

## CORRECTED PROOF

## Research Article

**Trophic role of marbled crayfish in a lentic freshwater ecosystem**Boris Lipták<sup>1, #</sup>, Lukáš Veselý<sup>1, #</sup>, Fabio Ercoli<sup>2, 3</sup>, Martin Bláha<sup>1</sup>, Miloš Buřič<sup>1</sup>, Timo J. Ruokonen<sup>2</sup> and Antonín Kouba<sup>1, \*</sup><sup>1</sup>University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátěší 728/II, 38925 Vodňany, Czech Republic<sup>2</sup>University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 Jyväskylä, Finland<sup>3</sup>Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Centre for Limnology, 51014, Kreutzwaldi 5, Tartu, EstoniaAuthor e-mails: [liptak.b@gmail.com](mailto:liptak.b@gmail.com) (BL), [veselyl@frov.jcu.cz](mailto:veselyl@frov.jcu.cz) (LV), [fabio.ercoli@emu.ee](mailto:fabio.ercoli@emu.ee) (FE), [blaha@frov.jcu.cz](mailto:blaha@frov.jcu.cz) (MaB), [buric@frov.jcu.cz](mailto:buric@frov.jcu.cz) (MiB), [timo.j.ruokonen@jyu.fi](mailto:timo.j.ruokonen@jyu.fi) (TJR), [akouba@frov.jcu.cz](mailto:akouba@frov.jcu.cz) (AK)

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**Abstract**

Species' introductions may cause severe adverse effects on freshwater ecosystems and their biota. The marbled crayfish, *Procambarus virginalis* Lyko, 2017, is an invasive parthenogenetically reproducing crayfish with rapid reproduction, maturation and tolerance to a wide range of environmental conditions, which was introduced to many sites across Europe during the last decade. Due to its recent speciation and limited number of field studies, the knowledge of trophic interactions of the marbled crayfish in freshwater food webs is scarce. An invaded area located in Central Europe was studied to identify the marbled crayfish food web interactions using analysis of carbon <sup>13</sup>C and nitrogen <sup>15</sup>N isotopes. This study brings the first insight into the trophic ecology of marbled crayfish in lentic freshwater ecosystems. Algae and detritus were identified as the most important food sources for the marbled crayfish, while zoobenthos and macrophytes were less important. Moreover, the marbled crayfish was found to be an important food source for top fish predators, but marginal for omnivorous fish. Being able to utilize energy from the bottom of the trophic food web, the marbled crayfish may have important roles in the ecosystem, transferring energy to higher trophic levels. It processes allochthonous and autochthonous matter in the ecosystem, thus being a competitor to other organisms with similar food preferences and impacting zoobenthos, algae and macrophytes through predation or direct consumption. To sum up, the marbled crayfish has a strong ability to utilize food sources from different trophic levels, and, thanks to its life history, can be a highly adaptable invader.

**Key words:** biological invasion, Central Europe, parthenogenetic species, *Procambarus virginalis*, stable isotope

**Introduction**

Crayfish (Decapoda: Astacidea) are a highly diverse taxonomic group of freshwater organisms, containing both critically endangered endemic species and highly invasive species, responsible for numerous cases of successful invasions in Europe and North America (Holdich et al. 2009; Lodge et al. 2012). Many populations of indigenous crayfish species (ICS)

in Europe have been lost or substantially reduced, largely due to direct or indirect effects of non-indigenous crayfish species (NICS) (Kouba et al. 2014). Furthermore, the entire functioning of an invaded ecosystem can be irreversibly altered by NICS (Lodge et al. 2000; Rodríguez et al. 2005), as they often exhibit higher population densities, faster life cycles and occupy a wider trophic niche when compared to ICS (Lodge et al. 2000). Additionally, North American NICS are also vectors of crayfish plague, caused by the oomycete *Aphanomyces astaci* (Schikora), which is lethal to other crayfish (Svoboda et al. 2017). Overall, North American NICS have been responsible for local extinctions in Europe, replacing ICS and affecting the food webs and communities of the invaded ecosystems through species-specific interactions (Rodríguez et al. 2005; Matsuzaki et al. 2009). Crayfish are large omnivorous macroinvertebrates often representing an important proportion of the benthos biomass, serving as a prey for a range of predators (Holdich 2002), and mediating nutrient and energy flow in freshwater ecosystems (Correia and Anastácio 2008; Grey and Jackson 2012; Ruokonen et al. 2012). Unsurprisingly, crayfish are considered not only as keystone species, but also strong ecosystem engineers (Reynolds and Souty-Grosset 2012).

Many examples of diverse negative effects of invasive crayfish on invaded ecosystems are known, with the red swamp crayfish *Procambarus clarkii* (Girard, 1852) being the most often studied species (Lodge et al. 2012; Twardochleb et al. 2013). For example, Nyström et al. (1996) compared ponds with presence or absence of signal crayfish *Pacifastacus leniusculus* (Dana, 1852). They found decreases in macrophyte biomass, cover and species richness, reduced benthic invertebrate taxa and biomass, shifts in invertebrate community and lower organic matter content in sediments in invaded localities. Ruokonen et al. (2014) and Ercoli et al. (2015) reported reduced aquatic invertebrate biodiversity (especially mollusc taxa) in boreal lakes as a consequence of signal crayfish introductions. The virile crayfish, *Faxonius virilis* (Hagen, 1870), impacted the phytoplankton abundance and metaphytic algae and had a dramatic effect on American bullfrog *Lithobates catesbeianus* (Shaw, 1802) tadpoles and benthic invertebrate biomass (gastropods) by disturbing breeding adults, destroying nest attachments or by feeding on their eggs, and direct predation, respectively. On the other hand, the virile crayfish also positively affected the zooplankton biomass due to highly reduced abundance of the bluegill larvae *Lepomis macrochirus* (Rafinesque, 1819) caused by egg predation (Dorn and Wojdak 2004).

The marbled crayfish, *Procambarus virginalis* Lyko, 2017, is one of the most invasive crayfish (Kawai et al. 2015; Nentwig et al. 2018), being included among the top invasive species of European Union concern (EU 2016). Even though the first known individuals of this species were found

in the German aquarist trade in the mid-90s (Scholtz et al. 2003), its exact native range remains uncertain. Its closest relative is the slough crayfish *Procambarus fallax* (Hagen, 1870), native to Florida and Georgia in the South-Eastern United States (Gutekunst et al. 2018). Unique among all decapods, the marbled crayfish reproduces through apomictic parthenogenesis and its global population likely represents a single clone (Vogt et al. 2015; Gutekunst et al. 2018). Originally kept in captivity, as a popular pet, the marbled crayfish quickly became established in the European wild (Chucholl et al. 2012; Patoka et al. 2016), owing to its fast growth, early maturation, high fecundity, short intervals between reproductive cycles, and competitiveness in behaviour interactions (Vodovsky et al. 2017 and references cited therein). The substantial ability of the marbled crayfish to withstand extreme environmental conditions (Veselý et al. 2015; Kouba et al. 2016; Veselý et al. 2017) may allow the species to spread and establish populations in many habitats, where it may cause water turbidity by disturbing fine sediment particles while burrowing for shelter, searching for food or escaping (Kouba et al. 2016; Pledger et al. 2016). Taken altogether, the marbled crayfish is a highly invasive species with potential to negatively affect ecosystem services and biodiversity.

The majority of the growing body of literature on the marbled crayfish deals primarily with laboratory experiments on its biology, establishment in the wild and use as a model organism (Patoka et al. 2016; Veselý et al. 2015). Other main targets are biogeography and pet trade, with risk assessment of the species (Uderbayev et al. 2017; Weiperth et al. 2018). However, data from the field are particularly scarce (Vogt 2018), leading to gaps in our basic knowledge of the species ecology. In this study, we hypothesized that (i) marbled crayfish utilize sources on multiple trophic levels and macroinvertebrates, macrophytes and detritus are likely the most important food items, (ii) marbled crayfish is an important food source for higher trophic levels, and (iii) it may act as a key species and transfer energy from the detritus to higher trophic levels. This study aims to provide the first insight into the trophic ecology of the marbled crayfish by investigating its trophic role in a recently colonized lentic freshwater ecosystem, through stable isotope analysis of carbon and nitrogen ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ).

## Materials and methods

### *Study site*

The flooded gravel pit in Leopoldov (48°27'2"N; 17°47'6"E) is a rather oligotrophic site, located in the south-western part of Slovak Republic. This lowland region (ca. 138 m a.s.l.) is characterized by a continental climate, with warm summers and cold winters. The water at the locality warms up to 23–25 °C in summer and freezes over in winter, with temperatures not

exceeding 4 °C. The surface area of the study site is ca. 13 ha, with maximum depth of 7 and 4 m in its southern and northern part, respectively. The shoreline is surrounded by terrestrial vegetation, including trees, and the majority of the bottom is covered by submerged macrophytes and algae. During the floods, the locality can be interconnected with the side channel (Drahovský kanál) of the Váh River that flows into the Danube River; the last such event occurred in 2010. The site is a frequently visited recreational and fishing ground, seasonally restocked with common carp *Cyprinus carpio* (Linnaeus, 1758) and has an established population of marbled crayfish. It was first recorded at the site in 2014, most likely introduced by hobby aquarists or fishermen, possibly from the system of flooded gravel pits near Koplotovce, where the marbled crayfish was first observed in the country in 2010 (Lipták et al. 2016).

### *Sample collection*

The locality was sampled for all potential food sources of each proposed ecosystem trophic level in mid-August 2016. Fish were collected by angling during the day and night. Crayfish were caught by manual hand search assisted with handheld nets, as well as with traps baited with fresh fish meat, placed along the shoreline in late afternoon and collected the following morning. Bulk zooplankton samples were collected using a net (mesh size 250 µm) pulled horizontally through the water column. Zoobenthos was collected, up to 1 m depth, using a hand net (mesh size 500 µm). Macrophytes, periphyton and autochthonous and allochthonous detritus were collected from the shoreline. All samples were kept frozen on dry ice after collection at the locality and transferred to the laboratory freezer (−30 °C) until further processing for stable isotope analysis (SIA) of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ).

### *Stable isotopes analysis*

In fish, a piece of white dorsal muscle tissue was taken, while in crayfish, a piece of abdominal muscle tissue was used as recommended by Stenroth et al. (2006). Samples of zoobenthos, terrestrial detritus and macrophytes were mostly separated to species or genus level for analysis. Later, to analyse energy flow in food web, fish and zoobenthos species were divided into functional groups (Supplementary material Table S1, Table S2 and Table S3). We also analysed macrophytes (*Myriophyllum aquaticum* (Vellozo) Verdcourt, *Chara vulgaris* Linnaeus, *Potamogeton obtusifolius* Mertens and W.D.J. Koch), algae, mosses and detritus (Table S4). All samples for SIA were dried at 50 °C for 48 h to constant weight and grounded to a fine homogenous powder. Approximately 0.5 mg of animal samples and 1.5 mg of plant and detritus samples were precisely weighed into tin cups. Stable isotope analyses were performed using a Carlo Erba

Flash EA 1112 elemental analyser connected to Thermo Finnigan DELTAplus and Advantage continuous-flow isotope ratio mass spectrometer (Thermo Electron Corporation, Waltham, MA, USA).

The standards used as reference materials were Vienna Pee Dee belemnite for carbon and atmospheric N<sub>2</sub> for nitrogen. Muscle tissue of pike *Esox lucius* Linnaeus, 1758 and potato *Solanum tuberosum* Linnaeus leaves of known isotopic compositions were run as internal working standards for animal and plant samples, respectively, after every 6 samples to control for instrument stability. Results are expressed using the conventional  $\delta$  notation as parts per thousand difference from the international standards. Analytical precision was < 0.1 ‰ for  $\delta^{13}\text{C}$  and < 0.3 ‰ for  $\delta^{15}\text{N}$ .

Trophic position of each species/functional group was calculated using the formula of Anderson and Cabana (2007):

$$T_p = ((\delta^{15}\text{N}_{\text{sample}} - \delta^{15}\text{N}_{\text{baseline}})/3.23) + E_p \quad (1)$$

where  $T_p$  is the trophic position of an organism,  $\delta^{15}\text{N}_{\text{sample}}$  represents the nitrogen isotope value of the given organism,  $\delta^{15}\text{N}_{\text{baseline}}$  is the isotopic ratio from several individuals of grazers (*Anopheles* spp. and *Physa* spp.), 3.23 is the nitrogen isotope fractionation between trophic levels (Vander Zanden and Rasmussen 2001) and  $E_p$  is the expected trophic position.

#### *Stable isotope mixing models*

The common carp was excluded from the analyses, as this species is the most popular game fish in the region and the fishing grounds are repeatedly stocked during fishing season, thus not reflecting the isotopic signal of the study site. To assess the contribution of the different food sources to the isotopic signature of each target organism or functional group, a separate Bayesian mixing model with a specific number of putative sources was run in SIAR-package (Parnell et al. 2010) in R (R Core Team 2016). For predatory fish, a four-source mixing model was produced (zooplankton, zoobenthos, crayfish, and omnivorous fish). For omnivorous fish, a five-source mixing model including autochthonous sources (autochthonous detritus, macrophytes, and algae), allochthonous sources (allochthonous detritus), zooplankton, zoobenthos and crayfish was applied. In crayfish, a five-source mixing model including algae, autochthonous detritus, allochthonous detritus, macrophytes, and zoobenthos was run. However, in final models for omnivorous fish and for crayfish, crayfish were omitted as a putative source in both cases due to their low contribution (2%). Lastly, for zoobenthos a two-source mixing model including autochthonous (autochthonous detritus, macrophytes, algae) and allochthonous sources (allochthonous detritus) was used. As recommended by Vander Zanden and Rasmussen (2001), fractionation factors assumed in the model were  $3.23 \pm 0.41$  ‰ for  $\delta^{15}\text{N}$  and  $0.47 \pm 1.23$  ‰

for  $\delta^{13}\text{C}$  for animals, and  $2.4 \pm 0.42 \text{ ‰}$  for  $\delta^{15}\text{N}$  and  $0.40 \pm 0.28 \text{ ‰}$  for  $\delta^{13}\text{C}$  for detritus and macrophytes (McCutchan et al. 2003).

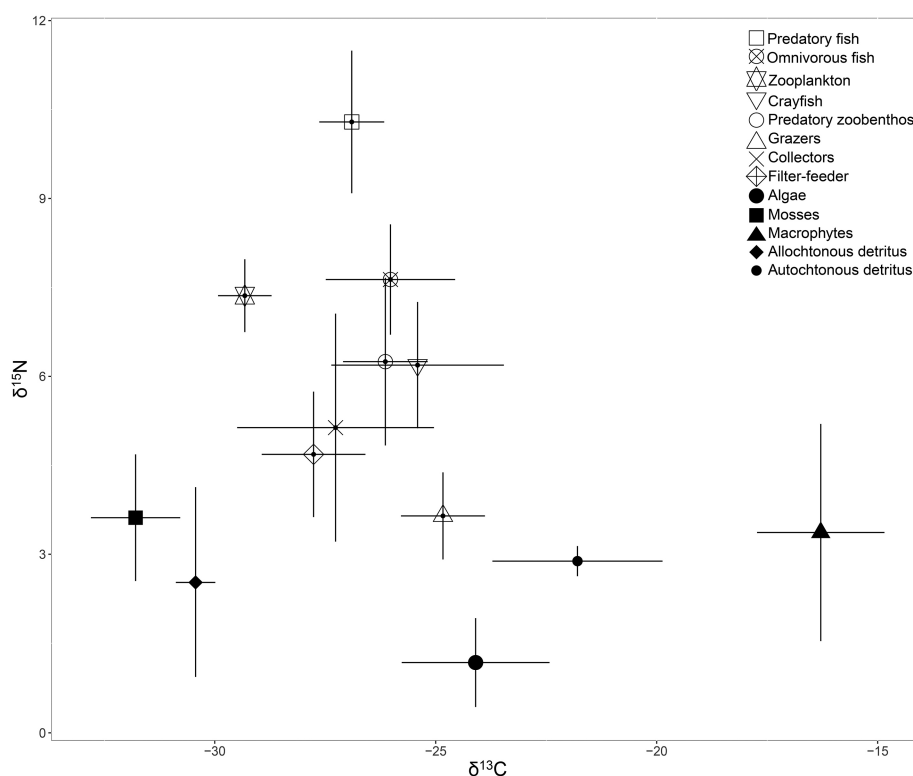
Recently, several experimental studies have examined crayfish specific isotopic turnover times and fractionation factors (e.g. Rudnick and Resh 2005; Carolan et al. 2012; Jussila et al. 2015; Glon et al. 2016). The results of these studies vary a lot, depending on the food sources (high or low protein, single diet etc.), condition of animals, and experimental conditions. Our unpublished results suggest that fractionation factors in marbled crayfish fed on either single plant or meat diet in both carbon and nitrogen could be twofold higher than some reported fractionation factors in crayfish (Veselý et al., *unpublished data*). Moreover, obtaining accurate values for all possible food source combinations is almost impossible. Thus, in our mixing models we decided to use the fractionation factors mentioned at the end of the previous paragraph (Vander Zanden and Rasmussen 2001; McCutchan et al. 2003), which are probably the most often applied in aquatic ecosystem stable isotope studies. These values fall within the range of those documented for crayfish specific factors. Another caveat to our study is that, due to isotopic turnover, the isotopic signatures may reflect feeding preferences earlier in the season. However, our unpublished studies suggest low isotopic turnover times in marbled crayfish tissue, thus samples taken in August should reflect the high vegetation season.

## Results

The trophic food web of the studied lentic ecosystem consists of three trophic levels, with predatory fish at the apex, primary producers on the bottom and crayfish together with grazers, filter-feeders, predatory zoobenthos, and omnivorous fish occupying an intermediate trophic level (Figure 1, Table S3). Omnivorous fish occupied a higher trophic position than crayfish, while zoobenthos occupied an intermediate trophic position between primary sources and crayfish.

The main food sources for predatory fish (Table 1; Figure S1) were zooplankton (mean 34%) and omnivorous fish (mean 28%), while the contribution of crayfish and zoobenthos amounted only to 19%. Omnivorous fish used most putative sources equally and all sources had a rather similar contribution of 30%, except for zooplankton that was slightly less preferred than other sources (mean 11%) (Table 1; Figure S2).

Crayfish used mostly allochthonous detritus (mean 30%), algae (mean 25%) and autochthonous detritus (mean 21%) as a food sources (Table 1, Figure S3), with zoobenthos and macrophytes on average contributing 9 and 14%, respectively. Zoobenthos showed a slightly higher utilization of allochthonous material (mean 59%) when compared to autochthonous material (mean 41%) (Table 1; Figure S4).



**Figure 1.** Mean values  $\pm$  SD of carbon and nitrogen stable isotope values (‰) of food web of the flooded gravel pit in Leopoldov, Slovak Republic.

**Table 1.** The relative contributions (means with upper and lower 95% highest density region – hdr) of putative food sources to the diets of marbled crayfish in the flooded gravel pit in Leopoldov, Slovak Republic.

Consumer	Source	Low 95% hdr	Mean % contribution	High 95% hdr
Predatory fish	Zooplankton	0.21	0.33	0.46
	Omnivorous fish	0.04	0.28	0.49
	Crayfish	0	0.19	0.38
	Zoobenthos	0	0.19	0.37
Omnivorous fish	Autochthonous sources	0.15	0.34	0.52
	Allochthonous sources	0.03	0.28	0.48
	Zoobenthos	0	0.28	0.53
	Zooplankton	0	0.11	0.24
Crayfish	Allochthonous detritus	0.13	0.29	0.46
	Algae	0	0.25	0.46
	Autochthonous detritus	0	0.21	0.42
	Macrophytes	0	0.15	0.29
	Zoobenthos	0.03	0.09	0.17
Zoobenthos	Autochthonous sources	0.49	0.59	0.70
	Allochthonous sources	0.30	0.41	0.51

## Discussion

This study indicates that the marbled crayfish utilizes a wide range of food sources, likely impacting and modifying the food web structure in the ecosystem. In line with our hypotheses, detritus (both autochthonous and allochthonous) was found to be the most important source in the diet of the marbled crayfish, while other sources such as zoobenthos, algae and macrophytes were utilized to a lesser extent. Moreover the marbled crayfish was also an important food source for predatory fish, which

supports our hypothesis of the multi-trophic functional role of this species in the ecosystem. As a result of its dominance in the benthic community, the marbled crayfish has a high potential to negatively impact local species diversity and ecosystem functioning (Creed et al. 2009; Ruokonen et al. 2014; Lipták et al. 2017), as documented for the red swamp crayfish (Gutiérrez-Yurrita et al. 1998; Souty-Grosset et al. 2016).

We revealed that the marbled crayfish serves as an important decomposer, processing allochthonous and autochthonous matter in the ecosystem. This concurs with Usio (2000), stressing the importance of a New Zealand crayfish *Paranephrops zealandicus* (White, 1847) in leaf processing in a temperate stream. Due to its preference for detritus, the marbled crayfish may compete with other organisms (e.g. native crayfish, collectors, shredders) utilizing allochthonous and autochthonous detritus (Dorn and Wojdak 2004; Ercoli et al. 2015). The marbled crayfish also utilizes algae and macrophytes, which is consistent with the behaviour of other NICS introduced to Europe, such as the signal crayfish and the red swamp crayfish (Nyström et al. 1999; Carreira et al. 2014). Although other studies reported especially strong negative effects or a high dependence of NICS on the mollusc taxa (e.g. Nyström et al. 1996, 1999; Glon et al. 2017), this was not confirmed in our research. Zoobenthos contributed little to the diet of the marbled crayfish, possibly due to the generally low population densities of zoobenthos at the locality, which is rather oligotrophic.

The marbled crayfish rapidly became a new element of the European fauna, but this invasive species may have negative effects to native species and communities. The utilization of a wide range of food sources by this highly plastic species may contribute to its successful establishment under a wide range of conditions. Moreover, marbled crayfish can form populations with high density and become very abundant in a short time (Janský and Mutkovič 2010; Lipták et al. 2017; Andriantsoa et al. 2019). As we hypothesized, marbled crayfish has the ability to exploit an ecosystem at various trophic levels potentially impacting on a large range of organisms within the community. As a large-bodied macroinvertebrate, the marbled crayfish acts as a predator as well as a prey for organisms at higher trophic levels. Thus, based on our results, it can be considered as a keystone species with ability to transport the energy from the bottom of the chain to top predators, potentially driving important changes in the invaded ecosystems, as demonstrated for the red swamp crayfish (Geiger et al. 2005). The impacts of NICS associated with their trophic role may vary, depending on the plasticity of the invading crayfish (Ruokonen et al. 2014), their life stages or thermal regimes experienced (Carreira et al. 2017). Thus, the negative effects on the invaded ecosystem can be established in many perspectives and fronts. Therefore, more studies on the feeding dynamics and seasonal behaviour of marbled crayfish are needed and we call for further field and experimental studies dealing with marbled crayfish under various biotic and abiotic conditions.



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### Supplementary material

The following supplementary material is available for this article:

**Table S1.** Biometry (mean  $\pm$  SD) of fish and crayfish used in stable isotope analysis.

**Table S2.** Composition of macroinvertebrate functional groups used in stable isotope analysis.

**Table S3.** The isotopic value, trophic position, and number on analysed samples of functional group of organism (mean  $\pm$  SD).

**Table S4.** The isotopic value and number of analysed samples of primary producers and detritus (mean  $\pm$  SD).

**Figure S1.** The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of predatory fish in the flooded gravel pit in Leopoldov, Slovak Republic.

**Figure S2.** The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of omnivorous fish in the flooded gravel pit in Leopoldov, Slovak Republic.

**Figure S3.** The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of marbled crayfish in the flooded gravel pit in Leopoldov, Slovak Republic.

**Figure S4.** The relative contributions (means with upper and lower 95% highest density region -hdr) of putative food sources to the diet of zoobenthos in the flooded gravel pit in Leopoldov, Slovak Republic.