Typology of a great river using fish assemblages: implications for the bioassessment of the Danube River

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Abstract

Matching habitat typology and ecological assemblages can be useful in environmental management. We examined whether *a priori* defined riverine sections correspond with distinct fish assemblage types along the >2000 km long course of the Danube River, Europe. We also tested whether different sampling methods (i.e. day and night inshore electric fishing and offshore benthic trawling) provide consistent typological results. Analysis of assemblage similarities, indicator species analysis, non-metric multidimensional scaling (NMDS) and k-means analyses indicated that fish assemblages of the *a priori* defined Upper-, Middle and Lower-Danubian sections differed slightly, within class variability was high. Although indicator species analysis showed that the Upper-Danube belongs to the barbel (*Barbus barbus*) zone and the Middle- and Lower Danube belong to the bream (*Abramis spp*) zone, indicator values of the character species were generally low. The NMDS analyses suggested a weak gradient in assemblage structure along the course of the river with relatively high variability between neighbouring sites. K-means analyses revealed that many sampling sites were in a different class than the *a priori* defined sections, and classifications at other group numbers did not lead to better classification outcome. Overall, the results do not suggest clearly distinguishable assemblage types with distinct boundaries in the potamal section of a great river. Nevertheless, the division of the potamon to smaller sections may explain some variability in fish assemblage structure, and could be used for bioassessment purposes. The study also shows the importance of multihabitat and multigear surveys in the typological assessment of great rivers.

Running head: typology of a great river

Introduction

Mmatching habitat typology and ecological assemblage types can be useful in environmental management and conservation (Dufrene and Legendre, 1997; Heino et al., 2003). For example, river typology can aid the reliable assessment of ecological status (Hering et al., 2010). It can account for natural variations in biological data caused by natural environmental gradients, and thereby helps to reveal the effect of anthropogenic disturbances (Heino et al, 2003). Different habitat types may also require specific management and conservation activities (Angermeier and Winston, 1999; Aarts and Nienhuis, 2003; Erős, 2007). The classification of assemblages and their corresponding habitat into well definable types, however, is not an easy task. The strength of the classification depends on several factors, including for example the abiotic heterogeneity of the environment and the responses of organisms to this heterogeneity. It can also depend on taxonomic resolution or sampling efforts and data evaluation methodology (Hawkins et al., 2000; Hughes et al., 2015). Delineation of assemblages can also be conducted at several hierarchical assemblage levels and spatial scales depending on the purpose of the study (Hawkins et al., 2000; Heino et al., 2003). Researchers agree that preliminary habitat classification schemes, which portray the environmental heterogeneity of the habitat, may help in the delineation of ecological assemblages to some degree, although no single organism group is likely to show a perfect match with any classification scheme (Hawkins et al, 2000; Heino and Mykrä, 2006; Hughes et al., 2015). In fact, ecologists have reported stronger associations between habitat conditions and assemblage level responses with the help of of predictive modelling than through the use of typological approaches for both macroinvertebrate (Moya et al., 2011; Chen et al., 2014; Mazor et al., 2016) and fish assemblages (Oberdorff et al., 2002; Pont et al., 2006; 2009; Esselman et al., 2013).

Delineation of assemblage types can be especially difficult in great rivers due to the extreme difficulty of collecting representative samples from the whole river body (Flotemersch et al., 2006; 2011). Most investigations are restricted to shoreline samples, although it is well known that shoreline sampling provides only limited information on patterns and processes in great rivers (Galat and Zweimüller, 2001; Wolter and Bischoff, 2001). Consequently, inferences about how offshore habitat data of the main channel contribute to the bioassessment of large rivers compared with exclusive shoreline monitoring data should be more precisely developed (de Leeuw et al., 2007; Flotemersch et al., 2006, 2011; Szalóky et al., 2014). It has been proven that different sampling methods show a different picture of the fish assemblage structure in great rivers (Dettmers et al., 2001; Wolter and Freyhof, 2004; Loisl et al., 2014).

It is not known, however, how differences in sampling methodology influence typological results (i.e. the match between habitat typology and fish assemblage structure). To increase the accuracy of typological systems it is a prerequisite to harmonize assemblage level classification results among different sampling methods.

Another difficulty of setting up a typology for great rivers is that the assemblages are distributed along the profile of hundreds or even thousands of kilometres long river sections. For the characterization of assemblage patterns transboundary surveys are needed with a consistent sampling methodology. However, most countries usually have their own monitoring scheme which can differ in several aspects (sampling, data evaluation) from their neighbours (Birk et al., 2012), and differences in sampling methodologies make the comparison of data usually unfeasible for bioassessment purposes (Wilson et al., 2015). This could be the reason why patterns in fish assemblages (e.g. zonation, responses to environmental gradients) are relatively well known in wadeable streams and in boatable rivers (Matthews, 1998; Kruk et al., 2007), whereas the typology of fish assemblages in great rivers is rather unclear (de Leeuw et al., 2007).

The purpose of this study is to establish a large scale typology of a great river (Danube River, Europe) using fish assemblages. With its 2872 km length, the Danube River is the second longest river in Europe. Although the river is the cradle of Europe's most diverse fish fauna (Reyjol, 2007), interestingly, no study so far has dealt with the large scale typology of Danubian fish assemblages. Here, we characterize fish assemblage types in the non-wadeable, more than 2000 km long main channel section of the Danube River using the results of the Joint Danube Survey 3 expedition (see methods for details). Our first objective was to determine whether large scale changes in abiotic conditions along the longitudinal profile of the river are mirrored in fish assemblages. Different habitat based typological systems have been established for the Danube River as part of the Water Framework Directive of the European Union (Literáthy et al., 2002; Birk et al., 2012). Analyses of different organisms groups (i.e. diatoms and macroinvertebrates) showed some separation according to these large scale typologies to some degree (see Birk et al., 2012). We assumed that fish assemblages would also respond to large scale changes in the habitat types (i.e. *a priori* established three habitat classes: Upper-, Middle- and Lower- Danube, which were set up based on geomorphological breaks that altered flow and substratum patterns see Fig 1.). Therefore, we predicted that distinct fish assemblage types would correspond with the habitat classes. Our second objective was to examine whether different sampling methodologies (day and night shoreline electric fishing and offshore benthic trawling) provide consistent typological results.

Finally, our third objective was to examine how variable the fish assemblages are along the longitudinal section of the river and whether gradual changes occur in the potamon both inshore and offshore. We assumed that the *a priori* established three classes would explain most of the variability in the structure of fish assemblages.

Materials and methods

Study area and *a priori* habitat classes

The Danube has a drainage area of approximately 796,250 km^2 and a mean discharge of 6,500 m^3 s^{-1} at its mouth. From source to mouth the Danube drains 19 countries, which makes the Danube basin the most international catchment in the world (http://www.icpdr.org/main/danube-basin). Large-scale river regulations starting from the second half of the nineteenth century, and paralleled by massive agricultural and industrial activites in the twentieth century, have substantially changed the natural Danubian riverscape and its fish fauna. The upstream sections were mainly affected by small-scale canalisation and reservoir construction, whereas the middle and more downstream sections by the separation of main channel and floodplain habitats. Beside pollution, river engineering and land use effects, overfishing also contributed to the deterioration of fish assemblages. As a result of these multiple stressor effects the abundance of floodplain fishes and long distance migratory sturgeon species decreased substantially in this riverscape (Schiemer et al., 2004).

At present, the river can be subdivided into three main sections based on its hydromorphological features, namely the Upper-, the Middle-, and the Lower-Danube (Fig. 1.; Literáthy et al., 2002; Schiemer et al., 2004). The Upper-Danube is highly regulated, especially by hydroelectric schemes, and contains only very short free-flowing stretches.The Middle-Danube is mainly free-flowing, although two large dams (i.e. Iron Gate I and II; Fig. 1.) heavily modify the flow of the river in its most downstream section. In the Middle-Danube the bank is relatively natural, although these natural banks are interrupted with hundreds to thousands of meters of rip-rap at many places for some hundred meters to even several kilometre long sections, especially in the vicinity of major cities. Finally, in the Lower-Danube the hydromorphology of the river is only slightly modified (Table I) and bank reinforcement covers only a very small percentage of the river. Although the Iron Gate dams clearly separate the Middle and Lower Danube (and virtually prevent the migration of fish, with the exception of shipping channels, which allow just a minimal rate of fish migrations) the two lowland stretches also largely differ in their hydromorphological features (Table I). In

the Middle-Danube, the bottom is mainly covered by gravel and sand and the river is faster flowing ($\sim$ 0.6-1.3 m s^{-1}), whereas in the Lower-Danube the bottom is covered almost exclusively by sand, and the river shows slow flow velocity ($\sim$ 0.4-0.7 m s^{-1}).

Field sampling and fish data

Fish data for this study were collected during the Joint Danube Survey 3 (JDS3), which was an international river research expedition organized by the International Commission for the Protection of the Danube River (ICPDR) between 13th of August to 26th of September 2013 (http://www.icpdr.org/jds/). The survey covered the sampling of several biotic and abiotic components of the Danube across ten countries from Regensburg, South Germany to the Danube Delta in Romania.

Sampling in inshore areas was based on the Austrian national guideline for fish sampling in running waters (Haunschmid et al., 2010), which is in agreement with international standards (EN 14011; CEN, 2003). The standardised sampling procedure for each site followed a habitat specific approach (Schmutz et al., 2010). The major mesohabitat types (i.e. rip-rap and natural stretches; see e.g Erős et al., 2008) were preliminary mapped and were sampled in their proportional distribution at the site level (i.e. fishing along a 5 km long section) both day and night to maximize the representativeness of the fish assemblage. Fishing was conducted from an electric fishing boat using a boom mounted anode (2.2 m width, 11 steel cables, 1m each; approx. 580V, 20A) going downstream. The fishing team consisted of two persons in the front of the boat. All fish showing electrotactic movement towards the anode or paralysis were sampled with dip nets (mesh size 6 mm), put in a tank, determined to species level, measured and released alive. Note, that although national teams also collected fish with different sampling protocols, we use only those data that were collected by the JDS3 core fishing team to maximize comparability of the data between sites.

Sampling in offshore areas was done by an electrified bentic frame trawl that consisted of a 2 m long and 1 m high stainless steel frame to which a knotted multifilament nylon net was attached (for details see Szalóky et al., 2014). The frame was electrified with a 40 m long electrode cable which was connected to a Hans-Grassl EL65 IIGI electric fishing device In this crew, 2 people handled the framed net (6 mm inner mesh size), one handled the electric fishing device and operated the boat. The direct current (approx. 350 V, 33 A) was given for 5-8 s with 3-5 s breaks between the operations to minimize fright bias and injury of fish. Each haul had a length of 500 m. Trawling was carried out during daytime. At each station 6 hauls

were conducted on average (min 3 max 9) along predefined transects, excluding the littoral, less than 2 m deep shoreline zone.

We used different kinds of presence/absence and relative abundance data to compare their effects on typology. Specifically, we used both presence/absence and relative abundance data of 1) day inshore electric fishing, 2) night inshore electric fishing, 3) combined day and night inshore electric fishing, 4) offshore trawling and 5) combined inshore electric fishing and offshore trawling collections. Bleak *Alburnus alburnus*, a common, surface oriented shoal forming species was so abundant in the samples (49.1% of the total catch) that it virtually homogenized the data, at least in case of shoreline electric fishing samples. Therefore, we decided to remove bleak from all statistical analysis in order to compare subtle changes in the composition of the fish fauna among habitats and methodological configurations more effectively.

Data analysis

We used analysis of similarities (ANOSIM), a non-parametric permutation procedure (Clarke and Warwick, 1994; O'Hara et al., 2008), to test for differences in fish assemblage composition and relative abundance between the three habitat classes. We used the Jaccard and the Bray and Curtis indices for compositional (presence-absence) and relative abundance data, respectively. The ANOSIM analyses were also augmented by indicator species analysis (Dufrene and Legendre, 1997) to find significant indicator species for the river sections. The best indicator species (i.e. symmetrical indicators) are those that occur only in a certain section with high occurrence and abundance (see e.g. Dufrene and Legendre, 1997 for more details).

Similarity of the sampling sites was also compared with two-dimensional Non-metric Multidimensional Scaling analysis (NMDS; Legendre and Legendre, 1998). Similar to ANOSIM, we used the Jaccard and the Bray and Curtis indices for compositional (presence-absence) and relative abundance data, respectively. Site symbols were coded according to the three main sections (Upper-, Middle-, Lower-Danube) and their separation and/or gradual changes in fish assemblages were evaluated visually in the ordination space.

We used k-means cluster analysis to further test the affinities between fish assemblage structure and Danubian sections. The k-means analysis is one of the most efficient and widely used non-hierarchical technique that places sites into user-defined numbers of groups such that the sum of squares from points to the assigned cluster centers is minimized (Hartigan and Wong, 1979; Watts and Worner, 2009; Pease et al., 2011). In this case, we were interested to

test how consistent is the classification of spatially neighbouring sites into distinct Danubian sections. If the large scale habitat typology induce strong fish assemblage responses the three group cluster model should classify neighbouring sites according to the *a priori* habitat typology. We performed the analyses at different cluster numbers (2-5 groups) as well, to examine whether other classifications give more consistent and better interpretable results.

Results

A total of 32268 fish specimens were collected during the survey and identified to 52 species (Table II).

The ANOSIM analysis generally indicated significant differences in assemblage composition (presence/absence data) between the three river sections (i.e. Upper-, Middle-, Lower-Danube) for both inshore and offshore samples, and for the combined data set (Table II). Consequently, the *a priori* defined habitat classes separated relatively well in their fish assemblage composition independently of sampling methodology. Only the Upper- and the Middle-Danubian sections and the Middle- and the Lower-Danubian sections did not differ in their fish assemblage composition for day and night inshore electric fishing data, respectively. For relative abundance data the differences were not so clear between the habitat classes (Table III), and, in addition, the separation of habitat classes showed some differences between inshore and offshore samples. Specifically, the Upper-Danubian assemblages differed significantly from both the Middle- and the Lower-Danubian sections in case of inshore samples (i.e. day, night and combined day and night data), but the Middle- and the Lower-Danubian sections did not differ for day, night or the combined inshore samples. Only the Upper- and Lower-Danubian sections differed in case of offshore trawling samples. Finally, both the Middle- and the Lower-Danubian sections differed from the Upper-Danube, but no differences were found between the Middle- and Lower-Danube in the combined inshore and offshore data set.

Many species proved to be the indicator of a certain section, sampling methodology or both (Table IV). The most characteristic species for the Upper Danube (section 1) were the eel *Anguilla anguilla*, the barbel *Barbus barbus* and the round goby *Neogobius melanostomus*. These species were indicator species of both inshore and offshore samples. The most characteristic species for the Middle Danube (section 2) were the different ruffe species and the ide *Leuciscus idus*. The most characteristic species for the Lower Danube (section 3) were the carp *Cyprinus carpio*, the monkey goby *Neogobius fluviatilis*, the pikeperch *Sander lucioperca*, the wels catfish *Silurus glanis*, and the black striped pipefish *Syngnathus abaster*.

These species were indicators of only a given section irrespective of the sampling methodology. Beside these, there were many species that proved to be the indicator of inshore areas within a certain section, which also contributed to the separation of the Upper-, Middle- and Lower-Danubian sections. For example, the burbot *Lota lota*, the spirlin *Alburnoides bipunctatus*, the dace *Leuciscus leuciscus*, the chub *Squalius cephalus* were the characteristic species of inshore samples in the Upper-Danube. The sterlet *Acipenser ruthenus* was the only species that was caught only in offshore areas, and, albeit relatively rare, it proved to be the indicator species of offshore Lower-Danubian samples. The Upper-Danubian section was clearly separated from the Middle- and Lower-Danubian sections based on indicator species. The separation of the Middle- and Lower-Danubian sections, however, was less explicit and though significant, the indicator values were low for most species.

The NMDS analyses generally confirmed the results of ANOSYM that the riverine sections differed to some degree in their fish assemblages. However, they also revealed gradual changes in fish assemblages to some extent along the course of the river (Figure 2). They also indicated that within class variability of fish assemblages was high, and the degree of overlaps among the classes in the ordination place depended on the data type and the sampling method. Separation of fish assemblages among the Upper-, the Middle- and the Lower-Danubian sections was relatively clear for presence/absence data (Figure 2 a,c, e, g, i), especially when both inshore and offshore data were pooled (Figure 2 i). Separation of the Upper-Danube was also relatively clear from the Middle- and the Lower-Danubian sections using relative abundance data in case of both inshore (day or night) and offshore samples (Figure 2 b, d, f, h, j). The Middle and Lower Danubian assemblages, however, showed high overlaps based on relative abundance data using any sampling methodology.

The k-means clustering pointed out that the correspondence between fish assemblage structure and the *a priori* defined habitat classes was weak (Figure 3). Although the visualization of clustering results revealed some patterns along the course of the river, variability in the classification of individual sites was high both between sampling methods (e.g. inshore vs offshore samples) and data type (composition vs relative abundance). This variability remained high at all cluster numbers (APPENDIX I).

Discussion

Fish zonations along the longitudinal profile of large rivers have long been recognized (e.g. Huet, 1959; Holčík, 1989; Rahel and Hubert, 1991). In Europe, several classifications schemes have been set up, which divide the rhithron and potamon part of the river into

characteristic fish assemblage types (e.g. Holčík, 1989; Aarts and Nienhuis, 2003). For example, the rhitrhon has been divided into the trout *Salmo trutta* and the grayling *Thymallus thymallus* zones, while the potamon part of the river into the barbel and bream zones (Huet, 1959; Holčík et al. et al., 1989). Most studies, however, evaluated fish assemblage responses to environmental gradients in the rhithron part of large river systems (e.g. Zalewsky et al., 1990), while the biological assessment of the typical potamon is much less developed. Our study of the >2000 km long potamal part of the Danube indicates that the Upper-Danube can be identified as the barbel zone, while the Middle- and Lower Danubian zones may belong to the bream zone, although it is clear that fish assemblages are too complex to be identified with only a single species in zonation models (Ibarra et al., 2005). Although subtle changes in fish assemblage composition and relative abundance further distinguished the Middle- and the Lower-Danube sections, the separation of these sections was less clear. In fact, we found more or less gradual changes in fish assemblage composition and relative abundance, but variability in fish assemblages was high within the same habitat classes. Overall, no defined boundaries in fish assemblages were observed (Figure 2), which would justify the clear separation of fish assemblages along the course of the river. Therefore, fish assemblage patterns only partly support the large scale habitat typology of the Danube, which will be discussed in more detail below.

The separation of the Upper-Danube from the Middle-Danube was relatively clear based on the characteristic fishes of the barbel zone, such as the barbel, the chub, the spirlin and the dace, which are the characteristic species of large highland rivers (Erős, 2007; Lasne et al., 2007). Note that the eel, which is not a characteristic species of the Danube is stocked in the Upper Danube and its tributaries. This explains why this species was an indicator species for the Upper-Danube. Although these characeristic species of the Upper-Danube also occur in the Middle-Danube, their abundance is lower (Erős et al., 2008; Kováć, 2015). The higher abundance of some native species, as the white eye bream, the asp *Leuciscus aspius*, the ide, the white finned gudgeon *Romanogobio vladykovi*, and the different ruffe species, which were characteristic species of the Middle-Danube also contributed to the separation of the Upper- and Middle-Danubian sections to some degree. On the contrary the separation of the Middle- and the Lower Danubian assemblages depended on the data type. The NMDS analyses, for example, showed a separation between the Upper-, Lower-, and Middle-Danube using compositional data, while it showed high overlaps using relative abundance data. The partial separation of the Middle- and the Lower-Danube for compositional data could not really be

justified with high differences in the relative abundance of some species, but rather with the overall contribution of many relatively rare species (Table II and IV).

Invasive species also contributed to the partial separation of the Upper-, the Middle-, and the Lower-Danubian sections. For example, the round goby and the monkey goby proved to be an indicator of the Upper-, and the Lower Danube respectively. Although, the round goby is very abundant along the longitudinal profile of the Danube (Szalóky et al., 2015; Kovać, 2015), the species is recently extremely abundant in its invasion front in the Upper-Danube (see also Brandner et al., 2013a,b). The monkey goby prefers sandy bottom (Erős et al., 2005), and this may explain why this species is more abundant in the Lower-Danube, especially in offshore areas (Szalóky et al., 2015). Beside the gobies, other invasive species (e.g. Prusian carp *Carassius gibelio*, stone moroko *Pseudorasbora parva*, pumpkinseed *Lepomis gibbosus*) were relatively rare in the main channel of the Danube, and their relative abundance was lower than it was observed in 2007 during the JDS2 survey (Kovać, 2015).

Analyses which were based on different collection methods yielded relatively consistent typological results, at least in the ANOSYM and NMDS analyses. For example, day and night inshore electric fishing data resulted in similar outcomes. In general the effect of data type (i.e. compositional vs relative abundance data) was more influential than the effect of time of day for inshore electric fishing. Nevertheless, the separation of habitat classes showed some differences based on inshore electric fishing and offshore trawling data (see Table III). The combination of different sampling methods contributed largely to a more effective detection of species and consequently, to a more reliable typological assessment. Not surprisingly, inshore electric fishing was more effective in detecting small inshore species, such as the bitterling *Rhodeus amarus*, the stone moroko *Pseudorasbora parva* and the rudd *Scardinius erythrophthalmus*. On the contrary, offshore trawling proved the occurrence of the sterlet and Danube bleak *Alburnus mento*, and also indicated the relatively high abundance of the Danube streber *Zingel streber*. Therefore, inshore electric fishing and offshore trawling complemented each other in assessing fish assemblages in this great river. In addition, pooling data of the different collection methods for a more reliable determination of species composition confirmed more convincingly the differences in the Upper-, the Middle- and the Lower-Danubian fish assemblages (Figure 2). Our large scale study along the Danube river thus confirms the results of smaller scale surveys, which stressed the importance of multihabitat and multigear surveys in the bioassessment of great rivers (Loisl et al., 2014; Szalóky et al., 2014). This study highlight, however that multigear surveys can be especially important for the detection of rare species and/or habitat specialist species, which can occur

only either inshore or offshore and that these species can have an influence on fish assemblage patterns (see e.g. Figure 2).

The absence of defined boundaries in fish assemblages along this >2000 km long potamal section can be related to several reasons. For example, variability of fish assemblages between individual sites may reflect human alteration effects, which may obscure the clearer realization of gradual changes or boundaries in assemblage structure. Some samplings inevitably happened at strongly modified reaches, for example in Austria, where impounded reaches were also sampled for reach scale representativeness. The effect of these human modifications should be explored in detail in further studies. Obviously, additional samples of the rhitron (wadeable) part of the Upper-Danube in Germany would reveal clearer changes in fish assemblages because of the longer environmental gradient (Heino et al. 2003; Lasne et al., 2007). For example, a similarly large scale study which included a longer environmental gradient (i.e. from source to mouth) found three distinct fish zones along the 2800 km long section of the Rio Chama and Rio Grande Rivers in North America (McGarvey, 2011). Other large scale studies, which identified 3-4 main assemblage types in European streams and rivers also support these findings (Ibarra et al., 2005; Kruk et al., 2007). Nevertheless, such changes in fish assemblages along the whole stream-riverine gradient are well-known (Aarts and Nienhuis, 2003; Lasne et al., 2007), and our specific purpose was to reveal differences in the potamal section of a great river.

The results of this study have clear management implications. Our study suggested that spatially very distant sections could serve as a reference to strongly human altered reaches. The Danube in Austria, for example, is heavily modified in most stretches; only two short free flowing sections remained due to ten consecutive hydropower dams along the river (Schiemer et al., 2004). These few free-flowing stretches (see Hirzinger et al., 2004), or alternatively, stretches from the Hungarian/Slovakian part of the Danube, can serve as a reference for dammed reaches, even if they are some hundred kilometers away from the heavily modified sections. This study also revealed that similarly to macroinvertebrates or diatoms (Birk et al., 2012), the division of the Danube into 3 or 4 sections according to contrasting habitat features may help in accounting for natural changes in fish assemblages to some degree. A habitat typology which fits commonly to multiple organism groups may ease the bioassessment of the river in determining the ecological status. It should be also kept in mind, however, that these sections do not separate strictly into distinct fish assemblage types. Analyses revealed that many sampling sites were classified to a different class than suggested by the *a priori* defined sections, and classifications at other group numbers did not lead to better classification

outcomes (Figure 3). Rather we revealed subtle gradual changes in fish assemblages along the course of the river, coupled with relatively large variability between neighbouring sites, which was comparable to between class variability. This means that other divisions, which divide the potamon to sections at other scales could also work well for the bioassessment of fish assemblages in the Danube River.

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Table I. Physical and chemical characteristics of the Upper-, Middle- and Lower Danubian sections. Hydromorphological classes are as folows. 1: near natural, 2: slightly modified, 3: moderately modified, 4: extensively modified, 5: severely modified. For further details see: www.icpdr.org/main/activities-projects/jds3 (Accessed: 03 June 2016)

	Upper	Middle	Lower
velocity (m s^{-1})	0.25-1.6	0.2-1.3	0.3-0.7
width (m)	106-322	343-1220	263-1036
depth (m)	2.3-15.0	5.7-14.2	7.0-22.6
discharge (m^3 s^{-1})	300-2000	1500-3500	3000-6000
bed material	gravel (cobbles)	gravel, sand	sand (gravel)
hydromorphology	1: 0%; 2: 0%; 3: 24%; 4: 38%; 5: 38%	1: 0%; 2: 13%; 3: 48%; 4: 29%; 5: 10%	1: 0%; 2: 42%; 3: 39%; 4: 16%; 5: 3%
conductivity (mikroS cm^{-1})	320-566	350-450	350-450
pH	7.9-8.4	7.8-8.1	8.0-8.3
Oxygen (mg l^{-1})	7.0-10	6.0-9.0	8.0-9.5
Total Nitrogen (mg l^{-1})	1.7-2.5	1.4-2.07	1.5-1.06
Total Phosphorous (mg l^{-1})	0.05-0.1	0.05-0.1	0.03-0.1
DOC (mg l^{-1})	1.8-4.8	1.8-3.8	1.9-4.0

Table II. List of fish species, their abbreviation code and their relative abundances (%) for daytime (DE) and for night time (NE) inshore electric fishing and for offshore trawling (OT) data

Scientific name	Common name	Code	DE	NE	OT
Abramis brama	Freshwater bream	abrbra	0.79	2.21	2.41
Acipenser ruthenus	Sterlet sturgeon	acirut	-	-	0.19
Alburnoides bipunctatus	Spirlin	albbip	0.18	0.16	-
Alburnus alburnus	Bleak	albalb	71.65	32.48	0.27
Alburnus mento	Danube bleak	albmen	-	-	0.02
Anguilla anguilla	European eel	angang	0.08	0.04	0.02
Babka gymnotrachelus	Racer goby	babgym	0.30	0.40	1.13
Ballerus ballerus	Zope	balbal	0.10	0.70	0.50
Ballerus sapa	White-eye bream	balsap	0.02	0.82	2.30
Barbus barbus	Barbel	barbar	0.13	0.79	0.57
Benthophilus stellatus	Stellate tadpole-goby	benste	0.01	-	0.43
Blicca bjoerkna	White bream	blicbjoe	0.73	16.60	9.26
Carassius gibelio	Prussian carp	cargib	0.41	1.08	0.33
Chondrostoma nasus	Nase	chonas	0.50	0.53	0.26
Cobitis elongatoides	Spined loach	cobelo	0.15	0.28	-
Cottus gobio	Bullhead	cotgob	0.02	-	0.07
Cyprinus carpio	Common carp	cypcar	0.09	0.16	0.05
Esox lucius	Northern pike	esoluc	1.89	2.26	0.07
Eudontomyzon mariae	Ukrainian brook lamprey	eudmar	0.02	0.06	-
Gymnocephalus baloni	Danube ruffe	gymbal	0.09	0.81	2.44
Gymnocephalus cernuus	Ruffe	gymcer	0.01	0.12	0.02
Gymnocephalus schraetser	Schraetzer	gymsch	0.06	1.81	5.98
Hucho hucho	Huchen	huchuc	0.01	-	-
Hypophthalmichthys molitrix	Silver carp	hypmol	-	0.04	-
Lepomis gibbosus	Pumpkinseed	lepgib	0.08	0.06	-
Leuciscus aspius	Asp	leuasp	3.71	1.86	0.14
Leuciscus idus	Ide	leuidu	1.26	1.42	0.05
Leuciscus leuciscus	Common dace	leuleu	0.01	0.02	-
Lota lota	Burbot	lotlot	0.45	0.42	-
Misgurnus fossilis	Weatherfish	misfos	0.02	0.02	-
Neogobius fluviatilis	Monkey goby	neoflu	0.40	1.21	10.94
Neogobius melanostomus	Round goby	neomel	9.24	11.90	36.91
Pelecus cultratus	Sichel	pelcul	0.23	0.23	0.09
Perca fluviatilis	European perch	perflu	1.22	2.52	0.36
Ponticola kessleri	Bighead goby	ponkes	2.57	1.62	0.89
Proterorhinus marmoratus	Tubenose goby	promar	0.09	0.05	0.14
Pseudorasbora parva	Stone moroko	psepar	0.03	0.02	-
Rhodeus amarus	Bitterling	rhoama	0.06	0.05	-
Romanogobio vladykovi	White-finned gudgeon	romvla	0.05	1.68	13.65
Rutilus pigus	Danube roach	rutpig	0.02	0.02	-
Rutilus rutilus	Roach	rutrut	2.24	6.77	0.28
Sabanejewia bulgarica	Golden loach	sabbul	0.01	-	2.60
Sander lucioperca	Pike-perch	sanluc	0.43	6.89	2.57
Sander volgensis	Volga pikeperch	sanvol	0.01	0.05	0.05
Scardinius erythrophthalmus	Rudd	scaery	0.10	0.04	-
Silurus glanis	Wels catfish	silgla	0.08	0.18	0.15
Squalius cephalus	Chub	squcep	0.28	0.64	-
Syngnathus abaster	Black-striped pipefish	synaba	0.10	0.20	0.75
Tinca tinca	Tench	tintin	-	0.01	-
Vimba vimba	Vimba bream	vimvim	0.01	0.66	0.34
Zingel streber	Danube streber	zinstr	0.03	0.02	2.89
Zingel zingel	Zingel	zinzin	0.02	0.13	0.84
Number of species			48	46	37
Number of individuals			17152	10903	4213

Table III. P values of the ANOSYM analyses for testing the separation of the Upper-, Middle- and Lower Danubian sections. Data types are as follows presence/absence data of day inshore

electric fishing (above diagonal), relative abundance data of day inshore electric fishing below diagonal), presence/absence data of night inshore electric fishing, relative abundance data of night inshore electric fishing, presence/absence data of combined (day and night) inshore electric fishing, relative abundance data of combined (day and night) inshore electric fishing, presence/absence data of offshore trawling, relative abundance data of offshore trawling, presence/absence data of combined inshore electric fishing and offshore trawling data relative abundance data of combined inshore electric fishing and offshore trawling.

	pres/abs inshore day			pres/abs inshore night			pres/abs inshore combined			pres/abs offshore trawling			pres/abs all data combined		
	Upper	Middle	Lower	Upper	Middle	Lower	Upper	Middle	Lower	Upper	Middle	Lower	Upper	Middle	Lower
Upper	–	0.116	0.002	–	≤0.001	≤0.001	–	≤0.001	≤0.001	–	0.009	0.002	–	≤0.001	0.002
Middle	≤0.001	–	0.001	≤0.001	–	0.146	≤0.001	–	0.015	0.079	–	0.003	0.006	–	≤0.001
Lower	0.003	0.682	–	0.009	0.839	–	0.002	0.880	–	≤0.001	0.226	–	0.002	0.340	–
	Upper	Middle	Lower	Upper	Middle	Lower	Upper	Middle	Lower	Upper	Middle	Lower	Upper	Middle	Lower
	relab inshore day			relab inshore night			relab inshore combined			relab offshore trawling			relab all data combined		

Table IV. Indicator values for the significant (P<0.05) indicator species of the Upper-, the Middle- and the Lower-Danubian sections for DE, day-time inshore electric fishing; NE, night-time inshore electric fishing; CIE, combined (day and night) inshore electric fishing; OT, offshore trawling; AD, combined inshore electric fishing and offshore trawlingdata.

Species	Upper Danube					Middle Danube					Lower Danube				
	DE	NE	CIE	OT	AD	DE	NE	CIE	OT	AD	DE	NE	CIE	OT	AD
abrbra						33.48			28.11			44.36	33.10		46.70
acirut														23.72	24.10
albbip	13.03	15.34	34.58		34.75										
albmen									11.11	11.11					
angang	33.33	33.33	50.00	16.67	50.00										
babgym							25.90	31.90		44.29	38.48			24.11	
balbal						37.33		13.22	66.67	64.78		11.20			
balsap						18.18	31.58	30.03						39.29	41.70
barbar	59.10	55.47	67.96	22.28	64.66										
benste											28.57		28.57	71.43	83.33
blicbjoe							44.97				38.68		45.97	50.52	48.20
cargib							54.86	51.16			45.97			34.71	62.57
chonas	38.42	57.32	55.67		57.05				27.27						
cobelo											38.27	50.09	44.44		37.85
cotgob	33.33		33.33	33.33	50.00										
cypcar											45.17	20.61	53.12	7.44	58.25
esoluc									9.19		52.64	49.95	56.21		64.70
eudmar											11.53	28.57	27.21		31.33
gymbal						14.25	51.15	51.71	37.48	67.91					
gymcer						9.09	7.28	7.16	11.11	18.08					
gymsch						5.99	50.21	47.41	53.87	55.24					
hypmol							18.18	18.18		22.22					
huchuc	16.67		16.67		16.67										
lepgib											25.23	16.85	40.52		36.63
leuasp						52.94	50.76	58.22		53.87					
leuidu						72.70	28.47	62.25	11.11	63.10					
leuleu	16.67	13.01	28.03		28.95										
lotlot	28.59	28.44	28.23		28.45										
misfos						9.09	9.09	9.09							
neoflu											97.65	67.83	77.68	95.12	87.07
neomel	67.57	77.49	73.06	55.60	64.06										
pelcul						21.33	24.71	38.76		33.66				8.80	
perflu						44.59		38.90				35.05		12.27	35.67
ponkes		45.96									44.94		39.55	27.03	37.37
promar						15.85		13.24				15.03		42.86	49.28
psepar						18.18	9.09	18.18		11.11					
rhoama						7.18	14.68	14.27							33.33
romvla							47.96	47.13	56.13	63.20	42.86				
rutpig	14.07		10.68		11.78		9.09								
rutrut						36.18						52.00	47.10	18.70	55.32
sabbul											14.29		14.29	84.05	96.25
sanluc											62.78	58.84	62.47	33.51	63.73
sanvol							18.18	15.98	11.11	30.67	14.29				
scaery							9.09	8.62			10.12				16.67
silgla											20.67	9.17	24.08	18.95	34.33
squcep	34.66	43.81	46.22		52.21										
synaba											57.14	57.14	57.14	42.86	66.67
tintin												14.29	14.29		16.67
vimvim			41.48				30.57	30.85		30.26	14.29				
zinstr			50.20							32.93	9.50	14.29	11.08		
zinzin	33.33						32.77	20.73	24.52	31.04					
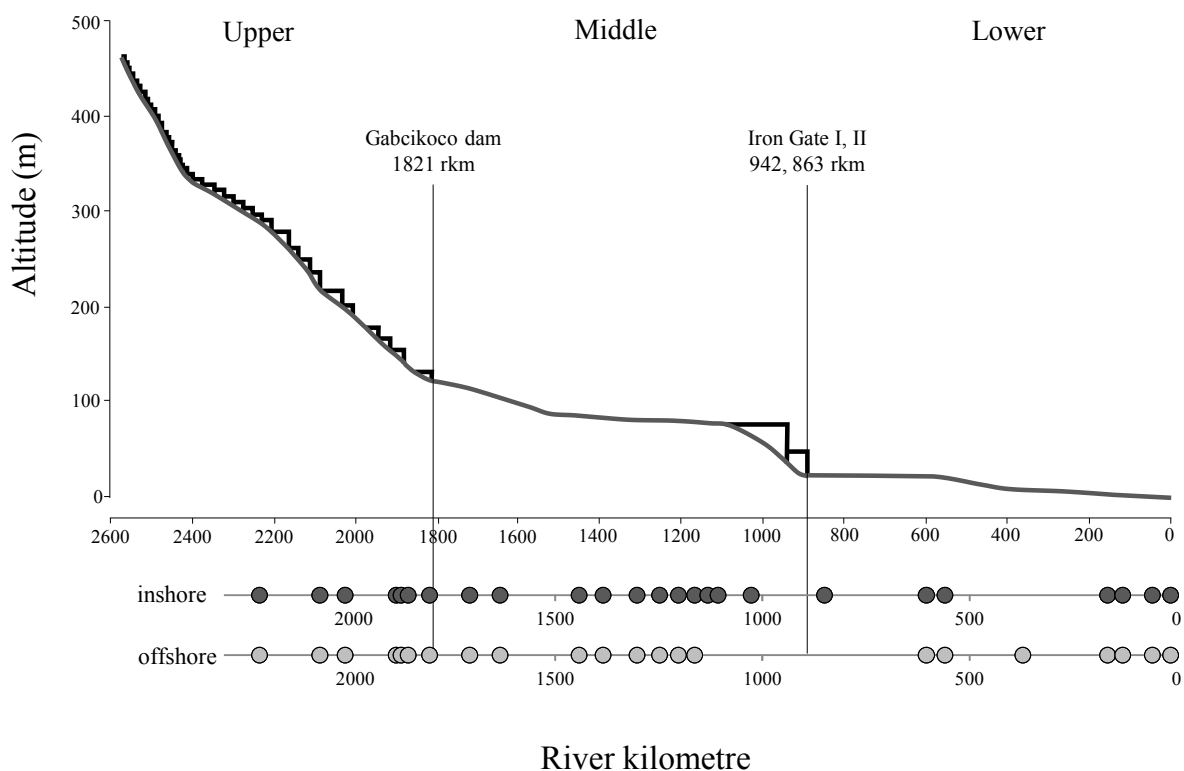
Figure 1. The separation of the Upper-, Middle-, and Lower-Danubian sections based on clear borders in human modifaction effects adopted from Literáthy et al. (2002) and Schiemer et al (2004). The distribution of sampling sites along the longitudinal profile of the river is indicated with black circles both for inshore (day and night) electric fishing and offshore benthic trawling samples.

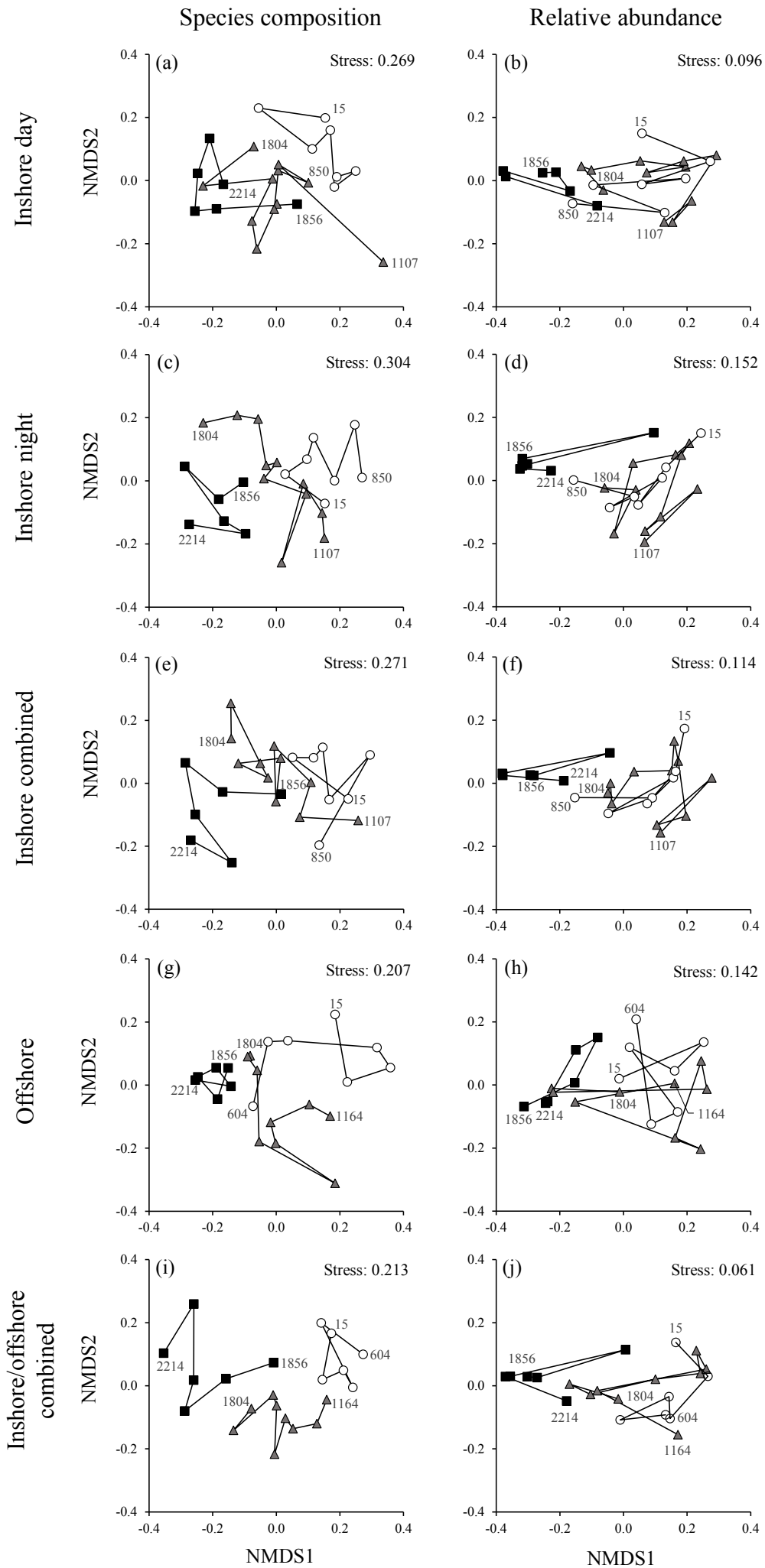Species composition   Relative abundance

Figure 2. Ordination of sites by Nonmetric Multidimensional Scaling (NMDS) using different fish assemblage data. Data types are as follows: (a) presence/absence data of day inshore electric fishing, (b) relative abundance data of day inshore electric fishing, (c) presence/absence data of night inshore electric fishing, (d) relative abundance data of night inshore electric fishing, (e) presence/absence data of combined (day and night) inshore electric fishing, (f) relative abundance data of combined (day and night) inshore electric fishing, (g) presence/absence data of offshore trawling, (h) relative abundance data of offshore trawling, (i) presence/absence data of combined inshore electric fishing and offshore trawling data (j) relative abundance data of combined inshore electric fishing and offshore trawling. The Upper-, Middle-, and Lower-Danubian sampling sites are indicated with black squares, grey triangles and open circles, respectively. Starting and ending river kilometre is also indicated for each section.

rkm	presence/absence 3					relative abundance 3				
	DE	NE	CIE	OT	AD	DE	NE	CIE	OT	AD
2214	1	1	1	1	1			1		1
2069	2	2	1	1	1	2	2	1		1
2008	1	1	2	1	1	2	2	1		1
1884	1	1	2	1	1	3		1		2
1873	1	2	2	1	1	2		1		1
1856	3	1	1	1	1	2	2	1		1
1804	3	1	2	2	1	3		1		2
1707	3	1	2	1	1	3		1		1
1634	2	1	2	2	1	3		1		2
1443	3	2	1	2	1	3	3	2	2	3
1385	1	2	1	3	1	3	3	2	2	3
1303	3	1	1	2	1	3	3	2	3	3
1248	1	2	1	2	1	3	3	2	3	3
1203	2	1	2	3	1	3	3	3		2
1164	2	3	3	3	2	3	3	3	2	3
1132	2	2	2	na	na	3	3	2	na	na
1107	2	2	1	na	na	3	3	3	na	na
850	2	3	3	na	na			1	na	na
604	3	3	3	1	2	3	3	3		3
559	2	3	3	1	2	3		1	3	2
371	na	na	na	2	na	na	na	na	3	na
167	2	3	2	3	3	3	3	2		3
130	2	3	2	3	3	3	3	3	2	3
58	1	2	2	3	3	3	3	2	2	3
15	2	2	3	3	3	3	3	2		3

Figure 3. Results of the k-means analysis showing the classification of the sampling sites at three cluster numbers in case of the different collection methods for presence/absence and relative abundance data. DE, day-time inshore electric fishing; NE, night-time inshore electric fishing; CIE, combined (day and night) inshore electric fishing; OT, offshore trawling; AD, combined inshore electric fishing and offshore trawling. Sites that belong to the same cluster

24

group are indicated with the same cell shade. Note that "na" indicates no data from the site due to logistical difficulties reasons.

APPENDIX I. Results of the k-means analysis showing the classification of the sampling sites at two, four, and five cluster numbers in case of the different collection methods for presence/absence and relative abundance data. DE, day-time inshore electric fishing; NE, night-time inshore electric fishing; CIE, combined (day and night) inshore electric fishing; OT, offshore trawling; AD, combined inshore electric fishing and offshore trawling. Sites that belong to the same cluster group are indicated with the same cell shade.

	presence/absence															relative abundance														
	2					4					5					2					4					5				
rkm	DE	NE	CIE	OT	AD	DE	NE	CIE	OT	AD	DE	NE	CIE	OT	AD	DE	NE	CIE	OT	AD	DE	NE	CIE	OT	AD	DE	NE	CIE	OT	AD
2214	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			1		1			1					1		
2069	1	2	1	1	1	1	1	2	1	1	1	1	2	1	2				1		2	2		2		2	1			2
2008	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			1	1		2	2	2	2		2	1	2	2
1884	1	1	1	1	1	1	2	3	1	1	1	2	3	1	1	2	1	2	2	2	2	2	1	2	1	2	1	2	2	3
1873	1	1	1	1	1	2	3	1	1	1	1	2	3	2	2		1	1	1	1	2	2	1	3	1	3	1	1	1	2
1856	2	1	1	1	2	2	1	4	1	2	2	1	4	1	2	1	1	1	1	1	2	2	1	3	1	2	1	1	1	2
1804	1	1	1	1	1	2	2	3	2	3	3	2	4	2	3	2	1	2	2	2	3	1	3	3	1	3	1	2	2	3
1707	1	1	2	1	1	1	2	3	2	3	1	2	5	2	3	2	1	2	1	1	3	2	3	1	1	2	1	2	1	1
1634	2	1	2	1	2	3	1	1	2	3	2	2	4	2	4	2	1	2	1	1	3	2	3	1	1	2	1	2	1	3
1443	2	1	2	1	2	2	3	2	1	2	4	2	4	3	4	2	2	2	2	2	2	1	3	4	4	2	4	2	3	4
1385	2	1	2	2	2	2	3	2	3	3	4	2	4	3	4	2	2	2	2	2	2	3	4	4	4	4	4	3	3	4
1303	2	1	2	1	2	2	1	2	4	2	4	2	4	2	3	4	2	2	2	2	4	4	4	4	4	4	4	3	4	4
1248	2	1	2	1	2	2	3	2	1	2	2	2	4	2	3	2	2	2	2	2	2	3	4	4	4	4	4	3	4	4
1203	2	1	2	1	2	3	1	2	4	3	2	3	4	4	5	2	2	2	1	1	3	3	4	1	1	2	4	3	1	3
1164	2	2	2	2	2	3	4	4	3	4	2	4	4	4	5	2	2	2	2	2	3	1	3	4	4	3	4	4	5	5
1132	2	2	1	na	na	3	3	4	na	na	2	3	1	na	na	2	2	2	na	na	2	4	4	na	na	5	4	5	na	na
1107	2	1	1	na	na	4	3	4	na	na	2	5	2	na	na	2	2	2	na	na	3	1	3	na	na	3	4	4	na	na
850	2	2	1	na	na	3	3	4	na	na	2	4	2	na	na	1	1	1	na	na	1	2	1	na	na	1	3	1	na	na
604	2	2	2	1	2	3	4	4	4	4	2	3	4	5	4	2	2	2	2	2	3	1	3	3	4	3	4	4	2	5
559	2	2	1	1	2	3	4	4	1	4	2	4	4	5	4	2	1	2	2	2	3	2	3	3	1	3	1	4	4	3
371	na	na	na	2	na	na	na	na	2	na	na	na	na	5	na	na	na	na	2	na	na	na	na	3	na	na	na	na	4	na
167	2	1	2	2	2	3	4	4	3	4	5	2	4	4	4	2	2	2	2	2	2	3	4	3	4	2	4	3	2	5
130	2	1	2	2	2	3	4	4	3	4	2	4	4	4	5	2	2	2	2	2	3	1	3	3	4	3	4	4	5	5
58	2	1	2	2	2	2	4	4	4	4	3	2	4	4	5	2	2	2	2	2	2	3	4	4	4	2	4	3	5	4
15	2	2	1	2	2	3	3	4	2	4	5	4	4	4	4	2	2	2	2	2	4	1	4	3	4	4	5	3	2	4