

The parasite community of round goby *Neogobius melanostomus* (Pallas, 1814) (Actinopterygii: Gobiidae) newly introduced into the upper Elbe

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Abstract – Round goby, *Neogobius melanostomus* (Pallas, 1814), an invasive Ponto-Caspian fish species, were registered in the upper Elbe for the first time in 2015, near the City of Ústí-nad-Labem (Czech Republic), apparently introduced by shipping. We sampled 53 individuals from this newly introduced population on May 18 (spring) and October 21 (autumn) 2016 in order to assess parasite load. Seven taxa were recorded, comprising two ciliates, one digenean (metacercariae), one acanthocephalan (cystacanth), two nematodes (larvae) and mollusc glochidia. No specific parasites were registered. Only the acanthocephalan *Pomphorhynchus tereticollis* was found in high numbers and occurred in both seasons, all other parasites occurring sporadically. Such a low parasite load is typical for Ponto-Caspian gobiids introduced far from their host range, and complies with the predictions of the ‘enemy release hypothesis’. All parasite species were probably acquired in the upper Elbe itself. According to the ‘parasite spillback’ concept, round goby could potentially play an important role in *P. tereticollis* distribution in the Elbe.

Keywords: enemy release hypothesis / *Neogobius melanostomus* / North Sea basin / parasite spillback / *Pomphorhynchus tereticollis*

Résumé – La communauté des parasites du Gobie à taches noires *Neogobius melanostomus* (Pallas, 1814) (Actinopterygii : Gobiidae) nouvellement introduit dans le cours supérieur de l’Elbe. Le Gobie à taches noires, *Neogobius melanostomus* (Pallas, 1814), une espèce invasive de poisson Ponto-Caspien, a été signalé pour la première fois en 2015 près de la ville d’Ústí-nad-Labem, en République tchèque. En mai (printemps) et octobre (automne) 2016, nous avons échantillonné 53 individus de cette population nouvellement introduite pour évaluer la charge parasitaire. Sept taxons ont été récoltés, comprenant deux ciliés, un digénien (métacercaires), un acanthocéphale (cystacanth), deux nématodes (larves) et des glochidies de mollusques. Aucun parasite spécifique n’a été récolté. Seul l’acanthocéphale *Pomphorhynchus tereticollis* a été trouvé en grand nombre et dans les deux saisons, tous les autres parasites se rencontrant sporadiquement. Cette faible charge parasitaire est typique des gobiidés nouvellement introduits et correspond aux prédictions de l’hypothèse ERH « enemy release hypothesis ». Toutes les espèces de parasites ont probablement été acquises dans le haut-Elbe lui-même. Selon le concept du « parasite spillback », le Gobie à taches noires pourrait jouer un rôle important dans la distribution de *P. tereticollis* dans l’Elbe.

Mots-clés : hypothèse ERH (enemy release hypothesis) / *Neogobius melanostomus* / Bassin de la mer du Nord / spillback du parasite / *Pomphorhynchus tereticollis*

1 Introduction

Ponto-Caspian gobies (Gobiidae: Benthophilinae) are one of the most successful invasive species in Europe and North America (Charlebois *et al.*, 1997; Corkum *et al.*, 2004; Copp *et al.*, 2005; Grabowska *et al.*, 2008). Over recent decades,

Ponto-Caspian gobies have extended their range more than any other fish species in Europe, having spread into both marine and freshwater habitats (Corkum *et al.*, 2004). The round goby, *Neogobius melanostomus* (Pallas, 1814), now registered as a species of high invasiveness risk (Gozlan *et al.*, 2010), began its range expansion in the 1980s, when it was recorded in the middle stretch of the River Dnieper (Pinchuk *et al.*, 1985). This species now has the widest invasive range of European gobies, having also spread to North America (Charlebois *et al.*, 1997),

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the Aegean Sea (Eryilmaz, 2002), the Baltic Sea (Sapota, 2004) and the Danube and Volga drainages (Sokolov *et al.*, 1989; Jurajda *et al.*, 2005). In North America, round gobies appear to have a negative influence on indigenous fish populations as they compete for food and habitat and have been recorded preying on eggs of local fishes (Corkum *et al.*, 2004). To date, round gobies do not appear to have had such a negative impact on the local fish fauna of Central Europe (e.g. Janáč *et al.*, 2016; Mikl *et al.*, 2017; and references therein) and there have been no reports of predation on eggs of native species (Vašek *et al.*, 2014).

Round goby were first recorded in the North Sea drainage in the River Lek (Netherlands) in 2004 (Van Beek, 2006). The species is now widespread throughout the Rhine Basin (Borcherding *et al.*, 2011; Kalchhauser *et al.*, 2013; Manné *et al.*, 2013) and has also spread into the lower reaches and tidal zones of a number of rivers, including the Schelde and Weser (Verreycken *et al.*, 2011; Brunken *et al.*, 2012). First recorded in the Elbe estuary and delta near the City of Hamburg (Germany) in 2013 (Hempel and Thiel, 2013), the species subsequently appeared in the upper Elbe in 2015 (Buřič *et al.*, 2015; Roche *et al.*, 2015), near the inland port at Ústí-nad-Labem (Czech Republic), having apparently been introduced *via* shipping.

Numerous studies have documented the parasites of round goby in their native range (e.g. Rădulescu and Vasiliu, 1951; Naidenova, 1974; Kvach, 2004b, 2005; Özer, 2007; Ondračková *et al.*, 2010; Krasnovyd *et al.*, 2012; Kvach *et al.*, 2014). Similarly, there have now been a number of parasite studies from its non-native range in the Black and Baltic Sea drainages (Mühlegger *et al.*, 2010 [species recorded as *Apollonia melanostoma*]; Ondračková *et al.*, 2010; Francová *et al.*, 2011; Kvach and Winkler, 2011; Kvach *et al.*, 2014; etc.) and the North Sea-Rhine drainage (Emde *et al.*, 2012; Ondračková *et al.*, 2015). Due to the very recent introduction of round goby into the River Elbe (Hempel and Thiel, 2013; Roche *et al.*, 2015), data for this region is still absent.

According to invasion theory, primary colonisation success may be facilitated by a temporary release from native parasites and pathogens in the new habitat (Torchin *et al.*, 2003), a concept forming an important part of the “enemy release hypothesis” put forward by Keane and Crawley (2002). On the other hand, invading species may be the source of new parasitic components that could parasitise indigenous fauna (Mack *et al.*, 2000). Following successful introduction, exotic host-species could also become infected with local parasites that naturally infest ecologically/phylogenetically similar species locally. Examples of these processes are found in the case of invasive gobiids utilising the Central (Kvach *et al.*, 2014) and Southern (Ondračková *et al.*, 2010, 2015; Francová *et al.*, 2011) European Invasion Corridors, where gobies appear to have partially escaped their native parasites but have continued to accumulate local parasite species. Over time, therefore, parasite load in the invasive population often increases until it reaches a level comparable with indigenous species. While this process has been relatively quick in European waters, round gobies in North America still host fewer parasites than local species, even after 20 years (Gendron *et al.*, 2012).

Very few parasite fauna studies have been undertaken on fish host populations soon after their introduction, most such populations only being registered some years after their first occurrence. As we regularly sample fish along the upper Elbe, we were able to register the appearance of round goby within

2 years of its first occurrence (for further details see Roche *et al.*, 2015). This allowed us to examine the species’ parasite community very soon after introduction, thereby contributing to our knowledge of parasite release and invasion success in this rapidly expanding fish species.

2 Materials and methods

A total of 53 round goby (19 males and 34 females) were sampled from the River Elbe at the site of first registration close to the City of Ústí-nad-Labem (50.649819N, 14.044797E) on May 18 and October 21, 2016 (Tab. 1). All fish were collected using backpack electrofishing equipment (LENA portable pulsed direct-current electrofishing device, Bednář, Czech Republic) and standard sampling methodology (Kvach *et al.*, 2016b). The time of sampling was chosen to cover both warm (May) and cold (October) seasons. The fish were transported live in aerated river water to the laboratory of the Institute of Vertebrate Biology, where they were held in open-air basins. All fish were dissected within 2 days (Kvach *et al.*, 2016a). Before dissection, the standard length (SL, mm) and total weight (WT, g) of each fish was measured (results presented as mean \pm standard deviation [SD]) and sex determined *via* the external urogenital papillae.

Living diplostomid metacercariae, glochidia and unicellular parasites were studied under an Olympus CX41 light microscope (Japan) with no further treatment. Nematodes were preserved in hot 4% formaldehyde and identified on glycerol temporary slides. Acanthocephalans were pressed between two slides, preserved in 70% ethanol and mounted in glycerol as temporary slides for light microscopy. All parasites were identified to species level or to the lowest possible taxon. Prevalence (%), intensity of infection (mean value and range) and mean abundance were presented for each species, according to Bush *et al.* (1997).

Parasite community similarity between seasons was evaluated using the Czekanowski-Sørensen Index (Ics; Sørensen, 1948). The importance of individual parasite species in the community was determined according to their abundance using the scale of Zander *et al.* (2000), *i.e.* >2 = core species, $0.6-2$ = secondary species, $0.2-0.6$ = satellite species and <0.2 = rare species.

For each season, we tested for association between overall abundance and abundance of individual species using the Spearman rank correlation test, thereby allowing us to determine the abundance of species defining overall abundance trends. The effect of fish size (covariate), season and sex on *Pomphorhynchus tereticollis* (Rudolphi, 1809) abundance was tested using a generalised linear model (GLM, negative binomial distribution). The effect of sex and season on fish condition was tested using analysis of covariance (ANCOVA), with weight as the response variable and fish size as the covariate. The validity of each model was evaluated through examination of residuals, according to Zuur and Ieno (2016).

3 Results

Fish SL varied from 59.4 to 86.3 mm (Tab. 1), with no difference between seasons (*t*-test, $df=51$, $P=0.32$). There was no effect of season or sex on fish condition, *i.e.* on

Table 1. Number of round goby, *Neogobius melanostomus*, sampled from the upper River Elbe in 2016. SL = standard length, mean \pm SD (mm); WT = total weight, mean \pm SD (g).

	Sex	Number	SL (min–max)	WT (min–max)
May 2016	Females	19	70.3 \pm 6.0 (61–82)	7.9 \pm 2.3 (5–13)
	Males	11	70.7 \pm 7.8 (61–86)	8.5 \pm 2.8 (5–14)
	Males + females	30	70.4 \pm 6.6 (60.9–86.3)	8.1 \pm 2.5 (4.7–14.4)
October 2016	Females	15	68.3 \pm 5.9 (60–82)	7.5 \pm 2.0 (5–13)
	Males	8	69.3 \pm 7.4 (59–81)	7.9 \pm 3.1 (4–13)
	Males + females	23	68.7 \pm 6.3 (59.4–82.3)	7.6 \pm 2.4 (4.2–13.0)
Total	Females	34	69.4 \pm 5.9 (60–82)	7.7 \pm 2.1 (5–13)
	Males	19	70.1 \pm 7.4 (59–86)	8.3 \pm 2.8 (4–14)
	Males + females	53	69.7 \pm 6.5 (59.4–86.3)	7.9 \pm 2.4 (4.2–14.4)

length–weight relationship (ANCOVA; season: $df=1,48$, $P=0.241$; sex: $df=1,48$, $P=0.343$).

Seven parasite taxa were recorded, including two ciliates, one digenean (metacercariae), one acanthocephalan (cystacanth), two nematodes (larvae) and mollusc glochidia (Tab. 2), with overall prevalence reaching 96.7% in spring and 95.7% in autumn. Overall parasite abundance was 24.1 ± 24.2 in spring and 17.4 ± 16.3 in autumn and was strongly correlated with abundance of *P. tereticollis* in both seasons (Spearman rank correlation; spring: $r_s=0.997$, $P<0.0001$, autumn: $r_s=0.995$, $P<0.0001$).

Only *P. tereticollis* occurred in both seasons, resulting in a similarity index of $I_{cs}=25\%$. This parasite was the only core species in the parasite community; two other taxa (*Diplostomum* spp.) and unionid glochidia being registered as satellite species and all other species present only sporadically (Tab. 2).

There was an increasing trend in the abundance of *P. tereticollis* with fish size (GLM; $df=1,50$, $P<0.001$; Fig. 1). No effect of fish sex or season was detected on number of *P. tereticollis* (GLM; both $df=1,50$, sex: $P=0.829$, season: $P=0.217$).

4 Discussion

A poor parasite fauna is typical of newly introduced populations (e.g. Torchin *et al.*, 2003) and a similar situation has been documented in other non-native Ponto-Caspian goby populations (Gendron *et al.*, 2012, Ondračková *et al.*, 2015). The decrease in parasite burden is particularly obvious at localities far from the original host area, with just three parasite species recorded from recently introduced round gobies in the Kiel Canal (Germany, North Sea drainage), for example (Kvach and Winkler, 2011). Likewise, the recently introduced population in the upper Elbe was characterised by a poor parasite community dominated by just one species and the ‘accidental’ presence of several local generalists (Tab. 2). These results are in accordance with the “enemy release hypothesis”, whereby newly introduced hosts escape from their natural parasites (Keane and Crawley, 2002; Torchin *et al.*, 2003). Nevertheless, an increase in both the abundance of local generalist parasites and infection by new local species can be expected in the near future (Ondračková *et al.*, 2015)

once the species becomes established in the new area as local parasites adopt the new host into their life cycles.

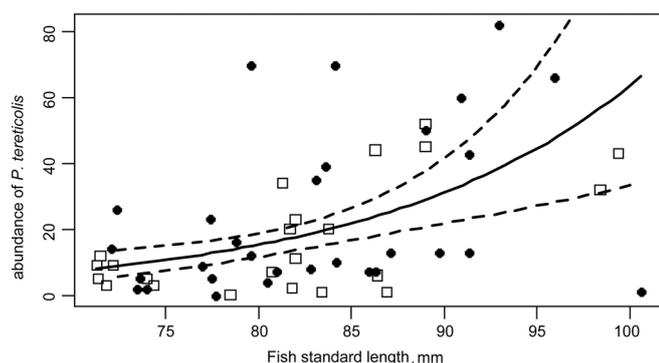
The acanthocephalan *P. tereticollis* dominated the parasite community, as demonstrated by the strong correlation between *P. tereticollis* abundance and overall parasite abundance. Moreover, this parasite was the only core species in both seasons. A similar situation was observed in the middle Rhine near Düsseldorf, where only *P. tereticollis* was numerous and two nematodes (*Raphidascaris acus*, *Paracuarua adunca*) occurred sporadically (Emde *et al.*, 2012). Amphipods, the first intermediate host of *P. tereticollis* (Perrot-Minnot *et al.*, 2007, Emde *et al.*, 2012), play an important role in the diet of round goby in freshwater and oligohaline habitats, both in their native range (Kudrenko and Kvach, 2005; Tkachenko and Demchenko, 2013) and in newly colonised areas (Polačik *et al.*, 2009; Emde *et al.*, 2012). In the upper Elbe, therefore, as in the lower Rhine (see Emde *et al.*, 2012), the round goby could be playing an important role in *P. tereticollis* distribution. This could also provide evidence for the ‘parasite spillback’ concept, whereby introduced species accept and distribute local parasites (Kelly *et al.*, 2009), resulting in an increased parasite load among native hosts.

The majority of other parasite species occurred only sporadically and at very low intensities, ranging from just one to three individuals. Glochidia occurred only in spring, reflecting the life cycle of bivalves that release larvae mainly in the spring or summer (Šlapanský *et al.*, 2016). A second satellite parasite, *Diplostomum* spp. metacercariae, only appeared in autumn. As release of cercariae increases with water temperature, the chances of fish infection increase during the summer.

The round goby is considered a new paratenic host in the life-cycle of the non-indigenous dracunculid nematode, *Anguillicoloides crassus* (Kuwahara *et al.*, 1974) in the River Elbe. This fish is already known to host this parasite in other parts of its invasive range, such as the Baltic Sea (Kvach, 2004a; Rolbiecki, 2006) and the River Rhine (Emde *et al.*, 2014). Despite the rare occurrence of *A. crassus* in our study, round gobies may play an important role in the transmission of this non-native nematode in the future, especially taking the current range expansion of the round goby into account and its potential role in the diet of European eel (Emde *et al.*, 2014). While *A. crassus* have been known from the upper Elbe since

Table 2. List of parasites of round goby, *Neogobius melanostomus*, from the upper River Elbe. *P*=prevalence, %; *MI*=mean intensity, *IR*=intensity range (minimum–maximum), *A*=mean abundance.

Indices	May 2016			October 2016			Total		
	<i>P</i>	<i>MI</i> ± <i>SD</i> (<i>IR</i>)	<i>A</i>	<i>P</i>	<i>MI</i> ± <i>SD</i> (<i>IR</i>)	<i>A</i>	<i>P</i>	<i>MI</i> ± <i>SD</i> (<i>IR</i>)	<i>A</i>
1	2	3	4	5	6	7	8	9	10
Ciliata									
<i>Trichodina</i> sp.	3.3	1.0	0.03				1.9	1.0	0.02
<i>Ichthyophthirius multifiliis</i>				4.3	1.0	0.04	1.9	1.0	0.02
Digenea									
<i>Diplostomum</i> spp. mtc.				26.1	1.7 ± 1.0 (1–3)	0.4	11.3	1.7 ± 1.0 (1–3)	0.2
Acanthocephala									
<i>Pomphorhynchus tereticollis</i>	96.7	24.6 ± 24.5 (1–82)	23.7	95.7	17.6 ± 16.6 (1–52)	16.8	96.2	21.5 ± 21.6 (1–82)	20.7
cystacanth									
Nematoda									
<i>Anguillicoloides crassus</i> larvae				8.7	1.0 ± 0.0 (1)	0.1	3.8	1.0 ± 0.0 (1)	0.04
<i>Contracaecum rudolphii</i> larvae				4.3	1.0	0.04	1.9	1.0	0.02
Bivalvia									
<i>Anodonta</i> sp. glochidia	23.3	1.4 ± 0.8 (1–3)	0.3				13.2	1.4 ± 0.8 (1–3)	0.2

**Fig. 1.** Relationship between *Pomphorhynchus tereticollis* abundance and fish size (SL). Black dots and empty squares represent spring and autumn samples, respectively. The predicted curve (solid line) and 95% confidence intervals (dashed lines) were produced using a generalised linear model (residual $R^2=0.201$).

at least the 1990s (Baruš *et al.*, 1999), round gobies could increase their chances of spreading.

The North Sea is the final meeting point of two aquatic invasion routes, the Central and Southern Corridors (Panov *et al.*, 2009). As such, gobies from both the lower Elbe and other rivers of the North-Sea drainage appear to have originated as a mixture of distant populations from the Danube and another, unspecified source (Mombaerts *et al.*, 2014; Janáč *et al.*, unpublished data). The new population in the upper Elbe, first found close to the inland port at Ústí-nad-Labem, remains isolated from the downstream population near Hamburg by approx. 600 km of open river (Roche *et al.*, 2015). This would tend to confirm their introduction *via* shipping, one of the main vectors of gobiid distribution in the North Sea drainage (Borcherding *et al.*, 2011; Kalchhauser *et al.*, 2013; Hirsch *et al.*, 2016). If the gobies were introduced as eggs and/or larvae, as has been suggested elsewhere (e.g. Corkum *et al.*, 2004), then they will probably have been introduced free

of metazoan parasites. This would explain the absence of native parasites and their tendency to become new hosts for the local parasite fauna.

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