

Research Article

Trophic relationships in Dutch reservoirs recently invaded by Ponto-Caspian species: insights from fish trends and stable isotope analysis

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Citation: Verstijnen YJM, Lucassen ECHET, van der Gaag M, Wagenvoort AJ, Castelijns H, Ketelaars HAM, van der Velde G, Smolders AJP (2019) Trophic relationships in Dutch reservoirs recently invaded by Ponto-Caspian species: insights from fish trends and stable isotope analysis. *Aquatic Invasions* 14(2): 280–298, <https://doi.org/10.3391/ai.2019.14.2.08>

Received: 10 August 2018

Accepted: 6 March 2019

Published: 23 April 2019

Handling editor: Michał Grabowski

Thematic editor: Ian Duggan

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Abstract

Invasive species can directly or indirectly alter (a)biotic characteristics of ecosystems, resulting in changing energy flows through the food web. This can potentially affect bottom-up or top-down control on resident species. The food web structure in the Biesbosch reservoirs (The Netherlands) was examined after recent invasions of the quagga mussel (*Dreissena rostriformis bugensis*) and round goby (*Neogobius melanostomus*), by means of stable isotope analysis. Quagga mussels reached relatively high densities after invasion, replacing the zebra mussel (*D. polymorpha*). The $\delta^{13}\text{C}$ signatures in the food web showed two distinct basic signatures of primary producers: 1) phytoplankton and *Elodea nuttallii*, and 2) more enriched submerged macrophytes (i.e. *Potamogeton* sp.). Quagga mussel was found to rely on zooplankton and phytoplankton. Mussel detritus seemed to be of importance for the gammarid *Dikerogammarus* spp. (both *D. villosus* and *D. haemobaphes*). $\delta^{15}\text{N}$ as a proxy for trophic level revealed that the largest specimens of ruffe (*Gymnocephalus cernuus*), pike-perch (*Sander lucioperca*) and perch (*Perca fluviatilis*) formed the top of the food chain. A stable isotope mixing model estimated that 43% of round goby diet consisted of *Dikerogammarus* spp. and approximately 27% of quagga mussels. Ruffe diet consisted of 29% of quagga mussels and only 12% of *Dikerogammarus* sp. Quagga mussels were less important as a food source for round goby than often has been described. Besides being a food source, mussel beds also provide a suitable habitat for macroinvertebrates (i.e. *Dikerogammarus* spp.). The invasive round goby and native ruffe seemed to compete for food in the benthic zone, where round goby possibly forced ruffe to use less nutritive or favourable food sources. Likely, this has contributed to the decline in ruffe abundance after the invasion of the highly competitive round goby. The altered and recent new links between species have changed the food web. The successful invasion in the reservoirs by the benthic invaders — quagga mussel and round goby — changed the benthic-pelagic coupling and has most likely increased the importance of the benthic food web in these reservoirs.

Key words: food web, mixing model, Biesbosch reservoirs, $\delta^{13}\text{C}$ & $\delta^{15}\text{N}$, quagga mussel, round goby, ruffe

Introduction

In ecology, food webs are intensively studied, because understanding of food web structure and functioning is important for ecosystem conservation and management. By studying food webs, the energy flows and species interactions such as predator-prey relationships can be unravelled (e.g. Thompson et al. 2012a). Food web studies typically combine the effects of community structure and ecosystem functioning (Doi 2009; Thompson et al. 2012b).

Invasive species, for instance, can directly alter the existing food web in an ecosystem by altering the trophic interactions, such as grazing and predation, and competition (David et al. 2017; Molles 2005). For example, a meta-analysis by Gallardo et al. (2016) showed that introduced benthic filter feeders can cause a collapse of the planktonic community and an increase in benthic invertebrates, while the benthic community densities can be decreased by introduced fish species. However, invaders not only modify trophic interactions by changing the community. Also, modifications in non-trophic interactions, such as ecosystem engineering, can have profound effects on the food web structure (van der Zee et al. 2016; David et al. 2017). So-called ‘ecosystem engineers’ modulate the availability of resources to other species by changing directly or indirectly their biotic or abiotic environment (Jones et al. 1994). Thus, invading ecosystem engineers can have profound effects on the food web by altering physical and biological characteristics of the system (Vitousek 1990). Invading engineers may therefore attain a prominent position in an ecosystem.

By directly or indirectly altering (a)biotic characteristics resulting in changed energy flows through the food web, invasive species can potentially affect either bottom-up or top-down control on resident species (van Riel et al. 2006; Gallardo et al. 2016) depending on their ecological function. To elucidate the trophic structure and to provide insight in energy flow in food webs, stable isotope analysis (SIA) can be an important tool (e.g. Layman et al. 2012). Often nitrogen and carbon are used for SIA. Nitrogen stable isotope ratios ($\delta^{15}\text{N}$) can be used for estimating trophic levels of species, whereas carbon stable isotope ratios ($\delta^{13}\text{C}$) can be used to determine their carbon source (Layman et al. 2012).

The quagga mussel (*Dreissena rostriformis bugensis* Andrusov, 1897) is an ecosystem engineer native to the Ponto-Caspian basin. The species has invaded large parts of North America and Europe since the 1940s (Karatayev et al. 2015; Matthews et al. 2014). The quagga mussels have been spreading, together with and/or after the spread of the invasive zebra mussel (*Dreissena polymorpha* (Pallas, 1771)). Many related changes in community structure and/or abiotic environment have been recorded with the invasion of dreissenid mussels (Kelly et al. 2010) such as a decrease in chlorophyll- α and total phosphorus in the water (Cha et al. 2013), changes

in benthic habitats (Karatayev et al. 2015) and replacement of zebra mussels by quagga mussels (Wilson et al. 2006; Matthews et al. 2014). A Ponto-Caspian species often invading waters together with the quagga mussel is the round goby (*Neogobius melanostomus* (Pallas, 1814)), which has a long evolutionary history with the dreissenids (Kornis et al. 2012; Naddafi and Rudstam 2014). Round goby is known to predate on quagga mussels when present (Corkum et al. 2004; Kornis et al. 2012; Perello et al. 2015) and can change fish abundance or population structure (e.g. Balshine et al. 2005; van Kessel et al. 2016).

In the south-west part of the Netherlands, three artificial freshwater reservoirs were constructed to store water from the River Meuse for drinking water production (Oskam 1982). Quagga mussels have invaded the reservoirs around 2008 and replaced the earlier established zebra mussels within one year. In the same period, the exotic round goby was increasingly observed and water quality variables changed (Wagenvoort 2014a, b; Jûza et al. 2018). The aim of the present study was to determine the current status of the food web in the reservoirs in order to elucidate the possible impacts of the recent invasive species on the community, by gaining insight into a) the structure of the food web (species composition and trophic relations) in the reservoirs, b) the change of the species composition and trophic relations after recent invasions, and c) the relative importance of the most recent and dominant invaders.

Materials and methods

Study location

The present study was carried out in the reservoir system, The Netherlands (51°44"N; 4°46"E) consisting of three (belowground) interconnected artificial reservoirs in the Biesbosch area (Figure 1): De Gijster (area 320 ha, average depth 12 m, maximum depth 27 m), Honderd en Dertig (area 219 ha, average depth 16 m, maximum depth 31 m) and Petrusplaat (area 105 ha, average depth 12 m, maximum depth 15 m). The three reservoirs were constructed in the 1970s for high volume storage (De Gijster) and natural prepurification of River Meuse water. Average retention of the water is 5–6 months, leading to considerable water quality improvements (Oskam 1982). The reservoirs are of major importance for the drinking, industrial, and agricultural water supply in the Rotterdam area. A littoral zone is lacking due to asphalt-concrete banks and there is no intrusion of surrounding ground water. The water in the reservoirs does not stratify in summer, as air injectors mix and aerate the water column from April–October (van Breemen and Ketelaars 1995). In general, water quality varies little between the three reservoirs. Overall, water quality improves gradually from De Gijster to Honderd en Dertig to Petrusplaat: increasing transparency, decreasing chlorophyll- α and nutrients (Table 1; variables during sampling period).

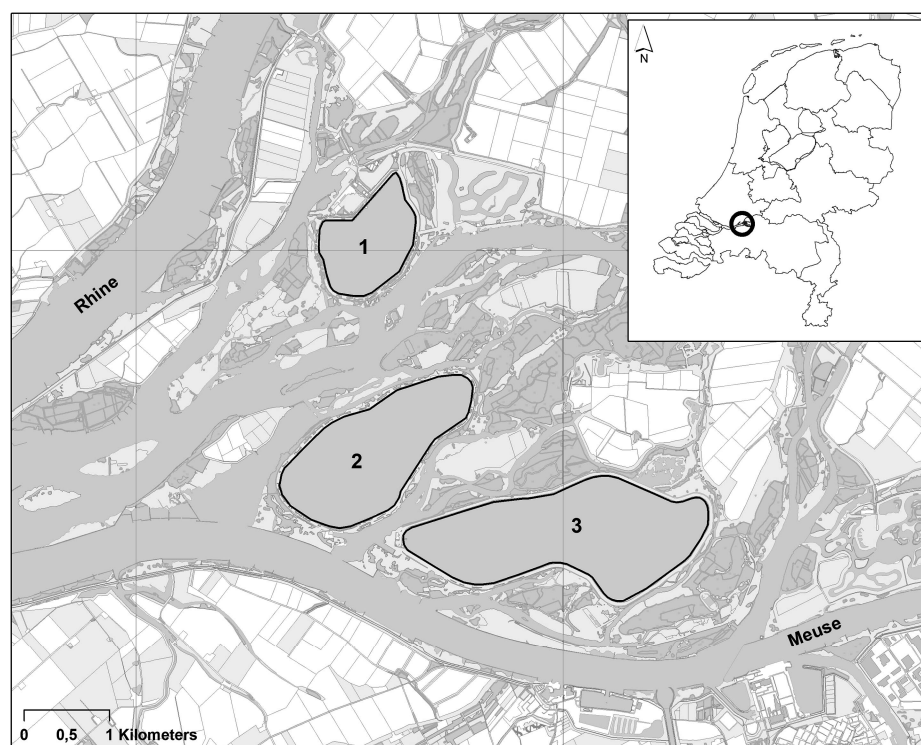


Figure 1. Locations of the three reservoirs in the Biesbosch: 1) Petrusplaat, 2) Honderd en Dertig and 3) De Gijster.

Table 1. Water quality parameters of the three reservoirs DG = De Gijster, HD = Honderd en Dertig and PP = Petrusplaat in summer 2016 (mean values of May–October $\pm$ SD).

	unit	DG	HD	PP
Temperature	$^{\circ}\text{C}$	18.2 ± 3.2	18.2 ± 2.9	18.4 ± 2.7
pH		8.4 ± 0.1	8.3 ± 0.1	8.6 ± 0.2
Chlorophyll- α	$\mu\text{g l}^{-1}$	7.5 ± 6.0	6.7 ± 7.0	4.8 ± 4.4
Transparency	m	3.3 ± 1.2	3.9 ± 1.7	5.6 ± 1.9
Total-phosphate	mg P l^{-1}	0.06 ± 0.02	0.06 ± 0.02	0.03 ± 0.01
Ammonium	mg N l^{-1}	0.05 ± 0.03	0.05 ± 0.04	0.04 ± 0.01
Nitrate	mg N l^{-1}	2.38 ± 0.34	2.22 ± 0.31	2.00 ± 0.27

Sampling

In 2016 (May, June, August) and 2017 (June, July) samples of the most abundant and/or potentially important organisms were collected on various locations in the three reservoirs, using different methods. Supplementary material Table S1 shows the methods of sampling per species/group.

Several algae and benthic Cyanobacteria were collected from the sediment from the shore or by snorkeling/scuba diving at depths < 6 m. Phytoplankton was collected by filtering a vast amount of water (mixed sample of the water column) over glass fiber filters ($0.45 \mu\text{m}$) and large zooplankton was efficiently removed from the filter by hand using a dissection microscope. Planktonic Cyanobacteria were manually collected from scum layers at the water surface. Macrophytes were in most cases manually collected by snorkeling or scuba diving and incidentally using an Eckman grab sampler at depths ≤ 6 m. Zooplankton was collected with

plankton nets (mesh size 30, 100 and 250 μm , respectively), and specimens of several species or groups were manually collected from water samples using a dissection microscope. Fishes were collected using seine and multi-mesh gill nets and trawls. Quagga mussels were sampled with an Eckman grab sampler (De Gijster) or by snorkeling (Honderd en Dertig and Petrusplaat). Crayfish were collected by seine fishing. Macroinvertebrates and other samples were in most cases manually collected from sediment samples that were collected with a sediment sampler (Eckman or Van Veen grab) or by a scuba diver at various depths (mainly < 6 m). Collected samples were separately stored in plastic bottles.

Sample processing

After sampling, identification took place, if possible to species level. Size (standard length of fish and shell length of mussels) and weight of fish and mussels were measured. After collection, samples were stored in the freezer ($-20\text{ }^{\circ}\text{C}$). In the laboratory, samples were rinsed with demineralized water, stored at $-80\text{ }^{\circ}\text{C}$ and freeze-dried for at least 48 hours. For fish, only muscle tissue without skin, taken from the flank of the fish (above the lateral lines and beneath the dorsal fin) was used and for mussels only the soft body tissue. A small piece of the phytoplankton glass fiber filters was cut out for use in isotope analysis. In the case of crayfish, only the soft muscle tissue collected from the claws was used. For macrophytes, stems and leaves were used as a pooled sample per species and for other small samples (macroinvertebrates etc.) whole individuals were used. After freeze-drying, the samples were ground to a fine powder in a bullet grinder (Retsch, Aartselaar, Belgium). The phytoplankton filters were not ground but used as a whole and only samples of April until August were used to diminish high seasonal variance. The data of all three reservoirs were lumped together for all further analyses as the reservoirs are interconnected so organisms can translocate over the reservoirs and water quality variables of the reservoirs are within the same order of magnitude. Furthermore, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the sampled species from the various reservoirs are largely overlapping.

Stable isotope analysis

Homogenized dried samples were weighed with an analytical balance and put into ultra-pure tin cups, which were rolled into pellets and subsequently analyzed for carbon and nitrogen stable isotope composition with a Flash 2000 elemental analyzer coupled online with a Delta V Advantage-isotope radiomonitoring mass spectrometer (IRMS, Thermo Scientific). The reference gasses which have been used were calibrated with the IAEA reference standards (IAEA-N-2 and IAEA-CH-6), with a maximum deviation of 0.15‰. The $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ were determined

(R in permil; ‰) of every sample and as an internal standard control, caffeine was used. Isotope ratios were expressed in the δ notation ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) relative to Vienna PDB and atmospheric N_2 using the equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = ([R_{\text{sample}}/R_{\text{standard}}] - 1) \cdot 1000 \quad \text{Eq. 1}$$

Per fish species a linear regression analysis was conducted to unravel correlations between fish length and $\delta^{15}\text{N}$ using SigmaPlot (version 14.0). After statistical analysis, consulting stomach content data (Evides, *unpublished data*) and information on length, diet and food items per fish species from Fishbase (Froese and Pauly 2018), several fish species were divided into size classes for analysis.

Diet source contribution modelling

The relative importance of prey species in the diet of the exotic round goby and native ruffe (*Gymnocephalus cernuus* (Linnaeus, 1758)) in the reservoirs was determined to unravel suspected diet overlap and the importance of mussels in their diets. This was conducted using stable isotope data of all sampled individuals of both consumers and their prey species in SIAR package (Parnell and Jackson 2011) in R Statistics (version 3.4.2) (R Core Team 2017). Also, roach (*Rutilus rutilus* (Linnaeus, 1758)) diet was analysed as this species showed a similar trend in abundance as ruffe. The input of diet sources was chosen on basis of empirically important consumer-resource interactions, based on stomach contents of the fish (Evides, *unpublished data*), overall data (i.e. Froese and Pauly 2018) and the biplots of isotope data. The model was first run (per consumer) with a wide range of possible diet sources. The sources which contributed least were then excluded. Eventually, stable isotope signatures of the four most important dietary items were included in the final model. Proportions of diet sources were modelled for the consumers on basis of four different stable isotope fractionation factors (ΔN and ΔC) and their standard deviations, namely A) $\Delta\text{N } 3.4 \pm 1.0$ and $\Delta\text{C } 0.4 \pm 1.3$ (Post 2002), B) $\Delta\text{N } 2.9 \pm 1.2$ and $\Delta\text{C } 1.3 \pm 1.3$ (McCutchan et al. 2003: based on fractionation in muscle tissue), C) $\Delta\text{N } 2.3 \pm 1.6$ and $\Delta\text{C } 0.4 \pm 1.2$ (McCutchan et al. 2003: based on aquatic consumers) and D) $\Delta\text{N } 3.1 \pm 1.6$ and $\Delta\text{C } 2.0 \pm 0.8$ (Caut et al. 2009: based on a selection of data of fractionation in muscle tissue of fish).

Historical monitoring data

Fish abundance data (kg per 1000 m²) for the reservoirs between 1998 and 2016 were obtained from Jůza et al. (2018), and transformed to obtain a general insight in fish trends in the reservoirs. Furthermore, macroinvertebrates were sampled twice a year in spring and summer in 2002 and between 2005–2017 on several locations in the reservoirs (3 locations

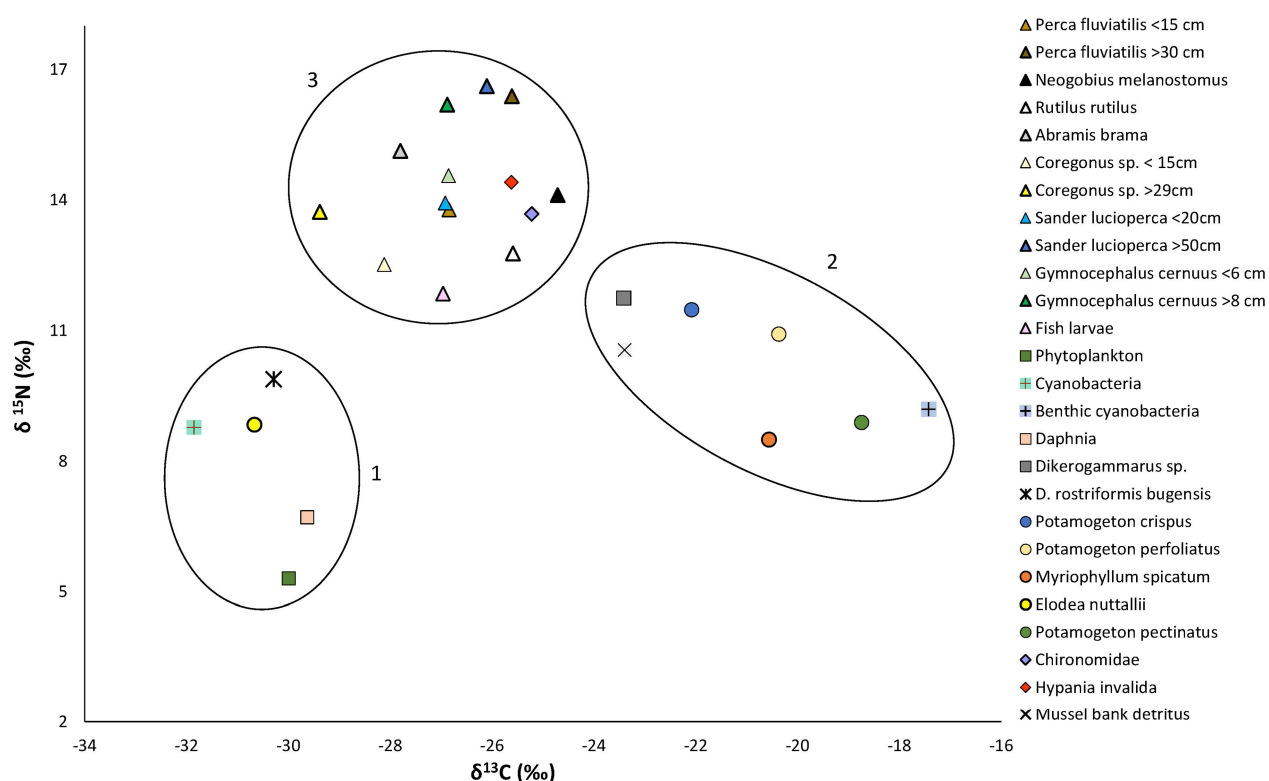


Figure 2. Food web of the Biesbosch reservoirs based on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (‰). The mean values per distinct group are shown. See table 2 for more detailed information (including standard deviation and range). With circles (1, 2 and 3) a division is made between groups with isotopic signatures in a certain range.

in De Gijster, 2 in Honderd en Dertig and 1 in Petrusplaat). Macroinvertebrate samples were collected manually from lake sediment sampled with an Eckman bottom grab (sampled area per location: 200 cm²). The fish and macroinvertebrate monitoring data were analysed after pooling the data of the three reservoirs.

Results

Food web composition

The food web at the basis showed a pelagic and a benthic component with two distinct basic signatures of primary producers (Figure 2: circle 1 and 2). The first consisted of phytoplankton with a mean $\pm$ SD $\delta^{13}\text{C}$ of -29.99 ± 2.37 ‰ and a mean $\pm$ SD $\delta^{15}\text{N}$ of 5.31 ± 2.86 ‰ (Figure 2 and Table 2). *Elodea nuttallii* (Planchon) St. John and Cyanobacteria showed a similar $\delta^{13}\text{C}$ value, while the $\delta^{15}\text{N}$ value was higher compared to phytoplankton (Table 2). The second base of primary producers showed a more enriched (less negative) $\delta^{13}\text{C}$ signature and consisted of *Myriophyllum spicatum* L., several *Potamogeton* species, *Chara contraria* Braun ex Kütz. 1845, *Vaucheria* and benthic Cyanobacteria (Figure 2: circle 2). *Daphnia* showed an N signature approximately 2‰ higher than phytoplankton (Figure 2, circle 1). Furthermore, several planktonic consumers occurred with a mean $\delta^{13}\text{C}$ value of -30.81 and a mean $\delta^{15}\text{N}$ of 9.80 (mixture of zooplankton species

Table 2. Sample size, mean isotopic signature, standard deviation and range of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (‰) per species or group of species and ordered by increasing $\delta^{15}\text{N}$ value.

Specimen	n	Mean		SD		Range	
		$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Fish							
Fish larvae	2	11.85	−26.96	2.41	0.40	10.14–13.56	−27.25 to −26.68
<i>Coregonus</i> sp. < 15 cm	9	12.52	−28.12	0.66	0.81	11.81–13.42	−28.94 to −26.84
<i>Rutilus rutilus</i> (< 12 cm)	12	12.77	−25.59	0.50	1.30	11.84–13.38	−28.98 to −24.21
<i>Osmerus eperlanus</i>	1	12.88	−31.28				
<i>Coregonus</i> sp. > 29 cm	9	13.73	−29.38	0.71	0.67	12.70–15.25	−30.24 to −28.12
<i>Perca fluviatilis</i> < 15 cm	33	13.77	−26.84	1.15	1.40	11.80–16.00	−28.77 to −24.05
<i>Sander lucioperca</i> < 20 cm	33	13.94	−26.92	0.74	0.89	12.56–16.52	−28.31 to −24.80
<i>Neogobius melanostomus</i>	45	14.11	−24.71	0.82	0.82	12.55–15.88	−28.85 to −20.74
<i>Perca fluviatilis</i> 15–30 cm	22	14.36	−26.87	0.90	1.01	12.44–16.14	−28.58 to −24.30
<i>Gymnocephalus cernuus</i> < 6 cm	11	14.56	−26.86	1.46	0.48	12.29–16.87	−27.46 to −25.88
<i>Abramis brama</i> (> 40 cm)	7	15.13	−27.80	0.78	1.68	14.51–16.26	−29.06 to −24.09
<i>Sander lucioperca</i> 20–30 cm	3	15.32	−26.61	0.56	0.38	14.92–15.96	−26.93 to −26.81
<i>Gymnocephalus cernuus</i> > 8 cm	16	16.19	−26.88	1.04	0.80	13.30–17.45	−28.07 to −25.42
<i>Perca fluviatilis</i> > 30 cm	11	16.39	−25.61	0.96	1.40	14.86–18.30	−28.08 to −23.52
<i>Sander lucioperca</i> > 50 cm	7	16.62	−26.11	1.15	0.84	15.65–19.04	−27.25 to −24.98
Macroinvertebrates							
<i>Dikerogammarus</i> spp. (<i>villosus</i> and <i>haemobaphes</i>)	28	11.74	−23.41	1.57	1.69	9.33–14.34	−26.72 to −20.20
Chironomidae	6	13.68	−25.22	1.29	2.25		
Oligochaeta	1	14.09	−28.02				
<i>Hypania invalida</i>	2	14.41	−25.62	0.06	0.77	14.37–14.46	−26.17 to −25.08
Dreissena and detritus							
<i>Dreissena rostriformis bugensis</i>	44	9.88	−30.29	1.06	1.45	7.35–11.86	−33.06 to −28.32
Detritus mussel banks	3	10.72	−20.05	2.14	5.80	8.43–12.67	−23.81 to −13.37
Zooplankton							
<i>Daphnia</i> sp.	5	6.71	−29.63	1.82	0.36	4.80–9.26	−30.02 to −29.14
Zooplankton (i.a. <i>Eudiaptomus</i> , nauplius larvae)	9	9.80	−30.81	1.43	4.80	7.83–11.81	−34.71 to −22.66
<i>Bythotrephes longimanus</i>	1	10.53	−30.44				
<i>Leptodora kindtii</i>	1	13.93	−30.87				
Cyanobacteria							
Cyanobacteria (i.a. <i>Microcystis</i>)	9	8.78	−31.85	2.15	1.75	6.02–11.48	−34.21 to −29.48
Benthic cyanobacteria (i.a. <i>Phormidium autumnale</i>)	4	9.20	−17.43	1.93	1.15	7.53–11.79	−18.84 to −16.34
Macrophytes							
<i>Myriophyllum spicatum</i>	5	8.49	−20.56	1.62	1.65	7.07–11.19	−21.99 to −17.84
<i>Elodea nuttallii</i>	8	8.83	−30.66	2.33	3.67	6.23–12.68	−35.30 to −25.12
<i>Potamogeton pectinatus</i>	6	8.88	−18.74	0.82	1.09	7.80–9.58	−19.66 to −16.78
<i>Potamogeton perfoliatus</i>	1	10.92	−20.36				
<i>Potamogeton crispus</i>	6	11.48	−22.08	0.47	1.34	10.75–11.94	−23.09 to −19.56
Algae							
Phytoplankton	21	5.31	−29.99	2.86	2.37	0.00–12.30	−34.56 to −22.77
<i>Vaucheria</i> sp.	4	8.58	−21.61	0.84	2.39	7.84–9.78	−24.00 to −19.37
<i>Chara contraria</i>	3	10.19	−19.88	1.19	2.00	9.29–11.54	−21.49 to −17.64
Other							
<i>Craspedacusta sowerbyi</i>	1	12.87	−31.27				
<i>Orconectes limosus</i>	2	12.98	−26.10	0.77	5.80	12.44–13.52	−26.60 to −25.61

i.a. *Eudiaptomus*, *Cyclops* and nauplius larvae). The signature of the quagga mussel (*D. rostriformis bugensis*) was similar to the mixed zooplankton with a mean value of -30.29 ± 1.45 and 9.88 ± 1.06 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Consumers above the C-enriched benthic base consisted of *Dikerogammarus haemobaphes* (Eichwald, 1841) and *D. villosus* (Sowinsky, 1894) (Table 2).

Higher in the pelagic food web other species were found (Figure 2: circle 3), such as the predatory water fleas *Leptodora kindtii* (Focke, 1844) and

Bythotrephes longimanus (Leydig, 1860) (Table 2). Crayfish (*Orconectes limosus* (Rafinesque, 1817)) showed a comparable isotopic composition as roach (*R. rutilus*). The worms (oligochaetes and the polychaete *Hypania invalida* (Grube, 1860)) and chironomids showed $\delta^{13}\text{C}$ values that lie between the phytoplankton and the more enriched food web base (Table 2). Fish species also showed intermediate $\delta^{13}\text{C}$ values ranging from -29.38 (*Coregonus* sp. > 29 cm) to -24.71 (*N. melanostomus*), suggesting a mixed diet based on species from both food chains (Figure 2). Whitefish (*Coregonus* sp.) showed the most depleted values of $\delta^{13}\text{C}$, while round goby was most enriched in ^{13}C (highest $\delta^{13}\text{C}$). Fish size correlated positively with $\delta^{15}\text{N}$ for Eurasian ruffe, perch (*Perca fluviatilis* (Linnaeus, 1758)), pike-perch (*Sander lucioperca* (Linnaeus, 1758)) and whitefish (Table S3). Larger sized fish occupied a higher trophic position in the food web (Figure 2 and Figure S1), with Eurasian ruffe (> 8 cm), perch (> 30 cm) and pike-perch (> 50 cm) being in the top of the food web. For round goby, roach and bream (*Abramis brama* (Linnaeus, 1758)), no significant correlation was found between isotopic values and the sizes of these species (Table S3).

Food web interactions

The data of fish monitoring revealed some clear changes in abundance of several species and showed a high abundance of round goby after 2008. Overall abundance of ruffe, pike-perch, bream and (older) roach decreased after 2008, while there was an increase of perch and whitefish (Jüza et al. 2018; Figure 3A and 3B). In 2012, round goby occurred in all depth zones (data transformed after Jüza et al. 2018). Ruffe strongly decreased after the invasion of round goby at all depths.

Monitoring revealed the occurrence of the quagga mussel in 2008. The rapid increase of the abundance in the following years coincided with a strong decrease of the zebra mussel (Figure 3C). Highest abundance was reached in 2012 and seemed to decrease after 2013. In the reservoirs alien gammarids (*Dikerogammarus* spp. (both *D. villosus* and *D. haemobaphes*)) were found since 2007, whereas other alien amphipod species (i.e. *Echinogammarus ischnus* (Stebbing, 1899) and *Chelicorophium curvispinum* (G.O. Sars, 1895)) were also found before 2007 (Wagenvoort, *unpublished data*). In 2009, relatively high numbers of *Dikerogammarus* species were found, which is reflected in high total numbers of sampled amphipods (Figure 3C).

The outcomes of the model with various fractionation factors overall showed a similarity in diet composition between round goby and ruffe (both benthic species). Based on the isotope data, diet of round goby mainly consisted of *Dikerogammarus* spp. with a diet proportion of 32–59%. Quagga mussels made up approximately 15–38% of the diet and subsequently

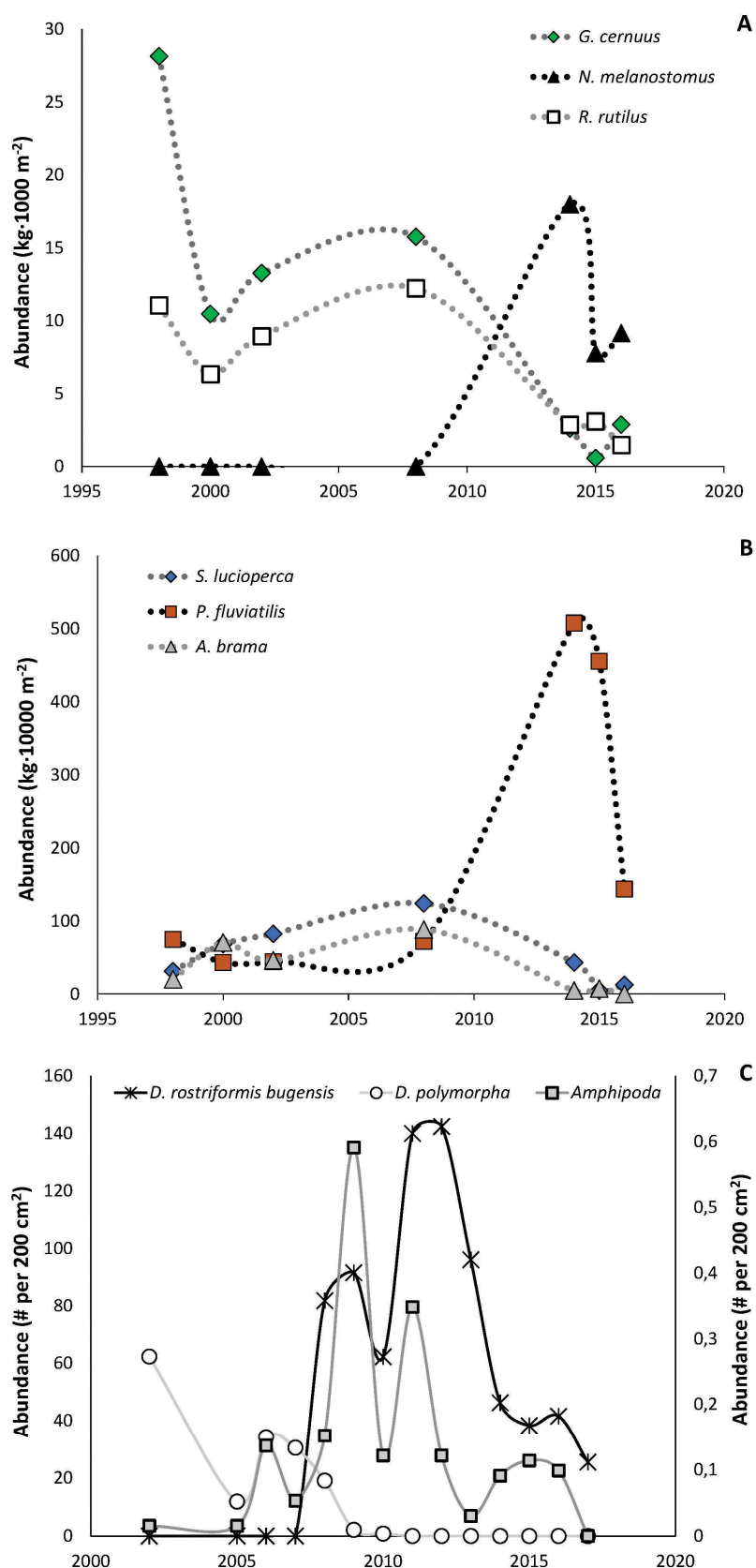


Figure 3. A and B) Biomass abundance (kg 1000 m⁻²) over time of several important fish species. Data was transformed after Jüza et al. (2018). and C) abundance of dreissenid mussels (left axis) and amphipods (right axis) (# per 200 cm²) in the reservoirs. Fish biomass as determined with gillnet sampling and mussel abundance as determined by individuals in one sediment grab.

Table 3. Proportion of round goby (*Neogobius melanostomus*) diet per dietary item (lower 95% interval-higher 95% interval) in the reservoirs. Values are given based on four different fractionation factors (A, B, C and D).

Dietary item <i>N. melanostomus</i>	Mean proportion per fractionation factor*			
	A	B	C	D
<i>Dreissena rostriformis bugensis</i>	0.28 (0.17–0.38)	0.31 (0.22–0.40)	0.15 (0.06–0.26)	0.38 (0.29–0.48)
<i>Dikerogammarus</i> spp.	0.59 (0.47–0.71)	0.39 (0.26–0.51)	0.41 (0.28–0.53)	0.32 (0.20–0.44)
Chironomidae	0.04 (0.00–0.11)	0.16 (0.04–0.28)	0.29 (0.16–0.42)	0.15 (0.02–0.26)
Fish larvae	0.09 (0.00–0.21)	0.14 (0.00–0.27)	0.15 (0.01–0.29)	0.15 (0.01–0.29)

*A) Post (2002); B) McCutchan et al. (2003) (Muscle); C) McCutchan et al. (2003) (Aquatic) and D) Caut et al. (2009) (Selection).

Table 4. Proportion of ruffe diet (*Gymnocephalus cernuus*) per dietary item (lower 95% interval-higher 95% interval) in the reservoirs. Values are given based on four different fractionation factors (A, B, C and D).

Dietary item <i>G. cernuus</i>	Mean proportion per fractionation factor*			
	A	B	C	D
<i>Dreissena rostriformis bugensis</i>	0.30 (0.21–0.39)	0.32 (0.22–0.41)	0.15 (0.05–0.26)	0.40 (0.32–0.50)
<i>Dikerogammarus</i> spp.	0.22 (0.12–0.33)	0.07 (0.00–0.15)	0.14 (0.04–0.25)	0.03 (0.00–0.07)
Chironomidae	0.15 (0.01–0.29)	0.11 (0.00–0.23)	0.16 (0.00–0.31)	0.04 (0.00–0.11)
Oligochaeta	0.33 (0.18–0.48)	0.50 (0.32–0.66)	0.56 (0.36–0.72)	0.53 (0.39–0.65)

*A) Post (2002); B) McCutchan et al. (2003) (Muscle); C) McCutchan et al. (2003) (Aquatic) and D) Caut et al. (2009) (Selection).

Chironomidae (4–29%) and fish larvae (9–15%) (Table 3). Table 4 showed that for ruffe, Oligochaeta were the most important food source (33–56%), followed by quagga mussel (15–40%) and similar proportions of *Dikerogammarus* spp. and Chironomidae. Diet analysis of roach showed that quagga mussel had the biggest share in the diet of roach (30–41%) (Table S2).

Discussion

Stable isotope analysis revealed the relative position in the aquatic food web of phytoplankton, macrophytes, zooplankton, macroinvertebrates, native fish species as well as the current position of the most recent invading species: the round goby and the quagga mussel. Both species are native to the Ponto-Caspian region (Gallardo et al. 2016) where they coexist since a long time. Via the river Meuse, a pathway for Ponto-Caspian invasive species (Leuven et al. 2009), the species probably ended up in the Biesbosch reservoirs. It is known that quagga mussels can have a strong bottom-up effect on the benthic community, whereas round goby can have a top-down effect (Pagnucco et al. 2016). Such changes in one or more trophic levels can have profound effects on other trophic levels along the cascades (Brett and Goldman 1996).

Stable isotope analysis

Besides prey isotopic signature, processes like food limitation and environmental variance may contribute to isotopic variance in consumers (Sweeting et al. 2007). Modelled diet proportion ranges give insight in possible diet overlap between the benthic fish species and their diet preferences. When modelling the diet composition of species based on isotopic data, however, the fractionation factors used affect the outcome of mixing models (Caut et al. 2009). For example, fractionation of N and C is often tissue specific (e.g. for liver, blood, muscle) (Caut et al. 2009; Ankjærø et al. 2012). By analysing muscle tissue of fish, a long-term diet assimilation is taken into account, rather than short-term shifts in diet (Ankjærø et al. 2012). By comparing several fractionation factors, we obtained a range of possible diet proportions. Although the outcomes differed when using alternative fractionation factors, the results were quite consistent and did not affect the interpretation towards the consequence for the food web.

During modelling the least important diet sources were left out of the final analysis, which possibly overestimate the current proportions. However, based upon the isotope data, pre-modelling and knowledge about food preferences and stomach content analysis, the most important diet sources chosen, give reliable information.

Structure of the food web

The base of the food web consisted of two main pathways of energy, namely phytoplankton ($\delta^{13}\text{C}$ around -30 to -32) and submerged macrophytes (except *Elodea nuttallii*) ($\delta^{13}\text{C}$ around -18 to -24). This division is apparent in more aquatic systems and can be explained by the main carbon source that is used (Mendonça et al. 2013). Some plants are capable of using HCO_3^- as a carbon source, which is more enriched (-7 to -11 ‰ less negative according to Keeley and Sandquist 1992) than CO_2 . In our study, in particular the rooted *Potamogeton* species and the benthic Cyanobacteria were more enriched in ^{13}C compared to pelagic phytoplankton and Cyanobacteria and *Elodea nuttallii*. Phytoplankton samples probably contained some Cyanobacteria next to algae. Relatively low $\delta^{15}\text{N}$ would have been expected in Cyanobacteria if they were N-fixing species as N_2 -fixation leads to low $\delta^{15}\text{N}$, resulting from atmospheric $\delta^{15}\text{N}$ (Bauersachs et al. 2009). Though, certain Cyanobacteria like *Microcystis* sp., which also occurred in the Cyanobacterial samples in de reservoirs, take up nitrate and ammonium (Lehman et al. 2015), resulting in highly variable $\delta^{15}\text{N}$ (Bauersachs et al. 2009). The relatively high $\delta^{15}\text{N}$ in rooted plants compared to phytoplankton can possibly be ascribed to the uptake of N from the pore water instead of dissolved N from the water column (Chappuis et al. 2017).

The quagga mussel (*D. rostriformis bugensis*) was linked to phytoplankton, which they filtrate from the water column as a food source (e.g. Cha et al. 2013; Karatayev et al. 2015). Also, the signature of the herbivorous *Daphnia* as primary consumer was linked to phytoplankton. The isotopic signatures of mixed zooplankton were similar to that of the mussels. They may directly compete with mussels for food, which was also found in North-American lakes (Garton et al. 2005). The dominance of quagga mussels led to a decrease in total zooplankton biomass during summer (Wagenvoort 2014b; Figure S2).

Gammarids rely on mussel banks like the formerly present zebra mussels and current quagga mussels (e.g. González and Downing 1999; Marescaux et al. 2016). Biodeposition (translocation of pelagic biomass to the benthic zone) in the form of (pseudo)faeces from the mussels, is thought to be an important food source for amphipods like *Dikerogammarus villosus* (Gergs and Rothhaupt 2008). This invasive species co-existed with *D. haemobaphes* in the Biesbosch reservoirs and both species can affect the macroinvertebrate community by predation, as their predation can be more effective than that of native gammarids (van Riel et al. 2006; Bacela-Spychalska and van der Velde 2013). The isotopic signature of *Dikerogammarus* spp. suggests a link with the mussel bank detritus/pseudofaeces, which is more enriched in ^{13}C than the mussels themselves (Figure 2). Bacela-Spychalska and van der Velde (2013) also found detritus to be the most important food source for *D. haemobaphes*, followed by animal remains. The highest positions ($\delta^{15}\text{N}$) in the food web, suggesting highest trophic level, were taken by fish and more specifically the larger specimens of pike-perch, perch and ruffe, which are top predators. This can be attributed to ontogenetic diet shifts (Kadye and Booth 2012). For pike-perch and perch, mainly fish was found in the stomachs of bigger specimens (Evides, unpublished data).

Food web interactions

The observed rapid increase and replacement of the zebra mussel by quagga mussel after 2008 has also occurred in other invaded areas (bij de Vaate et al. 2014; Matthews et al. 2014; Karatayev et al. 2015). The eco-engineering potential of dreissenid mussels can shape the food web via interactions beyond the trophic network (van der Zee et al. 2016). Concomitant with the invasion of the quagga mussel, water transparency increased (from circa 2.5–4 m to 3.3–5.6 m) and chlorophyll-*a* decreased (from circa 10 $\mu\text{g/l}$ to 6–7 $\mu\text{g/l}$) with a concomitant decrease in zooplankton in all three reservoirs (from $\pm 1.8 \text{ mm}^3/\text{l}$ to $< 0.4 \text{ mm}^3/\text{l}$ on average) (Wagenvoort 2014a, b). This is in line with previous findings in invaded areas (e.g. Higgins and Vander Zanden 2010; Cha et al. 2013; Pothoven and Fahnenstiel 2013). The decrease in chlorophyll was observed for four

to five years (Figure S2). As both dreissenid species have comparable filtration rates, increased filtering of the water column is probably the result of a higher abundance of the quagga mussels compared to the zebra mussels (Mei et al. 2016).

Stomach or gut analysis often suggest that quagga mussels are the primary food item for round goby (Corkum et al. 2004; Johnson et al. 2005). Round gobies have physical features to ingest shelled dreissenids (Marsden et al. 1996; own observations) and are known to decrease the abundance of quagga mussels or alter mussel-size composition in (newly) invaded lakes (Wilson et al. 2006; Lederer et al. 2008; Naddafi and Rudstam 2014). However, our stable isotope analysis and subsequent diet modelling, revealed that in the Biesbosch reservoirs quagga mussels were not the dominant prey item for round goby and that there was no correlation between goby length and isotopic signature. In accordance with our results, other (isotope) studies also found a less pronounced proportion of quagga mussels in the diet of round goby (Bauer et al. 2007; Brush et al. 2012) and no difference in diet preference of small and large sized gobies (Borcherding et al. 2013). Water clarity might be an important factor in explaining this difference in diet ingestion by enabling higher predation rates on gammarids at the cost of predation on dreissenid mussels (Diggins et al. 2002). Brush et al. (2012) determined that the dietary fraction of quagga mussels did not exceed 0.39 and that amphipods are an important food source (up to over 0.45) for round goby, which is in accordance with the results of our model (*Dikerogammarus*-fraction of 0.32–0.59).

In de Biesbosch reservoirs native ruffe decreased in absolute numbers and in biomass after quagga mussel and round goby invaded the reservoirs (Jüza et al. 2018; Figure 3A). In other waters, invasion of round goby has resulted in decreases of native fish species as well. Fish abundance of, among others, mottled sculpin (*Cottus bairdii* Girard, 1850), spoonhead sculpin (*Cottus ricei* (Nelson, 1876), logperch (*Percina* sp.) and johnny darter (*Etheostoma nigrum* Rafinesque, 1820) decreased following round goby invasions in the Laurentian Great Lakes (Balshine et al. 2005; Bergstrom and Mensinger 2009; Kornis et al. 2012). In the Dutch part of the River Meuse a decrease of the bullhead (*Cottus perifretum* Freyhof, Kottelat and Nolte, 2005) was observed after round goby invasion (van Kessel et al. 2016).

The reservoirs in the Biesbosch are quite homogenous in morphology and thus there is little variation in habitats. Therefore, food partitioning between the benthic ruffe and round goby (Marsden et al. 1996) in the benthic zone could explain the decrease in ruffe population in the Biesbosch reservoirs. The mussel beds are important shelter habitats for round goby and shunt organic matter and energy from the planktonic system into the benthic system leading to an increase in benthic macroinvertebrates (Mitchell et al. 1996), such as *Dikerogammarus* sp.

Empirical data based on stable isotope analysis showed the potential diet overlap between round goby and native ruffe (Rakauskas et al. 2013). In the Biesbosch reservoirs *Dikerogammarus* spp. were dominant in round goby diet, but were much less important in ruffe diet. This shows that the species currently do not feed primarily on the same prey. In an experiment by Bauer et al. (2007), round goby did grow faster than ruffe (both feeding on soft-bodied invertebrates) in an invasion scenario, suggesting that the round goby is competitively superior. Kakareko et al. (2013) found that racer gobies (*Babka gymnotrachelus* (Kessler, 1857)) are likely to displace the benthic European bullhead during feeding, which can have consequences for foraging efficiency. Next to that, Grabowska et al. (2016) found that invasive gobies can reduce the use of shelter (profitable habitats) by native bullheads. Round gobies can be opportunistic feeders (Ray and Corkum 1997; Rakauskas et al. 2013), aggressive (Savino et al. 2007) and in contrast to ruffe, they do defend their nests (Corkum et al. 1998; Ogle 1998). In the pre-invasion period in the Biesbosch reservoirs, ruffe diets consisted primarily of Gammaridae and Chironomidae. Likewise, Chironomidae and small crustaceans were the main diet items for ruffes in Finnish lakes (Tarvainen et al. 2008). In the post-invasion period, many ruffes in the Biesbosch reservoirs had empty stomachs (Jüza et al. 2018; Evides, *unpublished data*). Stomachs of round gobies up to 5 cm contained small crustaceans and gastropods, and bigger gobies contained i.e. quagga mussels which were often completely intact (Evides, *unpublished data*). Moreover, mussel shells are easily detectable in stomachs while they are hard to digest compared to soft-bodied prey, as discussed by Brush et al. (2012). Probably, round goby feeds in the reservoirs primarily on gammarids living between the quagga mussels and by being an efficient competitor for food and/or space, provoked a shift of ruffe's diet towards oligochaetes and quagga mussels.

Roach showed a similar decline as ruffe in the Biesbosch reservoirs. Though roach abundance can show between-year variability (Jüza et al. 2014), an eventual decrease in roach abundance might result from cormorant (*Phalacrocorax carbo* (Linnaeus, 1758)) predation, as roach can be an important food source for this bird species (Rakauskas et al. 2013), increased predation on larvae due to higher visibility, but possibly also because of the decline in zooplankton as a food source (mainly juveniles) (Karatayev et al. 2015) or competition with round goby. Perch increased massively in 2014 and 2015. Mainly 0+ (young of the year) perch became abundant, but their survival rate was very low (Jüza et al. 2018) possibly due to the overall decline in zooplankton biomass. As for 0+ yellow perch (*Perca flavescens* Mitchell, 1814) in Lake Michigan, competition for food with round goby could be a bottleneck (Houghton 2015). For adult perch in the reservoirs, round goby can be a food source (Evides, *unpublished data*).

Our study illustrates that relatively simple stable isotope analyses not only provide a good insight in the structure of aquatic food webs but also

help to unravel the possible competition for prey items between native and invasive fish species. In the case of the Biesbosch reservoirs we found a clear indication that ruffe has been forced to use less abundant and/or less nutritive/favourable food sources because of competition with the more aggressive and competitive round goby. This has very likely contributed to the observed decline of ruffe in these reservoirs. The quagga mussels itself can, by reaching high densities, change food web dynamics by efficiently filtering the water column and by providing a suitable habitat for macroinvertebrates. Quagga mussels appeared to be less important as a food source for the round goby than often has been described in literature. The new and altered links between species have changed the food web. The successful invasion in the reservoirs by the benthic invaders—quagga mussel and round goby—changed the benthic-pelagic coupling and has most likely increased the importance of the benthic food web in these reservoirs.

Acknowledgements

We would like to thank Deef van Houdt, Paul van der Ven and Sebastian Krosse for the preparation and conduction of the stable isotope analysis. Furthermore, we are grateful for the comments of Nils van Kessel and Martijn Dorenbosch during the process as well as the help of Tjisse van der Heide with the diet modelling. Also, we want to thank Frank Jonker, Afra Wagenvoort and the members of the Hydrobiological Institute of Ceska Budejovice for the help with sample collection. Lastly, we want to thank the anonymous reviewers for their useful comments.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Sampling methods and if applicable size and fresh weight ranges, per species or organism-group.

Table S2. Proportion of roach diet (*Rutilus rutilus*) per dietary item (lower 95% interval-higher 95% interval) in the reservoirs. Values are given based on four different fractionation factors (A, B, C and D).

Table S3. Results of linear regression analysis between fish length and $\delta^{15}\text{N}$ per fish species.

Figure S1. Length (mm) versus $\delta^{15}\text{N}$ (‰) of the sampled fish in the reservoirs.

Figure S2. Zooplankton ($\text{mm}^3 \text{ l}^{-1}$) and chlorophyll-a ($\mu\text{g l}^{-1}$) biomass in the reservoirs (average of May-October per year) (Wagenvoort, unpublished data).

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