

The current status of gobies (*Gobiidae*) in River Danube in Vienna, Austria

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by

Susanne Reisinger

Supervisors

A. o. Univ. Prof. Dr. phil. Herwig Waidbacher

Mentors

Paul Meulenbroek MSc

Dipl.-Ing. Silke-Silvia Drexler

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Abstract

Gobies (Teleostei: Gobiidae) are currently very abundant in the Austrian River Danube. Originally native to the Ponto-Caspian region they have been introduced by navigation and have colonized predominantly rip-raps.

Gobies are competitors for native fish species in terms of food and habitats. The ecological consequences of their invasion are not yet fully known.

The aim of this study is to determine the current distribution of gobies in the impounded section of the River Danube in Vienna. The first hypothesis states that the distribution of gobies in different zones of the impoundment is homogeneous. Another hypothesis assumes homogeneity in the structure of gobiid species in three types of habitats: rip-raps in the main channel, artificially created riparian structures on the Danube Island and natural side arms and backwaters.

Another objective is to analyse the historical development of gobies in the Austrian River Danube. Two hypotheses assume that the abundance as well as the structure of gobiid species is homogeneous in different time periods.

Electrofishing data of four sampling periods between 1993 and 2016 are used to test the hypotheses.

The results show that the abundance of gobies varies in different zones of the impoundment. The structure of gobiid species varies in the three habitat types.

A significant increase in gobies' abundance over the years and a change in species structure is visible. In 1993-1994 the western tubenose goby (*Proterorhinus semilunaris*, Heckel 1837) has been the only goby in the Danube. Ten years ago, the bighead goby (*Ponticola kessleri*, Günther 1861) has been predominant. Today, round gobies (*Neogobius melanostomus*, Pallas 1814) dominate over bighead gobies, western tubenose gobies and racer gobies (*Babka gymnotrachelus*, Kessler 1857).

The persistent success of the round goby can be justified with both high flexibility concerning feeding and high genetic variability in round goby populations.

Frequent monitoring of the fish fauna in the River Danube is recommended. This will help to follow the future development of gobies in the Danube as well as to evaluate their ecological impacts.

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Abbreviations

HPP	Hydro Power Plant
EF	Electro Fishing
MC	Main Channel
SA	Side Arm
HA	Habitats
RMDC	Rhine-Main-Danube Canal
CPUE	Catch per unit effort
Obs.	Observed
Exp.	Expected
α	Significance level
α^*	Significance level corrected after <i>Bonferroni</i>
NeMe	<i>Neogobius melanostomus</i> (round goby)
PoKe	<i>Ponticola kessleri</i> (bighead goby)
PrSe	<i>Proterorhinus semilunaris</i> (western tubenose goby)
BaGy	<i>Babka gymnotrachelus</i> (racer goby)

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1. Introduction

Neozoans are species that were introduced in Europe after the year 1492. Their dispersal happened directly or indirectly through human interventions and had different reasons (Kinzelbach 1996). In some cases Neozoans are more successful in their new homes than native species and they get invasive. One reason for their success can be indifferent preferences of Neozoans concerning habitats or food resources (Ahnelt et al. 1998).

If habitats are altered by humans, living conditions change and might shift to conditions that support population growth of non-native species (Kinzelbach 1995). Monotonous enforced river banks, so called rip-raps, are a common sight in European rivers. Regarding the Danube, only 17% of the banks of the total river course are classified as nearly natural and they are all located in the lower sections of the river. Especially the upper and middle reaches of the Danube are strongly affected by hydromorphological alterations (Schwarz and Kraler 2008). The destruction of lateral connectivity by the reinforcement of river banks with rip-raps and additional longitudinal interruptions (dams or weirs mainly for hydro power generation) lead to a decline of species biodiversity, species abundance and an alteration of population composition in the river (Schwarz and Kraler 2008). Native fish species get more and more replaced by species with indifferent habitat preferences (Straif et al. 2003).

The Danube is one of the European rivers with the highest diversity of fish species. 56 native fish and lamprey species can be found in the Austrian stretch of the Danube, which has an epipotamal character and is classified as Barbel zone. Anadromous migratory fish that come from the black sea are native as well as rhithral species that migrate into cold and oxygen-rich tributaries for spawning (Spindler 1997).

Since migration routes have been strongly altered in the past centuries and decades, active migration is no longer possible for many fish species. Especially the Iron Gate impoundments are an insurmountable barrier for fish in the Danube. Sturgeons, that used to migrate all the way from the Black Sea to the upper reaches of the Danube in Austria and Germany, disappeared completely. On the other hand, new species were introduced so that today a total

number of 70 fish species and 2 lamprey species can be found in the Austrian Danube (Jungwirth et al. 2014).

Especially during the last century a new vector for the passive dispersal of fish was established. New, non-native species arrived as “stowaways” in container ships. This happened with Ponto-Caspian gobies (*Gobiidae*), which originate from the Black Sea and have been transported in the ballast water tanks of ships or as eggs sticking to the ship hull to the Middle and Upper reaches of the Danube. But also other European rivers like the Rhine, canals and even the Laurentian Great Lakes in North America have been invaded by gobies (Jude et al. 1992, Ahnelt et al. 1998, Roche et al. 2013).

Several investigations have been made in the past few years concerning the distribution of gobies in the Danube (e.g. Ahnelt 1989, Moskal'kova 1996, Ahnelt et al. 1998, Wiesner 2003, Harka and Biró 2007, Konecná and Jurajda 2012, Roche et al. 2013). One of the most recent studies in the Austrian Danube was done by Wiesner in 2003.

1.1. Study Area

Study site is the Austrian Danube between km 1943.00 and 1917.00. The hydro power plant (HPP) Freudenu is situated in the studied section, at Danube-km 1921.05.

The HPP impoundment is divided into four zones: head of impoundment, transition zone, central impoundment and tailwater.

An overview over the study area is shown on the map in Figure 1. The borders of the four impoundment zones are visible on the map.

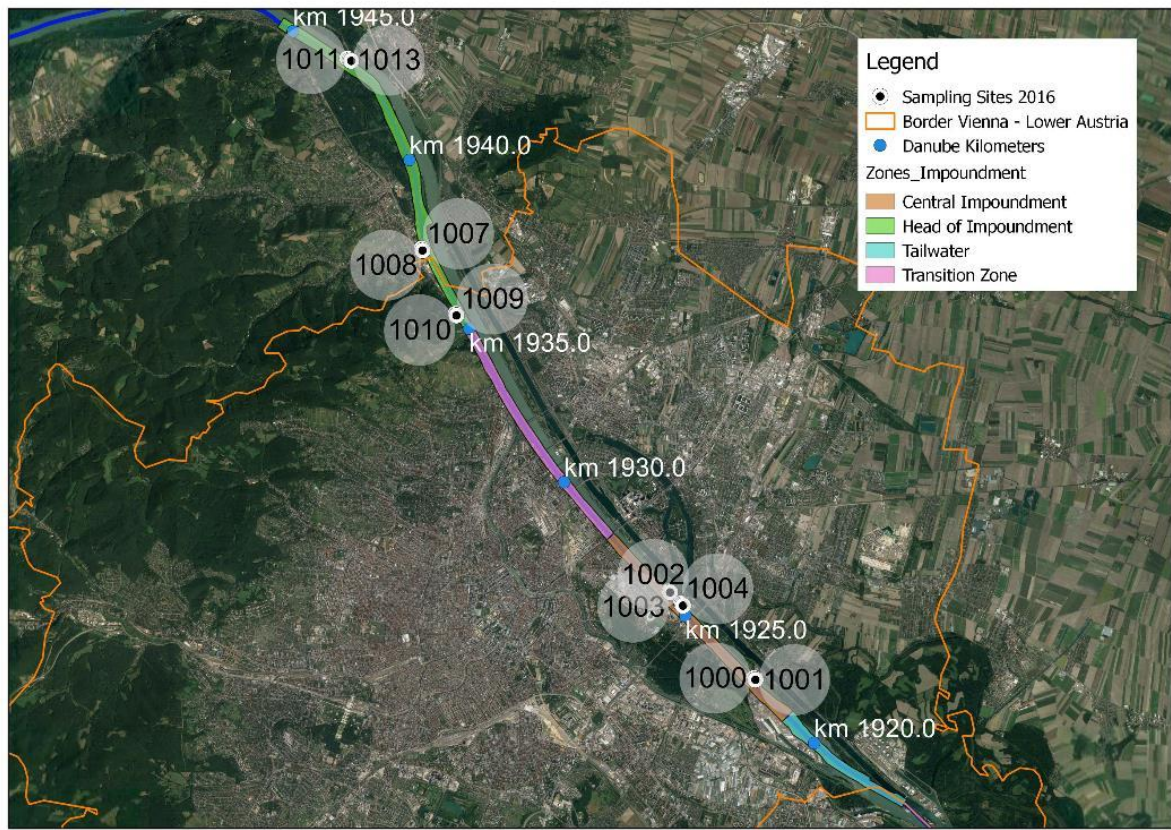


Figure 1: Study Area in Vienna, sites sampled in the year 2016 (Source: BEV, UBA, QGIS, Google Maps)

1.2. Gobiid species in Austria

Four different gobiid species have been detected so far in Austrian water bodies. The smallest of the four species is *Proterorhinus semilunaris* (Heckel 1837), commonly called **western tubenose goby** (Figure 2). It is the only gobiid species that is (by some authors) classified as native in Austria (Ahnelt 1989). Originally a saltwater species, it adapted to freshwater conditions and developed sustainable populations in many parts of the world. According to Ahnelt it immigrated to Austrian water bodies after the last ice age (Ahnelt 1989). The first tubenose gobies were reported in 1957 in Lake Neusiedl. In 1973 they were for the first time found in the Austrian Danube. The tubenose goby was probably present in the Danube already for a longer time, but being a small inconspicuous fish without any economic value, it was never reported earlier (Ahnelt 1989).

According to the same author, the western tubenose goby is mainly present in slow flowing streams and backwaters and they proliferate in reservoirs. By the construction of dams for

hydropower generation suitable conditions for the western tubenose goby are created. Spawning season is usually from April to August. They reach maturity after one to two years and usually spawn only one or two seasons. Eggs are deposited on hard surfaces like rocks or also on plants, males are guarding the nests (Ahnelt 1989).



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Figure 2: Appearance and range of the western tubenose goby (*Proterorhinus semilunaris*), red= native, green= not native

After being detected for the first time in 1994 in a backwater of the Danube downstream of Vienna, *Ponticola kessleri* (Günther 1861), also called **bighead goby** (Figure 3), was found in the main channel of the Austrian Danube east of Vienna (Regelsbrunn, Danube-km 1895-1905) in 1995 (Ahnelt et al. 1998). Repeated surveys showed that *P. kessleri* increased quickly in numbers from one year to the other. The authors characterized *P. kessleri* as eurytopic, since it was found in different habitat types with all kind of flow conditions (Ahnelt et al. 1998). They found evidence that the tubenose goby (*P. semilunaris*) was declining in habitats where the bighead goby was present. The bighead goby can therefore be considered as a competitor for benthic fish species that are native in the Danube (e.g. bullhead Cottus gobio) (Ahnelt et al. 1998). In 2001 and 2002, when Wiesner studied gobies in the Austrian Danube from the German to the Slovak border, *P. kessleri* was the most common gobiid species and it was found in every sampling station (Wiesner 2003). It was also in 2002 that it was declared as an invasive species (Mikschi 2002).

Bighead gobies usually spawn between March and May. The sticky eggs are deposited on rocks or other hard surfaces. They feed on benthic invertebrates, large individuals can be piscivorous. Bighead gobies are found in estuaries, lagoons and freshwater lakes as well as in big rivers, mostly on rock bottoms (Zweimüller et al. (1996) and Ahnelt et al. (1998) in

Kottelat and Freyhof 2007). Because of its indifferent preferences for habitats they are classified as eurytopic species (Ahnelt et al. 1998).



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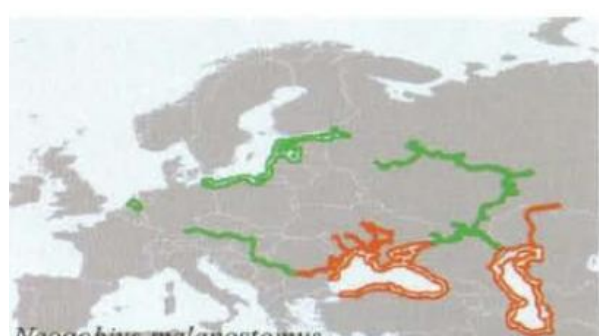
Kottelat and Freyhof (2007)

Figure 3: Appearance and range of the bighead goby (*Ponticola kessleri*), red=native, green=not native

The **round goby** (*Neogobius melanostomus*, Pallas 1814) was found by Wiesner et al. (2000) downstream of the HPP Freudenau in the year 1999. It occurred in high densities in a sampling site in the port of Lobau. No round gobies were reported in the Austrian Danube upstream of Vienna, except for one sampling site in the harbour of Krems, where *N. melanostomus* was very abundant in 2002 (Wiesner 2003). This is already an indicator that the expansion of gobies happens most likely via navigation.



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Figure 4: Appearance and range of the round goby (*Neogobius melanostomus*), red=native, green=not native

Round gobies have the longest spawning season of the species described in this study. They spawn between April and September. Males turn black during spawning season and usually

die when the spawning season is over. Females spawn repeatedly during a season. The adhesive eggs are stuck to rocks or other hard surfaces. Males guard the eggs during the incubation period, which lasts two to three weeks (Pinchuk et al. 2003b in Kottelat and Freyhof 2007).

Round gobies can similarly to the bighead goby be found in a variety of habitats. They live in brackish or freshwater lagoons as well as in lakes or big rivers. They prefer rocky or sandy substrates (Charlebois et al. 1997 in Kottelat and Freyhof 2007). Because of their indifferent preferences concerning habitats they are as well classified as eurytopic species.

Ahnelt et al. (2001) found **racer gobies** (*Babka gymnotrachelus*, Kessler 1857) in the year 1999 in the same backwater as *P. kessleri* was found in 1994 for the first time (Regelsbrunn, Danube-km 1895-1905). In 2002 *Babka gymnotrachelus* was found by Wiesner (2003) only downstream of the hydropower plant Freudenau. No evidence was given that racer gobies had spread to the Austrian Danube upstream of Vienna (Wiesner 2003).



Kottelat and Freyhof (2007)



Kottelat and Freyhof (2007)

Figure 5: Appearance and range of the racer goby (*Babka gymnotrachelus*), red= native, green= not native

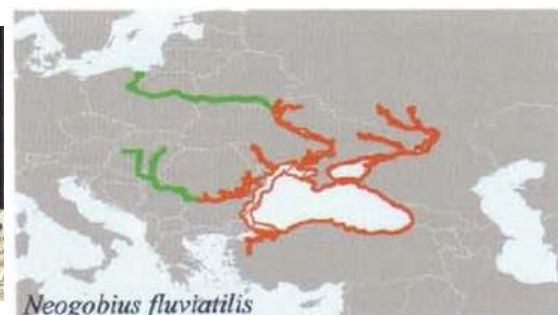
According to the Handbook of European Freshwater fish their habitat preferences are also quite broad, but they are mainly abundant in backwaters or channels with very low or zero flow velocity (Pinchuk et al. 2003a in Kottelat and Freyhof 2007). After the classification of Schiemer and Waidbacher (1992) they are classified as limnophilic.

They spawn between April and June and eggs are as well deposited to hard surfaces. Usually they spawn only for one season. Their food consists of benthic invertebrates, preferably molluscs (Kottelat and Freyhof 2007).

Up to today, **monkey gobies** (*Neogobius fluviatilis*, Pallas 1814) have not been reported in the Austrian Danube (Wiesner 2008, Roche et al. 2013, van Kessel et al. 2009, Konečná and Jurajda 2012). It is just mentioned here because there is evidence for monkey gobies in neighbouring countries, it has been reported in the Hungarian and Slovak parts of the Danube, even close to the Austrian border at the mouth of the river Raaba (Harka and Biró 2007). Van Kessel et al. (2009) reported *N. fluviatilis* in the Dutch Rhine in 2008. They have most probably been introduced via shipping to the harbour of Duisburg where monkey gobies were also reported in 2008 (Stemmer 2008). After introduction they might have migrated downstream to the Dutch parts of the Rhine (van Kessel et al. 2009).



Kottelat and Freyhof (2007)



Kottelat and Freyhof (2007)

Figure 6: Appearance and range of the monkey goby (*Neogobius fluviatilis*), red= native, green= not native

1.3. History of gobies' distribution in Central Europe

Gobies are small benthic fish species without a swimbladder. Since they live close to the river bottom they are not very much affected by the current. But still, their ability to swim upstream is limited (Ahnelt et al. 1998). Therefore it is hardly possible that they migrated actively to the middle and upper reaches of the Danube. Gobies of Ponto-Caspian origin are as well reported in the Rhine in Germany, the Netherlands and even in the uppermost reaches in Switzerland (Kalchhauser et al. 2013). Furthermore they were found in the Rhine-Main-Danube-Canal as well as in other canals in the Netherlands, Belgium and also in the river Moselle in France (Roche et al. 2013).

Ahnelt et al. (1998) already connected the dispersal of gobies to shipping activities, in particular to big cargo vessels. There is evidence for the passive dispersal of gobies to the

Laurentian Great Lakes in North America via the ballast water of cargo ships (Jude et al. 1992). Gobies like to colonize holes and depressions as can often be found in ship hulls. Sometimes big cargo vessels stay in harbours for a long time, sometimes even for several months. Algae, mussels etc. accumulate on the hull and provide a good shelter for small fish species. Gobies stick their eggs to hard surfaces and after fertilization the nests are protected by the males. When the ship moves upstream the gobies are transported together with the fertile eggs. Round gobies (*N. melanostomus*) have quite long incubation periods of two to three weeks. When freshly hatched, the young fish like to stay next to the egg membranes for a few days. Because of this behaviour they are able to travel long distances with a ship (Moskal'kova 1996).

Roche et al. (2013) analysed the main invasion corridors of gobiid species in Europe in a very interesting review. They linked the development of container transport on ships on the Rhine, the Danube and the Rhine-Main-Danube-Canal (RMDC) to the dispersion of gobies in these rivers. They identified three main “population centres” from where gobies have spread. One centre is the port of Vienna together with the port of Bratislava, another one is the port of Duisburg on the Rhine, which is the largest inland-port in Europe. And the third centre is the international sea port of Rotterdam in the Rhine Delta. They also considered geopolitical developments as a factor for the expansion of gobies. After the Second World War the Soviet Union controlled most of the Danube and container ships rarely passed to ports upstream of the Iron Curtain. When the Iron Curtain fell the economy of the former Soviet States and therefore also trading collapsed. But soon afterwards the new east-west trade route on the Danube was discovered for world shipping. It was at that time (starting from 1990) that gobies were introduced to the Laurentian Great Lakes in North America (Roche et al. 2013).

In 1999, during the Yugoslavian war, the bombing of bridges over the Danube in Yugoslavian countries was enforced by the NATO. The shipping route from the Black Sea to the North Sea was blocked. Shipping on the Danube was only possible upstream of Novi Sad. Transport from areas in the lower reaches of the Danube was transferred to rail and road but between Novi Sad and Vienna the shipping routes were still in use. Alternative shipping routes (longer ones, leading through the Mediterranean or the Baltic Sea) were established and shipping to and from Rhine Sea ports increased. It was in that period that populations of gobies

established in the Rhine. The expansion of the Rhine port at Duisburg from 1999 onwards might also have contributed to the spreading of gobies (Roche et al. 2013).

The RMDC, which was opened in 1992, provided the connection between the ports on the middle Danube reaches and Central Europe and served as an additional corridor for the expansion of gobies. However, the bighead goby *P. kessleri* and the monkey goby *N. fluviatilis* have been reported in the Rhine already before the opening of the RMDC. Probably the expansion happened in both directions, from the Danube into the Rhine and vice versa. The exact pathways are difficult to prove because of limited data availability (Roche et al. 2013).

1.4. Problem Statement

During the second Joint Danube Survey in 2007 rip-rap banks in the upper and middle reaches of the Danube were found to be heavily infested with gobies. In some places they were even the dominant species. In stretches downstream of the Iron Gate, where gobies are native, they were found to be much less abundant. The survey shows that particularly in the upper and middle reaches of the Danube hydromorphological alterations and navigation cause the main problems that affect the fish fauna (Wiesner et al. 2008).

The high abundances of gobies can have severe ecological impacts. Cucherousset and Olden (2011) studied the possible ecological impacts of non-native fish species on the native river fauna. The impacts can happen at different levels, starting from the genetic level to impacts that affect individuals, the population, the fish community or even the whole ecosystem.

As an example for the impact of *Gobiidae* they cite Steinhart et al. (2004) who studied the effects of the round goby (*N. melanostomus*) on the smallmouth bass (*Micropterus dolomieu*) in Lake Erie (Great Lakes basin in North America). The round goby was introduced to the Laurentian Great Lakes in North America from the Ponto-Caspian region via the ballast water of big transport ships. Round gobies are very well able to adapt to local conditions and especially to local food sources (Kornis et al. 2012). The smallmouth bass is a nest guarding species and it is native to the eastern part of North America. The nests are guarded by the male fish. In an experiment catch-and-release practices, as done in recreational fishing, were

mimicked. Smallmouth basses were removed via angling and their nests remained unguarded. Round gobies took advantage of the situation and consumed the fish embryos in the abandoned nests. When the smallmouth bass was released, it started to chase the round gobies. According to Steinhart et al. (2004) they chased them nine times more frequently than before being caught and released. This behaviour consumes a lot of energy and if energy reserves are low it can seriously affect the success of reproduction of the smallmouth bass.

Furthermore, gobies compete for food and habitat with native benthic fish species, e.g. the bullhead *Cottus gobio* and the streber *Zingel streber* (Nehring et al. 2010). Ahnelt et al. (1998) assumed already in 1998 that the expansion of the bighead goby will have consequences for several trophic levels in the Danube.

For the estimation of ecological consequences, the dispersion of gobies needs to be monitored frequently. Understanding the invasion process of *Gobiidae* will help to estimate the ecological consequences of the increasing populations of gobies in the Danube and its tributaries. Furthermore, if the negative impact of rip-rap banks on the composition of fish populations is proven in studies, they can serve as justification for restoration measures and the rehabilitation of natural habitats.

1.5. Historical development and human alterations of the Danube in Vienna

The regulation of the Danube has a long history. The first documents that showed regulation measures for the Danube date from the year 1652. Dams and cut-offs were constructed to embank the river arm (Hohensinner et al. 2013).

Later on, in the late 17th century, navigation gained more and more importance and the first attempts to improve the navigability of the Danube were made. The first flood protection measures were constructed at the end of the 18th century. But the main regulations finally took place between 1870 and 1875 when the Danube-cutoff was built (Hohensinner et al. 2013).

Although dikes and an inundation plain next to the straightened river channel were built, the safety of the protection system and the navigability had to be improved again in the 20th century. From 1972, a flood bypass, the “New Danube”, was created parallel to the river

channel. Between the main channel and the bypass, a 21 km long “Danube Island” was built (Hohensinner et al. 2013).

Concerning hydro power, the Danube is heavily used. In total, ten hydro power plants (HPP) can be found in the approximately 350 km long Austrian part of the Danube. The most recent impoundment was built for the hydro power plant Freudenau in Vienna, which went into operation in 1999. Ecological requirements had to be met and as a compensation for the degradation of the river, riparian structures were created on the Danube Island at the left bank of the Danube. Side arms, gravel bars, backwaters and shallow pools were built for the improvement of the ecological situation (Chovanec and Schiemer 2003).

The location of these inshore habitats is shown in Figure 7.

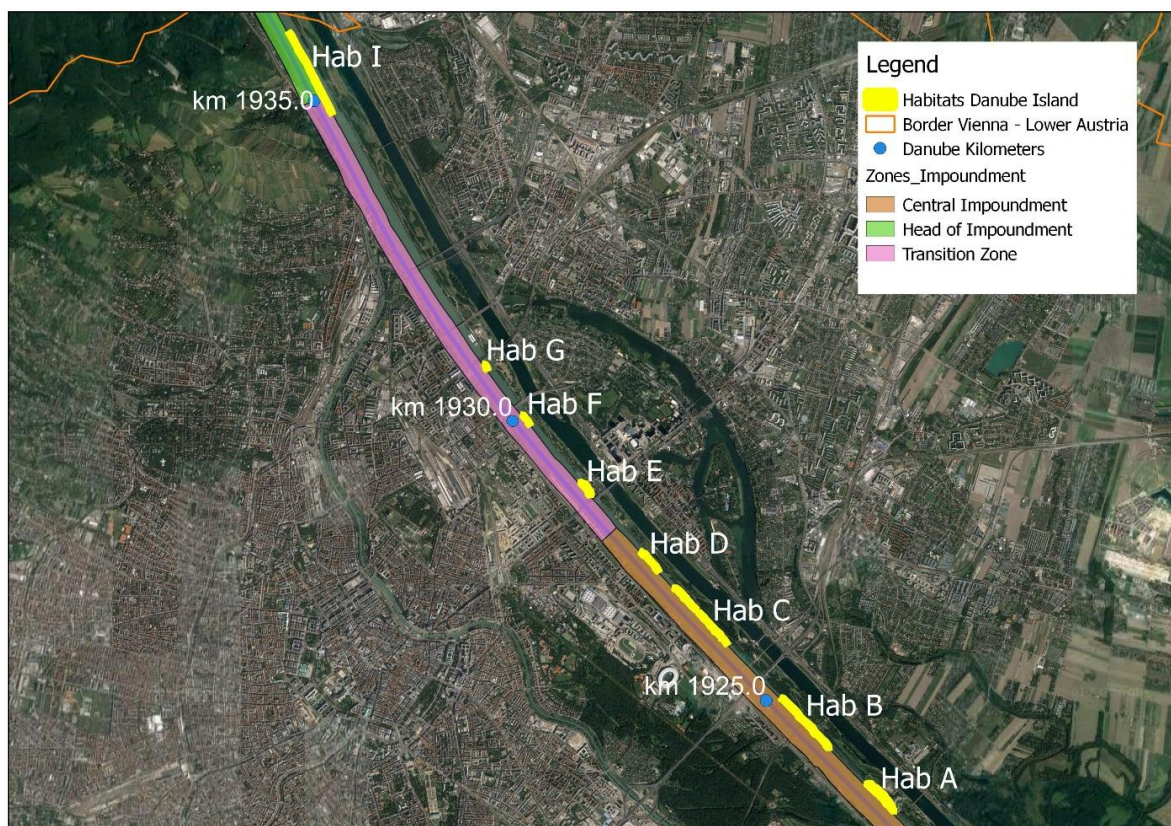


Figure 7: Location of artificially created inshore habitats on the Danube Island (BEV, UBA, QGIS, Google Maps)

The habitats are named with letters from A to I. Habitat H has silted up and is not shown any more in Figure 7. To give an idea about these habitats, pictures of Habitat C and Habitat E are given in Figure 8 and Figure 9.



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Figure 8: Habitat E on the Danube Island, the picture on the right shows the connection to the Danube on the downstream end of the habitat



Figure 9: Downstream end of Habitat C on the Danube Island, view in upstream direction, © Reisinger 2017

The construction of the HPP in Freudenua was accompanied by an intensive monitoring of fish biocenosis in the impounded river zones, the artificially created riparian structures on the Danube Island and some side arms, backwaters and oxbow lakes (Matschnig 1995, Chovanec

and Schiemer 2003, Straif et al. 2003, Waidbacher and Straif 2002, Waidbacher et al. 2016). Samples were collected with nets, longlines and also by electrofishing during three sampling periods to evaluate the impact of the HPP on the fish biocenosis. Further explanations are given in chapter 2.

1.6. General and Specific Objectives

One objective is to study the spatial distribution of gobies in the impounded section upstream of Freudenu in Vienna. Another aim of this study is to follow up previous studies and to examine the temporal dispersion of gobies in the Viennese Danube during the last years. Furthermore, the efficiency of two methods for the sampling of small benthic and speleophilic fish species is compared in this study.

- Objective 1: To determine the current distribution and structure of gobies in the Danube in Vienna
- Objective 2: To analyse the development of gobiid species abundance and structure in the Viennese Danube during the past years
- Objective 3: To evaluate the efficiency of two electrofishing methods for the fishing of benthic fish species

1.7. Research Questions (RQi) and Hypotheses (Hj)

1.7.1. RQ 1: How are gobies currently distributed in the impounded section of the Danube in Vienna?

- RQ1.1: Does the abundance of gobies in rip-raps vary in different zones of the impoundment (head of impoundment, transition zone, central impoundment, tailwater)?
 - *H1.1: The current distribution of gobies in the four impounded sections is homogeneous.*

DEPENDENT VARIABLES: “CPUE (n/10 min)”

INDEPENDENT VARIABLES:

Control variables:

“Family”: Gobiidae

“Habitat Type”: Main channel

“Sampling Period”: 14/15

“Life stage”: adult

Factors:

“River Bank”: left, right

“Zone”: Head of Impoundment, Transition Zone, Central Impoundment, Tailwater

- RQ1.2: Is there a difference in gobiid species structure between rip-raps of the Danube’s main channel, the Danube Island Habitats and the side arms/backwaters?
 - *H1.2: The species structure of gobies in rip-raps of the Danube’s main channel, the habitats on the Danube Island and the side arms and backwaters is homogeneous.*

DEPENDENT VARIABLES: Species presence/absence

INDEPENDENT VARIABLES:

Control variables:

“Species”: Round goby, Bighead goby, Racer goby, Western tubenose goby, Others

“Sampling Period”: 14/15

“Life stage”: adult

Factors:

“Habitat Type”: Main channel, Habitats Danube Island, Side Arms

1.7.2. RQ 2: How did the abundances of gobies in the Viennese Danube change over the past 24 years?

- RQ2.1: Did the abundance of Gobiidae (round goby, bighead goby, western tubenose goby and racer goby) in the impounded section of the Viennese Danube change during the last 24 years?

- *H2.1: The abundance of gobies in the Danube's main channel in the sampling periods 1993/1994, 1999/2000, 2002 and 2014/2015 is homogeneous.*

DEPENDENT VARIABLES: Species presence/absence

INDEPENDENT VARIABLES:

Control variables:

“Family”: Gobiidae

“Habitat Type”: Main channel

“Life stage”: adult

Factors:

“Sampling period”: 93/94, 99/00, 2002, 14/15

- RQ2.2: Did the species structure of gobies in the River Danube change during the years of invasion?

- *H2.2: The species structure of gobies in the periods 2014/2015, 1999/2000, 2002 and 2014/2015 is homogeneous.*

DEPENDENT VARIABLES: Species presence/absence

INDEPENDENT VARIABLES:

Control variables:

“Species”: Round goby, Bighead goby, Racer goby, Western tubenose goby, Others

“Life stage”: adult

Factors:

“Sampling period”: 93/94, 99/00, 2002, 14/15

“Habitat Type”: Main channel, Habitats Danube Island, Side Arms

1.7.3. RQ 3: Is there a difference in sampling efficiency between two electrofishing methods?

- RQ 3.1: Is the sampling efficiency of small benthic fish species in rip-raps higher when a portable backpack electrofishing unit is used in comparison to the use of a stationary electrofishing unit?

- *H3.1: The sampling efficiency of small benthic fish species is homogeneous for both methods (electrofishing with a portable backpack unit and with a stationary unit)*

DEPENDENT VARIABLES: “CPUE (n/10 min)”

INDEPENDENT VARIABLES:

Control variables:

“Year”: 2016

“Species”: benthic species

Factors:

“Method”: Electrofishing with a portable backpack unit, electrofishing with a stationary unit

2. Methods

Three major datasets were included in the analyses of this study. The first dataset contains sampling data collected for the limnologic preservation of evidence in the impoundment of the hydro power plant Freudenau. Therefore, samples were collected in three periods. Samples collected in the first period, from 1993 to 1994, reflect the limnologic situation in the Danube before construction of the HPP. The second batch of samples, collected in the period from 1999 to 2000, shows the situation right after the construction of the impoundment. Finally, from 2013 to 2015, another series of samples was collected to evaluate the impacts of the HPP and the ecological functioning of natural and artificially created habitats in the impounded zones (Waidbacher et al. 2016).

All samples from the first dataset can be associated with one of the four impounded zone (head of impoundment, transition zone, central impoundment and tailwater). Furthermore, they can all be assigned to one of three habitat types: the main channel, the artificially created riparian structures on the Danube Island (as described in chapter 1.5), and natural side arms, backwaters and oxbows in the Danube floodplain.

The second dataset that was included in some analyses of this study was established by Wiesner (2003). In his study he focussed on the distribution of gobies in the whole Austrian Danube. He collected 116 samples in 2002, of which 48 were collected from the Danube's main channel. The rest was collected from ports (Wiesner 2003). For this study, only the 48 samples from the main channel were considered.

Finally the third dataset was created exclusively for this study. Electrofishing samples were collected in the impounded zone of the HPP Freudenau in October 2016. The applied methods and the location of sampling sites are explained in chapter 2.3. For data analysis, these samples are considered as part of the last sampling period (2013-2015) of the limnologic preservation of evidence of the HPP Freudenau.

Sampling methods that were applied in October 2016 for this study, the chosen sampling sites, the relevant fishing parameter and also the applied statistical tests are explained in the following chapters.

2.1. Electrofishing

Electrofishing is a common method used by biologists for the sampling of fish in streams and rivers.

The principle of electrofishing is that fish react to a field of direct current (DC) in the water. At least two electrodes are needed to complete the electric circuit. The positive electrode is the anode and is held by the person who is fishing. The negative electrode is the cathode and usually consists of a copper strip that dives into the water. The fish are attracted by the anode and swim towards it. They get narcotized and are then caught with a net. After a while the narcotization stops and the fish are thrown back into the water (Bacon and Youngson 2007).

For the samples collected for this study in 2016, two different electrofishing gears were used. Every sampling site was divided into two sections. In the first section fishing was done with a stationary electrofishing unit (5 kW) and a 100 m long cable drum for the anode. In the other section, a portable backpack electrofishing unit (1.5 kW) was used. Every section was about 40 m long. Between the two sections a few meters were left out in order not to double-catch any individuals. The distance was measured and the sampling time was recorded. Flow velocity was measured at every site with a flowmeter.



Figure 10: Sampling with a portable electrofishing unit



Figure 11: Stationary electrofishing unit (Wiesner 2003)

The selected samples of the limnologic preservation of evidence of the HPP include as well samples that were collected with the use of a big or a small boat. These methods will not be explained here because they were not applied by the author of this study.

During electrofishing, small-sized fishes are likely to sink into the interstitial spaces of rip-raps when they are narcotized and therefore they can hardly be caught (Polačik et al. 2008). Additionally, some of them are narcotized inside the spaces and cannot be detected, not even visually. Benthic fish species are also likely to rest on the ground and are therefore not affected by the electric current (Wiesner 2003). Because of this behaviour, sampling of gobies by electrofishing is less efficient compared to other species that have swimbladders.

In 2016, special attention was paid to benthic fish species that like to stay on the bottom. By moving the anode across the ground the fish can be driven away from the bottom and sampling efficiency can be increased. It was also necessary to take enough time to let the small fish come out of the rip-rap. Every hole between the rip-rap stones had to be processed. The sampling of a stretch of 40 m took approximately 30 min.

Once the fish were narcotized by the anode they needed to be collected very quickly. In rip-raps it is often not possible to catch all fish with the net because they quickly sink into the spaces between the stones. Therefore, it is important to record as well all fish that were seen during the sampling to calculate the sampling efficiency. The sampling efficiency must be considered for the calculation of the CPUEs (see next chapter).

When sampling of one stretch was completed, the fish lengths were measured. Afterwards the fish were released back into the river.

2.2. Catch per Unit Effort (CPUE)

The numbers of each caught fish species and the fish lengths are used to analyse species abundance and structure. To make sampling points comparable, the different catches have to be expressed in the same unit. Therefore a standardized unit was developed by Bagenal in 1978 called “Catch per unit effort” (CPUE) (Bagenal 1978). It is a measure for the abundance of the different fish species in the catch. Considering the estimated sampling efficiency, the frequency of every species in the catch is converted to a standard period of time with a duration of 10 minutes. The CPUE can be used as an indicator that shows changes in the abundance of fish species.

2.3. Sampling Sites

The current study investigates the Danube River in the impounded section between km 1917.00 and 1945.50.

For this study the focus lies on rip-raps. In total 15 rip-rap samples were collected between 11.10.2016 and 19.10.2016. For the comparability with the results of Wiesner (2003), who sampled in 2001 and 2002, site-selection was done in accordance to his study. An advantage of this selection was as well the accessibility of the sites, which was one of Wiesner’s criteria (Wiesner 2003).

The sampling approaches for the samples collected in 2016 (sampling protocol, sampling gear, etc.) and the chosen rip-rap sections also correspond to the methods used for the limnologic preservation of evidence of the HPP.

The water temperature during the sampling period was 12.3°C. The discharge was below the mean annual discharge ($MQ=1910 \text{ m}^3/\text{s}$ at the gauging station Korneuburg) on all sampling days (noel.gv.at, 2016). The sampled stretches had lengths between 30 and 48 m. Sampling time was recorded and varied between 25 and 35 min.

Because of the low discharge the rip-rap bank structures and groynes in the tailwater section were dry and sampling was not possible (Figure 12).



Figure 12: Rip-rap and groyne in the tailwater at Danube-km 1918.30, © Reisinger 2016

The first samples were taken at Danube-km 1922.40 and 1922.34, upstream of the HPP, within the central impoundment (Figure 13).

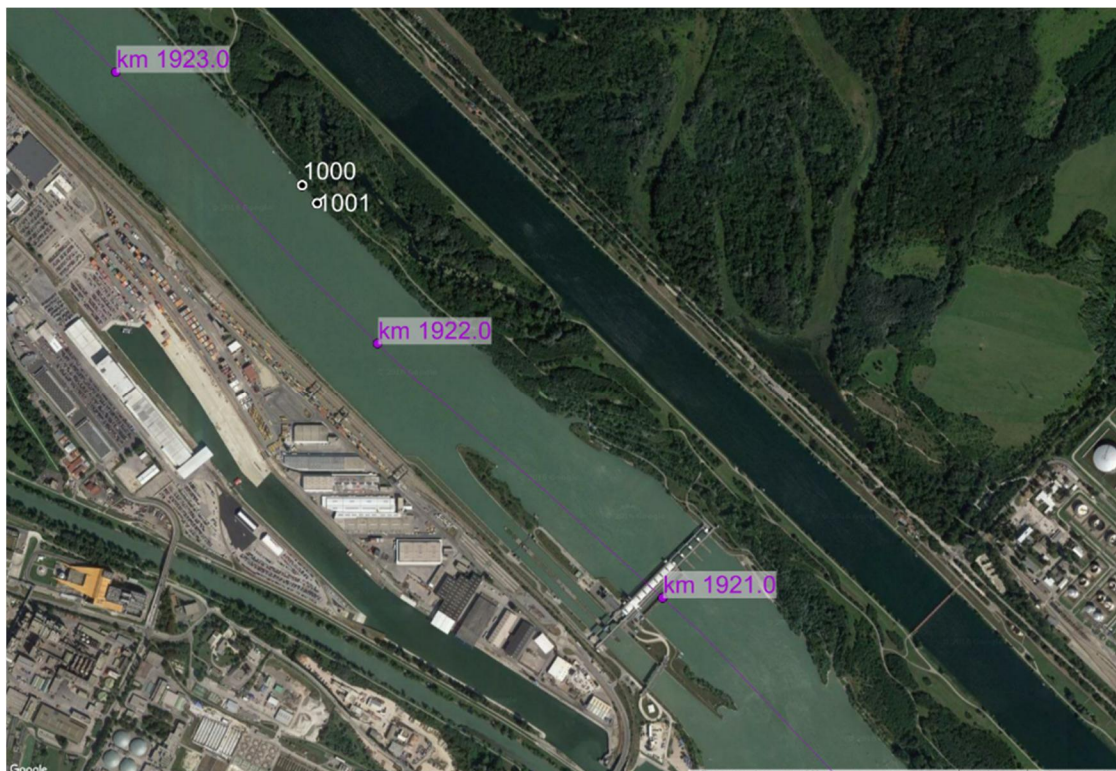


Figure 13: Sampling sites in the central impoundment at Danube-km 1922.40 and 1922.34 (Source: Google Maps, QGIS)

Fishing at km 1922.40 (Nr. 1000) was done with a stationary electrofishing unit (5 kW) and a 100 m long cable drum for the anode. The second sample (Nr. 1001) was collected a few meters downstream with a portable backpack electrofishing unit (1.5 kW).

On the same day two samples were collected at Danube-km 1925.80 and 1925.76 (Figure 14). Again two samples were taken, one with the stationary unit (Nr. 1002) and one with the portable electrofishing unit (Nr. 1003).

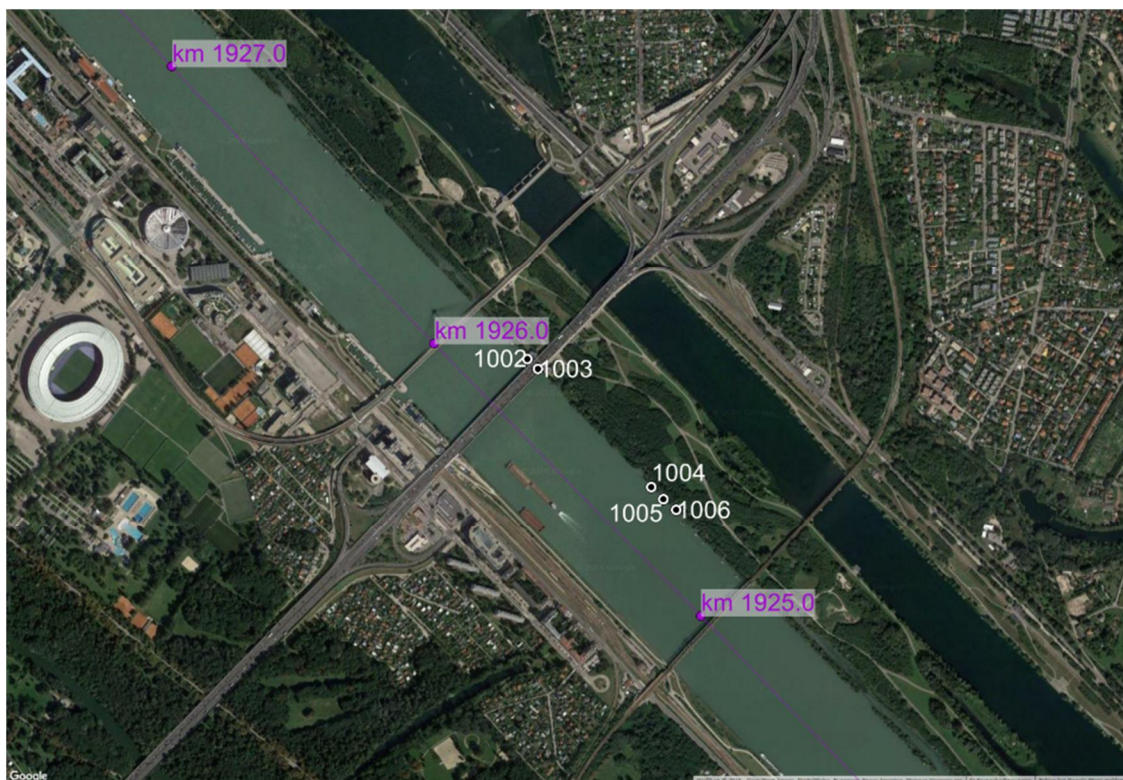


Figure 14: Sampling sites in the central impoundment at Danube-km 1925.80 and 1925.76 (collected on 11.10.2016) and at Danube-km 1925.33, 1925.29 and 1925.25 (collected on 12.10.2016) (Source: Google Maps, QGIS)

In the morning of the following day (12.10.2016) another three samples were taken in the central impoundment. The first one was collected at Danube-km 1925.33 with the stationary electrofishing unit (Nr. 1004), another one at Danube-km 1925.29 with the portable backpack unit (Nr. 1005, Figure 14 and Figure 15). A third sample was taken with the portable unit from a small bay on the left bank at km 1925.25 (Nr. 1006).



Figure 15: Rip-rap at Danube-km 1925.30, © Reisinger 2016

On the same day in the afternoon four samples were collected from the Danube's right bank, close to the port of Kuchelau (Figure 16). The same method was applied, first the stationary unit was used at km 1937.50 (Nr. 1007) and then, just subsequently downstream at km 1937.44, another sample was collected with the portable unit (Nr. 1008). Another two samples were taken from just upstream of the port's entry, at km 1935.50 (Nr. 1009, stationary electrofishing unit) and 1935.44 (Nr. 1010, portable electrofishing unit).



Figure 16: Sampling sites in the head of impoundment at Danube-km 1937.50, 1937.44, 1935.50 and 1935.44 (collected on 12.10.2016, Source: Google Maps, QGIS)



Figure 17: Rip-rap at Danube-km 1937.50, right bank, © Reisinger 2016

Four more samples from the left bank of the Danube in Korneuburg were collected on 19.10.2016 (Figure 18). The sites were chosen additionally sites samples by Wiesner (2003) because of their location at the outer side of the river bend and at the upper end of the impoundment, where flow velocity is high. The sites at km 1943.24 (Nr. 1011) and 1943.20 (Nr. 1013) were sampled with the stationary unit, the other two samples at km 1943.30 (Nr. 1012) and 1943.14 (Nr. 1014) were sampled with the portable unit.



Figure 18: Sampling sites in the head of impoundment at Danube-km 1943.24, 1943.30, 1943.20 and 1943.14 (collected on 19.10.2016) (Source: Google Maps, QGIS)



Figure 19: Danube at km 1943.30 (Korneuburg), © Reisinger 2016



Figure 20: Rip-rap at km 1943.30 (Korneuburg), left bank, © Reisinger 2016

2.4. Data Analysis

Hypothesis 1.1 and 1.2 are tested for significant differences, they both focus on homogeneity. In the first case (Hypothesis 1.1, addressing the distribution of gobies in the impounded zones) the dependent variable is metric, the independent variable (effect) is categorical. The hypothesis is globally tested with the median test, and also locally and pairwise with the post-hoc strategy of the median test (including Bonferroni-adjustment).

In Hypothesis 1.2 (concerning the structure of gobies in different habitat types) the dependent and the independent variable are categorical, therefore the analysis of cross tabulations has to be applied to test the hypothesis for homogeneity. This is done globally (for the whole table) with the classical χ^2 -test and locally (for every cell of the table) with the residual test (test statistic = $u(u_{ij}) = (\text{observed frequencies} - \text{expected frequencies}) / (\text{square root of expected frequencies})$, including Bonferroni-adjustment). The expected frequencies are based on the assumption that the dependent and the independent variables are totally independent.

In fact, several dependent variables are involved in Hypothesis 1.2 (four fish species). Actually, these fish species represent four one-dimensional variables. Because these four species have to be analysed simultaneously and also for their mutual interactions, a profile variable was created which reflects all possible combinations of the four fish species. This profile-variable is a so called meta-variable which is treated as a one-dimensional variable in the following statistical analysis, although it is possible to draw multiple conclusions from it.

Hypothesis 2.1 and 2.2, which address the temporal development of abundance and species structure of gobies, are as well tested for significant differences.

For the scientifically-based Hypothesis 2.1, two different statistical hypothesis are tested, whereby in the first case the dependent variable is on a binary scale, the independent variable is scaled categorically. The strategy of cross tabulation analysis, which was already explained for Hypothesis 1.2, was used for the analysis. In the second case, the dependent variable is metric and the independent variable is again categorical. Therefore, the global and local (Post-Hoc) median test was applied.

Hypothesis 3.1, which includes the evaluation of sampling efficiency of two different sampling methods, was in the same way tested for significant differences. The independent

variable is categorical. The metric dependent variable was analysed with the categorical independent variable in a median test.

Data collection was done with Microsoft Office Excel 2013. For the statistical analysis the program IBM SPSS Statistics, Version 21 (licence BOKU Vienna) with the addition of R-Subroutines was used.

3. Results

The current spatial distribution of gobies in the Danube will be analysed in the first chapter of this results section. Therefore, only samples collected from 2013 to 2016 will be considered. The analyses will address the abundance of gobies in the impoundment, regarding the main channel only (chapter 3.1.1). Furthermore, differences in the number of present gobiid species and species structure between the three habitat types (main channel, artificial riparian habitats on the Danube Island, side arms and backwaters) will be treated in chapter 3.1.2.

Afterwards, chapter 3.2 will focus on the historical development of gobies in the Viennese Danube. Again, the abundance as well as the number of species and the species structure will be analysed.

Finally, chapter 3.3 will address the sampling efficiency of two different methods.

The following abbreviations for the fish species have been used in the results chapter:

Table 1: Abbreviations of fish species used in the results chapter

Abbreviation	Scientific Name	Common Name
NeMe	<i>Neogobius melanostomus</i>	round goby
PoKe	<i>Ponticola kessleri</i>	bighead goby
PrSe	<i>Proterorhinus semilunaris</i>	western tubenose goby
BaGy	<i>Babka gymnotrachelus</i>	racер goby

3.1. Spatial distribution of gobies in the Viennese Danube – Current status

In this chapter, the current distribution of gobies in the Viennese Danube is analysed. 302 electrofishing samples, collected between 2013 and 2015 in the Danube's main channel, in the inshore habitats on the Danube Island and in side arms and backwaters, are included in the analysis. For the main channel only rip-rap-samples were used, gravel-bars and other structures are not analysed in this study. Additionally, in 2016, 15 samples from rip-rap

sections were added to the dataset (see chapter 2 for further explanations). For practical reasons the sampling period of samples collected from 2013 to 2016 will be called “14/15”, although samples from 2013 to 2016 are included in this period. In Table 2 the number of samples per habitat and year are shown. The applied methods are as well given in the table.

Table 2: Number of electrofishing samples per habitat and year and applied sampling methods in the sampling period 14/15

Habitat	2013	2014	2015	2016	Total
Habitats Danube Island	36	20	36	0	92
Portable EF unit, wading	12	8	14		34
Small boat + portable EF unit	20	11	22		53
Big boat	4	1			5
Main Channel	109	55	9	15	188
Big boat	74	52			126
Portable EF unit, wading	28	3	7	8	46
Small boat + portable EF unit	7		2		9
Stationary EF unit, wading					
*				7	7
Side Arms/Backwaters	7	11	19		37
Big Boat	2	10			12
Portable EF unit, wading	5	1	14		20
Small boat + portable EF unit			5		5
Total	152	86	64	15	317

The highest number of samples was collected in 2013, most of them from the main channel. Electrofishing with the big boat was the most frequent form of sampling, but many rip-raps have also been sampled by wading with a portable backpack unit.

In total, 50 different fish species were found in the three habitat types during the sampling period 14/15.

The most caught gobiid species in the sampling period was the round goby. The age structure of round gobies in each sampling year is shown in Figure 21. The threshold for adult individuals is 4 cm (oral comment Waidbacher 2017). It has to be considered that the proportion of juvenile gobies in electrofishing samples is generally low, because of their small body sizes they are hard to catch.

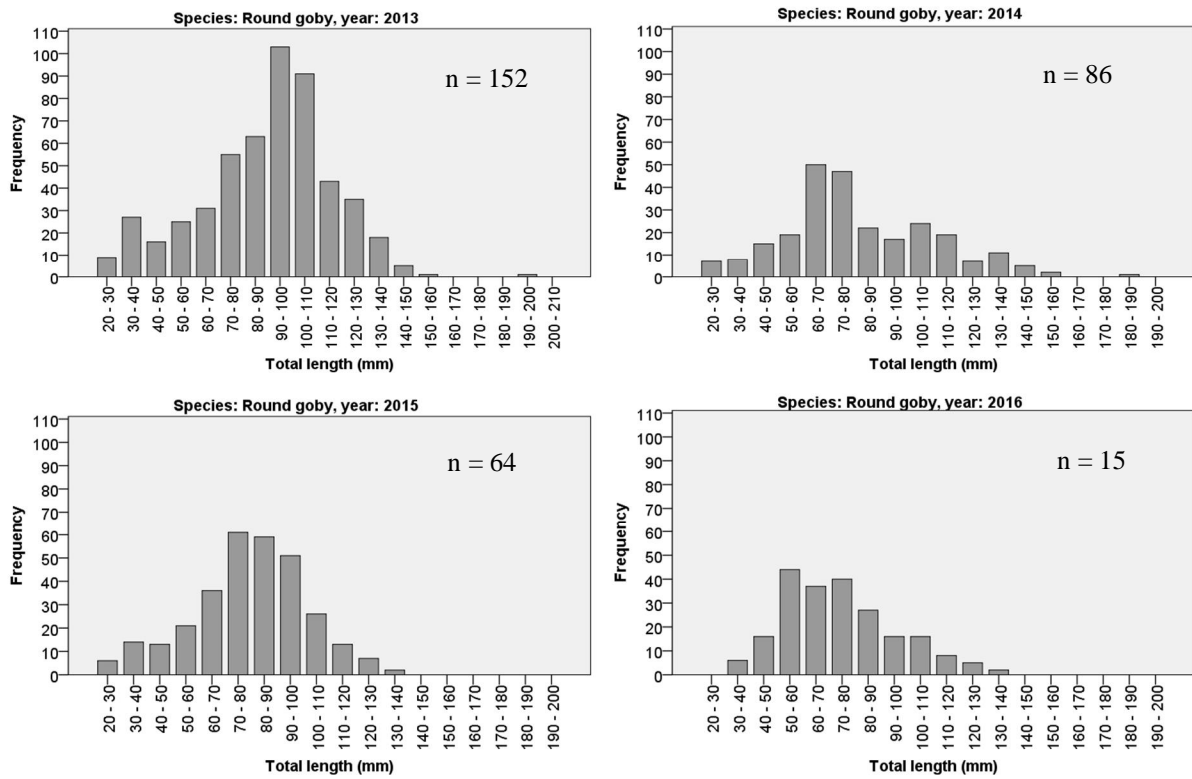


Figure 21: Length-Frequency plots of round goby for the years 2013, 2014, 2015, 2016 (n = number of samples)

The second most frequently caught gobiid species was the bighead goby. The same threshold of 4 cm for adults applies. The length-frequency plots of bighead gobies for every year are given in Figure 22.

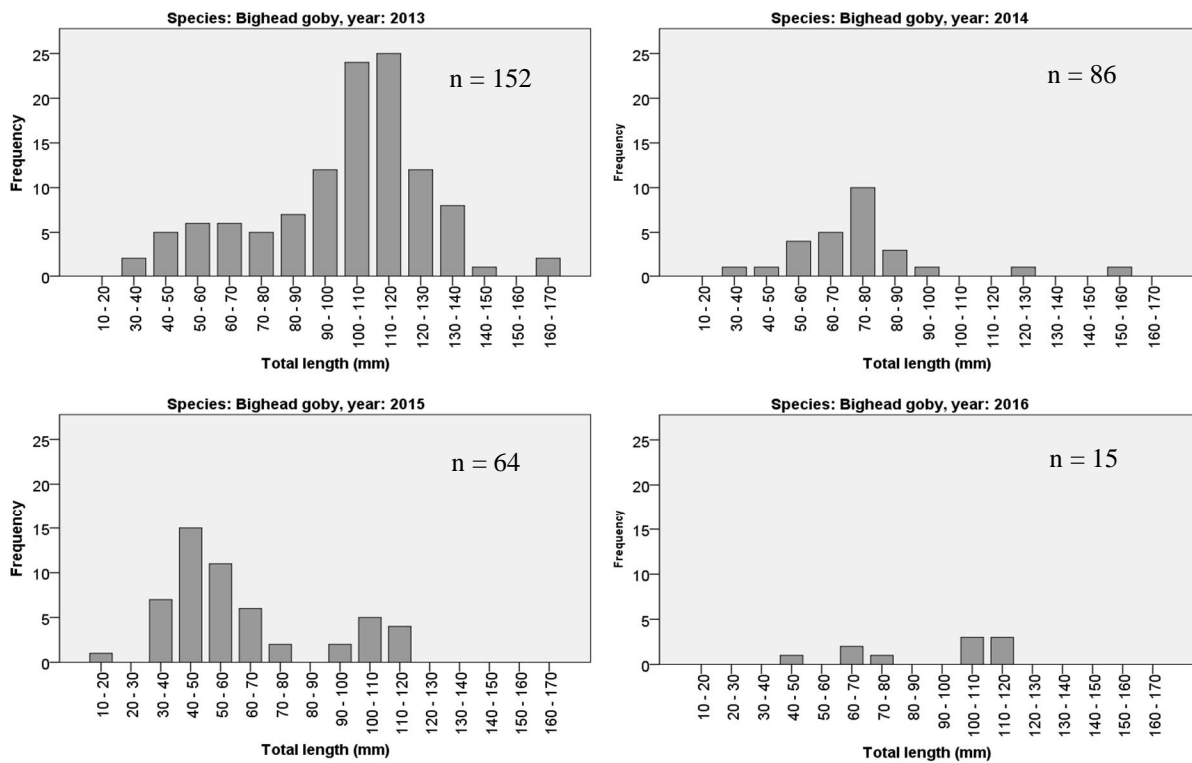


Figure 22: Length-Frequency plots of bighead goby, for the years 2013, 2014, 2015, 2016 (n = number of samples)

The number of samples varies with every year (Table 2) so the frequencies in the plots cannot be compared between the years, they just show the age structure of the species. Tubenose gobies and racer gobies were sampled in low numbers, therefore the length-frequency plots are not shown here.

3.1.1. Hypothesis 1.1

The first hypothesis addresses the current abundance of adult gobies (not differentiating between species) within the impounded section of the Danube's main channel. The impoundment is divided in four zones: head of impoundment, transition zone, central impoundment and tailwater. The four zones differ mainly in terms of flow velocity. The idea is to find out if the different conditions in the four zones influence the abundance of gobies. Since there are as well differences in flow velocity between the river banks (especially in the head of impoundment), the river bank is as well included as a factor.

Research Question 1.1: Does the abundance of gobies in rip-raps vary in different zones of the impoundment (head of impoundment, transition zone, central impoundment, tailwater)?

- *Hypothesis 1.1: The current distribution of gobies in the four impounded sections is homogeneous.*

The CPUE (n/10min, including sampling efficiency), which is a metric variable, is used as indicator for the abundance. The CPUE per species was calculated for every sample by summing up CPUE values of all individuals in the sample that belong to the same species. For Hypothesis 1.1, one CPUE value for all species belonging to the family *Gobiidae* was calculated for every sample. This value was then used as the dependent variable.

The data was filtered using two control variables, sampling period and habitat type (Table). For the analysis, only samples collected in period 14/15 from the main channel were used. Juvenile fish were not included in the analysis. The total number of samples used for the analysis of H1.1 is 188.

Table : Control variables for H1.1

Control variable	Filter
Sampling period	14/15
Habitat type	Main Channel
Life stage	Adult

A profile was created to test the influence of the two factors zone and river bank on the abundance of gobies. The profile has eight specifications, which are defined by combinations of the two factors. The specifications and their case numbers are listed in Table 3.

Table 3: Specifications of the profile

	Frequency of samples	Relative (%)	Cumulative (%)
1 Central Impoundment # Left	40	21.3	21.3
2 Central Impoundment # Right	20	10.6	31.9
4 Head of Impoundment # Left	30	16.0	47.9
5 Head of Impoundment # Right	26	13.8	61.7
7 Tailwater # Left	27	14.4	76.1
8 Tailwater # Right	11	5.9	81.9
9 Transition Zone # Left	27	14.4	96.3
10 Transition Zone # Right	7	3.7	100.0
Total	188	100.0	

Case numbers vary between the categories (Table 3) and the dependent variable CPUE (n/10min) is not normally distributed. Therefore the nonparametric Median test is used to test Hypothesis 1.1.

The zone “Transition Zone” was excluded from the tests because it is seen as a mixture between the head of impoundment and the central impoundment without clearly defined characteristics.

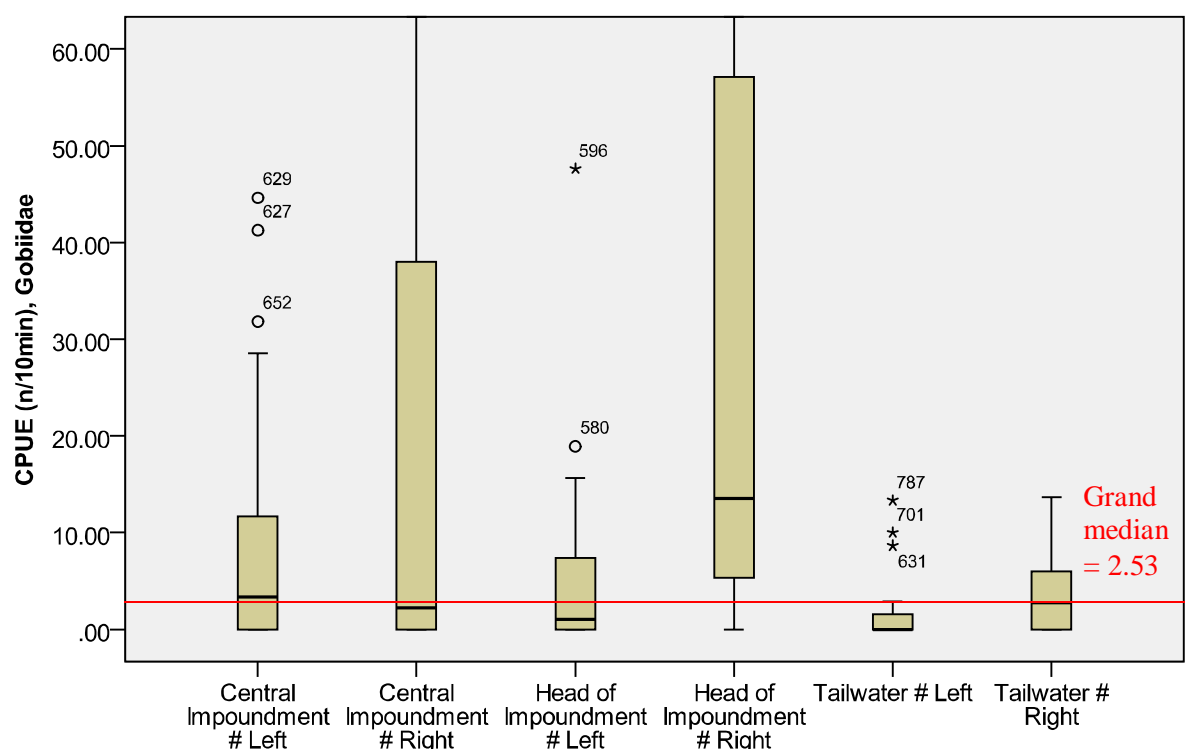


Figure 23: Boxplot, Distribution of Gobiidae by Zone and River ban, asterisks (*) indicate extreme values, dots (°) indicate outliers

The boxplot in Figure 23 shows the distribution of the CPUE (gobies/10min) by zone and river bank. The grand median over all profiles, as shown in the figure, is 2.53 gobies/10min. The boxplot indicates the deviations of the profile-medians from the grand median. Finally, the Median test shows that differences between the impounded zones and river banks exist. The result of the Median test is given in Table 4.

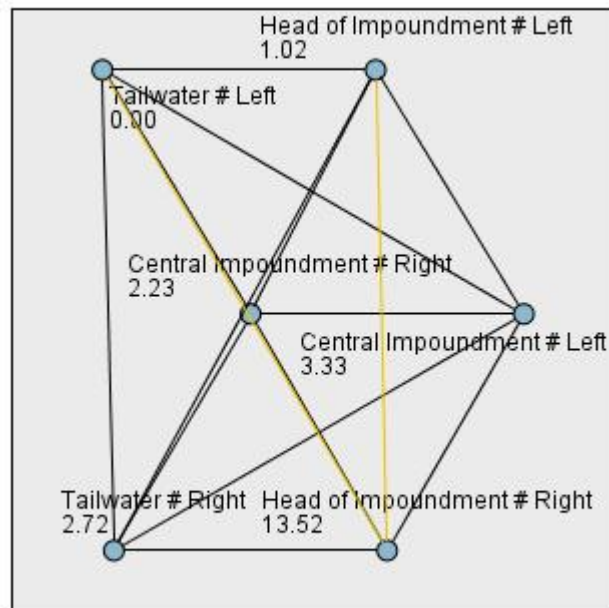
Table 4: Result of the Median test for H1.1

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The medians of Gobiidae are the same across categories of profile1_num.	Independent-Samples Median Test	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Pairwise Comparisons of profile1_num



Each node shows the sample median of profile1_num.

Figure 24: Median test for H1.1, sample medians for pairwise comparison, each profile category is represented by one node, yellow lines indicate significant differences between profile categories

In a Post-Hoc test medians of all profile-categories are compared pairwise. This test shows where significant differences can be found. In Figure 24 the median values of all profile categories are specified. Every category is compared with all other categories. Yellow lines connect categories that, according to the Post-Hoc test, are significantly different from each other.

Significant differences are visible between the left and right bank of the head of impoundment as well as between the left bank in the tailwater section and the right bank in the head of impoundment. All other comparisons show no significant differences in the abundance of gobies, according to the Post-Hoc test. Table 5 shows the test statistics of the Post-Hoc test as well as the significances. The significance level for the rejection of Hypothesis 1.1 is 0.05. In each row of Table 5 the hypothesis that CPUE (n/10min) of the compared profile categories is homogeneous, is tested.

Table 5: Median test of H1.1, pairwise comparison, significant differences are marked with orange colour

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
Tailwater # Left-Head of Impoundment # Left	1.521	.217	1.000
Tailwater # Left-Central Impoundment # Right	1.500	.221	1.000
Tailwater # Left-Tailwater # Right	2.237	.135	1.000
Tailwater # Left-Central Impoundment # Left	6.968	.008	.124
Tailwater # Left-Head of Impoundment # Right	20.538	.000	.000
Head of Impoundment # Left-Central Impoundment # Right	.333	.564	1.000
Head of Impoundment # Left-Tailwater # Right	.200	.655	1.000
Head of Impoundment # Left-Central Impoundment # Left	2.100	.147	1.000
Head of Impoundment # Left-Head of Impoundment # Right	10.338	.001	.020
Central Impoundment # Right-Tailwater # Right	.259	.611	1.000
Central Impoundment # Right-Central Impoundment # Left	.300	.584	1.000
Central Impoundment # Right-Head of Impoundment # Right	1.415	.234	1.000
Tailwater # Right-Central Impoundment # Left	.014	.904	1.000
Tailwater # Right-Head of Impoundment # Right	5.816	.016	.238
Central Impoundment # Left-Head of Impoundment # Right	6.346	.012	.176

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Remark: The significances (orange) are marked without adjustment. Because 15 pairwise Post-Hoc tests are done, the significance level would need to be adjusted after Bonferroni: $\alpha^* = \alpha / \text{Number of Post-Hoc tests} = 0.05/15 = 0.003$

According to the Median test, Hypothesis 1.1, which states that the distribution of gobies is homogenous in all zones and on both sides of the main channel, should be rejected.

3.1.2. Hypothesis 1.2

Hypothesis 1.2 was formulated to test the homogeneity of species structure of gobies in the three different habitat types (Main channel, habitats Danube Island, side arms). The assumption is that structures vary with habitat types.

Research Question 1.2: Is there a difference in gobiid species structure between rip-raps of the Danube's main channel, the Danube Island Habitats and the side arms/backwaters?

- *H1.2: The species structure of gobies in rip-raps of the Danube's main channel, the habitats on the Danube Island and the side arms/backwaters is homogeneous.*

The number of samples used for the analysis is given in Table 6.

Table 6: Number of samples by habitat type collected in the sampling period 14/15

Habitat Type	Number of Samples (period 14/15)
Side Arms/Backwaters	37
Habitats Danube Island	92
Main Channel	188
Total	317

The highest number of samples was collected from the main channel. The least samples are available for the side arms.

Four different goby species are present in period 14/15: western tubenose goby, bighead goby, round goby and racer goby.

In Figure 25 to Figure 27 all samples available for the period are plotted. The y-axis shows the CPUE in n/10min of each gobiid species in percent of the CPUE of all gobies. Only gobies are included in the graphs, species belonging to other fish families are not part of the analysis.

On the x-axis, data are arranged from downstream to upstream for the main channel and the Danube Island habitats. For the side arms the names of the sampled side arms, backwaters or oxbows are listed on the x-axis, not considering the sampling location.

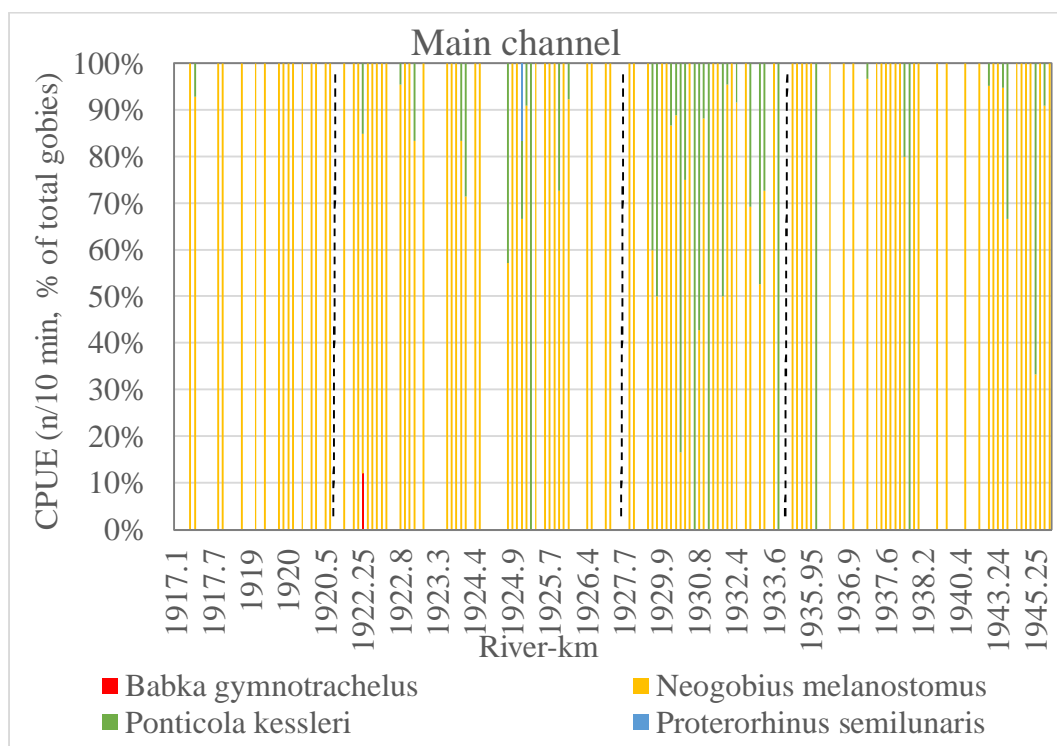


Figure 25: Species structure of gobies in the main channel in period 14/15, all samples arranged from downstream to upstream. Dashed lines indicate the borders between the four impoundment zones

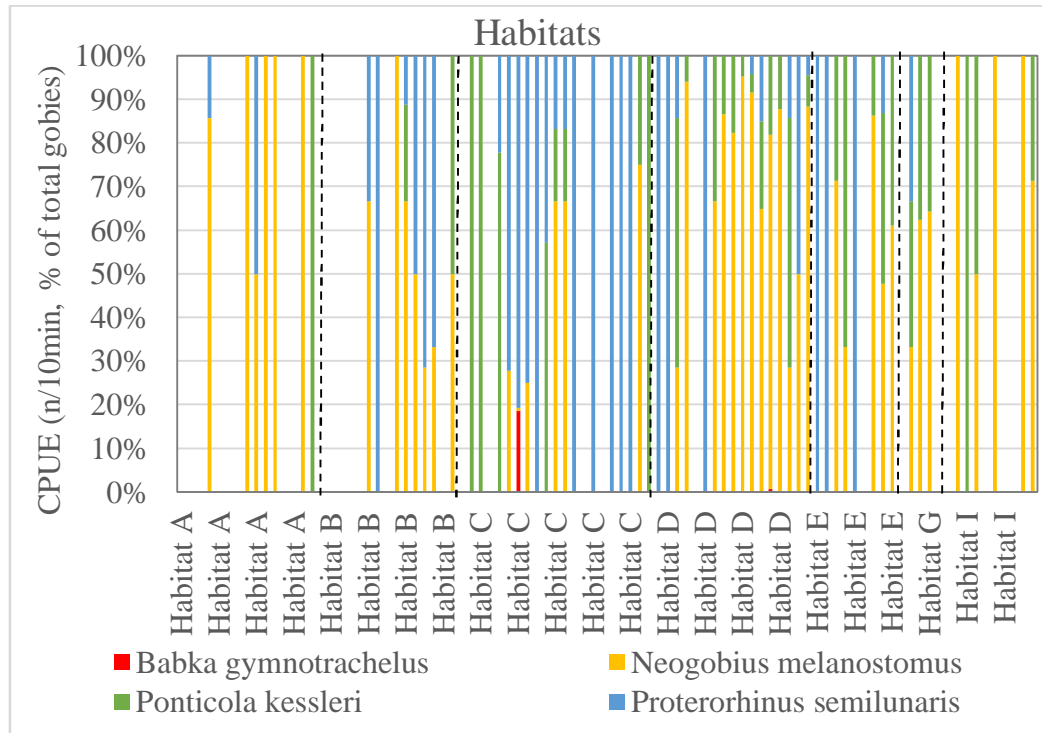


Figure 26: Species structure of gobies in the inshore habitats in period 14/15, all samples arranged from downstream to upstream. Dashed lines indicate the borders between the habitats

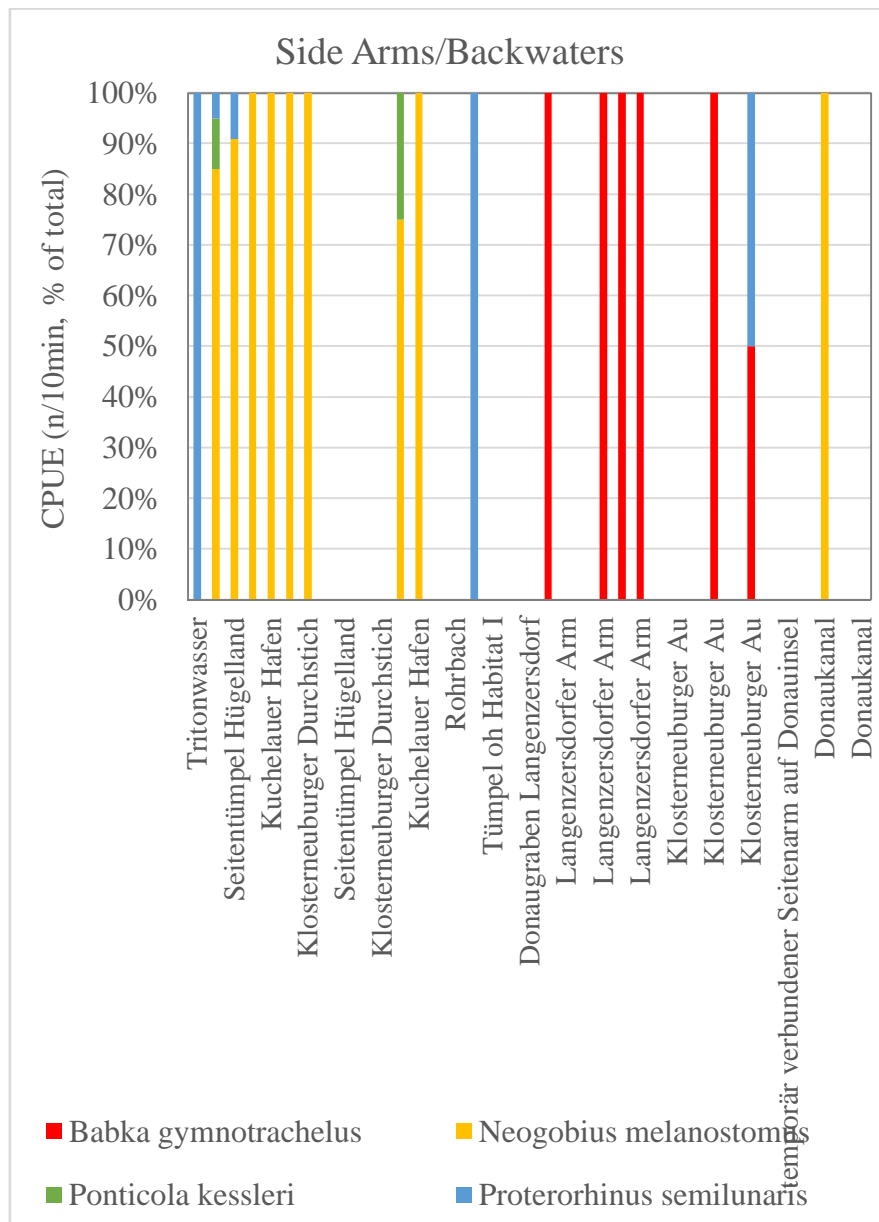


Figure 27: Species structure of gobies in the side arms and backwaters in period 14/15, all samples arranged from downstream to upstream

These figures give first ideas about the homogeneity of species structure in the three habitat types. In the main channel, the round goby is dominant. In some samples the bighead goby was found. The western tubenose goby and the racer goby were both found in only one sample.

To test the structure of gobiid species in the different habitat types, profiles were created for every sample. Each profile specification contains information about the presence or absence

of all four gobiid species in the sample. The information about species belonging to other fish families is included in the form of “no gobies”, so a differentiation between other fish species or families has not been made.

There are profiles where only one of the four gobiid species is present but also profiles where three species are combined. That means that sites where three different gobiid species occur, exist. There is no profile where all four gobiid species are present.

Table 7 shows the specifications of profile 2. For the following analysis all specifications with total case numbers less than ten were excluded.

Table 7: Specifications of profile 2 (=combination of gobiid species, absolute and relative counts per habitat type in the sampling period 14/15), abbreviations as explained in Table 1

Profile		Habitat Type			Total
		Habitats Danube Island	Main Channel	Side Arms /Backwaters	
1112 NeMe#PoKe#PrSe	Count	10	1	1	12
	% within Habitat Type	10.9%	0.5%	2.7%	3.8%
1121 NeMe#PoKe#BaGy	Count	1	1	0	2
	% within Habitat Type	1.1%	0.5%	0.0%	0.6%
1122 NeMe#PoKe	Count	16	30	1	47
	% within Habitat Type	17.4%	16.0%	2.7%	14.8%
1211 NeMe#PrSe#BaGy	Count	1	0	0	1
	% within Habitat Type	1.1%	0.0%	0.0%	0.3%
1212 NeMe#PrSe	Count	9	0	1	10
	% within Habitat Type	9.8%	0.0%	2.7%	3.2%
1222 NeMe	Count	8	81	6	95
	% within Habitat Type	8.7%	43.1%	16.2%	30.0%
2112 PoKe#PrSe	Count	2	0	0	2
	% within Habitat Type	2.2%	0.0%	0.0%	0.6%
2122 PoKe	Count	5	6	0	11
	% within Habitat Type	5.4%	3.2%	0.0%	3.5%
2211 PrSe#BaGy	Count	0	0	1	1
	% within Habitat Type	0.0%	0.0%	2.7%	0.3%
2212 PrSe	Count	13	0	2	15
	% within Habitat Type	14.1%	0.0%	5.4%	4.7%
2221 BaGy	Count	0	0	5	5
	% within Habitat Type	0.0%	0.0%	13.5%	1.6%
2222 No gobies	Count	27	69	20	116
	% within Habitat Type	29.3%	36.7%	54.1%	36.6%
Total	Count	92	188	37	317
	% within Habitat Type	100%	100%	100%	100%

Figure 28 to Figure 30 show how the different species structures appear in the three habitat types.

In the main channel the round goby as single gobiid species is the most common profile (almost half of the samples). The round goby in combination with the bighead goby is the second most frequent profile. There is no sample in the main channel where the tubenose goby appears as single goby (Figure 28).

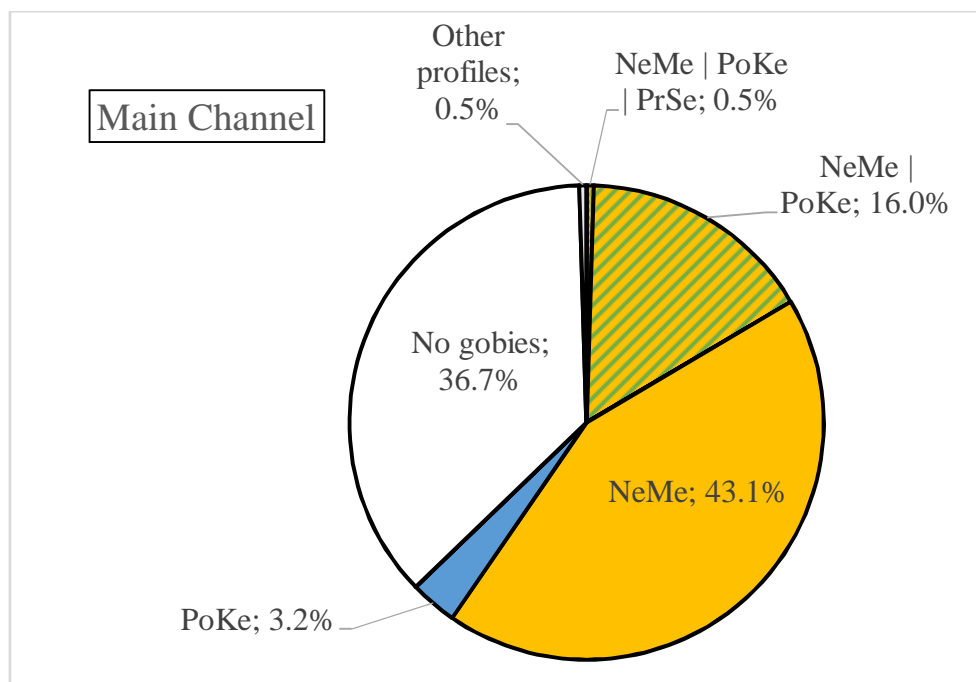


Figure 28: Species structure of gobies in the main channel, sampling period 14/15, abbreviations as explained in Table 1

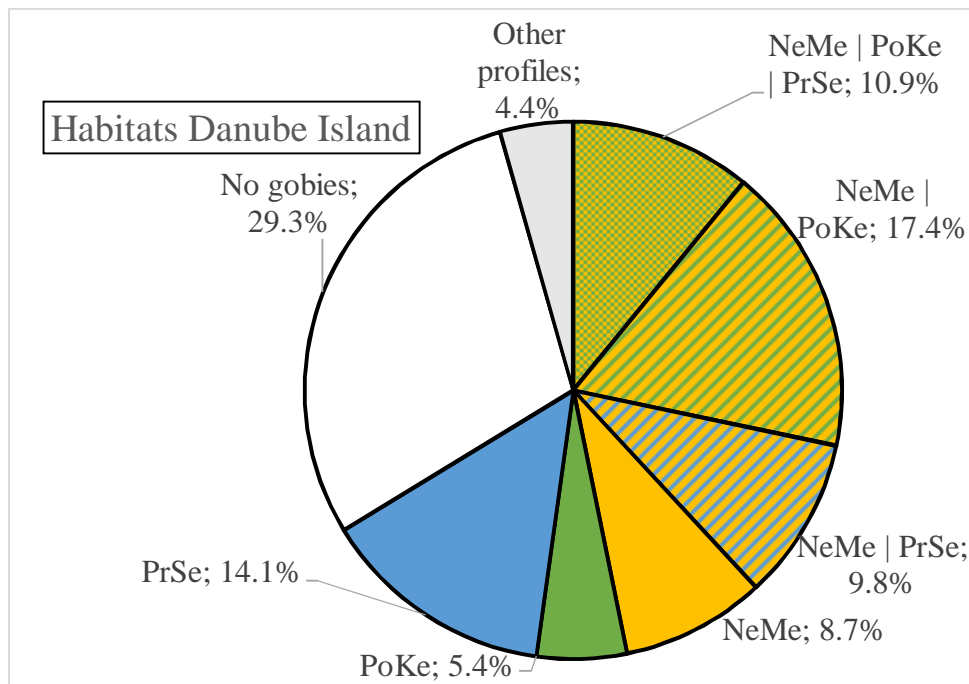


Figure 29: Species structure of gobies in the Danube Island habitats, sampling period 14/15, abbreviations as explained in Table 1

In the Danube Island habitats, the tubenose goby as single gobiid species appears in fourteen percent of the samples. More frequent is only the case “no gobies”, which represents almost one third of the samples. The combination of three gobies, round goby, bighead goby and tubenose goby forms as well a notable profile (Figure 29). Worth mentioning is the frequent occurrence of the western tubenose goby.

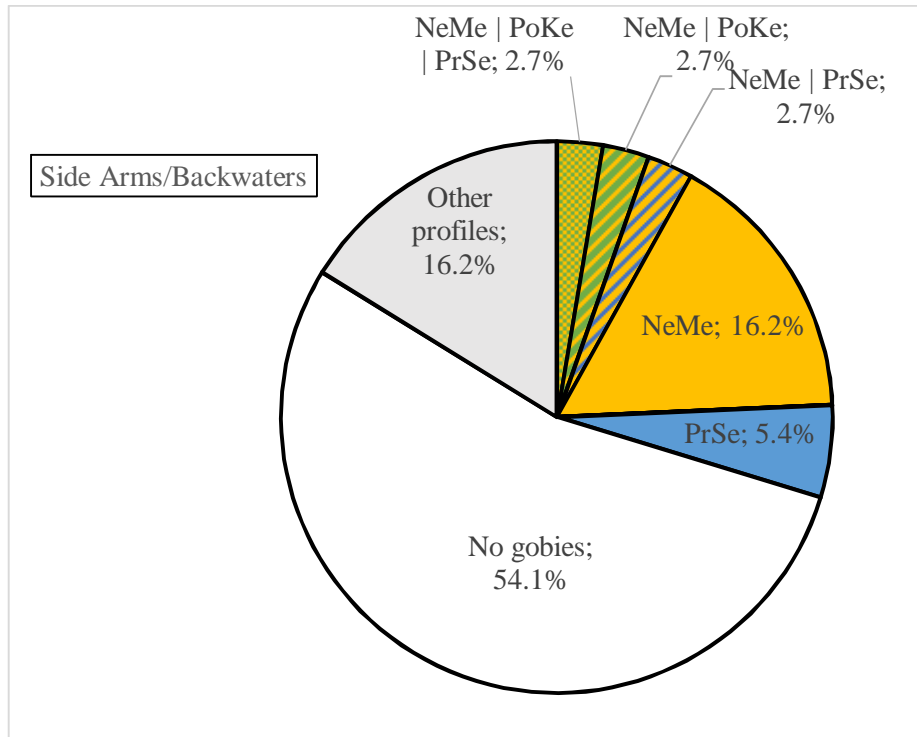


Figure 30: Species structure of gobies in the side arms and backwaters, sampling period 14/15, abbreviations as explained in Table 1

In the third habitat type, the side arms and backwaters, more than half of the samples did not contain any gobies. The round goby as single species was found in sixteen percent of the samples. The proportion of “other profiles” is quite big in the side arms as compared to the main channel and the Danube Island habitats (Figure 30). This is because the racer goby as single species, a profile which was not selected because of its low frequency regarding the global sampling pool of 14/15, appears in 13.5 % of the samples collected from the side arms.

The application of statistical tests for the hypothesis is limited because of the low and between habitat types strongly varying case numbers.

To clarify Hypothesis 1.2 typical features of every habitat type are pointed out in an explorative way. Therefore the residual test, which is usually used for the analysis of cross tabulations, is applied. Only the previously selected profile specifications, with a marginal distribution greater than ten, are included in the test.

Table 8: Standardized residuals showing typical and atypical profile specifications for the three habitat types, sampling period 14/15

	Habitats Danube Island			Main Channel			Side Arms		
	Obs.	Exp.	u[ij]	Obs.	Exp.	u[ij]	Obs.	Exp.	u[ij]
1112 NeMe PoKe PrSe	10	7	3.53	1	7	-2.34	1	1	-0.20
1122 NeMe PoKe	16	29	0.68	30	29	0.24	1	5	-1.72
1212 NeMe PrSe	9	6	3.61	0	6	-2.47	1	1	-0.01
1222 NeMe	8	58	-3.70	81	58	3.01	6	10	-1.17
2122 PoKe	5	7	1.03	6	7	-0.28	0	1	-1.06
2212 PrSe	13	9	4.18	0	9	-3.03	2	2	0.39
2222 No gobies	27	71	-1.10	69	71	-0.22	20	12	2.41

Significance level: $u(\alpha = 0.05) = |1.96|$ or $p = 0.05$

Interpretation of the local test statistics ($u[ij]$):

when $u[ij] > u[\alpha = 0.05] = 1.96$, then corresponding obs [ij] is statistically significantly over-represented ("typical" / "green").

when $-u[ij] < u[\alpha = 0.05] = -1.96$, then corresponding obs [ij] is statistically significantly under-represented ("atypical" / "red").

The residuals in Table 8 can be interpreted as typical and atypical profile specifications. Values greater than the test statistic of 1.96 (green) show typical profiles, values smaller than -1.96 (red) show atypical profiles for the three habitat types. As mentioned above, the round goby as only gobiid species ("1222 NeMe") can be called typical for the main channel, whereas the single tubenose goby ("2212 PrSe") is typical for the Danube Island habitats. In the side arms, only the profile "2222 No gobies" can be called typical.

Differences in species structures of gobies between the habitat types main channel, Danube Island habitats and side arms/backwaters could be observed in the sampling period 14/15. Therefore Hypothesis 1.2 should be rejected.

3.2. Temporal development of gobies abundance and structure in the Danube, 1993 – 2016

As mentioned already, the first non-native gobiid individual (not considering the western tubenose goby), which was a bighead goby (*Ponticola kessleri*), was found in a backwater at Regelsbrunn in 1993 (Ahnelt et al. 1998). The first racer goby (*Babka gymnotrachelus*) was found in the same water body in 1999 (Ahnelt et al. 2001). The round goby (*Neogobius melanostomus*) was also detected for the first time in the year 1999, in the port of Lobau (Wiesner et al. 2000). The monkey goby (*Neogobius fluviatilis*) has not yet been reported in the Austrian Danube.

These dates correspond well with the HPP Freudenu monitoring periods. The first sampling period went from 1993 to 1994, before establishment of the HPP. The second period went from 1999 to 2000, shortly after the HPP went into operation. During that period the inshore habitats on the left bank of the Danube already existed, since they were constructed as a compensation measure for the HPP. The last monitoring period went from 2013 to 2015, with some additional samples that were collected in the year 2016.

Regarding total species abundance, before construction of the HPP in 93/94, 49 different fish species were present in the Danube in Vienna. In 99/00, after the HPP went into operation, 50 species were found. In sampling period 14/15, 56 different fish species were found. The increase in species richness, which is caused by the arrival of neozoans, was accompanied by an overall decrease of abundance of most of the species (Waidbacher et al. 2016).

3.2.1. Hypothesis 2.1

Research Question 2.1: Did the abundance of *Gobiidae* (round goby, bighead goby, western tubenose goby and racer goby) in the impounded section of the Viennese Danube change during the last 24 years?

- *H2.1: The abundance of gobies in the Danube's main channel in the sampling periods 1993/1994, 1999/2000, 2002 and 2014/2015 is homogeneous.*

Hypothesis 2.1 addresses the temporal development of gobies' abundance in the Danube's main channel. For this purpose, samples collected by Wiesner in 2002 (Wiesner 2003) are included in the analysis. Wiesner collected samples from the Danube's main channel all over the Austrian part of the Danube and also from ports. Samples collected in ports were not considered here.

The sampling period "2002" is added to the dataset. The number of electrofishing samples collected from the main channel in the four sampling periods is given in Table 9.

Table 9: Number of electrofishing samples collected from the main channel and total number of collected samples in every sampling period

	Sampling_period				Total
	93/94	99/00	2002*	14/15	
Main Channel	300	98	48	188	634
Total Nr. of Samples	344	220	48	317	929

The highest number of samples was collected from the main channel. The presence of gobies in the main channel by sampling period is shown in Figure 31. The western tubenose goby was the only gobiid species present in the first sampling period, therefore it represents 100% of the present gobiid species. In period 99/00 the first "Neogobius" species were found.

In 2002, gobies were present in 100% of the samples. The percentage of samples containing the western tubenose goby increased as well in 2002. It is important to keep in mind that the samples collected in 2002 were part of a different project, so sampling methods might be slightly different. It is as well important to know that in the results of 2002, samples from different parts of the Austrian Danube are included.

Samples collected in 14/15 show a lower percentage of samples containing gobies and the western tubenose goby almost disappeared in the main channel, it was found in only one sample.

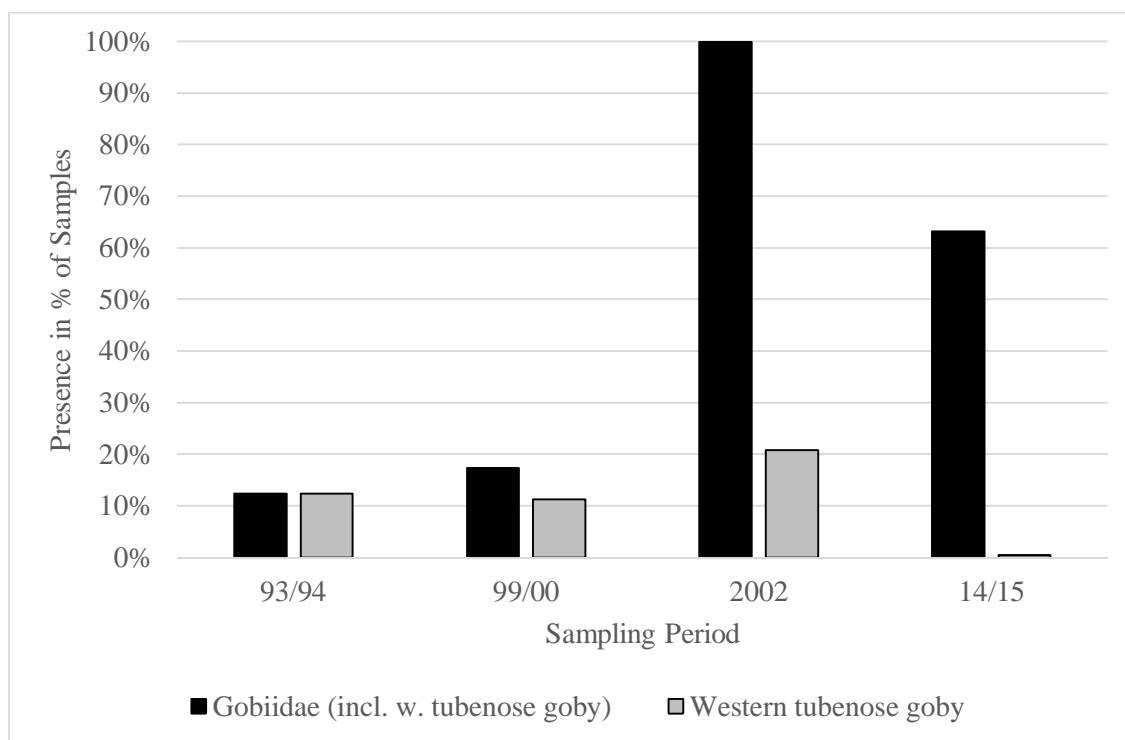


Figure 31: The presence of gobies and additionally the presence of the western tubenose goby in % of the samples by sampling period, main channel only.

Table 10: Presence/absence of gobies by sampling period, main channel only

		Sampling period				Total
		93/94	99/00	2002	14/15	
Gobies absent	Count (Obs.)	263	81	0	69	413
	%	87.7%	82.7%	0.0%	36.7%	70.5%
	u[ij]	3.5	1.4	-5.6	-5.5	
Gobies present	Count (Obs.)	37	17	48	119	221
	%	12.3%	17.3%	100%	63.3%	29.5%
	u[ij]	-5.5	-2.2	3.0	8.5	
Total	Count (Obs.)	300	98	48	188	634
	%	100%	100%	100%	100%	100%

Notes: Standardized residuals = **u[ij]** ;

Significance level (corrected after Bonferroni) :

$u(\alpha^* = \alpha / \text{cells number} = 0.05 / 8 = 0.00625) = |2.73|$

Interpretation of the local test statistics (**u[ij]**):

when $u[ij] > u[\alpha^* = 0.00625] = 2.73$, then corresponding obs [ij] is statistically significantly over-represented ("**typical**" / "green").

when $-u[ij] < u[\alpha^* = 0.00625] = -2.73$, then corresponding obs [ij] is statistically significantly under-represented ("**atypical**" / "red").

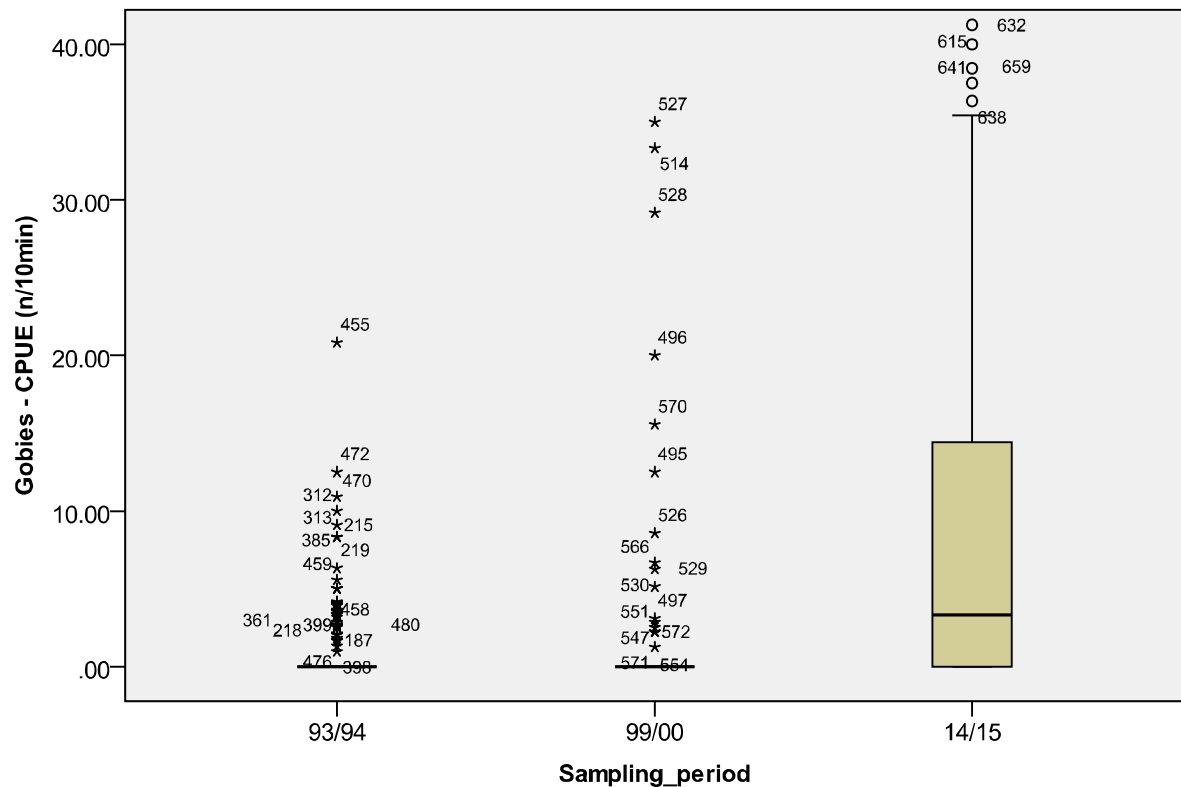
Table 10 shows the presence and absence of gobies in samples, not differentiating between species. The total number of samples varies with sampling periods, as does the number of samples where gobies are present. In 2002, gobies were found in all samples.

Considering the number of samples standard residuals can be calculated. According to the residuals, in the periods 2002 and 14/15 the presence of gobies was typical, whereas it was atypical in the period 93/94.

Additionally, the Median test was applied to test whether significant differences of gobies' abundance between the sampling periods can be found.

The sampling period 2002 could not be included into the test because the data were not available in the necessary form. The test results are shown for the periods 93/94, 99/00 and 14/15 only.

Samples with a CPUE = 0/10 min, which applies for the majority of samples from the first two periods, were included in the analysis.



Median-test, test-statistic 152.66, df = 2, p = 0.000

Figure 32: Boxplot Gobies (CPUE n/10min) by sampling period, asterisks (*) indicate extreme values, dots (°) indicate outliers

Figure 32 shows the distribution of CPUE (n/10min) for gobies by sampling period. It is visible that in period 93/94 and also in 99/00 the median of CPUE (n/10min) is practically zero.

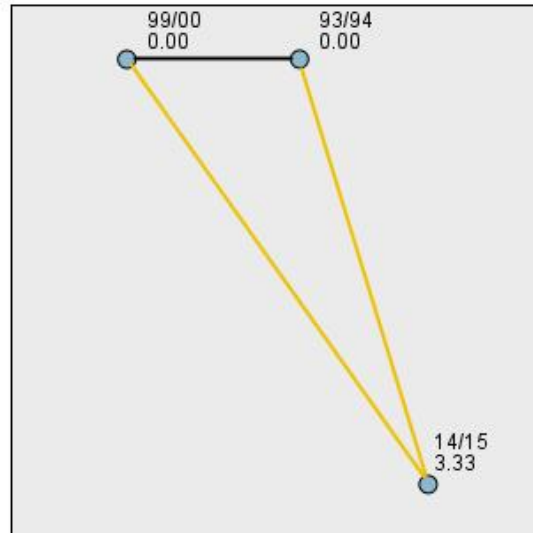
Table 11: Median-test result, dependent variable = CPUE (n/10min) for Gobiidae, factor = sampling period, only main channel samples are used

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The medians of (n/10min) are the same across categories of Sampling_period.	Independent-Samples Median Test	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Pairwise Comparisons of Sampling_period



Each node shows the sample median of Sampling_period.

Sample1-Sample2	Test Statistic	Sig.	Adj.Sig.
93/94-99/00	1.583	.208	.625
93/94-14/15	138.030	.000	.000
99/00-14/15	54.539	.000	.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

Figure 33: Pairwise comparison, Median test, dependent variable = CPUE (n/10min) for Gobiidae, factor = sampling period, only main channel samples are used

The Post-Hoc test of the Median test shows where significant differences can be found. Every sampling period is shown by one node in Figure 33. Significant differences between the nodes are indicated by yellow lines. There is no difference between the periods 93/94 and 99/00.

According to the residual test the presence of gobies is clearly typical for the periods 2002 and 14/15, contrary to the period 93/94 where the results show that the absence of gobies is typical.

The results of both statistical tests, the Median test and the residual test, reveal that Hypothesis 2.1 should be rejected.

3.2.2. Hypothesis 2.2

Research Question 2.2: Did the species structure of gobies in the River Danube change during the years of invasion?

- *H2.2: The species structure of gobies in the periods 1993/1994, 1999/2000, 2002 and 2014/2015 is homogeneous.*

Hypothesis 2.2 addresses the homogeneity of species structure in the different sampling periods. Therefore the composition of gobies is analysed in every habitat type (main channel, habitats Danube Island, side arms and backwaters). In Table 12 the number of samples for every habitat type in all sampling periods is given. The habitats on the Danube Island did not exist in sampling period 93/94, therefore the number of collected samples is zero.

Table 12: Number of electrofishing samples per habitat type and sampling period

	Sampling_period				Total
	93/94	99/00	2002*	14/15	
1 Habitats Danube Island	0	88	0	92	180
2 Main Channel	300	98	48	188	634
3 Side Arms/Backwaters	44	34	0	37	115
Total	344	220	48	317	929

* data collected by Wiesner (2003)

Figure 34 shows the presence of each gobiid species by period in the main channel. Wiesner's data of the sampling year 2002 are again included in this graph. Again it is important to keep in mind that samples collected by Wiesner include also samples collected from other parts of the Austrian Danube.

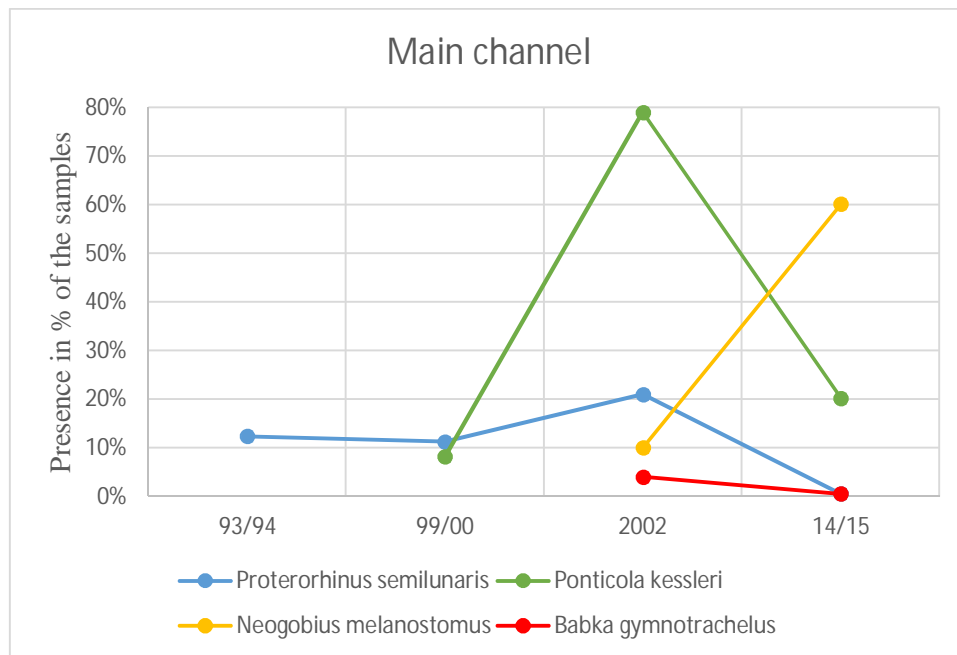


Figure 34: The presence of gobies in % of the samples by sampling period, main channel

The presence of tubenose goby decreased from the first to the last sampling period, with an exception in 2002, where it appeared in almost one quarter of the samples. Currently the tubenose goby can hardly be found in the main channel (it was present in only one sample).

The bighead goby was not present in 93/94 but it already appeared in some samples in 99/00. Conspicuous is the dominance of the bighead goby in 2002, which Wiesner found in more than three quarters of the samples. In period 14/15 the bighead goby was found in almost one quarter.

The currently dominant goby is the round goby. In 93/94 and 99/00 it was not present at all in the main channel. Wiesner found the round goby in ten percent of his samples (Wiesner 2003). In period 14/15 it was the most abundant gobiid species with a presence of sixty percent.

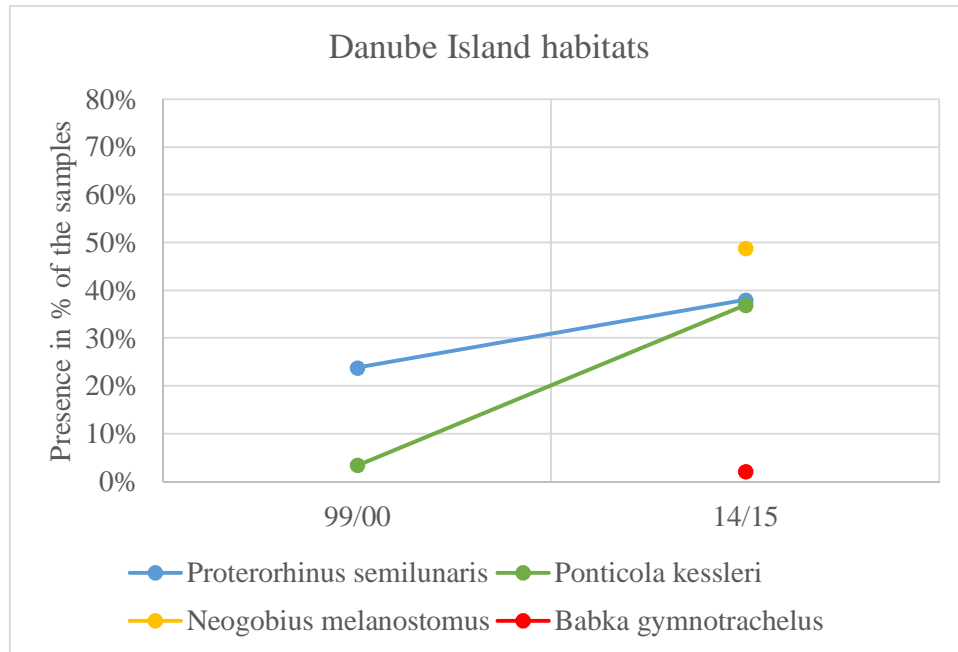


Figure 35: The presence of gobies in % of the samples by sampling period, Danube Island habitats

In Figure 35 the results for the presence in the Danube Island habitats are shown. The habitats did not exist in 93/94 and were not sampled in 2002, therefore these periods are missing. All four gobiid species were found in 14/15. The most abundant species was the round goby in period 14/15, it was found in almost half of the samples. Generally, samples that do not contain any gobies at all are more frequent in the Danube Island habitats than in the main channel.

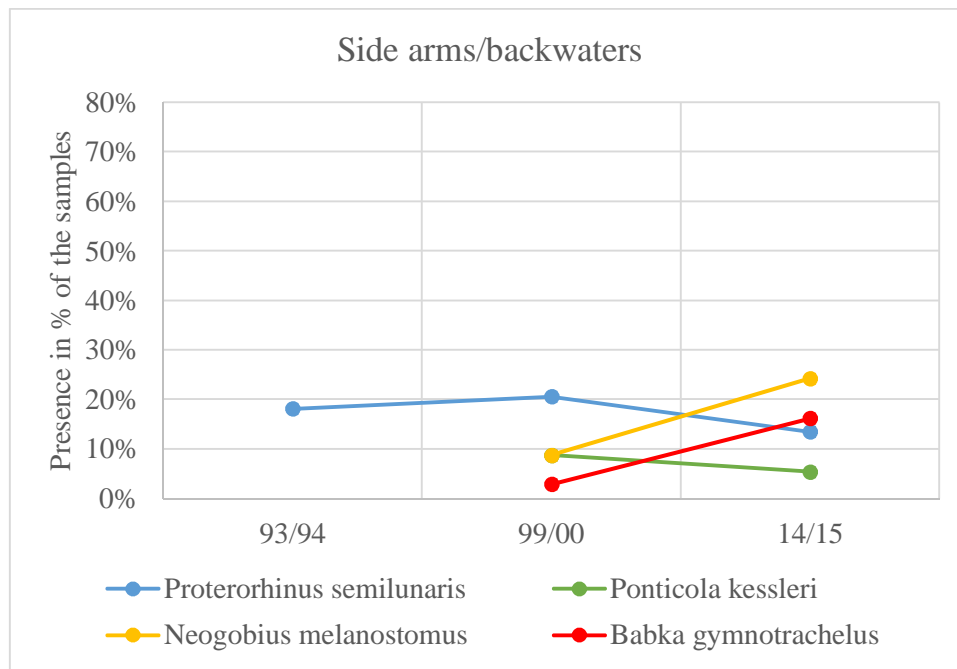


Figure 36: The presence of gobies in % of the samples by sampling period, side arms

Finally, in the side arms and backwaters, as shown in Figure 36, again the round goby shows the highest presence in period 14/15. It was found in one quarter of the samples. The side arms are the only habitat type in which several individuals of racer goby can be found. It is important to know that the side arms and backwaters are very heterogeneous. Racer gobies were found in the upper reaches of the Freudenu impoundment, in the Langenzersdorfer Arm and in some backwaters in the Klosterneuburger Au.

It is also important to mention and visible in Figure 36 that in all sampling periods, the majority of samples collected from the side arms were free of gobies.

The monkey goby (*Neogobius fluviatilis*) was not found in any of the three habitat types in any sampling period.

Figure 34 to Figure 36 show the CPUE in n/10min of each gobiid species in percent of the CPUE of all gobies. It is visible that the presence of gobies in period 93/94 and 99/00 was very low compared to period 14/15. In the majority of the samples no gobies were found, only the western tubenose goby was present in some. In 14/15 at least one of four gobiid species was found in almost two thirds of the samples.

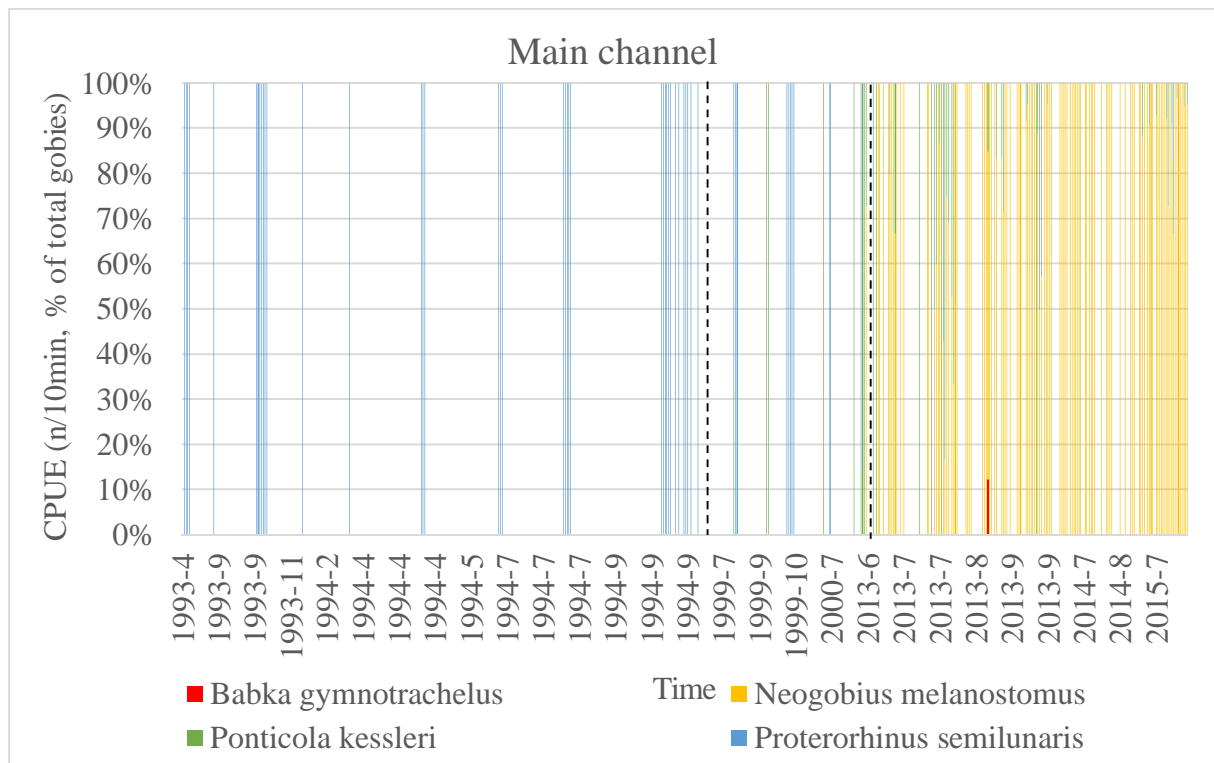


Figure 37: Species structure of gobies in the main channel, 1993 to 2016. Dashed lines indicate the borders between the periods

In Figure 37 all samples included in the analysis are arranged chronologically, starting from 1993. White areas show when gobies were absent. It is clearly visible that the density of gobies in the main channel is much higher in the last period than in the first and second period. The species structure changed as well and shifted from the western tubenose goby to a dominance of round goby. In between bighead gobies showed increased presence.

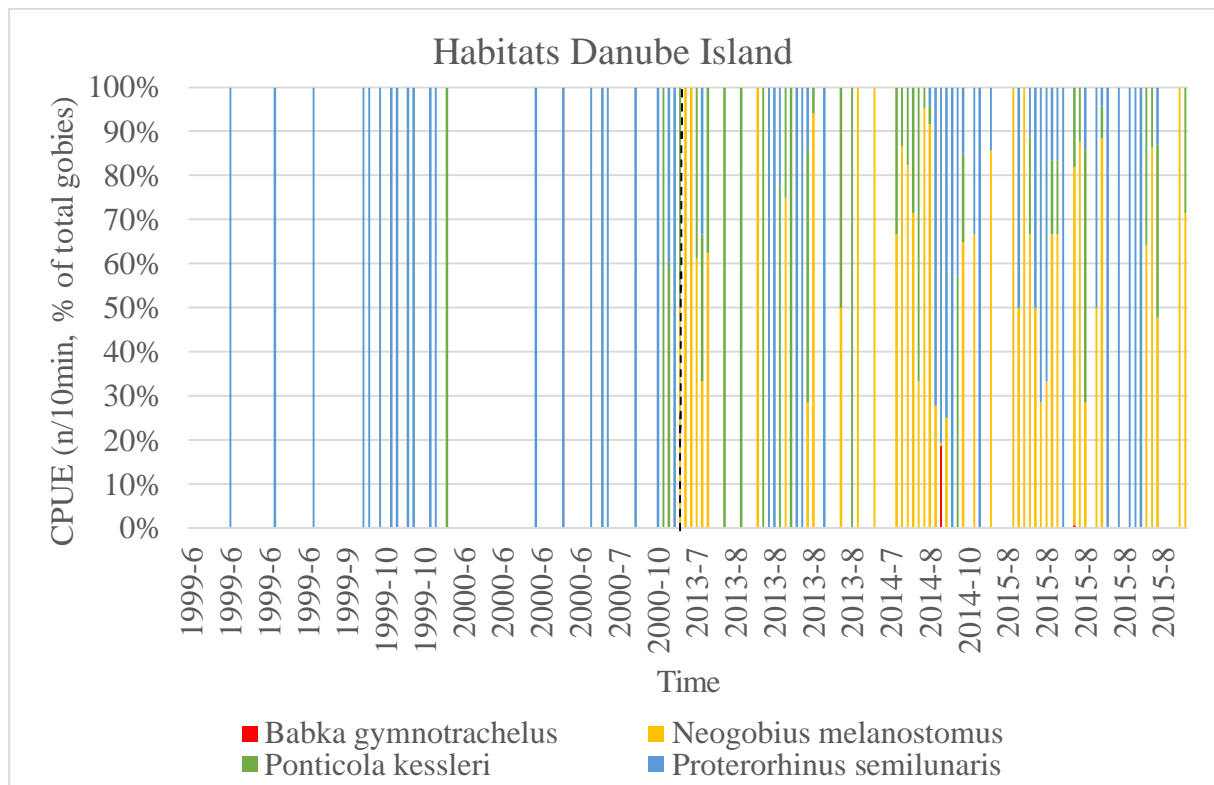


Figure 38: Species structure of gobies in the inshore habitats on the Danube Island, 1999 to 2016. Dashed lines indicate the borders between the periods

A similar result can be produced for the habitats on the Danube Island. In Figure 38 the relative CPUE of gobies is plotted chronologically for all samples collected from the Danube island habitats. The difference to the main channel is that the structure changed from the western tubenose goby as single goby to a mixture of three gobiid species in the last period, where the western tubenose goby is still present in many samples.

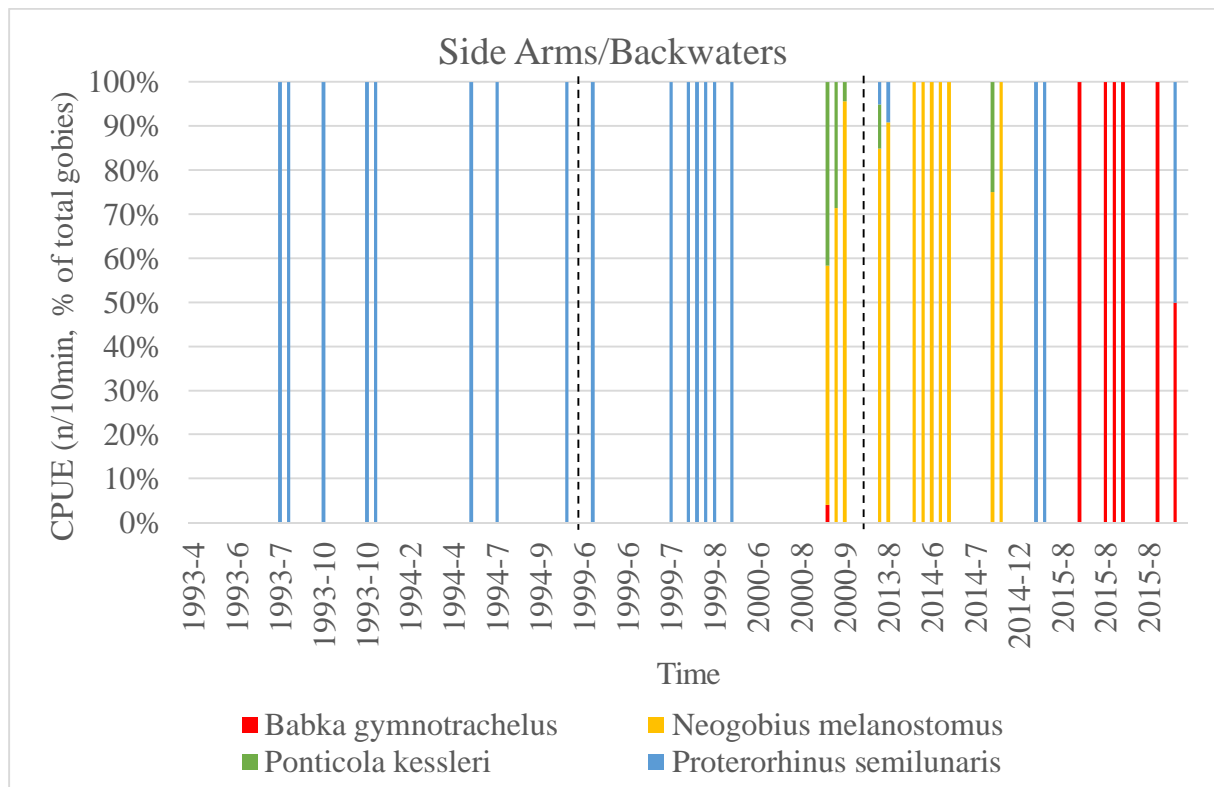


Figure 39: Species structure of gobies in the inshore side arms and backwaters, 1993 to 2016. Dashed lines indicate the borders between the periods

The chronological order of samples for the side arms and backwaters again shows a shift in the composition of species from western tubenose goby only to the presence of even four different gobies in the last period. Samples from the year 2000 already contained racer, bighead and round gobies (Figure 39). The plot cannot be interpreted in the way that the round goby, which was abundant in 2013 and 2014 was replaced by the racer goby in 2015. The reason for this “fake” chronological shift in Figure 39 is that samples were collected in different locations.

To evaluate Hypothesis 2.2 statistically, the same profiles as in Hypothesis 1.2 (Chapter 3.1.2) were used. A residual test was performed using only profiles of which total case numbers exceeded ten cases. In Table 14 all the profile specifications included in the residual test are shown.

Table 13: Profiles occurring in the side arms, all sampling periods

Profile	Gobiid species in the sample
PoKe	Only bighead goby
PrSe	Only western tubenose goby
NeMe	Only round goby
NeMe PoKe	Round goby and bighead goby in combination
NeMe PoKe PrSe	Round goby, bighead goby and western tubenose goby in combination
No gobies	No gobies

Table 14: Observed (obs.) and expected (exp.) values and standard residuals ($u[ij]$) showing typical and atypical profiles in every sampling period in the main channel

Main Channel		Sampling_period									
		93/94			99/00			14/15			
		obs.	exp.	$u[ij]$	obs.	exp.	$u[ij]$	obs.	exp.	$u[ij]$	Total
1122 NeMe PoKe	Count	0	15	-3.93	0	5	-2.22	30	10	6.59	30
1222 NeMe	Count	0	42	-6.46	0	13	-3.66	81	26	10.83	81
2122 PoKe	Count	0	6	-2.49	6	2	2.86	6	4	1.11	12
2212 PrSe	Count	37	24	2.73	9	8	0.51	0	15	-3.83	46
2222 No gobies	Count	263	213	3.43	81	68	1.56	69	132	-5.48	413
	Count	300			96			186			582

Notes: Standardized residuals = $u[ij]$;

Significance level (corrected after Bonferroni) :

$u(\alpha^* = \alpha / \text{cells number} = 0.05 / 15 = 0.0033) = |2.94|$

Interpretation of the local test statistics ($u[ij]$):

when $u[ij] > u[\alpha^* = 0.0033] = 2.94$, then corresponding obs [ij] is statistically significantly over-represented ("typical" / "green").

when $-u[ij] < u[\alpha^* = 0.0033] = -2.94$, then corresponding obs [ij] is statistically significantly under-represented ("atypical" / "red").

The test shows of course that in the first period, the absence of gobies is typical and in the last period, the presence of round goby and also the combination of round goby and bighead goby is typical (Table 14). What is interesting is that in period 93/94 only the absence of gobies was typical, the presence of the western tubenose goby was not. In period 14/15 the presence of western tubenose goby in the main channel is considered as atypical.

Table 15: Observed (obs.) and expected (exp.) values and standard residuals (u[ij]) showing typical and atypical profiles in every sampling period in the Danube Island habitats

Habitats Danube Island		Sampling_period						
		99/00			14/15			Total
		obs.	exp.	u[ij]	obs.	exp.	u[ij]	
1112 NeMe PoKe PrSe	Count	0	6	-2.37	10	4	2.69	10
1122 NeMe PoKe	Count	0	9	-3.00	16	7	3.41	16
2212 PrSe	Count	20	19	0.33	13	14	-0.37	33
2222 No gobies	Count	65	52	1.84	27	40	-2.08	92
		85.00			66.00			151.00

Notes: Standardized residuals = $u[ij]$;

Significance level (corrected after Bonferroni) :

$u(\alpha^* = \alpha / \text{cells number} = 0.05 / 8 = 0.00625) = |2.73|$

Interpretation of the local test statistics ($u[ij]$):

when $u[ij] > u[\alpha^* = 0.00625] = 2.73$, then corresponding obs [ij] is statistically significantly over-represented ("typical" / "green").

when $-u[ij] < u[\alpha^* = 0.00625] = -2.73$, then corresponding obs [ij] is statistically significantly under-represented ("atypical" / "red").

Interestingly, in the Danube Island habitats only the combination round goby with bighead goby is considered as typical in the last period. The combination of round goby, bighead goby and western tubenose goby is almost typical as well.

Table 16: Observed (obs.) and expected (exp.) values and standard residuals (u[ij]) showing typical and atypical profiles in every sampling period in the side arms and backwaters

Side Arms/Backwaters		Sampling_period									
		93/94			99/00			14/15			
		obs.	exp.	u[ij[]	obs.	exp.	u[ij[]	obs.	exp.	u[ij[]	Total
2212 PrSe	Count	8	8	0	7	5	0.67	2	4	-0.95	17
2222 No gobies	Count	36	36	0	24	26	-0.31	20	18	0.44	80
		44			31			22			97

Notes: Standardized residuals = $u[ij]$;

Significance level (corrected after Bonferroni) :

$u(\alpha^* = \alpha / \text{cells number} = 0.05 / 6 = 0.00833) = |2.64|$

Interpretation of the local test statistics ($u[ij]$):

when $u[ij] > u[\alpha^* = 0.00833] = 2.64$, then corresponding obs [ij] is statistically significantly over-represented ("typical" / "green").

when $-u[ij] < u[\alpha^* = 0.00833] = -2.64$, then corresponding obs [ij] is statistically significantly under-represented ("atypical" / "red").

According to the residual test, no typical and no atypical profiles could be identified for the side arms and backwaters in any sampling period (Table 16).

According to the results of the residual test, Hypothesis 2.2 should be rejected.

3.3. Evaluation of sampling efficiency of two electrofishing methods

During electrofishing, only a part of the present individuals is effectively caught. Especially in rip-rap sections fish are often seen after being narcotized but then they quickly disappear into the holes between the stones without being caught. To consider this side-effect in the results, the sampling efficiency is calculated at the end of the fishing activity. The number of sighted fish is added to the number of caught fish, then the CPUE is calculated to get a standardized value of individuals caught in a time span of 10 minutes ($n/10\text{min}$).

In Hypothesis 3.1 two different methods are evaluated regarding sampling efficiency of benthic fish species by comparing the CPUE ($n/10\text{min}$) of two methods.

3.3.1. Hypothesis 3.1

Research Question 3.1: Is the sampling efficiency of small benthic fish species in rip-raps higher when a portable backpack electrofishing unit is used in comparison to the use of a stationary electrofishing unit?

- *H3.1: The sampling efficiency of small benthic fish species is homogeneous for both methods (electrofishing with a portable backpack unit and with a stationary unit)*

Two different electrofishing methods were applied during sampling in the year 2016. In total, 15 samples containing 267 caught individuals were collected at seven sampling sites in the main channel. At each site one sample was collected with a stationary electrofishing unit and right next to it another sample with the portable backpack unit. There is just one site where only one sample with the portable unit was taken, this sample was not included in the test of Hypothesis 3.1.

The benthic species caught in 2016 were three gobiid species (round goby, bighead goby and western tubenose goby) and the bullhead.

To test the two methods for differences in sampling efficiency the Median test was applied. For every sample, the sum of the “Catch per unit effort” (CPUE, n/10min) of all benthic fish species was calculated. This CPUE including all benthic species was used as dependent variable.

At every sampling site, two samples were collected, one with each sampling gear. Hypothesis 3.1 assumes that the number of caught fish is the same for the samples collected at one site, despite the different gear.

The Boxplot in Figure 40 shows the distribution of CPUE (n/10min) for both methods.

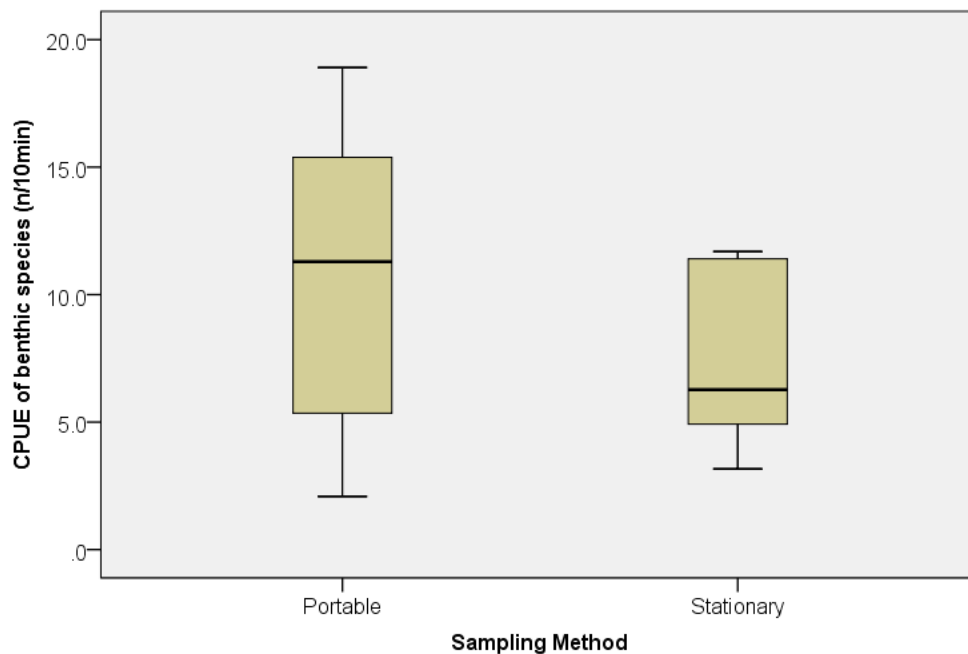


Figure 40: Boxplot of CPUE (n/10min) for two electrofishing methods, sampling year 2016

The Median test showed that the number of caught fish (CPUE) is statistically the same for both methods (sampling with a portable and a stationary electrofishing unit), therefore Hypothesis 3.1 should be retained. That means that the results of the two methods are comparable and differences in sampling efficiency are not related to different gear.

Table 17: Median test result for Hypothesis 3.1

Hypothesis Test Summary				
	Null Hypothesis	Test	Sig.	Decision
1	The medians of gobies_plus_benthic are the same across categories of method.	Independent-Samples Median Test	1.000 ^{1,2}	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

¹Exact significance is displayed for this test.

²Fisher Exact Sig.

4. Conclusion

Hypothesis 1.1 addresses the spatial distribution of gobies and states that their distribution in the impounded section of the Danube in Vienna is homogeneous. The impoundment was divided into eight parts combining the four zones of the impoundment with the two river banks. The results show that the distribution is not homogeneous. The right bank in the head of impoundment is significantly different from the left bank in the head of impoundment and also from the left bank in the tailwater zone. The hypothesis is rejected.

The analysis of species structure shows differences between the three habitat types. The occurrence of round gobies is typical for the main channel. In the riparian habitats on the Danube Island the combination of round gobies and western tubenose gobies and often also bighead gobies in one sample is typical. The occurrence of western tubenose goby alone is as well frequent in these habitats. In the side arms and backwaters the absence of gobies is typical. Therefore, Hypothesis 1.2 is also rejected.

The analysis of the historical development of gobies in the Danube in Vienna shows a clear increase in their abundance from 1993/1994 until today. In period 1993/1994 the presence of gobies is atypical, whereas it is typical in the year 2002 and the period 2013-2016. Hypothesis 2.1 assumes that the abundance of gobies is homogeneous in all sampling periods and is for this reason rejected.

Regarding species structure of gobies, Hypothesis 2.2 states that it is homogeneous in all sampling periods. The results show that the presence of the round goby as single gobiid species and also the combination of round goby and bighead goby is currently typical for rap-raps in the main channel and the riparian habitats on the Danube Island. In the first sampling period, only the absence of gobies is typical. In the side arms and backwaters, no typical profiles could be identified, neither in the first nor in the last period. Therefore Hypothesis 2.2 is rejected for the main channel and the riparian habitats on the Danube Island.

The assumption of Hypothesis 3.1, that sampling efficiencies of a portable and a stationary electrofishing unit are the same, could be confirmed.

5. Discussion

5.1. Spatial distribution of gobies in the Viennese Danube

Distribution of gobies in the impounded main channel

In the sampling period 2013 to 2016 four different gobies were found in the Viennese Danube. In general, the abundance of gobies was high in all rip-raps in the impoundment. Still, statistical analysis showed that the abundance of gobies varies with different zones of the impoundment. The highest abundances were found on the right bank in the head of impoundment, what might be explained by several reasons. The four zones of the impoundment and also the river banks can be distinguished mainly in terms of flow velocity and corresponding sedimentation. Most probably the varying availability of food in different zones plays a role for the distribution of gobies.

A master thesis project realized at IHG in 2014 treated the diet of round gobies in the impounded Danube-section in Vienna. The main food items found in gut-content analyses of 280 individuals of round goby were dipterans, trichoptera, molluscs and crustaceans. Piscivory did not occur. An ontogenetic shift toward molluscivory was confirmed. Molluscivory individuals could mainly be associated with the central impoundment, whereas individuals feeding on crustaceans were most common in the transition zone. The free flowing stretches were characterised by a diet dominated by dipterans (Ebm 2016).

The wetland “Klosterneuburger Au” is located on the right bank in the head of impoundment and is connected via side arms and backwaters to the main channel. The connection to the wetland might increase the availability of food items for gobies.

The lower abundances in the tailwater section might be related to higher flow velocities and also to varying water tables. In periods with low flow the rip-raps downstream of the hydro power plant can go dry, as it was the case during sampling in October 2016. These conditions might be unfavourable for a very intensive colonization by gobies.

Structure of gobies in different habitat types

The round goby is currently dominating the main channel. Artificially created inshore structures on the Danube Island showed a more diverse structure of gobiid species with higher abundances of bighead and western tubenose goby. The reason for the different structures might be related to different habitat and feeding preferences of the species as well as to competition between the species (Brandner et al. 2013).

In the lower parts of the river Rhine in Germany, four non-native gobies were reported in 2009, western tubenose goby, round goby, bighead goby and monkey goby. Round gobies were preferably found in rip-raps, whereas bighead gobies were present in all kind of different habitat types (Borcherding et al. 2011).

The same authors reported from lab experiments that when round and bighead gobies were directly competing in a habitat, the round goby would win in conflicts against the bighead goby, although it is smaller in body size. Concerning the competition for food, the bighead goby preferred fish while the round goby fed mainly on *Dikerogammarus spp.* Therefore, they assumed that by colonizing different niches a co-existence of both species in the river Rhine is possible (Borcherding et al. 2013).

Furthermore, the bighead goby shows an ontogenetic shift in diet, predation on fish as well as cannibalism increased when species were growing. Round gobies like to shift their diet to molluscs (Brandner et al. 2013, Ebm 2016).

The same study also revealed that invasive species were dominant in the diet of both species, bighead goby and round goby. The most important food items for both species were the Crustacean *Dikerogammarus spp.* and other amphipods (Brandner et al. 2013).

The invasive round goby might have led to the displacement of bighead gobies and also western tubenose gobies to the inshore habitats. Considering the piscivorous character of the bighead goby as described by Borcherding et al. (2013) the presence of fish larvae and other small fish species in these habitats could be favourable for the diet of the bighead goby.

5.2. Temporal development of gobiid distribution

The analysis of gobies' temporal dispersion in the Viennese Danube clearly shows a massive increase in abundance during the last 15 to 20 years. Regarding the number of species it increased from one present species in 1993/1994 (the western tubenose goby) to four species in 1999/2000, when the first individuals of bighead gobies, racer gobies and round gobies were detected in or around Vienna (Ahnelt et al. 1998, Wiesner 2000).

In 1999/2000, several individuals of bighead goby were found in the Danube's main channel and in inshore-habitats on the Danube Island. Therefore it was the only "new" gobiid species that was detected upstream of the HPP. Single racer and round gobies were detected in samples collected from the port in Lobau, which is located downstream of the Freudenau impoundment (Ahnelt et al. 1998, Wiesner 2000).

Even in 2002, no round gobies or racer gobies were present in samples collected from upstream the HPP. Going upstream, the next "distribution centre" for round gobies was the harbour of Krems, where the round goby was very abundant (Wiesner 2003). The racer goby was not detected in any samples of the Austrian Danube upstream of the HPP Freudenau (Wiesner 2003).

On the other hand, bighead gobies were present in all samples collected in 2002 and were overall the most abundant species in Danube rip-raps (Wiesner 2003). According to the dataset used in the present study, in 1999, bighead gobies were not found beyond the HPP, but in 2000, the first individuals were detected in the central impoundment. In the recent sampling period 2013-2016 bighead gobies had decreased a lot in numbers compared to the situation of 2002, but therefore the round goby was dominating rip-raps in the main channel.

In the inshore habitats on the Danube Island it is currently as well the round goby that dominates but not as clearly as it is the case in the main channel. Species structure in these habitats is much more diverse and higher frequencies of bighead and tubenose gobies are found.

In the same port, where round goby and bighead goby were detected in 1999, also the racer goby was present at that time (Ahnelt et al. 1998). This might be another indicator that ports act as centres of gobies' dispersal.

The development of racer goby populations

According to Kottelat and Freyhof (2007) the racer goby is a limnophilic species. In their “Handbook of European Freshwater Fish” it is described to be abundant mainly in backwaters and channels with low or zero flow velocity that are well vegetated. In its native range it is found mainly in habitats with sandy or muddy bottoms (Kottelat and Freyhof 2007).

In 2015, the racer goby was detected in oxbow lakes, side arms and backwaters located in the head of impoundment. It is not clear how the racer goby arrived in these water bodies, which are situated many kilometres upstream of the HPP. Waterfowl may play a major role in dispersion.

In 2002, Wiesner could not find any individuals of racer goby upstream of the HPP Freudenau at all. The only water bodies in Austria where the racer goby was present were all located east (so downstream) of Vienna (Wiesner 2003). Between 2013 and 2015, only one sample collected from the main channel contained racer gobies and two samples collected from the inshore habitats on the Danube Island.

Other surveys on fish species in the Austrian Danube between 2002 and 2013 showed that racer gobies were indeed present in other parts of the Austrian Danube. In the years 2005, 2007, 2010 and 2013 they were found in samples collected in Melk, Ardagger and also in the Bavarian part of the Danube (Jungwirth et al. 2014).

In Germany, the first individuals of racer gobies were found in the River Rhine (Borcherding et al. 2011) and shortly after in the year 2011 also in a backwater of the Danube at Regensburg (Haertl et al. 2012).

If the individuals found in the side arms and backwaters in the head of the Freudenau impoundment are related to downstream or upstream populations is not known.

Haertl et al. (2012) assume that the range of racer gobies might already be larger than what is known. In most studies on gobies, sampling effort focusses on the main channels. To find out more about the dispersion of racer gobies, backwaters as well as shallow, muddy habitats in rivers should be included in sampling activities more frequently.

The replacement of western tubenose goby by the bighead goby

In 1998, when Ahnelt et al. studied for the first time the occurrence of new gobies in the middle and upper Danube, they stated that the western tubenose goby and the bighead goby share the same habitat preferences. They found out that many rip-raps, which were formerly an ecological niche occupied by the western tubenose goby, were then dominated by the bighead goby. In all habitats where the bighead goby was abundant a decline of the western tubenose goby was observed (Ahnelt et al. 1998).

The bighead goby was not only a competitor for shelter but also for food. Since it is larger in body size and has as well a much bigger gape width it was considered to dominate over the western tubenose goby in terms of feeding. It was also assumed that the bighead goby, being a predator that feeds as well on small fishes, could predate on the western tubenose goby (Ahnelt et al. 1998).

In the lower parts of the river Rhine, the western tubenose goby was found for the first time in 1999 (Borcherding et al. 2011). It was reported to be widespread but concerns that it would become invasive did not get real. In 2010 it was mainly found in lentic backwaters and less in the main channel (Borcherding et al. 2011).

Western tubenose gobies in the Viennese Danube are probably the losers in competition against other gobiid species and therefore reduced a lot in number during the past years.

Possible reasons for the success of round goby and the decrease of bighead goby

Detailed studies on feeding behaviour revealed that the round goby was more able to adapt its diet to the local availability of food at the invaded sites than the bighead goby. It showed as well a clear shift in diet between early and late summer, meaning that the round goby is well able to adapt to seasonal variations of food. For the bighead goby, this shift was much less pronounced. Although both species, round and bighead goby, are omnivorous predators, bighead gobies can be classified as stenophag whereas round gobies are rather classified as euryphag species (Brandner et al. 2013). This might be a reason why the round goby finally succeeded over the bighead goby in the Austrian Danube.

Of course also genetic aspects play a role for the success in developing sustainable populations (Cerwenka et al. 2014, Kalchhauser et al. 2016).

Natural migration rates of gobies are very low, therefore their home ranges are narrow and gene flow is restricted. Passive downstream drift can increase the gene flow though. There are as well differences between species concerning the ability to swim upstream. The round goby is reportedly more able to swim upstream than other gobies. So their chances to mix with other populations are higher (Kornis et al. 2012). This ability could also contribute to the fact that in the Danube, round gobies are more successful invaders than bighead gobies.

Stepien et al. (2005) evaluated the success of invasive species by using DNA sequencing. They concluded that invasive gobies in the North American Great Lakes showed high genetic diversities. This and the fact that gobies that invaded the Great Lakes originated from multiple sources of invasion contributed to their persistent invasion success.

In Germany, the invasion of the Danube between the junction of the Rhine-Main-Danube Canal (RMDC) and Passau by gobiid species happened most likely in two directions. Rhine-populations spread via the RMDC and populations of the Danube joined from downstream. Cerwenka et al. (2014) hypothesized that populations of the bighead goby, which was less successful with the development of sustainable populations in the Danube, would show local population structures that are much less pronounced than the structure of the currently invasive and successful round goby. The results of AFLP (amplified length polymorphism) and mtDNA cytochrome B analysis showed that the overall genetic variability of round gobies in the Danube between the RMDC-junction and Passau is definitely higher than the variability of bighead gobies (Cerwenka et al. 2014).

This could explain the short peak of bighead gobies abundance in the Danube and the following rapid replacement of bighead gobies by round gobies in the past years. Their decline may be related to inbreeding rather than to interspecific competition. The two impoundments Freudenau and Greifenstein, which is the next hydro power plant after Freudenau when going upstream, could as well act as an anthropogenic barrier to gene flow. When Meulenbroek et al. (in prep.) investigated fish larvae drift between these two impoundments, the results of DNA barcoding showed that the within-species variation of bighead goby larvae was ten times less than of round goby larvae. Of all caught species, the bighead goby showed the lowest values concerning genetic distance within species. This is an indication that the round goby is able to develop more persistently in this area.

5.3. Outlook and Recommendations

The results of this study indicate that the degradation of European rivers, especially hydromorphological alterations, support the invasion by non-native species.

This agrees with the results of the second joint Danube survey where the high abundance of neozoa gobies in rip-raps of the Middle and Upper reaches of the Danube was also confirmed. Downstream of the Iron Gate, which is the first major fish barrier in the Danube coming from the Black Sea, gobies are much less abundant, although they are classified as native in this area. Hydromorphologically, the lower part of the Danube is much less degraded by impoundments. Rip-rap structures are rare and mainly sandy substrate is found on the river banks (Wiesner et al. 2008).

Also in 2010, Bammer reported in his Master thesis about benthic fish species in the Danube that gobies had clear preferences for groynes and rip-raps. Native species were most abundant at gravel bars where they were also predominant (Bammer 2010).

Long-time data series are necessary to study the impact of gobies on the native fish fauna. To follow the development of gobies or also other non-native species in the Danube, continuous monitoring of the fish biocenosis is necessary-

Finally, a possible strategy to support native species and to restrict spreading of neozoans could be the rehabilitation of natural habitats.

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