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Research Article

Assessment of biocontamination of benthic macroinvertebrate communities in European inland waterways

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Abstract

Introductions of alien species, regardless of their actual or potential impacts, can be considered as a biocontamination of the ecosystem. A simple method to assess biocontamination is described and tested on benthic macroinvertebrate communities from European inland waterways. This method includes calculations of abundance contamination and richness contamination at ordinal taxonomic rank, from which integrated estimations of biocontamination are derived. Our method can be applied to data collected during routine water quality monitoring, and allows estimation of biocontamination at specific study sites as well as integrated assessment of ecosystems or assessment units. Results clearly show that the main European inland waterways are highly biologically contaminated. They also indicate that richness contamination precedes abundance contamination, and that severe abundance contamination may be caused even by a single ecologically aggressive alien species. Comparison of biocontamination indices and ecological quality status by conventional methods suggests that these metrics are negatively correlated, and richness contamination has a stronger negative affect than abundance contamination. Biocontamination warrants inclusion within the development of holistic estimates of ecological quality status and should be considered in water management policy.

Key words: biocontamination, alien species, ecological status, benthic macroinvertebrates, European inland waterways, aquatic ecosystems

Introduction

Human-mediated introductions of invasive alien species in European inland and coastal waters are considered a serious environmental issue that requires development of relevant management approaches (Leppäkoski et al. 2002; Panov et al. 2002; Gherardi 2007). Alien species (AS) are those that take up residence in a biogeographical area, such as a river catchment, where they were previously unknown. In the context of the EU Water Framework Directive (European Community 2000), invasive AS represent a significant biological pressure. The assessment of such pressure should therefore be considered within an integrated catchment management strategy and should receive special attention within the context of ecological status assessments required by the EU Water Framework Directive (Cardoso and Free 2008).

Owing to adverse ecological and economic consequences, AS invasions are perceived as biological pollution of aquatic ecosystems. According to Elliott (2003), “biological pollution” is defined as the effects of introduced invasive species sufficient to disturb an individual, a population or a community; including the causation of adverse economic consequences. However, the quantitative assessment of negative impacts of AS is difficult and requires comprehensive research and database efforts (Molnar et al. 2008). Consequently, quantitative estimates of “biological pollution” sensu Elliott (2003) in aquatic ecosystems are lacking (Olenin et al. 2007). A more practical approach for assessing the impact of AS on the ecological status of water bodies, therefore, may be to assume that their affect is proportional to their occurrence and abundance within the invaded community. In such a case, AS would be considered as biological contaminants rather than biological pollutants, and “biological contamination” (i.e. biocontamination) means the presence of AS regardless of their abilities to cause negative ecological and/or socio-economic impacts (see also Panov et al. 2009).

The purpose of the present study is to address AS in ecological status assessments of water bodies, including specific locations within water bodies, considering the above “biocontamination” concept. We describe a simple method to measure the biocontamination of aquatic communities, which does not require sophisticated research and can be applied to water bodies for which routine monitoring data are available. This method is tested on benthic macroinvertebrate assemblages of European inland waterways. Since invaders affect the structural organisation of recipient communities (Simon and Townsend 2003), we hypothesize that the relative abundance of aliens within a community and the proportion of AS within a community at ordinal taxonomic rank are sufficient quantitative indicators to provide an integrated estimation of biocontamination. The ratio in numbers of AS to all species, i.e. abundance contamination, measures the community dominance by aliens. Whereas the proportion of alien orders within a community, i.e. ordinal richness contamination, can also be interpreted as a proxy of disparity contamination. Different macroinvertebrate orders represent particular ecomorphological groups associated with specific feeding patterns, therefore, the higher taxonomic richness provides an index of disparity (Foote 1997), and this concept is relevant to community structural organization. In parallel, richness contamination at familial and specific levels were investigated, when data were available, to test their utility for the assessment of alien contamination. Moreover, the relationship between biocontamination and ecological status in the inland waterways of Europe is analyzed and discussed.

Material and methods

Assessment of biocontamination

Biocontamination of sampling sites was assessed using a site-specific biocontamination index derived from two metrics: abundance contamination index (ACI) and richness contamination index (RCI) at ordinal rank. These indices were calculated as:

$$ACI = N_a/N_t$$

where N_a and N_t are numbers of specimens of alien taxa and total specimens in a sample, respectively, and

$$RCI = T_a/T_t \qquad (1)$$

where T_a is the total number of alien orders, and T_t is the total number of identified orders (for recorded AS and taxonomic resolution applied in this study see Annexes 1 and 2, respectively).

With values of ACI and RCI, the site-specific biocontamination index (SBCI) can then be derived from matrix in Table 1. Five classes of biocontamination ranging from 0 (“no” contamination) to 4 (“severe” contamination) are defined. Furthermore, these classes of SBCI directly correspond to five ecological quality classes sensu the Common Implementation strategy for the EU Water Framework Directive (2000/60/EC) (European Community 2000; European Communities 2003), and allow status ranking from “high” to “bad” (Table 1). The threshold for the lowest quality limit (“bad” class) is based on the assumption that when AS represent more than half the detected orders or when their abundance exceeds 50% of the individuals, the community/assemblage has developed as a consequence of the invasion by non-native species.

In those cases when multiple estimates of ACI and RCI are available for the same ecosystem or assessment unit (i.e. samples were collected at several sites), the integrated biocontamination index (IBCI) can be derived by averaging ACI and RCI of study sites, and ranking IBCI on mean values following Table 1.

Table 1. Assessment of site-specific and integrated biocontamination indices (SBCI and IBCI, correspondingly) based on abundance contamination index (ACI) and ordinal richness contamination index (RCI). SBCI and IBCI classes: 0 (no biocontamination, "high" ecological status, blue cell), 1 (low biocontamination, "good" ecological status, green cell), 2 (moderate biocontamination, "moderate" ecological status, yellow cells), 3 (high biocontamination, "poor" ecological status, orange cells), 4 (severe biocontamination, "bad" ecological status, red cells).

RCI	ACI				
	none	0.01 – 0.10	0.11 – 0.20	0.21 – 0.50	>0.50
none	**0**				
0.01 – 0.10		1	2	3	4
0.11 – 0.20		2	2	3	4
0.21 – 0.50		3	3	3	4
>0.50		4	4	4	4

Study sites and sampling

The method described above was tested on several extensive data sets from the main inland waterways of Europe. Samples of benthic macroinvertebrates for evaluation of biocontamination and ecological status of aquatic ecosystems were collected in selected assessment units (AUs) located within the three main European inland invasion corridors (sensu Bij de Vaate et al. 2002; Galil et al. 2007; see Figure 1): 1) two AUs within the Northern corridor (NC) - Neva Bay (NC5, 8 study sites, 1999) and Lake Ladoga (NC4, 8, 2000); 2) eight AUs within the Central corridor (CC) - Lower Pripyat River (CC8, 5, 2007), middle Pripyat River (CC9, 3, 2007), Pripyat-Bug canal (CC10, 5, 2007), middle Nemunas River (CC11, 5, 2007), lower Nemunas River (CC12, 7, 2007), Bug River (CC14a, 5, 2003), Vistula River (CC14b, 28, 2000), and Oder River (CC16, 14, 2001); and 3) nine AUs within the Southern corridor – lower Danube River (SC2, 3, 2007), middle Danube River (SC3, 3, 2007), Sava River (SC3a, 3, 2006), Tisa River (SC3b, 3, 2001), upper Danube River (SC4, 3, 2007), Main-Danube canal (SC5, 1, 1998), Main River (SC6, 2, 2001 and 2007), Rhine River (SC7, 3, 2006), and Rhine River Delta (SC8, 1, 1987-1999). In addition, the AU, Sukhoy Liman, was selected within the Southern Meridian corridor, as it links the southern parts of all three main invasion corridors (SMC1, 12, 2008) (Annex 3).

In most cases, sampling was performed by procedures comparable with AQEM methodology (AQEM 2002). In Neva Bay and Lake Ladoga, samples were collected in the shallow littoral (0.2-0.5 m depth) zone using a stovepipe sampler designed for quantitative collection of macroinvertebrates in reed beds (Panov 1996). Samples from the Pripyat River and Pripyat-Bug canal were collected using a dip net at depths between 0.2 and 0.5 m by sweeping a 5 m distance, in total. Samples from the Nemunas River were collected from shoreline to 1.2 m depth using a dip net over various substrates and vegetation for 15 min by two persons. In the Bug, Vistula and Oder rivers, a standard semi-quantitative procedure using a benthic dip net was applied (Grabowski et al. 2006). In the southern part of the Southern invasion corridor, sampling was also performed using a benthic dip net during three surveys: International Tisa Research (2001, organized by the International Commission for the Protection of the Danube River), Sava Survey (2006, supported by the Serbian Government) and Joint Danube Survey 2 (2007, supported by the International Commission for the Protection of the Danube River). Data for the Main-Danube canal, the Main River and the Rhine River were provided by the German Federal Institute of Hydrology. The sampling was performed from a ship by means of an orange-peel grab. Data on macroinvertebrates assemblages of the Rhine River Delta near Lobith (rkm 882) in the Netherlands were obtained from the Dutch Institute for Inland Water Management and Waste Water Treatment (RIZA). Over the period 1987-1999, macroinvertebrates were collected using artificial substrates (i.e. baskets filled with marbles). The annual taxa richness and relative abundance of

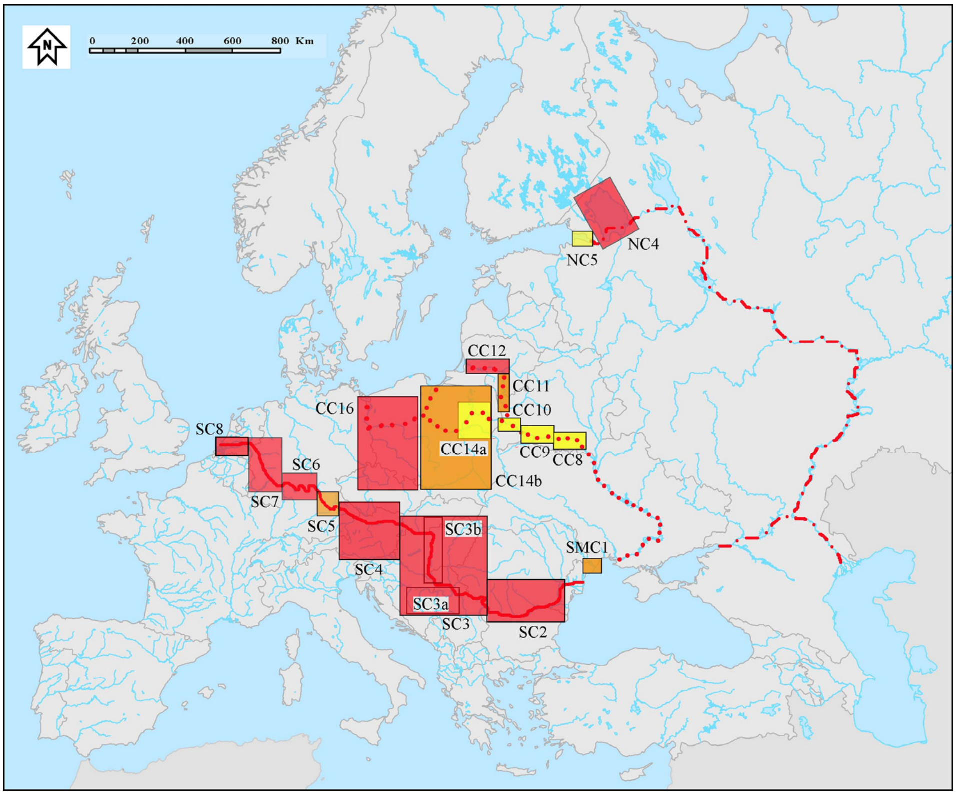

Figure 1. Selected assessment units (AUs) within European inland waterways (see Annex 3 for details). Dashed-dotted, dotted and solid lines indicate the Northern, Central and Southern invasion corridors, respectively. Colour fillings of AUs indicate integrated biocontamination estimates during the studied period (see Table 1 and Annex 3).

macroinvertebrates were based on pooled data (two baskets per sampling date; four to seven sampling dates from spring to autumn).

Macroinvertebrate samples were preserved in 4% formaldehyde or 70% alcohol. Animals were picked from whole samples or, when necessary, sub-sampling was applied.

Calculations and statistical analysis

Site-specific indices of abundance and ordinal richness contamination were calculated for all study sites. From these estimates, the SBCI for all study sites and the IBCI for all AUs were derived. Numbers of AS within study sites and AUs were counted for further analysis. In many cases, the richness contamination at familial rank was also evaluated following equation (1) and considering T_a and T_t as the number of alien families and the total number of families, respectively. If monitoring data existed at species-level resolution, the specific richness contamination indices were analogically calculated. Estimates of ordinal, familial and specific RCI were compared for their performance and utility for evaluation of biocontamination.

The relationship between biocontamination and ecological quality status was assessed using the SBCI (and metrics of its derivation, when appropriate) and BMWP (Biological monitoring working party) scores at sampling sites. The BMWP method is widely used in the EU and has proved to be among the best indicators of ecological quality of water bodies (Armitage et al. 1993; Leeds-Harrison et al. 1996; Semenchenko and Moroz 2005).

Continuous variables were analysed by ANCOVAs with studied waterways (which actually corresponded to countries) as a categorical predictor. This approach was taken because differences resulting from biogeographical factors and biocontamination level or applied sampling methodologies might be responsible for significant variation. Variation in continuous variables within biocontamination classes (categorical variable) was analysed by nested ANOVAs, controlling for waterway effect. Statistical calculations were performed using Statistica software (StatSoft, Inc., 2004, version 6).

Results

Annex 1 lists the AS recorded in various AUs. This list includes only those species that were identified in benthic samples from this study and were used in the calculation of biocontamination indices. Although the mysid *Paramysis lacustris* is known to occur over the entire middle section of the Nemunas River (CC11) (Arbačiauskas 2005), this species was not found in the dip net samples (probably due to its low density). Similarly, the polychaete *Hypania invalida* was found only in samples collected by Ponar grab in the port bay of Brest, the Pripyat-Bug Canal (CC10), but not in the dip net samples. Consequently, these two species were not indicated for those AUs. Therefore, it should be noted that Annex 1 does not represent a complete list of AS known from the European inland waterways included in this study.

Of the 43 AS listed in Annex 1, 24 are crustaceans, including13 species of amphipods, 3 species of mysids, isopods and decapods, and 2 species of balanids. Molluscs are represented by 8 snails and 7 mussels, while only one species each of flatworms, oligochaetes and polychaetes are included. In rivers of the Baltic and North Sea basins peracaridans prevail over other invaders with respect to species richness, whereas in the Black Sea basin rivers the diversity of alien molluscs is higher than that in more northern areas (Annex 1).

Site-specific and integrated estimates of abundance contamination and ordinal richness contamination (also familial and specific richness contamination when data were sufficient) as well as biocontamination assessments and BMWP scores at study sites are given in Annex 3.

The time series data that existed for the Rhine River Delta (SC8) allowed for the investigation of a temporal trend in biocontamination (Figure 2A). These data indicate that richness contamination preceded abundance contamination. When comparing RCI at different taxonomic ranks, the highest values were estimated at the ordinal level while the lowest values were estimated at specific level. In total, 17 non-indigenous macroinvertebrate species were recorded on artificial substrates in the River Rhine at Lobith (Annex 1). RCI estimates changed little during 1987-1991 but increased between 1992 and 1998. This increase in local fauna contamination could be mainly attributed to the invasions of Ponto-Caspian species after the opening of the Rhine-Main-Danube Canal, or so-called Southern invasion corridor, in 1992 (Van der Velde et al. 2000; Bij de Vaate et al. 2002).

In contrast to RCI estimates, the ACI indicated a rapid increase in the abundance of AS over the period 1987-1991, followed by a state of dynamic equilibrium (mean value of 0.83) during 1992-1999 (Figure 2A). The high ACI values could be mainly attributed to five AS that dominated the macroinvertebrate assemblages in those years: the isopod, *Jaera istri*, amphipods, *Chelicorophium curvispinum*, *Dikerogammarus villosus*, *Chaetogammarus ischnus* and *Gammarus tigrinus*, and bivalve molluscs, *Dreissena polymorpha* and *Corbicula fluminea*.

Based on BMWP scores, the ecological quality of the Rhine River Delta appeared to be moderate over the period 1987-1999 (Annex 3). However, the BMWP does not consider the contribution of AS to macroinvertebrate assemblages. The SBCI scores clearly indicate a decrease of ecological quality from poor (SBCI = 3) to bad (SBCI = 4), due to severe biocontamination of the river (Annex 3).

The surveys of the Nemunas River, Pripyat River and Pripyat-Bug Canal allowed an analysis of the spatial patterns of biocontamination. Eight and five AS were recorded in the lower and middle sections of the Nemunas River (CC11 and CC12), respectively (Annex 1). As indicated by the ACI estimates, biocontamination of the lower section of the river was severe (Figure 2B), resulting in bad IBCI-estimated ecological status (Annex 3). This low status mainly resulted from large numbers of the pontogammarid, *Pontogammarus robustoides*, the snail, *Lithoglyphus naticoides*, and mysids, *P. lacustris* and *Limnomysis benedeni*. The high abundance of these AS was expected because this section of the Nemunas River is located downstream of the Kaunas Water Reservoir into which Ponto-Caspian peracaridan species were intentionally introduced during the early 1960s (Arbačiauskas 2002). In contrast, the most biologically contaminated part of the middle section of the

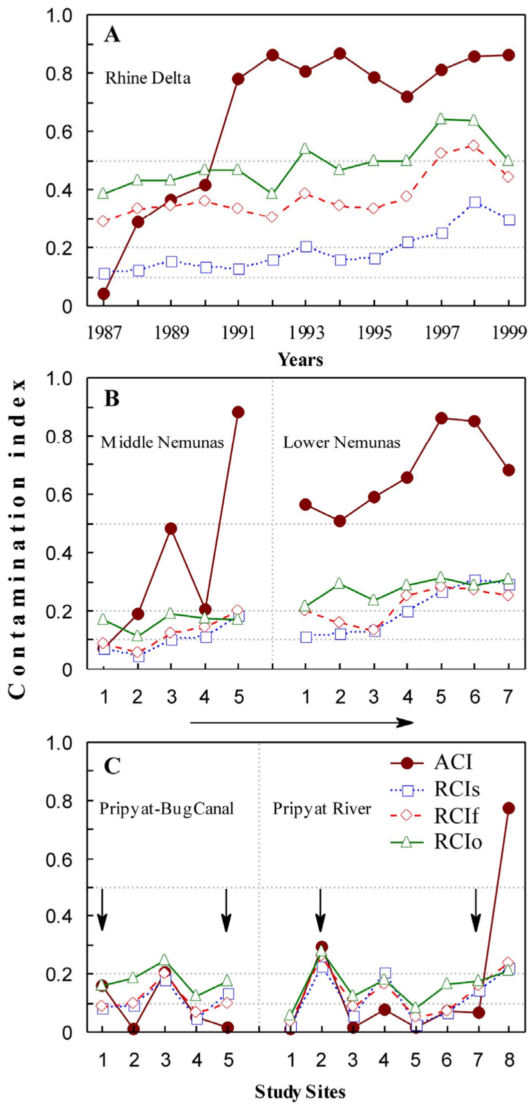

Figure 2. Temporal (A) and spatial (B, C) trends of abundance contamination (ACI) and richness contamination at specific (TCIs), familial (TCIf) and ordinal (TCIo) ranks in the Rhine River Delta (A), the Nemunas River (B) and the Pripyat River and Pripyat–Bug Canal (C), respectively. Study sites in (B) and (C) are arranged in upstream-downstream direction indicated by horizontal arrow (for coordinates see Annex 3). Vertical arrows in (C) indicate river ports.

Nemunas River was at the most downstream study site where the Kaunas Water Reservoir begins, whereas the presence and abundance of AS in macroinvertebrate communities decreased upstream (Figure 2B). The two most upstream study sites had SBCI scores of 2 (moderate biocontamination), whereas the ecological status of the entire middle section of the Nemunas River must be considered as poor (IBCI = 3) (Annex 3). Similarly to the Rhine River Delta, the highest values of RCI were obtained using ordinal taxonomic rank. However, at sites with high ACI, estimates of richness contamination were quite similar for the three different taxonomic resolutions (Figure 2B).

In the Pripyat River (CC8 and CC9) and Pripyat-Bug Canal (CC10), ten AS were recorded (Annex 1). Generally, the number of non-native species decreased with increasing distance from the potential donor area, the Kiev Water Reservoir located on the Dniepr River downstream from the inflow of the Pripyat River. Also there was an obvious increase in the number of AS at river ports compared to other study sites with the mean number being significantly higher even if the most downstream site was included (5.2 vs. 2.8 species, Kruskal-Wallis test: $H(1,n=13)=4.14$, $P<0.042$). The most common AS were the pontogammarids, *Dikerogammarus haemobaphes* and *D. villosus*, and the snail, *L. naticoides*. The latter species was responsible for the dramatic abundance contamination in the most downstream study site of the Pripyat River. This snail accounted for more than 1000 specimens per sample. Consequently, the highest SBCI was observed at that study site. The second highest biocontamination was observed at the Mykashevichy River Port (Site 2 on the Pripyat River, Figure 2C), which is characterized by a high number of ship calls.

One more aspect of biocontamination revealed itself in the Pripyat-Bug Canal. The highest contamination here was found at Site 3. Although only 2 AS were recorded, their share among native taxa was comparatively high because this part of the canal is artificial and the diversity of native species is low compared to other parts that are closer with respect to hydromorphology of natural rivers.

It should be noted that in most study sites of the Pripyat River and Pripyat-Bug Canal, the richness contamination indices received higher values than those for abundance contamination (Figure 2C). Such a pattern suggests that biocontamination of this waterway is ongoing and the decrease of ecological quality due to growth of abundance contamination may be expected. On the whole, two AUs within the Pripyat River and the Pripyat-Bug Canal were assessed as moderately biocontaminated systems (IBCI = 2) (Annex 3).

Neva Bay (NC5) and Lake Ladoga (NC4) were surveyed during 1999 and 2000, respectively. The littoral zone of Neva Bay, the freshwater part of the Neva River estuary, was contaminated by two alien amphipods, the Baikalian *Gmelinoides fasciatus* and Ponto-Caspian *P. robustoides*. They were present in most sampling locations. ACI and RCI at familial and ordinal ranks ranged 0.082-0.470, 0.067-0.200 and 0.083-0.250, respectively, resulting in estimation of IBCI of the littoral zone of Neva Bay as moderate (Annex 3). In Lake Ladoga, only one AS was recorded: the Baikalian amphipod, *G. fasciatus*. Still this single AS accounted for a dramatically large proportion of the organisms at most study sites. Consequently, SBCI estimates at most locations were high, and IBCI for Lake Ladoga littoral macroinvertebrate communities was estimated at 4, which indicates severe biological contamination (Annex 3).

During the survey of the Bug River (CC14a) in 2003, 4 alien macroinvertebrates, all of them amphipods of Ponto-Caspian origin, *C. curvispinum*, *C. ischnus*, *D. haemobaphes* and *D. villosus*, were recorded (Annex 1). These species occurred at two study sites located downstream from the Pripyat-Bug Canal. Their high abundances were responsible for high values of SBCI at these locations, while the remaining three sites were devoid of AS. The IBCI value for the Bug River was 3 (Annex 3).

Altogether 7 AS were found during the survey in the Vistula River (CC14b) (Annex 1). Among them were the snail, *Potamopyrgus antipodarum*, and the bivalve, *D. polymorpha*. All the other species were crustaceans, including amphipods of Ponto-Caspian origin, *C. curvispinum*, *C. ischnus*, *D. haemobaphes* and *D. villosus*, and the American crayfish, *Orconectes limosus*. AS occurred at 22 of 28 sampled locations. Generally, the observed pattern shows that AS were lacking in most upstream parts of the Vistula River, while their presence in macroinvertebrate communities increased downstream, resulting in the increase of SBCI values from 1 to 3 in the middle part of the river, and reaching 4 in the lowest part. The high biocontamination indices were mainly associated with a high abundance of *Dreissena* and alien amphipods (Annex 3).

A total of 7 alien macroinvertebrates were recorded from benthic samples in the Oder River (CC16) during 2001. There were two Ponto-Caspian molluscs, the gastropod, *L. naticoides*, and the bivalve, *D. polymorpha*. The remaining species were amphipods, including the North American *G. tigrinus*, South European *Gammarus roeselii* and Ponto-Caspian *C. curvispinum*, *D. haemobaphes* and *D. villosus* (Annex 1). AS occurred in 13 of 14 sampled locations. The only site free of aliens was a study site in the most upstream part of the river in Poland. At all the other locations, the dominant AS, in terms of abundance, were *C. curvispinum* and *G. tigrinus*, with poor representation of native fauna. Thus, with the exception of one and two sites with SBCI values of 2 and 3, respectively, the remaining 10 locations had high SBCI scores. As a result, the IBCI for the Oder River was also estimated at 4, indicating severe biocontamination and bad ecological status (Annex 3).

In the Danube River (SC2, SC3 and SC4), which belongs to the Southern invasion corridor, a total of 19 non-native species were found (Annex 1). All these species were recorded in the main channel of the Danube River. Along the investigated stretch of the Sava River (SC3a), 9 AS were identified, and 3 AS were recorded in the Tisa River (SC3b). The most frequent and abundant species was *L. naticoides*. This snail was recorded at all sampling sites, with relative abundance ranging from 0.8 to 54.4% of the total benthic community. Considerable occurrence and relative abundance were recorded for *D. villosus*. This pontogammarid was found in 73% of all samples, with relative abundance ranging from 0.4 to 43.8%. In addition to those species, the mussel, *C. fluminalis*, was present in all investigated AUs, except the Tisa River. The tubificid worm, *Branchiura sowerbyi*, was recorded in the middle Danube, along the entire lower Danube and in surveyed tributaries. The distribution of this species generally is associated with hydromorphological modification of rivers (Paunović et al. 2005). The Ponto-Caspian mysid, *L. benedeni*, was found to be limited to the lower and middle Danube River. Meanwhile the polychaete, *H.invalida*, was found in samples from the upper and middle Danube River. Its relative abundance varied between 0.4 and 3.9% of the total benthic community. This species was also recorded in the Sava River. Both dreissenid species were also detected among the non-native fauna of the Danube River. The zebra mussel, *D. polymorpha*, which is native for estuaries and coastal waters of the Ponto-Caspian and the Aral Sea basins and associated estuaries, was more abundant and outspread in the Danube River, while the quagga mussel, *Dreissena rostriformis bugensis*, which is native to the Dnieper and Bug

Limans of the northern Black Sea, was found limited to the lower Danube River (Annex 1). The majority of recorded non-native species are of Ponto-Casian origin (14 species). Beside these, aliens from New Zealand (the mudsnail, *P. antipodarum*) and Eastern Asia (the Chinese pond mussel, *Sinanodonta woodiana*, Asian clams, *C. fluminea* and *Corbicula fluminalis*, and the tubificid worm, *B. sowerbyi*) were recorded.

Assessments of the alien contamination and ecological status of the lower and middle Danube River and its tributaries (five AUs) suggest a high level of biocontamination and low status of ecological quality along the main course of the river, as well as in its tributaries, the Sava River and the Tisa River. With respect to IBCI, all AUs showed severe biocontamination and consequently indicated bad ecological status (Annex 3).

In the northwestward-located parts of the Southern invasion corridor, the Main-Danube Canal (SC5), Main River (SC6) and Rhine River (SC7), 3, 9 and 10 AS were recorded (Annex 1). In common to the Danube River, the Main and Rhine rivers indicated severe biological contamination and bad ecological status. Whereas in the Main-Danube canal, since 1992 connecting these rivers, the ecological status with respect to AS was estimated as poor, consequently one rank better than in the rivers (Annex 3). Such an estimate was derived from data collected in 1998 (since then no surveys were done). Within the last decade, at least 5 new AS from the Black Sea basin (including amphipods *Chelicorophium robustum* and *Chaetogammarus trichiatus*) have spread via the canal to the Main River, and further on to the Rhine River (Bernauer and Jansen 2006). The dispersal of AS in the opposite direction also is ongoing, and at least one macroinvertebrate species, the Chinese mitten crab, *Eriocheir sinensis*, has used the canal to penetrate into the Danube River (Rabitsch and Schiemer 2003). Currently the biocontamination of the Main-Danube Canal probably is substantially higher, and the ecological status of this water body has changed from poor to bad.

Assessment of biocontamination was applied also to Sukhoy Liman (SMC1), the AU which includes marine and freshwater environments. In marine part of Sukhoy Liman, which includes the nearby Commercial Sea Port of Illichivsk, that is known to harbor numerous non-indigenous species (Koshelev and Son 2007), low densities of AS were detected. In contrast, only the New Zealand mudsnail, *P. antipodarum*, was present in small rivers and streams emptying into the liman, however, this species was very abundant. As a result, ACI estimates, and consequently SBCI estimates, for freshwater locations were higher than those for marine locations (Annex 3).

The survey of main European waterways clearly suggests that benthic macroinvertebrate communities in all studied AUs are biologically contaminated, with integrated biocontamination indices ranging from 2 (moderate biocontamination or moderate ecological status) to 4 (severe biocontamination or bad ecological status). Highly biocontaminated water bodies include the littoral zone of Lake Ladoga, the lower section of the Nemunas River, the Oder River, the Rhine River and its delta, the Main River and the Danube River and its sampled tributaries, with mean relative abundance of AS exceeding 50% of the macroinvertebrate community. Only the Pripyat River, the Bug River, their joining canal, and the Neva Bay (during 1999 when sampling was performed) were found to be moderately biocontaminated systems (Figure 1, Annex 3).

The relationship between abundance contamination and richness contamination at different ranks was analysed over those AUs for which estimates on all taxonomic resolutions were available, i.e. the Rhine River Delta, Nemunas River, Pripyat River, Pripyat-Bug Canal, and the Danube River and its tributaries. A significant correlation was observed between abundance contamination and richness contamination for each taxonomic rank (Figure 3). As specific conditions within different waterways may influence these relationships, an ANCOVA with waterway as a fixed factor and ACI as a covariate was applied. Partial correlations between ACI and RCI were significant for specific ($r=0.52$, $P<0.001$) familial ($r=0.53$, $P<0.001$) and ordinal ($r=0.34$, $P<0.017$) ranks with the weakest correlation for ordinal level. The later may be interpreted also as the indication by ordinal richness contamination of different aspect of biocontamination in comparison to that measured by abundance contamination.

Of interest is how indices of abundance and richness contamination varied within different biocontamination classes. For this analysis, data were used from those AUs for which information on ordinal and familial RCI and spatial variation of site-specific estimates were available (see Annex 3). Variation of metrics showed that the separation between moderate, high and severe biocontamination resulted primarily from abun-

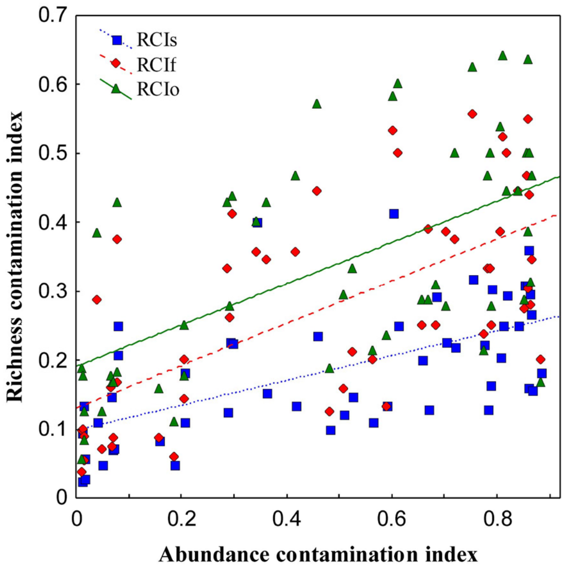

Figure 3. Relationships between abundance contamination index (ACI) and richness contamination index at specific (RCIs), familial (RCIf) and ordinal (RCIo) ranks. Lines indicate linear fit. Correlations between ACI and RCIs, RCIf and RCIo are 0.60, 0.65 and 0.59 (n=52, P<0.001), correspondingly.

dance contamination estimates, whereas, differentiation between good and moderate ecological status with respect to AS depended upon estimates of richness contamination (Figure 4). This is in accordance with above described temporal and spatial trends of biocontamination (see Figure 2) which definitely indicate that richness contamination precedes abundance contamination. Consequently, holistic assessment of biocontamination should consider and integrate both measures of contamination by AS. Variation of richness contamination at familial rank within SBCI classes suggests that this index actually may be used as a proxy SBCI for separation between low, moderate and high biocontamination (Figure 4). The threshold values between adjacent quality classes may be set as means between 75 and 25 percentiles of variation within higher and lower biocontamination classes, respectively. From current data, familial RCI values for good, moderate and poor ecological status range 0-0.07, 0.08-0.15 and >0.15, respectively. Such a procedure is in accordance with recommendations for the establishment of thresholds for ecological quality classes (see European Communities 2003).

When assessing biocontamination another important aspect is that severe abundance contamination of recipient communities may be caused by a single invasive AS. Although the correlation between the number of non-indigenous species in a community and abundance contamination was significant in the current study, the number of AS explained just 9% of total ACI variation (Figure 5). In particular, high abundance contaminations caused by just one or two AS were observed in Ladoga Lake, the Vistula River, the Oder River and the Sukhoj Liman (Annex 3).

The second purpose of this study was to investigate the relationship between biocontamination and ecological quality status assessed by conventional methods. Estimates of ecological status may depend upon biogeographical reasons, sampling methods and other local factors, therefore, BMWP estimates were subjected to nested ANOVA with SBCI ranks nested within waterway classes. Since the number of BMWP score estimates for high, good and moderate quality classes (with respect to biocontamination) were few, the BMWP estimates for those classes were merged into one group and only those waterways wherein estimates for at least 3 SBCI classes were available were involved in this analysis. As country effect when comparing BMWP scores for study sites located in Belarus and Poland was not significant (nested ANOVA, country effect: $F_{1,26}$=0.7, P=0.41), those BMWP estimates were further merged into one group. Results of the analysis showed that the ecological quality status estimated by BMWP method was significantly influenced by biocontamination (Table 2, Figure 6). Sites with higher biocontamination had lower ecological quality. Furthermore, the highly significant effect of waterway primarily reflects the different sampling effort (sampling method) used in different countries. It seems that the larger sampling effort applied in the Nemunas River resulted in larger numbers of recorded taxa and, consequently, higher BMWP estimates in comparison to other AUs.

The above analysis only revealed that study sites with higher biocontamination receive lower ecological quality estimates by BMWP method, however, it does not answer the question whether biocontamination directly affects ecological status estimates by conventional methods. Consequently, ANCOVAs using waterway (i.e. country) as a fixed factor and indices of biocontamination (ACI and ordinal and familial RCI) as covariates were conducted. To enable the traditional ANCOVA design, i.e. to remove

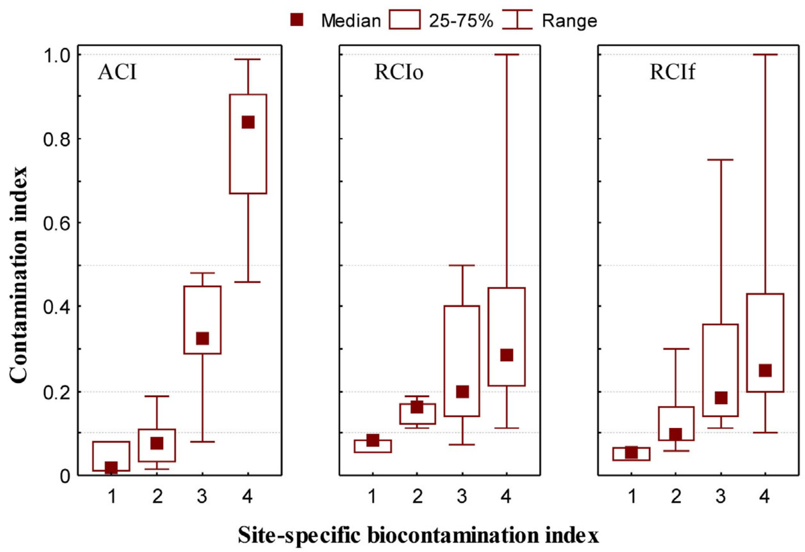

Figure 4. Variation of abundance contamination index (ACI) and richness contamination index at ordinal (TCIo) and familial (TCIf) ranks within different biocontamination classes: low (1), moderate (2), high (3) and severe (4).

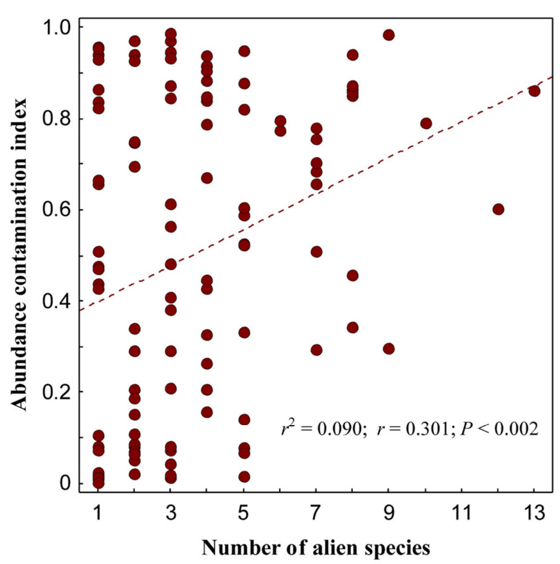

Figure 5. Relationship between number of alien species in a community and abundance contamination index. One estimates per study sites involved, i.e. for the Rhine River Delta only the last estimate was used to address pseudo-replication.

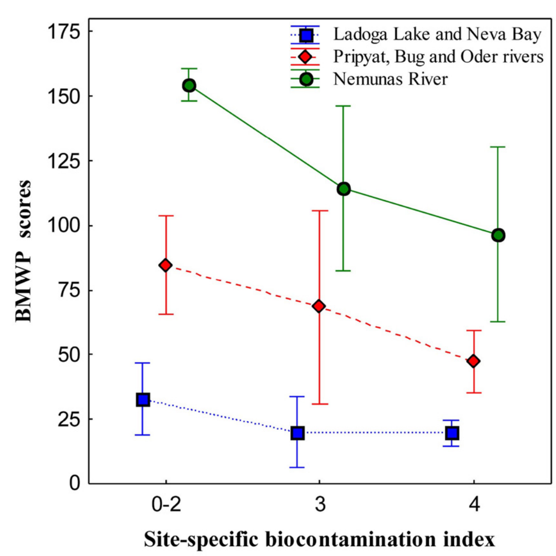

Figure 6. BMWP score weighted means in 3 groups of differently biocontaminated study sites (SBCI: 0-2, 3 and 4) located within Ladoga Lake and Neva Bay, the Pripyat, Bug and Oder rivers and Pripyat-Bug canal, and the Nemunas River. For results of nested ANOVA see Table 2. Vertical bars denote 0.95 confidence intervals.

Table 2. ANOVA assessing the impact of site-specific bio-contamination index (SBCI) class nested in waterway effect on ecological quality status measured as BMWP scores.

Effect	MS	df	F	P
Intercept	192397	1	250.0	<0.001
Waterway	24223	2	31.5	<0.001
SBCI	2482	6	3.2	0.009
Error	770	50		

Table 3. ANCOVAs assessing the impact of abundance contamination index (ACI) and richness contamination indices at ordinal (TCIo) and familial (TCIf) ranks, as covariates, on log-transformed BMWP scores measured in different waterways.

Effect	MS	df	F	P
ACI	0.3770	1	10.0	0.002
Waterway	0.9247	4	24.4	<0.001
Error	0.0378	68		
TCIo	1.0561	1	37.9	<0.001
Waterway	0.9777	4	35.1	<0.001
Error	0.0279	68		
TCIf	1.1354	1	42.5	<0.001
Waterway	0.9128	4	34.2	<0.001
Error	0.0237	68		

the interaction of continuous and categorical predictors, which was detected when using familial RCI as covariate (homogeneity-of-slope model, interaction effect: $F_{4,64}=5.26$, $P<0.002$), prior to analysis, BMWP scores were log-transformed. This analysis clearly shows that biocontamination significantly affects estimates of ecological status by BMWP method (Table 3, Figure 7). As in the previous analysis, the waterway effect also was significant. Estimates of ACI and RCI at familial and ordinal ranks were negatively correlated with BMWP scores (partial correlations were −0.36, −0.62 and −0.60, correspondingly), with a stronger negative correlation between indices of richness contamination and BMWP. The later suggests that the negative influence of richness contamination on BMWP estimates is stronger than that for abundance contamination.

Discussion

We attempted in this study to assess the biocontamination of benthic macroinvertebrate communities in various inland waterways of Europe. To achieve this it was first necessary to develop a method for assessing biocontamination. The method was designed to be simple and applicable to data collected during routine monitoring of water ecological quality. We hypothesized that an integrated index of biocontamination should include two measures characterizing different aspects of community structural organization, a measure of community dominance by AS which can be measured by relative abundance of alien individuals in the community (abundance contamination), and a measure of alien contribution to community disparity which can be assessed by the proportion of alien taxonomic orders in the community (ordinal richness contamination or disparity contamination). The utility of richness contamination at familial and specific ranks for biocontamination evaluation also was investigated.

Data on spatial and temporal variation of AS presence in benthic communities from different European inland waterways suggest that richness contamination precedes abundance contamination. During initial phases of invasions, ordinal RCI appears to be more sensitive to changes in comparison to estimates of richness contamination at familial or specific ranks (see Figure 2), and that is in accordance with the precautionary principle in environment management. Estimates of specific richness contamination (which also can be interpreted as diversity contamination) appeared to be of low value for assessment of biocontamination, at least with the method described here. Moreover, identification of all benthic macroinvertebrates to species level requires a substantial sample processing effort. This, however, does not imply that identification of AS to species is not required. Meanwhile, richness contamination at familial rank was capable to separate between classes of low, moderate and high biocontamination (see Figure 4), and after a more detailed analysis it may be found to be applicable in the assessment of biocontamination when only qualitative data are available or for the fast screening of previously collected information on benthic macroinvertebrates.

Our biocontamination index derived from estimates of abundance and richness composition

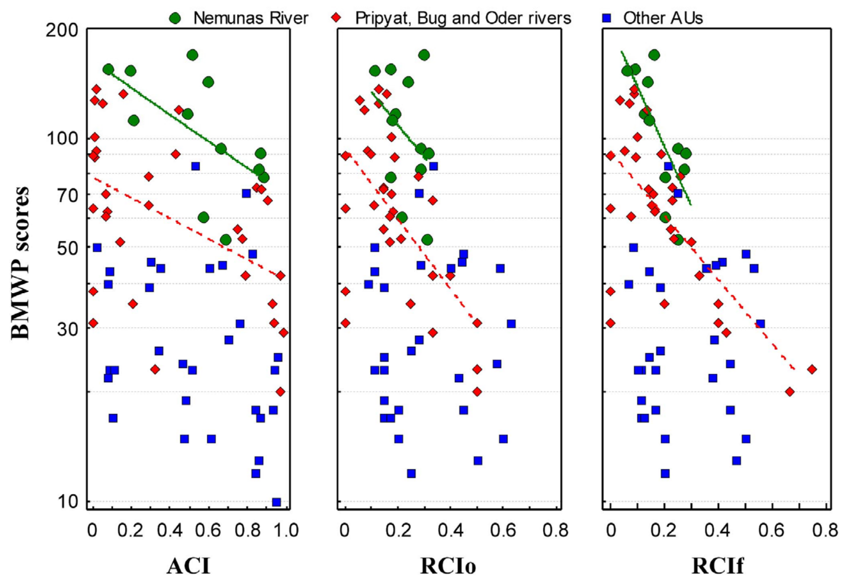

Figure 7. Relationships between abundance contamination index (ACI) and richness contamination indices at ordinal (RCIo) and familial (RCIf) ranks, and ecological status estimated by BMWP scores. Data for the Nemunas River (1), and the Pripyat, Bug and Oder river including Pripyat-Bug Canal (2) are highlighted. Note logarithmic scale. Solid and dashed lines indicate linear fit for 1 and 2, respectively.

allows a biocontamination evaluation of specific study sites as well as entire ecosystems or assessment units. It can be recommended for implementation in routine water ecological quality monitoring when multiple-habitat sampling is applied, and data are sufficient to reflect the taxonomic richness of benthic macroinvertebrates. While we applied this method to benthic macroinvertebrates, which are among the main indicators of ecological status of flowing waters, it may be extended to include other aquatic organisms such as fish or macrophytes in order to receive a more comprehensive assessment of biocontamination in aquatic ecosystems.

Our assessment of biocontamination clearly shows that the main inland waterways of Europe, at least with respect to benthic macroinvertebrates, are highly contaminated with AS. Since data in a few assessment units were collected some time ago, the current status of biocontamination therein may be even higher. Highly contaminated assessment units were identified in all three European invasion corridors (Figure 1). Severe biocontamination was observed in the littoral area of Lake Ladoga, the lower section of the Nemunas River, the Oder River, the Rhine River, the Main River, and the Danube River and its tributaries. In all these waterways, abundance contamination was over 50%, and bad ecological status with respect to biocontamination definitely can be stated. Only the Pripyat River, the Bug River and the Pripyat-Bug Canal can be considered to be just moderately biologically contaminated among the studied waterways.

This study also indicates that high abundance contamination of recipient communities may be caused by just a single ecologically aggressive AS (Figure 5). In Lake Ladoga, for example, the Baikalian amphipod, *G. fasciatus*, was capable alone of causing very high biological contamination of the littoral area. Other highly ecologically aggressive species include Ponto-Caspian amphipods such as *P. robustoides*, which frequently was responsible for severe biocontamination in the Nemunas River, and also *D. villosus* and *D. haemobaphes*. High abundance contamination caused by the Ponto-Caspian snail, *L. naticoides*, was also observed, e.g. in the Pripyat and Danube rivers. Information from the Pripyat River suggests that river ports may facilitate the spread of AS across inland waterways and promote richness contamination. Meanwhile, artificial water bodies, such as canals, are likely to be more susceptible to biocontamination relative to natural waterbodies.

The ecological quality of study sites assessed by the BMWP method significantly varied between studied waterways due not only to biogeographical factors, but also because of variation in sampling procedures applied in different countries. However, study results also clearly suggest that biocontamination and ecological status of water bodies assessed by conventional methods are in fact related. Study sites with higher biocontamination have lower estimates of ecological quality (Figure 6). This observation may suggest that sites of lower ecological status with respect to water quality and hydromorphology are more susceptible to biological invasions. The increase in industrial and agricultural pollution along large European rivers was hypothesized to be a trigger of mass AS invasions (e.g. Jazdzewski and Konopacka 2002). However, since indicators of biocontamination were negatively correlated with ecological quality by BMWP method (Figure 7), this may also indicate that biocontamination directly affects ecological quality. Moreover, the negative effect of richness contamination on BMWP estimates seems to be stronger than that originating from abundance contamination.

The negative correlation between biocontamination and ecological status does not imply that alien invaders are affecting water or hydromorphological quality of ecosystems (although this theory may not be totally excluded) for which metrics of ecological status, based on native fauna, have been developed. Instead, it implies that AS may impact native communities and may cause a decreased estimate of ecological status by suppressing local species and distorting the true quality status. Negative impacts of aliens on native species have been well documented. For example, a decline in the macroinvertebrate fauna following the arrival of *D. villosus* was reported from the waters of the Netherlands (Dick and Platvoet 2000; Van der Velde et al. 2000; Dick et al. 2002; Van Riel et al. 2006) and France (Devin et al. 2001). The extermination of a native amphipod by invaders was observed in a large number of water bodies (Jazdzewski et al. 2004; Arbačiauskas 2005; Grabowski et al. 2006). In experiments, Krisp and Maier (2005) have showed that *D. villosus* and *C. ischnus* effectively consume the larvae of Ephemeroptera that form the main indicator group for estimation of ecological water quality. Negative impacts by *P. robustoides* on abundance and diversity of lake littoral communities have also been documented (Arbačiauskas and Gumuliauskaitė 2007; Gumuliauskaitė and Arbačiauskas 2008).

Parts of European inland waterways that are highly biologically contaminated are probably irreversibly changed with respect to benthic fauna composition. Communities formerly consisting of native species are now alien-dominated communities. During the period of species composition change, they more properly may be defined as assemblages. In some water bodies, however, alien-dominated communities have shown very stable composition of dominant species for over a decade. When addressing the dominance of non-native species, such newly established communities may be defined as xenocommunities (in analogy to xenodiversity, sensu Leppäkoski and Olenin 2000). The improvement of ecological status, i.e. bad status with respect to biocontamination, in such water bodies with alien-dominated communities is unlikely, or too expensive. Meanwhile, the concept of water ecological status has been developed to consider the water physico-chemical parameters and the hydromorphological quality of river basins (European Community 2000). The water body status conferred by these characteristics may be substantially higher, with implementable management options in contrast to the status defined by biocontamination. Therefore, the water body status, with respect to biocontamination, is probably more appropriately defined as biological quality status, in order to exclude the dual interpretation of ecological quality status.

Although biocontamination in parts of main European inland waterways have irreversibly changed native communities, the problem of biological pressures from AS must be considered in water management strategies in order to prevent, as much as possible, the further spread of unwanted AS. These tasks are potentially manageable as dispersal of non-native aquatic species is by definition tightly associated with human activities. Other urgent tasks for the implementation of EU Water Framework Directive are to: 1) specify methods of water and hydromorphological quality assessment with respect to AS presence; and 2) develop holistic estimates of ecological quality status that incorporate biocontamination of aquatic ecosystems.

Only those impacts of AS invasions which cause “fitness for survival” decrease are to be considered the negative ecological impacts, i.e.

biological pollution (Elliott 2003). If we agree on that, then translating the presence of AS into an integrated ecological quality assessment of aquatic ecosystem is no simple challenge. A presence of AS may not only suppress but also enhance ecosystem functions and services. Hence, a rethink of the concept of ecological quality status of inland waters should not be excluded.

Conclusions

The biocontamination of study sites and aquatic ecosystems or selected assessment units can be assessed by site-specific biocontamination index and integrated biocontamination index, respectively, which classifies water bodies into 5 quality classes. These indices are derived from two metrics, abundance contamination index and richness contamination index at ordinal rank. Metrics of contamination in abundance and richness reflect the extent of alien contamination in the structural organisation of communities. This method of biocontamination assessment can be applied to data collected during routine water quality monitoring.

Severe biocontamination was observed in most parts of European inland waterways and, consequently, their status was classified as bad. The quality status with respect to biocontamination should be defined as biological quality status. Prevention of biocontamination should be considered in water management policy.

Estimates of ecological quality status determined by conventional methods appear to depend upon biocontamination. A specification of methods for water and hydromorphological quality assessment considering the presence of alien species, and an elaboration of holistic estimates of ecological quality status of aquatic ecosystems that incorporate biocontamination are warranted.

Acknowledgements

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Annex 1. Alien benthic macroinvertebrates identified in the studied assessment units (AUs). For abbreviations of AUs see Annex 3, for location see Figure 1.

Higher taxon /Family	Species	AUs
Turbellaria		
Dugesiidae	*Dugesia tigrina* (Girard, 1850)	SC8
Oligochaeta		
Tubificidae	*Branchiura sowerbyi* Beddard, 1892	SC2, SC3, SC3a, SC3b
Polychaeta		
Ampharetidae	*Hypania invalida* (Grube, 1860)	SC3, SC3a, SC4, SC6, SC7, SC8
Gastropoda		
Neritidae	*Theodoxus danubialis* (C. Pfeiffer, 1828)	SC3, SC3a
Hydrobiidae	*Lithoglyphus naticoides* (C. Pfeiffer, 1828)	CC8, CC9, CC10, CC11, CC12, SC2, SC3, SC3a, SC3b, SC4, SC8
	Potamopyrgus antipodarum (J.E. Gray, 1853)	CC14, SC3, SC6, SC7, SC8, SMC1
Physidae	*Haitia acuta* (Draparnaud 1805)	SC8
	Haitia heterostropha (Say, 1817)	SMC1
Ancylidae	*Ferrissia fragilis* (Tryon, 1863)	CC9,CC10
	Ferrissia wautieri (Mirolli, 1960)	SC8*
Bivalvia		
Unionidae	*Sinanodonta woodiana* (Lea, 1834)	SC3
Dreissenidae	*Dreissena polymorpha* (Pallas, 1771)	CC9, CC10, CC12, SC2, SC3, SC3a, SC5, SC6, SC7, SC8
	*Dreissena rostriformis bugensis (*Andrusov, 1897)	SC2
Corbiculidae	*Corbicula fluminalis* (O. F. Müller, 1774)	SC7, SC8
	Corbicula fluminea (O. F. Müller, 1774)	SC6, SC7, SC8
Sphaeriidae	*Musculium transversum* (Say, 1829)	SC7
Myidae	*Mya arenaria* Linnaeus, 1758	SMC1
Sessilia		
Balanidae	*Balanus amphitrite* Darwin, 1854	SMC1
	Balanus improvisus Darwin, 1854	SMC1
Amphipoda		
Corophiidae	*Chelicorophium curvispinum* (Sars, 1895)	CC8, CC9, CC10, CC11, CC12, SC2, SC3, SC3a, SC5, SC6, SC7, SC8
	Chelicorophium robustum (Sars, 1895)	SC3a, SC6, SC7
Gammaridae	*Chaetogammarus warpachowskyi* (Sars, 1894)	CC11, CC12
	Chaetogammarus ischnus (Stebbing, 1899)	CC8, CC9, CC14, CC16, SC2, SC3, SC4, SC8
	Chaetogammarus trichiatus (Martynov, 1932)	SC6
	Gammarus roeselii Gervais, 1835	CC16
	Gammarus tigrinus Sexton, 1939	CC16, SC8
	Gmelinoides fasciatus (Stebbing, 1899)	NC4, NC5
Pontogammaridae	*Pontogammarus robustoides* (Sars, 1894)	NC5, CC11, CC12
	Dikerogammarus haemobaphes (Eichwald,1841)	CC8, CC9, CC10, CC14, CC16, SC2, SC3, SC3b, SC4, SC5
	Dikerogammarus villosus (Sowinsky, 1894)	CC8, CC9, CC14, CC16, SC3, SC4, SC6, SC7, SC8
	Obesogammarus obesus (Sars, 1894)	SC4
	Obesogammarus crassus (Sars, 1894)	CC8, CC12
Mysida		
Mysidae	*Paramysis lacustris* (Czerniavsky, 1882)	CC12
	Limnomysis benedeni Czerniavsky, 1882	CC8, CC9, CC12, SC2, SC3
	Hemimysis anomala Sars, 1907	SC2, SC3
Isopoda		
Asellidae	*Proasellus meridianus* (Racovitza, 1919A)	SC8
	Proasellus coxalis (Dollfus, 1892)	SC8
Janiridae	*Jaera istri* Veuille, 1979	SC3, SC4, SC6, SC7, SC8
Decapoda		
Atyidae	*Athyaephyra desmarestii* (Millet, 1831)	SC8
Cambaridae	*Orconectes limosus* (Rafinesque, 1817)	CC11, CC12
Panopeidae	*Rhithropanopeus harrisii* (Gould, 1841)	SMC1
Trichoptera		
Hydropsychidae	*Hydropsyche bulgaromanorum* Malicky, 1977	SC8*

* alien status uncertain

Annex 2. Taxonomic resolution of aquatic macroinvertebrates by orders and families used for the assessment of biocontamination. Only taxa identified in present study are included. Higher rank taxonomy taken from http://www.faunaeur.org with addition of a few missing taxa. For classes Oligochaeta and Polychaeta only alien species were identified to a lower taxon.

Higher taxon	Order	Family
Phylum Porifera		
Class Demospongiae	Haploscerida	Spongiliidae
Phylum Cnidaria		
Class Leptolidaa	Hydroida	Hydridae
Phylum Platyhelminthes		
Subphylum Turbellaria	Seriata	Dugesiidae, Planariidae
Phylum Annelida		
Class Polychaeta		
Class Oligochaeta		
Class Hirudinea	Rhynchobdellida	Piscicolidae, Glossiphoniidae
	Arhynchobdellida	Haemopidae, Hirudinidae, Erpobdellidae, Salifidae
Phyllum Mollusca		
Class Gastropoda	Neritopsina	Neritidae
	Architaenioglossa	Viviparidae
	Neotaenioglossa	Bithyniidae, Hydrobiidae, Rissoidae
	Ectobranchia	Valvatidae
	Pulmonata	Lymnaeidae, Physidae, Planorbidae, Ancylidae
	Neogastropoda	Muricidae
Class Bivalvia	Unionoida	Unionidae
	Veneroida	Cardiidae, Corbiculidae, Dreissenidae, Sphaeriidae
	Myoida	Myidae
	Mytiloida	Mytilidae
Phyllum Arthropoda		
Subphyllum Crustacea		
Class Maxillopoda	Sessilia	Balanidae
Class Malacostraca	Amphipoda	Corophiidae, Gammaridae, Pontogammaridae
	Mysida	Mysidae
	Isopoda	Asellidae, Janiridae
	Decapoda	Atyidae, Cambaridae, Panopeidae
Subphyllum Hexapoda		
Class Insecta	Ephemeroptera	Ametropodidae, Baetidae, Heptageniidae, Ephemerellidae, Caenidae, Potamanthidae, Siphlonuridae
	Odonata	Calopterygidae, Platycnemididae, Coenagrionidae, Gomphidae, Aeshnidae
	Hemiptera	Gerridae, Aphelocheiridae, Nepidae, Naucoridae, Notonectidae, Corixidae, Pleidae
	Coleoptera	Gyrinidae, Dytiscidae, Noteridae, Haliplidae, Hydrophilidae, Elmidae
	Trichoptera	Hydropsychidae, Polycentropidae, Phryganeidae, Brachycentridae, Limnephilidae
	Megaloptera	Sialidae
	Diptera	Tipulidae, Chironomidae, Simuliidae, Athericidae, Tabanidae

Annex 3 . Assessment units (**AU**) and study sites (**SS**) within European inland water invasion corridors: Northern (**NC**), Central (**CC**), Southern (**SC**) and Southern Meridian (**SMC**) and site-specific estimates of biocontamination: number of alien species (**NA**), abundance contamination index (**ACI**), richness contamination index at specific (**RCIs**), familial (**RCIf**) and ordinal (**RCIo**) ranks, site-specific biocontamination index (**SBCI**), and **BMWP** scores. Integrated estimates of NA, ACI, RCIs, RCIf, RCIo and integrated biocontamination index (**IBCI**) for different AU (for geographical location see Figure 1) are indicated in bold. Sampling method, period and data ownership: **NC4** and **NC5** - stovepipe sampler, July 2000 and September 1999, respectively, V.E. Panov; **CC8, CC9** and **CC10** - dip net, August 2007, V.V. Vezhnovetz; **CC11** and **CC12** - dip net, August 2007, K. Arbačiauskas and S. Gumuliauskaitė; **CC14a, CC14b** and **CC16** - dip net, May 2003, April-November 2000 and May and September 2001, respectively, M. Grabowski, A. Konopacka and K. Jazdzewski; **SC2, SC3, SC3a, SC3b** and **SC4** - dip net, September 2007, August – September 2007, August 2006, September 2001 and August 2007, respectively, M. Paunović and B. Csányi; **SC5, SC6** and **SC7** - orange-peel grab, March 1998, July 2001 and July 2007, and May 2006, BfG (Federal Institute of Hydrology); **SC8** - artificial substrate, April – October of 1987 –1999, RIZA (Dutch Institute for Inland Water Management and Waste Water Treatment); **SMC1** - frame of fixed area, May 2008, M.O. Son .

AU/SS	Latitude, N	Longitude, E	NA	ACI	RCIs	RCIf	RCIo	SBCI/ IBCI	BMWP
NC4 Lake Ladoga			**1**	**0.692**		**0.128**	**0.156**	**4**	
1 Southern shore	60°01'04"	31°32'39"	1	0.838		0.200	0.250	4	12
2 Southern shore	60°13'17"	31°55'36"	1	0.940		0.111	0.143	4	23
3 Eastern shore	61°20'30"	31°39'51"	1	0.865		0.111	0.143	4	17
4 Eastern shore	61°24`03"	31°30`31"	1	0.017		0.083	0.111	2	50
5 Northern shore	61°37'16"	31°10'35"	1	0.509		0.100	0.111	4	23
6 Northern shore	61°29'08"	30°14'01"	1	0.476		0.111	0.143	3	19
7 Western shore	61°04'54"	30°05'50"	1	0.931		0.167	0.200	4	18
8 Western shore	60°06'39"	31°05'29"	1	0.957		0.143	0.143	4	25
NC5 Neva Bay			**2**	**0.195**		**0.154**	**0.139**	**2**	
1 Southern shore	59°54'50"	29°47'51"	2	0.292		0.182	0.143	3	39
2 Southern shore	59°54'35"	29°48'26"	2	0.082		0.143	0.111	2	43
3 Southern shore	59°54'22"	29°49'26"	2	0.086		0.167	0.143	2	23
4 Southern shore	59°53'23"	29°54'06"	2	0.109		0.167	0.143	2	23
5 Southern shore	59°53'25"	29°54'52"	2	0.340		0.182	0.250	3	26
6 Southern shore	59°56'54"	30°12'31"	1	0.105		0.125	0.167	2	17
7 Southern shore	59°56'27"	30°12'33"	1	0.470		0.200	0.200	3	15
8 Northern shore	59°59'38"	30°05'28"	1	0.080		0.067	0.083	1	40
CC8 Lower Pripyat River			**8**	**0.202**	**0.135**	**0.138**	**0.164**	**2**	
4 Pripyat River	52°11'44"	27°23'03"	5	0.079	0.208	0.167	0.182	2	63
5 Pripyat River	52°06'32"	28°32'43"	1	0.018	0.027	0.053	0.083	1	92
6 Pripyat River	52°07'02"	29°01'50"	2	0.070	0.069	0.074	0.167	2	61
7 Mozyr river port	52°08'46"	29°18'31"	5	0.067	0.147	0.160	0.176	2	70
8 Pripyat River	51°52'01"	29°29'13"	6	0.775	0.222	0.238	0.214	4	53
CC9 Middle Pripyat River			**9**	**0.107**	**0.102**	**0.129**	**0.153**	**2**	
1 Pripyat River	52°02'59"	26°09'49"	1	0.011	0.023	0.037	0.056	1	127
2 Mykashevichy river port	52°09'29"	27°20'19"	7	0.294	0.226	0.261	0.278	3	78
3 Pripyat River	52°11'44"	27°23'03"	3	0.017	0.057	0.088	0.125	2	137
CC10 Pripyat -Bug Canal			**6**	**0.089**	**0.108**	**0.110**	**0.179**	**2**	
1 Brest river port	52°04'56"	23°41'41"	4	0.158	0.082	0.086	0.158	2	132
2 Pripyat -Bug canal	52°06'34"	23°56'30"	3	0.013	0.094	0.097	0.188	2	88
3 Pripyat -Bug canal	52°08'53"	24°42'55"	2	0.207	0.182	0.200	0.250	3	35
4 Pripyat -Bug canal	52°03'06"	26°09'52"	2	0.050	0.048	0.069	0.125	2	124
5 Pinsk river port	52°06'18"	26°04'41"	5	0.015	0.135	0.100	0.176	2	101
CC11 Middle Nemunas River			**5**	**0.367**	**0.102**	**0.123**	**0.162**	**3**	
1 at Druskininkai	54°00'54"	23°56'05"	3	0.073	0.071	0.086	0.167	2	1 55
2 at Merkinė	54°09'16"	24°10'49"	2	0.188	0.047	0.059	0.111	2	154
3 at Alytus	54°21'60"	24°05'31"	3	0.483	0.100	0.125	0.188	3	117
4 above Balbieriškis	54° 29'27"	23°59'17"	4	0.207	0.111	0.143	0.176	3	112
5 above Prienai	54° 36'11"	24°00'08"	4	0.883	0.182	0.200	0.167	4	78
CC12 Lower Nemunas River			**8**	**0.674**	**0.205**	**0.220**	**0.276**	**4**	
1 at Kaunas	54°54'20"	23°50'03"	3	0.565	0.111	0.200	0.214	4	61
2 at Seredžius	55°04'34"	23°23'27"	7	0.509	0.121	0.158	0.294	4	170
3 at Skirsnemunė	55°05'04"	22°55'14"	5	0.590	0.135	0.133	0.235	4	143
4 at Viešvilė	55°03'18"	22°23'27"	7	0.657	0.200	0.250	0.286	4	94
5 at Bitėnai	55°04'19"	22°02'31"	8	0.864	0.267	0.280	0.312	4	91
6 at Pagėgiai	55°05'49"	21°51'37"	8	0.852	0.308	0.273	0.286	4	82
7 at Rusnė	55°17'44"	21°23'09"	7	0.684	0.292	0.250	0.308	4	53

Annex 3 (continued)

AU/SS	Latitude, N	Longitude, E	NA	ACI	RCIs	RCIf	RCIo	SBCI/ IBCI	BMWP
CC14a Bug River			**4**	**0.147**		**0.058**	**0.036**	**2**	
1 at Gołębie	50°38'39"	24°05'19"	0	0		0	0	0	31
2 at Matcze	50°56'46"	23°58'33"	0	0		0	0	0	89
3 at Włodawa	51°33'05"	23°33'59"	0	0		0	0	0	64
4 at Tonkiele	52°25'01"	22°33'33"	3	0.290		0.154	0.111	3	65
5 at Wyszków	52°35'25"	21°27'36"	4	0.447		0.136	0.071	3	119
CC14b Vistula River			**7**	**0.344**			**0.152**	**3**	
1 at Wawrzeńczyce	50°06'38"	20°20'25"	0	0			0	0	
2 at Ujście Jezuickie	50°14'33"	20°43'41"	0	0			0	0	
3 at Pawłów	50°16'00"	20°53'07"	0	0			0	0	
4 at Szczucin	50°19'27"	21°04'25"	0	0			0	0	
5 at Połaniec-Winnica	50°25'19"	21 18 '45"	1	0.015			0.100	1	
6 at Siedleszczany	50 31'08"	21°35'58"	1	0.024			0.091	1	
7 at Sandomierz	50°40'26"	21°45'25"	1	0.014			0.083	1	
8 at Zawichost	50°48'19"	21°51'53"	0	0			0	0	
9 at Zabełcze	50°50 02"	21°50'53"	1	0.003			0.077	1	
10 at Basonia	51°00'17"	21°49'12"	2	0.021			0.143	2	
11 at Solec nad Wisłą	51°07'29"	21°47'32"	1	0.657			0.125	4	
12 at Męćmierz	51°18'44"	21°54'30"	0	0			0	0	
13 at Świerże Górne	51°39'47"	21°28'56"	3	0.209			0.273	3	
14 at Mniszew	51°51'05"	21°17'33"	1	0.438			0.125	3	
15 at Góra Kalwaria	51°59'27"	21°14'06"	3	0.409			0.222	3	
16 at Falenica	52°09'01"	21°09'47"	2	0.064			0.125	2	
17 at Dziekanów Polski	52°22'37"	20°51'07"	2	0.152			0.091	2	
18 at Kępa Polska	52°25'54"	19°57'29"	2	0.695			0.083	4	
19 at Skoki Duże	52°36'35"	19°24'34"	4	0.849			0.571	4	
20 at Bachorzewo	52°38'32"	19°15'43"	5	0.606			0.375	4	
21 at Witoszyn	52°41'36"	19°01'19"	5	0.524			0.200	4	
22 at Kucerz	52°45'10"	18°57'20"	3	0.042			0.167	3	
23 at Złotoria	52°59'57"	18°41'25"	4	0.265			0.143	3	
24 at Topolinek	53°18'25"	18°19'29"	5	0.878			0.300	4	
25 at Zakurzewo	53°33'25"	18°45'49"	4	0.917			0.222	4	
26 at Gniew	53°49'59"	18°50'21"	4	0.939			0.200	4	
27 at Biała Góra	53°54'41"	18°52'49"	3	0.934			0.333	4	
28 at Kiezmark	54°15'22"	18°56'51"	5	0.949			0.222	4	
CC16 Oder River			**7**	**0.704**		**0.330**	**0.275**	**4**	
1 at Ligota Tworkowska	50°01'08"	18°16'09"	0	0		0	0	0	38
2 at Jeszkowice	51°03'05"	17°13'03"	3	0.972		0.333	0.333	4	42
3 at Brzeg Dolny	51°15'22"	16°43'16"	2	0.942		0.400	0.500	4	31
4 at Malczyce	51°31'34"	16°29'57"	3	0.986		0.429	0.333	4	29
5 at Chobienia	51°32'43"	16°27'02"	2	0.972		0.667	0.500	4	20
6 at Wyszanów	51°43'13"	16°14'52"	2	0.746		0.222	0.143	4	56
7 at Bytom Odrzański	51°44'03"	15°49'35"	2	0.926		0.400	0.250	4	35
8 at Milsko	51°56'57"	15°46'25"	4	0.905		0.231	0.333	4	67
9 at Brody	52°03'20"	15°25'42"	4	0.789		0.333	0.400	4	42
10 at Połęcko	52°03'05"	14°53'30"	3	0.845		0.231	0.143	4	73
11 at Kłopot	52°07'48"	14°40'58"	3	0.872		0.143	0.143	4	72
12 at Pławidło	52°26'30"	14°34'52"	4	0.428		0.186	0.100	3	90
13 at Porzecze	52°40'01"	14°27'44"	4	0.326		0.750	0.500	3	23
14 at Czelin	52°44'02"	14°22'42"	5	0.142		0.300	0.167	2	52
SC2 (Lower Danube River)			**11**	**0.646**	**0.323**	**0.452**	**0.490**	**4**	
1 at Donji Milanovac	44°28'42"	22°09'09"	4	0.840	0.250	0.444	0.444	4	18
2 at Tekija	44°40'10"	22°24'09"	7	0.754	0.318	0.556	0.625	4	31
3 at Iron Gate reservoir II	44°19'43"	22°32'38"	8	0.344	0.400	0.357	0.400	3	44
SC3 (Middle Danube River)			**16**	**0.573**	**0.311**	**0.482**	**0.488**	**4**	
1 at Szob	47°48'52"	18°52'02"	12	0.602	0.414	0.533	0.583	4	44
2 downstream Budapest	47°23'12"	19°00'31"	9	0.297	0.225	0.412	0.438	3	46
3 downstream Velika Morava confluence	44°44'16"	21°07'43"	5	0.820	0.294	0.500	0.444	4	48
SC3a (Sava River)			**9**	**0.744**	**0.221**	**0.414**	**0.355**	**4**	
1 at Jamena	44°52'42"	19°05'21"	7	0.704	0.226	0.385	0.278	4	28
2 at Sremska Mitrovica	44°57'55"	19°36'01"	8	0.858	0.308	0.467	0.500	4	13
3 at Ostruznica	44°43'20"	20°18'16"	4	0.670	0.129	0.389	0.286	4	45

Annex 3 (continued)

AU/SS	Latitude, N	Longitude, E	NA	ACI	RCIs	RCIf	RCIo	SBCI/ IBCI	BMWP
SC3b (Tisa River)			3	**0.547**	**0.417**	**0.625**	**0.676**	**4**	
1 at Martoonos	46º06'38"	20º04'44"	3	0.080	0.250	0.375	0.429	3	22
2 at Novi Becej	45º36'44"	20º06'35"	3	0.613	0.250	0.500	0.600	4	15
3 at Titel	45º11'32"	20º18'38"	3	0.947	0.750	1.000	1.000	4	10
SC4 (Upper Danube River)			10	**0.591**	**0.229**	**0.302**	**0.394**	**4**	
1 at Kelheim	48º54'53"	11º52'16"	5	0.525	0.147	0.211	0.333	4	84
2 at Niederalteich	48º46'48"	13º00'21"	10	0.790	0.303	0.250	0.278	4	71
3 at Oberloiben	48º23'04"	15º31'52"	8	0.459	0.236	0.444	0.571	4	24
SC5 (Main-Danube Canal)			**3**	**0.382**		**0.375**	**0.286**	**3**	
1 at Nürnberg	49°22'48"	11°03'44"	3	0.382		0.375	0.286	3	
SC6 (Main River)			**9**	**0.860**		**0.465**	**0.444**	**4**	
1 at Lohr	49°57'56"	09°35'40"	7	0.779		0.294	0.333	4	
2 at Aschaffenburg	49°58'38"	09°07'31"	8	0.942		0.636	0.555	4	
SC 7 (Rhine river)			**10**	**0.884**		**0.645**	**0.602**	**4**	
1 at Eltville	50°01'46"	08°09'00"	9	0.985		0.700	0.625	4	
2 at Koblenz	50°24'45"	07°29'15"	6	0.797		0.636	0.625	4	
3 at Xanten	51°39'46"	06°31'50"	8	0.873		0.600	0.555	4	
SC8 (Rhine Delta)									
1 Rhine at Lobith (1987)	51°07'97"	05°55'26"	5	0.040	0.111	0.286	0.384	3	57
idem (1988)	idem	idem	7	0.288	0.125	0.333	0.429	3	74
idem (1989)	idem	idem	10	0.363	0.152	0.346	0.429	3	98
idem (1990)	idem	idem	10	0.418	0.135	0.357	0.467	3	90
idem (1991)	idem	idem	10	0.782	0.128	0.333	0.467	4	89
idem (1992)	idem	idem	8	0.860	0.160	0.304	0.385	4	72
idem (1993)	idem	idem	11	0.806	0.204	0.385	0.538	4	93
idem (1994)	idem	idem	11	0.868	0.157	0.345	0.467	4	94
idem (1995)	idem	idem	12	0.787	0.164	0.333	0.500	4	98
idem (1996)	idem	idem	12	0.720	0.218	0.375	0.500	4	92
idem (1997)	idem	idem	13	0.811	0.250	0.524	0.642	4	61
idem (1998)	idem	idem	13	0.859	0.361	0.550	0.636	4	69
idem (1999)	idem	idem	13	0.862	0.295	0.440	0.500	4	73
SMC1 (Sukhoy Liman)			7	**0.336**			**0.172**	**3**	
1 Illichivsk Sea Commercial Port	46°21'02"	30°38'34"	5	0.333			0.081	3	
2 Estuary middle part	46°22'11"	30°38'31"	1	0.001			0.166	2	
3 Estuary upper freshwater part	46°23'48"	30°38'08"	2	0.750			0.200	4	
4 Dalnik River floodplains	46°23'57"	30°35'45"	0	0			0	0	
5 Dalnik River	46°24'01"	30°35'32"	1	0.824			0.333	4	
6 Upper Dalnik River Reservoir	46°24'04"	30°35'28"	0	0.000			0.000	0	
7 Freshwater gulf of estuary	46°21'07"	30°35'52"	0	0.000			0.000	0	
8 Akkarzha Stream, Site 1	46°20'53"	30°35'44"	1	0.428			0.333	3	
9 Akkarzha Stream floodplains	46°20'52"	30°35'44"	1	0.072			0.200	2	
10 Akkarzha Stream, Site 2	46°20'50"	30°35'42"	1	0.666			0.250	4	
11 Akkarzha Stream Reservoir	46°20'49"	30°35'41"	0	0			0	0	
12 Akkarzha Stream, Site 3	46°20'47"	30°34'50"	1	0.955			0.500	4	