is subject to larger water level variation than e.g. reservoirs devoted to hydropower supply.

Water level fluctuations (WLFs) are among the most important stressors in lentic environments (Leira and Cantonati, 2012 and references therein). They have the greatest impact on shallow lakes and on the littoral zone, by means of top-down and bottom-up mechanisms (Zohary et al., 2011). WLFs are also related to loss of biodiversity and degradation of littoral community as well as an increase in prevalence of alien species (Zohary et al., 2011).

External inputs from watershed are relevant during the wet season, or “filling phase”; while internal recycling processes support biological communities during the drought season (Zoary et al., 2011). The emergence and dry-out of lake shoreline for a long time period, prevents growth of a well structured macrophyte community; indeed, in this man-made environments, autotrophic primary producers are generally phytoplankton.

Pelagic food sources available for primary consumers are composed both by autochthonous (auto- and hetero-trophic organisms) and allochthonous (i.e. terrestrial detritus) suspended particulate matter (SPM). Carbon and nitrogen stable isotope signatures allowed for investigating contribution of sources of different origin to support pelagic food web of SCR. SPM was collected from different stations and depths and along the seasons (ca. every month throughout the year). On each sampling date water chemistry and physical variables were measured. Phytoplankton samples were also analyzed. Water Level (WL) markedly varied from fall (27 m in October 2010) to late winter months (37 m in February 2011). SPM carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures were quite comparable between stations (i.e. riverine-shallow (RIV) and deep station (DEEP) during the low WL. This result suggested a homogeneous composition of SPM in SCR during this phase.

Since February, when WL rapidly increased, SPM C and N isotopic signatures differed between surface (0-7.5 m depth; SUR) and deep water (below 10 m depth; DEEP and from SPM of RIV (ca. 8 m). The new water inputs from watershed upward hypolimnetic water, recorded with more depleted $\delta^{13}C$ signature in SPM in SUR. Since February to June, $\delta^{13}C$ in SUR were more depleted than in DEEP and RIV-SPM. Commonly, depleted $\delta^{13}C$ were reported for carbon dioxide from respiration activity uptake by organisms living in deep waters. When water temperatures increased, reaching their maximum in July (20°C), $\delta^{13}C$ tended to be more enriched. $\delta^{13}C$ signature is related to temperature, influencing phytoplankton activity, as well known in temperate lakes (Caroni et al. 2012; Perga and Gerdeax, 2006). SPM $\delta^{13}C$ mostly composed by phytoplankton are influenced by species composition and their nutrient uptake activity. Phytoplankton community in SCR was dominated, in terms of biomass, by mixotrophic dinoflagellates throughout the year, in particular by Gymnodinium uberrimum. SPM concentration (µg d.w./L) on SUR was correlated to phytoplankton biomass as well as to chlorophyll-a concentration, suggesting that G. uberrimum constituted a large amount of SPM in SUR. Thus, this peculiar distribution of $\delta^{13}C$ SPM signatures along the water column was mainly related to presence of dinoflagellates and their carbon uptake behavior.

Phytoplankton also influences $\delta^{15}N$; a relationship between nitric-nitrogen and dinoflagellate biomass also being observed in surface waters. SPM $\delta^{15}N$ seasonally changed. It was enriched and quite similar between stations in fall; since February till June constantly decreased, and increased again since July to September, mainly in DEEP. Phytoplankton composition also influences nitric-nitrogen concentration and consequently, $\delta^{15}N$ signature. The distribution of *G. uberrimum* in the water column is led by irradiance; hence, nitric-nitrogen distribution can be influenced by its metabolic activity (Tilzer, 1973; Cullen and Horrigan, 1981). In SCR, nitric-nitrogen concentration along the water column tended to decrease during the hottest month in SUR, while being rather similar throughout the season in DEEP. Accordingly, during cool months dinoflagellates were mainly close to the surface, while settled down at 5 m during summer, when depth of the photic-zone increased. Whereas SUR $\delta^{13}C$ and $\delta^{15}N$ was strong influenced by phytoplankton activity, $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures recorded in DEEP and RIV SPM were mainly affected by annual hydrological dynamics of SCR.

In lacustrine systems, $\delta^{13}C$ and $\delta^{15}N$ signatures vary over time (Zohary et al. 1994; Lehman et al., 2004), and this variability is related to external loading, phytoplankton species composition, primary productivity, as well as sources and concentration of dissolved inorganic C and N (Caroni et al., 2012; Grey et a., 2001). In SCR, SPM showed a visible annual cycle of $\delta^{13}C$ and $\delta^{15}N$ signatures. During the "still-water" phase, in fall months, depleted $\delta^{13}C$ and enriched $\delta^{15}N$ were recorded. During the "dynamic" phase, since February till May $\delta^{13}C$ tended to be more depleted, and $\delta^{15}N$ more enriched than in the pother months. In hotter months, mainly in July, $\delta^{13}C$ increased and $\delta^{15}N$ decreased in SUR, while DEEP being closer to June. At the end of summer, in September, values approached those measured in fall the previous year.

To estimate seasonal changes in trophic behavior and sources supporting zooplakotn secondary production, SPM signature was used as baseline.

SIA was therefore performed on cladocerans (*Ceriodaphnia* spp. and *Daphnia* spp.; calanoid (*C. numidicus*) and cyclopoid copepods (*C. abyssorum* gr. and *M. albidus*). Seasonality in feeding behavior could be identified by means of changes in isotopic signatures. They were attributed to changes in sources as well as in developmental stages and body size of individuals contributing to the populations in different stations and periods of the year.

During fall, spatial homogeneity of SPM $\delta^{13}C$ and $\delta^{15}N$ was also detected in zooplankton taxa, suggesting that they were exploting available pelagic sources. *Ceriodaphnia* and *Daphnia* were the primary consumers, while being copepods on a higher trophic position, likely exploting cladocerans. Whereas for large cyclopoids species, such as *C. abyssorum* gr. and *M. albidus*, the predation on cladocerans was well documented (Dussart and DeFaye, 2001; Fernando, 1998), little is known on the feeding behavior of *C. numidicus*. Calanoids are taste-selective feeders, omnivory is also reported for different species, among which *C. numidicus* (namely, in a Portuguese estuary; Gonçalves et al., 2012).

In February, water level rapidly increased by ca 10 m. Copepods were more enriched in δ^{15}N, suggesting an increase in body lipid content (El-Sabaawi et al. 2009). Storage lipids provide energy for reproduction, periods of low food supply, escaping predation and for vertical and horizontal migrations (Lee et al., 2006). In heterogeneous or temporary habitats many copepods have evolved adaptive mechanisms that guarantee synchronization in time and space between growth, reproduction and favorable environmental conditions (Santer, 1998). Whereas in cyclopoids dormant copepodites and dormant and diapausing adults are known, freshwater calanoids mainly produced resting or diapausing eggs. In SCR, C. numidicus resting eggs were not found in surface sediments (cfr. Chapter 1). In February, *C. numidicus* population was mainly composed by adults and copepodites, thus corroborating that specimens analyzed for SIA were likely in diapause.

During diapause, copepods may migrate in deep cool water layers (Vanderploeg, 1998), thus the upward of hypolimnetic waters may have transported diapaused copopepods which carried isotopic signatures of bottom sediments.

This interpretation was also supported by isotopic signature of oligochaetes collected from lake sediments (used as a bottom-deep sediment proxy in February).

From March till July analysis of zooplankton and SPM isotopic signatures at different stations and depths suggested the possibility of Diel Horizontal Migration (DHM), in response to zooplanktivorous fish. Zooplankton is not totally at the mercy of the water currents and is known to migrate vertically and horizontally. Diel horizontal migration has been observed in shallow lakes, where zooplankton settle down near to the littoral zone during the day and migrate into pelagic open waters during the night (Wicklum 1999, Masson et al, 2001; Burks et al., 2002 and Romare and Hansson 2003). We may hypothesize that a similar DHM was responsible for having cladocerans, collected in shallow-station from March to May δ13C and δ15N isotopic signatures close to SPM of the deep station. In July, δ13C and δ15N signatures recorded on open-water zooplankon were closer to isotopic signatures measured in the littoral (on shredder decapods: *Atyaephyra desmaresti*, used as a littoral stable isotope baseline). In Sos Canales a voracious zooplanktivourous fish, the mosquitofish lives. This fish is known to be able to strongly affect species and size of zooplankton communities (George et al. 2008 and references therein). In the cool season, mosquitofish usually reduce their populations (Fernandez-Delgado et al., 1989; 1997; Hughes, 1985; Haynes, 1993). During this period, of low fish predation, cladocerans migrated in deep waters for feeding, and part of them moved on shallow-riverine areas during the day. The increase of temperature drove suitable conditions for mosquitofish in the littoral; zooplankters might have migrated to the littoral during the night for feeding, and moved into open water during day.

Two fish species were introduced in SCR, a North-Atlantic brown trout (*Salmo trutta*) and the mosquitofish (*Gambusia holbrooki*). To study their feeding behavior SPM and zooplankton were collected in open water (pelagic) and macroinvertebrates along the reservoir shoreline (littoral). Trophic position of fish and the proportion of exploited sources (pelagic vs littoral) were calculated through a Dynamic Baseline Mixing Model (DBMM) recently proposed by Woodland et al. (2012). Analysis of gut content was also applied. While gut content analysis provides an estimate of the last meals, $\delta^{13}C$ and $\delta^{15}N$ signatures integrated food uptake to detect assimilated carbon and nitrogen sources. Fish populations were constituted by few adults of brown trout, likely affected by reproductive trail, and mosquitofish with a littoral abundance of 46 ind. m^{-2} in fall. Analysis of gut contents recorder a prevalence of *A. desmaresti* and aquatic insects in brown trout. As expected, zooplankton organisms were the only prey of mosquitofish. Isotopic baseline of littoral and pelagic zones followed a seasonal trend, with depleted $\delta^{13}C$ in cool month (February-March) that constantly increased in summer (June-July), while $\delta^{15}N$ had an inverse trend. DBMM results corroborated that brown trout was a littoral feeder, while mosquitofish changed feeding zone seasonally. Mosquitofish since winter to summer fed in the littoral, while mainly feeding on pelagic food sources in fall. Mosquitofish is a voracious predator and an invasive species introduced in Europe to control mosquito larvae. One fertilized female is able to replace a new mosquitofish population with little or no damaging founder effect (Chesser et al., 1984; Allemadi and Jekins, 2008). Results of the present study highlighted predation pressure on pelagic zooplankton restricted to fall, when a shift from littoral to pelagic sources can result from favorable hydrodynamic conditions. Mosquitofish mainly inhabit slow flow or “standing-waters” and are able to survive in a wide range of environmental conditions (Peterson and Peterson, 1990; Rupp, 1997). At the end of summer e water inputs were negligible and the little differences in SPM $\delta^{13}C$ and $\delta^{15}N$ of deep vs. shallow stations, highlighted homogenization of the system. The prolongation of this hydrodynamic condition, as well as the lack of effective predators might enhance exponential development of mosquitofish population, in turn able to affect planktonic crustacean diversity of SCR. Spreading ability of non indigenous species (NIS) like mosquitofish, are improved in stressed environments such as the reservoirs.

Investigation on one recently invader non indigenous species, roach (*Rutilus rutilus*), and two naturalized fish species, the whitefish (*Coregonus lavaretus*) and the shad (*Alosa agone*), through the carbon and nitrogen SIA, allowed to recorded a seasonality on their isotopic signatures as well as on their potential prey in Lake Maggiore. The analysis was performed applying the recently development of linear Dynamic Baseline Mixing Model (DBMM), as was applied on man-made Lake Sos Canales. Carbon and nitrogen of primary and secondary zooplanktonic consumer allowed to see change in their trophic position throughout the year. Thus, predatory zooplankters, in particular *Bithotrephes longimanus*, seem to occupy the same trophic position of fish in pelagic food web in winter, when the latter were exploiting littoral food sources. By applying a DBMM (Woodland et al., 2012) we were able to link temporal fluctuations in food preferences of the three most important planktivorous fish in Lake Maggiore with those of littoral and pelagic baseline. The two naturalized species (whitefish and shad) highlighted a prevalence of planktonic feeding behaviors for almost of the year. On latter, between February and April, carbon isotopic signature became closer to that recorded on littoral area before spawning time. Instead, roach was tight dependence on littoral food sources was detected during most part of the year, although the contribution of pelagic sources was far from being negligible during the late summer-early autumn. Results suggested that the feeding niche of the roach does not overlap with that of the two other species. Indeed, whilst roach spawn in March-May, the spawning season of the shad is in June-July and that of whitefish in winter. Thus , within the lake there is a natural rhythm of movement of fish species between the inshore and offshore regions minimizing their competitive overlap and maintaining a transfer of nutrients (and pollutants) between the systems. Furthermore, along the food chains the biomagnifications was largely estimated from nitrogen stable isotope signatures (Broman et al., 1999; Fisk, Hobson and Norstrom, 2001; villa, Bizotto and Vighi, 2011). In Lake Maggiore whitefish the bioconcentration and biomagnifications were estimated for three different POPs (DDT, DDD, DDE) in 1998 (Broman et al. 1999). Thus, the contribution of this, highlighting and quantifying seasonal changes in both food sources and trophic position of fish and of their potential prey might be also useful for exploring relations and mechanisms responsible on the relationship between fish biomagnifications potential (Broman et al., 1999) and concentration detected in the water column (Borga et al., 2004; Borga and Di Guardo, 2005). Finally, this study has contribute not only to a better knowledge of the food web dynamics in Lake Maggiore, but a has shed light on ecological displacement amongst displacement amongst competitors (Whitefish, shad and roach) common to many of the subalpine larger lakes, provides insight into pathways of exchange between the littoral and pelagic subsystems with potential importance in these larger lakes, and provided refinement for trophic models of organic biomagnifications.

REFERENCES

Cattaneo A., M. Manca and J.B. Rasmussen. 2004. Peculiarities in the stable isotope composition of organisms from an alpine lake. Aquat. Sci., 66: 440–445.

E. Stella, Calanoidi (Crustacea, Copepoda, Calanoida). Guide per il riconoscimento delle specie animali delle acque interne italiane. Edizioni Calederini, Bologna, 1982.

E. Stella, I Diaptomidi della Sardegna, Rend. Ist. Lombardo Sc. e Lett., 104 (1970), pp. 69-87.

F. G. Margaritora, Cladoceri (Crustacea:Cladocera). Guide per il riconoscimento delle specie animali delle acque interne italiane, Officine Grafiche Calderini, Bologna, 1983.

Fadda A., S. Marková, P. Kotlík, A. Lugliè, B.Padedda, P. Buscarinu, Ni. Sechi and M. Manca. First record of planktonic crustaceans in Sardinian reservoirs. Biologia 66(5): 856-865. 2011.

Fadda A., S. Marková, P. Kotlík, A. Lugliè, B.Padedda, P. Buscarinu, Ni. Sechi and M. Manca. First record of planktonic crustaceans in Sardinian reservoirs. Biologia 66(5): 856-865.

Finlay and Kendall, 2008. In “ Stable Isotopes in Ecology and Environmental Science, second edition,. Finlay J.C. and Kendall C.- Stable Isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems, pp. 283-324.

Finlay and Kendall, In “ Stable Isotopes in Ecology and Environmental Science, second edition,. Finlay J.C. and Kendall C.- Stable Isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems, pp. 283-324, 2008.

France R.L. (1995) Carbon-13 enrichment in benthic compared to pelagic algae: food web implications. Marine Ecology Progress Series, 124, 307–312.

G. Cabana and J.B. Rasmussen, Comparision of aquatic food chains using nitrogen isotopes. Proceeding of the National Academy of Sciences of the United States of America, 93: 10844-10847, 1996.

Grey J. Trophic fractionation and the effects of diet switch on the carbon stable isotopic “signatures” of pelagic consumer. Verh. Internat.Verein. Limnolol. 27: 3187-3191, 2000.

Grey J., R.I. Jones, D. Sleep. Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state. Oecologia, 123: 232–240, 2000.

Gu B., A.D. Chapman, C.L. Schelske. Factors controlling seasonal variations in stable isotope composition of particulate organic matter in a soft water eutrophic lake. Limnol. Oceanogr., 51(6): 2837-2848, 2006.

Gu B., D.M. Schell and V. Alexander. Stable Carbon and Nitrogen Isotopic Analysis of the Plankton Food web in a Subarctic Lake. Can. J. Fish. Aquat., 51: 1338-1344, 1994.

J. Armengol, Colonizaciòn de los embalses espanoles por crustàceos planctònicos y evoluciòn de la estructura de sus comunidades, Oecologia acquatica, 4 (1980), pp. 45-70.

J. G. Tundisi, O. Rocha, T. Matsumura-Tundisi and B. Braga, Reservoir management in South America, Water Resources Development, 14-2 (1998), pp. 141-155.

L. Cioglia, M. Cottiglia, M. Tagliasacchi-Masala, B. Rotta and A. Atzeni, Osservazioni su un lago artificiale a usi multipli della Sardegna meridionale. Il lago di Monti Pranu. Rend. Sem. Fac. Scienze Univ. Cagliari, 39(1969) pp. 1-32.

M. Cherbi, Contribution à l'etude du peuplement zooplanctonique de trios lacs de barrage: Hamiz, Boughzoul et Ghrib. Thèse de Doctorat, Universitè d'Alger, pp. 216, 1984.

M.J. de Niro and S. Epstein. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimca Acta. 42: 495-506, 1978.

Marchetto, B.M Padedda, M. Mariani, A. Lugliè, N. Sechi, A numerical index for evaluating phytoplankton response to changes in nutrient level deep mediterranean reservoirs, J.Limnol., 68-1 , pp. 106-121, 2009.

Massidda, P.; Conti, G.; Loddo, A.; Cau, A., 2008: Pesci dacqua dolce della Sardegna. Aı`sara, Cagliari, Italia, pp. 93.,

Minagawa M. & Wada E. (1984) Stepwise enrichment of 15N along food chains: Further evidence and the relation between δ15N and animal age. Geochimica et Cosmochimica Acta, 48, 1135-1140

Mulholland P.J., Marzolf E.R., Webster J.R., Hart D.R. & Hendricks S.P. (1997) Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams. Limnology and Oceanography, 42,443-451.

OECD (1982) Eutrophication of waters. Monitoring, assessment and control. Organization for Economic Co-operation and Development. Paris, France

Orrù F., Deiana A.M., Cau A., Introduction and distribution of alien freshwater fishes on the island of Sardinia (Italy): assessment on the basis of existing data sources. J. App. Ichthyol. 26 (2): 46-52, 2010.

Perga, M.E. & D. Gerdeaux. Seasonal variability in the δ13C and δ15N values of the zooplankton taxa in two alpine lakes. Acta Oecologica, 30: 69-77, 2006.

Peterson B. J. & Fry B. (1987) Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics, 18, 293-320

Post. D. M. Using stable isotopes to estimate trophic position: models, methods, and assumption. Ecology 83: 703-718, 2000.

Sabatini, A.; Orru` , F.; Cannas, R.; Serra, P.; Cau, A., 2006: Conservation and management of Salmo (trutta) macrostigma. in Sardinian freshwaters: first results of genetic characterization. J. Freshw. Biol. (Quaderni ETP) 34, 335–340.

Sechi N. and A. Lugliè, Limnlogical studies on man-made lakes in Sardinia (Italy). Mem. Ist. Ital. Idrobiol., 50, pp. 365-381, 1992.

Sechi N. and A. Cossu, Prime valutazioni sui livelli trofici di alcuni bacini artificiali della Sardegna, Mem. Ist. Ital. Idrobiol., 37, pp. 259-276, 1979.

Sechi N. and A. Lugliè, Limnlogical studies on man-made lakes in Sardinia (Italy). Mem. Ist. Ital. Idrobiol., 50: 365-381, 1992.

Sechi N., and A. Luglié, Phytoplankton in Sardinian reservoirs.Giornale Botanico Italiano, 130, pp. 977-994, 1996.

Straškraba, M., Tundisi, J. G. (1999) Reservoir water quality management. Guidelines of lake management, volume 9. Ed. International Lake Environment Committee, Kusatsu. 1999.

Straskraba, M., Tundisi, J.G., Duncan, A. (1993) Comparative Reservoir Limnology and Water Quality Management. Developments in Hydrobiology. Volume DH 77, Kluwer Academic Publishers, Boston.

Sulzman E.. In “ Stable Isotopes in Ecology and Environmental Science, second edition,. Finlay J.C. and Kendall C.- Stable Isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems, pp. 283-324. , 2008.

Zoary T. and Ostrovsky I., Ecologica impacts of excessive water level fluctuations in stratified freshwater lakes. Inland Water 1,: 47-59, 2011.

Zoary T., Erez J., M. Gophen, I. Berman-Frank, M. Stiller. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret,. Limnology and Oceanography, 39: 1030-1043, 1994.

Thornton K. W., Kimmel B.L., Payne F.E. (1990) Reservoir limnology: Ecological perspectives. John Wiley & Sons, Inc., Somerset, NewJersey.

THORNTON, K. W., B. L. KIMMEL, AND F. E. PAYNE [EDS.]. 1990. Reservoir limnology: Ecological per- spectives. John Wiley & Sons, Inc., Somerset, New Jersey. 246 p. $49.95. ISBN 0-471-88501-O.

Tundisi G. and Matzumura-Tundisi J., Integration of research and management in optimizing multiple uses of reservoirs: the experience in South America and Brazilian case studies. Hydrobiologia. Vol. 500, no. 1-3, pp. 231-242, 2003.

Tundisi J. G. et al. Theoretical basis for reservoir management .In Tundisi J.G and Straskraba M, Theoretical Reservoir Ecology and Its Applications s.l.: IIE. BAS, Backhuys Publishers, pp. 505, 1999.

Tundisi J. G., O. Rocha, T. Matsumura-Tundisi and B. Braga, Reservoir management in South America, Water Resources Development, 14-2, pp. 141-155, 1999.

Vander Zanden, M.J. & J.B. Rasmussen. Primary consumer 13C and 15N and the trophic position of aquatic consumers. Ecology, 80: 1395-1404, 1999.

Wetzel R.G. 2001. Limnology: Lake and river ecosystems. 2nd edition. Academic Press, London (Pages 1006).

Woodland R.J., Rodrìguez M.A., Magnan P., Glèmet H. and Cabana G.. Incorporating temporally dynamic baselines in isotopic mixing models. Ecology, 93, 131-144, 2012.

AKNOWLEDGMENT

I would thank my family to believe in me for all of this time and for their invaluable support;

Prof. Nicola Sechi and *Prof.* Antonella Lugliè ….where it all began;

Dr. Marina Manca for invaluable aid;

Prof. Sergio Vacca to believe in me;

Dr. Federica Camin to believe in this project;

Marina & Piero family to, welcoming me as one of the family;

the ISE Library to help me for all of this time, thank you so much Laura Puppieni and Mariagrazia Cuochi…. greats!!

the ISE team and particulary Anna Visconti, Benedetta Ponti, Sara Bovio, Roberta Piscia, Silvia Zaupa, Stefano Gerli and Angela Boggero;

the Enas team and in particular the "*best sailor* " Stefano Cadelano, Oriana Soru, Barbara Begliutti, Licia Orrù, Luisa Dessi, Micol Vaschellari, Ignazio Ghironi, Simona Deidda, Tomasa Virdis, Marcella Ferralis, Paola Buscarinu, Gabriele Marras and Mariantonietta Dessena;

the sampling team of University of Sassari;

Silvia Gabba for zooplankton sorting;

Giovanna Fadda to careful sewn the plankton-net……

and so on…..

ANNEX

Verso un approccio funzionale allo studio della diversità: risultati di una prima indagine sullo zooplancton e la rete trofica pelagica del Lago Maggiore attraverso analisi d'isotopi stabili di carbonio e azoto*

Anna Visconti, Amedeo Fadda, Rossana Caroni, Igor Cerutti, Pietro Volta, Marina Manca

CNR ISE Largo Tonolli 50 28922 Verbania (VB)

* Lavoro realizzato con il contributo della Commissione Internazionale per la Protezione delle Acque Italo-Svizzere nell'ambito delle ricerche sugli aspetti limnologici del Lago Maggiore, Campagna 2008.

Riassunto

In ambito internazionale si va sempre più affermando la necessità di studiare in chiave funzionale gli ecosistemi, primi fra tutti quelli d'acqua dolce. Questi ultimi infatti dovrebbero essere tra gli ambienti maggiormente influenzati dai mutamenti globali e dall'invasione di specie aliene. L'impatto di questi grandi cambiamenti sulla biodiversità e sul funzionamento degli ambienti dulcacquicoli non può prescindere da una caratterizzazione degli organismi mirante alla definizione delle fonti da essi utilizzate e del loro ruolo trofico nell'ecosistema. In quest'ottica è stato condotto nel 2008 uno studio sul pelago del Lago Maggiore, uno dei laghi maggiormente studiati in Europa. Scopo dell'indagine era quello di caratterizzare le variazioni stagionali nelle fonti di carbonio e nel ruolo trofico di organismi pelagici, sì da mettere in luce eventuali contributi del litorale alla produzione secondaria pelagica e le vie principali del trasferimento di materia ed energia dai produttori primari ai consumatori secondari, fino ai pesci planctivori. Campioni raccolti in diversi momenti stagionali da tre stazioni sono stati analizzati al fine di identificarne i *fingerprint* isotopici di carbonio e azoto. I risultati ottenuti confermano quanto osservato nel Lago Lemano, suggerendo l'esistenza di meccanismi comuni ai laghi profondi subalpini relativamente alle variazioni stagionali del *fingerprint* del carbonio e un ruolo trofico dei cladoceri molto simile. Il ruolo di fonti litorali di carbonio non sembra trascurabile: il *fingerprint* dei pesci zooplanctivori è risultato infatti molto vicino a quello del litorale in alcuni momenti dell'anno. Inoltre, i dati ottenuti relativamente ai copepodi sembrano suggerire per essi un ruolo di carrier del carbonio litorale verso il pelago. Ulteriori studi attualmente in corso consentiranno di approfondire questi due aspetti emersi da questa prima indagine.

PAROLE CHIAVE: diversità funzionale / fingerprint isotopico / zooplancton / seston / pesci

Towards a functional approach of diversity: preliminary results of a first study on the Lake Maggiore pelagic trophic web using stable carbon and nitrogen isotopes

The need of functional studies in freshwater ecology is increasing at international level. Freshwater ecosystems are considered among the most affected by climate change and alien species invasions. In order to evaluate the impact of these factors on biodiversity and ecosystem functioning it is important to define the organism's trophic position in the ecosystem and their food sources. For this purpose we have carried out a study on the pelagic web in Lake Maggiore, one of the most studied lakes in Europe. The main aim of the study was the evaluation of seasonal variations in carbon sources and the trophic position of the pelagic organisms. Additionally, it was intended to investigate the possible contribution from the littoral zone to secondary pelagic production and to identify the energy flow from primary producers up to planktivorous fish. Stable isotopes analysis of carbon and nitrogen were used from lake samples taken in different seasons and at three different sites. Our results are comparable with studies conducted in Lake Leman, suggesting that subalpine deep lakes have similar seasonal variations in carbon and nitrogen fingerprints. Input from the littoral zone appeared to be significant, as the planktivorous fish fingerprint was occasionally very close to littoral values. Moreover, our results seem to suggest that zooplankton copepods could be carbon 'carriers' from the littoral to the pelagic zone. Further ongoing studies will allow us to better understand the processes observed in this preliminary study.

KEY WORDS: functional diversity / isotopes fingerprint / zooplankton / seston / fish

INTRODUZIONE

Aspetti generali e delle tecniche di analisi degli isotopi stabili

Le sostanze biogeniche in natura contengono quantità significative di isotopi stabili (IS) di elementi leggeri quali idrogeno, carbonio, azoto e ossigeno. Ad esempio un corpo umano di 50 kg contiene circa 225 g di isotopi più pesanti (Wada, 2009). Sebbene gli IS di un particolare elemento abbiano simile comportamento chimico, i loro parametri termodinamici specifici e le costanti di velocità nelle reazioni chimiche e biologiche differiscono.

Gli IS delle sostanze biogeniche variano in dipendenza delle composizioni isotopiche dei reagenti, dei percorsi e delle modalità cinetiche delle dinamiche di reazione, e delle condizioni fisiche e chimiche. Di conseguenza, ogni materiale biogenico ha la sua propria, unica, composizione isotopica, nota come "*fingerprint* dinamico in isotopi stabili", determinato dalla sua funzione e dalla posizione nel flusso di materia dell'ecosistema in un dato momento (Minagawa e Wada, 1984; Wada, 2009).

I recenti progressi nelle scienze della vita hanno messo in evidenza come gli organismi viventi posseggano tre fondamentali *fingerprint* all'interno del loro corpo.

Il primo *fingerprint* è quello dell'informazione biologica ottenuta nel corso dell'evoluzione della vita nell'arco degli ultimi 3,5 miliardi di anni, il "DNA *fingerprint*", trasferito dai genitori alla prole.

Il secondo *fingerprint* comunemente accettato è rappresentato dalla memoria immagazzinata nel cervello: man mano che cresce, un animale accumula memorie differenti nelle diverse circostanze. Oggigiorno, l'elasticità delle trasmissioni sinaptiche è considerata come una possibile chiave dei meccanismi della memoria del cervello, e molto vi è ancora da scoprire relativamente ai dettagli con i quali tali processi avvengono.

Il terzo *fingerprint*, come sopra anticipato, è l'"IS *fingerprint*", ovvero il modello isotopico caratteristico di un individuo, dato dalle abbondanze relative degli isotopi stabili in esso presenti, sostanzialmente determinate dalle abitudini alimentari e dalle modalità di escrezione. Determinazioni precise dei rapporti isotopici degli organismi consentono, in linea di principio, di ricostruire dati fondamentali quali la storia di una molecola o di un materiale.

Secondo l'approccio degli studi sugli isotopi stabili, un ecosistema può essere trattato come la somma di complesse reazioni chimiche mediate da organismi viventi a diversa funzione e da fattori fisico-chimici. Accanto alle variazioni nella temperatura, nella pressione e nell'energia prodotta e consumata nel corso delle reazioni metaboliche, in questo tipo di studi si tiene conto del cosiddetto "effetto isotopico", o "frazionamento isotopico", vale a dire delle possibili differenze nei rapporti degli IS nei reagenti e nei prodotti. L'effetto isotopico che accompagna i processi metabolici consente la comprensione delle sequenze di reazione implicate nella fisiologia degli organismi vegetali e della funzione e posizione di organismi animali nella rete trofica. Esso può essere utilizzato a diversi livelli di complessità, da quello molecolare a quello ecosistemico, e può essere considerato come un parametro interdisciplinare degli ecosistemi naturali.

Il frazionamento isotopico può essere suddiviso in due categorie. La prima categoria è quella delle reazioni di equilibrio di scambio isotopico, la più rappresentativa della quali è la reazione di scambio che consente di mantenere il rapporto isotopico del carbonio nell'atmosfera costante quasi su tutto il pianeta:

$$^{13}CO_{2(aria)} + H^{12}CO_{3(acqua)} \leftrightarrow {}^{12}CO_{2(aria)} + H^{13}CO^{-}_{3\,(acqua)} \qquad (1)$$

La seconda categoria è il cosiddetto effetto isotopico cinetico, che può aver luogo in quasi tutte le reazioni biologiche, in dipendenza dal meccanismo di reazione. In generale, la molecola isotopica a più basso peso molecolare ha una costante di velocità di reazione più grande rispetto a quella della molecola più pesante. Il grado di frazionamento dipende dalle caratteristiche proprie dell'enzima interessato nella reazione. Ad esempio, è noto che la denitrificazione ha un fattore di frazionamento grande, fino a 1,04 (4%), mentre la fissazione biologica dell'N_2 ha un fattore di frazionamento piccolo, di 1,002 (0,2%). Il fattore di frazionamento di una data reazione in due specie differenti è in generale della stessa grandezza se i siti enzimatici sono simili. Di conseguenza, il rapporto $^{15}N/^{14}N$ della biosfera nel suo insieme è leggermente maggiore di quello dell'azoto atmosferico. D'altro canto, si osserva un fattore di frazionamento isotopico grande -di 1,03 (3%)- nella carbossilazione delle piante C_3, e questo risulta in un rapporto $^{13}C/^{12}C$ più basso nella biosfera nel suo insieme rispetto all'HCO_3^- delle acque.

Poiché le variazioni nei rapporti isotopici di carbonio e azoto negli ecosistemi naturali sono lievi, il contenuto in ^{13}C e ^{15}N è espresso come deviazione ‰ rispetto a uno standard, secondo le formule seguenti:

$$\delta^{13}C\ (‰)=[(^{13}C/^{12}C_{campione})/(^{13}C/^{12}C_{PDB})-1]x1000 \qquad (2)$$

$$\delta^{15}N\ (‰)=[(^{15}N/^{14}N_{campione})/(^{15}N/^{14}N_{aria})-1]x1000 \qquad (3)$$

Come standard vengono utilizzati, per il carbonio e l'azoto rispettivamente, la belemnite Pee Dee (PDB), un carbonato di calcio fossile avente un contenuto di ^{13}C quasi identico a quello della HCO_3^- delle acque, e l'azoto atmosferico (N_2). Valori positivi del δ ‰ stanno ad indicare che il campione ha un contenuto di ^{13}C e ^{15}N maggiore di quello dello standard corrispondente; al contrario, quando esso ne sia impoverito, il valore del δ ‰ del corrispondente isotopo risulta negativo.

Naturalmente:
$\delta^{13}C\,(HCO^-_3)_{(acqua)} = 0{,}0‰$
e $\delta^{15}N(N_{2(aria)}) = 0{,}0‰$.

Molte differenti sostanze biologiche e biogeniche sono state raccolte da diversi ecosistemi. I loro rapporti isotopici sono stati misurati mediante spettrometri di massa con metodologie descritte in dettaglio. È così possibile identificare i principali fattori che governano le distribuzioni isotopiche in un ecosistema. Essi sono: 1) le composizioni isotopiche dei substrati utilizzati dai vegetali, quali la CO_2, l'H_2O e i nitrati inorganici; 2) gli effetti cinetici degli isotopi durante i processi di assorbimento; 3) effetti trofici durante i processi di nutrizione; 4) metabolismo gassoso durante la mineralizzazione.

In generale, le leggi empiriche che governano l'impiego degli isotopi stabili di carbonio e azoto nelle analisi ecosistemiche sono le seguenti:

1. Negli organismi vegetali, il $\delta^{13}C$ è determinato dalla dinamica della fissazione di CO_2 durante la fotosintesi. Le piante C_3 e C_4 e i vegetali terrestri presentano differenti contenuti in ^{13}C, e risultano a loro volta differire dal fitoplancton. Quest'ultimo a sua volta differisce dal fitobentos.
2. Il rapporto $^{13}C/^{12}C$ di un organismo animale è molto prossimo a quello della sua dieta.
3. In una gran varietà di invertebrati e vertebrati nel passaggio da preda a predatore si osserva un arricchimento in ^{15}N.
4. Una correlazione tra il rapporto $^{15}N/^{14}N$ e il livello trofico corrispondente è stata dimostrata chiaramente in diversi ecosistemi. Il flusso di sostanza organica lungo la catena alimentare dai produttori primari (costituenti la base alimentare) agli animali di livelli trofici più elevati può essere ricostruito attraverso la misura dei loro $\delta^{15}N$ e $\delta^{13}C$. I valori di $\delta^{13}C$ e di $\delta^{15}N$ degli animali durante un singolo processo di nutrizione, il cosiddetto effetto trofico, vengono dunque descritti dalle seguenti equazioni:

$$\delta^{13}C_{(animale)}(‰) = (1{,}0\sim2{,}0)(TL-1) \quad (4)$$

$$\delta^{15}N\,(‰)_{(animale)} = 3{,}3\,(TL-1) + \delta^{15}N_{(alghe)} \quad (5);$$

nelle quali TL indica il livello trofico (TL alghe=1).

5. un aumento di ^{15}N in un ecosistema è causato dall'evaporazione dell'NH_3 e dalla denitrificazione ($NO_3 \rightarrow N_2$).

L'uso degli isotopi stabili di C e N in ambienti lacustri

Gli isotopi stabili sono uno strumento efficiente per rivelare la diversità e la complessità della rete trofica zooplanctonica dei laghi (Post, 2002). Gli organismi zooplanctonici utilizzano fonti di carbonio autoctono, attraverso il grazing del fitoplancton, dei batteri e di altri organismi che dipendono dalla produzione fitoplanctonica. I batteri dipendono anche dal pool del carbonio organico disciolto prodotto attraverso molte altre fonti, ivi inclusi il fitoplancton, il perifiton, le macrofite e il carbonio alloctono. Gli studi sugli isotopi stabili sono stati utilizzati per distinguere tra fonti fitoplanctoniche e non-fitoplanctoniche di carbonio (bentoniche, litorali e alloctone) in diversi ecosistemi lacustri, dimostrando che il grado di dipendenza dello zooplancton da fonti non fitoplanctoniche dipende dal grado di trofia, dalla stagione, e dal rapporto N/P (Karlsson *et al.*, 2003).

Differenze nel $\delta^{13}C$ e nel $\delta^{15}N$ dello zooplancton possono risultare da uno *sfhift* nella linea di base pelagica, comunemente definita come la composizione isotopica delle fonti primarie di rifornimento della rete trofica pelagica. La linea pelagica comprende sia fonti fitoplanctoniche sia fonti non fitoplanctoniche: entrambe possono contribuire alla produzione secondaria della rete trofica pelagica. La linea di base pelagica di $\delta^{13}C$ e $\delta^{15}N$ varia a seconda del lago e, in uno stesso lago, negli anni e a seconda della stagione (Mattews e Mazumder, 2003). Le variazioni tra laghi sono tendenzialmente legate alle dimensioni (Post *et al.*, 2000; Perga e Gerdeaux, 2006). In uno stesso lago o in un gruppo di laghi simili il segnale della linea di base pelagica è influenzato dallo stato trofico (Perga e Gerdeaux, 2006). Input di azoto e di nutrienti inorganici δ ^{15}N possono alterare la linea di base del $\delta^{15}N$ (Lake *et al.*, 2001; Cole *et al.*, 2004; Lehmann *et al.*, 2004). Fattori ambientali quali temperatura, produzione primaria, e riciclo dei nutrienti inorganici possono influenzare il $\delta^{13}C$ e il $\delta^{15}N$ del fitoplancton. Nell'arco dell'anno si possono osservare differenze nel $\delta^{13}C$ e nel $\delta^{15}N$ dei diversi taxa zooplanctonici in relazione a modificazioni nelle abitudini alimentari (vale a dire nella posizione trofica) e nella disponibilità delle risorse.

Con questo studio ci si è proposti di:

- dare una prima caratterizzazione del segnale isotopico di carbonio e azoto e del suo propagarsi lungo la rete trofica pelagica del Lago Maggiore, dalla base alimentare fino ai pesci, nei quattro momenti stagionali. Come evidenziato nella parte introduttiva, l'intervallo di valori del $\delta^{13}C$ lacustre ne riflette principalmente la tipologia e dunque, nel caso del Maggiore, ci si attende che esso sia raffrontabile a quello di altri laghi profondi subalpini. Il $\delta^{15}N$ dei laghi è invece principalmente legato allo stato trofico e tende ad innalzarsi in acque di scarico urbano (CABANA e RASMUSSEN, 1996); il termine di paragone, nel caso del Maggiore, dovrebbe essere dunque quello di ambienti oligotrofi;

- analizzare le variazioni nel tempo (con la stagione) e nello spazio (attraverso il confronto tra stazioni rappresentative dell'interferenza, nel pelago, di apporti di materiale alloctono, dal Fiume Toce, Stazione di Baveno e dal litorale, Stazione di Lesa) del segnale isotopico del carbonio;
- individuare il possibile contributo alla rete trofica pelagica di fonti di carbonio non pelagiche;
- identificare i rapporti trofici e il loro variare nel corso dell'anno, di organismi pelagici, dai consumatori primari zooplanctonici ai pesci zooplanctivori.

MATERIALI E METODI

È questo il primo studio sul Lago Maggiore di questo tipo, inteso a dare una prima caratterizzazione della linea di base (pelagica, litorale, ed eventualmente del Fiume Toce), e del propagarsi del segnale isotopico lungo la catena alimentare dallo zooplancton fino ai pesci. Pertanto, il campionamento è stato previsto, oltreché nella stazione pelagica al largo di Ghiffa, corrispondente al punto di massima profondità del lago, da sempre utilizzata per rappresentare il pelago lacustre, anche in altri due siti, scelti in quanto indicativi, stando ai risultati di precedenti studi, dell'influenza del Fiume Toce (Stazione denominata "Baveno"; latitudine 45°54'28''N; longitudine 8°31'44''E) e dell'ambiente litorale (Stazione denominata "Lesa"; latitudine 45°49'70''N; longitudine 8°34'70''E), rispettivamente. I campionamenti sono stati effettuati con cadenza all'incirca mensile e i dati ottenuti raggruppati in modo tale da rappresentare, secondo quanto previsto dal progetto, i quattro momenti stagionali. Campioni d'acqua sono stati raccolti dalle tre stazioni entro i primi 50 m (lo strato usualmente campionato per lo zooplancton) per la determinazione del segnale isotopico del materiale sestonico (<76 µm) e per la misura dell'abbondanza e della biomassa del fitoplancton (misure di clorofilla *a* e biovolume). A ogni data di campionamento organismi appartenenti ai diversi taxa zooplanctonici venivano raccolti in quantità sufficienti a consentire analisi del segnale isotopico. Per la descrizione dettagliata dei metodi di preparazione dei campioni si veda MANCA *et al.* (1994 e 1997). Nel contempo, campioni quantitativi prelevati con le usuali metodiche servivano a determinare la densità di popolazione e la biomassa zooplanctonica nei quattro differenti momenti e nelle tre diverse stazioni. Venivano inoltre misurate la temperatura dello strato d'acqua campionato mediante termometro termistore e la trasparenza delle acque mediante Disco di Secchi. Al termine della campagna d'indagine sono stati inoltre prelevati campioni dal litorale (perifiton e anfipodi), utili a caratterizzarne il segnale isotopico, in tre diverse stazioni situate lungo l'asse maggiore del lago.

Onde ottenere campioni adatti a garantire risultati attendibili delle analisi strumentali è stato necessario prelevare, di volta in volta, e per ogni replica, un numero d'individui compreso tra un minimo di 40 e un massimo di 500, in dipendenza dalla taglia individuale. I dati che qui verranno presentati, dunque, sono rappresentativi, nel loro complesso, di circa 13000 individui zooplanctonici.

I campioni relativi a tre diverse specie ittiche (coregone lavarello, agone, gardon) sono stati ottenuti mediante trattamento di campioni di muscolo prelevati dalla regione dorsale compresa tra il capo e l'inizio della pinna dorsale di cinque individui per ogni data di campionamento, prelevati, nelle quattro diverse stagioni, con reti branchiali in zona pelagica (coregone lavarello, *Coregonus lavaretus* e agone, *Alosa fallax lacustris*) e litorale (gardon, *Rutilus rutilus*) nella regione centrale del Lago Maggiore, all'incirca nelle vicinanze dell'abitato di Ghiffa. Dei singoli pesci è stata misurata la taglia (lunghezza totale e peso totale) e determinata l'età mediante lettura delle scaglie con la finalità di costituire campioni di taglia ed età il più possibile omogenea per limitare la variabilità del segnale isotopico legata a possibili differenti abitudini trofiche.

I campioni e gli standard sono stati pesati in capsulini di stagno e caricati in analizzatore elementare (Vario EL III) interfacciato con uno spettrometro di massa per il rapporto isotopico (G. G. Hatch Isotope Laboratories, University of Ottawa, Faculty of Science, Ottawa, Canada). I campioni e gli standard sono stati combusti a circa 1800°C (combustione di Dumas) e i prodotti gassosi risultanti trasportati in elio attraverso colonne di ossido-riduzione ottimizzate per CO_2 e N_2. I gas venivano separati mediante colonne di assorbimento a purificazione e cattura, inviate all'interfaccia (Conflo II) e all'IRMS (Isotope Ratio Mass Spectrometer della Delta XP Plus Advantage). La precisione analitica delle analisi è stata basata su uno standard interno (C-55, acido glutamico, $\delta^{15}N$, $\delta^{13}C$= 3,98 e -28,53, rispettivamente) con precisione analitica solitamente inferiore allo 0,2 ‰.

I valori di $\delta^{15}N$ sono riferiti all'N_2 dell'aria e normalizzati con standard interni calibrati all'International standard IAEA-N1 (+0,4‰), IAEA-N2 (+20,3‰), USGS-40 (-4,52‰) e USGS-41 (47,57‰). I valori di $\delta^{13}C$ sono riferiti alla PDB e normalizzati con standard interni calibrati presso l'International standard IAEA-CH-6 (-10,4‰), NBS-22 (-29,91‰), USGS-40 (-26,24‰) e USGS-41 (37,76‰). La deviazione standard dei valori relativi alle analisi di ^{13}C e ^{15}N nei campioni di seston, zooplancton e pesci, è risultata, rispettivamente pari a 0,05 e 0,12; 0,12 e 0,06; 0,12 e 0,06.

Così come suggerito da studi analoghi effettuati in precedenza in laghi simili (ad esempio, il Lago Lemano; Perca e Gerdaux, 2006), i dati relativi alla composizione isotopica del materiale sestonico sono stati confrontati con quelli ottenuti per *Daphnia*, sì da verificare la possibilità di utilizzare la composizione isotopica di quest'ultima come tracciante della linea di base pelagica, in virtù del suo comportamento alimentare non selettivo nelle diverse stagioni. A tal fine, i valori del $\delta^{13}C$ e del $\delta^{15}N$ del seston (frazione ≤ 76 µm entro lo strato 0-50 m) sono stati confrontati con quelli misurati alle stesse date di campionamento e nelle medesime stazioni, in *Daphnia*. La validità di *Daphnia* come integratore del segnale isotopico del carbonio pelagico è stata anche saggiata attraverso il confronto con il rapporto isotopico misurato in *Dreissena polymorpha* a conclusione della campagna di rilevamenti. Molluschi bivalvi di questo genere sono infatti spesso utilizzati come integratori e indicatori della linea di base pelagica (Post, 2002).

I diversi taxa zooplanctonici presi in esame nel corso dell'anno comprendevano, oltre a *Daphnia*, anche i copepodi, con distinzione in diaptomidi e ciclopidi, e i cladoceri di grosse dimensioni *Leptodora kindtii* e *Bythotrephes longimanus*. I segnali isotopici dei diversi taxa nelle diverse stazioni e nei diversi periodi dell'anno sono stati analizzati e comparati con quelli ottenuti per *Daphnia*. Alla base di tale approccio vi sono due importanti assunzioni:

1) qualora un dato taxon sia supportato dalla linea di base pelagica, il suo $\delta^{13}C$ sarà simile a quello di *Daphnia*, il che significa che la varianza residua sarà minore o uguale a quella dell'arricchimento atteso per livello trofico (cfr. materiali e metodi, equazione 4);

2) qualora il $\delta^{13}C$ di un taxon zooplanctonico risulti statisticamente diverso da quello di *Daphnia* e/o la varianza residua maggiore di quella attesa, è possibile ipotizzare che vi siano fonti multiple di alimentazione della comunità zooplanctonica;

3) le modificazioni nelle posizioni trofiche relative dei taxa zooplanctonici sono ottenute a partire dalla comparazione tra il loro $\delta^{15}N$ e quello di *Daphnia*. Se un dato taxon occupa la stessa posizione trofica (relativamente a quella di *Daphnia*) lungo l'arco dell'anno, allora la differenza $\Delta N=\delta^{15}N_{taxon}-\delta^{15}N_{Daphnia}$ dovrebbe rimanere costante, e presentare una variabilità minore di quella determinata dall'arricchimento per livello trofico (cfr. materiali e metodi, equazione 5). Variazioni importanti nel ΔN nel corso dell'anno potrebbero indicare sia che il taxon non utilizza sempre le medesime fonti primarie, e che pertanto utilizza fonti alternative a segnale isotopico differente, ovvero che non occupa la stessa posizione trofica durante tutto l'arco dell'anno.

Le relazioni tra i taxa e la

Tab. I. Risultati del test Kruskal-Wallis per il confronto dei dati relativi ai segnali isotopici ($\delta^{13}C$ e $\delta^{15}N$) e del rapporto C/N dei diversi campioni di materiale prelevato dalle tre stazioni (Ghiffa, Baveno e Lesa). N= numero di campioni; P= livello di significatività. NS = non significativo

		N	P	risultato
Daphnia	$\delta^{13}C$	14	0,85	NS
	$\delta^{15}N$	13	0,21	NS
	C/N	10	0,39	NS
Leptodora	$\delta^{13}C$	12	0,73	NS
	$\delta^{15}N$	10	0,73	NS
	C/N	10	0,76	NS
Bythotrephes	$\delta^{13}C$	20	0,61	NS
	$\delta^{15}N$	17	0,95	NS
	C/N	14	0,40	NS
Seston 0-20m	$d^{13}C$	11	0,90	NS
	$\delta^{15}N$	11	0,39	NS
	C/N	8	0,08	NS
Seston 25-50m	$\delta^{13}C$	12	0,87	NS
	$\delta^{15}N$	12	0,78	NS
	C/N	9	0,84	NS

linea di base sono state investigate mediante regressioni lineari. La significatività delle differenze rispetto al valore 1 dei coefficienti angolari delle rette di regressione è stata testata mediante t-test. I valori della variabilità residua, ottenuti mediante modello ANOVA sono stati comparati a quelli previsti per il frazionamento trofico di^{13}C e ^{15}N (deviazione standard= 1,3 e 1,0‰, rispettivamente).

RISULTATI

Confronto tra le diverse stazioni del pelago lacustre

I dati relativi al segnale isotopico dei campioni raccolti nelle tre stazioni (Ghiffa, Baveno e Lesa) sono stati testati statisticamente (test Kruskal-Wallis non parametrico, one way) al fine di verificare se, e in che misura, le differenze osservate fossero statisticamente significative. I risultati, riportati in tabella I, dimostrano chiaramente come le differenze osservate tra i diversi siti campionati non siano statisticamente significative.

Interessanti differenze tra il segnale isotopico del carbonio del materiale sestonico dei primi 20 m e quello dello strato più profondo (25-50m) sono state osservate relativamente alla stazione di Baveno e a seguito di intense precipitazioni, con valori più negativi nella zona più profonda. Esse potrebbero essere indicative dell'influenza di un apporto di materiale dal Fiume Toce, in accordo con quanto osservato in studi precedenti (Ambrosetti *et al.*, 1980 ; de Bernardi *et al.*, 1988).

Tuttavia, nel loro complesso, le variazioni tra le diverse stazioni non sono risultate statisticamente significative (test Kruskal-Wallis per raffronto multiplo; H (1, N= 23) =3,185606 p =0,074).

Anche le differenze tra il δ^{13}C del seston dello strato più superficiale e quelle dello strato più profondo nelle tre stazioni sono risultate non significative dal punto di vista statistico. Tale dato può essere spiegato a partire dall'analisi del contributo dei diversi gruppi alla biomassa totale (Fig. 1): quando legate al fitoplancton, le differenze nel segnale isotopico possono essere in linea generale relazionabili non tanto alla maggiore o minore biomassa raggiunta, quanto a importanti modificazioni nella struttura del popolamento, in particolar modo nella geometria degli organismi presenti (Popp *et al.*, 1998). I dati riportati nei grafici in figura 1 dimostrano come i gruppi dominanti siano sostanzialmente gli stessi nei due strati d'acqua considerati, mentre differenze anche impor-

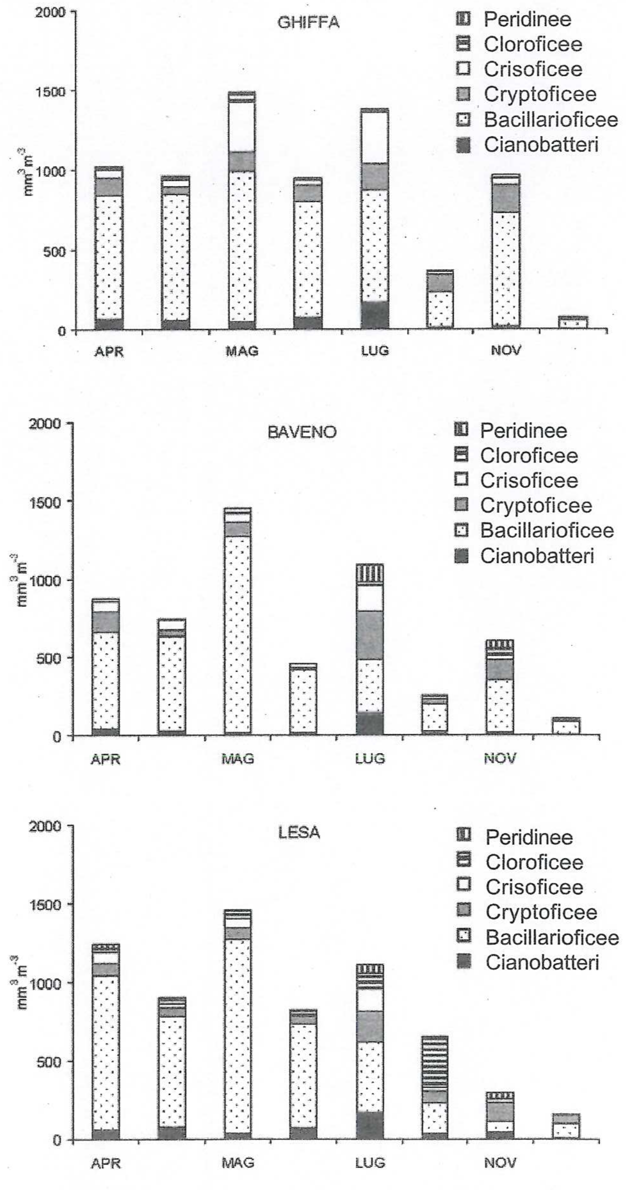


Fig. 1. Biovolume fitoplanctonico misurato nelle tre stazioni del Lago Maggiore in diversi periodi dell'anno e nei due diversi strati (epilimnico e ipolimnio) campionati per le analisi degli isotopi stabili di carbonio e azoto.

tanti sono state osservate, com'è logico attendersi, nei valori complessivi di biomassa raggiunti, soprattutto nelle fasi di maggiore sviluppo, primaverile estivo e autunnale, del popolamento. Le differenze di biomassa sono risultate rilevabili anche dalla comparazione dei valori di clorofilla *a* nei due strati campionati (test di Wilcoxon; p= 0,008; N=18).

I risultati delle analisi relative al segnale isotopico del carbonio nei quattro periodi dell'anno e per le diverse componenti prese in esame sono riassunti nel grafico in figura 2. La scala del $\delta^{13}C$ comprende valori rappresentativi dell'ambiente litorale (meno negativi) e di quello pelagico, di laghi profondi, quali il Maggiore. Organismi bentonici e di acque profonde presentano generalmente valori del $\delta^{13}C$ più bassi rispetto a quelli del pelago (Cattaneo *et al.*, 2004). Il grafico può dunque essere letto, dall'alto verso il basso, come una graduale transizione dal litorale alle zone più profonde del lago.

Il segnale isotopico del carbonio delle acque pelagiche è rappresentato, nel grafico in figura, dai punti corrispondenti a *Daphnia*. Filtrando non selettivamente il materiale sestonico, questo cladocero rappresenta nel Maggiore, così come in laghi ad esso assimilabili per tipologia e livello di produttività, un indicatore della cosiddetta linea di base pelagica (Perga e Gerdaux, 2006). Effettivamente, in linea generale, il segnale isotopico del $\delta^{13}C$ di *Daphnia* misurato nel corso del 2008 ha riflettuto fedelmente quello del materiale sestonico ($r = 0,86$; $p<0,01$; N=13). Secondo le assunzioni sulle quali si basa il principio del metodo degli isotopi stabili, organismi che rispecchiato il segnale isotopico del carbonio di *Daphnia* sono ad essa relazionabili in termini di fonte alimentare, e dunque definibili come tipicamente legati al pelago. La conferma dell'idoneità di *Daphnia* a rappresentare il segnale pelagico è anche emersa dai risultati delle misure effettuate a conclusione del lavoro di monitoraggio su *Dreissena polymorpha*, il mollusco bivalve generalmente utilizzato come integratore della linea di base del pelago ($\delta^{13}C$ medio= -35,14, N= 72; SE= 0,28‰).

I dati del Lago Maggiore rappresentati in figura 2 dimostrano chiaramente come il segnale isotopico del $\delta^{13}C$ di cladoceri zooplanctonici, quali *Bythotrephes* e *Leptodora*, e dei ciclopidi (*Cyclops abyssorum* e *Mesocyclops leuckarti*), ben si adatti a una situazione tipica dell'ambiente pelagico. In altre parole, i valori del $\delta^{13}C$ dei tessuti di questi organismi sono del tutto assimilabili a quelli componenti *Daphnia*. Così come osservato nel Lago Lemano, in particolare, la correlazione tra i valori del $\delta^{13}C$ di *Daphnia* e quelli di *Bythotrephes* è risultata molto elevata ($r =0, 89$) e statisticamente altamente significativa ($p<0,001$; N=18). La pendenza della retta di regressione ottenuta dai dati di $\delta^{13}C$ relativi ai due taxa non è risultata significativamente diversa da 1 e il valore dell'intercetta non diverso da 0.

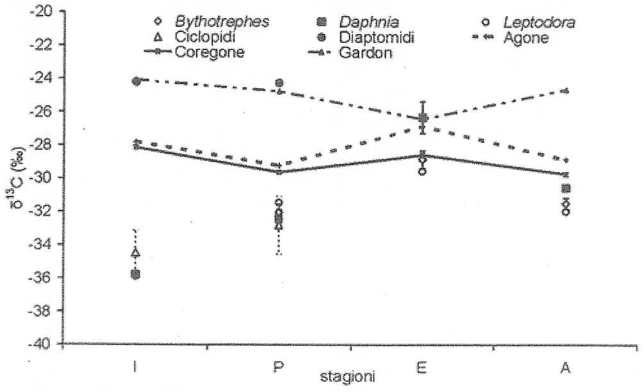


Fig. 2. Valori del rapporto isotopico del carbonio ($\delta^{13}C$‰) ottenuti nel corso del 2008 da diversi taxa componenti lo zooplancton pelagico del Lago Maggiore e in tre diverse specie di pesci. Le barre verticali rappresentano l'errore standard calcolato sulle repliche dei campioni.

Il grafico in figura 2 mostra anche molto bene come il segnale isotopico del carbonio dei diversi componenti del sistema cambi al variare delle stagioni, muovendosi verso valori meno negativi durante le stagioni calde per ritornare, in autunno, verso valori più negativi, che preludono a quelli del periodo invernale. Le variazioni stagionali nel segnale isotopico pelagico del carbonio sono risultate in accordo con le variazioni nella temperatura media ambientale (Fig. 3). La correlazione fra le due variabili su tutti i dati (N=12) è alta ($r=0,74$) e statisticamente significativa ($p< 0,05$). La spiccata stagionalità osservata nel Lago Maggiore ricalca molto bene quella riportata per laghi simili, ad esempio per il Lemano. Di tutta una serie di variabili fisiche, chimiche e biologiche prese in esame, solamente la temperatura ambientale, espressa come la media delle temperature entro lo strato d'acqua campionato, è risultata correlata in modo statisticamente significativo con il segnale isotopico del $\delta^{13}C$ della linea di base pelagica (Perga e Gerdeaux, 2006).

Dal grafico in figura 2 emerge anche, e piuttosto chiaramente, come il $\delta^{13}C$ dei copepodi diaptomi-

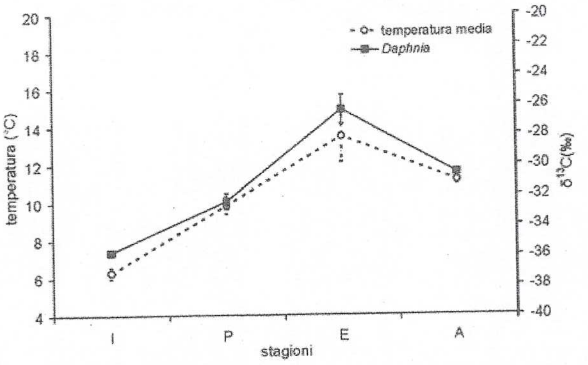


Fig. 3. Variazioni stagionali del segnale isotopico pelagico del carbonio e della temperatura nelle tre stazioni nello strato d'acqua campionato (0-50m) (valori medi ± Errore Standard).

di (della specie *Eudiaptomus padanus*) si discosti nettamente da quello misurato negli altri taxa zooplanctonici. In particolare, questi organismi prelevati in acque pelagiche sono risultati occupare, nel grafico relativo al segnale isotopico del carbonio, una posizione che ne suggerisce una dipendenza da fonti alimentari di origine litorale. Come evidenziato in precedenza, valori del $\delta^{13}C$ relativamente alti (meno negativi rispetto a quelli del segnale pelagico) caratterizzano gli organismi di ambiente litorale. Il riferimento litorale, per il Lago Maggiore, è rappresentato dal segnale isotopico del carbonio da noi misurato nel perifiton e in anfipodi bentonici prelevati in zona litorale, in occasione di una serie di prelievi in tre diverse stazioni lungo l'asse principale del lago ($\delta^{13}C$= -24,41‰, valore medio ±0,028). I dati relativi ai diaptomidi del Lago Maggiore sembrano inoltre suggerire come, contrariamente a quanto rilevato per gli altri taxa zooplanctonici esaminati, il segnale isotopico del carbonio in questi organismi tenda a mantenersi più stabile nel tempo. Questi due aspetti emersi dall'attività di monitoraggio del 2008 saranno, con tutta probabilità oggetto d'indagine più approfondita nel prossimo futuro.

Molto interessante risulta, sempre nel grafico in figura 2, l'analisi del segnale isotopico del carbonio nelle tre differenti specie di pesci da noi prese in esame. In aggiunta all'agone, specie ittica autoctona, e del coregone, specie alloctona ma da tempo acclimatatasi con successo, si è ritenuto opportuno analizzare anche il gardon, specie che, di recente, è stata protagonista di una vera e propria invasione nelle acque del Lago Maggiore (VOLTA e JEPSEN, 2008). Risulta evidente il carattere litorale del segnale isotopico del gardon, particolarmente in inverno e primavera, quando i valori sono molto simili a quelli misurati nei diaptomidi del lago, e anche coerenti con il segnale litorale del perifiton e degli anfipodi. Nella stagione estiva, il segnale isotopico del gardon si avvicina molto a quello di *Daphnia*, suggerendo un importante cambiamento nell'alimentazione e il passaggio da fonti litorali a fonti pelagiche di supporto alimentare. Se per le specie pelagiche, quali coregone e agone, è sostanzialmente corretto affermare che non vi possano essere sostanziali differenze nel regime alimentare in relazione alla taglia e all'età, altrettanto non è lecito supporre per il gardon. Tale specie è onnivora e opportunista e, almeno oltralpe, mostra una spiccata plasticità nella dieta in relazione al periodo stagionale e alla taglia. I pesci considerati in queste analisi erano soggetti adulti di 3 o 4 anni. I risultati dunque, benché di assoluta rilevanza scientifica, risultano limitati a una o due classi di età e non esaustivi per la caratterizzazione della specie e del suo ruolo trofico nell'ecosistema lacustre. Una più approfondita indagine potrebbe essere condotta in futuro per definire con maggior dettaglio questi elementi, possibilmente corroborata dalla stima del contenuto alimentare.

L'analisi del grafico in figura 2 mostra inoltre come il segnale isotopico dei pesci sia, in generale, meno variabile nel corso dell'anno di quello dello zooplancton. Tale risultato conferma come la variabilità sia principalmente legata alla velocità di turnover metabolico e dunque tenda a essere più elevata negli organismi più piccoli rispetto a quelli di taglia maggiore. Soprattutto in inverno, i segnali isotopici del carbonio di agone e coregone si discostano in maniera significativa da quelli della linea di base pelagica, e dei diversi taxa zooplanctonici ad essa relazionati, suggerendo come fonti diverse da quelle pelagiche possano costituire una componente importante dei tessuti di queste due specie di pesci durante la stagione iniziale dell'anno. In estate, i valori del $\delta^{13}C$ di coregone e agone tendono a incontrare quelli, divenuti meno negativi, della linea di base pelagica, rappresentata da *Daphnia*, e degli organismi da essa dipendenti (cladoceri predatori): in questa fase, lo zooplancton d'acque libere rappresenta il costituente principale dei tessuti di queste due specie di pesci.

Il pattern stagionale, sostanzialmente invertito nel caso del gardon, potrebbe anche riflettere modificazioni nella linea di base litorale, governata da fattori ambientali diversi rispetto a quella pelagica.

Indagini ulteriori sull'ambiente litorale dovrebbero servire a chiarire questo punto, ancora poco discusso in letteratura.

I dati relativi al $\delta^{15}N$ di diversi taxa zooplanctonici sono risultati coerenti con un quadro generale di frazionamento trofico; l'arricchimento nell'isotopo più pesante in *Daphnia* rispetto al seston ($\leq$76µm, pool 0-50m, valori del $\delta^{15}N$ compresi tra 3,5 e 5,5 ‰) è risultato compreso tra 2 e 3 (valore medio= 2,58), con valori più bassi in estate e più elevati in primavera); *Bythotrephes* è risultato in media arricchito di circa il 3,6 ‰ rispetto a *Daphnia*, con valori invernali più elevati (4,56 ‰) rispetto a quelli estivi (2,59 in media). Le differenze stagionali potrebbero rispecchiare una dipendenza del ruolo trofico di questo predatore invertebrato dalla sua taglia corporea, confermando la possibilità che individui più grossi possano esercitare un'azione di predazione paragonabile a quella dei pesci planctivori (Manca *et al.*, 2000, Manca *et al.*, 2008; Manca e De Mott, 2009). I valori del $\delta^{15}N$ di *Leptodora* e dei ciclopidi si collocano entro un intervallo del 2,8-2,9 ‰ superiore a quelli di *Daphnia*.

Delle tre diverse specie di pesci, l'agone è risultato quella a minor livello di frazionamento del $\delta^{15}N$, con valori mediamente molto simili a quelli dei predatori invertebrati (2,84 in media, con un intervallo compreso tra 3,6 in inverno, e 2,1 in primavera). I massimi livelli di frazionamento dell'azoto sono stati riscontrati nei coregoni, con valori di $\delta^{15}N$ intorno al 12 ‰ in inverno e del 10-11 ‰ in primavera e estate, rispettivamente. Durante il periodo invernale e primaverile, il gardon sembra occupare un posto intermedio tra i coregoni e i predatori zooplanctonici, con valori di $\delta^{15}N$ abbastanza stabili, di 8,8-8,9 ‰ e lievemente più bassi (8,3‰) in estate.

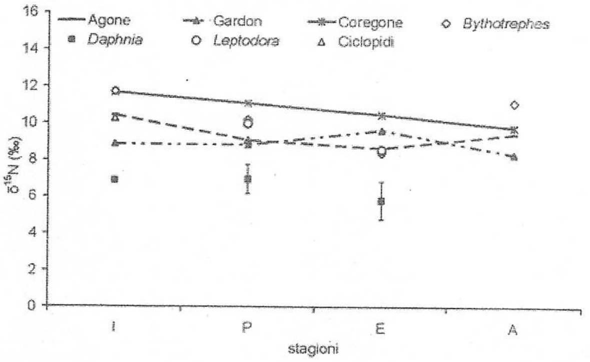


Fig. 4. Valori isotopici del $\delta^{15}N$ misurati nel corso dell'anno in diverse componenti dello zooplancton, e in tre diverse specie di pesci del Lago Maggiore.

CONCLUSIONI

I principali risultati di questo studio preliminare sono riassumibili nei seguenti punti:

- il segnale isotopico della linea di base pelagica del carbonio del Lago Maggiore, ben rappresentato da *Daphnia* (confermato dal buon accordo con *Dreissena* e con i dati relativi al seston della frazione dimensionale $\leq$76 µm nello strato 0-50 m) è del tutto raffrontabile a quello misurato in laghi della stessa tipologia del Lago Maggiore;
- nel corso dell'anno, la linea di base del carbonio pelagico si sposta da valori di $\delta^{13}C$ più alti (meno negativi) a valori più bassi (più negativi), e le variazioni osservate sono correlate con la temperatura ambientale;
- alcuni organismi zooplanctonici, quali i cladoceri predatori *Bythotrephes longimanus* e *Leptodora kindtii* e i ciclopidi adulti (delle specie *Cyclops abyssorum* e *Mesocyclops leuckarti*) ricalcano fedelmente le variazioni stagionali nella linea di base pelagica; questo risultato sta a indicare una dipendenza dalle stesse fonti alimentari utilizzate da *Daphnia*;
- i diaptomidi adulti pelagici recano un segnale isotopico del carbonio molto diverso da quello tipico del pelago, ma caratteristico dell'ambiente litorale; quest'ultimo, rilevato su perifiton e anfipodi con una precisione molto buona, è pari a quello riportato per laghi a tipologia simile; i diaptomidi adulti del lago (della specie *Eudiaptomus padanus*) sono di fatto interpretabili come carrier per il rifornimento di fonti non pelagiche alla produzione secondaria del pelago;
- nelle tre stazioni prese in esame, indicate in studi pregressi come rappresentative dell'impatto del Fiume Toce e dell'ambiente litorale, il segnale isotopico di carbonio e azoto delle diverse componenti da noi analizzate non presenta differenze significative. Evidentemente, le variazioni locali non sono tali da influenzare in maniera significativa la composizione isotopica della linea di base e il suo propagarsi lungo la rete trofica pelagica;
- il segnale isotopico del carbonio dei pesci, in generale meno variabile di quello degli zooplanctonti, indica una loro dipendenza alimentare, oltreché da fonti pelagiche, anche da fonti litorali, in dipendenza dalla stagione. Delle tre specie, è il gardon quella maggiormente legata al litorale del lago.

Sviluppi futuri

L'esperienza maturata consente di tracciare alcune linee di pianificazione dell'attività futura, anche in un'ottica di ottimizzazione del rapporto costi/benefici. Nel prossimo futoro si cercherà di estendere le analisi anche a quei taxa zooplanctonici per i quali non è stato possibile ottenere materiale sufficiente a causa del loro basso peso individuale. In particolare, è auspicabile ottenere i segnali istotopici di *Bosmina* e *Diaphanosoma*, estremamente interessanti sia per l'individuazione di fonti non pelagiche nella dieta, sia per valutarne il ruolo trofico. Si prevede anche di migliorare le conoscenze relative ai segnali isotopici degli apporti di materiale alloctono attraverso il campionamento di invertebrati acquatici (anfipodi e larve di ditteri chironomidi) provenienti dal Fiume Toce e di organismi zooplanctonici prelevati anche in prossimità della Foce del Torrente San Bernardino. Questo, al fine di meglio caratterizzare i segnali isotopici non pelagici e la loro variabilità spaziale.

Bibliografia

Ambrosetti W., Barbanti L., de Bernardi R., Libera V., 1980. Alcune risposte limnologiche ad un evento meteorologico eccezionale: il nubifragio del 7 Agosto 1978 nel bacino del Lago Maggiore. *Atti 3° Congresso A.I.O.L.*: 389-406.

Cabana G., Rasmussen J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. *Ecology* **93**: 10844-10847.

Cattaneo A., Manca M., Rasmussen J.B., 2004. Peculiarities in the stable isotope composition of organisms from an alpine lake. *Aquat. Sci.* **66**: 440–445.

Cole M.L. Valiela I., Kroeger K.D., Tomasky G. L., Cebrian J., Wigand C. McKinney R.A., Grady S.P., da Silva M.H.C., 2004. Assesment of a $\delta^{15}N$ isotopic method to indicate anthropogenic eutrophication in acquatic ecosystem. *J. Envirom. Qual.* **33**: 124-132.

de Bernardi R., Manca M., Giussani G., 1988. Dinamica di popolazione e distribuzione verticale di cladoceri planctonici del Lago Maggiore in relazione a parametri ambientali. *Verh. int. ver. Limnol.* **23**: 530-534.

Karlsson J., Jonsson A., Meili M., Jansson M., 2003. Control of zooplankton dependence on allochtonus organic carbon in humic and clearwater lakes in northern Sweden. *Limnol. Oceonagr.* **48**: 269-276.

Lake J.L., McKinney R.A., Osterman F.A., Pruell R.J., Kiddon J., Ryba S.A., Libby A.D., 2001. Stable nitrose isotopes as indicators of anthropogenic activities in small freshwater system. *Can. J. Fish. Aquat. Sci.* **58**: 870-878.

Lehmann M.F., Bernasconi S., McKenzie., Barbieri A., Simona M., Veronesi M., 2004. Seasonal variation of the $\delta^{13}C$ and $\delta^{15}N$ of particulate and dissolved carbon and nitrogen in Lake Lugano: constraints on biogeochemical cycling in a eutrophic lake. *Limol. Oceanogr.*, **49**: 415-429.

Manca M., Spagnuolo T., Comoli P., 1994. Variations in carbon and nitrogen content with body length of *Daphnia hyalina-galeata* s.l. from laboratory and field observations. *Journal of Plankton Research*, **16** (10): 1303-1314.

Manca M., Comoli P., Spagnuolo T., 1997. Length-specific carbon content of the *Daphnia* population in a large italian subalpine lake, Lago Maggiore (Northern Italy): the importance of seasonality. *Aquat. Sci.*, **59**: 48-56.

Manca, M., Ramoni C., Comoli P., 2000. The decline of *Daphnia hyalina galeata* in Lago Maggiore: a comparison of the population dynamics before and after oligotrophication. *Aquat. Sci.* **62**: 142-153.

Manca M., De Mott W.R., 2009. Response of the invertebrate predator *Bythotrephes* to a climate-linked increase in the duration of a refuge from fish predation. *Limnol. Oceanogr.* **54**(4): 2506-2512.

Manca M., Vijverberg J., Polishchuk L.V., Voronov D.A., 2008. *Daphnia*_body size and population dynamics under predation by invertebrate and fish predators in Lago Maggiore: an approach based on contribution analysis. *J. Limnol.*, **67**(1): 15-21.

Matthews B., Mazumder A., 2003. Compositional and inter-lake variability of zooplankton affect baseline stable isotope signatures. *Limnol. Oceanogr.* **48**: 1977-1987.

Minagawa M., Wada E., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between ^{15}N and animal age. *Geochim. Cosmochim. Acta* **48**: 1135–1140.

Perga M.E., Gerdeaux D., 2006. Seasonal variability in the $\delta^{13}C$ and $\delta^{15}N$ values of the zooplankton taxa in two alpine lakes. *Acta Oecologica*, **30**: 69-77.

Popp B.N., Laws E.A., Bidigare R.R., Dore J.E., Hanson K.L., Wakeham S.G., 1998. Effect of phytoplankton cell geometry on carbon isotopic fractionation. *Geochimica e Cosmochimica Acta*, **62**: 69-77.

Post D.M., 2002. Using stable isotopes to estimate trophic position: Models methods, and assumptions. *Ecology* **83**: 703-718.

Post D.M., Pace M.L., Hairston N.G., 2000. Ecosystem size determines food-chain length in lakes. *Nature* **405**: 1047-1049.

Volta P., Jepsen N., 2008. The recent invasion of *Rutilus rutilus* (L.) (Pisces: Cyprinidae) in a large South-Alpine lake: Lago Maggiore. *J. Limnol.* **67**: 163-170.

Wada E. 2009. Stable $\delta^{13}C$ and $\delta^{15}N$ isotope ratios in aquatic ecosystems. *Proc. Jpn Acad., ser B*, **85**: 98-107.

Indagini sulla rete trofica pelagica del Lago Maggiore mediante analisi d'isotopi stabili di carbonio e azoto (SIA): risultati della campagna 2009[§]

Amedeo Fadda, Anna Visconti, Benedetta Ponti, Marina Manca[*]

CNR ISE, Largo Tonolli, 50 - 28922 Verbania (VB)

[*] Referente per la corrispondenza: m.manca@ise.cnr.it

[§] Lavoro realizzato con il contributo della Commissione Internazionale per la Protezione delle Acque Italo-Svizzere nell'ambito delle ricerche sugli aspetti limnologici del Lago Maggiore, Campagna 2009. Funded by the International Commission for the Protection of Swiss-Italian Waters on Lake Maggiore.

Riassunto

Gli studi sulla rete trofica pelagica del Lago Maggiore mediante SIA sono stati estesi nel 2009 a cladoceri di piccola taglia e a organismi litorali. La spiccata stagionalità di *Daphnia* riflette quella della linea di base del pelago, e da essa sembra dipendere la maggior parte degli zooplanctonti analizzati. L'analisi del *fingerprint* isotopico ha permesso di evidenziare come *Diaphanosoma* sia vicariante di *Daphnia* nel periodo autunnale. In primavera-estate *Leptodora* e *Eudiaptomus* sembrano svolgere un ruolo trofico confrontabile. *Bythotrephes* e i ciclopidi sembrano avere a maggio e novembre un ruolo paragonabile a quello dell'agone. In generale, non solamente *Rutilus*, ma anche *Alosa* e *Coregonus* sembrano dipendere in misura non trascurabile da fonti non pelagiche di alimento. Nell'insieme, i risultati ottenuti offrono un elemento importante verso la definizione di quella *operational diversity* che è alla base di una gestione sostenibile dell'ambiente.

PAROLE CHIAVE: laghi / reti alimentari pelagiche / fingerprint isotopico / zooplancton / pesci

Pelagic food web of Lake Maggiore: results of SIA in 2009

During 2009, studies on Lake Maggiore pelagic food web were extended to small sized cladocerans and to littoral organisms. We were able to reconstruct seasonal changes in isotopic fingerprint and in the trophic relations of pelagic organisms. We found that *Daphnia* seasonality, related to changes the signature of the food, was also reflected by other organisms, thus suggesting a main dependence from pelagic carbon sources to different taxa. During winter, *Diaphanosoma* replaced *Daphnia* in its trophic role, while during spring and summer *Eudiaptomus* appeared to play the same role as *Leptodora*. Along the year, the signatures of littoral and pelagic food chains changed, overlapping during summer to be clearly separated in spring and autumn. *Bythotrephes* and the cyclopoids seemed vicariate of zooplantkivorous fish, particularly *Alosa*, in November. Not only *Rutilus*, but also *Alosa* and *Coregonus* relied from littoral food sources more than expected. Overall, the study gave a contribution for a characterization of *operational diversity*, which represents the basis for a sustainable environmental management.

KEY WORDS: lakes / pelagic food web / isotopic fingerprint / zooplankton / fish

Nel 2009 le indagini relative alla caratterizzazione della rete trofica pelagica del Lago Maggiore mediante analisi d'isotopi stabili di carbonio e azoto sono proseguite con due scopi principali: 1) estendere le analisi anche agli organismi zooplanctonici di piccola taglia e basso peso individuale, quali *Bosmina* e *Diaphanosoma*, per i quali è necessario un elevato numero d'individui; 2) dare una prima caratterizzazione dei segnali isotopici di organismi di zone litorali e prossime ai corsi d'acqua.

In diversi momenti dell'anno sono stati realizzati campionamenti di materiale zooplanctonico in zona pelagica, con una frequenza maggiore rispetto a quella stagionale prevista, tale da permettere la raccolta, oltre che degli organismi consueti, anche dei cladoceri di piccole dimensioni che, essendo soggetti ad improvvise e poco durature fasi di sviluppo numerico, possono sfuggire ad un campionamento su base stagionale. Detti campionamenti sono stati affiancati da raccolte di organismi in diverse zone del litorale lacustre.

Il materiale zooplanctonico è stato raccolto nelle tre diverse stazioni a suo tempo prescelte come tendenzialmente rappresentative di diverse situazioni: la stazione ubicata al largo di Ghiffa (latitudine 45°57'30"N; longitudine 8°39'09" E), sito elettivo per il monitoraggio del pelago lacustre; quella denominata "Baveno" (latitudine 45°54'28" N; longitudine 8°31'44"E), per la quale studi pregressi indicavano l'influenza del trasporto di materiale alloctono ad opera del Fiume Toce; e quella di Lesa (latitudine 45°49' 70"N; longitudine 8°34'70" E), ove maggiore è l'influenza del litorale. In aggiunta a queste, raccolte di altri organismi (perifiton, larve di ditteri chironomidi, oligocheti e anfipodi) sono state effettuate a marzo e a giugno in diverse zone del litorale lungo l'asse principale del lago e in prossimità della foce del Fiume Toce.

Ad ogni data di campionamento, organismi appartenenti ai diversi taxa zooplanctonici sono stati raccolti in quantità sufficienti a consentire analisi del segnale isotopico (Manca *et al.*, 1994; 1997). Onde ottenere campioni adatti a garantire risultati attendibili delle analisi strumentali è stato necessario prelevare, di volta in volta, e per ogni replica, un numero d'individui compreso tra un minimo di 40 e un massimo di 500, in dipendenza dalla taglia individuale.

Nel contempo, campioni quantitativi prelevati con le usuali metodiche sono serviti a determinare la densità di popolazione e la biomassa zooplanctonica nei differenti momenti stagionali e nelle tre stazioni. Sono state inoltre misurate la temperatura dello strato d'acqua campionato mediante termometro termistore e la trasparenza delle acque mediante Disco di Secchi.

Nel 2009 è stato inoltre completato il quadro relativo alla caratterizzazione del *fingerprint* isotopico, e delle sue modificazioni nel corso dell'anno, delle tre diverse specie di pesci previste dal progetto: agone (*Alosa fallax lacustris*), coregone (*Coregonus lavaretus*) e gardon (*Rutilus rutilus*), campionati nel 2008, catturati mensilmente con reti branchiali in zona pelagica (coregone e agone) e litorale (gardon) nella regione centrale del Lago Maggiore. Campioni di muscolo dorsale sono stati ottenuti da cinque individui per specie. Dei singoli pesci è stata misurata la taglia (lunghezza totale e peso totale) e determinata l'età mediante lettura delle scaglie, al fine di ottenere campioni il più possibile omogenei per taglia ed età, sì da limitare la variabilità legata a possibili differenti abitudini alimentari. In totale sono stati analizzati circa 1500 campioni di organismi zooplanctonici, 300 di pesci e un centinaio di organismi di zone litorali.

Tutti i campioni sono stati essiccati, polverizzati e pesati al fine di ottenere un peso idoneo a garantire il livello di precisione richiesto dalle analisi isotopiche (0,7-1 mg). I campioni, posti in capsulini di stagno, sono stati caricati in analizzatore elementare (Vario EL III) interfacciato con uno spettrometro di massa per il calcolo del rapporto isotopico (G. G. Hatch Isotope Laboratories, University of Ottawa, Faculty of Science, Ottawa, Canada). I campioni e gli standard sono stati combusti a circa 1800 °C (combustione di Dumas) e i prodotti gassosi risultanti trasportati in elio attraverso colonne di ossido-riduzione ottimizzate per CO_2 e N_2. I gas, separati mediante colonne di assorbimento a purificazione e cattura, venivano inviati all'interfaccia (Conflo II) e all'IRMS (Isotope Ratio Mass Spectrometer della Delta XP Plus Advantage). La precisione analitica delle analisi era basata su uno standard interno (C-55, acido glutamico, $\delta^{15}N$, $\delta^{13}C$ = 3,98, -28,53, rispettivamente) solitamente con precisione analitica inferiore allo 0,2 ‰.

I valori di $\delta^{15}N$ sono stati riferiti all'azoto atmosferico e normalizzati con standard calibrati all'International standard IAEA-N1(+0,4 ‰), IAEA-N2(+20,3 ‰), USGS-40(-4,52 ‰) e USGS-41(47,57 ‰). I valori di $\delta^{13}C$, riferiti alla PDB (Belemnite di Pee Dee) sono stati normalizzati con standard interni calibrati presso l'International standard IAEA-CH-6(-10,4 ‰), NBS-22(-29,91‰), USGS-40(-26,24 ‰) e USGS-41(37,76‰). La deviazione standard dei valori relativi ai campioni di invertebrati, perifiton e pesci è risultata compresa tra 0,05-0,06 e 0,12 per ^{13}C e ^{15}N, rispettivamente.

Nel pelago del Lago Maggiore, indipendentemente dalla stazione di campionamento, il *fingerprint* isotopico di *Daphnia* è risultato correlato a un elevato livello di significatività statistica con quello del materiale sestonico (<76 µm) che ne costituisce la base alimentare (Manca *et al.*, 2009). Pertanto, *Daphnia* rappresenta un buon integratore della linea di base pelagica e delle sue variazioni nel corso dell'anno. Sono quindi state investigate le relazioni tra i taxa e la linea di base rappresentata da *Daphnia*, e i valori delle prime sono stati comparati con quelli previsti per il frazionamento trofico di ^{13}C e ^{15}N. Un *taxon* è stato considerato supportato dalla linea di base pelagica quando il suo $\delta^{13}C$ differiva meno di 1-2 volte rispetto a quello misurato per *Daphnia*. Qualora esso fosse differente, si è ipotizzato l'utilizzo di fonti multiple di alimentazione da parte della comunità zooplanctonica. In particolare, il segnale di organismi provenienti da zone diverse da quella pelagica, prelevati in diversi punti del litorale lacustre, sono serviti a identificare l'importanza di fonti non pelagiche di supporto alla produzione secondaria. Anche la posizione trofica relativa dei diversi *taxa*, e le sue variazioni nel corso dell'anno, sono state riferite a *Daphnia*. Perché la posizione trofica possa essere ritenuta costante, l'arricchimento rispetto a *Daphnia* deve mantenersi costante nel corso dell'anno.

I risultati delle analisi relative al segnale isotopico del carbonio nei diversi mesi dell'anno e per le diverse componenti prese in esame sono riassunti nel grafico in figura 1. La scala del $\delta^{13}C$ comprende valori rappresentativi dell'ambiente litorale (meno negativi) e di quello pelagico di laghi profondi, quali il Maggiore. Generalmente, organismi di acque più profonde presentano valori del $\delta^{13}C$ più negativi rispetto a quelli di acque più superficiali. Il grafico può dunque essere letto, dall'alto verso il basso, come una graduale transizione da zone più prossime alla superficie a zone più profonde del lago.

La linea di base del segnale isotopico del C presenta una spiccata stagionalità, con una fase di aumento graduale fino al raggiungimento del valore massimo in agosto e un successivo, più lento, declino in autunno. Questo andamento ricalca fedelmente l'aumento delle temperature medie nella colonna d'acqua campionata (i primi 50 metri) con il procedere della stagione calda, e questo fa sì che le due variabili, il $\delta^{13}C$ della linea di base e la temperatura, siano fra loro correlate a un alto livello di significatività statistica (Manca *et al.*, 2009). Tale correlazione rispecchia l'aumento del valore di $\delta^{13}C$ del fitoplancton durante la stratificazione termica (Zohary *et al.*, 1994), conseguente ad un maggiore frazionamento del $\delta^{13}C$ del fitoplancton, dovuto al graduale esaurimento della CO_2 disciolta in epilimnio, con l'incremento dei produttori primari. In autunno e in inverno, la diminuzione del segnale isotopico del carbonio è il risultato della graduale de-stratificazione che porta ad un rinnovato apporto del carbonio inorganico disciolto dall'ipolimnio, culminante nel minimo stagionale, solitamente osservato durante il mescolamento invernale (Zohary *et al.*, 1994). La buona correlazione tra il segnale isotopico di *Daphnia* e la temperatura può essere ritenuta come l'indicazione di una prevalente dipendenza di questo cladocero da fonti alimentari fitoplanctoniche. Al contrario, in situazioni nelle quali tale correlazione sia più debole, s'ipotizza un ruolo non trascurabile di fonti non fitoplanctoniche di materia ed energia per lo sviluppo e la crescita nel corso dell'anno della popolazione.

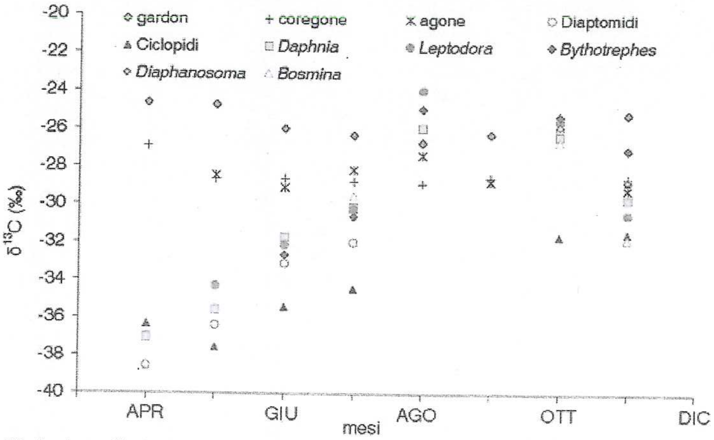


Fig. 1. Valori medi del rapporto isotopico del carbonio ($\delta^{13}C$‰) misurati nel corso del 2009 per diversi taxa zooplanctonici pelagici del Lago Maggiore e in tre diverse specie di pesci.

La dipendenza dalla linea di base pelagica è abbastanza evidente per la maggior parte dei *taxa* zooplanctonici analizzati nella maggior parte dell'anno. La sovrapposizione è particolarmente evidente a giugno, luglio ed ottobre per tutti i *taxa* ad eccezione dei ciclopidi. Questi ultimi, inizialmente molto prossimi a *Daphnia* nel segnale isotopico, se ne differenziano in modo significativo durante la maggior parte dell'anno, durante la quale si registrano valori nettamente più negativi rispetto a quelli misurati per gli altri componenti dello zooplancton pelagico. I valori più negativi dei ciclopidi rispetto a quelli misurati in *Daphnia* (valore medio di $\delta^{13}C$ inferiore del 6 ‰ in ottobre) potrebbero essere anche legittimati da una loro dipendenza dal segnale isotopico di acque più profonde rispetto a quelle rappresentate da *Daphnia*. Le misure su *Dreissena polymorpha*, il mollusco bivalve solitamente utilizzato come integratore conservativo della linea di base del pelago, indicano per il Maggiore valori medi di $\delta^{13}C$ di -35,14 ‰, molto simili a quelli misurati nei ciclopidi a luglio.

Nel 2009 sono stati campionati, oltre ai cladoceri di grandi dimensioni, anche quelli di piccola taglia, dei generi *Bosmina* e *Diaphanosoma*. Come si evince dall'esame del grafico in figura 1, in luglio ed ottobre, il *fingerprint* isotopico del carbonio di *Bosmina* ha sostanzialmente coinciso con quello di *Daphnia*, indicando un'identità di fonti alimentari; diversa è la situazione rilevata a novembre, quando il segnale di *Bosmina* coincide con quello misurato per i ciclopidi, indicando una dipendenza da zone del pelago più profonde rispetto a quelle abitate da *Daphnia*.

Molto prossimi a quelli di

Daphnia sono risultati i *fingerprint* isotopici del carbonio di *Diaphanosoma* in ottobre e novembre, fatto che sta ad indicare una dipendenza di quest'ultimo dalle stesse fonti alimentari utilizzate da *Daphnia*.

Il segnale isotopico di *Bythotrephes* è apparso invece discostarsi a novembre da quello di *Daphnia*, e questo potrebbe essere indicativo di una dipendenza da fonti più prossime al litorale. Un legame di *Byhtotrephes* con il litorale lacustre è stato ipotizzato in passato come possibile spiegazione alle modificazioni nella durata della presenza di quest'organismo zooplanctonico nel pelago, diminuita in anni d'intense precipitazioni e in regime di piena (Manca e DeMott, 2009).

Nel corso del 2009 sono state completate anche le analisi relative alle tre specie di pesci del Maggiore. I dati, rappresentati nel grafico in figura 1 indicano una generale dipendenza da fonti non pelagiche di alimento, con valori di $\delta^{13}C$ caratteristici di zone poco profonde e di organismi litorali. Tale dato è nettamente evidente per il gardon, specie ad abitudini litorali, il cui fingerprint si colloca intorno a valori di $\delta^{13}C$ -26 ‰, ma è anche abbastanza chiaro per le altre due specie, coregone e agone, ad abitudini più pelagiche. La scarsa dipendenza da fonti pelagiche di coregone e agone sussiste fino a giugno, ed è solamente a luglio che il loro segnale isotopico si approssima a quello degli zooplanctonti pelagici, indicandone da essi una stretta dipendenza alimentare. Si osserva inoltre come, queste due specie siano perfettamente sovrapponibili, quanto a base alimentare, fatta eccezione per agosto, quando il segnale isotopico di coregone sembra indicare anche l'utilizzo di fonti di carbonio da acque più profonde rispetto a quelle utilizzate dai principali organismi costituenti lo zooplancton al momento del campionamento.

Il segnale isotopico dell'azoto, rappresentato nel grafico in figura 2, mette in luce come varino i rapporti trofici tra gli organismi zooplanctonici, e di quelli delle tre

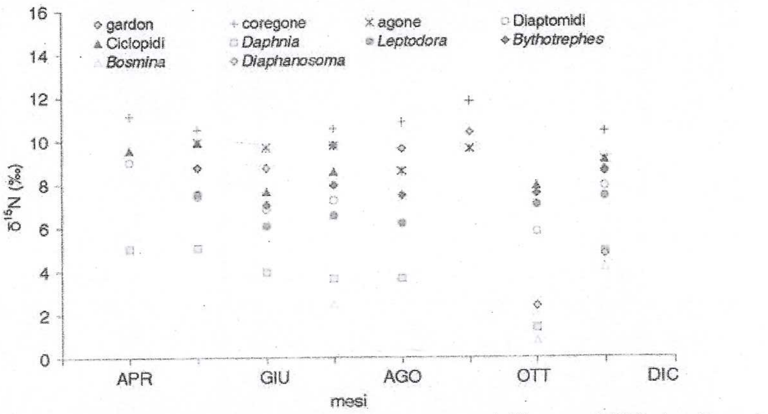


Fig. 2. Valori isotopici del $\delta^{15}N$ misurati nel corso dell'anno 2009 in diversi organismi zooplanctonici, e in tre specie di pesci, del Lago Maggiore.

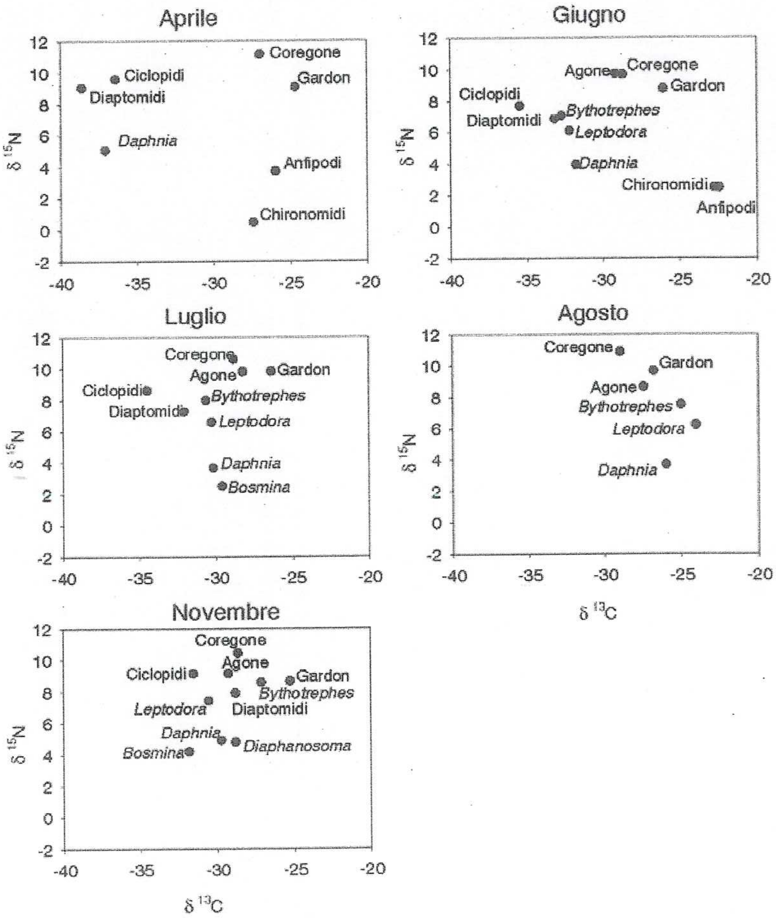


Fig. 3. Grafici trofochimici relativi ai segnali isotopici rilevati in diversi mesi del 2009 nei principali organismi zooplanctonici, del bentos litorale, ed in tre diverse specie di pesci del Lago Maggiore.

diverse specie ittiche, nel corso dell'anno.

Tendenzialmente, *Bosmina* presenta valori di arricchimento lievemente più bassi di quelli di *Daphnia*, in tutti e tre i mesi nei quali è stata rinvenuta. Quest'ultima presenta valori di $\delta^{15}N$ più elevati in primavera, tendenzialmente più bassi in estate, e minimi in ottobre. Un arricchimento tra il 2 e il 3 ‰ rispetto a *Daphnia* caratterizza *Leptodora* nei periodi primaverile ed estivo, mentre valori ben più elevati sono stati rilevati nel mese di ottobre, quando il *fingerprint* isotopico di *Daphnia* risultava molto basso, prossimo a quello di *Bosmina*.

Tra gli organismi zooplanctonici, *Leptodora* è risultata arricchita in $\delta^{15}N$ in modo significativo solamente a ottobre, con una differenza rispetto a *Daphnia* di oltre il 5 ‰, e dell'1,5 ‰ rispetto ai diaptomidi, non sufficiente a giustificarne l'attribuzione ad un livello trofico più elevato rispetto a questi ultimi.

Il segnale isotopico dell'azoto di *Bythotrephes* è risultato, in generale, superiore rispetto a quello di *Leptodora* (Fig. 2), con un arricchimento rispetto a *Daphnia* compreso tra un minimo del 3 ‰ (a maggio) e un massimo di circa il 6 ‰ (in ottobre). I copepodi ciclopidi sono risultati caratterizzati da valori di $\delta^{15}N$ prossimi a quelli di *Bythotrephes*, in taluni casi sovrapponibili a quelli di agone e coregone (i.e. a maggio ed a novembre).

Molto interessante è apparso anche il fingerprint del gardon, inferiore rispetto a quelli di coregone e agone da aprile a giugno, coincidente con quello del secondo a luglio e compreso tra i due in agosto e settembre. L'arricchimento massimo in ogni data di campionamento è risultato quello del coregone, valori compresi tra un minimo del 5,5 ‰ (a maggio) e un massimo del 7,2 ‰ (in agosto).

Il complesso dei dati raccolti nel 2009 (Fig. 3) consente di caratterizzare le modificazioni stagionali nella rete trofica pelagica e di dare una prima descrizione quantitativa di quella litorale, identificando alcune possibili interazioni tra le due. I dati dimostrano la necessità di investigare i *fingerprint* isotopici del bentos litorale in diversi momenti dell'anno e su substrati sia inorganici sia fitofili, sì da poter meglio caratterizzare il segnale misurato nelle tre specie di pesci, indicativo di un ruolo non trascurabile, e per certi versi inatteso, di fonti non planctoniche di alimento. Da ultimo, la perfetta sovrapponibilità dei valori ottenuti per *Diaphanosoma* e *Daphnia* a novembre, suggerisce come il primo possa avere, nella rete trofica pelagica del Lago Maggiore, un ruolo vicariante rispetto alla seconda. Questo, e altri risultati emersi durante i primi due anni d'indagine, dimostrano come l'approccio di tipo funzionale, alla base delle analisi di isotopi stabili di carbonio e azoto, sia di fondamentale importanza per la definizione di quella *operational diversity* sulla quale si fonda una gestione sostenibile dell'ambiente, finalizzata al mantenimento e, ove necessario alla riabilitazione, delle strutture ecologiche (Moss *et al.*, 2009).

BIBLIOGRAFIA

Manca M., DeMott W.R., 2009. Response of the invertebrate predator *Bythotrephes* to a climate-linked increase in the duration of a refuge from fish predation. *Limnol. Oceanogr.*, **54**(6, part 2): 2506-2512.

Manca M., Spagnuolo T., Comoli P., 1994. Variations in carbon and nitrogen content with body length of *Daphnia hyalina-galeata* s.l. from laboratory and field observations. *J. Plankton Res.*, **16**(10): 1303-1314.

Manca M., Comoli P., Spagnuolo T., 1997. Length-specific carbon content of the *Daphnia* population in a large Italian subalpine lake, Lago Maggiore (Northern Italy): the importance of seasonality. *Aquat. Sci.*, **59**: 48-56.

Manca M., Visconti A., Fadda A., Caroni R., Cerutti I., Volta P., 2009. Indagini sullo zooplancton e sulla rete trofica pelagica attraverso analisi d'isotopi stabili di carbonio e azoto. In: C.N.R.-I.S.E. Sede di Verbania. *Ricerche sull'evoluzione del Lago Maggiore. Aspetti limnologici. Programma quinquennale 2008-2012. Campagna 2008.* Commissione Internazionale per la protezione delle acque italo-svizzere (Ed.): 34-52.

Moss B., Hering D., Green A.J., Aidoud A., Becares E., Beklioglu M., Bennion H., Boix D., Brucet S., Carvalho L., Clement B., Davidson T., Declerck S., Dobson M., van Donk E., Dudley B., Feuchtmayr H., Friberg N., Grenouillet G., Hillebran H., Hobaek A., Irvine K., Jeppesen E., R Johnson., Jones I., Kernan M., Lauridsen T.L., Manca M., Meerhoff M., Olafsson J., Ormerod S., Papastergiadou E., Penning W.E., Ptacnik R., Quintana X., Sandin L., Seferlis M., Simpson G., Trigal C., Verdonschot P., Verschoor A.M., Weyhenmeyer G.A., 2009. Climate change and the future of freshwater biodiversity in Europe: a primer for policy-makers. *Freshwater Reviews*: **2**: 103-130.

Zohary T., Erez J., Gophen M., Berman-Frank I., Stiller M.. 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. *Limnol. Oceanogr.*, **39**: 1030-1043.

Indagini sulla rete trofica pelagica del Lago Maggiore mediante analisi d'isotopi stabili di carbonio e azoto (SIA): risultati della campagna 2009§

Amedeo Fadda, Anna Visconti, Benedetta Ponti, Marina Manca*

CNR ISE, Largo Tonolli, 50 - 28922 Verbania (VB)

* Referente per la corrispondenza: m.manca@ise.cnr.it

§ Lavoro realizzato con il contributo della Commissione Internazionale per la Protezione delle Acque Italo-Svizzere nell'ambito delle ricerche sugli aspetti limnologici del Lago Maggiore, Campagna 2009. Funded by the International Commission for the Protection of Swiss-Italian Waters on Lake Maggiore.

Riassunto

Gli studi sulla rete trofica pelagica del Lago Maggiore mediante SIA sono stati estesi nel 2009 a cladoceri di piccola taglia e a organismi litorali. La spiccata stagionalità di *Daphnia* riflette quella della linea di base del pelago, e da essa sembra dipendere la maggior parte degli zooplanctonti analizzati. L'analisi del *fingerprint* isotopico ha permesso di evidenziare come *Diaphanosoma* sia vicariante di *Daphnia* nel periodo autunnale. In primavera-estate *Leptodora* e *Eudiaptomus* sembrano svolgere un ruolo trofico confrontabile. *Bythotrephes* e i ciclopidi sembrano avere a maggio e novembre un ruolo paragonabile a quello dell'agone. In generale, non solamente *Rutilus*, ma anche *Alosa* e *Coregonus* sembrano dipendere in misura non trascurabile da fonti non pelagiche di alimento. Nell'insieme, i risultati ottenuti offrono un elemento importante verso la definizione di quella *operational diversity* che è alla base di una gestione sostenibile dell'ambiente.

PAROLE CHIAVE: laghi / reti alimentari pelagiche / fingerprint isotopico / zooplancton / pesci

Pelagic food web of Lake Maggiore: results of SIA in 2009

During 2009, studies on Lake Maggiore pelagic food web were extended to small sized cladocerans and to littoral organisms. We were able to reconstruct seasonal changes in isotopic fingerprint and in the trophic relations of pelagic organisms. We found that *Daphnia* seasonality, related to changes the signature of the food, was also reflected by other organisms, thus suggesting a main dependence from pelagic carbon sources to different taxa. During winter, *Diaphanosoma* replaced *Daphnia* in its trophic role, while during spring and summer *Eudiaptomus* appeared to play the same role as *Leptodora*. Along the year, the signatures of littoral and pelagic food chains changed, overlapping during summer to be clearly separated in spring and autumn. *Bythotrephes* and the cyclopoids seemed vicariate of zooplantkivorous fish, particularly *Alosa*, in November. Not only *Rutilus*, but also *Alosa* and *Coregonus* relied from littoral food sources more than expected. Overall, the study gave a contribution for a characterization of *operational diversity*, which represents the basis for a sustainable environmental management.

KEY WORDS: lakes / pelagic food web / isotopic fingerprint / zooplankton / fish

Nel 2009 le indagini relative alla caratterizzazione della rete trofica pelagica del Lago Maggiore mediante analisi d'isotopi stabili di carbonio e azoto sono proseguite con due scopi principali: 1) estendere le analisi anche agli organismi zooplanctonici di piccola taglia e basso peso individuale, quali *Bosmina* e *Diaphanosoma*, per i quali è necessario un elevato numero d'individui; 2) dare una prima caratterizzazione dei segnali isotopici di organismi di zone litorali e prossime ai corsi d'acqua.

In diversi momenti dell'anno sono stati realizzati campionamenti di materiale zooplanctonico in zona pelagica, con una frequenza maggiore rispetto a quella stagionale prevista, tale da permettere la raccolta, oltre che degli organismi consueti, anche dei cladoceri di piccole dimensioni che, essendo soggetti ad improvvise e poco durature fasi di sviluppo numerico, possono sfuggire ad un campionamento su base stagionale. Detti campionamenti sono stati affiancati da raccolte di organismi in diverse zone del litorale lacustre.

Il materiale zooplanctonico è stato raccolto nelle tre diverse stazioni a suo tempo prescelte come tendenzialmente rappresentative di diverse situazioni: la stazione ubicata al largo di Ghiffa (latitudine 45°57'30"N; longitudine 8°39'09" E), sito elettivo per il monitoraggio del pelago lacustre; quella denominata "Baveno" (latitudine 45°54'28" N; longitudine 8°31'44"E), per la quale studi pregressi indicavano l'influenza del trasporto di materiale alloctono ad opera del Fiume Toce; e quella di Lesa (latitudine 45°49' 70"N; longitudine 8°34'70" E), ove maggiore è l'influenza del litorale. In aggiunta a queste, raccolte di altri organismi (perifiton, larve di ditteri chironomidi, oligocheti e anfipodi) sono state effettuate a marzo e a giugno in diverse zone del litorale lungo l'asse principale del lago e in prossimità della foce del Fiume Toce.

Ad ogni data di campionamento, organismi appartenenti ai diversi taxa zooplanctonici sono stati raccolti in quantità sufficienti a consentire analisi del segnale isotopico (Manca *et al.*, 1994; 1997). Onde ottenere campioni adatti a garantire risultati attendibili delle analisi strumentali è stato necessario prelevare, di volta in volta, e per ogni replica, un numero d'individui compreso tra un minimo di 40 e un massimo di 500, in dipendenza dalla taglia individuale.

Nel contempo, campioni quantitativi prelevati con le usuali metodiche sono serviti a determinare la densità di popolazione e la biomassa zooplanctonica nei differenti momenti stagionali e nelle tre stazioni. Sono state inoltre misurate la temperatura dello strato d'acqua campionato mediante termometro termistore e la trasparenza delle acque mediante Disco di Secchi.

Nel 2009 è stato inoltre completato il quadro relativo alla caratterizzazione del *fingerprint* isotopico, e delle sue modificazioni nel corso dell'anno, delle tre diverse specie di pesci previste dal progetto: agone (*Alosa fallax lacustris*), coregone (*Coregonus lavaretus*) e gardon (*Rutilus rutilus*), campionati nel 2008, catturati mensilmente con reti branchiali in zona pelagica (coregone e agone) e litorale (gardon) nella regione centrale del Lago Maggiore. Campioni di muscolo dorsale sono stati ottenuti da cinque individui per specie. Dei singoli pesci è stata misurata la taglia (lunghezza totale e peso totale) e determinata l'età mediante lettura delle scaglie, al fine di ottenere campioni il più possibile omogenei per taglia ed età, sì da limitare la variabilità legata a possibili differenti abitudini alimentari. In totale sono stati analizzati circa 1500 campioni di organismi zooplanctonici, 300 di pesci e un centinaio di organismi di zone litorali.

Tutti i campioni sono stati essiccati, polverizzati e pesati al fine di ottenere un peso idoneo a garantire il livello di precisione richiesto dalle analisi isotopiche (0,7-1 mg). I campioni, posti in capsulini di stagno, sono stati caricati in analizzatore elementare (Vario EL III) interfacciato con uno spettrometro di massa per il calcolo del rapporto isotopico (G. G. Hatch Isotope Laboratories, University of Ottawa, Faculty of Science, Ottawa, Canada). I campioni e gli standard sono stati combusti a circa 1800 °C (combustione di Dumas) e i prodotti gassosi risultanti trasportati in elio attraverso colonne di ossido-riduzione ottimizzate per CO_2 e N_2. I gas, separati mediante colonne di assorbimento a purificazione e cattura, venivano inviati all'interfaccia (Conflo II) e all'IRMS (Isotope Ratio Mass Spectrometer della Delta XP Plus Advantage). La precisione analitica delle analisi era basata su uno standard interno (C-55, acido glutamico, $\delta^{15}N$, $\delta^{13}C$ = 3,98, -28,53, rispettivamente) solitamente con precisione analitica inferiore allo 0,2 ‰.

I valori di $\delta^{15}N$ sono stati riferiti all'azoto atmosferico e normalizzati con standard calibrati all'International standard IAEA-N1(+0,4 ‰), IAEA-N2(+20,3 ‰), USGS-40(-4,52 ‰) e USGS-41(47,57 ‰). I valori di $\delta^{13}C$, riferiti alla PDB (Belemnite di Pee Dee) sono stati normalizzati con standard interni calibrati presso l'International standard IAEA-CH-6(-10,4 ‰), NBS-22(-29,91‰), USGS-40(-26,24 ‰) e USGS-41(37,76‰). La deviazione standard dei valori relativi ai campioni di invertebrati, perifiton e pesci è risultata compresa tra 0,05-0,06 e 0,12 per ^{13}C e ^{15}N, rispettivamente.

Nel pelago del Lago Maggiore, indipendentemente dalla stazione di campionamento, il *fingerprint* isotopico di *Daphnia* è risultato correlato a un elevato livello di significatività statistica con quello del materiale sestonico (<76 µm) che ne costituisce la base alimentare (Manca *et al.*, 2009). Pertanto, *Daphnia* rappresenta un buon integratore della linea di base pelagica e delle sue variazioni nel corso dell'anno. Sono quindi state investigate le relazioni tra i taxa e la linea di base rappresentata da *Daphnia*, e i valori delle prime sono stati comparati con quelli previsti per il frazionamento trofico di ^{13}C e ^{15}N. Un *taxon* è stato considerato supportato dalla linea di base pelagica quando il suo $\delta^{13}C$ differiva meno di 1-2 volte rispetto a quello misurato per *Daphnia*. Qualora esso fosse differente, si è ipotizzato l'utilizzo di fonti multiple di alimentazione da parte della comunità zooplanctonica. In particolare, il segnale di organismi provenienti da zone diverse da quella pelagica, prelevati in diversi punti del litorale lacustre, sono serviti a identificare l'importanza di fonti non pelagiche di supporto alla produzione secondaria. Anche la posizione trofica relativa dei diversi *taxa*, e le sue variazioni nel corso dell'anno, sono state riferite a *Daphnia*. Perché la posizione trofica possa essere ritenuta costante, l'arricchimento rispetto a *Daphnia* deve mantenersi costante nel corso dell'anno.

I risultati delle analisi relative al segnale isotopico del carbonio nei diversi mesi dell'anno e per le diverse componenti prese in esame sono riassunti nel grafico in figura 1. La scala del δ^{13}C comprende valori rappresentativi dell'ambiente litorale (meno negativi) e di quello pelagico di laghi profondi, quali il Maggiore. Generalmente, organismi di acque più profonde presentano valori del δ^{13}C più negativi rispetto a quelli di acque più superficiali. Il grafico può dunque essere letto, dall'alto verso il basso, come una graduale transizione da zone più prossime alla superficie a zone più profonde del lago.

La linea di base del segnale isotopico del C presenta una spiccata stagionalità, con una fase di aumento graduale fino al raggiungimento del valore massimo in agosto e un successivo, più lento, declino in autunno. Questo andamento ricalca fedelmente l'aumento delle temperature medie nella colonna d'acqua campionata (i primi 50 metri) con il procedere della stagione calda, e questo fa sì che le due variabili, il δ^{13}C della linea di base e la temperatura, siano fra loro correlate a un alto livello di significatività statistica (Manca *et al.*, 2009). Tale correlazione rispecchia l'aumento del valore di δ^{13}C del fitoplancton durante la stratificazione termica (Zohary *et al.*, 1994), conseguente ad un maggiore frazionamento del δ^{13}C del fitoplancton, dovuto al graduale esaurimento della CO_2 disciolta in epilimnio, con l'incremento dei produttori primari. In autunno e in inverno, la diminuzione del segnale isotopico del carbonio è il risultato della graduale de-stratificazione che porta ad un rinnovato apporto del carbonio inorganico disciolto dall'ipolimnio, culminante nel minimo stagionale, solitamente osservato durante il mescolamento invernale (Zohary *et al.*, 1994). La buona correlazione tra il segnale isotopico di *Daphnia* e la temperatura può essere ritenuta come l'indicazione di una prevalente dipendenza di questo cladocero da fonti alimentari fitoplanctoniche. Al contrario, in situazioni nelle quali tale correlazione sia più debole, s'ipotizza un ruolo non trascurabile di fonti non fitoplanctoniche di materia ed energia per lo sviluppo e la crescita nel corso dell'anno della popolazione.

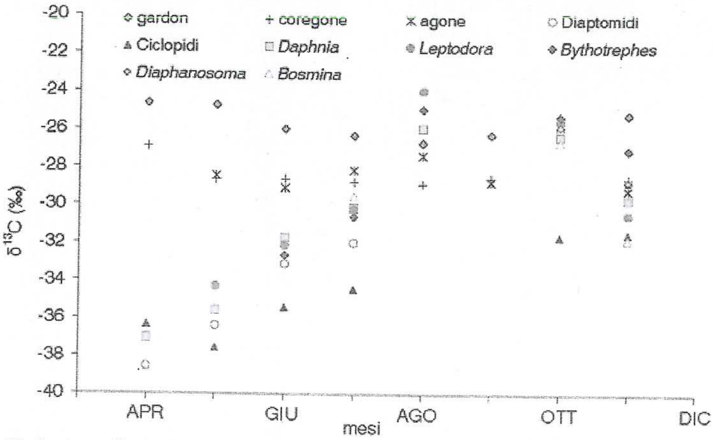


Fig. 1. Valori medi del rapporto isotopico del carbonio (δ^{13}C‰) misurati nel corso del 2009 per diversi taxa zooplanctonici pelagici del Lago Maggiore e in tre diverse specie di pesci.

La dipendenza dalla linea di base pelagica è abbastanza evidente per la maggior parte dei *taxa* zooplanctonici analizzati nella maggior parte dell'anno. La sovrapposizione è particolarmente evidente a giugno, luglio ed ottobre per tutti i *taxa* ad eccezione dei ciclopidi. Questi ultimi, inizialmente molto prossimi a *Daphnia* nel segnale isotopico, se ne differenziano in modo significativo durante la maggior parte dell'anno, durante la quale si registrano valori nettamente più negativi rispetto a quelli misurati per gli altri componenti dello zooplancton pelagico. I valori più negativi dei ciclopidi rispetto a quelli misurati in *Daphnia* (valore medio di δ^{13}C inferiore del 6 ‰ in ottobre) potrebbero essere anche legittimati da una loro dipendenza dal segnale isotopico di acque più profonde rispetto a quelle rappresentate da *Daphnia*. Le misure su *Dreissena polymorpha*, il mollusco bivalve solitamente utilizzato come integratore conservativo della linea di base del pelago, indicano per il Maggiore valori medi di δ^{13}C di -35,14 ‰, molto simili a quelli misurati nei ciclopidi a luglio.

Nel 2009 sono stati campionati, oltre ai cladoceri di grandi dimensioni, anche quelli di piccola taglia, dei generi *Bosmina* e *Diaphanosoma*. Come si evince dall'esame del grafico in figura 1, in luglio ed ottobre, il *fingerprint* isotopico del carbonio di *Bosmina* ha sostanzialmente coinciso con quello di *Daphnia*, indicando un'identità di fonti alimentari; diversa è la situazione rilevata a novembre, quando il segnale di *Bosmina* coincide con quello misurato per i ciclopidi, indicando una dipendenza da zone del pelago più profonde rispetto a quelle abitate da *Daphnia*.

Molto prossimi a quelli di

Daphnia sono risultati i *fingerprint* isotopici del carbonio di *Diaphanosoma* in ottobre e novembre, fatto che sta ad indicare una dipendenza di quest'ultimo dalle stesse fonti alimentari utilizzate da *Daphnia*.

Il segnale isotopico di *Bythotrephes* è apparso invece discostarsi a novembre da quello di *Daphnia*, e questo potrebbe essere indicativo di una dipendenza da fonti più prossime al litorale. Un legame di *Byhtotrephes* con il litorale lacustre è stato ipotizzato in passato come possibile spiegazione alle modificazioni nella durata della presenza di quest'organismo zooplanctonico nel pelago, diminuita in anni d'intense precipitazioni e in regime di piena (Manca e DeMott, 2009).

Nel corso del 2009 sono state completate anche le analisi relative alle tre specie di pesci del Maggiore. I dati, rappresentati nel grafico in figura 1 indicano una generale dipendenza da fonti non pelagiche di alimento, con valori di $\delta^{13}C$ caratteristici di zone poco profonde e di organismi litorali. Tale dato è nettamente evidente per il gardon, specie ad abitudini litorali, il cui fingerprint si colloca intorno a valori di $\delta^{13}C$ -26 ‰, ma è anche abbastanza chiaro per le altre due specie, coregone e agone, ad abitudini più pelagiche. La scarsa dipendenza da fonti pelagiche di coregone e agone sussiste fino a giugno, ed è solamente a luglio che il loro segnale isotopico si approssima a quello degli zooplanctonti pelagici, indicandone da essi una stretta dipendenza alimentare. Si osserva inoltre come, queste due specie siano perfettamente sovrapponibili, quanto a base alimentare, fatta eccezione per agosto, quando il segnale isotopico di coregone sembra indicare anche l'utilizzo di fonti di carbonio da acque più profonde rispetto a quelle utilizzate dai principali organismi costituenti lo zooplancton al momento del campionamento.

Il segnale isotopico dell'azoto, rappresentato nel grafico in figura 2, mette in luce come varino i rapporti trofici tra gli organismi zooplanctonici, e di quelli delle tre

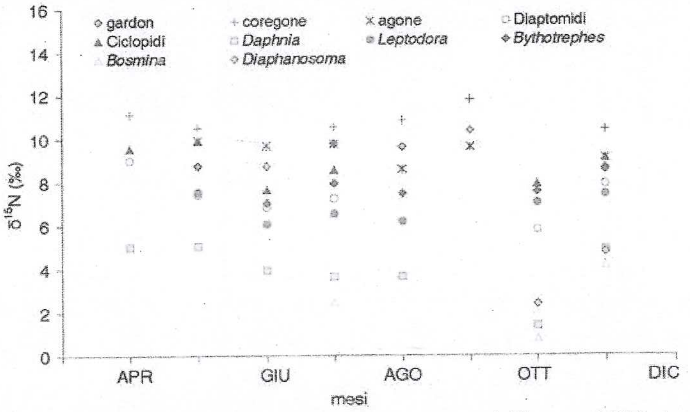


Fig. 2. Valori isotopici del $\delta^{15}N$ misurati nel corso dell'anno 2009 in diversi organismi zooplanctonici, e in tre specie di pesci, del Lago Maggiore.

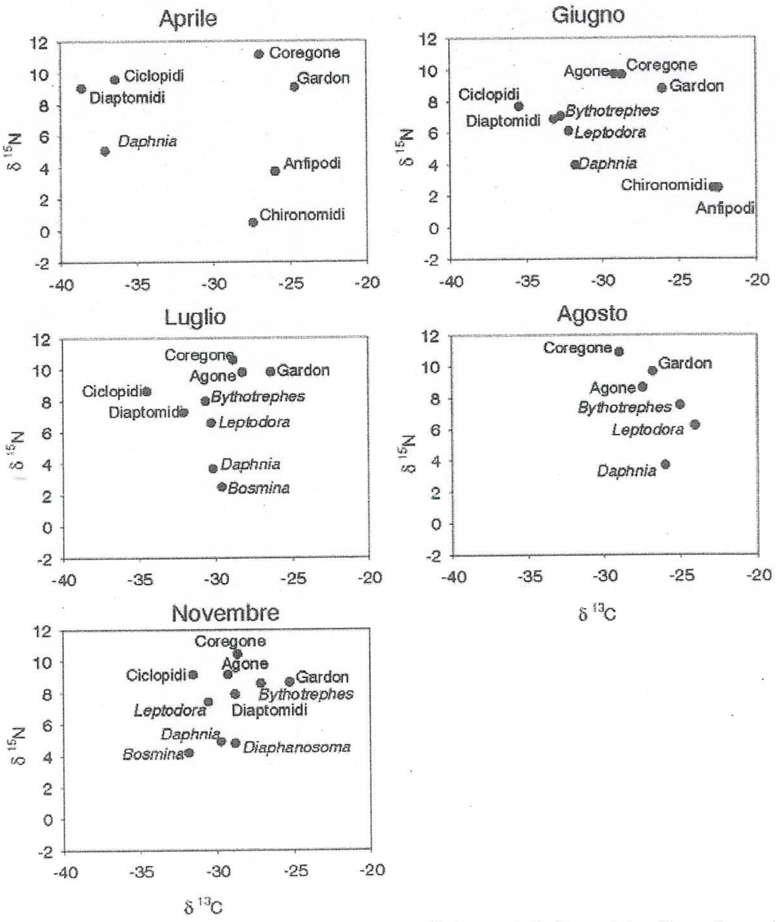


Fig. 3. Grafici trofochimici relativi ai segnali isotopici rilevati in diversi mesi del 2009 nei principali organismi zooplanctonici, del bentos litorale, ed in tre diverse specie di pesci del Lago Maggiore.

diverse specie ittiche, nel corso dell'anno.

Tendenzialmente, *Bosmina* presenta valori di arricchimento lievemente più bassi di quelli di *Daphnia*, in tutti e tre i mesi nei quali è stata rinvenuta. Quest'ultima presenta valori di $\delta^{15}N$ più elevati in primavera, tendenzialmente più bassi in estate, e minimi in ottobre. Un arricchimento tra il 2 e il 3 ‰ rispetto a *Daphnia* caratterizza *Leptodora* nei periodi primaverile ed estivo, mentre valori ben più elevati sono stati rilevati nel mese di ottobre, quando il *fingerprint* isotopico di *Daphnia* risultava molto basso, prossimo a quello di *Bosmina*.

Tra gli organismi zooplanctonici, *Leptodora* è risultata arricchita in $\delta^{15}N$ in modo significativo solamente a ottobre, con una differenza rispetto a *Daphnia* di oltre il 5 ‰, e dell'1,5 ‰ rispetto ai diaptomidi, non sufficiente a giustificarne l'attribuzione ad un livello trofico più elevato rispetto a questi ultimi.

Il segnale isotopico dell'azoto di *Bythotrephes* è risultato, in generale, superiore rispetto a quello di *Leptodora* (Fig. 2), con un arricchimento rispetto a *Daphnia* compreso tra un minimo del 3 ‰ (a maggio) e un massimo di circa il 6 ‰ (in ottobre). I copepodi ciclopidi sono risultati caratterizzati da valori di $\delta^{15}N$ prossimi a quelli di *Bythotrephes*, in taluni casi sovrapponibili a quelli di agone e coregone (i.e. a maggio ed a novembre).

Molto interessante è apparso anche il fingerprint del gardon, inferiore rispetto a quelli di coregone e agone da aprile a giugno, coincidente con quello del secondo a luglio e compreso tra i due in agosto e settembre. L'arricchimento massimo in ogni data di campionamento è risultato quello del coregone, valori compresi tra un minimo del 5,5 ‰ (a maggio) e un massimo del 7,2 ‰ (in agosto).

Il complesso dei dati raccolti nel 2009 (Fig. 3) consente di caratterizzare le modificazioni stagionali nella rete trofica pelagica e di dare una prima descrizione quantitativa di quella litorale, identificando alcune possibili interazioni tra le due. I dati dimostrano la necessità di investigare i *fingerprint* isotopici del bentos litorale in diversi momenti dell'anno e su substrati sia inorganici sia fitofili, sì da poter meglio caratterizzare il segnale misurato nelle tre specie di pesci, indicativo di un ruolo non trascurabile, e per certi versi inatteso, di fonti non planctoniche di alimento. Da ultimo, la perfetta sovrapponibilità dei valori ottenuti per *Diaphanosoma* e *Daphnia* a novembre, suggerisce come il primo possa avere, nella rete trofica pelagica del Lago Maggiore, un ruolo vicariante rispetto alla seconda. Questo, e altri risultati emersi durante i primi due anni d'indagine, dimostrano come l'approccio di tipo funzionale, alla base delle analisi di isotopi stabili di carbonio e azoto, sia di fondamentale importanza per la definizione di quella *operational diversity* sulla quale si fonda una gestione sostenibile dell'ambiente, finalizzata al mantenimento e, ove necessario alla riabilitazione, delle strutture ecologiche (Moss *et al.*, 2009).

BIBLIOGRAFIA

Manca M., DeMott W.R., 2009. Response of the invertebrate predator *Bythotrephes* to a climate-linked increase in the duration of a refuge from fish predation. *Limnol. Oceanogr.*, **54**(6, part 2): 2506-2512.

Manca M., Spagnuolo T., Comoli P., 1994. Variations in carbon and nitrogen content with body length of *Daphnia hyalina-galeata* s.l. from laboratory and field observations. *J. Plankton Res.*, **16**(10): 1303-1314.

Manca M., Comoli P., Spagnuolo T., 1997. Length-specific carbon content of the *Daphnia* population in a large Italian subalpine lake, Lago Maggiore (Northern Italy): the importance of seasonality. *Aquat. Sci.*, **59**: 48-56.

Manca M., Visconti A., Fadda A., Caroni R., Cerutti I., Volta P., 2009. Indagini sullo zooplancton e sulla rete trofica pelagica attraverso analisi d'isotopi stabili di carbonio e azoto. In: C.N.R.-I.S.E. Sede di Verbania. *Ricerche sull'evoluzione del Lago Maggiore. Aspetti limnologici. Programma quinquennale 2008-2012. Campagna 2008.* Commissione Internazionale per la protezione delle acque italo-svizzere (Ed.): 34-52.

Moss B., Hering D., Green A.J., Aidoud A., Becares E., Beklioglu M., Bennion H., Boix D., Brucet S., Carvalho L., Clement B., Davidson T., Declerck S., Dobson M., van Donk E., Dudley B., Feuchtmayr H., Friberg N., Grenouillet G., Hillebran H., Hobaek A., Irvine K., Jeppesen E., R Johnson., Jones I., Kernan M., Lauridsen T.L., Manca M., Meerhoff M., Olafsson J., Ormerod S., Papastergiadou E., Penning W.E., Ptacnik R., Quintana X., Sandin L., Seferlis M., Simpson G., Trigal C., Verdonschot P., Verschoor A.M., Weyhenmeyer G.A., 2009. Climate change and the future of freshwater biodiversity in Europe: a primer for policy-makers. *Freshwater Reviews*: **2**: 103-130.

Zohary T., Erez J., Gophen M., Berman-Frank I., Stiller M.. 1994. Seasonality of stable carbon isotopes within the pelagic food web of Lake Kinneret. *Limnol. Oceanogr.*, **39**: 1030-1043.