Variation in the structure of food webs related to climate change and anthropogenic disturbance

Department of Environmental Biology
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Candidate
Simona Sporta Caputi
13145380

Tutor
Prof. Loreto Rossi
Prof. Maria Letizia Costantini

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Food webs: the hard core of ecology

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Abstract

Climate change and anthropogenic pressure are deeply altering the global environments dramatically threating the persistence of biodiversity and ecosystems processes including the primary production, matter cycling and energy flux. However, the still scarce knowledge on the biodiversity organization in the food web structure, and not only on the species number, limits our power to predict the effects of environmental change and the possible response mechanism of biological community to environmental variations. This work aims to understand the mechanism underlying the community structure and its stability under different natural or anthropogenic pressures conditions. The food web structure and its metrics, the main carbon pathways and the taxa that play a key role in the community organisation in aquatic ecosystems were identified and quantified. The extensive field sampling and stable isotope analysis of each individual collected in the study ecosystems allowed to reconstruct and compare highly detailed food webs and trophic niche at both spatio-temporal and hierarchical scale (from individuals to community). In addition, the new consideration of trophic specie based on restricted interval of two elements isotopic signals, proved to be an efficient approach to overcome the current limits of the classical food web reconstruction, that occur when the biodiversity and the possible trophic interactions between species within an ecosystem are particularly complex. Our results highlight that modifications in the availability and quality of basal resources alters the interactions among organisms at different hierarchal scale with cascade effects on the entire food web structure, complexity, and stability. In accordance with optimal foraging theory, the availability of food sources has imposed change of link density already starting from single individuals with important variation of recurring patterns in the food web structure. Specifically, the consumers diet breadth decreased when the per capita availability and quality of resources increase. This, in turn, led to a relatively simpler food web characterized by lower link density affects the stability of the community against biodiversity loss and biological invasions. Our results are of particular relevance for ecology and for methodology, providing the key information for understanding the mechanisms that structure the community and its possible response to the different disturbance conditions. The results support that the expected changes in the habitat physical conditions could be reflected in food web architecture and organisms with greater generalism and trophic plasticity may be able to persist after a disturb playing a key role in community stability. The structure and function of food webs could be an efficient measure to predict and mitigate the effect of induced both physical and biological change on natural communities, both in the short and medium-long term (including climate change).

Introduction

Global changes coupled with anthropic pressures are strongly reshaping both terrestrial (Latour, 2014; Waters et al., 2016) and aquatic (Fiorentino et al., 2017; Amundrud and Srivastava; 2019; Malhi et al., 2020) ecosystems (Pimm et al., 1995; Sala et al., 2000; Dirzo et al., 2014). This poses a concern since change driving factors as the temperature, the invasion of species and the increase in anthropogenic activities and land use, affect the biodiversity and, thus, the ecosystem functioning (Sala et al., 2000; Clark et al., 2013, Chown et al., 2015).

Nevertheless, the current and future effects of environmental changes on biodiversity remain uncertain (Rubenstein, et al., 2020). Limited is our knowledge on the biodiversity organization and on the possible responses of communities to environmental variations (Dirzo et al., 2014; Rossi et al., 2019; Rubenstein, et al., 2020). This particularly affects successful decision-making measures for the conservation and management of natural resources (Rubenstein, et al., 2020).

The main actions to safeguard biodiversity are often aimed at managing and maintaining one or a few flagship species (Thompson et al., 2012; Sibarani et al., 2018). However, the latter are not isolated entities but are connected to the physical environment in which reside with other species, including humans (Hutchinson, 1959; Cohen et al., 1993; Tylianakis et al., 2010; Lang et al., 2014). Interactions between species in a food web play a key role in regulating, both directly (predation) and indirectly (competition), several ecosystem processes (Chapin lii et al., 2000). These include the primary production, the energy and matters fluxes between ecosystem compartments and decomposition rates, all dependent by the architecture of biodiversity in the food web (Chapin lii et al., 2000; Rooney and McCann, 2012; Thompson et al., 2012; Costantini et al., 2014; Calizza et al., 2015; Rossi et al., 2015).

Understanding and describing the food webs means highlighting all the connections existing between populations and the characteristics of the community structure (Cohen, 1989). For this, the food webs theory represents still today the *hard core* of Ecology (Odum and Biever, 1984).

Changes in space-time environmental dynamics driving the availability and quality of resources, could alter the food web structure in many ecosystems (Polis and Strong, 1996; Chapin lii et al., 2000; Dunne et al., 2002; Calizza et al., 2012; McMeans et al., 2015). The ability of communities to oppose or adapt following a perturbation is closely linked to the complexity of their architecture (Elton, 1927; Lindeman 1942; MacArthur, 1955; Paine, 1966; May, 1972;1973 Chapin lii et al., 2000; Montoya et al., 2009; Costantini and Rossi, 2010; Thompson et al., 2012~~; Wagner et~~

~~al., 2012~~). Understanding the link between the food web structure and community stability is still one of the greatest challenges of ecology (Elton, 1927,1958; MacArthur, 1955; May, 1972; Yodzis, 1981; McCann, 2000; Rooney and McCann, 2012; Allesina et al., 2015; Pringle and Hutchinson, 2020).

Analysis of several random food webs showed that the more complex the community, the less stable it is (May, 1972). However, species distribution and interactions are not random in natural systems (Yodzis, 1981; Pimm et al., 1991; Dunne et al., 2002; Allesina et al., 2015; Rossi et al., 2015) and are affect by environmental conditions (Yodzis, 1981; Pimm et al., 1991; Chapin Iii et al., 2000; Rooney et al., 2006).

Investigating the structure and dynamics of food webs under different spatio-temporal stress conditions could be the key to highlight the mechanisms underlying the communities' persistence and its possible response to environmental change (Rooney and McCann, 2012, Rossi et al., 2015, Rosenblatt and Schmitz, 2016; Calizza et al., 2018).

The continuous development of methodologies based on quantitative and statistical approaches, has led to a notable increase in knowledge on food webs structures and metrics (Speirs et al., 2000; Saito, 2001; Petchey et al., 2008; Steele, 2009). Among these, Stable Isotope Analysis of Carbon ($\delta^{13}C$) and Nitrogen ($\delta^{15}N$) is an increasingly useful tool for providing clear information, space and time integrated, on diet and relationships between organisms (Lajtha and Michener, 1995; Williams and Martinez, 2000; Post, 2002; Layman et al., 2007). This because the isotopic signatures of consumer tissues predictably reflect those of the assimilated resources (DeNiro and Epstein, 1978; Peterson and Fry, 1987; Post, 2002; Inger and Bearhop, 2008). $\delta^{13}C$ signatures widely vary among primary producers but are stable in consumers allowing to detect and quantify the different carbon sources contribution to the food web (DeNiro and Epstein, 1978; Peterson and Fry, 1987; Post, 2002; Inger and Bearhop, 2008; Careddu et al., 2015). On the contrary, $\delta^{15}N$ values gradually increase along food chain, providing information on individuals or populations trophic position in the food web (Minagawa et al., 1984; DeNiro and Epstein, 1978; Post, 2002; McCutchan et al., 2003; Careddu et al., 2015). Bayesian mixing models applied to organisms' isotopic signatures are useful statistic tool to estimate the consumers' diets (Phillips et al., 2014; Parnell et al., 2013; Calizza et al., 2018).

However, assigning and quantifying all possible trophic links between species and reconstruct the community architecture is still difficult (Petchey et al., 2008). This mainly due to the extraordinary trophic diversity (also within a population) and the complexity of the potential trophic links between species (Polis and Strong, 1996; Layman et al., 2012). Although the taxonomy approaches are important and in continuous evolution (Monckton et al., 2020), giving a unique identity to organisms is still not straightforward (Martinez, 1993; Fulton et al., 2003; Bates et al., 2015; Monckton et al., 2020). Moreover, species with high trophic plasticity, generalism and omnivory degree can explore high variety of resources, vary their diet in according to age and body size and perform different trophic functions in different space or times (McMeans et al., 2015; Feiner et al., 2019). This makes their trophic role still uncertain or completely unknown (De Broyer and Danis, 2011; Martinez, 1993; Fulton et al., 2003; Bates et al., 2015). The difficulty of analysing all the community interactions, especially when the number of interacting species and the uncertainty about their taxonomic identity are high, places limits on the food webs reconstruction (Polis and Strong, 1996; Petchey et al., 2008; Layman et al., 2012; Monckton et al., 2020).

The present dissertation aims to understand the mechanisms underlying the structure and stability of communities under different stress conditions. A new food web approach has been introduced to describe, in unprecedented detail, the complex architecture of communities emphasising the role of the individuals within populations, overcoming the current limits of the reconstruction of food web.

We tested the hypothesis that food web architecture can changes towards the variation of density of feeding link, affecting the community stability, the biodiversity loss and the vulnerability to biological invasions. The change of feeding links can follow the availability of basal resources and/or environmental complexity.

The dissertation focuses on the study of structure and of variation of food webs in relation to three kinds of widespread environmental stress: climate change, cultural eutrophication and biological invasions, that are the main causes of biodiversity loss (Chapin lii et al., 2000; Sala et al., 2000; Hooper et al., 2005; Malhi et al., 2020). Following this, respectively communities of pristine ecosystems (**Chapters 1-2,** Ross Sea, Antarctica), Mediterranean coastal brackish lakes (**Chapters 1-3,** Caprolace, Lake Fogliano and Sabaudia) and a large volcanic lake (**Chapter 4,** Lake Bracciano) have been analysed.

The Ross Sea (Antarctica) is the largest marine protected area in the world (a biodiversity hotspot) and the most pristine marine ecosystem on our planet (Peck, 2005; Leihy et al., 2020). It is also a critical climate-change reference area, and a climate refugium for ice-dependent species. Significant variation in environmental physical conditions linked to climate change are already recorded in coastal Antarctic marine ecosystems and the future expected scenarios in this region are still uncertain and dramatic (Michel et al., 2019; Bracegirdle et al., 2020; Leihy et al., 2020; Roach et al., 2020; Rogers et al., 2020).

The light and sea-ice coverage cycle drive the structure and functioning of both terrestrial and aquatic food webs, in which most of the Antarctic biodiversity lies (Eicken, 1992; Poloczanska et al., 2016). Such factors influence key ecological processes of Antarctic ecosystems (Gutt and Piepenburg, 2003; Ingels et al., 2012; Clark et al., 2013) as the phytoplankton blooms, the reproductive and recruitment cycles of population, the availability of food sources and trophic interactions of a wide range of species (Massom and Stammerjohn, 2010; Clark et al., 2013; Constable et al., 2014). Changes in seasonal sea-ice thickness and consequent light could be reflected in the Antarctic food webs and compromise, directly and indirectly, the biodiversity persistence (Clark et al., 2013; Hughes et al., 2020). Following this, the investigation of the organization of pristine biodiverse communities in the Ross Sea may represent a unique research opportunity to explore and describe the stability mechanisms of communities in seasonally forced food webs that are not affected by human pressure (Norkko et al., 2007; Chown et al., 2015; Cummings et al., 2018; Rossi et al., 2019; Leihy et al., 2020). In addition, it also could provide a unique opportunity to study the effects and the possible biological response of the community organisation to environmental variation closely related to the climate change.

However, Antarctic biodiversity is still largely unexplored or poorly understood (De Broyer and Danis, 2011). Great is the difficulty of taxonomic determinations on the very abundant species. At the same time, the high degree of omnivory and Intra-guild predation that characterize these communities (Carscallen and Romanuk, 2012; Norkko et al., 2007; Dunlop et al., 2014), make it difficult to apply the classical methods of food web investigations, based mostly on literature research. Hence the need for a new approach to reconstruct complex food webs, the *isotopic trophospecies*, inspired by the concept of *trophospecies* sensu Cohen and Briand (1984). The Isotopic trophospecies, expressed in Isotopic Trophic Units (ITUs), are identified by groups of individuals with similar isotopic signature, exploring the same spectrum resources and occupying the same trophic position on the isotopic space ($\delta^{13}C$ and $\delta^{15}N$). (Further details are available in the following chapters).

If on the one hand extreme and pristine environments such as Antarctica are the first to be directly affected by climate change, on the other in Mediterranean environments the climate driving factors often interacting with the pressures related to the increasing human activities (Calizza et al., 2012, 2015; Santoro et al., 2014; Costantini et al., 2018; Fiorentino et al., 2017, 2020; Rossi et al., 2019; Calizza et al., 2020). The latter include the increase in eutrophication levels due to the release in anthropogenic nitrogen inputs and the introduction and diffusion of potentially invasive species (Gritti et al., 2006, Hellmann et al., 2008).

Water ecosystems are indispensables source of ecosystem services (Costanza et al., 1997;

Barbier, 2017). The high exploitation of them to continuous external disturbances, often linked to human activity carried out in the surrounding area (Orlandi et al., 2014; Jona-Lasinio et al., 2015; Fiorentino et al., 2017; 2020). Aquatic ecosystems are extremely productive and complex ecosystems (Basset et al., 2006; Fiorentino et al., 2017). They provide habitats, refuge areas and food sources for a wide range of aquatic animals and important services for the human benefits (Costanza et al., 1997; Teixeira et al., 2019). The latter include the good water quality, fisheries, aquaculture, tourism, as well as agricultural activities in their watersheds.

Lake Caprolace, Lake Fogliano and Sabaudia are three neighbouring Mediterranean brackish costal lakes located on the Tyrrhenian coast of central Italy (Circeo National Park, Latium). Various anthropogenic disturbances related to organic and inorganic nitrogen inputs from human activities widespread in the surrounding areas affect these water bodies (Santoro et al., 2014; Jona-Lasinio et al., 2015). The anthropogenic disturbances include the urban treated sewage, livestock farming and agricultural activities. Lake Caprolace and Lake Fogliano are Sites of Community Importance (SCIs). They are characterised respectively by low and intermediate levels of eutrophication (Santoro et al., 2014; Jona-Lasinio et al., 2015). Higher anthropogenic pressures insist on Lake Sabaudia compared to other lakes. These mainly due to runoff from both the namesake city and cultivated fields in the surrounding areas, as well as fishing and mussel farming (Santoro et al., 2014; Jona-Lasinio et al., 2015).

Agricultural and urban activities are the main cause of release of anthropogenic nitrogen input which poses potential threats to biodiversity and ecosystem functioning (Howarth and Marino, 2006; Santoro et al., 2014; Calizza et al., 2020). High N-loads can significantly compromise the water quality, promoting the development algal blooms and altering the structure and feeding behaviour of the aquatic animal community, from primary consumers to top predators, with effects on the stability and structure of the entire food web (Santoro et al., 2014; He et al., 2019; Martínez-Durazo et al., 2019). The increase in anthropogenic N-loads could thus also compromise, either directly or indirectly, also the persistence of ecologically and economically important fish species.

Fish species with high omnivory and trophic plasticity can directly reflect variations in the inputs determining the trophic status of the waters and thus the quality and availability of potential prey. Understanding the patterns underlying their trophic choices and associated food webs under different eutrophication level could be crucial for ecosystem management and the habitat conservation.

On the other hand, changes in physical and chemical habitat conditions due to climate warming and human action could also promote the success of biological invasions, increasing the risk of biodiversity and ecosystem services loss (Ricciardi et al., 2017; McClelland et al., 2018). Biological invasions are a widespread phenomenon and one of the main causes of biodiversity loss around the world, with negative ecological and economic impacts on invaded ecosystems including the production of fish stocks (Iguchi et al., 2004; Leunda 2010Costantini et al., 2018). Although several are the field research carried out during recent years (Dick et al., 2017; Laverty et al., 2017; Corrales et al., 2019) scarce are our abilities to measure ecological and economic impact of invasion on the invaded ecosystem (Vander Zanden et al., 1999; Crystal-Ornelas and Lockwood, 2020).

Lake Bracciano is a volcanic lake located in the Central Italy (Lazio) and part of the regional park of Bracciano Martignano (Fiorentino et al., 2017; Rossi D. et al., 2019). Considerable are the services for the human benefit provided by this ecosystem and its surrounding areas. These include the drinking water production mostly for the city of Rome, tourism, agriculture, and fisheries.

A decline in water level has been observed in the recent years (Rossi D. et al., 2019; Fiorentino et al., 2020) due to the climatic variations and anthropogenic water withdrawal. The water crisis could threaten this lacustrine ecosystem affecting the communities' structure and ecosystem functioning (Wantzen et al., 2008). Changes in physical and chemical habitat conditions could further threaten native species with important ecological and economic values (Chapin lii et al., 2000), already greatly affected by the presence of the invasive species *Micropterus salmoides,* a generalist and omnivorous predator fish, introduced into Lake Bracciano as game in recent decades (Costantini et al., 2018).

The introduction and diffusion of new species can affect the structure and function of the invaded communities by altering the interactions between populations and the energy pathways between the ecosystem compartments (Maezono et al., 2005; Leunda, 2010; Hughes et al., 2020). The structure of invaded food web, in turn, can plays a key role in the containment of the invasion through biotic resistance mechanisms both by competitors (competitive resistance) and predators (consumptive resistance) species (Britton 2012; Alofs and Jackson 2014). Following this, measuring both ecological and economic impacts produced by the diffusion of an invasive species is necessary for effective managing invaded food webs. The food web approach could be a useful tool also to overcoming these limits and thus improving our strategies of ecosystem management (Gozlan et al., 2010; Latombe et al., 2017).

The present dissertation is organized in chapters as follow:

The **Chapter 1** contains the detailed description of the food web architecture of Antarctic coastal marine communities of medium-deep waters, in order to improve our understanding of its variations in different conditions of ice-sea-cover and consequently, of availability of basal resources. By individual stable isotopes analysis and Bayesian mixing models, a new food web reconstruction approach, the "Isotopic-Trophic-Units", was developed. "*Space for time*" experimental design was used to investigate the food web reshaping under different sea-ice coverage conditions in different place in the same time. The food web approach at the individual level revealed a change in the feeding strategies of Antarctic organisms over time closely related to seasonal input from primary producers. The release of algae associated with seasonal sea-ice dynamics promoted the optimization of the foraging by benthic consumers by drastically modifying the architecture and stability of Antarctic coastal biodiversity of medium-deep waters.

The **Chapter 2,** starting from the concept that the niche width depends by link density I track seasonal changes in both the trophic niche of populations and food web structure in the species-rich benthic Antarctic community of shallow waters (Tethys Bay, Ross Sea), before and after the sea-ice break-up. The increased availability of primary producers (sympagic algae) following the sea-ice break-up affected the trophic niche of benthic species with important effects on the food web architecture and stability. Specifically, the foraging optimization by consumers led to simpler food web, in which the potential competition for resources was lower and the food chains shorter. Species with a key role in the biodiversity maintenance in relation to the seasonal sea-ice dynamics, which is expected to change with global warming, were also identified.

The **Chapter 3** investigates the trophic response of water community to eutrophication and its effect on the feeding behaviour and food choices of ecologically and economically important omnivorous fish species. The diet of the eel, *Anguilla anguilla* and the seabream, *Diplodus annularis,* was descripted and analyses in three neighbouring Mediterranean brackish coastal lakes (on Tyrrhenian coast of central Italy) characterised by different eutrophication levels.
Anthropogenic nutrient inputs propagated along food chains from primary producers to top predators, including fish of ecologically and economically interest. The diet of the two species was obtained started from the individual choice within each population of each lake. The piscivory reduction, especially in eel, following the increase in the eutrophication levels of the lake, led to an increase in the niche overlap, and thus in potential competition, between the two fish species.

The **Chapter 4** quantify the resistance against an invasive and its impact on the autochthonous species, at the population- and food web-level in two communities differing each other in species richness and environmental topography, thus, in food web complexity. Based on abundance, biomass and stable isotope data of species, a new analytical method to quantify the trophic interaction strengths was introduced. Following this, the carrying capacity of fish populations and the resilience of food web to perturbations were estimate.

In addition, the impact of invasive species in terms of potential economic loss on lake fish stocks was also measured and the specie that play a key role to limit the invader identified. The more complex and species rich food web promoted high resistance of community to invasion and lower ecological and economic impact of invader.

Chapter 1: Antarctic food web architecture under varying dynamics of sea ice cover (published in Scientific Report, IF:3.998)

Loreto Rossi[1,2], **Simona Sporta Caputi[1]**, Edoardo Calizza[1,2,5], Marco Oliverio[3], Stefano Schiaparelli[4], Maria Letizia Costantini[1,2].

[1] Department of Environmental Biology, Sapienza University of Rome, Via dei Sardi 70, Rome (IT)

[2] CoNISMa-Consorzio Nazionale Interuniversitario per le Scienze del Mare, Piazzale Flaminio 9, Rome (IT)

[3] Department of Biology and Biotechnologies ‘‘Charles Darwin’’, Sapienza University of Rome, Zoology–Viale dell’Universita` 32, Rome (IT)

[4] Department of Earth, Environmental and Life Sciences (DISTAV), University of Genoa, Corso Europa
26, Genoa (IT)
[5]Corrispondent author

Corresponding Author: edoardo.calizza@uniroma1.it (E. Calizza).

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Antarctic food web architecture under varying dynamics of sea ice cover

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Loreto Rossi[1,2], Simona Sporta Caputi[2], Edoardo Calizza[1,2], Giulio Careddu[2], Marco Oliverio[3], Stefano Schiaparelli[4,5] & Maria Letizia Costantini[1,2]

In the Ross Sea, biodiversity organisation is strongly influenced by sea-ice cover, which is characterised by marked spatio-temporal variations. Expected changes in seasonal sea-ice dynamics will be reflected in food web architecture, providing a unique opportunity to study effects of climate change. Based on individual stable isotope analyses and the high taxonomic resolution of sampled specimens, we described benthic food webs in contrasting conditions of seasonal sea-ice persistence (early vs. late sea-ice break up) in medium-depth waters in Terra Nova Bay (Ross Sea). The architecture of biodiversity was reshaped by the pulsed input of sympagic food sources following sea-ice break up, with food web simplification, decreased intraguild predation, potential disturbance propagation and increased vulnerability to biodiversity loss. Following our approach, it was possible to describe in unprecedented detail the complex structure of biodiverse communities, emphasising the role of sympagic inputs, regulated by sea-ice dynamics, in structuring Antarctic medium-depth benthic food webs.

The Ross Sea (Antarctica), the largest marine protected area in the world[1], is considered a pristine ecosystem and an important biodiversity hotspot[2]. It is also a critical climate-change reference area, and a climate refugium for ice-dependent species. The protection of this area is thus essential for ethical reasons and because of its environmental and scientific value.

In the Ross Sea, biodiversity is strongly influenced by temperature and the consequent extent of sea-ice cover[3–5], which is characterised by marked spatio-temporal variations[5–7]. The expected changes in seasonal sea-ice dynamics will be reflected in the structure of food webs, providing a unique opportunity to study the effects of climate change on biodiversity organisation[4,5,7,8]. However, food web complexity in polar ecosystems, along with under-sampling, has limited efforts to predict future modifications due to climate change[9]. Specifically, the lack of scientific information on Antarctic communities and the ecological roles of their component species[10] makes it difficult to predict how changes will affect mechanisms regulating key ecological processes, such as productivity and species interaction, and the associated community stability and vulnerability to species loss[11–13]. Understanding biodiversity architecture is thus necessary for a proper mechanism-based approach to the management and conservation of Antarctic coastal ecosystems under a climate change scenario.

One major factor impeding the reconstruction of Antarctic food web structure is the difficulty of correctly depicting the taxonomic basis of Antarctic biodiversity. Recent integrative approaches have revealed unexpectedly high levels of endemic and cryptic species, most of which have not yet been described, making classification of Antarctic biodiversity challenging. For this reason, as well as for the high degree of omnivory, the trophic role of many species remains uncertain or completely unknown[14]. In this context, stable isotope analysis (SIA) can provide clear information, integrated in space and time, about the trophic relationships that are established between organisms[5,15,16] and can therefore be used to develop models of trophic structures[13,17,18]. With respect to other techniques of food web reconstruction, such as feeding experiments and stomach contents analysis, the stable isotope method has several advantages because, depending on the tissue analysed, it reflects the resources

[1]CoNISMa-Consorzio Nazionale Interuniversitario per le Scienze del Mare, Piazzale Flaminio 9, Rome, Italy. [2]Department of Environmental Biology, Sapienza University of Rome, Via dei Sardi 70, Rome, Italy. [3]Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Zoology–Viale dell'Università 32, Rome, Italy. [4]Department of Earth, Environmental and Life Sciences (DISTAV), University of Genoa, Corso Europa 26, Genoa, Italy. [5]Italian National Antarctic Museum (Section of Genoa), University of Genoa, Viale Benedetto XV 5, Genoa, Italy. Correspondence and requests for materials should be addressed to E.C. (email: edoardo.calizza@uniroma1.it)

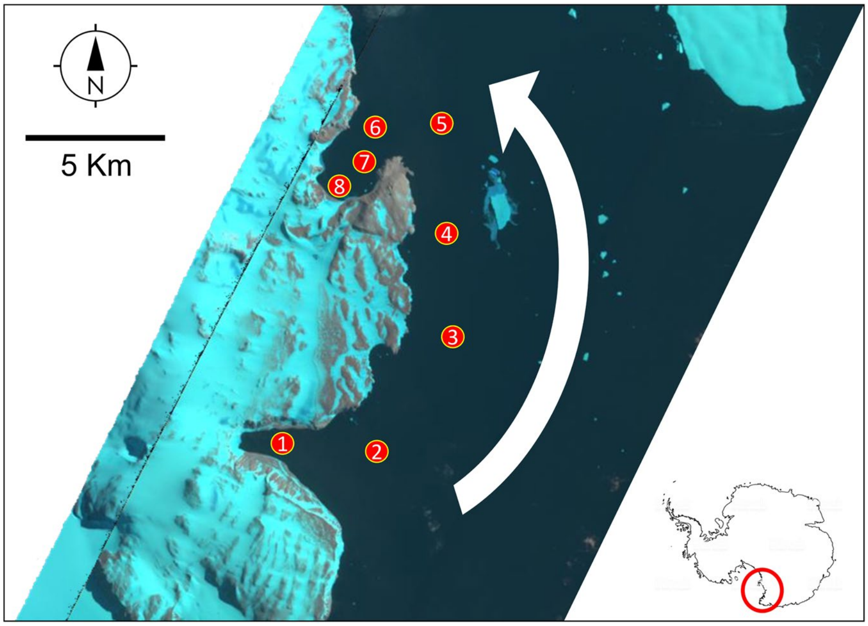

Figure 1. Sampling sites in Terra Nova Bay (Ross Sea). The background image of Terra Nova Bay was obtained by the Earth Observing-1 Satellite, Path: 62, Row: 113. The white arrow indicates the temporal gradient of seasonal sea-ice break up, which typically proceeds northwards, i.e. from sites 1 and 2, in the area of Adelie Cove, to sites 7 and 8, in the area of Tethys Bay. The red circle in the small map shows the position of Terra Nova Bay (Ross Sea) in Antarctica. Please refer to Table S1 for coordinates and the number of days elapsing between sea-ice break up and sampling at each site. Satellitary image of Terra Nova Bay from 03/02/2012 was obtained from https://lpdaac.usgs.gov/ maintained by the NASA EOSDIS Land Processes Distributed Active Archive Center (LP DAAC), USGS/Earth Resources Observation and Science (EROS) Center, Sioux Falls, South Dakota, 2018.

really assimilated by organisms in the short, medium and long term[7,15,18]. This can provide information on key food web characteristics such as the distribution of organisms and feeding links across trophic levels, and energy flows and matter circulation in the food web[5,13,15,17,18] in a dynamic framework. However, due to trophic generalism and omnivory in populations, the currently used analytical methods can be ineffective. Since energy flows and nutrient transfer depend firstly on the individual foraging choices of organisms within the community, the concept of "trophospecies" (sensu Cohen and Briand[19]) can be adopted as part of a new mechanistic approach to determining trophic interactions in complex environments such as Antarctica. Here, based on the isotopic signatures of single individuals in the web, we introduce the concept of isotopic trophospecies (i.e. a group of individuals that share a similar position in the trophochemical bidimensional graph and the food web), reported as an Isotopic-Trophic-Unit (hereinafter: ITU).

The main purpose of this study was to describe the architecture of the food web of Antarctic coastal marine communities at the kilometre scale and to determine its variations under varying conditions of coastal sea-ice persistence. It has been observed that the spatio-temporal dynamics of sea-ice cover influences basal resource inputs[8,20], with increased availability of sympagic algae during and soon after sea-ice break up[5,18,21–24]. In accordance with optimal foraging theory, which predicts a decrease in consumer diet breadth as the per capita availability of resources increases[13,18,25], we tested the hypothesis that food web architecture changes towards a reduction in feeding link density, and consequent web simplification, following the seasonal input of sympagic algae associated with sea-ice melting[5,18,24]. To this end, we used a "space for time" approach, sampling across multiple sites in Terra Nova Bay (TNB, Ross Sea) (Fig. 1 and Table S1), which we expected to experience varying sympagic inputs due to differences in the timing of local sea-ice break up and sea current circulation patterns[5,18,24,26,27]. Thus, based on the analysis of stable isotopes and Bayesian mixing models, the ITU-food webs were described in two adjacent areas: one where sea-ice break up occurred a few days before sampling and its possible effects on the isotopic signatures of benthic organisms were not yet detectable[18] (Before-Breaking Diet, hereafter *BEFORE*), and the other where sea-ice break up started more than one month before sampling (reflecting the After-Breaking Diet, hereafter *AFTER*).

Comparisons were extended from web to species level, focusing on the sea urchin *Sterechinus neumayeri*, an opportunistic omnivorous feeder, which is also an important prey for abundant predator taxa such as asteroids and anemones[7,18,28,29]. *S. neumayeri* is among the most common benthic species in Antarctic coastal waters. It plays a key role in matter recycling and energy flows through food webs[18,28,29], being able to feed both on vegetal and animal matter, and to vary its diet over space and time according to resource availability[18,28,29].

Results

Isotopic niches. Both the $\delta^{13}C$ and $\delta^{15}N$ values of the basal resources differed significantly among guilds (i.e. sympagic algae, plankton, benthic algae, epiphytes, and sediment organic matter) but not between *BEFORE* and *AFTER* areas (Fig. 2; two-way PERMANOVA, Guild: $F = 56.1$, $p < 0.0001$; Location: $F = 0.50$, n.s.; Interaction:

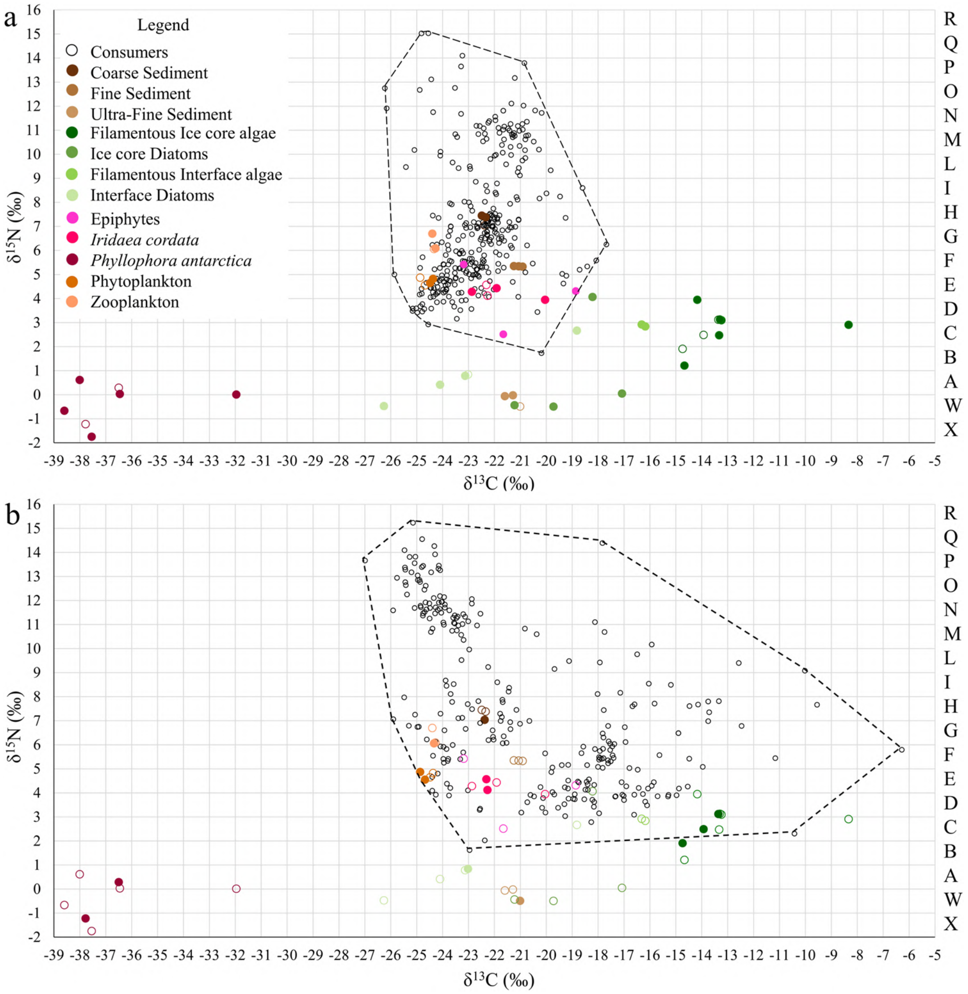

Figure 2. Isotopic niche biplot of BEFORE (**a**) and AFTER (**b**) communities. Each point represents a sample. The legend shows the respective colours of consumers and basal resources. Full symbols represent the basal resources of the respective study location. Empty coloured symbols represent the basal resources of the other study location. The isotopic niche space was divided into Isotopic Trophic Units (ITUs), i.e. squares having 1 × 1‰ ranges of $\delta^{15}N$ and $\delta^{13}C$ values, which were defined as groups of individuals occupying the same position in the $\delta^{13}C$-$\delta^{15}N$ niche space. Dashed-line polygons delimit the convex hull (Total Area) of consumers.

$F = 0.43$, n.s). Specifically, sympagic algae and the red alga *Phyllophora antarctica* had the highest and lowest $\delta^{13}C$ values respectively, while sediment organic matter, the red alga *Iridaea cordata* and epiphytes had intermediate $\delta^{13}C$ values. The isotopic values of Adelie penguin guano were outside the isotopic spectrum of both resources and consumers ($\delta^{13}C = -28.4 \pm 0.2‰$, and $\delta^{15}N = 11.3 \pm 0.6‰$, $n = 16$) and hence were not considered for the Mixing Models.

A total of 327 and 273 individuals belonging to 86 and 41 zoobenthic taxa were respectively identified in *BEFORE* and *AFTER* sites and underwent SIA (Table S2).

The mean $\delta^{13}C$ values of benthic animals differed among sampling sites (One-way ANOVA, $F = 72.3$, $p < 0.0001$), being positively correlated with the number of days since sea-ice break up ($n = 8$, $y = 0.09 \times -23.05$, $r = 0.94$, $p = 0.0004$, permutation $p = 0.0033$, 95% bootstrapped confidence interval on intercept $= -23.53$, -22.47). Mixture model clustering grouped the *BEFORE* and *AFTER* sites into two clearly distinct clusters (sites 5–8 and sites 1–4) with more variable and higher average $\delta^{13}C$ values in the latter than the former (Table 1 and Table S1; mixture model clustering based on the EM algorithm, Bayesian Information Criterion $= -4477.5$). Higher $\delta^{13}C$ in *AFTER* was also observed at the taxon level (Fig. 3), with the Location factor having a dominant effect (Two-way ANOVA, Location: $F = 126.8$, Taxon: $F = 11.3$, Interaction: $F = 6.3$, p always < 0.0001). Specifically, consumer $\delta^{13}C$ values in *AFTER* were distributed along the entire isotopic spectrum of basal resources, including values typical of sympagic algae (Fig. 2).

As regards $\delta^{15}N$ values, although no differences were found in mean and variance, distribution varied significantly, being normally distributed in *BEFORE* and right-skewed in *AFTER* (Shapiro-Wilk test, p-normal > 0.05; Skewness $= 0.07$, kurtosis $= -1.05$ vs. p-normal < 0.05; Skewness $= 0.47$, kurtosis $= -0.61$).

The community occupied a larger isotopic niche space (TA) and individuals were isotopically more widely distributed (MND) in *AFTER* than in *BEFORE* (Table 1 and Fig. 2). Therefore, a much larger number of ITUs,

	Before	After
Isotopic niche metrics		
$\delta^{13}C^{\circ\circ\circ}$	−22.3 ± 0.2‰	−19.3 ± 0.5‰
$\delta^{15}N$	8.3 ± 0.4‰	7.5 ± 0.3‰
Variance $\delta^{13}C^{\$\$\$}$	4.0‰	20.2‰
Variance $\delta^{15}N$	11.0‰	10.8‰
CR	8.5‰	20.7‰
NR	13.3‰	13.6‰
TA	80.4‰^{2}	186.1‰^{2}
MND$^{\circ\circ\circ}$	4.8 ± 0.1‰	6.9 ± 0.2‰
Food web properties (fraction)		
Basal Level	0.19	0.15
Intermediate*	0.42	0.59
Top*	0.39	0.26
Link properties (complexity)		
L/S$^{\bullet\bullet\bullet}$	7.5 ± 0.5	5.7 ± 0.3
Cmin***	0.18	0.11
Links (fraction) between		
Basal resources-consumers**	0.27	0.41
Predators-prey**	0.73	0.59
Chain properties		
Mean chain length	2.23	2.49
Mean trophic position$^{\bullet}$	4.2 ± 0.2	3.7 ± 0.2
Degree of Omnivory	0.77	0.77
Degree of Intraguild Predation**	0.42	0.23
Fraction of competitors$^{\bullet\bullet}$	0.40 ± 0.01	0.28 ± 0.01
Robustness to primary extinctions*	0.45	0.38

Table 1. Isotopic niche and food web metrics in BEFORE and AFTER areas in Terra Nova Bay, Ross Sea. CR: Carbon Range, NR: Nitrogen Range, TA: Total Area, MND: Mean Neighbour Distance, L: number of feeding links and S: number of nodes (i.e. Isotopic Trophic Units, ITUs, in the food web). Mean values are presented as mean ± standard error. Superscript symbols indicate a significant difference between locations (*χ^2 test; $^{\bullet}$Mann-Whitney test; $^{\circ}$t-test for unequal variances, $^{\$}$F test for variances). One, two and three superscript symbols indicate p values of <0.05, <0.01 and <0.001 respectively.

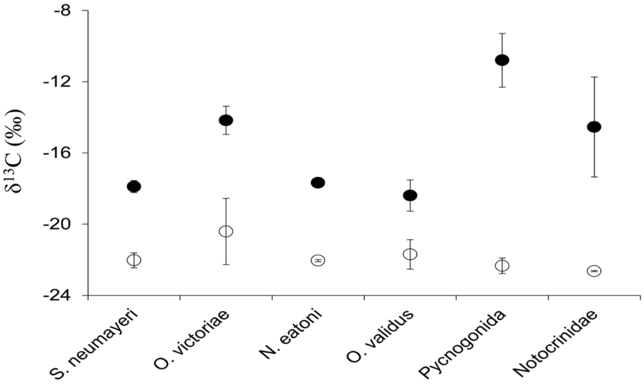

Figure 3. $\delta^{13}C$ values of benthic organisms. Comparison between areas (i.e. BEFORE sea-ice break up, empty symbols, and AFTER sea-ice break up, black symbols) of the mean ± s.e. $\delta^{13}C$ values of six generalist taxa commonly found in Antarctic benthic communities.

together with a lower average number of taxa/individuals per ITU, was found in the former than the latter: 108 ITUs (1.54 ± 0.11 taxa and 3.01 ± 0.33 individuals per ITU) vs. 83 ITUs (2.94 ± 0.39 taxa and 4.88 ± 0.77 individuals per ITU) (Figs 2 and S1).

Food web structures. In both food webs, the isotopic distances between ITUs significantly reflected differences in the composition of their diets (Mantel test, *BEFORE*: $R = 0.56$, $p < 0.00001$; *AFTER*: $R = 0.47$, $p < 0.00001$).

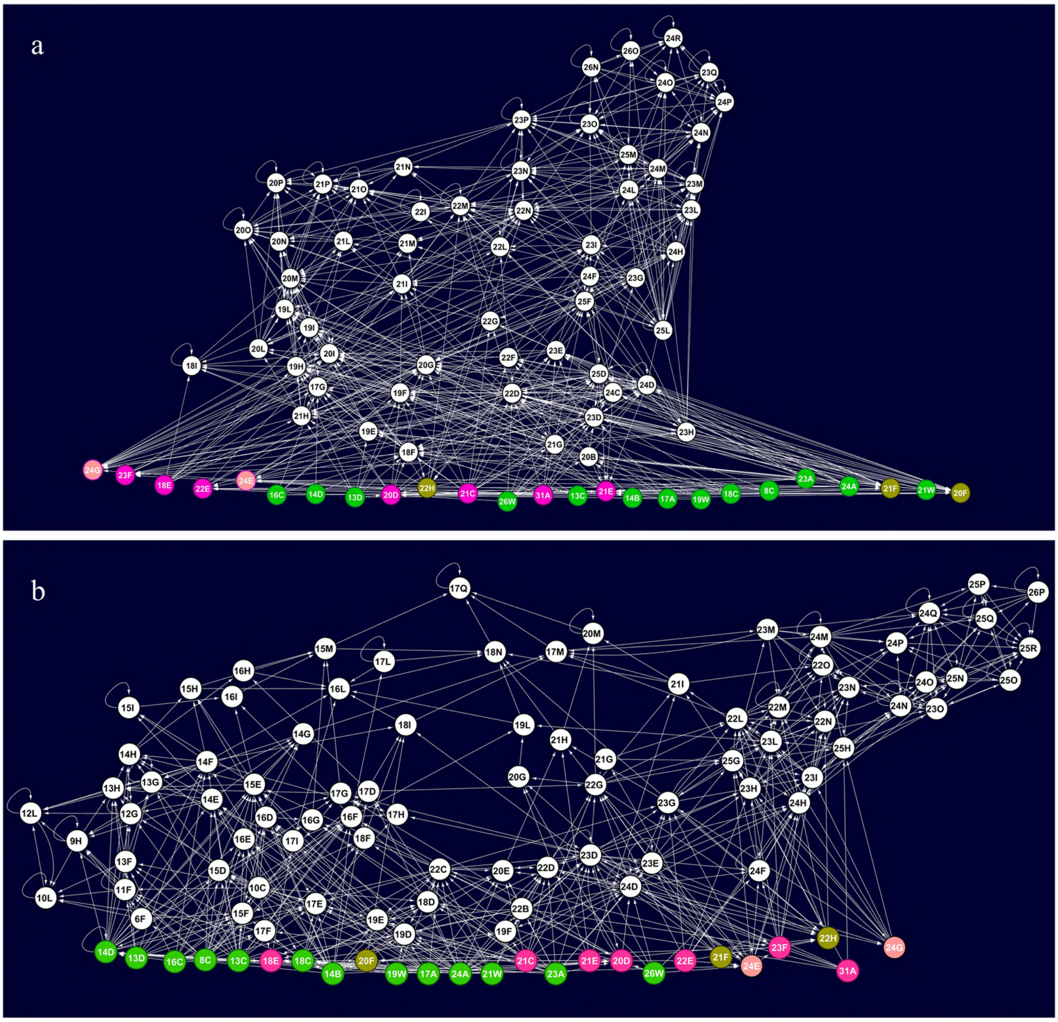

Figure 4. Benthic food web structure in Terra Nova Bay, Antarctica: BEFORE (**a**) and AFTER (**b**) sea-ice break up. Each node represents one Isotopic Trophic Unit (ITU) in the community. Isotopic Trophic Units (ITUs) i.e. squares having 1 × 1‰ ranges of δ^{15}N and δ^{13}C values, were defined as groups of individuals occupying the same position in the δ^{13}C-δ^{15}N niche space. Arrows point from a resource to its consumer. Nodes containing basal food sources are highlighted in different colours: green = sympagic algae, brown = organic matter in sediments, pink = plankton, violet = macroalgae. Letters and numbers identify each node as displayed in Fig. 2 and Fig. S1. The food web graphs were developed using Cytoscape software. For the list of taxa in each ITU please refer to Table S2.

Several food webs characteristics differed between areas. Specifically, the food web was more complex in *BEFORE* than in *AFTER*, as denoted by the higher feeding linkage density, which was associated with higher minimum connectance (Cmin), intraguild predation and competition between ITUs (Table 1 and Fig. 4).

The distribution of ITUs and feeding links across trophic levels also differed between areas (Table 1). While the degree of omnivory was similar and high in both food webs, only 27% of total links were with basal food sources in *BEFORE* (Table 1) compared to 41% in *AFTER*, where the mean trophic position of ITUs was correspondingly lower (Table 1), largely due to increased assimilation of sympagic algae (12% vs. 28% of total links in the web, χ^2 test, $\chi^2 = 48.9$, $p < 0.0001$; Fig. 5). Specifically, in *BEFORE* sympagic algae were consumed by only 18% of individuals, including those of *Ophionotus victoriae* (Echinoidea) and *Echinopsolus mollis* (Holothuroidea), which were found to assimilate this food source in both areas. In *AFTER*, sympagic algae were consumed by 41% of individuals, including those of Pycnogonida and *Staurocucumis turqueti* (Holothuroidea), which were not found to assimilate this food source in *BEFORE*.

Lower linkage density implies lower robustness of the web to bottom-up biodiversity loss (*sensu* Dunne *et al.*[30]) (Table 1). The percentage of ITUs secondarily lost following simulated primary ITU extinctions in *BEFORE* was half what it was in *AFTER* (18% vs. 37%, χ^2 test, $\chi^2 = 8.3$, $p < 0.01$). However, both food webs were more vulnerable to top-down than bottom-up propagation of disturbance, as indicated by the direct correlation between δ^{15}N and the closeness centrality of ITUs (*BEFORE*: $r = 0.68$, $p < 0.0001$; *AFTER*: $r = 0.45$, $p < 0.0001$), which quantifies their topological proximity in the web and, thus, the potential for disturbance propagation. This implies that taxa at higher trophic levels, such as the common predators *Trematomus bernacchii*, *T. hansoni* and *Crionodraco*

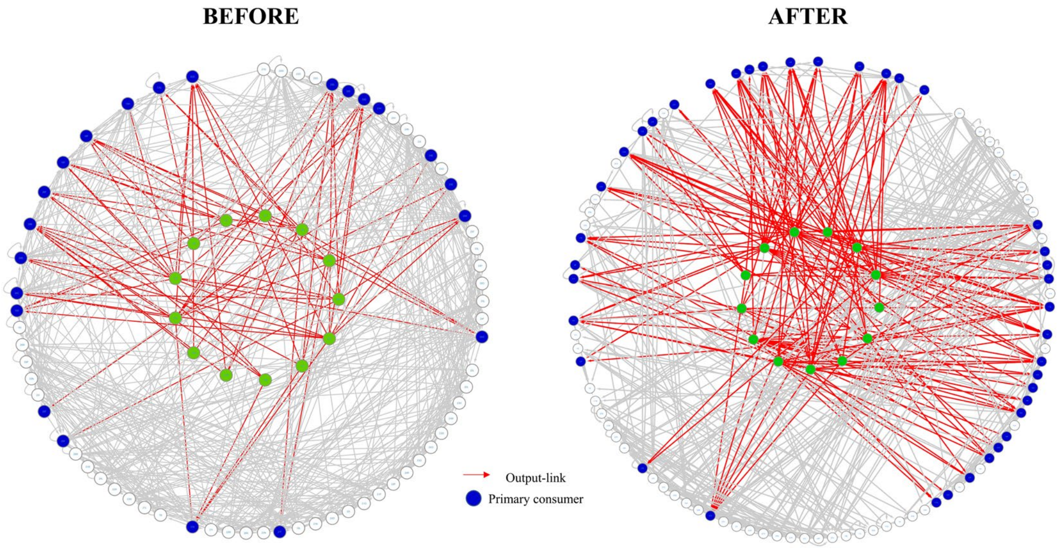

Figure 5. Sympagic algae-based food webs in BEFORE and AFTER areas. Each node represents one Isotopic Trophic Unit and each edge represents one trophic link. Isotopic Trophic Units (ITUs), i.e. squares having 1 × 1‰ ranges of δ^{15}N and δ^{13}C values, were defined as groups of individuals occupying the same position in the δ^{13}C-δ^{15}N niche space. Nodes at the centre of each network represent the ITUs containing sympagic algae. Peripheral nodes represent direct (blue) and indirect consumers (white, i.e. included in the source network but not feeding directly on nodes at the centre). Red edges represent direct links between basal resources and consumers, grey edges the other links in the source network. The food webs were developed using Cytoscape software.

hamatus, had a higher potential to propagate disturbance than other organisms. On average, the closeness centrality of ITUs was higher in *BEFORE* than in *AFTER* (0.55 ± 0.02 vs. 0.49 ± 0.02; Mann-Whitney, $U = 3537.5$, $p = 0.01$). The 10 most central ITUs in the *BEFORE and AFTER* food webs included 21 and 9 animal taxa respectively, having in common, as primary consumers, the demosponge *Haliclona dancoi* and the bivalve *Adamussium colbecki* and, as predators, the gastropod *Neobuccinum eatoni* and the fish *T. bernacchii*. In *AFTER*, the 10 most central ITUs included three basal resources (filamentous sympagic algae, sympagic diatoms and epiphytes).

Diet of the key species *Sterechinus neumayeri*. *Comparisons between areas.* As observed, on average, at the food web level, the sea urchin *S. neumayeri* displayed lower δ^{13}C values and higher δ^{15}N values in *BEFORE* than in *AFTER* (δ^{13}C: −21.8‰ ± 0.49 vs. −17.55‰ ± 0.50; δ^{15}N: 5.19‰ ± 0.50 vs. 4.14‰ ± 0.19, Man Whitney test, $p < 0.05$ for both), and its omnivorous diet varied remarkably between areas. Specifically, the ITUs occupied by the sea urchin had a lower proportion of links with basal resources in the former than in the latter (43% vs. 79%; χ^2 test, $\chi^2 = 67.2$, $p < 0.0001$), where the consumption of sympagic algae was higher (accounting for 32% and 64% of the animal's trophic links respectively; χ^2 test, $\chi^2 = 23.2$, $p < 0.0001$). Accordingly, its trophic position was significantly lower ($TP_{BEFORE} = 2.97 \pm 0.23$ and $TP_{AFTER} = 2.28 \pm 0.07$; t-test, $t = 3.3$, $p < 0.01$). Some other food resources were used in both areas (e.g. epiphytes, ascidians and the macroalga *Phyllophora antarctica)*, whereas others were consumed only in *BEFORE* (e.g. polychaetes and carcasses of the abundant sea-star *Odontaster validus)* (Fig. 4 and Table S2). On the other hand, the description of trophic links between ITUs allowed to observe predatory links affecting *S. neumayeri*. As an example, specimens of *O. validus* and *O. victoriae*, which occupied ITUs 21H and 17G in BEFORE, and ITUs 16H and 13H in AFTER, fed on specimens of *S. neumayeri* contained in ITUs 19F and 20D (in BEFORE) and ITUs 17D and 14E (in AFTER).

Comparisons among approaches. The diet of the sea urchin population of *AFTER* extrapolated by the mixing models from the single ITU values (individual approach) was compared with the diet determined with the two population-level approaches, which take into account all the isotopic values of *S. neumayeri* and the mean and standard deviations of its potential food sources. Thirteen food sources were identified and included in the diet by the individual approach (Table 2), while only 5 food sources were found by the classical population-level approach (Table 2). Although both of these approaches indicated that feeding on basal resources prevailed, in the individual approach the assimilation of animal-derived food was more pronounced (12% of the diet) than in the classical approach (3%). This difference was also found when a population-level approach based on the means and standard deviations of the 13 sources extrapolated in the individual-based diet was followed (Table 2).

Discussion

This study examines for the first time the food web structure of Antarctic benthic communities in medium-deep waters, where most of the Antarctic biodiversity lies[8,31] and where the spatio-temporal sea-ice dynamics are expected to affect basal resource inputs[7,18,24,32–34]. In these highly dynamic environments, the isotopic characterisation of single individuals was crucial for describing the functional organisation of the community, including the

Resources	Classical approach (Population level)	ITU approach (Individual level)	*a posteriori* approach (Population level)	References
Notocrinidae (Crinoidea)	—	5.34% ± 0.001	1.42%	[80]: reporting the congeneric *S. antarcticus* feeding on Crinoidea
Polyeunoa (Polichaeta)	2.33%	1.64% ± 0.002	0.30%	[7,55,81]
Demospongiae	—	0.31%	0.49%	[7,29,55]
Echinopsolus mollis (Holoturoidea)	—	3.3% ± 0.004	1.12%	First report by this study
Cnemidocarpa verrucosa (Ascidiacea)	—	0.6% ± 0.001	0.37%	[28]: reporting the congeneric *S. antarcticus* feeding on Ascidiacea
Ophionotus victoriae (Ophiuroidea)	—	0.35%	0.60%	[36]
Filamentous ice core algae	26.98%	28.74% ± 0.001	24.80%	[7,28]
Filamentous interface algae	—	7.46% ± 0.003	2.13%	[7,28]
Ice core Diatoms	27.27%	13.05% ± 0.001	34.19%	[7,28,55]
Interface Diatoms	—	25.79% ± 0.001	6.63%	[7,28,55]
Epiphytic diatoms	—	6.79% ± 0.003	0.64%	[28]
Ultra-fine Sediment	33.54%	4.79% ± 0.001	26.70%	[7,28,55]
Phyllophora antarctica (Florideophyceae)	—	1.84% ± 0.003	0.62%	[28,55]
Iridaea cordata (Florideophyceae)	9.88%	—	—	[7,28,55,81]

Table 2. Diet of *Sterechinus neumayeri*. Proportional contribution of food sources to the diet of *Sterechinus neumayeri* in AFTER (i.e. after sea-ice break up) obtained from mixing models using various procedures for food source selection. In the ITU approach, the contribution of each food source is reported as the mean (±S.E.) contribution of that source to the ITUs of *S. neumayeri* (for details please refer to the methods section). The classical approach refers to the proportional contributions obtained at the population level starting from the isotopic mean and S.D. of potential prey taxa and resources. In the *a posteriori* approach, the mixing model at population level was applied using the 13 food sources identified with the ITU approach. The References column lists bibliographical references (as numbers) to previous observations of selected food sources, either alive or as carrion.

topology of the feeding network that underlies nutrient and energy transfer across trophic levels[12,13,15,17]. Indeed, the observed complex trophic structures, which were consistent with what is expected in Antarctic benthic communities where omnivory, trophic generalism, necrophagy and intraguild predation are frequent[7,35,36], were supported by variation in resource use between individuals within local species populations. Although it is difficult to disentangle these complex interactions, neglecting trophic variability among individuals is thus a potential source of bias in depicting food webs and energy pathways, leading to oversimplification[37]. Furthermore, the presence of a large number of potential food sources, including some with strong isotopic overlap, can reduce the discriminatory power of the mixing model and thus the effectiveness of Stable Isotope Analysis in food web reconstruction[38]. From this perspective, the concept of 'trophospecies' based on SIA applied at the individual level (giving rise to the ITUs in this study) represented a useful approach to the reconstruction of Antarctic food webs.

Although this approach does not allow to directly depict trophic links between taxa, it allows (i) to extrapolate trophic links between taxa starting from the information provided by the ITU-based food web, as we demonstrated for the sea urchin *S. neumayeri*, and (ii) to better compare food webs characterised by inconsistent taxonomic resolution[37], provided that the isotopic analysis of specimens is performed at the individual level. On the other hand, our approach may be sensitive to undersampling or differences in sampling effort, when communities are compared, given that a low sampling effort can fail to consider the whole isotopic variability of populations. In our study, the similar high number of individuals sampled within each area gives us confidence on the results from the comparison between the two food webs. Furthermore, unambiguous extrapolation of trophic links between taxa may be difficult when a single ITU contains a high number of taxa for which previous information on diet is scarce. This risk is expected to increase with increasing size of ITUs, while it is avoided by selecting an isotopic grid of 1×1‰ in size as in our case (Fig. S2).

ITUs of 1×1‰ in size represent minimum isotopic niche units that made it possible to compare two communities where consumers showed a different variability in their isotopic signatures. It also allowed obtaining food webs composed by nodes representative of an equal portion of the community niche space, while excluding ITUs with an extremely large and variable number of individuals (Fig. S2). Since 1‰ is among the highest carbon isotopic discrimination factors expected for aquatic animals[39], differences exceeding 1‰ on the $\delta^{13}C$ axis can be considered indicative of differences in diet between individuals. This is corroborated by the strong linear correlation observed between the similarity in feeding links and the isotopic distance between ITUs. While the nitrogen isotopic discrimination factor is generally wider (~2.3‰ and ~3.4‰ for invertebrates and fishes respectively[39]), consideration of a range of 1‰ on the $\delta^{15}N$ axis allowed to split individuals belonging to omnivorous populations into different trophic units, thus preserving information on food web complexity and vertical distribution of individuals along food chains.

Consideration of the mean and variance of the single ITUs' isotopic signatures, defined by a common restricted δ-space, together with the multiple steps adopted for the selection of resources to be used in isotopic mixing models, allowed us to overcome the limits of Bayesian mixing when applied to complex communities[38,40]. Following our approach, it was thus possible to describe in unprecedented detail the food web structure of these biodiverse communities as well as the diet of single species, such as the omnivorous key species *S. neumayeri*, starting from individual feeding preferences without *a priori* reducing information on population diets. Finally, spatial comparisons, supported by cluster analysis and the correlation between benthic isotopic carbon signatures and sea-ice persistence, emphasised the importance of sea-ice algae input, which is affected by sea-ice extent and/or duration[5,18], in structuring medium-depth Antarctic benthic food webs. The marked spatial effect of sea-ice break up on the isotopic signatures of organisms and food web structure suggest that the potential redistribution of sympagic material across sites due to sea currents was not relevant. Furthermore, the isotopic signatures excluded any effect of the guano of Adélie penguins, as organic input, on the isotopic composition of basal resources and benthic organisms, either before or after sea-ice break up.

Our results showed that the input of sympagic food sources following coastal sea-ice melting affected the foraging choices of benthic individuals. In turn, this reshaped the entire food web by modifying its architecture and functioning. Specifically, following the seasonal sympagic inputs, the food web was characterised by a relaxed structure with the δ^{13}C values of consumers distributed along the entire isotopic spectrum of basal resources, while the mean δ^{13}C shifted towards the values of sympagic algae[5,18]. The food web appeared less complex, having a higher number of trophospecies and trophic diversity (*sensu* Layman *et al.*[16]) but lower linkage density, than when the ice-coverage persisted and sea-ice algae were poorly assimilated in the food web. Before the sea-ice break up, the δ^{13}C values of individuals were highly overlapping and their narrow distribution was centred on the isotopic values of benthic food sources. Indeed, Calizza *et al.*[18] and Pusceddu *et al.*[32,41] demonstrated that in the study area sympagic algae become available to the benthos during and soon after the seasonal sea-ice break up (see also Wing *et al.*[5], Leu *et al.*[24]). It has also been noted that such sympagic inputs can reach great depth in just a few days through vertical deposition[21,42]. Since the isotopic analysis of soft tissues in organisms sampled soon after sea-ice break up (as in *BEFORE* conditions) describes their diet preceding the seasonal input of sympagic algae[7,18], while it includes sympagic algae assimilation where sea-ice breaks up earlier (as in *AFTER* conditions), the differences in benthic diets and the consequent structural changes in the food web are clearly related to the different resource inputs.

Although rarely considered in food web descriptions, seasonal fluctuations in the availability of basal resources are directly reflected in the structure of the food web, given the trophic plasticity of its constituent species[43]. Here, in response to these changes, consumers specialised on the abundant basal resources after their seasonal release. This is in agreement with what has been observed spatially[5,7] and temporally[18,43] in some polar species. It is also consistent with what is expected from the foraging optimisation theory[44,45] and what has been observed at the food web level in aquatic communities at lower latitudes[13,43,46]. Increased selectivity with regard to resources, coupled with decreased intraguild predation and competition, under conditions of increased resource availability can result in greater efficiency in terms of energy use by consumers and its delivery to top predators[25,47,48], supporting the summer reproductive season[49]. In contrast, greater trophic generalism, as denoted by the higher linkage density observed before sea-ice break up, makes consumers more resistant to temporal/local extinction or reduction in single resources[12,13,30], an advantage during the long and severely resource-limiting Antarctic winter and early spring. It is thus evident that by modulating food inputs, the seasonal growth and decline of sea ice is an important process in the persistence of marine Antarctic communities. It dynamically influences species interactions and food web structure, which in turn are both strictly related to community stability and resilience to perturbation[45,50,51].

Despite the continuing difficulties in providing a solid taxonomic basis for Antarctic biodiversity, the efforts made here to identify specimens at the lowest possible taxonomic level yielded an unprecedented dataset, increasing our knowledge of the autoecological features of a large number of species and also enabling the description of new ones[52]. We found the sponge *Haliclona dancoi* and the bivalve *Adamussium colbecki*, the gastropod *Neobuccinum eatoni* and the fish *Trematotus bernacchii* among the 10 most central ITUs at both locations, while sympagic algae were found among the 10 most central ITUs only after sea-ice melting. We observed that topological centrality increased with trophic level, up to the fish that can be part of the diet of higher Antarctic predators, such as the tootfish *Dissosticus mawsoni* and its predator, the weddel seal[53,54]. This indicates that higher-level benthic consumers play a key role in the Antarctic food web, and suggests that the inclusion of top-predators in future research may represent a useful step to depict benthic-pelagic trophic coupling in Antarctic coastal ecosystems. In this context, identifying topological keystone populations that occupy central positions in the food web, either as a whole or some ITUs only, may help to identifying specific targets for management actions aimed at preserving food web stability.

Changes at the food web level following sympagic inputs reflected individual diet changes observed at the species population level. Here, we focused on the key species *Sterechinus neumayeri*. Among the most common benthic species in Antarctic coastal waters, the sea-urchin *S. neumayeri* is an omnivorous and opportunistic grazer and scavenger that plays a key role in Antarctic community structure and energy and matter flows[18,28,29]. It is known that *S. neumayeri* opportunistically feeds on debris and animal prey, live or as carrion, during the less productive season, but adopts a predominantly herbivorous diet, feeding mainly on sympagic algae when these are available[7,18,36,55]. As observed for the community as a whole, the isotopic values of *S. neumayeri* indicated increased assimilation of sympagic algae and a lower trophic position (due to decreased assimilation of animal-derived food) where sea-ice had already broken up, coupled with a decreased mean number of food sources. Despite the species' acknowledged omnivory and marked trophic plasticity, the ITU-based approach was effective in describing its diet with a high level of detail. Specifically, the ITU-based food web highlighted the presence of resource types that are known to be consumed (live or as carrion) by *S. neumayeri* (see references reported in Table 2). Previously known food sources included Demospongiae, Notocrinidae and sympagic algae growing at the interface between sea-ice and water, which could not be detected by the classic population mixing model approach. In contrast, high-resolution local diets and feeding links can be extrapolated for the sea urchin and any other species in the community from the ITU-based food web. Indeed, this method made it possible to consider the entire spectrum of resources, without pooling them by mean isotopic similarity as in the classic method[38], which also gives more weight to central values than marginal ones. In the case of the sea urchin, for example, the ITU approach highlighted the use (by some specimens) of the alga *Phyllophora antarctica*, which was characterised by markedly lower C isotopic values and would thus have been excluded by the classic population approach. Such approach also detected the consumption of *Echinpsolus mollis* (Holoturoidea), which was not reported before, and *Cnemidocarpa verrucosa* (Ascidiacea), which was reported to be consumed by *Sterechinus antarcticus* but not by *S. neumayeri*[28]. In parallel, the detailed ITU-food web description allowed to observe predatory links affecting the sea urchin, and to trace it back to its predators, as the sea star *Odontaster validus* and the ophiuroid *Ophionotus victoriae*.

In conclusion, our approach revealed the development of Antarctic organisms' food strategies over time, which made it possible to scale the effects of seasonal resource input from the individual to the whole food web level[37]. Accordingly, it was shown that after sea ice break up, sympagic algae were assimilated by a greater number of benthic consumers in medium-depth waters, contributing to about one third of the links in the food web as a whole. The release of sympagic algae associated with seasonal sea-ice dynamics promoted foraging optimisation by benthic consumers, which specialised on a restricted number of resources, drastically modifying the architecture of coastal Antarctic biodiversity. This implies that changes in the sympagic compartment could significantly affect medium-depth benthic food web structure and functioning.

In the Southern Ocean, sympagic algae blooms can differ considerably from phytoplankton blooms on both spatial and temporal scales[23]. In parallel, the space-time sea-ice variations expected under current global climate change scenarios[56,57] suggest that changes in sea-ice dynamics will modify the quantity and timing of resource availability to the benthic food web[23,24]. Thus, a potential phenological mismatch between resource availability and consumer demand due to climate change may produce unprecedented pressures (both bottom-up and top-down), threatening the persistence of Antarctic biodiversity[34].

As concluding remarks, we can affirm that the ITU approach improved our understanding of trophic energy pathways along food chains in complex and biodiverse food webs. This approach should be applicable to any other habitat, starting from the individual isotopic characterisation of organisms. Notably, we presented unprecedented evidence that Antarctic food web architecture and metrics change predictably towards web simplification in accordance with optimal foraging theory, which expects a decrease in consumer diet breadth as the per capita availability of resources increases[13,25,45,46]. This improves our ability to model the expected effects of climate change on Antarctic biodiversity structure and functioning, as well as enabling comparison of future observations with present baseline conditions. In this sense, field experiments performed in areas naturally subject to marked seasonal sea-ice dynamics (as in the exceptional natural laboratory of Terra Nova Bay in the Ross Sea) represent a unique opportunity to make predictions regarding the ecological responses of communities in other polar areas where sea-ice persistence and resource inputs will be affected in the near future.

Materials and Methods

Ethics statement. All experimental protocols and methods were agreed with the PNRA (Italian National Antarctic Research Program), which also issued permits to collect samples in the study area on behalf of the Italian Ministry of Foreign Affairs. Permits were issued in compliance with the "Protocol on Environmental Protection to the Antarctic Treaty", Annex II, art.3.

Sampling area and samples collection. The study was carried out at various sites in Terra Nova Bay (TNB, Ross Sea) (Fig. 1 and Table S1). Samples were collected during PNRA (Italian National Antarctic Research Program) expeditions XXVII (mid-late January 2012) and XXVIII (mid-late January 2013). The Ross Sea is characterised by strong seasonality in sea-ice cover and primary productivity, which produces marked spatio-temporal variations in the availability of food[5,7,24,58]. The same seasonality is found in TNB[18,27,32,59]. Sampling was carried out along a coastal tract located between Adelie Cove (Southward) and Tethys Bay (Northward), nearly 15 km apart (Fig. 1). Along this tract, seasonal sea-ice break up typically proceeds northwards, implying that sea-ice coverage persists longer in the area of Tethys Bay. In order to quantify the number of days elapsing between sea-ice break up and sampling at each site, ground observations were coupled with satellite images (Earth Observing Satellite-1 and Landsat 7, target paths: 62–63, target row: 113). Sea ice break up occurred 57 days before sampling at sites 1 and 2 in the area of Adelie Cove, when sea-ice in the interior of the bay also showed evident cracking, and 62 and 36 days before sampling at sites 3 and 4 respectively. Sites 5–8, in the area of Tethys Bay, were characterised by longer seasonal sea-ice persistence and were sampled between five and nine days after sea-ice break up (Table S1). A cluster analysis (see Data analysis for details) grouped the individuals from different sites in two clusters: the first group ("*AFTER*") included sites 1,2,3 and 4, while the second group ("*BEFORE*") included sites 5, 6, 7 and 8 (Table S1). The dominant surface current circulation proceeds northwards in TNB, being affected by coastal morphology and sea-ice extent[26]. Sites 5-8 in the area of Tethys Bay were thus expected to be less affected by the dominant surface current than the others.

Adelie Cove is V-shaped. Tethys Bay in contrast is a small bay located further North along the coast of TNB. A colony of Adélie penguins (*Pygoscelis adeliae*) inhabits the inner part of Adelie Cove. The colony has around 11,200 breeding pairs, considered to be part of a metapopulation that includes colonies located at Inexpressible Island and Edmonson Point[60] (in the southernmost and northernmost parts of our study area respectively). Foraging trips in this species can extend for more than 100 km[61], and foraging penguins are also observed in Tethys Bay, both before and after sea-ice break up. Thus, given the potential flow of organic material from penguins to coastal waters through inputs of guano, fresh guano samples were collected at the rockery and considered for isotopic analyses. Further details of the study area can be found in Faranda *et al.*[6] and Norkko *et al.*[7]. Benthic invertebrate sampling was carried out by dredging at depths ranging from 21 m to 240 m (Table S1). Fish samples were collected by net and line in the areas situated between sites 5 and 7 (samples assigned to the *BEFORE* food web) and between sites

1 and 3 (samples assigned to the *AFTER* food web). A few fish samples were also coincidentally collected while dredging for invertebrate sampling. Samples of detritus (organic matter in coarse, fine and ultra-fine sediments), macroalgae (*Iridaea cordata* and *Phyllophora antarctica*) and associated epiphytes were also collected by dredging in the areas of Tethys Bay and Adelie Cove. Similarly, sympagic algae (diatoms and filamentous algal aggregates, referred to as "filamentous algae", contained within the ice core or at the ice-water interface) were sampled by coring the ice-pack before sea-ice break up. Plankton was collected by sampling the whole water column to a depth of 100 m with a plankton net (20-μm mesh size). Since the bulk sample was composed almost exclusively of copepods, zooplankton was carefully separated from the rest by hand under a stereoscope. To obtain the phytoplankton, the remaining sample was filtered at 100 μm and collected on pre-combusted Whatmann GF/F filters.

All individual samples used for Stable Isotopes Analysis (SIA) were identified to the finest possible level of taxonomic resolution (ideally to species level). Most Mollusca, and all Porifera, Arthropoda and Chordata were determined by morphology (the complete sponge dataset from Terra Nova Bay is available in Ghiglione *et al.*[62]). The other metazoans were determined by integrating the preliminary morphological identification with a DNA-barcoding approach. For part of the Mollusca, Echinodermata and Annelida (Polychaeta), partial cytochrome c oxidase subunit I (COI) sequences were produced at the Canadian Centre for DNA Barcoding (University of Guelph, Ontario, Canada) with LCOech1aF1/HCO2198 primers[63]. For Cnidaria, partial COI sequences were produced with COII8068F/COIOCTR primers[64], and Internal Transcribed Spacer 2 (ITS2) sequences were produced with 5.8S-436/28S-663 and ITS2-3d/ITS2-4r primers[65,66] at the Molecular Systematics lab, Dept. BBCD, Sapienza University of Rome. DNA-Barcodes were compared with the BLAST algorithm against the GenBank database (www.ncbi.nlm.nih.gov/nuccore), and within the BOLD Barcode Index Number system against the BOLD database (www.boldsystems.org), where there is also a large, still unpublished DNA-Barcode database of the Italian National Antarctic Museum (MNA) under the project BAMBi (Barcoding of Antarctic Marine Biodiversity, PNRA 2010/A1.10 project). Accession numbers are provided in the supplementary material, Annex 1.

Laboratory procedures and stable isotope analysis. Specimens were individually stored at −80 °C and freeze-dried for 24 hours. Before the analysis, each specimen was pulverised in a ball mill (Mini-Mill Fritsh Pulverisette 23: Fritsh Instruments, Idar-Oberstein, Germany). Where necessary, in order to eliminate inorganic carbon, samples were pre-acidified using 1M HCl according to the drop-by-drop method[67]. To prevent acidification interfering with Nitrogen analysis, $\delta^{15}N$ signatures were measured in un-acidified powders. After acidification, the sampled powders were re-dried (60 °C) for 72 hours to remove the remaining moisture. Aliquots of 0.25 ± 0.10 mg for the animals and 1.00 ± 0.10 mg for detritus, phytoplankton and benthic and sympagic algae were pressed into tin capsules for the Stable Isotopes Analysis. Samples were analysed in two replicates using an Elementar Vario Micro-Cube elemental analyser (Elementar Analysensysteme GmbH, Germany) coupled with an IsoPrime100 continuous flow mass spectrometer (Isoprime Ltd., Cheadle Hulme, UK), at the Trophic Ecology laboratory (Dept. of Environmental Biology, Sapienza University of Rome). Carbon (C) and Nitrogen (N) isotopic signatures were expressed in δ units ($\delta^{13}C$; $\delta^{15}N$) as parts per-thousand (‰) deviations from international standards: Vienna Pee Dee Belemnite (PDB) for C and atmospheric N_2 for N. Isotopic ratios were computed according to the equation: $\delta X (‰) = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3$, where X is the Carbon or Nitrogen isotope and R is the heavy-to-light isotope ratio of the respective element ($^{13}C/^{12}C$; $^{15}N/^{14}N$). Finally, $\delta^{13}C$ values were corrected for lipid content based on the C/N ratio of each sample in accordance with Post *et al.*[68]. The internal laboratory standard used was IAEA600 Caffeine. $\delta^{13}C$ and $\delta^{15}N$ measurement errors were typically smaller than ±0.05‰.

Data analysis. Mixture model clustering[69] was applied in order to group sampling sites into different classes based on the log-transformed number of days elapsing between sea-ice break up and sampling and the $\delta^{13}C$ and $\delta^{15}N$ values of benthic organisms. Cluster parameters were estimated using an EM algorithm[70]. Model selection was based on the Bayesian Information Criterion[71] (BIC), and the model assumes that each class is described by a multivariate Gaussian distribution with full covariance matrix. In order to confirm the observed grouping of sites into the two broader "locations" (i.e. *AFTER* and *BEFORE*), a two-way ANOVA testing the effect of Location, Taxon and their interaction was applied to the $\delta^{13}C$ values of six generalist benthic taxa commonly found in Antarctic benthic communities, i.e. the sea urchin *Sterechinus neumayeri*, the brittle star *Ophionotus victoriae*, the gastropod *Neobuccinum eatoni*, the seastar *Odontaster validus* and specimens of Pycnogonida and Notocrinoidae.

Isotopic data of collected organisms were used to reconstruct the food webs at each location, where nodes represented isotopic trophospecies. Isotopic trophospecies are defined as groups of individuals with very similar isotopic signatures, which therefore occupy the same position in the $\delta^{13}C$-$\delta^{15}N$ niche space. In this sense, the bi-dimensional isotopic space was subdivided into ITUs (Isotopic-Trophic-Units), i.e. squares having 1 × 1‰ ranges of $\delta^{15}N$ and $\delta^{13}C$ values, starting from the lowest $\delta^{13}C$ value present in the dataset and a $\delta^{15}N$ value of zero and proceeding in ascending order. The ITUs were univocally identified by a number on the $\delta^{13}C$ axis and a letter on the $\delta^{15}N$ axis (Fig. 2 and Fig. S1). Within the bi-dimensional isotopic space, basal resources, invertebrates and fish were identified and labelled. This was necessary for assigning the correct isotopic fractionation to vertebrates (i.e. $\delta^{15}N$: 2.1 ± 0.7‰, $\delta^{13}C$: 0.4 ± 0.2‰, based on observation in Antarctic fish[72]) and invertebrates (i.e. $\delta^{15}N$: 2.3 ± 0.4‰, and $\delta^{13}C$: 0.4 ± 0.2‰). Based on a broader literature analysis by McCutchan *et al.*[39], these values produced meaningful mixing model outputs when applied to benthic invertebrates in our study area[7,18]. The result was a grid (in a trophochemical graph) in which each ITU could be a consumer and/or a resource of organisms (Fig. S1) whose taxonomic classification is known in detail.

The diet of each ITU was calculated by means of Bayesian Mixing Models[73] (SIAR package in R-statistics software). The SIAR mixing models return probable resource proportion values with credibility intervals plotted at 95, 75 and 50%. This allows direct identification of the food sources in the diet of each consumer and therefore robust food web reconstruction[13,17,18,38,40,46]. For each consumer ITU, we considered a set of potential ITU food sources on the entire $\delta^{13}C$ axis and within a given range on the $\delta^{15}N$ axis, i.e. with values within ± 3‰ of the value of the consumer after subtracting its TEF[13,40]. Furthermore, given the high number of ITUs in each food web, the food sources were split into two subsets (A and B). Similar to white and black cells in a chessboard, the two subsets were divided so that within each subset there were no two contiguous ITUs on the $\delta^{13}C$ and $\delta^{15}N$ axes, while the ITUs within each subset covered the whole range of $\delta^{13}C$ values observed. Based on the mixing model outputs for subsets A and B, a set of selected food sources was retained for a third mixing model. Based on the output of this model, a pool of real (i.e. likely to be assimilated) food sources was then selected in accordance with Phillips *et al.*[38] and Costantini *et al.*[40]. At each step, a food source was retained if (i) the 95% confidence interval (CI) did not include 0, or if (ii) the 75% CI did not include 0 and its modal contribution was $\geq$5%, and/or (iii) it was necessary in order to obtain a mixing space encompassing the consumer[40]. Lastly, a fourth mixing model was run including only these real food sources in order to quantify the proportional contribution of each source to the diet of a consumer[40]. After the assignment of trophic links, the food web structures were reconstructed, the basal resource inputs were described and the respective food web metrics were quantified using the "foodweb" package in R and Cytoscape software[74].

In order to validate the diet of the sea-urchin *Sterechinus neumayeri* obtained with the procedure described above, we assessed its diet and reconstructed its sub-web by a traditional approach, i.e. starting from the isotopic mean and SD of potential prey taxa and resources as selected by the literature and SIA[38,40].

Food web metrics. To compare the food webs of the two locations, trophic chain length was estimated from the $\delta^{15}N$ range (NR) and the diversity of basal resources exploited by the community was estimated from the $\delta^{13}C$ range (CR) and variance[13,15,75]. NR and CR were both calculated as the Euclidean distance between the highest and the lowest respective isotopic values ($\delta^{15}N$, $\delta^{13}C$). Total area (TA) was calculated as the convex hull encompassing the consumer data points and was expressed as isotopic area[16] (‰^{2}). Total area provides a measurement of the total niche space occupied by the community at each sampling location, while the mean isotopic distance between all ITUs in each location provides a measure of trophic-functional diversity within the community.

Trophospecies richness (S) was estimated as the number of total nodes (i.e. ITUs) present in the food web. Linkage density (L/S) was measured as the average number of feeding links (L) per ITU. Minimum Connectance (Cmin) was calculated as: $C = 2L/S^2$. For each ITU, the trophic position (TP) and its closeness centrality were obtained using the foodweb package in R. The TP of a consumer takes into account the TP of the food sources it assimilates and their relative contribution to its diet, starting from TP = 1 assigned to basal food sources. The closeness centrality Cc(n) of a given node n is defined as the reciprocal of the average shortest path length. It is computed as follows: $Cc(n) = 1/avg(L(n,m))$, where L(n,m) is the length of the shortest path between two nodes n and m. The Cc(n) of each node is a measure, ranging between 0 and 1, of how fast information spreads from a given node to other nodes in the network[76]. Thus, it can be related to the vulnerability of a food web to perturbation propagation across nodes (ITUs in our case) and can help to identify which ITUs/species display the highest centrality in the community[77]. The importance of omnivory and intraguild predation were estimated using the foodweb package[78] in R. Given the potential presence of different taxa within the same ITU, we refer to "intraguild predation" instead of "cannibalism", intending that individuals contained within an ITU fed either on conspecifics and/or on individuals belonging to other taxa contained within the same ITU. The evidence for including an "intraguild" link was the same as for the selection of all the other links in the food web.

Food web robustness to ITU loss was estimated following simulated deletion of ITUs from the food web, proceeding from the most to the least connected ITU and considering all the ITUs in the food web in accordance with Calizza *et al.*[12]. The simulated deletion of an ITU is considered as a primary extinction (sensu Dunne *et al.*[30]), while a secondary extinction occurs when a non-basal ITU loses all of its prey/resource ITUs except itself. Lastly, a Mantel test was applied to assess if isotopic signatures of ITUs were predictive of the feeding links they made in the food web, considering both the identity and the relative contribution of each food source to each ITU. The Mantel test, as a permutation test for correlation between two distance or similarity matrices, allowed us to compare multivariate data with different similarity measures[79]. Here, the Euclidean distance and the Bray-Curtis dissimilarity were selected to quantify isotopic distances between ITUs and pairwise differences in feeding links respectively[72,79].

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Author Contributions

L.R., M.L.C. conceived the study; E.C., S.S. performed sampling in Antarctica; G.C., E.C., S.S.C. performed Stable Isotope Analyses; S.S., M.O. performed genetic analysis and taxonomic identification of specimens; L.R., M.L.C., E.C., G.C., S.S.C. analysed data; L.R., M.L.C., E.C., S.S.C. wrote the paper; all Authors revised the paper.

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Chapter 2: Seasonal food web dynamics in the Antarctic benthos of the Ross Sea: implications for biodiversity persistence under climate change (Under review in Frontiers in Marine Science, IF:3.661)

Sporta-Caputi Simona[1], Careddu Giulio[1], Calizza Edoardo[1*], Fiorentino Federico[1], Maccapan Deborah[1], Rossi Loreto[1,2] and Costantini Maria Letizia [1,2].

[1]Department of Environmental Biology, Sapienza University of Rome, via dei Sardi 70, 00185 Rome, Italy

[2] CoNISMa, piazzale Flaminio 9, 00196 Rome, Italy

*** Correspondence: Calizza Edoardo**
edoardo.calizza@uniroma1.it

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Seasonal Food Web Dynamics in the Antarctic Benthos of Tethys Bay (Ross Sea): Implications for Biodiversity Persistence Under Different Seasonal Sea-Ice Coverage

Simona Sporta Caputi[1], Giulio Careddu[1], Edoardo Calizza[1]*, Federico Fiorentino[1], Deborah Maccapan[1], Loreto Rossi[1,2] and Maria Letizia Costantini[1,2]

[1] Department of Environmental Biology, Sapienza University of Rome, Rome, Italy, [2] CoNISMa, Rome, Italy

Edited by:
Susana Carvalho,
King Abdullah University of Science
and Technology, Saudi Arabia

Reviewed by:
Ian Hawes,
University of Waikato, New Zealand
Lee W. Cooper,
University of Maryland Center
for Environmental Science (UMCES),
United States

***Correspondence:**
Edoardo Calizza
edoardo.calizza@uniroma1.it

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Determining food web architecture and its seasonal cycles is a precondition for making predictions about Antarctic marine biodiversity under varying climate change scenarios. However, few scientific data concerning Antarctic food web structure, the *species* playing key roles in *web* stability and the community responses to changes in sea-ice dynamics are available. Based on C and N stable isotope analysis, we describe Antarctic benthic food webs and the diet of species occurring in shallow waters (Tethys Bay, Ross Sea) before and after seasonal sea-ice break-up. We hypothesized that the increased availability of primary producers (sympagic algae) following sea-ice break-up affects the diet of species and thus food web architecture. Basal resources had distinct isotopic signatures that did not change after sea-ice break-up, enabling a robust description of consumer diets based on Bayesian mixing models. Sympagic algae had the highest $\delta^{13}C$ ($\sim-14‰$) and red macroalgae the lowest ($\sim-37‰$). Consumer isotopic niches and signatures changed after sea-ice break-up, reflecting the values of sympagic algae. Differences in food web topology were also observed. The number of taxa and the number of links per taxon were higher before the thaw than after it. After sea-ice break-up, sympagic inputs allowed consumers to specialize on abundant resources at lower trophic levels. Foraging optimization by consumers led to a simpler food web, with lower potential competition and shorter food chains. However, basal resources and Antarctic species such as the bivalve *Adamussium colbecki* and the sea-urchin *Sterechinus neumayeri* were central and highly connected both before and after the sea-ice break-up, thus playing key roles in interconnecting species and compartments in the web. Any disturbance affecting these species is expected to have cascading effects on the entire food web. The seasonal break-up of sea ice in Antarctica ensures the availability of resources that are limiting for coastal communities for the rest of the year. Identification of species playing a key role in regulating food web structure in relation to seasonal sea-ice dynamics, which are expected to change with global warming, is central to understanding how these communities will respond to climate change.

Keywords: Antarctica, climate change, food webs, keystone species, population-wide metrics, seasonal sea-ice dynamics, stable isotopes, trophic interactions

INTRODUCTION

The persistence of biodiversity in seasonally variable environments is linked to the ability of populations to adapt to variable and/or temporally constrained resource inputs (McMeans et al., 2015). This ability is directly dependent on their trophic plasticity, a key functional trait that allows populations to persist in complex food webs (Rossi et al., 2019).

Among other factors, seasonality is a major force structuring ecological communities (Thrush and Cummings, 2011; Clark et al., 2013). Nevertheless, our knowledge of how temporal variations in the trophic niches of populations affect food web structures in real ecosystems is scarce. This limits our understanding of stability mechanisms in temporally forced ecosystems (*sensu* McMeans et al., 2015), thus impairing their management and conservation.

The effects of seasonality are expected to increase with latitude, with tremendous changes observed in polar areas (Clark et al., 2013; Chown et al., 2015; Ingels et al., 2020), where light and sea-ice dynamics drive the structure and functioning of aquatic food webs (Poloczanska et al., 2016; Wing et al., 2018). Sea-ice influences key ecological processes in polar ecosystems, such as phytoplankton blooms, physical disturbance of the seabed due to iceberg scouring (Gutt and Piepenburg, 2003; Arrigo, 2014), reproductive cycles, recruitment, and trophic interactions among a wide range of species (Constable et al., 2014). Specifically, sea-ice shrinking during spring, together with sea-ice break-up in summer, allows a massive increase in ice-bound primary production (sympagic production), which is subsequently released into the water column, fuelling both pelagic and benthic communities (Pusceddu et al., 1999; Lizotte, 2001; Leu et al., 2015). Under these conditions, sympagic algae represent the main direct (via vertical flux) and indirect (after sedimentation) food input for a large part of Antarctic marine diversity (Lizotte, 2001; Michel et al., 2019; Rossi et al., 2019).

The sympagic algae that live inside or on the lower surface of the sea-ice produce at least 20% of the primary biomass of the Arctic and Antarctic Oceans (Stoecker et al., 2000; Arrigo, 2014). The seasonal availability of this high-quality energy source favors trophic specialization among a large number of taxa in both pelagic and benthic communities, which are specifically adapted to the physical and biological conditions characteristic of polar environments (Knox, 2006; McMahon et al., 2006). In contrast, short but intense phytoplankton blooms occur mostly during open-sea periods (Pusceddu et al., 1999; Lizotte, 2001; Leu et al., 2015). The increased availability of basal resources after sea-ice break-up allows consumers to exploit a range of carbon sources in both the water column and on the bottom, reinforcing benthic-pelagic coupling (Cattaneo-Vietti et al., 1999; Kaehler et al., 2000; Dunton, 2001; Knox, 2006). The seasonal supply of sympagic algae thus becomes crucial to energy exchanges between ecosystem compartments, also affecting the structure and stability of communities (Hobson et al., 1995; Søreide et al., 2010; Leu et al., 2015; McMeans et al., 2015; Michel et al., 2019).

Expected changes in seasonal light and sea-ice dynamics due to climate change could directly and indirectly compromise biodiversity persistence (Clark et al., 2013; Rossi et al., 2019). These changes, together with ocean warming, could also favor the entry and success of new faster-moving and more generalist species, one of the main causes of biodiversity loss worldwide (Aronson et al., 2007; Morley et al., 2019; Hughes et al., 2020). This in turn could affect the structure and function of polar communities by altering the interactions between populations and the energy pathways between benthic and pelagic compartments (Fossheim et al., 2015; Hughes et al., 2020). Biodiversity management and conservation efforts have generally focused on the study of individual species (Jordán, 2009; McDonald-Madden et al., 2016). However, failure to consider interactions with all the other species within the community and with the physical environment can lead to ineffective management and conservation measures in the medium and long term (Trebilco et al., 2020).

In the Ross Sea (Antarctica), a biodiversity hotspot and the most pristine marine ecosystem on our planet (Rossi et al., 2019; Leihy et al., 2020), seasonal changes in sea-ice coverage are particularly marked (Wing et al., 2018). Expected changes in sea-ice dynamics due to climate change will modify the extent and intensity of algal blooms, with possible effects on benthic food webs and pelagic top predators, thus altering biodiversity architecture (Clark et al., 2013; Poloczanska et al., 2016; Mangoni et al., 2019; Hughes et al., 2020). Although our knowledge of biodiversity in the region is increasing (Chown et al., 2015), little is known about species' functional roles in the food web, particularly in the benthic habitat, where the majority of Antarctic biodiversity lies (Rossi et al., 2019). This represents a major gap, limiting our ability to conserve biodiversity and anticipate the effects of changes in sea-ice dynamics on Antarctic food webs.

Recently, application of stable isotope analysis to Antarctic benthic communities has proved useful for describing space-time variations in nutrient fluxes associated with sea-ice dynamics (Michel et al., 2019; Rossi et al., 2019). Studies in the Ross Sea have demonstrated that sympagic production is readily incorporated into benthic biomass following sea-ice break-up (Norkko et al., 2007; Calizza et al., 2018), supporting between 30 and 80% of benthic secondary production (Wing et al., 2018). Accordingly, pulsed sympagic inputs during summer (Pusceddu et al., 2000, 2009) produce significant changes in the diet and trophic position of benthic taxa (Norkko et al., 2007; Calizza et al., 2018; Wing et al., 2018; Rossi et al., 2019), with cascade effects on predators at higher trophic levels (Leu et al., 2015; Poloczanska et al., 2016) and contaminant redistribution throughout the food web (Signa et al., 2019).

At present, quantitative data on feeding and competition links in Antarctic benthos are extremely scarce, and quantitative descriptions of the complex food web structures and their dependence on sea-ice coverage are even scarcer (Rossi et al., 2019). However, key ecological properties including stability, energy flux, vulnerability to biodiversity loss and invasibility are closely related to the complexity of food webs and the distribution of links among species (Aronson et al., 2007; Carscallen and Romanuk, 2012; Rossi et al., 2015; Calizza et al., 2019; Trebilco et al., 2020). While challenging, the quantitative analysis of food webs thus represents a necessary step toward understanding the persistence mechanisms of Antarctic communities and improving our ability to conserve Antarctic biodiversity during climate change.

In our study, for the first time we had the opportunity to track seasonal changes at the same site in both the trophic niches of populations and food web structure in the species-rich benthic Antarctic community. By means of C and N stable isotope analysis and Bayesian mixing models, we quantified (i) the isotopic niche metrics of populations, (ii) the strength of feeding and competitive interactions, and (iii) the food web properties of shallow-water benthic communities both before (hereafter BEFORE) and after (hereafter AFTER) sea-ice break-up in Tethys Bay, an inlet inside Terra Nova Bay in the Ross Sea. We sought to understand how the expected increase in resource availability following summer sea-ice break-up (Pusceddu et al., 1999; Lizotte, 2001; Leu et al., 2015) affects the trophic niches of populations and how this results in a rewiring of feeding links in the food web.

For this purpose, we measured variations in the food web structure of the shallow-water Antarctic benthic community under two different sets of sea-ice coverage conditions. Since the number of species in shallow-water Antarctic benthic communities is subject to natural seasonal variation (Gutt and Piepenburg, 2003; Thrush and Cummings, 2011; Clark et al., 2013; Ingels et al., 2020), to avoid any bias arising from species number in the interpretation of niche and food web metrics, we also considered taxa common to both study periods.

Lastly, based on food web structure analysis, we sought to quantify food web vulnerability to biodiversity loss, identifying those taxa that occupied the most critical positions in the food web and quantifying their sensitivity to changes in resource availability associated with sea-ice dynamics. In accordance with optimal foraging theory (Pyke et al., 1977; Rossi et al., 2015), we hypothesized that changes in the trophic niches of populations resulting from the increased availability of primary producers following sea-ice break-up drive the simplification of food web architecture, with effects on its stability and vulnerability to species loss.

MATERIALS AND METHODS

Study Area and Sampling Activities

The study was carried out in Tethys Bay (74° 41′8″S, 164° 04′8″E), an inlet inside Terra Nova Bay in the Ross Sea (**Figure 1**), during the 32nd expedition of the PNRA (Italian National Antarctic Research Program).

Tethys Bay is characterized by marked seasonality in sea-ice dynamics, with coverage generally persisting until mid-December, leaving the area ice-free during the austral summer. This produces marked spatio-temporal variations in the availability of food for benthic communities (Pusceddu et al., 1999; Lizotte, 2001; Leu et al., 2015; Calizza et al., 2018). The chlorophyll-a concentrations in this area generally range between 0.1 and 4 mg m^{-3}, with even higher values observed between January and February in association with phytoplankton blooms (Lazzara et al., 1997; Pusceddu et al., 2009; Mangoni et al., 2019). This trend was also observed in Aqua MODIS satellite images generated during our sampling periods (sources: worldview.earthdata.nasa.gov). Further information on the study area can be found in Calizza et al. (2018) and Rossi et al. (2019) and the literature cited therein.

Samplings were performed before and after sea-ice break-up, which started around 18th December 2016, leading to ice-free open water around 10th January 2017. The first round of sampling (i.e., BEFORE) was performed from 14th November to 15th December 2016. During this period, the whole study area was characterized by dark and non-turbid waters. The second round of sampling (i.e., AFTER) was performed from 19th January to 7th February 2017. During this period, icebergs flowed into and around the bay (personal observation), as usually observed in the area (Melchiori and Cincotti, 2018). The number of days elapsing between sea-ice break-up and sampling time were quantified by means of direct observations on the ground coupled with reference to satellite images (Landsat 8, target paths: 61, target row: 113).

Three sampling stations in shallow waters were chosen (**Figure 1**), characterized by mixed rocky and soft bottoms and varying slopes in order to take full account of the environmental heterogeneity of this area (Cattaneo-Vietti et al., 1999). All possible invertebrate organisms and basal resources were collected both BEFORE and AFTER. The collection and analysis of samples was carried out by the same field and laboratory research team in both sampling periods using similar sampling methods.

Benthic invertebrates, detritus (organic matter in coarse, fine and ultra-fine sediments) and macroalgae (*Iridaea cordata* and *Phyllophora antarctica*) with associated epiphytes were collected by SCUBA divers at a depth of 10–30 m. Small invertebrates, such as polychaetes and amphipods, were collected by carefully separating them from sediments or other sampled benthic specimens, mainly sponges.

Sediment samples were sieved and grouped on the basis of particle diameter into Coarse (>1 mm), Fine (between 1 and 0.56 mm) and Ultra-fine (<0.56 mm) fractions in accordance with Calizza et al. (2018).

Sympagic algae (diatoms and filamentous algal aggregates present in the ice core and at the ice-water interface) were collected by coring the ice-pack three times at each sampling site. Three replicates of sympagic algae per ice-core (i.e., a total of 27 samples) were collected. The filamentous and diatom fractions were separated from each other by sieving and filtering through Whatman GF/F filters. The sympagic diatoms and filamentous algal aggregates in Tethys Bay are mainly composed of species belonging to the genera *Amphiprora* and *Nitzschia*, respectively (Lazzara et al., 1995; Saggiomo et al., 2017 for further details).

Phytoplankton and zooplankton were collected by vertically sampling the water column at a depth of 20 m with a plankton net (20-μm mesh size) from sea-ice holes BEFORE and in open waters AFTER. Each zooplankton specimen was carefully separated from the bulk sample under a stereoscope. Phytoplankton was collected by filtering the remaining water sample at 100 μm and then collected on pre-combusted Whatman GF/F filters. In our study area, the phytoplankton

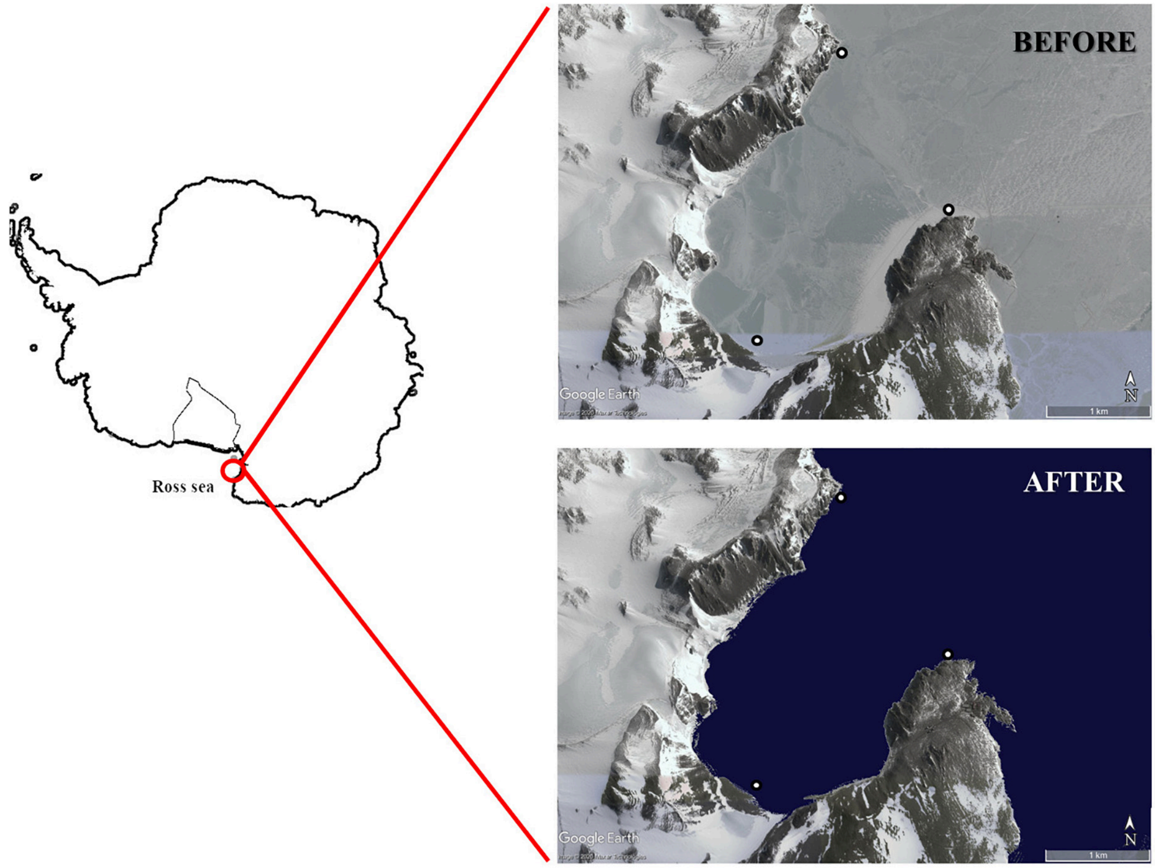

FIGURE 1 | Sampling sites in Tethys Bay (Terra Nova Bay, Ross Sea). The background image of Tethys Bay was obtained from Google Earth.

assemblages are dominated by *Phaeocystis antarctica* (Fabiano et al., 1997; Mangoni et al., 2019).

Since coprophagy is a common strategy among Antarctic benthic invertebrates (McClintock, 1994; Norkko et al., 2007), samples of seal feces from a colony of Weddell seals (*Leptonychotes weddellii*, Lesson, 1826) were also collected. The seal colony consisted of around 80–100 specimens usually stationed in the sampling area. Basal resources (including the upper 5 cm layer of sediments and seal feces) were collected within the first week of sampling activities in both sampling periods. They were analyzed in order to measure differences between BEFORE and AFTER in the isotopic baseline of consumer diets and food web architecture.

All collected samples were sorted, counted, identified to the finest possible taxonomic level and transported to Italy to be properly processed for the stable isotope analysis.

Laboratory Procedures and Stable Isotope Analysis

Samples were individually stored at −80°C and then freeze-dried for 24 h before Stable Isotope Analysis (SIA).

Specific soft tissues were taken from the large invertebrates (Norkko et al., 2007; Careddu et al., 2017; Calizza et al., 2018; Rossi et al., 2019). For small invertebrates (such as amphipods and polychaetes), the whole body was used. These samples were individually analyzed. In addition, samples of very small organisms (e.g., nematodes) were grouped in order to obtain sufficient biomass for stable isotope analyses (Hobson et al., 1995; Rossi et al., 2015). The dry sample was homogenized to a fine powder using an agate mortar and pestle or a ball mill (Mini-Mill Fritsch Pulverisette 23: Fritsch Instruments, Idar-Oberstein, Germany). Where necessary, samples were pre-acidified using 1M HCl drop-by-drop (Hobson et al., 1995) in order to eliminate inorganic carbon, which can interfere with the $\delta^{13}C$ signature, and were then re-dried at 60°C for 72 h. $\delta^{15}N$ was measured in non-acidified powders to avoid possible interference of HCl with the nitrogen signatures (Calizza et al., 2018; Rossi et al., 2019).

Aliquots of 0.50 ± 0.10 mg for animals and 4.00 ± 1.00 mg for basal resources were placed in tin capsules for the carbon and nitrogen SIA. Samples were then analyzed in two replicates using a continuous flow mass spectrometer (IsoPrime100, Isoprime Ltd., Cheadle Hulme, United Kingdom) coupled with an elemental analyzer (Elementar Vario Micro-Cube, Elementar Analysensysteme GmbH, Germany). Carbon (C) and Nitrogen (N) isotopic signatures were expressed in δ units ($\delta^{13}C$ and $\delta^{15}N$, respectively) as parts per thousand (‰) deviations from international standards (atmospheric N_2 for N; Vienna-PDB for C). The isotope ratios were calculated relative to the respective standard following the equation: δX (‰) = $[(R_{sample} - R_{standard})/R_{standard}] \times 10^3$ (McKinney et al., 1950; Vander Zanden and Rasmussen, 1996), where X is ^{13}C or ^{15}N and R is the corresponding ratio of heavy to light isotope for the element ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Caffeine IAEA-600 ($C_8H_{10}N_4O_2$) was used as the internal laboratory standard. Measurement errors associated with the linearity and stability of the mass spectrometer were typically smaller than 0.05‰, while the standard deviation of repeated measurements of lab standard material (one replicate every 10 analyses) was typically ± 0.1‰ for $\delta^{13}C$ and ± 0.2‰ for $\delta^{15}N$.

Isotopic Niche Metrics

The isotopic niche metrics of populations at both sampling times were calculated in accordance with Layman et al. (2007) and Jackson et al. (2011). These metrics, originally proposed for application at community level, can also be used at population level to obtain information on trophic diversity within a single population (Layman et al., 2007; Jackson et al., 2011; Careddu et al., 2017; Sporta Caputi et al., 2020). Carbon Range (CR) and Nitrogen Range (NR) were measured as the difference between the lowest and highest $\delta^{13}C$ and $\delta^{15}N$ values, respectively. CR provides information about the variety of food sources exploited by the population and hence its degree of trophic generalism. NR quantifies the degree of omnivory of the population, accounting for inter-specimen variation in trophic position.

The isotopic niche width was measured as the total area (TA) of the convex hull encompassing all the isotopic values of individuals within each population. It is expressed as the isotopic area (‰^{2}) and allows comparison of isotopic niches between populations. The overlap between the isotopic niches of populations was calculated and expressed both as a percentage of TA and as the number of overlaps for each taxon (R software ver. 3.5.3, SIBER analysis package, Jackson et al., 2011). Both TA and isotopic niche overlap were measured for populations common to BEFORE and AFTER with a sample size greater than or equal to 4 individuals (18 animal taxa in total). Although low, this sample size allowed us to include naturally scarce taxa in the comparison of isotopic niche metrics between study periods. The number was also less limiting for species diet reconstruction based on Bayesian Mixing Models.

The overall competitive effect was expressed as the diffuse overlap (*sensu* MacArthur, 1972 and Pianka, 1974), measured as the average overlapping TA multiplied by the average number of overlapping taxa.

Food Webs

The diet of each taxon was estimated by means of Bayesian Mixing Models (SIMMr package, R software ver. 3.5.3, Parnell et al., 2010; Parnell and Inger, 2016). The inputs of the model were: (i) the isotopic signatures of the target consumer, (ii) the mean isotopic signatures and relative standard deviation of potential food sources, and (iii) the Trophic Enrichment Factor (TEF) of the consumer. The TEFs between consumers and their potential food sources (expressed as the mean ± standard deviation) were 0.4 ± 0.2‰ for $\delta^{13}C$ and 2.3 ± 0.4‰ for $\delta^{15}N$ (Calizza et al., 2018; Costantini et al., 2018; Rossi et al., 2019). The models return the probability distribution of the proportional contribution of each food source to the consumer's diet, taking account of the uncertainty in the source and Trophic Enrichment Factor (TEF) values (Parnell et al., 2010; Jackson et al., 2011; Parnell and Inger, 2016; Careddu et al., 2017; Costantini et al., 2018; Rossi et al., 2019). The set of initial potential food sources for each consumer was assigned based on literature data and/or its position in the bi-dimensional isotopic space with respect to that of the target consumer (Costantini et al., 2018; Rossi et al., 2019). Since a high number of resources can reduce the discriminatory ability of mixing models and thus the effectiveness of Stable Isotope Analysis in food web reconstruction, for each taxon, a multiple-step procedure, in accordance with Costantini et al. (2018), was applied. Based on the output of the Bayesian isotopic Mixing Models, the pool of real (i.e., likely to be assimilated) food sources for each consumer was then selected in accordance with Costantini et al. (2018) and Rossi et al. (2019). At each step in the mixing model, a food source was maintained if either (i) its modal contribution was at least 5% and a contribution of 0% did not lie within the 75 or 95% confidence intervals or (ii) the food source was necessary in order to obtain the mixing space that enclosed the consumer.

Finally, following the assignment of all the trophic links, the structures and the respective metrics of the food webs were obtained using the software (Cytoscape 3.7.2 and R-Statistical 3.5.3). Although a generally accepted classification of Antarctic organisms into trophic guilds is limited by their marked trophic generalism and omnivory, purely in order to improve food web representation, taxa were grouped into broad "feeding modes" based on bibliographical information (McClintock, 1994; Dunton, 2001; Gillies et al., 2013). These included: "suspension feeder," "deposit feeder," "omnivorous/grazer," "omnivorous/predator," and "predator."

Food Web Metrics and Data Analysis

The food web metrics of the BEFORE and AFTER communities were calculated and compared using the Cheddar and NetworkExtinction packages in R-software (Hudson et al., 2013; Corcoran et al., 2019) and Cytoscape 3.7.2.

In the food web, the nodes are the taxa (S) and the links (L) are the feeding links from resources to consumers. The Linkage density (L/S) was measured as the average number of feeding links per taxon present in the food web. The minimum Connectance (Cmin) was calculated as: $Cmin = 2L/S^2$. The trophic position (TP) of each taxon (i) was calculated in accordance with the formula: $TP_i = 1 + \Sigma_j (TP_j \times f_{ij})$, where TPj is the trophic position of food item j and f is the proportional consumption of food item j by consumer i (Odum and Heald, 1975). TP = 1 was assigned to basal food sources. The proportional similarity of diet among taxa was measured by the Bray–Curtis similarity index. The index is based on the contribution of each resource to the diet of each consumer and it ranges from 0, when no common resources are found for the compared groups, to 1, when the compared groups have the same resources in the same proportions (Calizza et al., 2018; Costantini et al., 2018; Sporta Caputi et al., 2020).

The potential competition for resources was measured in accordance with Levins' (1968) equation:

$$\alpha_{i,j} = \frac{\sum_k pi_k \times pj_k}{\sum_k \left(pi_k\right)^2}$$

in which the competition strength $\alpha_{i,j}$ is expressed as the effect of species j on species i, where pi_k and pj_k are the proportional contributions of any given resource k to species i and j, respectively.

Topological food web metrics were also measured. These included Betweenness Centrality (BC), Neighborhood Connectivity (NC) and food web robustness to taxon (i.e., node) loss. BC is a measure of the centrality of a taxon in the food web. This parameter indicates the importance of a taxon in terms of the role it plays in connecting other nodes in the network (Freeman, 1977). Taxa with BC > 0 play a central role in connecting others within the food web (Martín González et al., 2010). Neighborhood Connectivity (NC) indicates the average connectivity of each taxon in the food web and provides a measure of interconnection (direct and indirect) among all taxa in the food web. Food web robustness to loss of taxa was estimated by simulating primary extinctions (*sensu* Dunne et al., 2002). Taxa were sequentially removed from the most to the least connected, in accordance with Calizza et al. (2012) and Rossi et al. (2019). Secondary extinction occurs when a taxon loses all of its food items.

Statistics

If not specified otherwise, the results are reported as mean ± standard error (SE). The Shapiro–Wilk normality test was applied to each dataset to verify that it followed a Gaussian distribution.

Differences between sampling periods were tested using the Student's *t*-test for variables that fulfilled the normality assumption (similarity of diet, competition strength, number of competitors) and the Mann-Whitney test when normality was not observed (mean trophic position and food chain, Neighborhood Connectivity). The same tests were also used to test differences in the contribution of resources to the diet of BEFORE and AFTER communities.

Two-way PERMANOVA and associated *post hoc* comparisons were used to test for the effect of taxon and period (i.e., BEFORE and AFTER) on the isotopic signatures of basal resources and consumers. A Mantel test was performed in order to assess the correlation between the matrices of isotopic Euclidean distances and the Bray-Curtis diet similarity (Signa et al., 2019).

Finally, the difference between BEFORE and AFTER in terms of the proportion of the total number of links in the web accounted for by feeding links between resources and consumers was assessed using Chi-square (χ^2) tests, while ANCOVA was used to test the difference in total extinction distribution. In order to avoid the potential confounding effects of differences in taxonomic composition between the BEFORE and AFTER communities, further comparisons of isotopic and food web metrics included only consumers common to both sampling periods and were performed using paired *t*-tests and Wilcoxon paired tests for inter-period comparison of variables with normal and non-normal distribution, respectively.

RESULTS

Isotopic Niche and Species Diet

A total of 1732 specimens belonging to 61 animal taxa were sampled, 34 of which were common to the BEFORE and AFTER samples (**Table 1** and **Figure 2**). Sediments, sympagic algae and animal-derived food (live prey or carrion) were the resource items that most contributed to the diet of benthic organisms in both sampling periods (**Figure 3**).

Basal resources were isotopically distinct from each other (although interface diatoms had similar values to the ultra-fine fraction), and their BEFORE and AFTER values did not differ (**Figure 2**; Two-way PERMANOVA, $F_{resource} = 99.17$, $p < 0.0001$; $F_{time} = 2.32$ and $F_{interaction} = -2.20$, n.s.; pairwise *post hoc* comparisons). Among the basal food sources, the red macroalga *Phyllophora antarctica* and sympagic algae (especially filamentous algae) had the lowest and highest $\delta^{13}C$ values (respectively, −36.7 ± 0.3‰ and −13.7 ± 0.5‰), while the ultra-fine sediment fraction and seal feces had the lowest and the highest $\delta^{15}N$ values (respectively, −0.04 ± 0.02‰ and 13.6 ± 0.1‰; **Figure 2**).

While no differences between sampling periods were found in the $\delta^{13}C$ distribution within the community as a whole, the $\delta^{13}C$ values of taxa common to the two periods were higher in the AFTER sample (Wilcoxon paired test, $z = 2.2$, $p < 0.05$), with values closer to those typical of sympagic algae (**Table 1** and **Figure 2**). Yet differences between groups were also observed. Zooplankton and Amphipoda were the most ^{13}C-depleted consumers BEFORE, with values denoting dependence on phytoplankton and detritus chains. Amphipoda, which occupied the outermost area of the isotopic niche space close to the benthic compartment resources, had more enriched $\delta^{13}C$ values, similar to those of sympagic algae, AFTER. The sea urchin *Sterechinus neumayeri* had the highest $\delta^{13}C$ among consumers, showing dependence on sympagic algae in both periods, although this dependence was more pronounced AFTER. Other Antarctic key species such as the predaceous sea star *Odontaster meridionalis* and the anemone *Urticinopsis antarctica* occupied a central position in the isotopic space, with intermediate $\delta^{13}C$ and high $\delta^{15}N$ values, indicating consumption of materials mainly of animal origin, both BEFORE and AFTER (**Table 1** and **Figure 2**).

The mean isotopic niche width (Total Area) of taxa common to both periods decreased from 7.60 ± 1.3‰^{2} to 6.05 ± 1.3‰^{2} (**Table 2**), and the Carbon Range decreased from 5.2 ± 0.8‰ to 3.5 ± 0.5‰ (paired *t*-test, t: 1.78, $p < 0.01$), but individual taxa displayed distinctive trends. While the brittle star *Ophionotus victoriae,* the sea-star *Diplasterias brucei* and the anemone *Urticinopsis antarctica* mirrored the general trends, with decreasing Total Area and CR after the sea-ice break-up, the

TABLE 1 | Community carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic signatures (mean ± SE) BEFORE and AFTER.

Phylum-Group	Class	Order	Taxon	Before					After					Food web ID
				n	δ^{13}C (‰)	δ^{15}N (‰)	FL	TP	n	δ^{13}C (‰)	δ^{15}N (‰)	FL	TP	
Annelida														
	Polychaeta													
		–	*Leitoscoloplos kerguelensis*	5	−21.2 ± 0.6	10.5 ± 0.5	4	2.0	–	–	–	–	–	1
		Phyllodocida	*Barrukia cristata*	7	−21.9 ± 0.7	8.2 ± 0.5	7	3.3	–	–	–	–	–	2
			Hesionidae	12	−21.3 ± 0.5	9.6 ± 0.3	9	3.3	–	–	–	–	–	3
			Polynoidae	5	−20.8 ± 0.4	9.4 ± 0.1	9	3.2	–	–	–	–	–	4
			Psamathe fauveli	2	−19.6 ± 0.5	6.7 ± 0.5	2	2.8	–	–	–	–	–	5
		Sabellida	*Perkinsiana sp.*						3	−22.5 ± 2.3	7.2 ± 0.2	3	2.1	6
			Serpulidae	4	−21.9 ± 0.7	7.1 ± 0.5	7	2.2	3	−22.6 ± 0.1	7.1 ± 0.2	5	2.2	7
		Spionida	Spionidae	2	−23.1 ± 0.5	7.5 ± 0.5	3	2.0	–	–	–	–	–	8
		Terebellida	*Flabelligera mundata*	2	−19.7 ± 0.5	7.8 ± 0.5	5	2.2	–	–	–	–	–	9
			Terebellida	19	−23.8 ± 0.3	6.8 ± 0.1	3	2.4	7	−22.7 ± 0.4	6.9 ± 0.3	7	2.1	10
Arthropoda														
	Malacostraca													
		Amphipoda	*Amphipoda (alia)*	4	−24.6 ± 0.4	10.5 ± 0.3	3	2.9	2	−21.9 ± 0.5*	9.8 ± 0.5	4	2.1	11
			Orchomenella sp.	6	−21.9 ± 0.5	8.2 ± 0.6	4	2.5	–	–	–	–	–	12
			Paramoera walkeri	13	−17.5 ± 1.2	6.2 ± 0.6	4	2.0	–	–	–	–	–	13
		Isopoda	Arcturidae	2	−21.9 ± 0.5	5.2 ± 0.5	5	2.0	–	–	–	–	–	14
			Munna sp.	5	−20.3 ± 0.4	7.4 ± 0.2	6	2.4	–	–	–	–	–	15
			Paramunnidae	9	−20.4 ± 0.2	4.4 ± 0.2	3	2.0	–	–	–	–	–	16
		Tanaidacea	*Nototanais dimorphus*	3	−19.0 ± 1.0	8.8 ± 0.3	7	2.7	–	–	–	–	–	17
	Pycnogonida													
		Pantopoda	*Achelia* sp.	11	−24.1 ± 0.3	9.4 ± 0.3	4	3.1	7	−24.1 ± 0.2	10 ± 0.3	4	2.5	18
			Ammothea sp.	23	−22.9 ± 0.1	11.3 ± 0.2	6	3.3	17	−22.8 ± 0.4	12.4 ± 0.2*	6	3.2	19
			Colossendeis sp.	2	−23.2 ± 0.5	10.3 ± 0.5	5	3.1	5	−22.9 ± 0.2	9.2 ± 0.2	5	2.6	20
			Nymphon australe	12	−23 ± 0.3	8.1 ± 0.5	6	3.2	–	–	–	–	–	21
Bryozoa														
	Gymnolaemata													
		Cheilostomatida	*Camptoplites* sp.	8	−18.1 ± 0.8	6.2 ± 0.2	3	2.0	3	−21.0 ± 0.7*	5.0 ± 0.0*	4	2.0	22
Chordata														
	Ascidiacea													
Cnidaria			Ascidiacea	8	−23.3 ± 0.5	6.7 ± 0.2	5	2.2	4	−23.2 ± 0.2	6.8 ± 0.3	3	2.3	23
	Anthozoa													
		Actinaria	*Urticinopsis antarctica*	7	−20.3 ± 0.8	9.3 ± 0.4	7	3.4	8	−18.9 ± 0.3	10 ± 0.2	5	3.5	24
		Alcyonacea	*Alcyonium antarcticum*	11	−22.2 ± 0.6	7.0 ± 0.1	7	2.2	9	−21.7 ± 0.5	7 ± 0.1	6	2.1	25
			Clavularia frankliniana	3	−20.4 ± 0.3	8.3 ± 0.2	5	2.2	–	–	–	–	–	26
Echinodermata														
	Asteroidea													
		Forcipulatida	*Diplasterias brucei*	22	−19.2 ± 0.4	8.2 ± 0.2	3	3.2	27	−18.9 ± 0.4	8.6 ± 0.2	3	3.1	27

(Continued)

TABLE 1 | Continued

Phylum-Group	Class	Order	Taxon	Before					After					Food web ID
				n	δ^{13}C (‰)	δ^{15}N (‰)	FL	TP	n	δ^{13}C (‰)	δ^{15}N (‰)	FL	TP	
			Diplasterias brucei (adults)	3	−16.5 ± 0.2	9.2 ± 0.2	5	3.3	–	–	–	–	–	28
		Valvatida	*Acodontaster hodgsoni*	2	−18.0 ± 0.5	11.7 ± 0.5	4	3.1	–	–	–	–	–	29
			Odontaster meridionalis	8	−19.4 ± 0.5*	12.6 ± 0.6	5	3.6	3	−21.2 ± 0.9*	10.3 ± 0.5*	5	3.2	30
			Odontaster validus	20	−17.0 ± 0.4	9.7 ± 0.1	7	3.2	22	−17.7 ± 0.4	9.6 ± 0.2	6	3.0	31
			Perknaster fucus antarcticus	2	−17.9 ± 0.5	11.4 ± 0.5	3	3.5	–	–	–	–	–	32
	Echinoidea													
		Camarodonta	*Sterechinus neumayeri*	25	−13.6 ± 0.4	7.2 ± 0.1	8	2.5	25	−13.6 ± 0.4	7.6 ± 0.3	8	2.2	33
	Holothuroidea													
		Dendrochirotida	*Cucumaria* sp.	3	−21.5 ± 1.6	5.5 ± 0.2	5	2.0	–	–	–	–	–	34
			Staurocucumis turqueti	14	−22.7 ± 0.2	7.6 ± 0.3	11	2.2	26	−22.3 ± 0.1*	7.7 ± 0.2	3	2.2	35
	Ophiuroidea													
		Ophiurida	*Ophionotus victoriae*	21	−18.6 ± 0.7	8.2 ± 0.2	17	2.8	21	−18.1 ± 0.5	8.4 ± 0.2	9	2.4	36
			Ophioplinthus gelida	9	−21.3 ± 0.7	7.7 ± 0.3	4	2.1	6	−20.5 ± 1.0	8.2 ± 0.7	9	2.2	37
Mollusca														
	Bivalvia													
		-	*Laternula elliptica*	3	−22.0 ± 0.3	5.7 ± 0.8	8	2.1	3	−22.2 ± 0.3	6.6 ± 0.3	6	2.2	38
		Limida	*Limatula hodgsoni*						2	−21.5 ± 0.5	7.9 ± 0.5	5	2.3	39
		Nuculanida	*Aequiyoldia eightsii*	2	−17.3 ± 0.5	8.0 ± 0.5	2	2.0	2	−16.1 ± 0.5	6.3 ± 0.5	2	2.0	40
		Pectinida	*Adamussium colbecki*	24	−21.7 ± 0.1	5.7 ± 0.2	8	2.1	16	−21.8 ± 0.1	6.4 ± 0.9	9	2.1	41
	Gastropoda													
		Neogastropoda	*Neobuccinum eatoni*	24	−18.9 ± 0.2	10.3 ± 0.4	4	2.8	34	−18.5 ± 0.2	11.1 ± 0.2	4	2.6	42
			Trophonella longstaffi	2	−20.0 ± 0.5	9.0 ± 0.5	3	3.1	–	–	–	–	–	43
		Nudibranchia	*Tritoniella belli*	4	−23.3 ± 0.2	9.9 ± 0.8	4	2.6	–	–	–	–	–	44
		Pteropoda	*Clione limacina antarctica*	17	−25.7 ± 0.2	5.4 ± 0.2	3	2.3	9	−25.9 ± 0.2	5.2 ± 0.3	3	2.9	45
			Limacina helicina	50	−23.6 ± 0.3	4.2 ± 0.4	2	2.0	5	−21.7 ± 0.2*	4.8 ± 0.0*	3	2.0	46
Nematoda														
			Nematoda	9	−23.0 ± 0.2	9.9 ± 0.3	3	2.0	7	−21.9 ± 0.3*	8.8 ± 0.2*	4	2.0	47
Nemertea														
	Pilidiophora													
			Parborlasia corrugatus	7	−20.5 ± 0.2	8.5 ± 0.2	10	2.8	–	–	–	–	–	48
Porifera														
	Demospongiae													
		Dendroceratida	*Dendrilla antarctica*	9	−23.2 ± 0.1	5.7 ± 0.1	5	2.3	2	−23.5 ± 0.5	5.5 ± 0.5	3	2.3	49
		Haplosclerida	*Haliclona dancoi*	4	−23.1 ± 0.1	10.1 ± 0.2	3	3.4	–	–	–	–	–	50

(Continued)

TABLE 1 | Continued

Phylum-Group	Class	Order	Taxon	Before					After					Food web ID
				n	δ13C (‰)	δ15N (‰)	FL	TP	n	δ13C (‰)	δ15N (‰)	FL	TP	
			Haliclona sp.	8	−23.7 ± 0.2	8.1 ± 0.6	3	2.7	5	−23.8 ± 0.4	8.2 ± 0.6	5	2.3	51
			Haliclona tenella	2	−22.9 ± 0.5	10.7 ± 0.5	3	3.5	3	−22.9 ± 0.0	10 ± 0.1	5	2.2	52
			Hemigellius fimbriatus	3	−24.0 ± 0.4	7.4 ± 0.6	3	2.3	2	−23.5 ± 0.5	10.3 ± 0.5*	4	2.3	53
			Hemigellius sp.	7	−23.8 ± 0.2	8.0 ± 1.0	6	2.5	5	−23.4 ± 0.4	6.8 ± 0.1*	4	2.1	54
		Poecilosclerida	*Kirkpatrickia variolosa*	3	−23.2 ± 0.0	6.5 ± 0.4	6	2.2		-	-			55
			Mycale sp.	6	−23.2 ± 0.1	7.1 ± 0.4	5	2.6	6	−23.0 ± 0.8	7.4 ± 0.6	6	2.2	56
		Polymastiida	*Polymastia invaginata*	2	−21.6 ± 0.5	8.6 ± 0.5	4	2.9	4	−20.6 ± 0.5	8.9 ± 0.2	6	2.4	57
			Sphaerotylus antarcticus	6	−21.8 ± 0.1	10.6 ± 0.2	4	3.1	8	−21.9 ± 0.2	10.2 ± 0.2	3	2.6	58
		Suberitida	*Homaxinella balfourensis*	5	−21.8 ± 0.3	6.4 ± 0.3	5	2.1	–	–	–	–	–	59
			Suberites topsenti	10	−24.0 ± 0.2	9.8 ± 0.7	3	3.1	5	−23.9 ± 0.2	9.2 ± 1.5	5	2.2	60
Benthic algae														
			Benthic diatoms	3	−21.0 ± 1.3	4.1 ± 0.8		1.0	3	−20.5 ± 1.0	4.1 ± 0.8		1.0	R1
			Iridaea cordata	3	−21.4 ± 0.8	4.2 ± 0.1		1.0	3	−21.0 ± 1.0	4.0 ± 0.6		1.0	R7
			Phyllophora antarctica	17	−37.0 ± 0.4	0.7 ± 0.3		1.0	16	−35.9 ± 0.1	2.2 ± 0.1		1.0	R14
Epiphytes														
			Epiphytes	6	−23.8 ± 0.1	6.0 ± 0.3		1.0	5	−24.3 ± 0.5	5.7 ± 0.3		1.0	R3
Plankton														
			Phytoplankton	6	−25.1 ± 1.3	4.4 ± 0.2		1.0	5	−24.5 ± 0.0	4.4 ± 0.0		1.0	R8
			Zooplankton	4	−25.7 ± 0.5	6.4 ± 0.7	6	2.0	6	−21.3 ± 0.1*	4.3 ± 0.1*	4	2.0	61
Seal Feces														
			Seal Feces	7	−26.4 ± 0.7	13.6 ± 0.2		1.0	4	−26.6 ± 0.0	14 ± 0.0		1.0	R4
Sediment														
			Coarse Sediment	3	−22.2 ± 0.1	7.4 ± 0.0		1.0	3	−22.0 ± 0.1	7.4 ± 0.1		1.0	R9
			Fine Sediment	3	−20.8 ± 0.1	5.3 ± 0.0		1.0	3	−20.7 ± 0.1	5.2 ± 0.1		1.0	R10
			Fine-Ultra-Fine Sediment	15	−22.9 ± 0.2	4.4 ± 0.2		1.0	6	−22.0 ± 0.6	5.0 ± 0.3		1.0	R11
			Ultra-Fine Sediment	3	−21.2 ± 0.2	0.0 ± 0.0		1.0	6	−21.8 ± 0.9	0.83 ± 0.06		1.0	R12
Sympagic algae														
			Diatoms (ice core)	27	−18.8 ± 0.9	0.8 ± 1.1		1.0	–	–	–		1.0	R13
			Diatoms (interface)	26	−22.8 ± 1.6	0.9 ± 0.7		1.0	–	–	–		1.0	R2
			Filamentous algae (ice core)	27	−13.7 ± 0.5	1.8 ± 0.3		1.0	–	–	–		1.0	R5
			Filamentous algae (interface)	20	−16.0 ± 0.1	2.9 ± 0.0		1.0	–	–	–		1.0	R6

A dash (–) indicates that the taxon was not found. Superscript letters indicate the top ten central nodes (i.e., the topological keystone taxa) in the food web BEFORE (^{B}) and AFTER (^{A}).
Asterisks indicate significant differences between BEFORE and AFTER (two-way PERMANOVA, $p < 0.01$).
n, number of isotopic samples; FL, Feeding links; TP, Trophic position; Food web ID, identification code as shown in ***Figure 4****.*
Underlined IDs indicate taxa common to BEFORE and AFTER for which isotopic niche metrics were compared (for details, refer to section "Materials and Methods").

scallop *Adamussium colbecki*, the brittle star *Ophioplinthus gelida* and the sea-urchin *Sterechinus neumayeri* increased their Total Area along with NR.

The closer the taxa in the isotopic niche space, the more similar their diet according to the Bayesian mixing models (Mantel test, Correlation R: 0.5, $p < 0.001$). Within trophic guilds,

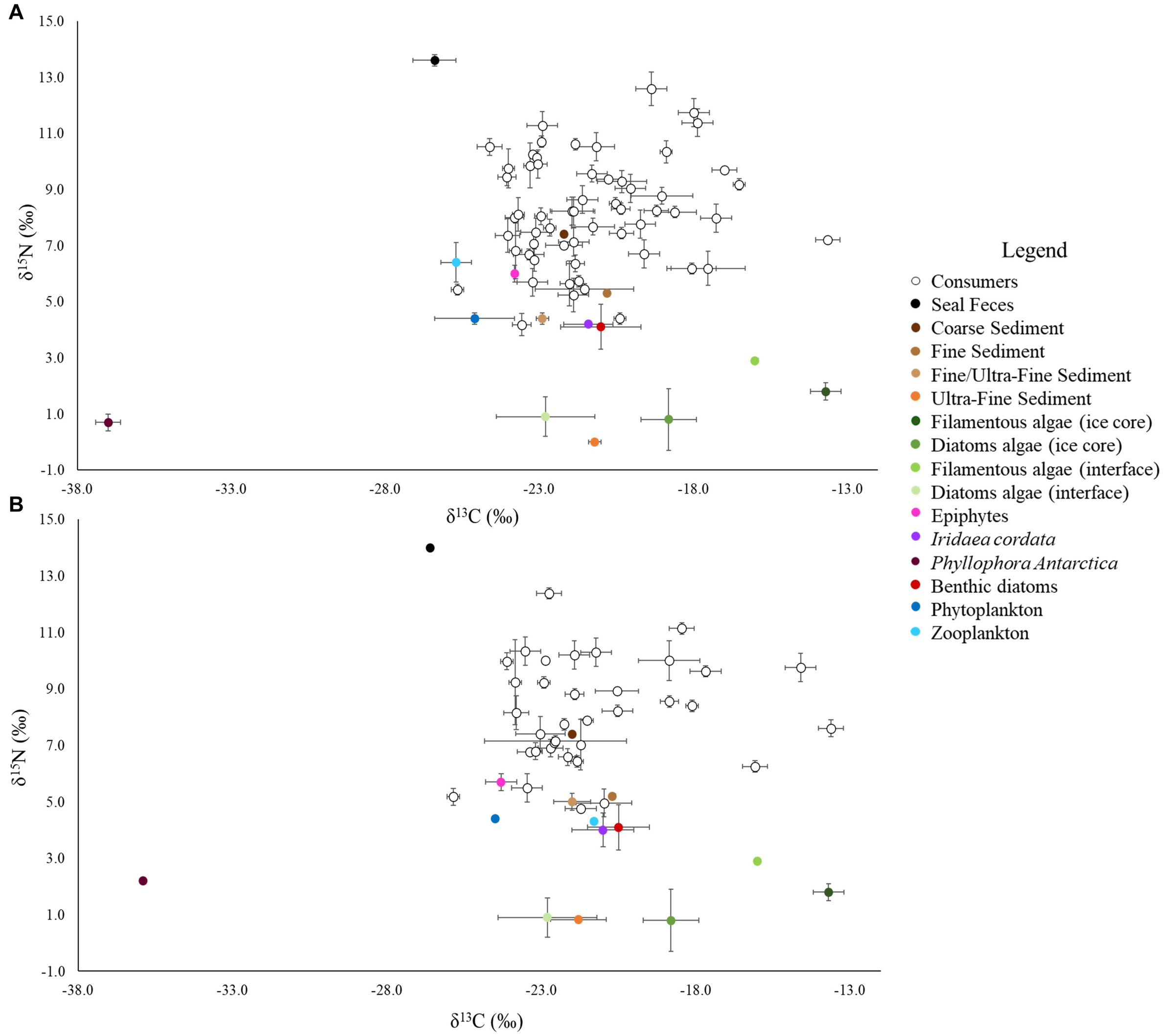

FIGURE 2 | Isotopic biplots of the BEFORE **(A)** and AFTER **(B)** communities. Each point represents a taxon. Isotopic signatures were expressed as the mean ± SE. The legend shows the respective colors of consumers and basal resources.

the isotopic signatures of taxa were rather heterogeneous (e.g., the two congeneric seastars *Odontaster meridionalis* and *O. validus* and the two bivalves *Aequiyoldia eightsii* and *Adamussium colbecki*, **Table 1**). Consistent with the changes in the isotopic distribution of animal taxa, the contribution of sympagic algae to their diet increased after the sea-ice break-up (t-test, t: 2.49, $p < 0.05$), while that of animal-derived food decreased (Mann-Whitney test, U: 466, $p < 0.01$, **Figure 3**). As a result, the diet composition of the various taxa became more similar (Bray-Curtis: 15.07 ± 0.86% and 22.58 ± 1.86%; t-test, $p < 0.01$). Among other resources, the contribution of seal feces to consumers' diets was low in both BEFORE and AFTER.

The mean potential competition strength based on isotopic niches increased but the number of competitor taxa decreased (t-test, t at least: 2.37, $p < 0.01$, **Tables 1**, **3**). The lower number of competitors, together with the generally narrower isotopic niche in AFTER (**Table 2**), was reflected in a lower diffuse niche overlap between taxa (from 235.9 ± 36.4% to 196.1 ± 33.6%; paired t-test, t: 2.3, $p < 0.05$.

Food Web Structures

The two periods were characterized by different food web topology and metrics (**Table 3**). Both the total number of feeding links and the number of food chains in the web were higher BEFORE than AFTER (**Figures 4**, **5A** and **Table 3**). Specifically, the feeding links between consumers and basal food sources increased from 52% to 66% of the total (**Table 1**, χ^2-test, χ^2:10, $p < 0.01$) and Compartmentalization slightly increased from 0.10 to 0.13 (**Table 3**). Furthermore, although a similarly, high degree of omnivory was found in the BEFORE and AFTER food webs, the food chains were shorter on average (**Figures 4**, **5A**) and the mean trophic position was thus lower AFTER than BEFORE (**Table 1** and **Figure 4**; Mann-Whitney test, U:1323.5, $p < 0.05$).

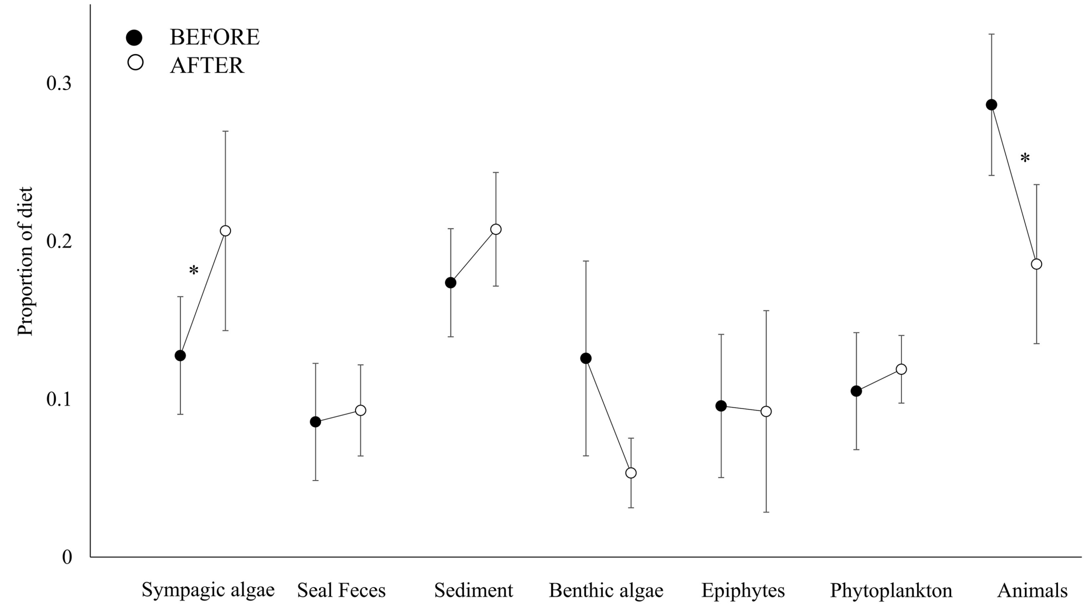

FIGURE 3 | Contribution of basal resources (Sympagic algae, Seal feces, Sediment, Benthic algae, Epiphytes and Phytoplankton) and animal-derived resources (Animals) to the diet BEFORE (full symbols) and AFTER (empty symbols). The proportional contribution of each item is reported as the mean ± SE. Superscript symbols (*) indicate a significant difference between periods (see also section "Results").

The trophic position of consumers varied between 2.0 and 3.6 (**Figure 4**). *Urticinopsis antarctica*, *Odontaster meridionalis*, *Ammothea* sp., *D. brucei,* and *O. validus* had a high trophic position, equal to or greater than 3, in both sampling periods. Another 14 taxa had a similarly, high trophic position BEFORE but, of those species present in both periods, the sea-spiders *Colossendeis* sp. and *Achelia* sp. and the sponges *Sphaerotylus antarcticus, Haliclona tenella,* and *Suberites topsenti* were found at lower trophic positions AFTER (**Table 1**). More generally, at the guild level, the common suspension feeders, including sponges, had significantly lower trophic positions AFTER (**Table 1** and **Figure 4**; Wilcoxon test, W: 263, $p < 0.01$).

TABLE 2 | Isotopic niche and food web metrics of taxa common to BEFORE and AFTER.

	Before	After
Isotopic niche metrics		
CR**	5.24 ± 0.77	3.47 ± 0.55
NR	3.63 ± 0.54	3.37 ± 0.51
TA	7.60 ± 1.35	6.05 ± 1.32
S in overlap**	8.56 ± 0.85	5.17 ± 0.75
% overlap	25.52 ± 2.79	34.23 ± 5.67
Food web metrics		
Links per species (2L/S)**	9.39 ± 1.01	7.26 ± 0.70
Feeding links per species (L/S)	3.90 ± 0.36	3.60 ± 0.39
No of competitors***	38.28 ± 2.56	26.28 ± 1.72
Competition strength (α)	0.32 ± 0.03	0.35 ± 0.04

Each parameter was expressed as the mean ± SE of the taxa sampled in each study period.
CR, Carbon Range (‰); NR, Nitrogen Range (‰); TA, Total Area (‰^{2}).
For details of metrics, please refer to the "Materials and Methods" section.
*Superscript symbols indicate a significant difference between periods (*Wilcoxon paired test).*
One, two, and three superscript symbols indicate p values of <0.05, <0.01, and <0.001, respectively.

As observed for the community as a whole (**Table 3**), the taxa common to both sampling periods were less connected (both directly and indirectly) with others in the AFTER food web (NC: 14.37 ± 0.73 vs. 11.39 ± 0.60, Wilcoxon test, W: 1009, $p < 0.001$). The mean linkage density decreased (**Table 2**, Wilcoxon test, W: 659, $p < 0.01$), together with both the number of food items in the diet and potential competitors (**Tables 1**, **2**; Wilcoxon test, W: 594, and W: 638, $p < 0.0001$, respectively). Specifically, the most generalist taxa BEFORE saw the greatest reductions in the number of consumed resources AFTER (e.g., *O. victoriae* and the sea cucumber *Staurocucumis turqueti*), while a small number of specialist taxa had a higher number of trophic links AFTER (e.g., Terebellidae and the brittle star *Ophioplinthus gelida*).

These differences influenced the topology of the food web and its robustness to the loss of highly connected nodes, which was higher in the AFTER food web (**Table 3** and **Figure 5B**, ANCOVA test, F: 125.1, $p < 0.001$). Highly connected nodes BEFORE included zooplankton and Amphipoda (including *Paramoera walkeri*), whereas AFTER they included *Ophioplintus gelidae, Aequiyoldia eightsii,* Terebellidae and *D. brucei*. Basal resources and *S. neumayeri* were highly connected in both periods. Except for *A. eightsii* and basal resources, these taxa also ranked among the 10 most central nodes in the food web structure in terms of their Betweenness Centrality (**Figure 4**), with much higher consumption of sympagic algae AFTER.

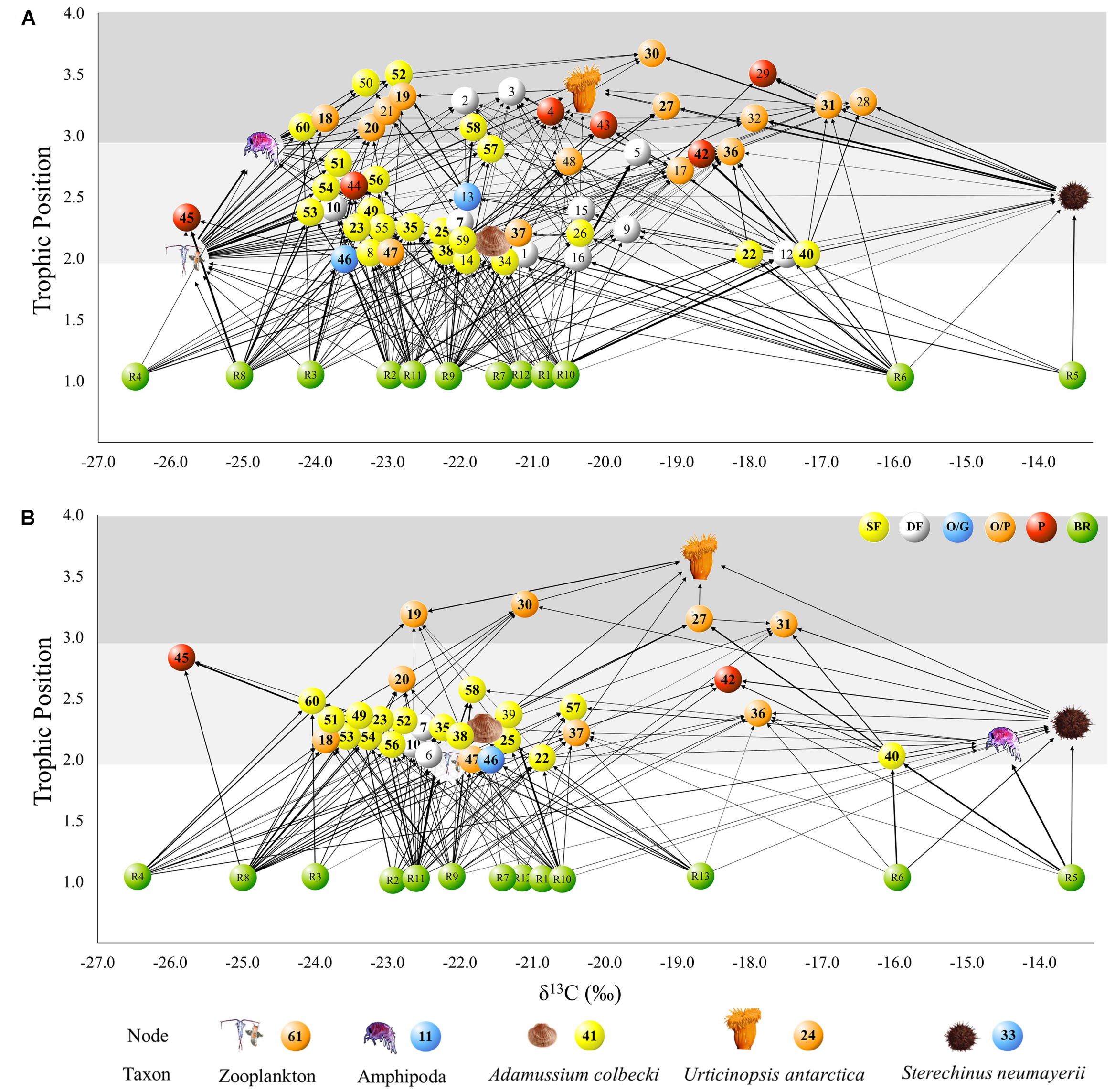

FIGURE 4 | Benthic food web structure in Tethys Bay, Terra Nova Bay (Ross Sea): BEFORE **(A)** and AFTER **(B)**. Each node represents one taxon in the community. Arrows point from a resource to its consumer and their thickness is proportional to the linkage strength. The position of each node represents its $\delta^{13}C$ and Trophic Position (TP). Colors indicate the dominant feeding mode of each taxon: yellow: suspension feeder; white: deposit feeder; blue: omnivorous/grazer; orange: omnivorous/predator; red: predator. Basal resources are shown in green. The food web graphs were developed using Cytoscape software. Bold IDs indicate taxa sampled in both study periods. For the list of taxa please refer to **Table 1**.

DISCUSSION

Antarctic benthic organisms are markedly adapted to their extreme environment, which is characterized by long-term low availability of food resources, which are closely linked to seasonal sea-ice dynamics (Pusceddu et al., 2000; Clark et al., 2013; Calizza et al., 2018; Rossi et al., 2019). Thus, changes in sea-ice spatio-temporal dynamics, which are expected in all climate change scenarios (Schofield et al., 2010; Constable et al., 2014), will determine changes in resource inputs, affecting biodiversity persistence and ecosystem structure and functioning (Pusceddu et al., 2000; Norkko et al., 2007; Clark et al., 2013; Constable et al., 2014; Calizza et al., 2018; Michel et al., 2019; Rossi et al., 2019).

In this study, community isotopic characterization, coupled with diet and food web reconstruction, provided crucial support for understanding and describing the response of Antarctic coastal benthic communities to the availability of basal resources under two different sea-ice coverage conditions (Calizza et al., 2015; Rossi et al., 2015, 2019). The results highlighted significant changes in the trophic niches of Antarctic benthic populations dwelling in shallow waters, as well as in food web architecture, after the sea-ice break-up and its associated input of sympagic algae. In addition, they allowed us to identify

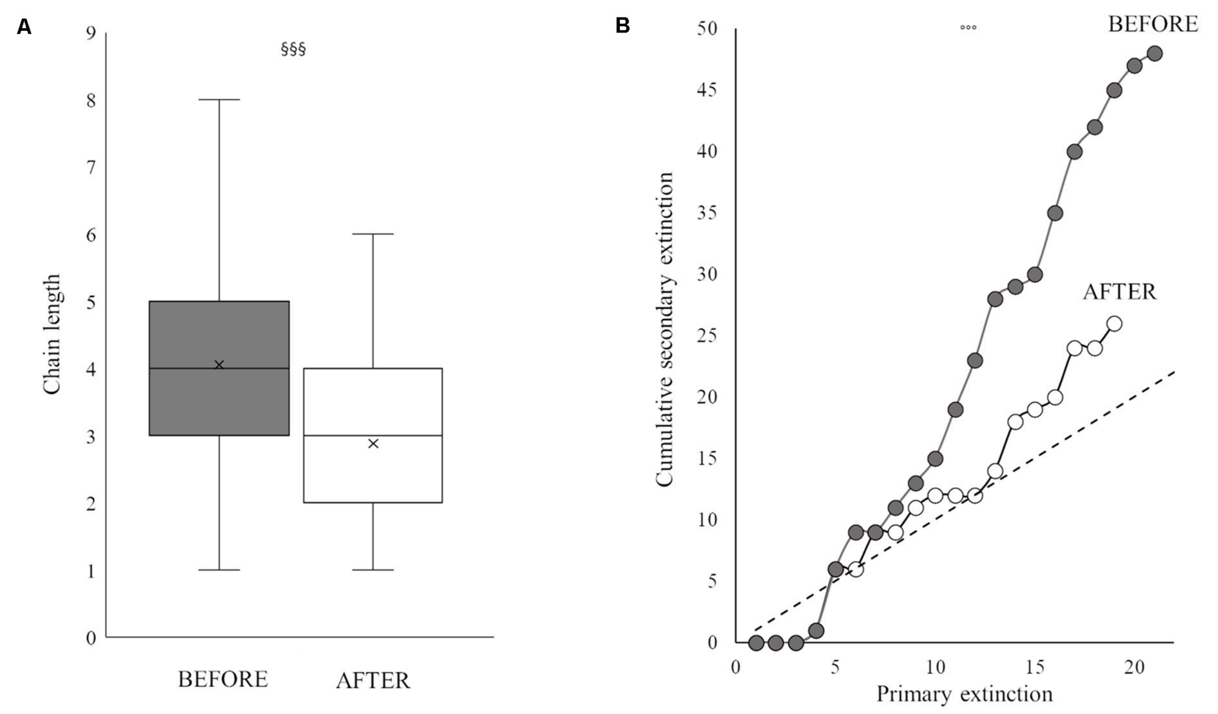

FIGURE 5 | Box plot of mean chain length **(A)** and robustness trend (primary extinction against cumulative secondary extinction) **(B)** BEFORE (gray) and AFTER (white). Superscript symbols (§ and ∘) indicate a significant difference between periods (see also "Results" section). One, two and three superscript symbols indicate *p* values of <0.05, <0.01, and <0.001, respectively.

the main carbon pathways that support the benthic populations, including taxa that play a key role in the organization of these complex communities.

The isotopic signatures of basal resources were clearly distinct and highly conserved over time. This allowed a robust description of consumers' diets based on isotopic Bayesian Mixing Models. In addition, values were similar to what has been observed in other study areas (Kaehler et al., 2000; Gillies et al., 2013) and in Tethys Bay in different seasons and years and at a range of depths (Norkko et al., 2007; Calizza et al., 2018; Rossi et al., 2019), demonstrating the slow pace of biochemical transformation. The observed isotopic similarity of phytoplankton over time suggests that samples collected in dark waters before the sea-ice break-up are the result of horizontal transport to the coast from open waters, as already reported for the study area (Pusceddu et al., 1999; Norkko et al., 2007). The isotopic signature of the ultra-fine sediment fraction (particle diameter <0.56 mm) did not differ significantly from that of sympagic diatoms. Other studies have found isotopic similarity between sediments and sympagic algae, suggesting that these algae are available to benthic organisms both as fresh material when released into the water column and as a small but constant supply of organic matter in the sediment (Lizotte, 2001; Wing et al., 2018). The latter constitutes an important food bank in the unproductive season (Mincks et al., 2008), consistent with several studies carried out in the study area (Cattaneo-Vietti et al., 1999; Pusceddu et al., 2000). Biochemical studies show that the organic matter on the bottom in the neritic zone of the area was largely undegraded (Fabiano and Pusceddu, 1998; Pusceddu et al., 1999). The isotopic similarity suggests negligible biogeochemical transformations of organic carbon and nitrogen following production, and thus minimal change in food quality, which might impact the proportions of carbon and nitrogen sources and their apparent incorporation in benthic communities over time. By comparing the short- and long-term diets of the sea urchin *Sterechinus neumayeri*, Calizza et al. (2018) demonstrated the rapid availability of sympagic material for the benthos within a few days of sea-ice break-up. Similarly, Wing et al. (2018) observed that the contribution of sympagic algae to the secondary productivity of Antarctic benthos increased near the edge of the sea ice soon after the summer break-up. However, this may not represent a general pattern in polar areas (Gibson et al., 1999; Lovvorn et al., 2005). Biochemical processes in sediments associated with the short- and long-term deposition of sympagic and pelagic matter after sea-ice break-up thus require further studies in order to improve our understanding of carbon and nitrogen pathways and recycling in the Antarctic, especially in the light of climate change.

Overall, sediments, sympagic algae and animal-derived matter contributed most to the diet of organisms, both before and after the sea-ice break-up. This is consistent with what is generally observed in Antarctic benthic communities (Dunton, 2001; Norkko et al., 2007; Smale et al., 2007; Gillies et al., 2012; Michel et al., 2019), including in our study area but at a greater depth (Rossi et al., 2019). The change in diet following the sea-ice break-up was taxon-dependent and was reflected in changes in

TABLE 3 | Food web metrics BEFORE and AFTER in Terra Nova Bay, Ross Sea.

Food web metrics	Before	After
Food web properties		
S (nodes)	71	49
Trophic links	304	176
Basal Level fraction	0.17	0.27
Intermediate fraction	0.52	0.41
Top fraction	0.31	0.33
Trophic position§	2.35 ± 0.09	2.01 ± 0.10
Link properties (complexity)		
Linkage links (L/S)	4.28 ± 0.36	3.59 ± 0.39
C_{min} ($2L/S^2$)	0.12	0.15
Fraction of links		
Basal resource-consumer**	0.52	0.66
Consumer-consumer**	0.48	0.34
Top-intermediate	0.20	0.18
Top-basal	0.21	0.24
Intermediate-intermediate	0.28	0.15
Intermediate-basal	0.31	0.42
Chain properties		
N° chains	1563	361
Mean chain length§§§	4.05 ± 0.04	2.88 ± 0.07
Maximum chain length	8	6
Competition		
N of competitors §§§	35.39 ± 1.52	24.83 ± 1.29
α	0.33 ± 0.01	0.40 ± 0.03
Vulnerability to biodiversity loss		
Secondary extinctions$^{\circ\circ\circ}$	0.32	0.24
Robustness	0.17	0.24
Network properties		
Neighborhood Connectivity (NC)§§§	14.07 ± 0.52	11.67 ± 0.60
Compartmentalization	0.10	0.13

S, number of nodes (i.e., taxa); L, number of feeding links.
The minimum connectance (C_{min}) is expressed as $2L/S^2$.
Mean values are presented as the mean ± the standard error.
Superscript symbols indicate a significant difference between periods ($^{*}\chi^2$ test; §Mann–Whitney test; $^{\circ\circ}$ANCOVA test for equality of means).
One, two and three superscript symbols indicate p values of <0.05, <0.01, and <0.001, respectively.

both the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) signatures and in the trophic niche of the populations, regardless of their feeding mode (ranging from filter feeders to predators). Indeed, while isotopic similarity among taxa predicted similarity in their diet, taxa that are generally grouped within broad trophic guilds in the classification of Antarctic benthos were not always isotopically similar (i.e., the composition of their diet differed), as in the case of the two omnivorous predators *Odontaster meridionalis* and *O. validus* and the two suspension feeders *Aequiyoldia eightsii* and *Adamussium colbecki*.

Before the sea-ice break-up, consumers showed ^{13}C-depleted values typical of benthic resources (Norkko et al., 2007; Calizza et al., 2018; Michel et al., 2019; Rossi et al., 2019). Their wide isotopic niches and Carbon Ranges suggested high trophic generalism within the community in this period (Layman et al., 2007; Jackson et al., 2011; Careddu et al., 2017; Sporta Caputi et al., 2020). After the sea-ice break-up, the increased availability of sympagic algae allowed consumers to specialize on this carbon source, making the coupling of distinct energy channels less marked (Kaehler et al., 2000; Dunton, 2001; Knox, 2006). Specifically, the opportunistic brittle star *Ophionotus victoriae* and sea-star *Diplasterias brucei,* the micro-predators/suspension feeders *Alcyonium antarcticum* (soft coral) and Ascidiacea, and the predator/scavenger anemone *Urticinopsis antarctica* are characterized by the highest trophic plasticity among Antarctic invertebrates (McClintock, 1994; Norkko et al., 2007; Marina et al., 2018). These taxa had a narrower range of resources in their diet after the sea-ice break-up than before. On the other hand, the trophic niches of the sea-star *Odontaster validus* and the sea-urchin *Sterechinus neumayeri* widened after the sea-ice break-up, and their $\delta^{13}C$ indicated that sympagic algae made an important contribution to their diets in both sampling periods, in accordance with previous observations (Corbisier et al., 2004; Jacob et al., 2006; Norkko et al., 2007; Rossi et al., 2019).

During the Antarctic winter, benthic communities in shallow waters obtain large quantities of carbon from benthic algae, detritus accumulated in sediment (Kaehler et al., 2000; Dunton, 2001; Mincks et al., 2008; McMeans et al., 2015) and animal-derived matter (McClintock, 1994; Gillies et al., 2012). Predation and necrophagy, which are common in these communities, are considered successful strategies above all when basal resource inputs are limiting and in the presence of disturbance caused by ice-scouring (McClintock, 1994; Smale et al., 2007; Dunlop et al., 2014). The latter reduces the diversity and abundance of benthic animals and algae (McClintock, 1994; Smale et al., 2007; Dunlop et al., 2014; Ingels et al., 2020), with a substantial impact on the local availability of food sources. The incidence of ice-scouring is generally known to be higher in shallow than in medium-depth waters (Gutt and Piepenburg, 2003), although the availability of sympagic algae is greater, and can explain the lower number of taxa found after than before the sea-ice break-up. Both factors can result in lower competitive pressure and greater stability of the community in shallow waters. Benthic consumers, mainly Antarctic echinoderms, may opportunistically feed on seal feces when this resource is available (McClintock, 1994 and literature cited therein). Considering that Weddell seals forage mainly in medium-depth waters (more than 100 m) (Burns et al., 1998), we hypothesize that the observed low consumption of seal feces was related to their low availability for shallow-water benthic consumers in our study area. The low consumption of feces was also evident from the distinct isotopic signatures of this resource compared to those of consumers both before and after sea-ice break-up. However, further studies of the seasonal availability of this resource for Antarctic benthic organisms are necessary.

Compared to other basal resources for polar benthic organisms, sympagic algae are higher quality (McMahon et al., 2006; Pusceddu et al., 2009). The higher consumption of sympagic algae led to a reduction in both the overall trophic position and the length of food chains in the web, as also observed in other studies based on stable isotope analyses (Gillies et al., 2012; Michel et al., 2019; Rossi et al., 2019). This is consistent with expectations from theory

(Kondoh and Ninomiya, 2009), observations from temperate aquatic systems (Calizza et al., 2012) and previous observations in our study area (Norkko et al., 2007; Rossi et al., 2019), and may be related to increased community resilience, theoretically associated with shorter food chains (Pimm and Lawton, 1977).

Sympagic algae are both released in small quantities from sea ice during their growth (i.e., before sea-ice break-up), and in a strong pulse for a short time during and soon after the melting of sea ice (Clarke, 1988; Cattaneo-Vietti et al., 1999; Pusceddu et al., 2009; Leu et al., 2015). After the ice melt, this abundant food source quickly becomes available to consumers (in a few hours in shallow waters and in a few days in medium-depth waters), which then adopt a more specialized diet and increase feeding (Calizza et al., 2018; Michel et al., 2019; Rossi et al., 2019) and reproductive activities (Pearse et al., 1991; Knox, 2006). This was observed both at the level of the entire food web and in species common to the two study periods. Generalist taxa reduced their trophic niches and the number of links after the sea-ice break-up, when the availability and consumption of basal resources increased. However, the fact that certain more specialist taxa increased the number of trophic links after the sea-ice break-up suggests that the lower number of taxa did not generate bias in the linkage density measurements.

Changes in foraging behavior following the ice melt affected the entire food web, leading to its simplification. Specifically, before the sea-ice break-up, the community was composed of a high number of densely linked species. The mean numbers of trophic positions and potential competitors per taxon were found to be high, in accordance with the literature (McMeans et al., 2015; Michel et al., 2019). Web simplification observed after the sea-ice break-up is consistent with the findings of Rossi et al. (2019). In their "space for time" study of the effects of sea-ice dynamics on the medium-depth benthic communities, the food webs were based on isotopic trophospecies, or Isotopic-Trophic Units (i.e., groups of individuals that share a similar position in the food web). After the sea-ice break-up, linkage density decreased and the trophic diversity within populations increased, lowering competitive interactions but increasing the vulnerability to the loss of highly connected ITUs. In contrast, in our study, the lower connectivity between species observed when resources were less limiting made food webs *less* vulnerable to the loss of taxa (Elton, 1927; Pimm and Lawton, 1977; Dunne et al., 2002; Kondoh and Ninomiya, 2009). Indeed, lower connectivity can reduce the risk of disturbance propagation (Calizza et al., 2019). In this context, highly interconnected populations could accelerate disturbance propagation along food chains via both direct effects, such as predation, and indirect effects, such as competition (Estrada, 2007; Lai et al., 2012; Marina et al., 2018). Specifically, when simulating a scenario of species loss from the most to the least connected species (Dunne et al., 2002; Staniczenko et al., 2010), secondary extinctions were boosted by the loss of central nodes in the food web. This suggested that the topological position of the species in the food web plays a greater role in the propagation of disturbance and hence in maintaining the stability of the community than the number of trophic links (Allesina and Pascual, 2009; Staniczenko et al., 2010). Keystone species, which generally dominate the Antarctic benthic communities (sea urchins, scallops, anemones, and amphipods) were key topological nodes in our food webs. Disturbance arising from changes in seasonal sea-ice dynamics directly and indirectly affecting these topological keystone populations can spread rapidly via trophic cascades, leading to food web collapse or abrupt rewiring (Estrada, 2007).

Bayesian mixing models proved to be a useful tool for tracking changes in food web structure based on shifts in species' diets. However, the presence of a large number of possible food sources linked to the high degree of generalism and trophic plasticity of Antarctic benthic species (Norkko et al., 2007; Carscallen and Romanuk, 2012; Dunlop et al., 2014) may limit the models' power of discrimination and thus the reconstruction of food webs in complex communities (Rossi et al., 2019). Our previous knowledge of the studied system, along with the results of the multi-step mixing models and the rigorous selection of each resource based on the probability of its contribution to a consumer's diet, made it possible to disentangle the diets of species belonging to this complex community. Nevertheless, the combination of stable isotopes with other biochemical tracers (e.g., fatty acids) (North et al., 2019) and the isotopic analysis of limiting amino-acids (Larsen et al., 2013) would further improve our ability to discern and understand the contribution of the various carbon sources to Antarctic food webs and their vulnerability in the face of changes in sea-ice coverage.

CONCLUDING REMARKS

In conclusion, the marked seasonality of sea-ice dynamics in Antarctica ensures the summer availability of resources that are limiting for the rest of the year (Chown et al., 2015; Rossi et al., 2019). Spatio-temporal changes in sea-ice dynamics can affect the trophic choices of species populations within the community, with cascade effects on the entire food web (Pusceddu et al., 2000; Leu et al., 2015; Poloczanska et al., 2016; Calizza et al., 2018; Rossi et al., 2019). Coastal marine Antarctic ecosystems, which are biodiversity hotspots closely associated with sea-ice, are seeing significant changes in physical and environmental conditions as a result of ongoing climate change (Michel et al., 2019; Leihy et al., 2020; Rogers et al., 2020). In these complex ecosystems, identifying key species in terms of community stability under a range of sea-ice coverage conditions requires the isotopic characterization of the community and the quantification of intra- and interspecific links between benthic populations, providing useful information for the management and conservation of biodiversity. Our study location (Tethys Bay, in Terra Nova Bay) is in the middle of the recently established Ross Sea marine protected area, and it is an Antarctic biodiversity hotspot. Our comprehensive dataset, in which several taxa were characterized by analyzing a substantial number of specimens, enabled a very robust estimation of species' isotopic niches and diet composition, useful for the reconstruction of the food web.

This dataset includes taxa spanning multiple feeding modes and trophic levels, as well as abundant circumpolar species playing key ecological roles in Antarctic coastal ecosystems.

In addition, the study of pristine biodiverse communities in the Ross Sea also represents a unique opportunity to explore stability mechanisms in seasonally forced food webs that are not affected by human pressure (Norkko et al., 2007; Chown et al., 2015; Cummings et al., 2018; Wing et al., 2018; Rossi et al., 2019; Leihy et al., 2020). Some genera and families included in this study are not limited to polar areas, being also found at lower latitudes and in anthropised coastal areas. Hence, studying them in the Ross Sea makes it possible to observe mechanisms of trophic plasticity and adaptation to temporal variations in food sources under undisturbed conditions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LR and MLC: conceptualization, supervision, and funding acquisition. EC and GC: field sampling. SSC, EC, GC, FF, and DM: investigation. SSC and GC: formal analysis and visualization. LR, MLC, and SSC: writing – original draft preparation. All authors contributed to the writing – review and editing.

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Chapter 3: Changing Isotopic Food Webs of Two Economically Important Fish in Mediterranean Coastal Lakes with Different Trophic Status (published in Applied Sciences, IF: 2.474)

Simona Sporta Caputi 1, Giulio Careddu 1 , Edoardo Calizza 1,2,*, Federico Fiorentino 1, Deborah Maccapan 1, Loreto Rossi 1,2 and Maria Letizia Costantini 1,2

1 Department of Environmental Biology, Sapienza University of Rome, via dei Sardi 70, 00185 Rome, Italy;
simona.sportacaputi@uniroma1.it (S.S.C.); giulio.careddu@uniroma1.it (G.C.);
federico.fiorentino@uniroma1.it (F.F.); deborah.maccapan@uniroma1.it (D.M.);
loreto.rossi@uniroma1.it (L.R.); marialetizia.costantini@uniroma1.it (M.L.C.)
2 CoNISMa, piazzale Flaminio 9, 00196 Rome, Italy
***** Correspondence: edoardo.calizza@uniroma1.it

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Changing Isotopic Food Webs of Two Economically Important Fish in Mediterranean Coastal Lakes with Different Trophic Status

Simona Sporta Caputi [1], Giulio Careddu [1], Edoardo Calizza [1,2,*], Federico Fiorentino [1], Deborah Maccapan [1], Loreto Rossi [1,2] and Maria Letizia Costantini [1,2]

[1] Department of Environmental Biology, Sapienza University of Rome, via dei Sardi 70, 00185 Rome, Italy; simona.sportacaputi@uniroma1.it (S.S.C.); giulio.careddu@uniroma1.it (G.C.); federico.fiorentino@uniroma1.it (F.F.); deborah.maccapan@uniroma1.it (D.M.); loreto.rossi@uniroma1.it (L.R.); marialetizia.costantini@uniroma1.it (M.L.C.)

[2] CoNISMa, piazzale Flaminio 9, 00196 Rome, Italy

* Correspondence: edoardo.calizza@uniroma1.it

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Abstract: Transitional waters are highly productive ecosystems, providing essential goods and services to the biosphere and human population. Human influence in coastal areas exposes these ecosystems to continuous internal and external disturbance. Nitrogen-loads can affect the composition of the resident community and the trophic relationships between and within species, including fish. Based on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses of individuals, we explored the feeding behaviour of two ecologically and economically important omnivorous fish, the eel *Anguilla anguilla* and the seabream *Diplodus annularis*, in three neighbouring lakes characterised by different trophic conditions. We found that *A. anguilla* showed greater generalism in the eutrophic lake due to the increased contribution of basal resources and invertebrates to its diet. By contrast, the diet of *D. annularis,* which was mainly based on invertebrate species, became more specialised, focusing especially on polychaetes. Our results suggest that changes in macroinvertebrate and fish community composition, coupled with anthropogenic pressure, affect the trophic strategies of high trophic level consumers such as *A. anguilla* and *D. annularis*. Detailed food web descriptions based on the feeding choices of isotopic trophospecies (here Isotopic Trophic Units, ITUs) enable identification of the prey taxa crucial for the persistence of omnivorous fish stocks, thus providing useful information for their management and habitat conservation.

Keywords: food webs; Mediterranean coastal lakes; nitrogen pollution; stable isotopes; trophic relationships; *Anguilla anguilla*; *Diplodus annularis*

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1. Introduction

Transitional waters are extremely complex ecosystems [1–3]. The Water Framework Directive of the European Communities (European Communities, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000) defines them as "superficial bodies of water near the mouths of rivers which have a partially saline character due to their proximity to coastal waters, but which are substantially influenced by freshwater flows". Their high productivity provides habitats, refuge areas and food sources for a wide range of aquatic animals from resident brackish to freshwater and marine migratory species [4]. Transitional waters support important ecosystem services, including good water quality, fisheries, aquaculture and tourism, as well as agricultural activities in their watersheds [5]. Anthropic activities expose these ecosystems to continuous internal and external disturbance [2,6–8], including nitrogen (N) pollution arising from agricultural and urban activities, which poses potential threats to biodiversity and ecosystem functioning [3,9,10]. In addition, an increase in N-loads can significantly compromise water quality, promoting the development of micro and macroalgal blooms [11,12]. This, in turn, could alter the species composition and feeding behaviour of the aquatic animal community, from primary consumers to top predators. Changes in the availability and quality of basal food sources can affect the distribution of organisms and the feeding links between trophic levels, with effects on the stability and structure of the entire food chain [2,12,13]. Increased N-loads could thus also compromise, either directly or indirectly, the persistence of ecologically and economically important fish species [14].

In the Mediterranean area, the European eel, *Anguilla anguilla* (Linnaeus, 1758), and the annular seabream, *Diplodus annularis* (Linnaeus, 1758), are widespread and among the most important fishery resources [15,16]. However, in the last two decades, European eel populations have collapsed due to low recruitment and habitat alteration, and the species has been classified as 'critically endangered' since 2014, according to the International Union for Conservation of Nature [17]. It is known that both fish species are generally characterised by a high degree of omnivory and trophic plasticity depending on the composition and abundance of the available prey [18,19]. Specifically, the annular seabream, *Diplodus annularis*, is a demersal omnivorous species, feeding opportunistically on a wide variety of prey including zoobenthos, algae and plants. The European *Anguilla anguilla* is a generalist predator feeding mainly on invertebrates and fish but it also exhibits scavenger behaviour, feeding on dead animals including fish. These trophic traits can be expressed differently by individuals within the population [18,20,21]. Due to their omnivory, the trophic strategies of these species can directly reflect variations in the inputs determining the trophic status of the waters and thus the quality and availability of potential prey. Thus, understanding the patterns underlying the trophic choices of these fish species and their associated food webs is crucial for ecosystem management and the conservation of their habitats.

Several studies have been carried out on the diet of eels and seabream, often based on gut content analysis [19,22–25]. However, gut content analysis provides only a snapshot of a consumer diet, which is assumed to vary over time [7,26,27]. Furthermore, individuals often have no recognisable prey in their stomach, and description of the trophic links between species thus requires large samples [28].

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis is increasingly becoming useful tool for detecting organic and inorganic matter sources and understanding species' foraging behaviour and the relationships between organisms. It is thus useful for reconstructing food webs in aquatic ecosystems [7,29–31]. The isotopic ratio of these elements in consumer tissues reflects that of the assimilated food sources in a predictable way [7,32]. $\delta^{13}C$ signatures vary considerably among primary producers, generally with lower values in marine than terrestrial aquatic vegetation. This makes it possible to disentangle the contribution of various basal sources to food networks [7,31,33–36]. The $\delta^{15}N$ values gradually increase with each trophic level, thus providing information on the position of organisms in the food web [31,37,38]. In parallel, the $\delta^{15}N$ values of primary producers reflect the nature (organic or inorganic) and the source of nitrogen inputs (natural or anthropogenic) in a predictable way. $\delta^{15}N$ is thus also useful for tracking anthropogenic N pollution in water bodies and across trophic levels in food webs [11,39–41].

The main purpose of this study was to describe and analyse the diets and food webs of the eel *Anguilla anguilla* and the annular seabream *Diplodus annularis* in three neighbouring Mediterranean coastal lakes characterised by different eutrophication levels. It is known that energy flows and the transfer of nutrients depend primarily on the foraging choices of each organism within the community [31]. Similarly, the high trophic generalism and omnivory generally observed in *A. anguilla* and *D. annularis* [19,24,25,42,43] can be the result of different foraging strategies adopted by each individual within their respective populations.

In order to obtain highly detailed information and to consider variability in the use of resources by *A. anguilla* and *D. annularis*, the diet of the two species was obtained from trophic links of each individual within a population as determined by means of the Isotopic Trophic Unit (ITU) approach [31].

Isotopic Trophic Units are defined as groups of individuals with similar isotopic signatures occupying the same position in the δ^{13}C-δ^{15}N niche space [31].

We studied the diet of each population in detail without excluding a priori any food source in the area. We hypothesised that differences in invertebrate and fish assemblages across lakes with differing trophic status could determine patterns of variation in the trophic niche width of the two fish species depending on their specific feeding habits. Specifically, we sought to verify whether a lower abundance and diversity of species at higher trophic levels caused *A. anguilla* to become more generalist and *D. annularis* to become more specialized.

2. Materials and Methods

2.1. Study Area

The samplings were carried out in three neighbouring Mediterranean brackish costal lakes located on the Tyrrhenian coast of central Italy (42°28′00″ North–12°51′00″ East): Lake Caprolace, Lake Fogliano and Lake Sabaudia (Figure 1). The three lakes respectively have a surface area of about 3 km^2, 4 km^2 and 3.9 km^2, and mean depths of 3 m, 2 m and 10 m. They are classified as non-tidal lagoons with a maximum tidal excursion of 0.21–0.23 m [44–46]. Salinity generally varies between 33.7 and 38.1 PSU in Caprolace, 29.9 and 39.2 PSU in Fogliano, and 28.8 and 33.7 PSU in Sabaudia. The annual average was 36.3 ± 0.8 PSU in Caprolace, 35.3 ± 0.8 PSU in Fogliano and 31.7 ± 0.9 PSU in Sabaudia in 2006–2010 [44]. Data are expressed as mean ± standard error.

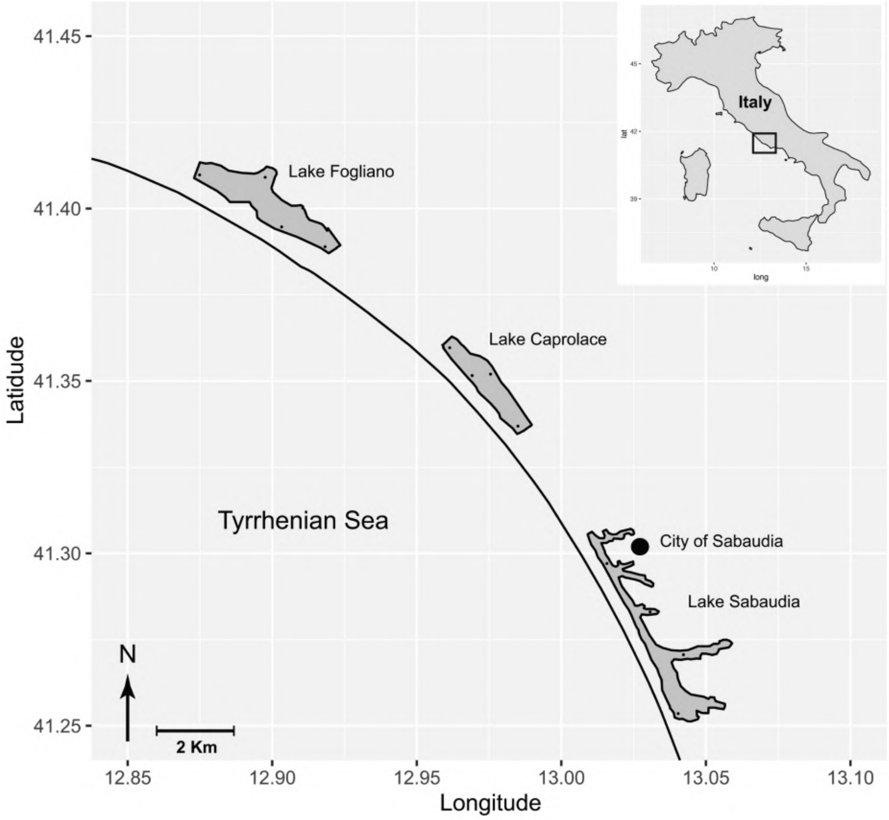

Figure 1. Map of the sampling area. The map shows the costal lakes of Caprolace (LP), Fogliano (IP) and Sabaudia (HP) located on the Tyrrhenian coast of central Italy (42°28′00″ North–12°51′00″ East).

The lakes are affected by various forms of anthropogenic disturbance related to organic and inorganic nitrogen inputs from urban treated sewage, livestock farming and agricultural activities, which are widespread in the surrounding areas [2,3].

On average, the mean concentration of total nitrogen was 383.6 ± 23.21 µg/L in Caprolace, 662.6 ± 66.70 µg/L in Fogliano and 1006.1 ± 49.97 µg/L in Sabaudia in 2006–2010. Santoro et al. [2] found the same trend in nitrate concentrations, with 12.2 ± 2.9 µg/L, 42.4 ± 61.3 µg/L and 91.9 ± 70.24 µg/L in Caprolace, Fogliano and Sabaudia respectively in the same period.

Lake Caprolace and Lake Fogliano (hereafter respectively LP and IP), characterised respectively by low and intermediate levels of eutrophication [3,47], are Sites of Community Importance (SCIs) located within the Circeo National Park (Lazio).

Lake Caprolace does not receive water inputs from the hinterland, while Lake Fogliano is affected by nutrient inputs from both the River Rio Martino and the livestock breeding activities practised in the surrounding areas. The annual concentration of *Chlorophyll a* was generally lower in Caprolace (2.1 ± 0.4 µg/L) than Fogliano (5.8 ± 1.2 µg/L) in 2006–2010.

Lake Sabaudia, the southernmost lake (hereafter HP), is affected by the highest anthropogenic pressure [3], mainly due to runoff from both the city of Sabaudia and cultivated fields in the surrounding areas as well as fishing and mussel farming. In this lake, freshwater inputs are present throughout the year.

Annual algal biomass and *Chlorophyll a* concentrations in this lake vary from 10.2 to 40.9 µg/L, with an average recorded value in 2006-2010 of 24.2 ± 6.15 µg/L. Further details regarding the study area can be found in Santoro et al. [2] and Jona-Lasinio et al. [3].

2.2. Field Collections

Samples of basal resources (primary producers and detritus), invertebrates and fish were collected in 4 sites per lake between April and May 2012, when primary productivity and invertebrate abundances were high. The sampling sites within each lake were selected from areas with heterogeneous physical and biotic characteristics and a range of anthropogenic impacts deriving from the surrounding areas [2,3]. The sampling sites were located at the northern and southern ends of each lake, and both on the landward and seaward sides (see also Santoro et al. [2]). Macrophytes, algae, and detritus samples were collected by hand and invertebrates by Van Veen grab (volume: 3.5 L) in three replicates per sampling site. The dominant macrophytes were *Ruppia* sp. and *Cymodocea nodosa* (Ucria) Ascherson, while the macroalgae were represented by taxa of the genera *Chetomorpha*, *Chondria*, *Gracilaria*, *Rytiphloea* and *Ulva*. The detritus was mostly composed of fragments of dead leaves delicately scraped to remove any epibionts and rinsed in distilled water. Phytoplankton samples were collected using a plankton net (20-µm mesh size) and concentrated by centrifugation (2000 rpm for 20 min).

Samples of fish were collected once a day for 3 days in each site. In order to collect pelagic, benthic, resident and migratory fish species, fish samples were collected using fixed weirs and fishing traps placed on the bottom. The fishing traps, made of very fine mesh (0.5 cm), were 1.5 m in diameter at the mouth and were composed of four consecutive chambers of decreasing diameter with a total length of 3.6 m. In addition to *A. anguilla* and *D. annularis*, the sampled fish community included the sand smelt *Atherina boyeri* (Risso, 1810), black goby *Gobius niger* (Linnaeus, 1758) and the mullets *Chelon ramada* (Risso, 1827), *C. aurata* (Risso, 1810), *C. saliens* (Risso, 1810) and *C. labrosus* (Risso, 1827), which are known to be prey species of *A. anguilla* and *D. annularis* [19,24,25,42,43]. Further fish samples included species belonging to the Sparidae, Scorpaenidae, Clupeidae, Cyprinodontidae, Blenniidae and Belonidae, Gobiidae, Labridae, Moronidae, Mugilidae, Soleidae and Syngnathidae families. Standard length measured in centimetres was recorded for each fish specimen. For each fish species, individuals of different sizes were collected in order to reduce the effects of size variability on isotopic signals. From the sampled fish specimens, including *A. anguilla* and *D. annularis*, samples of dorsal white muscle were taken. This tissue provides a long-term (several months) integrated indicator of food sources due to its slow turnover with respect to other tissues (e.g., liver and blood) [32].

After collection, all samples were transported to the laboratory, where specimens were sorted, counted, and identified to the lowest possible taxonomic level and processed for the stable isotope analysis.

2.3. Stable Isotope Analysis (SIA)

Samples were individually stored at −80 °C and freeze-dried for 24 h. Fish specimens were considered individually for isotopic analysis. Muscle samples were also taken from large invertebrates such as crustaceans, for which the tissue was taken from the claws, and bivalves and sea snails, whose tissue was taken from the feet [7]. When present, shells, valves and other exoskeletal parts of animals were removed under dissection microscopes in order to avoid tissue acidification before the stable isotope analysis. For small invertebrates (such as amphipods and polychaetes), the whole body was used. Samples were individually analysed. Plankton biomass was analysed as a whole due to the difficulty of obtaining sufficient biomass for isotopic analysis.

Before the stable isotope analysis, each sample was homogenised to a fine powder using a ball mill (Mini-Mill Fritsch Pulverisette 23: Fritsch Instruments, Idar-Oberstein, Germany). When necessary, samples were pre-acidified using 1M HCl according to the drop-by-drop method [48] in order to eliminate inorganic carbon and re-dried (60 °C) for 72 h to remove the remaining moisture. $\delta^{15}N$ signatures were measured in un-acidified powders to prevent acidification from interfering with the nitrogen analysis [30,49].

Aliquots of 0.25 ± 0.10 mg for the animals and 2.00 ± 0.10 mg for basal resources were placed into tin capsules for C and N stable isotopic analysis (SIA). Each sample was analysed in two replicates. The analyses were carried out using a continuous flow mass spectrometer (IsoPrime100, Isoprime Ltd., Cheadle Hulme, United Kingdom) coupled with an elemental analyser (Elementar Vario Micro-Cube, Elementar Analysensysteme GmbH, Germany).

The isotopic signatures of each sample were expressed in δ units ($\delta^{15}N$; $\delta^{13}C$) as parts per thousand (‰) deviations from international standards (atmospheric N_2 for N; PD-belemnite (PDB) carbonate for C), in accordance with the formula:

δX (‰) = [(Rsample − Rstandard)/Rstandard] × 10^3 [50], where X is ^{13}C or ^{15}N and R is the corresponding ratio of heavy to light isotope for the element ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Outputs were standardised with the internal laboratory standard Caffeine IAEA-600 ($C_8H_{10}N_4O_2$). Measurement errors were found to be typically smaller than 0.05‰.

2.4. Data Analysis

Differences between lakes in terms of animal community composition (considering both fish and benthic invertebrates) were tested using contingency tables based on chi-square (χ^2) tests, Monte Carlo permutation tests and the associated Cramer's V index (a measure of the strength of association among communities; Past 3.0 software package). Specimens collected in each sampling site (and replicates) were grouped by type or taxon (respectively for basal resources and animals) for each lake.

The Shannon diversity index (Hs) of invertebrate fauna for each lake was calculated at family level considering a total abundance of the taxa collected in each lake. Given that assessing Shannon diversity is only possible at the level of equal identification of all taxa, the few individuals belonging to the Gastropoda, Oligochaeta, Nematoda, and Nemertea classes (together accounting for less than 1.5% of total fauna) were excluded from the Shannon diversity index computation. Hutcheson's diversity *t*-test and the associated bootstrap procedure (9999 replicates), both available in the Past 3.0 software package, were applied to Hs values to test for significant differences [51]. Hutcheson's diversity *t*-test is a modified version of the classic *t*-test and is based on comparison of Hs variances. The *t* statistics of Hutcheson are defined as:

$$t = \frac{\left|Hs_i - Hs_j\right|}{\sqrt{var(Hs_i) + var\left(Hs_j\right)}} \tag{1}$$

which follows Student's *t* distribution. In the equation, *i* and *j* referred to the invertebrate communities of the lakes in paired comparisons, *Hs* represents the Shannon diversity index and *var(Hs)* its variance.

The isotopic values of collected organisms were used to reconstruct the diets of the eel *Anguilla anguilla* and the annular seabream *Diplodus annularis* in each lake. The diets were estimated on the basis of the Isotopic Trophic Unit (ITU) method [31]. The isotopic signatures of single basal resources, invertebrates, and fish were represented in the bi-dimensional isotopic space (Figure S1). This was subdivided into squares (ITUs) corresponding to 1 × 1‰ δ^{15}N and δ^{13}C values, starting from the lowest δ^{13}C value in the dataset and a δ^{15}N value of zero. The ITUs were thus identified and labelled (Figure S1).

The diets of each ITU containing individuals of the two fish species were calculated by means of Bayesian Mixing Models (R software ver. 3.5.3, SIMMr package) [52] considering a Trophic Enrichment factor (TEF) of 3.4 ± 1.0‰ for δ^{15}N and 1.0 ± 0.5‰ for δ^{13}C [18,37,49,53–56] and uninformative priors. These TEF values (expressed as mean ± standard deviation) are considered a robust and widely applicable assumption in the presence of multiple trophic pathways and different types of food sources [37,56]. For all SIMMr models, we ran three Markov Chain Monte Carlo chains of 300,000 iterations each with a burn-in of 200,000 and a thinning rate of 100 iterations. We assumed that all incoming food items had the same probability of being included in the consumer's diet. The model considers both variance in the isotopic signatures of the resources and uncertainty regarding the trophic enrichment of the consumer (TEF). The model results were expressed in the form of a probability distribution of plausible contribution values. The central tendency values of the distribution (mode, mean, median) allowed us to identify the most important food sources, while the upper and lower limits of the credibility ranges (CI: 50%, 75%, 95%) revealed the range of feasible contributions. The pool of food sources was selected based on the mixing model outputs in accordance with Rossi et al. [31]. Since the *A. anguilla* and *D. annularis* diet was obtained by starting from the foraging choice of each individual, the overall contribution of some food sources, important at individual level (>5%), could be relatively small (<5%) if considered at the population level (see also [31]). In order to obtain detailed information on the diet of the eel and the seabream these contributions were also considered.

Individuals other than *A. anguilla* and *D. annularis*, including basal resources and invertebrates, were excluded from ITU-consumers (but not from potential ITU food sources) before performing the Bayesian mixing models. This was done in order to correctly estimate the diet of *A. anguilla* and *D. annularis*. The set of potential ITU food sources was considered on the entire δ^{13}C axis and within a given range on the δ^{15}N axis, i.e., within ±3.4‰ (the TEF) of the value of the consumer [31]. The Bray–Curtis similarity index (BC), based on the contribution of each resource to the diet of the two fish, was also calculated in order to quantify the diet similarity among lakes [36,56]. BC is expressed as proportional similarity ranging from 0, when no common food sources are found for the compared groups, to 1, when the compared groups have the same food sources in the same proportions [36,56].

The symmetric overlap in resource use [57–59] was measured in accordance with the Pianka equation [59]:

$$O_{jk} = \frac{\sum_{i=1}^{n} p_{ij} p_{ik}}{\sqrt{\sum_{i=1}^{n} \left(p_{ij}\right)^2 \sum_{i=1}^{n} \left(p_{ik}\right)^2}} \tag{2}$$

where the Pianka index (O_{jk}) represents a symmetric measure of overlap between species j and k, and p_{ij} and p_{ik} are the proportional contributions of any given resource i used by species j and species k. The Pianka index ranges from 0 (overlap absent) to 1 (complete overlap).

Chesson's selectivity index [60] was calculated for each food item to determine possible preferences for particular food sources among those offered:

$$\alpha_i = \frac{r_i / n_i}{\sum_{i=1}^{m} \left(r_i / n_i\right)} \tag{3}$$

where α_i is Chesson's selectivity index, m is the number of food source types, r_i is the proportion of food type i in the diet and n_i is the proportion of food type i in the environment. The value of α_i ranges from 0 to 1, with 0 indicating complete avoidance, values above 1/m indicating preference and 1 indicating absolute preference [61]. Since consumer isotopic ratios provide an integrated measure of prey assimilated over time, we hypothesized that the composition of the taxon in each lake did not vary considerably over the course of a season. Therefore, the Chesson index based on the relationship between assimilated prey and its abundance in the environment could measure the selectivity of food products with a good approximation.

χ^2 tests were performed to test for differences between lakes in terms of the relative abundance of fauna and differences between food sources in terms of their proportional contribution to the diet of the fish population in each lake. Although it is not possible to establish a theoretical expected value, a χ^2 test was performed to test for possible differences between IP and LP and between HP and LP, considering the least polluted lake as the reference value.

Differences between lakes in both the δ^{15}N and δ^{13}C isotopic signatures of basal resources and fauna were tested by one-way ANOVA for comparisons between normal distributions (Shapiro–Wilk normality test, $p > 0.05$) while the Mann–Whitney with Bonferroni correction in cases of multiple comparisons and Kruskal–Wallis tests were used if non-normality was observed (Shapiro–Wilk normality test, p-value < 0.05). Levene's test for variances was used to test for differences within and between lakes in the δ^{13}C variance of primary producers. Kruskal–Wallis tests and associated Mann–Whitney pairwise comparisons were also used to compare the proportional contribution of food items to the diet of *A. anguilla* and *D. annularis*.

The niche metrics for both species in each lake were also calculated [62–64]. These metrics, originally proposed by Layman et al. [62] for application at community level, can be used at population level to obtain information about trophic diversity within a single population [35,63,64]. These included the ranges (highest to lowest) of δ^{13}C (Carbon Range, CR) and δ^{15}N (Nitrogen Range, NR) values. CR provides information about the variety of food sources exploited by the population (i.e., its trophic generalism), while NR indicates the number of trophic levels (i.e., degree of omnivory) of the population. The isotopic niche widths of both *A. Anguilla* and *D. annularis* were calculated as SEAc (Standard Ellipse Area corrected by degree of freedom) using R software ver. 3.5.3, SIBER analysis package [64,65]. The SEAc encompasses the core (about 40%) of the population's isotopic observations. This is a solid metric for comparing the isotopic niche of populations regardless of sample size and any isotopic outliers in the data [62,64]. Linkage density (L/S) was measured as the average number of feeding links (L) per ITU (S). Finally, based on the proportional contribution of each food source, the trophic niche width (TNW) of each population was measured as the diversity of resources consumed (Hs) by each population and compared among lakes. If not specified otherwise, the results are reported as mean ± standard error (s.e.).

3. Results

3.1. Community Composition and Isotopic Signatures

A total of 8752 samples comprising basal resources, invertebrates and fish were collected from the three lakes, 8645 (148 taxa) of which were invertebrates and fish (Table 1, Table S1).

Malacostraca (Amphipoda, Decapoda and Isopoda), Gastropoda, Anthozoa, Bivalvia, Polychaeta and Ophiuroidea together made up 93.09 ± 3.86 % of invertebrates.

Invertebrate abundance was lower in IP than the other two lakes (Table 1, paired-χ^2 test, χ^2 at least 20.46, p-value always <0.0001, Table S2). The composition of both the invertebrate and fish community also varied (contingency table, χ^2 at least 170.2, p always <0.001, Cramer's V at least 0.46, Tables S2 and S3). The abundance of some taxa, such as Decapoda and Anthozoa, decreased, while that of others (such as Amphipoda) increased with the pollution level of the lake (paired-χ^2 test, χ^2 at least 48.36, p-value always <0.0001). The number of fish taxa varied, i.e., 23 in LP, 17 in IP and 7 in HP. The relative abundance of fish differed between lakes (paired-χ^2 test, χ^2 at least 34.21 p-value always <0.0001) and was lowest in HP. The standard length of *Anguilla anguilla* was lower in HP (35.10 ± 3.63 cm) than the other two lakes (48.81 ± 6.07 cm in LP, 47.66 ± 2.12 cm in IP) (Mann–Whitney test with Bonferroni correction in cases of multiple comparisons, U = 13.0, p-value always <0.05). Similarly, *Diplodus annularis* had an average standard length of 6.25 ± 0.14 cm in HP, which was lower than LP

(8.76 ± 0.31 cm) and IP (9.43 ± 0.41 cm) (Mann–Whitney test with Bonferroni correction in cases of multiple comparisons, U = 2.0, *p*-value <0.05).

Table 1. Parameters describing the communities in each Lake. LP, IP and HP: low, intermediate and high eutrophication. N° indicates the sample size. Numbers in parentheses indicate the number of samples analysed. Community indicates both fish and benthic invertebrates. Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ are reported as mean (‰) ± s.e. For each parameter, different superscript letters (a,b,c) indicate differences between lakes (one-way ANOVA or Mann–Whitney test; $p < 0.05$).

	LP	**IP**	**HP**
N°			
Community	2942 (417)	2777 (502)	2926 (340)
Basal resources	28 (28)	51 (51)	28 (28)
Invertebrates	2793 (268) ^{a}	2526 (251) ^{b}	2848 (262) ^{a}
Fish	149 (145) ^{a}	251 (251) ^{b}	78 (78) ^{c}
$\delta^{13}C$ (‰)			
Community	−13.34 ± 0.17 ^{a}	−15.96 ± 0.13 ^{b}	−14.77 ± 0.15 ^{c}
Basal resources	−15.83 ± 0.78 ^{a}	−18.84 ± 0.80 ^{b}	−22.03 ± 1.04 ^{c}
Invertebrates	−12.65 ± 0.22 ^{a}	−15.63 ± 0.19 ^{b}	−14.42 ± 0.18 ^{c}
Fish	−14.67 ± 0.21 ^{a}	−16.30 ± 0.17 ^{b}	−16.09 ± 0.15 ^{b}
$\delta^{15}N$ (‰)			
Community	5.94 ± 0.16 ^{a}	8.36 ± 0.14 ^{b}	10.54 ± 0.14 ^{c}
Basal resources	3.65 ± 0.51 ^{a}	4.46 ± 0.38 ^{a}	6.70 ± 0.57 ^{b}
Invertebrates	4.42 ± 0.16 ^{a}	7.31 ± 0.21 ^{b}	9.82 ± 0.14 ^{c}
Fish	8.79 ± 0.17 ^{a}	9.41 ± 0.15 ^{b}	13.21 ± 0.16 ^{c}

Among the basal resources, detritus showed depleted $\delta^{13}C$ values, while primary producers were $\delta^{13}C$-enriched (Figure 2). $\delta^{13}C$-enrichment was also observed in pelagic fish with specialist diets such as *Atherina boyeri* ($\delta^{13}C$ = −15.34 ± 0.08‰ in LP, −17.45 ± 1.35‰ in IP and −16.14 ± 0.90‰ in HP). Since neither the mean nor the variance (σ^2) of $\delta^{13}C$ in the primary producers differed significantly either within each lake or between lakes (one-way ANOVA and associated Levene's test for homogeneity of variances, F at least 0.1412, *p*-value always >0.05), we concluded that the presence of a salinity gradient within a lake could not have an effect on the isotopic variability of the baseline. $\delta^{15}N$ values of primary producers increased with eutrophication (one-way ANOVA, F: 5.80, p <0.01).

The isotopic differences observed in basal resources reflected those observed in the whole community (Table 1, Figure 3; Kruskal–Wallis, Hc at least 127.1, *p*-value < 0.001; for $\delta^{13}C$ Mann–Whitney with Bonferroni correction for multiple comparisons, U at least 45,590, *p*-value always <0.001 and for $\delta^{15}N$ Mann–Whitney, U at least 421.57 with Bonferroni correction for multiple comparisons, *p*-value always <0.001, Figure 3) and in *A. anguilla* and *D. annularis*.

The $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of *Anguilla anguilla* and *Diplodus annularis* differed between lakes (Kruskal–Wallis, Hc at least 35.5, *p*-value <0.001). $\delta^{13}C$ values were higher in the least polluted lake (Table 2; Mann–Whitney with Bonferroni correction for multiple comparisons, U at least 3392.5, *p*-value always <0.001), while $\delta^{15}N$ values increased with eutrophication (Table 2; Mann–Whitney with Bonferroni correction for multiple comparisons, U at least 555, *p*-value always <0.001).

Specifically, in *A. anguilla*, the more generalist of the two species, $\delta^{13}C$ values reflected the shift of inputs from marine to terrestrial origin passing from the least to the most eutrophic lake (Figure 2 and Tables 1 and 2).

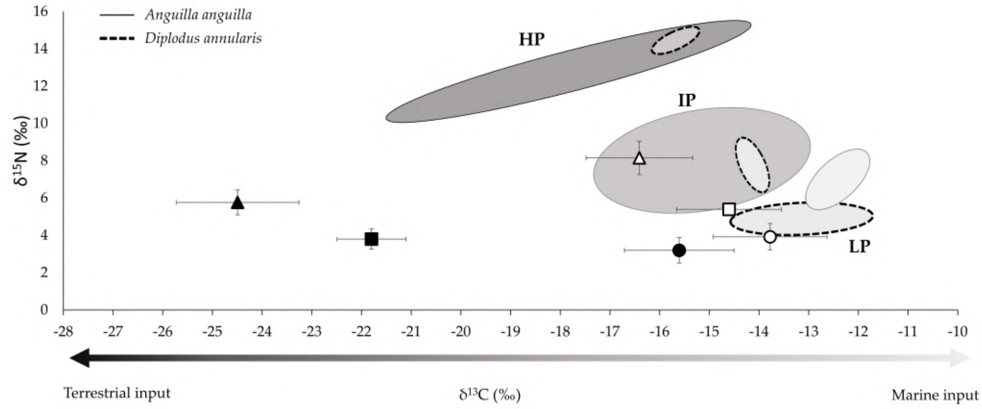

Figure 2. Isotopic standard ellipse areas (SEAcs) of *Anguilla anguilla* (continuous line) and *Diplodus annularis* (dashed line) in lakes with low (LP), intermediate (IP) and high (HP) eutrophication. Isotopic signatures (Mean ± s.e.) of primary producers (empty symbols) and detritus (full symbols) in lakes with low (circle), intermediate (square) and high (triangle) eutrophication. The greyscale reflects the origin of the main organic matter inputs from terrestrial (dark grey, left), to marine (light grey, right).

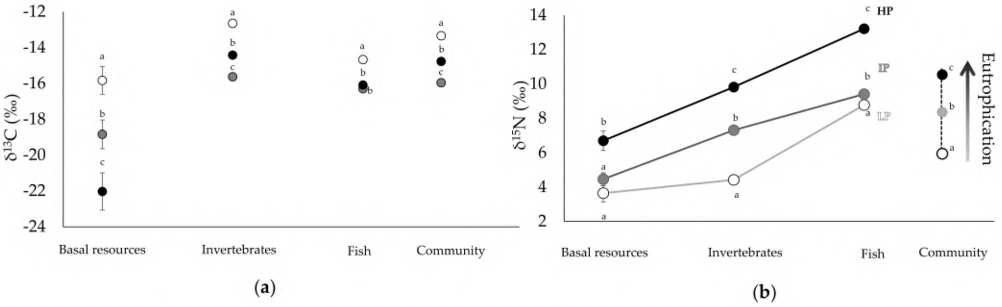

Figure 3. $\delta^{13}C$ **(a)** and $\delta^{15}N$ **(b)** values (‰) of basal resources, invertebrates, fish and the whole animal community in each lake. LP, IP and HP: low, intermediate and high eutrophication. Isotopic values are reported as mean ± s.e. Greyscale indicate degrees of eutrophication: LP (white), IP (grey), HP (black). Arrow indicates increasingly eutrophic conditions. Different letters (a, b, c) within panels indicate differences between lakes (Mann–Whitney test with Bonferroni correction for multiple comparisons; *p*-value <0.05).

Table 2. Isotopic niche and food web metrics of the eel *Anguilla anguilla* and the seabream *Diplodus annularis* in each lake. LP: low, IP: intermediate, HP: high eutrophication. N: sample size, ITUs: Isotopic Trophic Units, $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) (mean ± s.e.), CR: Carbon Range, NR: Nitrogen Range, L: number of feeding links, S: number of ITUs in the diet, L/S: Linkage density, SEAc: Standard Ellipse Area "corrected" (SEAc) by degree of freedom, TNW: Trophic Niche Width. For details of metrics, please refer to the materials and methods section. For each parameter, different superscript letters (a,b,c) indicate differences between lakes (Mann–Whitney test with Bonferroni correction for multiple comparisons; *p*-value <0.05).

	Anguilla anguilla			*Diplodus annularis*		
	LP	**IP**	**HP**	**LP**	**IP**	**HP**
N	8	16	10	8	6	8
ITUs						
$\delta^{13}C$ (‰)	$-12.76 \pm 0.11\ ^{a}$	$-15.07 \pm 0.15\ ^{b}$	$-16.31 \pm 0.16\ ^{c}$	$-13.49 \pm 0.16\ ^{a}$	$-16.23 \pm 0.14\ ^{a}$	$-15.34 \pm 0.19\ ^{b}$
$\delta^{15}N$ (‰)	$8.27 \pm 0.19\ ^{a}$	$9.62 \pm 0.12\ ^{a}$	$13.58 \pm 0.11\ ^{b}$	$5.81 \pm 0.15\ ^{a}$	$7.99 \pm 0.14\ ^{b}$	$10.24 \pm 0.15\ ^{c}$
CR	2.05	9.52	6.19	2.17	0.35	0.65
NR	3.15	5.79	3.51	0.67	0.96	0.74
Taxa						

Table 2. *Cont.*

	Anguilla anguilla			*Diplodus annularis*		
	LP	**IP**	**HP**	**LP**	**IP**	**HP**
δ^{13}C (‰)	−12.29 ± 0.19 ^{a}	−15.01 ± 0.53 ^{b}	−17.20 ± 1.12 ^{c}	−13.00 ± 0.49 ^{a}	−14.00 ± 0.10 ^{a}	−15.57 ± 0.16 ^{b}
δ^{15}N (‰)	9.08 ± 0.32 ^{a}	9.50 ± 0.36 ^{a}	12.96 ± 0.51 ^{b}	7.73 ± 0.17 ^{a}	9.59 ± 0.29 ^{b}	13.87 ± 0.16 ^{c}
CR	1.55	4.66	6.19	2.17	0.35	0.65
NR	2.54	2.59	2.86	0.67	0.96	0.74
L	30	84	33	28	13	14
S	19	42	22	21	10	11
L/S	1.6	2.0	1.5	1.3	1.3	1.3
SEAc	1.46	9.62	4.84	1.55	0.45	0.32
TNW	1.81	2.06	2.32	2.15	2.28	1.98

3.2. Niche Metrics and Diet of *Anguilla anguilla*

The isotopic signatures and niche metrics of *Anguilla anguilla* varied among lakes (Table 2, Figures 2–4; Kruskal–Wallis, Hc least 12.06, *p*-value <0.001). The highest δ^{15}N values were observed in HP (Table 2; Mann–Whitney with Bonferroni correction in cases of multiple comparisons, U at least 0.1, *p*-value always <0.001). The Carbon Range increased with eutrophication (Figure 4, Table 2) and the largest Nitrogen Range was observed in the eutrophic lake (Table 2).

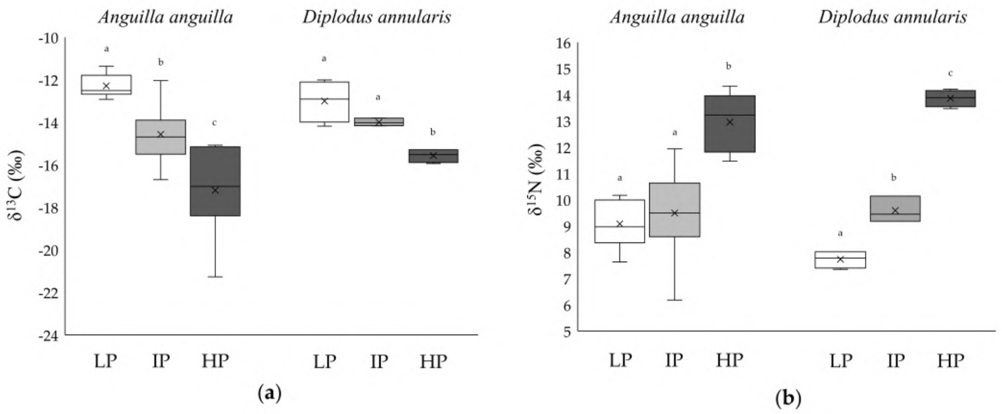

Figure 4. Boxplot of the distribution of δ^{13}C (**a**) and δ^{15}N (**b**) isotopic signature of *Anguilla anguilla* and *Diplodus annularis* in each lake: LP, IP and HP: low, intermediate and high eutrophication. For each lake, the thick horizontal line represents the median of the distribution, the box includes 50% of the data, the symbol (x) represents the mean and the whiskers reach the highest and lowest value within 95% of the distribution. Different letter (a, b, c) within panels indicates differences among lakes (Mann–Whitney test with Bonferroni correction in cases of multiple comparisons; *p* <0.05).

Overall, no correlation between the body length and δ^{13}C (‰) of *Anguilla anguilla* was observed in any lake (Pearson correlation, *p* >0.05).

Anguilla anguilla had 5 ITUs in LP and HP and 12 ITUs in IP, where the eel-resource ITU linkage density was highest (Table 2). ITU-based mixing models showed no differences between lakes in terms of the overall contribution of invertebrates to the eels' diet (Figure 5a).

By contrast, the consumption of basal resources increased and piscivory decreased with increasing levels of pollution (i.e., from LP to HP; Table 3 Figure 5a).

A. anguilla showed a generalist diet including 20 different categories of food source (Table 3, Figures 6 and 7). Some of these were common to the three lake populations (e.g., Actinopterygii, Bivalvia, Gastropoda, Decapoda and Polychaeta) but their consumption varied. The Bray–Curtis index (BC) applied to diet showed a lower similarity between the HP population and the others (76% similarity between LP and IP vs. 41% between LP and HP, and 54% between IP and HP). Specifically, in LP the diet of *A. anguilla* was mostly based on Actinopterygii (34.76% ± 1.90), Decapoda (27.84% ± 4.60) and Gastropoda (13.06% ± 0.40), in IP on Actinopterygii (30.65% ± 0.50) and Decapoda (26.13% ±

1.90) and in HP on Polychaeta (30.41% ± 0.60), Actinopterygii (12.18% ± 1.50), Bivalvia (10.35% ± 0.90), detritus (9.03% ± 0.50) and Decapoda (9.11% ± 1.90) (Table 3, Figure 6).

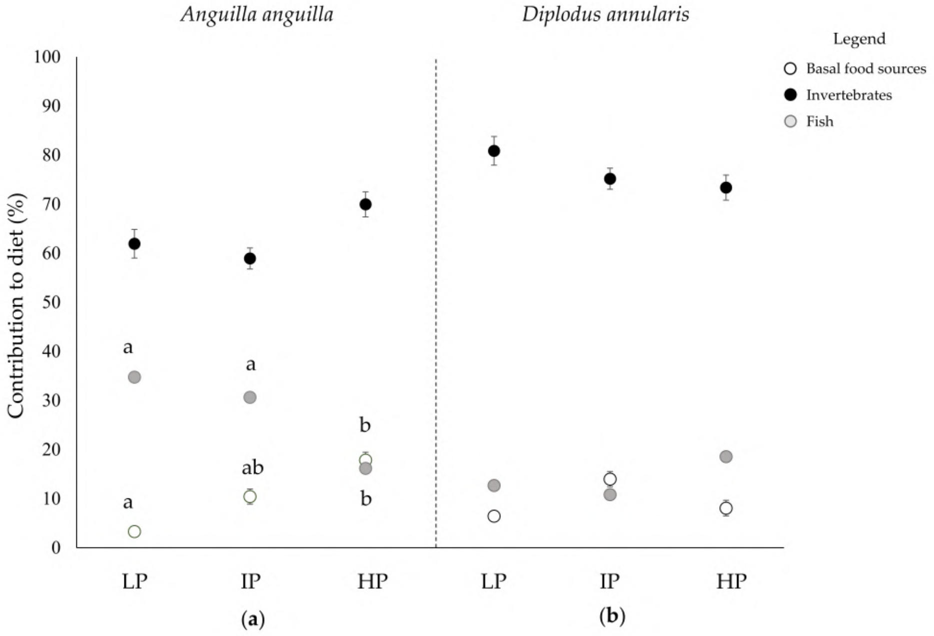

Figure 5. Contribution to the diet of *Anguilla anguilla* (**a**) and *Diplodus annularis* (**b**) of basal food sources (white), invertebrates (black) and fish (grey) in the lakes with low (LP), intermediate (IP) and high (HP) eutrophication. The overall contribution of basal resources, invertebrates and fish is reported as the mean (%) ± s.e. Different letters (a,b,c) within panels indicate differences between lakes in the contribution of food sources to the diet (χ^2-test, *p*-value <0.001).

Table 3. Proportional contribution (in %) of food sources to the diet of *A. anguilla* in each lake, obtained from ITU-based mixing models. LP: low anthropogenic pressure, IP: intermediate anthropogenic pressure, HP: high anthropogenic pressure. The contribution of each food source is reported as the mean (±s.e.). "Taxa" indicates the number of taxa belonging to the respective group in the diet of *A. anguilla.* The overall contribution of basal resources, invertebrates and fish is reported as the mean (%) ± s.e. Different superscript letters (a,b,c) indicate differences between lakes in the contribution of categories of food sources to the diet (χ^2-test, *p*-value <0.05). For details please refer to the methods section.

	LP		IP		HP	
Food Sources	**Taxa**	**Contribution**	**Taxa**	**Contribution**	**Taxa**	**Contribution**
TELEOSTS						
Actinopterygii	7	34.76 ± 1.90	12	30.65 ± 0.50	3	12.18 ± 1.50
CNIDARIANS						
Anthozoa	2	4.40 ± 0.60	4	3.17 ± 0.10	2	3.39 ± 0.20
Hydrozoa	-	-	-	-	1	1.67 ± 0.10
ASCIDIANS						
Ascidiacea	-	-	-	-	1	1.82 ± 0.10
BASAL RESOURCES						
Algae	1	0.75 ± 0.10	-	-	1	1.55 ± 0.10
Detritus	2	1.81 ± 0.40	4	5.33 ± 0.60	2	9.03 ± 0.50
Phytoplankton	-	-	1	0.42 ± 0.10	1	3.10 ± 0.10
Aquatic plants	1	0.75 ± 0.10	4	4.68 ± 0.40	1	4.19 ± 0.10
MOLLUSCS						
Bivalvia	1	0.43 ± 0.10	4	5.68 ± 0.40	4	10.35 ± 0.90
Gastropoda	8	13.06 ± 0.40	3	1.57 ± 0.20	2	2.95 ± 1.00
ANELLIDA						
Clitellata (Oligochaeta)	-	-	-	-	1	2.60 ± 0.10
Polychaeta	6	5.62 ± 0.20	5	6.99 ± 0.20	13	30.41 ± 0.60

Table 3. *Cont.*

	LP		IP		HP	
Food Sources	**Taxa**	**Contribution**	**Taxa**	**Contribution**	**Taxa**	**Contribution**
ECHINODERMS						
Eleutherozoa (Asteroidea)	1	0.29 ± 0.10	-	-	-	-
Euechinoidea (Echinoidea)	1	4.40 ± 0.10	-	-	-	-
Ophiuroidea	-	-	1	2.01 ± 0.10	-	-
ARTHROPODS						
Insecta	-	-	1	1.42 ± 0.10	1	1.62 ± 0.10
Malacostraca						
Amphipoda	4	4.74 ± 0.30	4	5.10 ± 0.40	2	4.13 ± 0.50
Decapoda	4	27.84 ± 4.60	7	26.13 ± 1.90	2	9.11 ± 1.90
Isopoda	2	1.15 ± 1.70	4	5.78 ± 0.30	-	-
NEMERTEANS						
Nemertea	-	-	1	1.08 ± 0.10	1	1.91 ± 0.10
BASAL RESOURCES		3.31 ± 0.35 ^{a}		10.43 ± 1.54 ^{ab}		17.87 ± 1.60 ^{b}
INVERTEBRATES		61.93 ± 2.92		58.93 ± 2.15		69.96 ± 2.56
FISH		34.76 ± 1.90 ^{a}		30.65 ± 0.50 ^{a}		16.18 ± 1.50 ^{b}

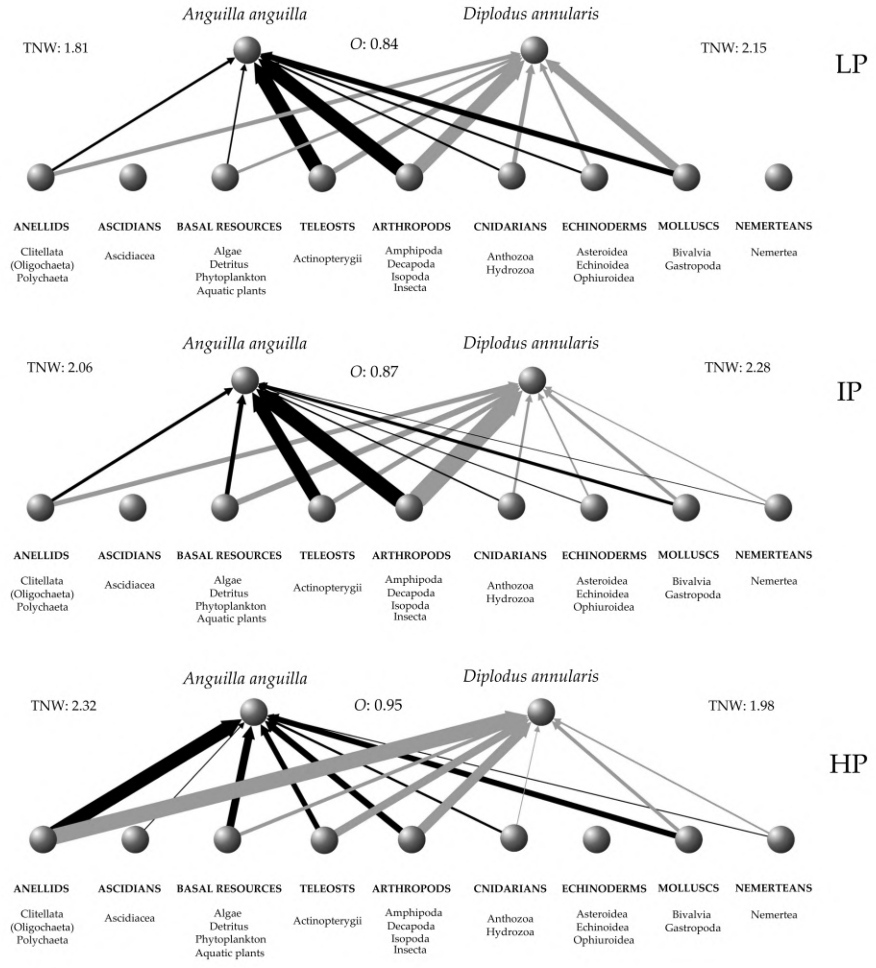

Figure 6. *Anguilla anguilla* and *Diplodus annularis* food webs in lakes with low (LP), intermediate (IP) and high (HP) eutrophication. Each node at the base of the food web represents a food source (in terms of class and respective families). Arrows point from each food item to its consumer: *Anguilla anguilla* (black arrows) and *Diplodus annularis* (grey arrows). The arrows' thickness is proportional to the trophic interaction strength. TNW indicates trophic niche width. *O* indicates the niche overlap between *D. annularis* and *A. anguilla*. For details of metrics, please refer to the results section.

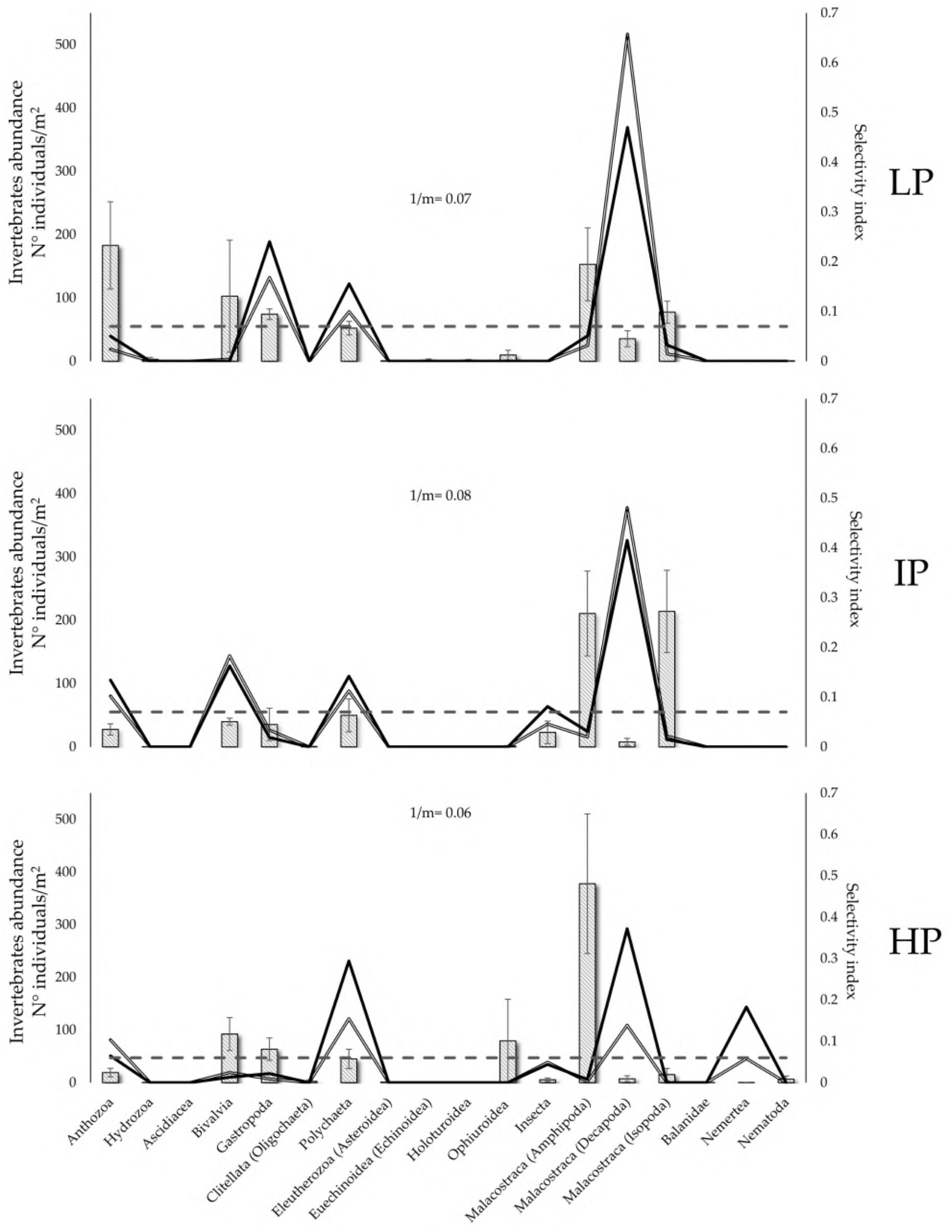

Figure 7. Invertebrate abundance in the environment (histograms), and selectivity values for each invertebrate group in the diet of *Anguilla anguilla* (double line) and *Diplodus annularis* (single thick line) in LP, IP and HP (low, intermediate and high eutrophication). Selectivity values greater than 1/m (dotted line) indicate preference.

The difference in resource use was associated with a difference in trophic niche width (Figures 2–6), which increased with eutrophication (TNW: 1.81, 2.06 and 2.32 in LP, IP and HP respectively), with significant differences between HP and LP (bootstrap comparison among populations, $p < 0.0001$).

3.3. Niche Metrics and Diet of Diplodus annularis

The δ^{13}C and δ^{15}N of *Diplodus annularis* varied across lakes (Figures 2–4, Kruskal–Wallis for both δ^{13}C and δ^{15}N, Hc at least 35.5, p-value <0.001). In addition, no significant correlation between the body length and δ^{13}C (‰) of *Diplodus annularis* was observed in any lake (Pearson correlation, $p > 0.05$).

δ^{15}N increased with the level of pollution (Table 2, Figure 4). The Carbon Range was highest in LP while no differences in Nitrogen Range were observed between lakes (Table 2). The seabreams had four ITUs in LP and two ITUs in both IP and HP (Table 2). No differences in the linkage density (L/S) of the ITUs or in SEAc were observed between lakes (Table 2).

Mixing models based on the single ITU values showed similar average contributions of basal resources, invertebrates and fish in the lakes (Figure 5b; paired-χ^2 test, p-value always >0.05).

However, when taxa in each category were distinguished, diet similarity between LP and HP was 55%, while between IP and both LP and HP it was 61% (Bray–Curtis index, BC).

Overall, the diet of *D. annularis* was based on 17 different taxa, and invertebrates represented more than 70% of it in all lakes (Table 4, Figures 5 and 6). Among these, Decapoda (22.03% ± 1.37), Gastropoda (20.65% ± 1.06) and Actinopterygii (12.69% ± 0.49) contributed most to the diet of *D. annularis* in LP (Figure 6); Decapoda (27.86% ± 1.69), Actinopterygii (10.84% ± 0.28) and Amphipoda (9.86% ± 1.54) in IP (Figure 5); and Polychaeta (34.76% ± 1.12), Actinopterygii (18.56% ± 0.78) and Decapoda (14.67% ± 1.03) in HP.

Table 4. Diet composition of *Diplodus annularis* in each lake. Proportional contribution (in %) of food sources to the diet of *D. annularis* obtained from ITU-based mixing models. LP: low anthropogenic pressure, IP: intermediate anthropogenic pressure, HP: high anthropogenic pressure. The contribution of each food source is reported as the mean (± s.e.). "Taxa" indicates the number of taxa belonging to the respective group in the diet of *D. annularis.* The overall contribution of basal resources, invertebrates and fish is reported as the mean (%) ± s.e.

	LP		**IP**		**HP**	
Food Sources	**Taxa**	**Contribution**	**Taxa**	**Contribution**	**Taxa**	**Contribution**
TELEOSTS						
Actinopterygii	4	12.69 ± 0.49	5	10.84 ± 0.28	3	18.56 ± 0.78
CNIDARIANS						
Anthozoa	2	10.21 ± 1.31	3	5.15 ± 0.69	1	1.28 ± 4.75
Hydrozoa	-	-	-	-	-	-
BASAL RESOURCES						
Algae	1	2.01 ± 0.10	-	-	-	-
Detritus	1	1.56 ± 0.58	2	5.91 ± 0.29	1	3.02 ± 5.00
Phytoplankton	-	-	-	-	-	-
Aquatic plants	2	2.88 ± 0.91	4	8.07 ± 0.10	1	5.05 ± 0.28
MOLLUSCS						
Bivalvia	-	-	2	6.24 ± 0.76	2	3.37 ± 0.44
Gastropoda	6	20.65 ± 1.06	1	1.08 ± 5.00	2	4.29 ± 1.23
ANELLIDS						
Clitellata (Oligochaeta)	-	-	-	-	1	2.73 ± 5.00
Polychaeta	8	9.71 ± 0.55	3	10.89 ± 0.69	8	34.76 ± 1.12
ECHINODERMS						
Eleutherozoa (Asteroidea)	1	0.99 ± 5.00	-	-	-	-
Euechinoidea (Echinoidea)	1	6.04 ± 5.00	-	-	-	-
Ophiuroidea	-	-	1	3.54 ± 0.75	-	-
ARTHROPODS						
Insecta	-	-	1	3.1 ± 1.06	1	0.87 ± 5.00
Malacostraca						
Amphipoda	3	8.43 ± 1.13	3	9.86 ± 1.54	2	7.8 ± 1.10
Decapoda	5	22.03 ± 1.37	5	27.86 ± 1.69	2	14.67 ± 1.03
Isopoda	3	2.8 ± 0.31	4	4.85 ± 0.20	-	-
NEMERTEANS						
Nemertea	-	-	1	2.61 ± 0.71	1	3.6 ± 0.74
BASAL RESOURCES		6.45 ± 0.39		13.98 ± 1.08		8.07 ± 1.02
INVERTEBRATES		80.86 ± 2.7		75.18 ± 2.46		73.37 ± 3.61
FISH		12.68 ± 0.49		10.82 ± 0.28		18.57 ± 0.78

The trophic niche was significantly narrower in HP than LP and IP (Figure 6; TNW: 1.98 vs. 2.15 and 2.28 respectively; bootstrap comparison among populations, p <0.05). The niche overlap between *D. annularis* and *A. anguilla* decreased from the eutrophic to the unpolluted lake (O = 0.95, 0.87 and 0.84 in HP, IP and LP respectively).

4. Discussion

Our results indicate that anthropogenic inputs affected the composition and abundance of the lake animal community. Specifically, the diversity of fish decreased, and trophic choices of the eel *Anguilla anguilla* and the seabream *Diplodus annularis* changed, with increasing eutrophication. *A. anguilla*, which was more piscivorous than the seabream at low and intermediate eutrophication, increased its consumption of invertebrates and basal resources. On the other hand, the seabream, which fed more on invertebrates, increased its preference for polychaetes. The high selectivity for polychaetes in the highly eutrophicated lake could be due to the facilitated capture of these preys and their good contribution to the energy supply of their predators compared to other aquatic invertebrates [66–68]. Changes in the feeding choices of the two fish species resulted in increased interspecific niche overlap, suggesting that eutrophication may have strong bottom-up effects on interspecific interactions [69,70].

Previous research demonstrates that the δ^{15}N values of the aquatic biota reflect anthropogenic nitrogen inputs from the surrounding terrestrial areas [7,10,71,72]. In our study, increased eutrophication was associated with higher δ^{15}N in the fish community, as previously observed by Santoro et al. [2] and Jona-Lasinio et al. [3] for invertebrates and primary producers respectively. Our results indicate that in transitional waters, individual fish δ^{15}N could be a useful indicator of anthropogenic N transfer along food webs [10,73], while the range of δ^{13}C in the fish population could reflect the diversity of C inputs, emphasising the need for ecological monitoring in these productive ecosystems.

The distinct carbon isotopic signatures (δ^{13}C) of primary producers (e.g., terrestrial vs. aquatic vegetation) allowed us to discern the origin of the organic matter contributing to the nutrient pool of water bodies [7,33,34,74]. Specifically, depleted δ^{13}C organic matter values indicated the contribution to the organic matter pool of allochthonous (terrestrial) carbon, while enriched δ^{13}C indicated autochthonous primary production, as also observed in other aquatic ecosystems [7,34,35,74]. Although it was not in the remit of this study to investigate the cause, carbon enrichment was also visible in pelagic and strictly specialist species such as the sand smelt, *A. boyeri*. In addition, neither the mean nor the variance of δ^{13}C of primary producers differed significantly between sites within the same lake or between different lakes. This allowed us to exclude possible interferences in the isotopic baseline arising from environmental parameters such as the salinity and oxygenation levels of the respective area. The larger contribution of terrestrial organic matter in HP may be due to the large input of fresh water that this lake receives from the hinterland, as indicated by the low salinity generally observed in this lake compared to the other two. These results are consistent with what has been observed in similar environments [7,74,75].

Large Carbon Ranges suggest multiple carbon sources at the base of the food web [7,35,62–64]. The supply of organic matter from multiple sources in the three lakes was evident in the δ^{13}C values of *A. anguilla*, which shifted from marine to terrestrial input with the increasing pollution. Its larger Carbon Range in the eutrophic lake indicates that this species integrated both autochthonous and allochthonous carbon pathways, while in the unpolluted lake it relied mainly on the autochthonous one.

It is acknowledged that increased N-loads promote significant changes in aquatic productivity [76] that could potentially affect the composition of the prey community [77,78]. This in turn might be reflected in the feeding behaviour of consumers at all trophic levels [2,7,79].

In our study, increased N pollution resulted in altered community composition, with decreasing diversity, which seemed to affect the feeding preference and niche width of *Anguilla anguilla* and *Diplodus annularis*. The feeding regime of the two species is known to be characterised by marked generalism and trophic plasticity [19,22,42,43,80]. However, while the eel enlarged its trophic niche, feeding off multiple resources at various trophic levels in eutrophic conditions, the seabream concentrated on a small number of invertebrates. Although dietary changes are known to depend primarily on size and growth stage [81–83], in our study neither *A. anguilla* nor *D. annularis* showed a relationship between body size and δ^{13}C signatures. The greater trophic generalism and omnivory of eels with eutrophication may be due to the different density, accessibility and availability of the prey at higher trophic levels [19,22,24,42,43] as well as to an altered presence of potential competitors for the same food sources [78]. This was also confirmed by the results of the Bayesian mixing models, which showed a shift with eutrophication in the diet of *Anguilla anguilla* mostly from pelagic (fish) to benthic (invertebrates) prey, regardless of their abundance. Bouchereau et al. [24,25] reported Teleosts, amphipods and decapods as the predominant prey in the diet of *A. anguilla* in two North-Mediterranean lagoons, and that prey selection could be linked to the activity and accessibility of the prey itself. Rosecchi [42], Pita et al. [23] and Chaouch et al. [19] indicated molluscs (bivalves and gastropods), crustaceans, polychaetes and Teleosts as the main items in the diet of *D. annularis* in lagoons and coastal waters. Lammens et al. [84] and Dörner et al. [80] identified *A. anguilla* as belonging to the piscivorous community in many European lakes. In our study, the adoption of piscivory in the least eutrophic lake allowed the eel to reduce niche overlap [80,84,85] and therefore potential competition with other fish.

However, the trophic behaviour of the two species can be expressed differently by individuals within populations [18,20,21], enabling *A. anguilla* and *D. annularis* to include several food sources in their diet even in a single area [19,25]. In this context, the individual isotopic characterisations of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), coupled with diet reconstruction at the isotopic trophic unit level (ITU, as recently proposed by Rossi et al. [31]) were crucial to the detailed estimation of the diets of the two fish species with high trophic generalism and omnivory. This allowed us to consider the possibility that each individual consumer could draw on the whole spectrum of potential food sources available within each lake [31]. In this way, we are able to describe the trophic plasticity and generalism of two ecologically and economic important fish species, and hence the real variation of the diet within the same populations under a range of eutrophication conditions.

5. Concluding Remarks

Understanding the trophic response of the community to eutrophication and depicting the structure of food webs in coastal lakes is still problematic [14], mainly due to the extraordinary biological diversity and complexity of the potential trophic links between species [2,86,87]. In our study, the stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) provided an effective approach with which to (1) track the propagation of anthropogenic nutrient inputs along food chains, (2) evaluate the relative contributions of food sources to fish diets, and (3) quantify the trophic relationships between organisms [31,37,62,87–89]. Here, the diet resulting from the application of the method recently proposed by Rossi et al. [31] substantially improved our ability to understand the response of communities to increasing eutrophication, as well as its effect on the feeding behaviour and food choices of important fish species in Mediterranean coastal lakes. Together, our results confirm food web theory as a powerful approach for obtaining valuable information for the management and conservation of these complex and productive ecosystems.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/8/2756/s1, Figure S1: Isotopic niche biplots of the community in each lake, Table S1: Carbon (δ13C) and Nitrogen (δ15N) Isotopic signature and abundances (n° Ind./m2) of invertebrate in each lake, Table S2: Contingency table for Invertebrates (Total Individuals) community composition in each lake, Table S3: Contingency table for fish (Total individuals) community composition in each lake.

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Chapter 4: A novel approach to quantifying trophic interaction strengths and impact of invasive species in food webs (Under review in Biological Invasions, IF: 3.137)

E. Calizza[1,2], L. Rossi[1,2], G. Careddu[1], S. Sporta Caputi[1,*], M. L. Costantini[1,2]

[1]Department of Environmental Biology, Sapienza University of Rome, via dei Sardi 70, 00185 Rome, Italy

[2]CoNISMa, piazzale Flaminio 9, 00196, Rome, Italy

*Corresponding Author: simona.sportacaputi@uniroma1.it

Corresponding Author: simona.sportacaputi@uniroma1.it (S. Sporta Caputi).

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ORIGINAL PAPER

A novel approach to quantifying trophic interaction strengths and impact of invasive species in food webs

Edoardo Calizza · Loreto Rossi · Giulio Careddu · Simona Sporta Caputi · Maria Letizia Costantini

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Abstract Measuring ecological and economic impacts of invasive species is necessary for managing invaded food webs. Based on abundance, biomass and diet data of autochthonous and allochthonous fish species, we proposed a novel approach to quantifying trophic interaction strengths in terms of number of individuals and biomass that each species subtract to the others in the food web. This allowed to estimate the economic loss associated to the impact of an invasive species on commercial fish stocks, as well as the resilience of invaded food webs to further perturbations. As case study, we measured the impact of the invasive bass *Micropterus salmoides* in two lake communities differing in food web complexity and species richness, as well as the biotic resistance of autochthonous and allochthonous fish species against the invader. Resistance to the invader was higher, while its ecological and economic impact was lower, in the more complex and species-rich food web. The percid *Perca fluviatilis* and the whitefish *Coregonus lavaretus* were the two species that most limited the invader, representing meaningful targets for conservation biological control strategies. In both food webs, the limiting effect of allochthonous species against *M. salmoides* was higher than the effect of autochthonous ones. Simulations predicted that the eradication of the invader would increase food web resilience, while that an increase in fish diversity would preserve resilience also at high abundances of *M. salmoides*. Our results support the conservation of biodiverse food webs as a way to mitigate the impact of bass invasion in lake ecosystems. Notably, the proposed approach could be applied to any habitat and animal species whenever biomass and diet data can be obtained.

Keywords Alien species · Biotic resistance · Competition · *Micropterus salmoides* · Predation · Food web resilience

Introduction

Invasive species are among the main causes of biodiversity loss and changes in ecosystems (Gozlan et al. 2010; Gallardo et al. 2016), and their impact is expected to increase worldwide due to climate warming and human action (McClelland et al. 2018; Ricciardi et al. 2017). Resource consumption by invaders in relation to resource availability (i.e. the

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E. Calizza · L. Rossi · G. Careddu ·
S. Sporta Caputi (✉) · M. L. Costantini
Department of Environmental Biology, Sapienza
University of Rome, via dei Sardi 70, 00185 Rome, Italy
e-mail: simona.sportacaputi@uniroma1.it

E. Calizza · L. Rossi · M. L. Costantini
CoNISMa, piazzale Flaminio 9, 00196 Rome, Italy

functional response by invaders, sensu Dick et al. 2017a) is a central element in several hypotheses predicting their success in invaded habitats (Dick et al. 2017a). Nevertheless, measurements of functional responses are often performed under simplified conditions. Specifically, they do not take into account the multiple biotic interactions that constrain invasive species in food webs, and may thus fail to predict their impact in real ecosystems (see Vonesh et al. 2017 for a discussion on this point). Thus, while research in the field has increased during recent years (Dick et al. 2017b; Laverty et al. 2017; Corrales et al. 2019), our ability to measure the ecological and economic impact of invaders in natural communities is still limited (Kulhanek et al. 2011; Crystal-Ornelas and Lockwood 2020).

In this context, the quantification of the impact of biological invasions on complex food webs and key ecosystem services (including the productivity of commercially exploited species) is necessary to formulate effective management strategies (Gozlan et al. 2010; Davies and Britton 2015; Latombe et al. 2017). In parallel, conservation biological control strategies would benefit from a quantitative measure of the resistance by autochthonous and allochthonous species against recently established invaders, thus enabling the identification of meaningful conservation targets (Frost et al. 2019). Indeed, invaders' success can vary greatly depending on the structure of invaded food webs (Dzialowski et al. 2007; Jackson et al. 2013; Smith-Ramesh et al. 2017; Vonesh et al. 2017), through mechanisms of biotic resistance by competitors (competitive resistance) and predators (consumptive resistance) (Britton 2012; Alofs and Jackson 2014; Smith-Ramesh et al. 2017; Rehage et al. 2019). However, field studies comparing consumptive and competitive resistance at the whole food web level are lacking (Alofs and Jackson 2014). Similarly, there is a lack of information about the effects of invaders on the dynamic properties of food webs, including web resilience to further perturbations. This hinders an effective conservation and management of invaded communities, which may be exposed to other environmental stressors (e.g. habitat degradation) that interact with invasion, impairing the persistence of food webs (Didham et al. 2007; Prior et al. 2017; Norbury and van Overmeire 2019).

In recent years, the study of invaded food webs has benefited from the application of stable isotope analysis coupled with Bayesian mixing models to the description of trophic links between species (Bond et al. 2016; Costantini et al. 2018; Ferguson et al. 2018). However, isotopic Bayesian mixing models quantify interspecific interactions as the proportional contribution of each prey to the predator's diet, while they do not provide information on the biomass and/or number of individuals subtracted to prey populations by predators. This represents a crucial limitation when the food web description has to be used to quantify the effect of invasive species on (1) the population dynamics of other species (Ferguson et al. 2018), (2) food web resilience to perturbations (May 1974; Allesina and Tang 2012; Landi et al. 2018), and (3) potential economic losses associated to the reduction of commercially exploited species.

To fill these gaps, we proposed a novel approach for the quantification of trophic interaction strengths in terms of number of individuals and biomass that each predator and/or competitor subtracts to other species in invaded food webs. Based on abundance, biomass and stable isotope data of species, the proposed method allowed us to calculate the resistance against an invasive species at the population and the food web levels, as well as the impact of the invader on the remaining populations. The method also allowed us to estimate the carrying capacity of fish populations and, thus, to calculate food web resilience to perturbations (May 1974; Allesina and Tang 2012).

As a case study, we considered the invasion of lake food webs by the largemouth bass, *Micropterus salmoides* (Lacépède) (Costantini et al. 2018), one of the 100 most invasive species in the world (Welcomme 1992; Brown et al. 2009). We compared two communities invaded by the bass and including both autochthonous and allochthonous fish species introduced into the lake before *M. salmoides*. The two food webs differed in the number of species and trophic links, and we related differences in the impact of the invasive species to their structures. Specifically, we calculated the consumptive and competitive resistance by competitors and predators to the invader, and the biomass that it subtracted to each prey. This also allowed to quantify the potential economic loss associated to the impact of the invader on lake fish stocks, and to simulate the effect of a reduction in its abundance on food web resilience. While we have focused on lake food webs, the proposed approach can be applied to any other habitat and animal species if biomass and diet data (either obtained through stable isotopes or other methods) can be obtained. This virtually includes all the invertebrate and vertebrate animal taxa.

Materials and methods

Interspecific interaction strengths and biotic resistance to an invasive species

Using food web data from Costantini et al. (2018), we calculated the strength of all species-pair trophic interactions in the food web. Species' diets were assessed through Bayesian mixing models (SIAR package for R software) based on stable isotope analysis of fish species and their potential food sources. Abundance and biomass of fish were also measured (Appendix S1: Table S1). Analytical methods for the description of species' diet in food webs based on isotopic data have been widely addressed and implemented in recent years, and details can be found in Costantini et al. (2018), Rossi et al. (2019), and in the literature cited therein.

Competition

Following Levins (1968) and Calizza et al. (2017), the strength of interspecific competition between species pairs was calculated with reference to the overlap in resource use, accounting for the proportional contribution of each prey to the diet of each predator. Competition strength is indicated by α_{ij}, i.e. the effect of species *j* on species *i*, in accordance with the Levins's formula:

$$\alpha ij = \frac{\sum_h pih \times pjh}{\sum_h (pih)^2} \tag{1}$$

where p_{ih} and p_{jh} are the proportional consumptions of a resource *h* by species *i* and species *j* respectively.

The strength of interspecific competition was corrected for the biomass ratio between competitors, i.e., the measured value of α_{ij} was multiplied for the ratio between the mean body mass of species *j* and that of species *i*. Such product will be identified with β_{ij}, intending the competitive effect of species *j* on species *i*. This was necessary because competition is expected to decrease with increasing difference in body mass between competitors, i.e. the smaller a competitor *j* with respect to a species *i*, the weaker the effect of *j* on *i*, due to mass-related differences in energetic requirements, food-size selection and foraging habitat exploration (Basset 1995; Emmerson and Raffaelli 2004; Brose et al. 2006; Neutel et al. 2007). This also has the potential to create asymmetric competition for similar values of α due to body mass differences between species.

Then, the limiting effect of a given competitor *j* on a species *i* was calculated as:

$$\text{Limiting effect} \ = \beta_{ij} \times N_j \tag{2}$$

where N_j is the number of individuals of species *j* (Calizza et al. 2017 and literature cited therein). According to Eqs. 1 and 2, both α_{ii} and β_{ii} (i.e. the intraspecific interaction terms) give always 1. Thus, the intraspecific limiting effect is directly dependent on the population size, i.e. the number of individuals (N) of species *i*.

Predation

The effect of a predator species on its prey species was also calculated. To achieve this, the biomass density of each predator species (BD_P) was calculated as:

$$BD_P = N_P \times B_P \tag{3}$$

where N_P and B_P are the mean density and body mass of a given predator *P* respectively. Then, the biomass of a given prey *m* subtracted by a given predator *P* (B_{mP}) was estimated as:

$$B_{mP} = (BD_P \times F_{mP})/\text{Eff} \tag{4}$$

where F_{mP} is the proportional contribution of the prey to the diet of a predator, and Eff is the efficiency of transformation of prey biomass into predator biomass, with Eff varying between 0 and 1 (see below).

Then, the number of individuals of a prey *m* subtracted by a predator *P* (N_{mP}) was obtained as:

$$N_{mP} = B_{mP}/B_m \tag{5}$$

where B_m is the mean body mass of the prey.

Carrying capacity and biotic resistance against the invasive species

Based on Eqs. 2 and 5, the carrying capacity of a given species *i* (K*i*) was thus calculated with reference to Lotka-Volterra models (Cohen et al. 1990; Zeeman 1995; Calizza et al. 2017), as:

$$\mathrm{K}i = \mathrm{N}i + \sum_{j=1}^{C} \beta ij\mathrm{N}j + \sum_{P=1}^{S} \mathrm{N}mP \tag{6}$$

where $\beta_{ij}\mathrm{N}_j$ and N_{mP} are the effects of competitors and predators of species *i* respectively, as reported above, and *C* and *S* are the numbers of competitors and predators of species *i*.

After the calculation of its K, food web-scale competitive and consumptive resistance against an invasive species can be obtained as:

$$\text{Competitive resistance} = \left.\sum_{j=1}^{C} \beta ijNj\right) / Ki \times 100 \tag{7}$$

$$\text{Consumptive resistance} = \left.\sum_{P=1}^{S} NmP\right) / Ki \times 100 \tag{8}$$

Hence, resistance to an invasive species is measured as the percentage of individuals that competitors and predators subtract to its population with respect to its carrying capacity.

Table S2 in Appendix 1 includes all the variables used in Eqs. 1 to 9, their units and ecological meaning.

Food web structure and resilience to perturbations

Food webs were described with reference to: the number of species (S), the number of feeding links (L), connectance (Cmin), measured as 2L/(S x (S−1)), and connectivity, measured as S x Cmin. The Shannon diversity (Hs) was used to quantifying the diversity of fish communities excluding the invasive species *M. salmoides*. A bootstrap procedure, available in the Past 3.0 software, was applied to compare Hs values between food webs.

In order to account for the effects of both direct and indirect interactions between species, food web resilience to perturbations (i.e., the local Lyapunov stability) was investigated with reference to the inverse of the classical Jacobian matrix (J^{-1}) (May 1974; Montoya et al. 2009; Calizza et al. 2017). Here, we focused on competitive interactions because they represented the majority of interactions between fish species (Costantini et al. 2018), they have a strong destabilising effect on species assemblages (Allesina and Tang 2012) and because invaders are expected to have strong competitive effects in invaded communities (Marchetti et al. 2004; Gozlan et al. 2010). The classical Jacobian matrix (J) is obtained by multiplying species densities with the interspecific interaction matrix containing pair-wise interaction coefficients (see Appendix 1: Fig. S1 for details). The diagonal of the Jacobian matrix contains the intraspecific interaction terms. The inverse Jacobian matrix (J^{-1}) is obtained by multiplying J for its transpose matrix (J'). Each element of J^{-1} describes the net effect of species A on species B, taking into account all indirect pathways that link species A and B via intermediate species (Montoya et al. 2009). This implies that species A may have an effect on species B even if the two species do not interact directly. The stability of a given n-species matrix can be inferred from its eigenvalues, with stability (i.e. resilience) being expected for matrices having only negative eigenvalues (in their real part) (May 1974; Allesina and Tang 2012). Here, stability is defined by Re(λmax) of J^{-1}, which is the real part of the maximum eigenvalue, and by its sign. The system will return to the equilibrium for negative Re(λmax), while it will move away from the equilibrium for positive Re(λmax). In both cases, the rate of return to or 'escape' from the equilibrium is defined by the absolute value of Re(λmax). The mean real part of all eigenvalues of the matrix, Re(λmean), is a function of the mean diagonal elements of the matrix itself. Increasing the absolute value of the negative terms on the diagonal moves the Re(λmean) towards more negative values (Johnson et al. 2014; Jacquet et al. 2016). Given that the diagonal contains the intraspecific interaction terms, and according to the stability criterion applied, i.e. $\mathrm{Re}(\lambda\mathrm{max}) < 0$, the value of Re(λmax) (hereafter referred to as λ) can thus be seen as a measure of the intraspecific regulation that is needed to stabilize the system considering both direct and indirect interactions between species in the food web.

Application to a case study: fish invasion in lake food webs

Study area and sampling activities

Lake Bracciano is located 32 km north-west of Rome (Lazio, Italy), and it is an oligo-mesotrophic volcanic lake (Rossi et al. 2010). It has a perimeter of 31.5 km, a surface area of 57 km^2 and its maximum depth is 165 m. Bass invasion (i.e. *Micropterus salmoides*) was first reported in 1998 (Marinelli et al. 2007).

Two sampling locations were selected in the north-western (hereafter: North) and south-eastern (hereafter: South) littoral areas of the lake. The South location was characterised by a gently sloping bottom, in contrast to the sharper slope observed in North. Previous samplings along 100 m linear transects indicated that the coverage and diversity of both riparian and submerged vegetation were nearly double on gently sloping bottom (Rossi et al. 2010; Costantini et al. 2018). Accordingly, we considered the North and South locations to be characterised by low and high habitat complexity respectively. Consistently, the percentage of organic matter in sediment (2.2 ± 0.2% in North vs. 17.3 ± 4.2% in South) and macroinvertebrate density were higher in South (311.4 ± 34.9 individuals per sampling site in North vs. 711.9 ± 123.8 in South) (Costantini et al. 2018). In both locations, measured temperature, pH and oxygen concentrations fall within the optimal range for the growth and activity of *M. salmoides* (Scott and Crossman 1973; Brown et al. 2009; Costantini et al. 2018). Fish were sampled with the help of professional fishermen from local cooperatives. Catches were performed for three days in each location, in the last week of June. A modified version of a surrounding net without a purse line (40 m linear length) and two fishing traps were used in each location and at each sampling occasion. A very fine mesh (0.5 cm) was used in order to include small fish specimens in the catches. Sampling locations and sampling activities are described in detail in Costantini et al. (2018).

Based on a recent checklist of the Italian freshwater fish fauna (Lorenzoni et al. 2019), fish species were indicated as “autochthonous” or “allochthonous”, intending those allochthonous species introduced in central Italy in the past (i.e. before the half of the twentieth century) that naturally persist within the lake (Fig. 1 and Appendix 1: Table S1).

Impact of Micropterus salmoides on lake fish stocks and food web resilience

Original of North America, *M. salmoides* is a successful invader worldwide (Brown et al. 2009), producing severe impacts on biodiversity and ecosystem functioning in invaded habitats (Jackson 2002; Leunda 2010; Ribeiro and Leunda 2012). The bass can survive between 10 °C and 32 °C, while optimal temperature ranges from 24 to 30 °C. It normally inhabits waters with an oxygen concentration > 3.0 mg/l, while a concentration between 1.5 mg/l and 3.0 mg/l can be tolerated at optimal temperature. Optimal pH ranges from 6.5 to 8.9 (Scott and Crossman 1973; Brown et al. 2009). Regarding its diet, *M. salmoides* is known to feed both on invertebrate and fish prey. The bass starts consuming fish when it reaches a standard length of 7–10 cm (Olson 1996; Marinelli et al. 2007), and it is able to adapt its diet depending on habitat complexity and prey availability (Brown et al. 2009; Britton et al. 2010; Costantini et al. 2018).

Various studies provided detailed measurements of physiological parameters of *M. salmoides*. Specifically, an ingestion rate of 3.0% (range: 2.2–3.9%) of its body mass per day, and an efficiency of prey transformation into body mass (Eff) of 27–28% have been reported (Markus 1932; Hunt 1960; Scott and Crossman 1973; Brown et al. 2009). Together, these values allow estimating that a complete turnover of a given standing biomass of *M. salmoides* would take 121.4 days (min: 95.0 days, max: 168.3 days, based on the reported range of ingestion rate). This implies a biomass turnover rate (TO) of 3.0 per year on average (range: 2.3–3.7 per year).

Given the TO of *M. salmoides*, we estimated the year-round body mass that *M. salmoides* subtracted to each prey population *m* at each location as:

$$B_{mP\text{year}} = B_{mP} \times \text{TO} \tag{9}$$

where B_{mP} is the biomass subtracted to each prey as calculated with Eq. (4). The calculation was repeated by accounting for the different number of specimens of *M. salmoides* captured at each sampling occasion and location.

For *Perca fluviatilis* and *Atherina boyeri*, the two most commercialized fish species of the lake, the biomass subtracted by *M. salmoides* was converted into economic value by considering a cost of 18 €

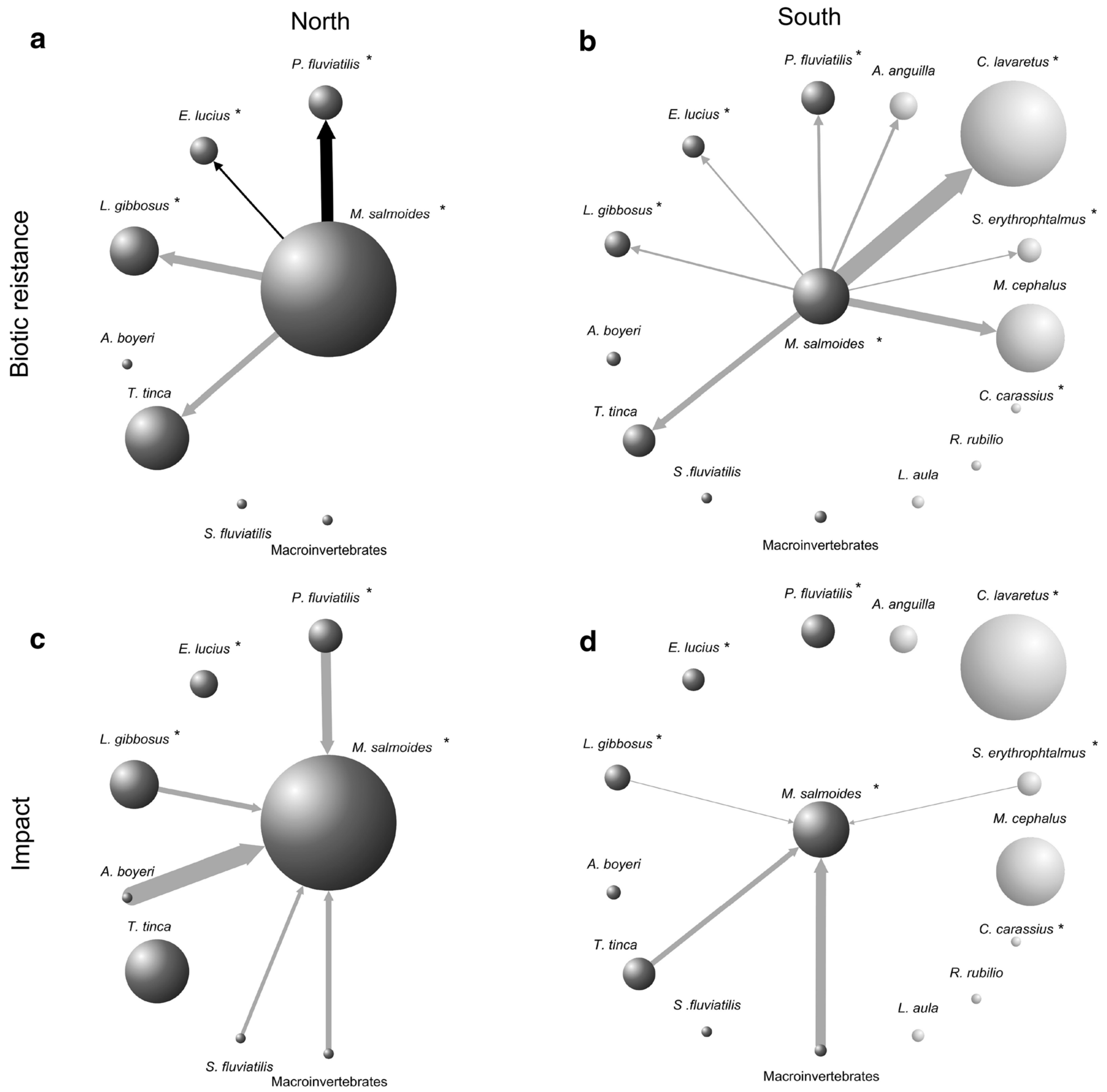

Fig. 1 Panels **a**–**b**: Biotic resistance of fish communities against the invasive species *Micropterus salmoides* in the North and South littoral area of Lake Bracciano (Italy). Arrow width is proportional to the limiting effect, measured in terms of number of individuals subtracted to *M. salmoides* (limiting effects < 1 are excluded). Grey arrows: competition; black arrows: both competition and predation contributed to the limitation on *M. salmoides*. Panels **c**–**d**: Impact of *M. salmoides* on invaded communities. Arrow width is proportioanl to the estimated biomass that *M. salmoides* subtracted to each prey. Nodes' size is proportional to the biomass density of each population. The size of nodes symbolising invertebrates is not to scale with fish. Asterisks denote allochthonous fish species. Dark grey nodes: species found in both locations; light grey nodes: species found only in South. See Tables S1 and S4 in Appendix 1 for details

Kg^{-1} for the former, and 6 € Kg^{-1} for the latter in accordance with the local fish market.

M. salmoides was preyed by *Perca fluviatilis* and *Esox lucius* only in North. Here, the three species shared a very similar trophic position, having a mainly piscivorous diet (Costantini et al. 2018). *P. fluviatilis* had a nearly complete overlap in resource use with *M. salmoides*. In parallel, while *E. lucius* had a relatively different use of specific prey, the applied Eff falls within values reported for this species (Diana 1983). Accordingly, Eff = 0.27 (known for *M. salmoides*) was applied also to *P. fluviatilis* and *E. lucius* to quantifying the biomass and number of individuals of *M. salmoides* subtracted by the two predators.

In order to test the effect of *M. salmoides* on food web resilience, we simulated a progressive reduction in its abundance up to eradication. Based on Lotka-Volterra population models, at each step, the equilibrium abundances of all remaining fish species were recalculated by taking into account the number of individuals of *M. salmoides* eliminated, and the number of individuals that it subtracted to each competitor (Eq. 2) and/or prey according to the contribution to the diet of *M. salmoides* (Eq. 5). Then, the value of λ was recalculated.

To explore the effect of increased fish diversity, i.e. increased evenness of fish populations' abundances, on food web resilience, we repeated the above-mentioned simulations by considering an equal abundance of fish species while maintaining stable the overall number of fish specimens sampled at each location. This had the effect to maximize the evenness of fish populations and thus the Shannon diversity value (Hs) of the two fish communities. This allowed us to evaluate (1) the potential effect of lake management strategies aimed at improving fish diversity, and (2) the theoretical effect of diversity on the resilience of invaded food webs.

Lastly, based on the observed relation between the abundance of *M. salmoides* and the value of λ, we used linear models to calculate the increase of *M. salmoides* (as % of the observed abundance within each food web) which should be expected to destabilize the food web (i.e. which led to $\lambda \geq 0$). The Akaike Information Criterion (AIC) was used to select the best model of fit.

Results

Food web structure and biotic resistance against *M. salmoides*

Richness, abundance and diversity of fish species were all higher in South than in North (Table 1 and Appendix 1: Table S1). Accordingly, the total number of trophic links and food web connectivity were both higher in the former location (Table 1). In contrast, *M. salmoides* was more abundant in North (45 ± 7 vs. 18 ± 2 individuals, t-test, t = 6.9, $p < 0.01$). Excluding *M. salmoides*, 3 autochthonous and 3 allochthonous fish species were found in North, while 7 autochthonous and 6 allochthonous species were found in South. Allochthonous species represented the 79.9% of all the individuals sampled in North and the 40.4% in South (χ^2 test, $\chi^2 = 45.1$, $p < 0.0001$), and their mean body mass was generally higher than that of autochthonous species (Appendix 1: Table S1).

Table 1 Number (S), abundance (N) and diversity (Hs) of fish species being part of the communities invaded by *M. salmoides* in the North and South littoral lake area of Lake Bracciano (Italy)

	North	South
S	6	13
$N^{\S}$	169 ± 11	267 ± 29
Hs^{*}	1.1 ± 0.4	2.3 ± 0.2
Links	26	68
Cmin	0.26	0.23
Connectivity	2.6	4.1

Values exclude *M. salmoides*. Links, Cmin (i.e. minimum connectance) and Connectivity refer to the number of trophic links and complexity in the two food webs. Superscript symbols indicate a significant difference ($p < 0.05$, §: Wilcoxon test, *: Bootstrap comparison)

The contribution of fish prey to the diet of *M. salmoides* was higher in North (87% vs. 41% of diet). In particular, *P. fluviatilis* and *A. boyeri* constituted the 23% and 43% of its diet respectively in North, while they were not consumed in South (Fig. 1). In turn, *M. salmoides* contributed to the diet of *P. fluviatilis* (26%) and *E. lucius* (7%) in the former location, while it was not preyed in the latter (Fig. 1). Given the low biomass density of *P. fluviatilis* and *E. lucius* with respect to *M. salmoides* (Fig. 1), and the relatively low contribution of *M. salmoides* to their diet, varying the Eff value had a largely negligible effect on the estimated number of individuals of *M. salmoides* subtracted by the two predators (Appendix 1: Table S3).

Mean competition strength (i.e. α_{ij}) suffered by *M. salmoides* from the other fish species was higher in South (0.71 ± 0.06 vs. 0.34 ± 0.04, t-test, t = 3.2, $p < 0.01$) (Appendix 1: Table S4), and the biotic resistance against the invader was higher in this location (Fig. 2). Indeed, the observed abundance of

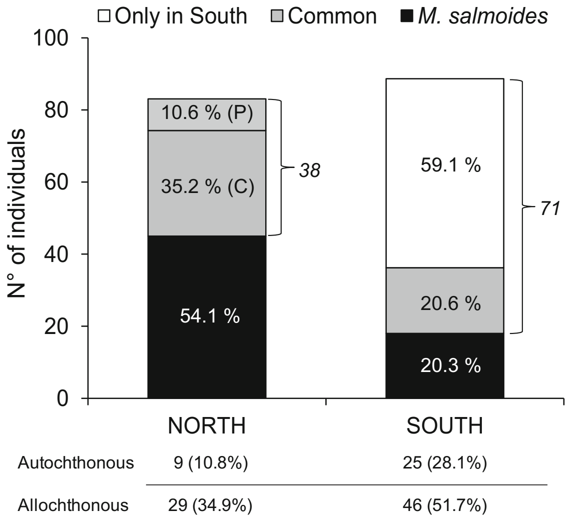

Fig. 2 Abundance of *Micropterus salmoides*, as well as the biotic resistance (i.e. the limiting effect, in terms of n° of individuals as a percentage of its carrying capacity) undergone by the bass in the North and South littoral area in Lake Bracciano (Italy). "Common" refer to the biotic resistance by fish species found in both locations; "Only in South" refers to the biotic resistance by fish species found only in South. P and C refer to the effect of predation and competition respectively. Only competition was observed in South. Numbers within each area represent the percentage of the total, while numbers on the right of brackets quantify the n° of individuals subtracted to *M. salmoides*. The total number of specimens (i.e. the bar height) is the estimated carrying capacity (K) of *M. salmoides*. The n° of individuals subtracted to *M. salmoides* by autochthonous and allochthonous fish species (and the percentage on its K) is shown below the two bars

M. salmoides represented the 54.1% of its carrying capacity (K) in North and the 20.3% in South (χ^2 test, $\chi^2 = 20.5$, $p < 0.0001$). Competitive resistance was the main component of biotic resistance in North, and it was the only component observed in South (Fig. 2). Here, resistance against *M. salmoides* was mainly dependent on those species found in this location only (Fig. 2). The top predator *P. fluviatilis* in North, and the whitefish *Coregonus lavaretus* in South, were the two species that most limited the invader (Fig. 1). In both locations, allochthonous species had a stronger limiting effect than autochthonous ones on *M. salmoides* (Fig. 2, North, $\chi^2 = 13.6$, $p < 0.001$; South, $\chi^2 = 10.3$, $p < 0.01$).

Ecological and economic impact of *M. salmoides*

Based on our sampling effort (i.e. on the standing biomass density of *M. salmoides* sampled), the biomass subtracted by *M. salmoides* to fish prey was markedly higher in North (North: 19.8 ± 0.7 kg vs. South: 3.4 ± 0.2 kg), while that subtracted to macroinvertebrates was higher in South (North: 3.1 ± 0.3 kg vs. South: 5.6 ± 0.3 kg) (Fig. 1 and Appendix 1: Table S4). The two most impacted fish species were *P. fluviatilis* and *A. boyeri* (Fig. 1 and Table 2). Both predation and competition contributed to the impact of *M. salmoides* on *P. fluvaitlis* in North, while only competition (at a reduced level) contributed in South (Table 2). Predation in North represented the main cause of impact of *M. salmoides* on *A. boyeri* (Table 2).

Given the estimated biomass turn-over rate (TO) of *M. salmoides* and an economic value of 18 € Kg^{-1} and 6 € Kg^{-1} for *P. fluviatilis* and *A. boyeri* respectively, it is thus possible to estimate that the biomass subtracted by the invader was equivalent to a potential economic loss of 688.2 ± 21.6 € per year and 144.9 ± 10.2 € per year in North and South respectively (Table 2). All individuals of *M. salmoides* were captured with the linear net (40 m in length), and no individuals were found in fish traps. By standardizing the measured impact over 100-m shoreline, it is thus possible to estimate a potential loss of 1720.5 ± 53.7 € 100 m^{-1} per year in North and 362.1 ± 25.5 € 100 m^{-1} per year in South due to the impact of *M. salmoides* on *P. fluviatilis* and *A. boyeri.*

Food web resilience and simulated eradication of the invasive species

The observed value of λ was comprised between -0.82 (South) and -0.77 (North) (Fig. 3). Increased resilience (i.e. decreased expected time of recovery after a perturbation, for increased absolute value of λ) was observed when simulating the eradication of *M. salmoides*, as well as when simulating a more even distribution of abundances among fish populations (Fig. 3 and Appendix 1: Fig. S2). The tight correlation between the relative abundance of *M. salmoides* and λ allowed to calculate the increase of *M. salmoides* (as % of the observed abundance) which should be expected to destabilize the food web (i.e. which led to $\lambda \geq 0$) (Appendix 1: Fig. S2). Such threshold value

Table 2 Biomass subtracted by *M. salmoides* to the predator *Perca fluviatilis* and the forage fish *Atherina boyeri* in the North and South littoral lake area of Lake Bracciano (Italy) according to our sampling effort, i.e. according to the mean standing biomass of *M. salmoides* sampled

	Biomass Loss (g)			Potential Economic Loss per year (€)		
NORTH	*P. fluviatilis (f)*	*A. boyeri (b)*	Sum (*f* + *b*)	*P. fluviatilis*	*A. boyeri*	Sum (*f* + *b*)
Predation (P)	5244 ± 178	9918 ± 337	15,162 ± 515	283.2 ± 6.9	178.2 ± 6.3	461.4 ± 13.2
Competition (C)	4096 ± 148	320 ± 11	4416 ± 159	221.1 ± 8.1	5.7 ± 0.3	226.8 ± 8.4
Total (P + C)	9340 ± 326	10,238 ± 348	19,578 ± 674	504.3 ± 15.0	183.9 ± 6.6	688.2 ± 21.6
SOUTH						
Predation (P)	0	0	0	0	0	0
Competition (C)	2321 ± 161	1078 ± 76	3399 ± 237	125.4 ± 8.7	19.5 ± 1.5	144.9 ± 10.2
Total (P + C)	2321 ± 161	1078 ± 76	3399 ± 237	125.4 ± 8.7	19.5 ± 1.5	144.9 ± 10.2

The effect of competition and predation is reported

The values of biomass are converted to a potential economic loss per year according to a commercial value of 18 € Kg^{-1} for *P. fluvatilis* and 6 € Kg^{-1} for *A. boyeri* and the estimated biomass turn-over rate of *M. salmoides* (see methods)

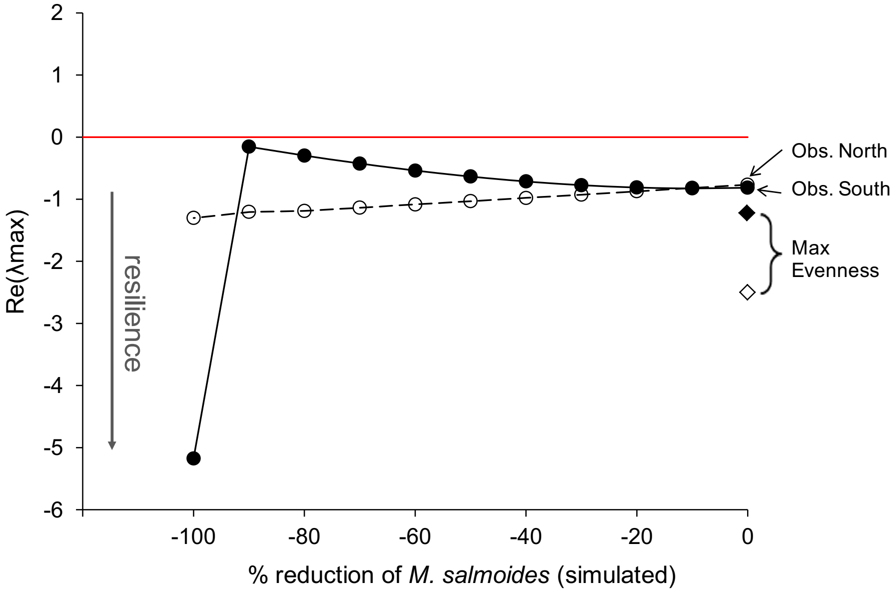

Fig. 3 Impact of *M. salmoides* on food web resilience to perturbations. Resilience is expected for negative real values of the maximum eigenvalue, Re(λmax) of the inverse Jacobian matrix. "Obs. North" and "Obs. South" point to the two values of Re(λmax) measured for the food web in the North and South location of Lake Bracciano, based on the observed abundance of *M. salmoides* at each location. "Max Evenness" (diamonds) refers to the values of Re(λmax) expected under simulated conditions of equal abundances of all fish populations in the food web except *M. salmoides,* which was maintained at the observed abundance. For each food web, a gradual reduction in the abundance of *M. salmoides* was simulated and values of Re(λmax) were recalculated accordingly (symbols from − 10% to − 100% of simulated reduction of *M. salmoides*). Empty symbols: North; Black symbols: South

was higher in North (+ 152%) than in South (+ 88%) ($\chi^2 = 50.5$, $p < 0.0001$), and increased to + 365% (North, $\chi^2 = 24.2$, $p < 0.0001$) and + 199% (South, $\chi^2 = 50.5$, $p < 0.0001$) when simulating a more even distribution of abundances among fish populations (Appendix 1: Fig. S2).

Discussion

Based on abundance, biomass and diet data of species, we proposed a method for quantifying trophic interaction strengths in terms of number of individuals and biomass that each species subtracts to the others through competition and/or predation in invaded food webs. In our study case, this method was useful to measure the impact of an invasive fish species on the invaded communities, as well as the biotic resistance to the invader. Specifically, our approach allowed us to quantify (1) the ecological and economic impact of the invasive species, considering its effect both on commercial fish stocks and on food web resilience, and (2) the competitive and consumptive resistance of autochthonous and allochthonous fish species. This kind of information can provide an ecological theory-based support to the management and conservation of invaded ecosystems.

The knowledge of the ingestion rate and prey transformation efficiency by the invasive species allowed us to estimate the biomass that it subtracted to each prey. Furthermore, the effect of competition on the invader by autochthonous fish species was weighed according to interspecific differences in body mass. This implies that a competitor smaller than the invader will have a small limiting effect on its abundance, regardless of the similarity of resources used by the two species, as expected by ecological theory, model and empirical evidence (Basset 1995; Emmerson and Raffaelli 2004; Brose et al. 2006; Neutel et al. 2007). While ingestion rates and efficiency of prey transformation into body mass of *M. salmoides* were available, this may not be the case for other species. Since metabolic and tissue turnover rates are related to the body mass of organisms (Peter 1983; Brown et al. 2004; Vander Zanden et al. 2015), published allometric coefficients may be used to estimate the parameters starting from body mass data. Noteworthy, the efficiency of prey transformation into body mass is the only metabolic parameter necessary to quantify the impact of a predator on its prey according to the proposed method. It may be calculated as the ratio between the amount of food provided and the net increase in weight of a consumer.

Biotic resistance to *Micropterus salmoides*

Starting from the realized trophic links in the food web, we quantified all the competitive and predatory interactions that limited the invader. In both food webs, competitive resistance had a stronger limiting effect on *M. salmoides* than consumptive resistance. The latter was only observed in the less complex food web. Here, lower habitat complexity and invertebrate prey availability may have increased the predation of *M. salmoides* by other predatory fish (Costantini et al. 2018). Notably, the fish species present only in the more complex and species-rich food web provided an important contribution to the higher competitive resistance observed. This supports a positive relation between species richness and community resistance to invasion, consistently with theory and field observations (e.g. Stachowicz et al. 1999; Kennedy et al. 2002). Increased fish abundance and species richness were associated to higher habitat complexity, as often observed in freshwaters (Willis et al. 2005; Smokorowski and Pratt 2007; Thomas and Cunha 2010). Thus, our results suggest a bottom-up effect of habitat features on the invasibility of lake communities. In parallel, recent modelling and field food web studies have demonstrated that increased food web complexity may decrease communities' invasibility (Hui et al. 2016; Smith-Ramesh et al. 2017; Romanuk et al. 2017). This was ascribed to a higher probability of an invader to undergo competition in highly interconnected webs, as it was observed in our study.

Our approach allowed us also to rank fish species according to their ability to limit the invader that can be considered in biological control strategies. In the study lake, the two species that most limited *M. salmoides* (*P. fluviatilis* and *C. lavaretus*) also represent valuable commercial fish species. This raises management issues since an unavoidable trade-off between the exploitation of these fish species and the maintenance of biotic resistance against the invader exists. Notably, allochthonous species represented half of the fish species sampled and had a strong limiting effect on *M. salmoides*, particularly at low habitat complexity. This can be explained by their higher abundance, mean body mass and competitive effect, consistently with the expected high adaptability and competitiveness of allochthonous species in non-native habitats. Our data are in line with a recent review of the Italian freshwater fish fauna (Lorenzoni et al. 2019), which found that nearly one out of two species is allochthonous. Thus, our results suggest that the management of invaded ecosystems should carefully consider the ecological role and the potential ecosystem services provided by allochthonous not invasive species.

Impact of *Micropetrus salmoides* on invaded food webs

High habitat complexity and invertebrate prey availability reduced the impact of *M. salmoides*, as biomass subtracted to fish with respect to invertebrates, on the invaded fish communities. This may have strong implication in the top-down control exerted by the invader on lower trophic levels (Mancinelli et al. 2007; Jackson et al. 2013). Indeed, comparative lake studies highlighted important effects of bass invasion on native community composition via predation and competition, with cascade effects on local biodiversity (Jackson 2002; Maezono et al. 2005; Leunda 2010) and key ecosystem processes, including the production of fish stocks (Iguchi et al. 2004; Leunda 2010).

While the abundance of invertebrates generally increases with vegetation coverage in freshwaters (Godinho et al. 1994, 1997; Olson et al. 1995; Batzer and Wissinger 1996; Dala-Corte et al. 2020), the higher consumption of invertebrates by *M. salmoides* in the more complex habitat should not be solely related to their higher abundance. Indeed, the abundance of fish (an energetically richer prey type) increased in a similar manner in this location. According to optimal foraging theory (Pyke et al. 1977; Rossi et al. 2015), we thus hypothesis that the interaction between increased availability of invertebrates and increased energetic cost of fish predation underlies the shift from a fish- to an invertebrate-dominated diet by *M. salmoides* in the more complex habitat. Consistently, reduced consumption of fish with increasing habitat complexity due to reduced attack success rate (Gotceitas and Colgan 1989), increased handling time of fish prey (Alexander et al. 2015), modification of habitat use by fish prey (Jackson 2002) and increased invertebrate abundance (Godinho et al. 1994, 1997) has been reported for *M. salmoides* as well as for other invasive fish (Moyle and Light 1996; MacRae and Jackson 2001; Nasmith et al. 2010; Hanisch et al. 2012). The results presented in this case study may thus provide useful information for the management of habitats invaded by alien predatory fish and by *M. salmoides* in particular, which represents a worldwide ecological and management issue (Welcomme 1992; Jackson 2002).

In the study lake, the measured impact of *M. salmoides* on other fish species has important economic implications. Indeed, the two most profitable commercial fish in the lake (*P. fluviatilis* and *A. boyeri*) were also the two most impacted fish species, and local professional fishermen reported drops in catches following invasion by the bass. Our approach made it possible to estimate the impact of *M. salmoides* on these two species over a year-round basis. Although our estimations assume no changes in the relative importance of prey to the diet of *M. salmoides* over the year, they are based on the description of diet composition over a medium-long term period (4–5 months) thanks to the stable isotope analysis of fish muscle (Weidel et al. 2011; Winter et al. 2019). The difference in the potential economic loss observed between locations ($\sim$ 1360 € 100 m^{-1} per year) may thus be considered a useful measure to quantifying the specific ecosystem service provided thanks to the presence of a biodiverse and complex food web.

Scaling from the population- to the food web-level, we quantified the expected resilience to perturbations of invaded communities. To our knowledge, this is the first attempt to quantify food web resilience, as firstly proposed by May (1974), starting from the isotope-based description of realized trophic links between species. Notably, observed values of λ lie in the range expected for real and model ecological networks (Allesina and Tang 2012), implying that resilience after a perturbation can be expected in both food webs, but at a relatively slow rate of recovery (i.e. λ was negative but relatively close to 0).

Simulated eradication of *M. salmoides* led to increased resilience. In parallel, increased evenness in the abundances of populations also led to increased resilience, and our simulations showed that increased evenness was associated to a higher potential of invaded food webs to maintain stability at high density of *M. salmoides*. The stability criterion applied (i.e. $\lambda < 0$) implies that food web resilience is achieved if all the populations can be expected to recover following a perturbation (May 1974; Allesina and Tang 2012). The differences between simulated (i.e. increased evenness) and observed communities may be explained by considering that under observed conditions poorly abundant species may experience weak intraspecific regulation and thus local extinction after a perturbation due to interspecific competition (Arnoldi et al. 2016). This can also explain the observed slighter increase in the value of λ in South at simulated low abundances of *M. salmoides*, given the strong interspecific competition suffered by the bass in this location.

The successful eradication of *M. salmoides* has been reported in small and well-defined habitats, e.g. ponds (Tsunoda et al. 2010) and streams (Ellender et al. 2015), while it is often considered unfeasible in large ecosystems (Britton et al. 2011). In Lake Bracciano, *M. salmoides* is subject to professional and recreational fishing, and the releasing of captured specimens is not allowed. Nevertheless, this is the only method applied for the control of the bass, and its successful eradication from the lake seems unrealistic. In this context, while the simulated eradication of the bass allowed us to estimate its effect on the stability of invaded food webs, the proposed approach may allow to estimate the economic and ecological impact of *M. salmoides* at progressively reduced abundances, thus supporting the identification of science-based thresholds for its regulation.

Concluding remarks

Improvements in the quantification of trophic interaction strengths in real ecosystems represent an important step towards a better understanding of the structure and functioning of invaded and pristine ecological communities. Indeed, theoretical and experimental evidences show that the effects of one species on the population dynamics of others (Levins 1968; Montoya et al. 2009; O'Gorman et al. 2010; Calizza et al. 2017) and key food web properties, including resilience (May 1974; Allesina and Tang 2012), robustness to biodiversity loss (Eklöf et al. 2013), energy transfer (Bellingeri and Bodini 2016), and vulnerability to disturbance propagation (Montoya et al. 2009; Calizza et al. 2019), are tightly related to the strength and distribution of interspecific interactions.

Here, the quantification of trophic interactions in invaded food webs provided important insights into ecological mechanisms behind the ability of autochthonous and allochthonous species to resist invasion by a recently introduced species, as well as on the direct and indirect effects of the invader on ecological communities. Notably, our results provided quantitative evidence supporting the conservation of biodiverse and complex food webs, associated to vegetated and productive littoral lake habitats (Costantini et al. 2018), as a way to mitigate bass invasion, its impact on commercial fish stocks and on food web stability.

Species invasions are increasing worldwide, boosted by human activities and climate change (McClelland et al. 2018; Ricciardi et al. 2017; Frost et al. 2019). In parallel, applications of stable isotopes in food web studies are flourishing, and include an increasing number of taxa and habitats, from small invertebrates to aquatic and terrestrial megafauna, from temperate to polar regions (Vander Zanden et al. 2004, 2015; Fry 2006; Michel et al. 2019; Rossi et al. 2015, 2019; Sporta Caputi et al. 2020). Thus, the proposed approach could be applied to a broad array of ecosystems and species if biomass and diet data (either obtained through stable isotopes or other methods) are available, improving our ability to conserve and manage invaded food webs.

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Author contribution E.C., L.R., M.L.C. conceived the study. L.R., M.L.C. provided food web data, E.C., G.C., S.S.C. analysed data. EC, LR, MLC wrote the paper. All Authors revised the paper.

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Data availability Data will be made available on reasonable request.

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SUPPLEMENTARY MATERIAL: Appendix 1

A novel approach to quantifying trophic interaction strengths and impact of invasive species in food webs

E. Calizza[1,2], L. Rossi[1,2], G. Careddu[1], S. Sporta Caputi[1,*], M. L. Costantini[1,2]

[1]Department of Environmental Biology, Sapienza University of Rome, via dei Sardi 70, 00185 Rome, Italy.

[2]CoNISMa, piazzale Flaminio 9, 00196, Rome, Italy

*Corresponding Author: simona.sportacaputi@uniroma1.it

Table S1. Mean abundance (n) and mean (min-max) body weight (g) of fish species in the North and South littoral lake area of Lake Bracciano (Italy) (data from Costantini et al., 2018). Species are indicated as allochthonous or autochthonous with reference to central Italy, where the study lake is located, and according to a recent check list of the Italian freshwater fish fauna (Lorenzoni et al. 2019).

a) NORTH	n (±s.d.)	Body weight (g)	b) SOUTH	n (±s.d.)	Body weight (g)
Allochthonous			Allochthonous		
L. gibbosus	116±10	16.5 (1.8-79.3)	*L. gibbosus*	50±5	15.6 (0.9-77.2)
M. salmoides	45±7	136.8 (80.4-200.8)	*M. salmoides*	18±2	126.9 (85.3-195.9)
P. fluviatilis	15±2	79.2 (56.1-118.6)	*S. erythrophtalmus*	18±3	38.8 (1.5-122.5)
E. lucius	4±1	221.4 (219.8-225.4)	*P. fluviatilis*	16±3	72.6 (59.4-80.7)
Autochthonous			*E. lucius*	12±2	51.4 (20.7-135.9)
A. boyeri	15±4	1.6 (0.5-3.8)	*C. lavaretus*	10±4	469.2 (243.4-695.0)
T. tinca	10±2	265.2 (2.5-618.0)	*C. carassius*	2±1	1.9 (1.4-2.2)
S. fluviatilis	9±1	1.2 (0.4-2.9)	Autochthonous		
			A. boyeri	53±9	4.1 (1.0-7.5)
			S. fluviatilis	40±5	0.9 (0.5-1.2)
			T. tinca	30±4	37.1 (2.6-217.7)
			R. rubilio	14±3	0.7 (0.6-0.9)
			M. cephalus	10±2	286.7 (1.4-1417)
			A. anguilla	10±2	87.8 (13.7-368.0)
			L. aula	2±1	64.8 (59.9-70.5)

Table S2. Variables used in the main text (equations 1 to 9), with their symbols, units, and ecological meaning. “Equation” indicates the first equation in which each variable is reported in the main text. “per 40m” in the “Unit” column refers to our sampling effort. Indeed, samplings were performed with linear nets of 40m in length.

Symbol	**Unit**	**Meaning**	**Equation**
α_{ij}	adimensional, varying between 0 and positive values	strength of interspecific competition between species *j* and species *i*, sensu Levins (1968)	eqn 1
β_{ij}	adimensional, varying between 0 and positive values	competitive effect of species *j* on species *i*, obtained as α_{ij} multiplied for the ratio between the mean body mass of species *j* and that of species *i*	eqn 2
N_j	n° individuals per 40m	density of a competitor species *j*	eqn 2
N_P	n° individuals per 40m	density of a predator species *P*	eqn 3
B_P	grams per individual	mean body mass of a predator species *P*	eqn 3
B_{mP}	grams per 40m	biomass of a prey species *m* subtracted by a predator species *P*	eqn 4
F_{mP}	adimensional, varying between 0 and 1	proportion of a prey species *m* in the diet of a predator species *P*	eqn 4
Eff	adimensional, varying between 0 and 1	proportion of prey biomass transformed into predator biomass after ingestion	eqn 4
N_{mP}	n° individuals per 40m	n° of individuals of a prey species *m* subtracted by a predator species *P*	eqn 5
B_m	grams per individual	mean body mass of a prey species *m*	eqn 5
K_i	n° individuals per 40m	carrying capacity of species *i* according to our sampling effort, i.e. the n° of individuals of *i* expected to be found if in the absence of predators and competitors.	eqn 6
C	n° of species	n° of competitor species of species *i* within the food web	eqn 6
S	n° of species	n° of predator species of species *i* within the food web	eqn 6
$B_{m\ \mathrm{year}}$	grams per 40m per year	year-round body mass that *Micropterus salmoides* subtract to a prey species *m*	eqn 9
TO	n° of times per year	biomass turn-over rate of *M. salmoides*	eqn 9

Table S3. Effect of varying the efficiency of transformation (Eff) of prey body mass into predator body mass on the estimated number of individuals of *Micropterus salmoides* (N_m) subtracted by *Perca fluviatilis* and *Esox lucius*. ΔN_m refers to the difference between results obtained with Eff = 0.27 (in bold, applied for the calculation of values shown in the main text) and other values of Eff.

	Perca fluviatilis		*Esox lucius*	
Eff_P	N_m	ΔN_m	N_m	ΔN_m
0.27	**7.3±0.5**	**-**	**1.6±0.2**	**-**
0.20	9.9±0.7	2.6±0.2	2.2±0.3	0.6±0.1
0.25	7.9±0.6	0.6±0.1	1.8±0.2	0.1±0.0
0.30	6.6±0.5	-0.7±0.1	1.5±0.2	-0.2±0.0
0.35	5.6±0.4	-1.7±0.1	1.3±0.2	-0.4±0.1
0.40	4.9±0.4	-2.4±0.2	1.1±0.2	-0.5±0.1
0.45	4.4±0.3	-2.9±0.2	1.0±0.1	-0.7±0.1
0.50	3.9±0.3	-3.4±0.2	0.9±0.1	-0.8±0.1

Table S4. Biomass lost: estimated biomass (mean ± s.e.) that *M. salmoides* subtract to each prey in the North and South littoral lake area of Lake Bracciano (Italy) according to our sampling effort. α_{ij} and β_{ij} measure the interspecific competition between *M. salmoides* (i) and its competitors (j) solely based on the overlap on resource use (α_{ij}) and once taking into account differences in body mass between species (β_{ij}). Resource use is determined by Bayesian isotopic mixing models. "-": species not found. 0: species found but not preyed by *M. salmoides*.

	Biomass lost (g)		α_{ij}		β_{ij}	
Prey	North	South	North	South	North	South
P. fluviatilis	5244 ± 103	0	0.71	1.10	0.49	0.24
L. gibbosus	2932 ± 254	466 ± 30	0.57	0.63	0.09	0.06
E. lucius	0	0	0.47	0.85	0.58	0.19
T. tinca	0	2892 ± 185	0.13	0.72	0.86	0.27
A. boyeri	9918 ± 195	0	0.02	0.52	0.00	0.02
S. fluviatilis	2040 ± 177	0	0.16	0.65	0.00	0.00
A. anguilla	-	0	-	1.12	-	0.38
M. cephalus	-	0	-	0.63	-	1.09
C. lavaretus	-	0	-	0.72	-	3.53
C. carassius	-	0	-	0.59	-	0.01
R. rubilio	-	0	-	0.64	-	0.00
S. erythrophtalmus	-	453 ± 29	-	0.41	-	0.09
L. aula	-	0	-	0.58	-	0.26
Macroinvertebrates	3085 ± 267	5589 ± 358				

$$J = \begin{vmatrix} -d_1 & 0 & 0 & 0 \\ 0 & -d_2 & c_{2,3} & c_{2,4} \\ 0 & c_{3,2} & -d_3 & c_{3,4} \\ 0 & c_{4,2} & c_{4,3} & -d_4 \end{vmatrix}$$

Fig. S1. Example of a generic Jacobian matrix J = [c_{ij}] for a four-species community, in which all species compete among each other, except species 1. Given the equilibrium assumption, and according to Lotka-Volterra competition models, the off-diagonal elements of J are obtained by multiplying the interspecific interaction matrix A = [β_{ij}] with species density (N). The diagonal of J contains the intraspecific interactions terms. The diagonal element for the i^{th} species can be obtained as: $Ki - 2Ni - \sum_{j=1}^{C} \beta ij \mathrm{N} j$. Thus, the diagonal contains negative terms that are directly dependent on the species density. Once constructed the Jacobian matrix (J), the inverse Jacobian matrix (J^{-1}) is obtained by multiplying J with its transpose matrix (J'), i.e. J^{-1} = J x J'.

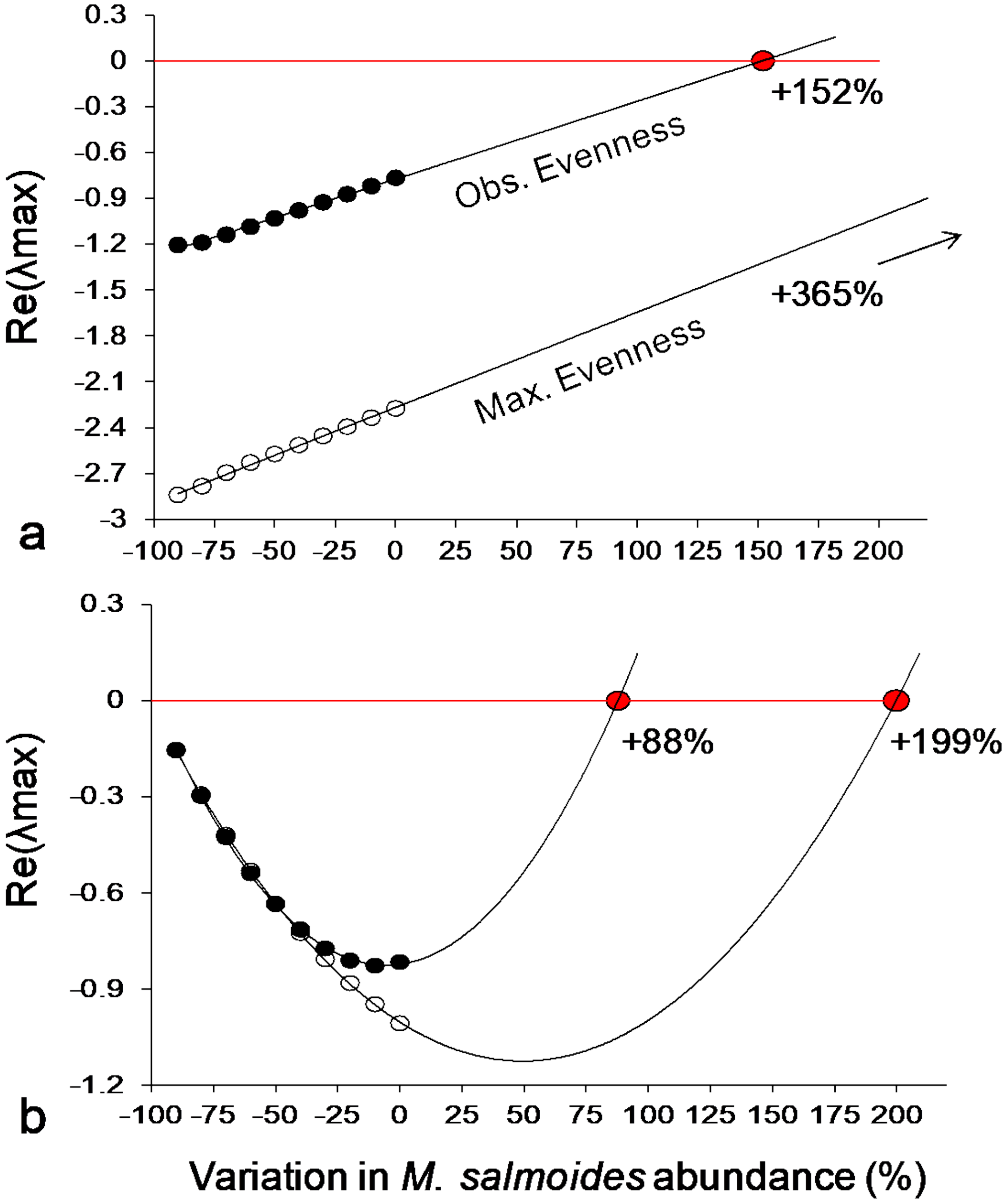

Fig. S2. Food web resilience to perturbations in the North (a) and South (b) food web. Food web resilience is expected for Re(λmax) < 0, where Re(λmax) is the real part of the maximum eigenvalue of the inverse Jacobian matrix. For each food web, a gradual reduction in the abundance of *M. salmoides* was simulated. 0 on the horizontal axis indicates the observed abundance of *M. salmoides* at each location. Simulations were repeated both considering observed (“Obs”, black symbols) and simulated (i.e. “Max”, empty symbols) evenness of fish populations. Red dots and associated percentage values represent the estimated increase in the abundance of *M. salmoides* (with respect to the observed value) that should be expected to destabilise the food web. North: 1st order polynomial models; Obs. Evenness: AIC= 5.7, R^2= 0.995, p value= 8.4E-11; Max. Evenness: AIC= 5.5, R^2= 0.999, p value= 7.9E-19. South: 2nd order polynomial models; Obs. Evenness: AIC= 10.0, R^2= 0.999, p value= 1.6E-12; Max. Evenness: AIC= 9.4, R^2= 0.999, p value= 1.9E-17.

Discussion

In the present dissertation I reported my original studies (from the sampling in fields to the chemical and numerical analyses and publication) that aim to understand the mechanisms underlying the structure and stability of communities in both pristine, directly impacted by climate change, and in the anthropized environments.

The principal results of present dissertation can be summarized in two crucial points: (1) the variation of recurring patterns in the food web structure under different disturbed conditions (both natural or anthropogenic) are affect by the availability of food sources that imposes change of link density, and (2) the current limits of the food web reconstruction has been overcome.

The first and second point derived from extensive field sampling and stable isotope analysis of every single specimen sampled for each population of species that inhabits the study ecosystems, and from the new consideration of trophic specie based on restricted interval of isotopic signal.

Reconstructed food webs and their metrics were confirmed a valuable tool for understanding the mechanism underline the biodiversity structure and stability**,** and its possible response to environmental changes linked to climate drivers **(Chapter 1-2)**, anthropogenic activities **(Chapter 3)**, and the invasion of species (**Chapter 4).**

From the result showed, it was possible to estimate the diets of consumers across different spatial, temporal and hierarchical scale (from individuals to community) (**Chapters 1-4)**. In addition, the niches of populations, the food web structures and their spatio-temporal variations in relation to different habitat complexity and thus supply of food sources both in undisturbed and disturbed conditions, were profitable investigation.

Stable isotopes analysis provided clear information about the carbon sources and trophic relationships that are established between organisms (as in Post, 2002; Layman et al., 2012). Notably, the individual stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) has proven to be an effective approach with which to: (i) assess and quantify the relative contributions of food sources to diet of consumers at different taxonomic level; (ii) highlight the trophic role and quantify the trophic relationships between organisms (iii) track the role of propagation of natural and anthropogenic inputs along food chains.

Understanding how disturbance can affect community stability and thus ecosystem functioning is of increasing importance as climate change and human-induced impacts become increasingly widespread (Chapin Iii et al., 2000; Hooper et al., 2005; Thompson and Williams, 2017; Calizza et al., 2015; 2019). To this end, a functional study of the food webs organization and how they can be affected by different space and time availability of resources is crucial. Food webs represent the complex trophic patterns of communities that change both seasonally and geographically (Paine, 1980; Pimm et al., 1991; Rossi et al., 2015; Calizza et al., 2019).

In all my studies, the initial hypothesis related to the role of food availability in the construction of links between the nodes of food web was confirmed, and recurrent food web patterns found. In accordance with optimal foraging theory, we observed a decrease in consumer diet breadth as the per capita availability and quality of resources increases. As a consequence, the optimization of foraging by consumers, regardless of their hierarchical scale (from individual to species), led to a relatively simpler food web, with lower density of feeding links and potential competition, influencing the stability of the community against the loss of biodiversity and biological invasions.

Describing the mechanism that structures the ecological communities is crucial to predict their response to environmental change and provide key information for an efficient management measures in different spatial and temporal scale (Thompson et al., 2012; Rossi et al., 2015; Tam et al., 2017).

The Ross Sea (Antarctica), undisturbed ecosystem and rich in biodiversity, is a good model to understand the mechanism structuring the community and predict it responses of climate induced-environmental changes. Here biodiversity is markedly adapted to the extreme environment in which the seasonal regime of light and sea-ice persistence regulate the availability of food sources limiting the benthic compartment (Norkko et al., 2007; Clark et al., 2013; Constable et al., 2014; Calizza et al., 2018). The study of its pristine communities allowed us to explore stability mechanisms of food webs, as well as trophic plasticity and adaptation of populations to temporal variations of food sources.

Although our biodiversity knowledge in the region is increasing (Clarke, 2008; Griffiths et al., 2010; Chown et al., 2015), the presence of a strong cryptism, high endemism, omnivory degree and number of species not yet described (Clarke, 2008; Griffiths et al., 2010; De Broyer and Danis, 2011; Chown et al., 2015) limits our knowledge about the organization of these complex communities, especially the benthic compartment in which the majority of Antarctic biodiversity lies (Brandt et al., 2007; Chown et al., 2015; Xavier et al., 2016; Rossi et al., 2019). Hence the need to develop a novel, efficient and generalizable quantitative approach to describe food webs of complex communities, in all their variability.

The ability to assign links and locate single organisms within a food web are the key to describing the structure of communities in all their complexity (Thompson et al., 2012; Bates et al., 2015). However, the extraordinary biological diversity of ecosystems and the multiple possible interactions between species make it difficult to achieve this goal (Martinez, 1993; Fulton et al., 2003; Bates et al., 2015). Indeed, individual belonging to different species as well as individual belonging to the same species can occupy the same ITU and different ITU respectively. This fact means that biodiversity could be measured non only by species counting. This represents an important point that limits our ability to understand mechanisms underlying the stability of complex communities, conserve biodiversity (in the classic sense) and predict the effects of the expected environmental changes on food webs (Schofield et al., 2010; Rooney and McCann, 2012; Chown et al., 2015; Xavier et al., 2016; Michel et al., 2019).

Considering the populations from the tropho-functional standpoint, developing and adopting a novel quantitative approach to determine the trophic interactions in complex communities, allowed us to overcame these limits and obtain information about the diet and the role of species in the food web structure in unprecedented detail. The "trophospecies" (*sensu* Cohen and Briand) applied to the isotope signatures of carbon and nitrogen of organisms were the basic unit (ITU, Isotopic-Trophic-Units) of this approach.

Cohen and Briand (1984) defined the trophospecies as groups of individuals that share both the same set of resources and predators. The classical aggregation of taxa by common trophic guild was based on qualitative information as bibliographic information or direct observations. (Cohen and Briand, 1984; Martinez, 1993; Martinez et al., 1999; Phillips et al., 2014). However, the potential uneven and biased both taxonomic resolutions and aggregations of organisms even within the same community affects the quality of the food webs description (Martinez, 1993) and its metrics, as well as, make the structure of communities belonging to different environments hardly comparable (Martinez, 1993; Martinez et al., 1999).

We extend the classical trophospecies concept by providing an objective and quantitative measure of such groups since that the isotopic signature of each individual reflects those of its resources in predicable way (Peterson and Fry, 1987; Careddu et al., 2015). Notably, we define the isotopic trophospecies are as groups of individuals with similar isotopic signatures, that share the same position along food chains and resources axis (based on $\delta^{15}N$ and $\delta^{13}C$ values respectively). This implies that an ITU can include within it several taxonomic populations and, at the same time, the same population can be distributed over a wide spectrum of ITUs.

The Isotopic-Trophic-Units approach is a step forward in studies on the food web, as it allows us to focus on organisms as real tropho-functional and not as single and homogeneous taxonomic entities within community. Although this method does not allow to directly represent trophic links at the level of individual taxa, it allows to (y) consider the possibility that each individual consumer can explore the full spectrum of potential food sources available in his habitat, (yy) extrapolate the diet of each population starting from the information provided by the food web based on ITU (Isotopic-Trophic-Units), (yyy) better compare the food webs characterized by an inconsistent taxonomic resolution.

The robustness of our approach makes it a useful and promising tool to describe and compare complex structures of the food web, as well as the trophic role of their component, including those belonging to different ecosystems.

The role of individuals in food web stability

The consideration of the individual ecological role based on the study of Carbon and Nitrogen isotopic signatures of single organisms, has allowed us to overcome the actual difficulties of food web reconstruction and to describe the organization of communities, as well as the diet of single, in all their complexity (**Chapter 1, 3)**.

The detailed diet description of ecologically and economic important species with marked generalism allowed us to identify the set of resources crucial for the persistence of populations both in undisturbed and disturbed habitat (**Chapter 1, 3)**.

Several studied show that the generalist consumers play a fundamental role in the stability of the food web, since, by exploring food sources belonging to one or more chains, they may or may not strengthen the coupling between different ecosystem compartments (McCann, 2000; Rooney et al., 2006; Rossi et al., 2015). Due to their trophic strategy, these trophic groups can be a useful indicator of environmental states as they can directly reflect changes in both natural and anthropogenic inputs that determine the quality and availability of potential resources in their diet **(Chapters 1-3)** and contribute to the stability of the community **(Chapters 1-4)**. However, their trophic role has been little investigated empirically and considering populations mostly as homogeneous entities (Svanbäck et al., 2015). This could be reductive and misleading since there are variations between individuals of the same population both in habitat and resources use (Svanbäck et al., 2015).

We observed that changes in the availability of basal resource inputs due to different spatio-temporal environmental conditions, affected the trophic choices of organisms, and thus their linkage density, with cascade effects on the entire food web structure, complexity and stability (**Chapter 1-4)**. Pulsed inputs of sympagic algae, regulated by the seasonal sea-ice dynamics, played a fundamental role in the diet of individuals and therefore in structuring Antarctic benthic food of medium-depth waters (**Chapter 1)**. The reduction of prey placed at higher trophic levels, instead, was implicated in the piscivory reduction observed in the eel *Anguilla anguilla* with increasing eutrophication in coastal Mediterranean lakes (**Chapter 3)**. The persistence of biodiversity in variable environments was thus closely linked to the ability of organisms to adapt to different spatio-temporal availability in resource inputs (**Chapter 1-4)**. Notably, in low availability of basal resource conditions, the adoption of generalism and plasticity as trophic strategies, allowed to consumers to exploit a wide range of carbon sources, promoting both the species coexistence and persistence (Rossi et al., 2015; Schalk et al., 2016). This was observed at both different hierarchical and spatio-temporal scale (**Chapter 1-4)**.

Our results are of particular relevance because provide key information linking traits and ecological function of individual within food web, an ecological still open challenge (Thompson et al., 2012). Indeed, although it is known that the food web structure and complexity could be affected by individual responses to environmental changes, the latter is still litter understood (Thompson et al., 2012; Svanbäck et al., 2015; Feiner et al., 2019). In accordance with Petchey et al., (2008), we demonstrated that the trophic choices of individuals can determine not only the diet populations but also play a key role in the stability of food web whose structure is based on the sum of these individual decisions.

Variation of food web structure in highly variable and disturbed environments

Food web and tropho-functional approach highlights the ecological role of food web components helping to forecast potential ecological effects of environmental pressures linked to climate change, human activity, and biological invasions on the organization of biodiversity (**Chapter 1-4)**. This allows us to add crucial information for understanding the mechanisms that structure ecological communities, to predict their response to environmental change and to provide key information for efficient management of measures on different spatial and temporal scales.

The different spatio-temporal availability of basal resources linked to environment dynamics and complexity, as presences vs absent of sea-ice coverage and increasing of eutrophication level, led to a reshape of trophic interactions at a different hierarchical scale (i.e. from individual to community level) (**Chapters 1-4).** Notably, when the abundance and quality of the basal resource were higher, the consumers focused their foraging mostly on few resources, reducing both their trophic niche and the linkage density (**Chapters 1-3)**. The reduction in the variety of carbon sources explored made the coupling of distinct energy channels (both in the benthic-pelagic and marine-terrestrial compartments) less marked (Kaehler et al., 2000, Dunton 2001, Poulin et al., 2002, Knox, 2006) **(Chapter 1-3).**

The shift in the diet of consumers both following the sea-ice break-up (in Antarctica, **Chapter 1-2**) and the increased eutrophication level (brackish Mediterranean lakes , **Chapter 3**), was reflected in changes to both the carbon (δ^{13}C) and nitrogen (δ^{15}N) signatures and in their trophic niche (**Chapter 1-3)**. In addition, the closer the taxa are within the isotopic niche space, the more similar their diet is according to Bayesian mixing models, also by analysing the food web based on populations as homogeneous taxonomic units. **(Chapter 2-4).** This is further support that isotopic trophospecies can be a novel and useful indicator of the functional role of organisms within the community (**Chapter 1, 3)**. Changes in food web structure affected the potential disturbance propagation, the vulnerability to biodiversity loss and biological invasion **(Chapters 1-4)** as also observed in other studies (as Araújo et al., 2011, Calizza et al., 2017).

The link underlying the stability of communities, the structure and complexity of the food web and biodiversity is a much-discussed topic in ecology (McCann, 2000; Rossi et al., 2015; Xu et al., 2020). Ecosystems could respond to biotic and abiotic perturbations through two inversely proportional stability strategies: resistance and resilience. Both abilities are closely linked to community structure and complexity. The latter is measured as Connectance, i.e. the number of achieve links (L) with respect to all possible ones (S^2), and size (richness in species) of the food web (Gardner and Ashby, 1970; May, 1972; Dunne et al., 2002).

We found that food web with higher connectance and lower diversity were less vulnerable to temporal local loss of taxa or tropho-functional groups (Elton, 1927; Hutchinson, 1959; Pimm and Lawton 1977; Pimm, 1981; Vander Zanden et al., 1999; Post, 2002; Dunne et al., 2002; Kondoh and Ninomiya, 2009) **(Chapters 1-3)**. This suggests that changes in physical habitat characteristics could have devastating effects on ecosystem functioning by compromising both the structure, through species loss, and the function, such as communities' biotic resistance to invading, of biodiversity (Chapin lii et al., 2000; Sala et al., 2000; Eklöf et al., 2012; Dee et al., 2019).

Of particular note, we also observed the presence of a hard core of the food web components that persisted even after both natural and anthropogenic disturbances **(Chapters 1-4)**. This would suggest the presence of natural “hub-communities”, composed by keystone populations with high trophic plasticity, generalism and therefore linkage density, which could play a key role in food web structure and functioning and thus in the maintaining or restoring ecosystem functions (Jordàn, 2009; Kortsch, et al., 2015).

Highly interconnected populations within food web could accelerate disturbance propagation along food chains (Dunne et al., 2002; Estrada, 2007; Dobson et al., 2009; Lai et al., 2012; Calizza et al., 2017). Based on food web topology we identified hub populations that played a central role in interconnecting others in the food web **(Chapter 2)**. The extinction of keystone species, in a simulation of loss from the more to the less connected population (Dunne et al., 2002; Staniczenko et al., 2010), enhanced the number and rate of the secondary extinctions observed. In addition, starting from the trophic connections made in the food web, we identified and ranked the key species that most competitively and predatory restricted the invader with respect to its carrying capacity, which were present only in the more complex food web **(Chapter 4**). Following this, we suggested that also the topological population positions within the food web structure play a key role to the biodiversity maintenance in relation to disturb affecting the availability and quality of food sources (Allesina and Pascual 2009; Staniczenko et al., 2010).

Current biodiversity management and conservation efforts are generally focused on studying individual species or traits of them (Chapin lii et al., 2000; Jordàn et al., 2007, 2009; McDonald-Madden et al., 2016; Griffith et al., 2018). This could be reductive as species are not single and homogeneous entities but are trophic closely influenced by both intra and inter population interactions, including the environment in which they live and coexist with other species (Hutchinson, 1959; Cohen et al., 1993; Tylianakis et al., 2010; Lang et al., 2014; Svanbäck et al., 2015).

Our results support that the conservation of structure and function of biodiverse food webs could be an efficient measure to predict and mitigate the impact of induced environmental and biological change on natural communities.

Based on individual stable isotope analysis and food web reconstruction, it was possible to identify some recurring patterns in the architecture of biodiversity that underlie the mechanisms of persistence and stability of communities, regardless of the hierarchical and space-time scale analysed. In addition, it allowed the identification of keystone species that could most limit both the loss and the invasion of species, representing significant objectives on which to focus attention for efficient strategies of biological control and the conservation of biodiversity both in the short and medium-long term.

Conclusions

Changes in habitat characteristics induce substantial reshaped of ecological communities via introduction or losses of species, triggering cascading effects on the entire ecosystem structure and functioning (Chapin Iii et al., 2000; Clark et al., 2013; Hughes et al., 2020; Malhi et al., 2020). This raise concerns as biodiversity and ecosystem processes are linked by a reciprocal relationship, affecting the availability of goods and services essential for human well-being (Thompson et al., 2012; Chapin Iii et al., 2000). Although it is recognized that biodiversity plays an important role in the structure and function of ecosystems (Costanza et al., 1997; De Groot et al., 2002; Chapin Iii et al., 2000), the mechanisms underlying its stability, the functional role of species and the possible management measures to preserve it are not yet fully understood (McDonald-Madden et al., 2016; Xu et al., 2020). This poses limits as it is the interactions between species, rather than their presence or absence, that determine the characteristics and stability of the community (Jordàn et al., 2007, 2009; Thompson et al., 2012; Chapin Iii et al., 2000).

Present PhD dissertation quantified changes in feeding interactions and in food web metric under different space-time disturb conditions (both natural or anthropogenic) and identified food web recurring patterns.

The individual isotopic characterization of organisms and the food web reconstruction was crucial for describing and analysing the architecture of complex communities, characterized by high generalism and omnivory degree. In addition, this allowed to quantify the main energy flows and the role of the different basal resources in the food web topology in unprecedented detail (Post, 2002, Rossi et al., 2015, Calizza et al., 2015, Careddu et al., 2015). The linkage density decreased with increasing availability and quality of resources. Similarly, the more biodiverse food webs, the more complex they were.

The results shown suggest that physical changes in habitat that lead to a reduction of resources availability promote significant changes in the trophic choices of community already starting with individuals. In this condition, organisms with greater generalism and trophic plasticity may be better able to persist after a disturb.

Although the trophic niche analysis of populations is fundamental to understanding population foraging, their single study may not be sufficient to explain the mechanisms underlying community stability. The individuals and populations choices are reflected on the entire food web, affecting the persistence and resilience of the entire community. Less complex communities in terms of connectivity were the most able to limit the impact of invasive species but, at the same time, the most vulnerable to biodiversity loss. This suggest that taxonomically and functionally biodiverse food webs can be capable buffers for the propagation of disturbances towards a hard core with a crucial role for the persistence and functioning of the local community.

The isotopic characterisation of organisms and the quantification of intra- and interspecific links between populations provided crucial data for identifying key species for community stability under a range of biotic and abiotic disturbances. Disturbs that directly and indirectly affect the keystone topological populations can rapidly spread via trophic cascades, leading to the collapse of the food web.

The food web theory is the *hard core* of Ecology and its structures represent the biodiversity architecture (Odum and Biever, 1984). In the present PhD dissertation it was confirmed as a powerful tool for understanding and managing complex ecosystems, providing important information about the interaction between its components (individuals, populations, and species) and the possible response of community to biotic and abiotic perturbations.

The field study of biodiversity architecture represents a fundamental step towards understanding the mechanisms underlying the persistence of communities under different environmental pressures and improving our ability to preserve biodiversity and ecosystem functioning in a changing environment in the medium and long term.

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