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# New Localities and Habitat Suitability Modelling for the Riverine Water Bug *Aphelocheirus aestivalis* (Fabricius, 1794) (Heteroptera: Aphelocheiridae) in Northern and Eastern Bulgaria

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**Abstract:** *Aphelocheirus aestivalis* is a stenobiotic species with low dispersion ability. It is the only member of the family Aphelocheiridae found in most of Europe. Hydromorphological changes and water pollution threaten its subpopulations. Considering the vulnerability of the species, more accurate data about its occurrence and geographic distribution are needed. We present here 65 localities of *A. aestivalis* in 27 rivers in Bulgaria, including the southernmost record of the species in Europe (Maritsa River near the town of Svilengrad). Of those, 23 are in the Danube River Basin, two flow directly into the Black Sea and two belong to the Aegean Sea watershed. In the subpopulations of *A. aestivalis* in the Lower Danube River (Bulgarian section) and the rest of the studied rivers, all developmental stages were present during all sampling seasons. Remarkable downstream drift of nymphs and adults of *A. aestivalis* was observed in the Danube River (Bulgarian section) during spring high water levels. In order to identify potential suitable habitats for the species in the Black Sea Basin, we built a habitat suitability model using Maxent software. Our models suggested (with high probability) that there could be suitable habitats in the basins of six more rivers in the Black Sea Basin with no current records of *A. aestivalis*.

**Key words:** *Aphelocheirus aestivalis,*Nepomorpha, distribution, Ecoregion 12, Maxent

## Introduction

The bugs of the family Aphelocheiridae (benthic water bugs), in contrast to most aquatic insects, are able to spend their entire life under the water surface because of their plastron respiration (Polhemus & Polhemus 1988). Unlike them, the majority of species of the same heteropteran infraorder (Nepomorpha) live in or close to aquatic biotopes but need to resurface and renew their oxygen reserves (Kriska 2013). In most parts of the Western Palaearctic (except for the Iberian Peninsula, Middle East and Caucasus), the only reported species of this monotypic family is *Aphelocheirus aestivalis* (Fabricius, 1794) (see Kanyukova 1995, Coulianos 2005, Živić et al. 2007). This water bug is a predator living mostly in lowland rivers (Živić et al. 2007) with β-mesosaprobic waters (Živić et al. 2007,Wegl 1983), high oxygen content (Aukema 2002, Živić et al. 2007) and hard substratum, i.e. gravel and/or sand (Hoffmann 2008).

The specimens of *A. aestivalis* collected in Central Europe and Western Europe are predominantly micropterous (Aukema 2002, Papáček 2012). As very few capable of flight specimens have been collected (reviewed in Hoffmann 2008), it has been assumed that *A. aestivalis* disperses mainly through the water courses (Aukema 2002, Tachet et al. 2010, Papáček 2012).

Human activities like water pollution and stream regulation can cause extinction of the subpopulations (Papáček 2012) of this stenobiotic species (Hoffmann 2008) with low dispersion ability (Papáček 2012). Because of its vulnerability, *A. aestivalis* has been included in the Red Data Lists of some European countries (Papáček 2012). Considering the vulnerability of the species, more accurate data about the occurrence of *A. aestivalis* and its geographic distribution are required. Therefore, in the current paper we present data about 61 new localities of *A. aestivalis* in Bulgaria within Ecoregion 12 and four in Ecoregion 7. Most of the localities in Ecoregion 12 are in the Danube River Basin, but there are also few in rivers directly flowing into the Black Sea. In order to predict if there are more habitats in the Black Sea Basin, which might be potentially suitable for *A. aestivalis*, we have built a habitat suitability model. In this paper we also discuss the species' phenology in the studied area.

# Materials and Methods

### Study area and species occurrence data

For delimiting the ecoregions we followed the Water Framework Directive 2000/60/EC (WFD) but with the changes discussed in Cheshmedjiev et al. (2010). Basin districts (Danube River and Black Sea), belonging to the Black Sea catchment area are united as Ecoregion 12 (Cheshmedjiev et al. 2010). The other two districts (East and West Aegean River Basin Districts) are within Ecoregion 7 (Eastern Balkans).

The occurrence data for *A. aestivalis* used in this paper came from 85 hydrobiological samples from 65 localities (Table 1). Samples were collected between 2009 and 2016 (except for two samples, collected in 2005 and 2006).

The sampling was carried out mainly using the adapted version of the multi-habitat sampling method (Cheshmedjiev et al. 2011) according to the accepted standard procedures (EN 16150: 2012, EN ISO 10870: 2012). Five samples were collected with bottom rectangular ichthyoplankton nets (area of 0.25m$^2$) from depth between 6 and 10 m in the main course of the Danube River.

The instar of the nymphs was determined following the key of Indrova (2008).

The material is stored in the collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia.

### Environmental predictors

Our models for predicting the habitat suitability were built at the scale of a river segment and the connected to it primary catchment *sensu* Vogt et al. (2007). Based on the published information about the ecology of *A. aestivalis* (see Wesenberg-Lund 1943, Thorpe 1965, Krajewski 1966, Kanyukova 1974, 2006, Nieser 1982, Savage 1989, Statzner et al. 1998, Vercauteren et al. 2002, Papáček 2006, Papáček & Bauer 2006, Zivic et al. 2007, Papáček et al. 2009, Tachet et al. 2010, Lock et al. 2013), we assumed the following variables should be accounted for building a habitat suitability model for this species: flow velocity, water depth, substratum composition, stream size and levels of pollution from point and diffuse sources. As there are no available data about these variables for the river segments in the studied area, we used proxy variables associated with those listed above.

Catchment area, mean slope and cumulative length of the upstream flow network were considered as predictor variables which represented the physical characteristics of primary catchments (from here on catchments) or river segments. In addition, yearly wastewater discharge per catchment and six land cover variables were also included in the models (Table 2). We used the slope as a proxy for flow velocity (following Pilière et al. 2014). In this way, we partly took into account also other variables connected to flow velocity, e.g. water depth and substratum composition (for references see Ahmadi-Nedushan et al. 2006). We used the cumulative length and catchment area as proxies for stream size and variety of physical habitats in the catchment, respectively. For the use of catchment area we followed Pilière et al. (2014). To account for the influence of pollution sources on the habitat suitability for *A. aestivalis*, we used: for point sources – yearly discharge of wastewater; for diffuse sources (agriculture) – the percent of the catchment area occupied by each of six classes of agricultural land cover (similar approach has been used in Kuemmerlen et al. 2014, 2016).

### Environmental dataset

We prepared, using the CCM2 geodatabase (link in references), a GIS layer consisting of catchments in Bulgaria within Ecoregion 12. This layer was

**Table 1.** Data for localities of *Aphelocheirus aestivalis* in Bulgaria collected during this study. Abbreviations: CS – code of sample; WT – Waterbody type; Methods of collection: MM – Multihabitat method; IN – Ichtyoplankton net; T – all collected specimens in a sample. The number of the collected juvenile specimens in every sample is separately given for each larva age (marked with Roman numerals).

| CS | Drainage basin | Used for modelling | Locality | Latitude (N) | Longitude (E) | Alt. (m) | WT | Date | Leg. | Number of specimens | | | | | | | | Method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | juveniles | | | | | adults | | | |
| | | | | | | | | | | I | II | III | IV | V | ♀ | ♂ | T | |
| 1 | Danube | yes | Baniski Lom River, Shirokovo Vill. | 43.55072 | 25.94289 | 95 | R8 | 18.8.2009 | Y. Vidinova | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | MM |
| 2 | Danube | yes | Beli Lom River, Pisanets Vill. | 43.66717 | 26.18122 | 110 | R8 | 20.9.2014 | M. Kerakova, R. Soufi | 6 | 2 | 2 | 0 | 0 | 3 | 1 | 14 | MM |
| 3 | Danube | no | Baniski Lom River, Shirokovo Vill. | 43.55072 | 25.94289 | 92 | R8 | 11.8.2015 | R. Soufi | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | MM |
| 4 | Danube | yes | Botunya River, Golyamo Babino Vill. | 43.36862 | 23.45628 | 138 | R8 | 26.7.2014 | R. Soufi | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | MM |
| 5 | Danube | yes | Botunya River, Ohrid Vill. | 43.45611 | 23.37115 | 104 | R8 | 4.8.2015 | R. Soufi | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | MM |
| 6 | Danube | no | Botunya River, Ohrid Vill. | 43.45611 | 23.37115 | 104 | R8 | 27.7.2014 | R. Soufi | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | MM |
| 7 | Danube | yes | Cherni Lom River, Svetlen Vill. | 43.31529 | 26.29133 | 193 | R8 | 19.9.2014 | M. Kerakova, R. Soufi | 12 | 2 | 1 | 0 | 0 | 2 | 0 | 17 | MM |
| 8 | Danube | yes | Cherni Lom River, Cherven Vill. | 43.62103 | 26.02461 | 66 | R7 | 27.8.2009 | V. Tyufekchieva | 1 | 2 | 1 | 0 | 0 | 1 | 0 | 1 | MM |
| 9 | Danube | yes | Cherni Lom River, Cherven Vill. | 43.61258 | 26.02452 | 74 | R7 | 19.9.2014 | M. Kerakova, R. Soufi | 3 | 2 | 1 | 0 | 1 | 6 | 1 | 14 | MM |
| 10 | Danube | yes | Cherni Lom River, Ostritza Vill., infusion of Banishki Lom River | 43.53939 | 25.96047 | 101 | R8 | 19.8.2009 | V. Tyufekchieva | 0 | 13 | 25 | 0 | 0 | 4 | 0 | 42 | MM |
| 11 | Danube | yes | Danube River after infusion of Yantra River | 43.65628 | 25.59867 | 13 | R6 | 28.8.2013 | M. Ihtimanska | 0 | 0 | 1 | 2 | 2 | 0 | 0 | 5 | MM |
| 12 | Danube | yes | Danube River Lower Tsibar | 43.82413 | 23.50583 | 28 | R6 | 22.8.2013 | M. Ihtimanska | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | MM |
| 13 | Danube | yes | Danube River near Vidin | 43.94038 | 22.85538 | 32 | R6 | 21.8.2013 | M. Ihtimanska | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | MM |
| 14 | Danube | yes | Danube River, after Vardim Island | 43.64232 | 25.50827 | 15 | R6 | 14.5.2013 | T. Stefanov | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 4 | IN |
| 15 | Danube | no | Danube River, Baikal Vill. | no data | no data | no data | R6 | 17.4.2014 | B. Velkov | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | MM |
| 16 | Danube | no | Danube River, front of Kozloduy Island | no data | no data | no data | R6 | 24.9.2012 | M. Ihtimanska, S. Kazakov | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | MM |
| 17 | Danube | no | Danube River, Krivina Vill. | 43.64643 | 25.57900 | 18 | R6 | 19.8.2015 | Y. Vidinova | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | MM |
| 18 | Danube | no | Danube River, near Kamadino Island | 43.77019 | 25.86894 | 16 | R6 | 15.5.2013 | T. Stefanov | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | IN |
| 19 | Danube | yes | Danube River, near Vardim Island | 43.62506 | 25.44791 | 22 | R6 | 3.11.2010 | M. Pavlova | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | MM |
| 20 | Danube | yes | Danube River, near the rock church at Nikopol | 43.71214 | 24.91792 | 24 | R6 | 12.4.2014 | T. Stefanov | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | IN |
| 21 | Danube | yes | Danube River, near the rock church at Nikopol | 43.71158 | 24.91603 | 23 | R6 | 19.4.2014 | T. Stefanov | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | IN |
| 22 | Danube | no | Danube River, Nikopol before Cherkvitsa | 43.709711 | 24.831888 | no data | R6 | 21.4.2014 | B. Velkov | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | MM |

**Table 1.** Continuation.

| CS | Drainage basin | Used for modelling | Locality | Latitude (N) | Longitude (E) | Alt. (m) | WT | Date | Leg. | Number of specimens | | | | | | | | Method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | juveniles | | | | | adults | | | |
| | | | | | | | | | | I | II | III | IV | V | ♀ | ♂ | T | |
| 23 | Danube | no | Danube River, right riverbank, Nebuna Iland | 43.803472 | 23.140306 | 27 | R6 | 23.7.2012 | M. Ihtimanska, S. Kazakov | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | MM |
| 24 | Danube | yes | Danube River, the tail of the Magarets Island | 43.65580 | 25.12865 | 15 | R6 | 11.5.2013 | T. Stefanov | 0 | 3 | 1 | 1 | 1 | 2 | 0 | 8 | IN |
| 25 | Danube | yes | Dzhulyunska River, Djulyunitsa Vill. | 43.13828 | 25.90639 | 70 | R7 | 6.8.2009 | V. Tyufekchieva | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 3 | MM |
| 26 | Danube | yes | Dzhulyunska River, Djulyunitsa Vill. | 43.13900 | 25.90652 | 70 | R7 | 10.8.2015 | R. Soufi | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 4 | MM |
| 27 | Danube | yes | Eliyska River at Polski Trumbesh before it flows into Yantra River | 43.36567 | 25.64211 | 35 | R8 | 9.10.2009 | V. Tyufekchieva | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | MM |
| 28 | Danube | yes | Iskar River, Gigen Vill., bridge to Baikal Vill. | 43.70258 | 24.45558 | 40 | R7 | 25.8.2009 | Y. Vidinova | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 3 | MM |
| 29 | Danube | yes | Iskar River, near Orehovitsa Vill., bridge to Bregare Vill. | 43.58761 | 24.35883 | 41 | R7 | 25.8.2009 | V. Tyufekchieva | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | MM |
| 30 | Danube | no | Iskar River, Orehovitsa Vill. | 43.586630 | 24.35893 | 37 | R7 | 16.5.2005 | L. Kenderov | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | MM |
| 31 | Danube | yes | Iskar River, Orehovitsa Vill. | 43.58523 | 24.35858 | 41 | R7 | 8.8.2015 | R. Soufi | 1 | 1 | 4 | 0 | 0 | 2 | 1 | 9 | MM |
| 32 | Danube | yes | Lefedzha River, Bryagovitsa Vill. | 43.19091 | 25.88172 | 64 | R7 | 28.7.2011 | T. Stefanov | 0 | 7 | 5 | 0 | 0 | 2 | 2 | 16 | MM |
| 33 | Danube | yes | Lefedzha River, Bryagovitsa Vill. | 43.19072 | 25.88030 | 60 | R7 | 22.9.2014 | M. Kerakova, R. Soufi | 2 | 1 | 0 | 2 | 0 | 1 | 0 | 6 | MM |
| 34 | Danube | no | Lefedzha River, Bryagovitsa Vill. | 43.19072 | 25.88030 | 60 | R7 | 10.8.2015 | R. Soufi | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 4 | MM |
| 35 | Danube | yes | Lefedzha River, Bryagovitsa Vill. before infusion into the Yantra River | 43.19031 | 25.88539 | 56 | R7 | 2.8.2009 | Y. Vidinova | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 2 | MM |
| 36 | Danube | no | Lefedzha River, Dzhulyuntsa Vill. | 43.138253 | 25.906329 | 95 | R7 | 22.9.2014 | M. Kerakova, R. Soufi | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | MM |
| 37 | Danube | yes | Lefedzha River, Kesarevo Vill. | 43.16594 | 25.93250 | 70 | R7 | 6.9.2009 | V. Tyufekchieva | 0 | 2 | 1 | 0 | 0 | 0 | 1 | 4 | MM |
| 38 | Danube | yes | Lom River near Roglets Vill | 43.67184 | 23.92673 | 132 | R8 | 28.9.2011 | T. Stefanov, M. Ihtimanska | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | MM |
| 39 | Danube | yes | Lom River near Zemfir Vill. | 43.78537 | 23.23725 | 36 | R8 | 25.9.2011 | T. Stefanov | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | MM |
| 40 | Danube | yes | Lom River, between Drazhintsi Vill. and Ruzhintsi Vill. | 43.62359 | 22.86464 | 161 | R8 | 29.9.2011 | T. Stefanov, M. Ihtimanska | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | MM |
| 41 | Danube | yes | Lom River, railway bridge near Vasilovtsi Vill. | 43.70621 | 23.12648 | 70 | R8 | 26.9.2011 | M. Kerakova | 0 | 3 | 4 | 1 | 0 | 0 | 0 | 8 | MM |
| 42 | Danube | yes | Lom River, Traykovo Vill. | 43.75201 | 23.19274 | 41 | R8 | 25.9.2011 | M. Kerakova | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | MM |
| 43 | Danube | yes | Malak Iskar River before outfall into the Iskar River, Town of Roman | 43.13911 | 23.92492 | 149 | R4 | 24.8.2009 | V. Tyufekchieva | 1 | 6 | 2 | 0 | 0 | 1 | 1 | 11 | MM |

**Table 1.** Continuation.

| CS | Drainage basin | Used for modelling | Locality | Latitude (N) | Longitude (E) | Alt. (m) | WT | Date | Leg. | Number of specimens | | | | | | | | Method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | juveniles | | | | | adults | | | |
| | | | | | | | | | | I | II | III | IV | V | ♀ | ♂ | T | |
| 44 | Danube | yes | Malki Lom River, Nisovo Vill. | 43.65242 | 26.06857 | 68 | R7 | 20.9.2014 | M. Kerakova, R. Soufi | 1 | 1 | 0 | 0 | 1 | 2 | 1 | 6 | MM |
| 45 | Aegean Sea | yes | Maritsa River between Svilengrad and Lyubimets | 41.80340 | 26.15677 | 49 | R12 | 26.7.2011 | V. Tyufekchieva, M. Vasilev | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | MM |
| 46 | Aegean Sea | yes | Maritsa River, Harmanli | 41.92993 | 25.93130 | 76 | R12 | 27.7.2011 | V. Tyufekchieva, M. Vasilev | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 3 | MM |
| 47 | Aegean Sea | no | Maritsa River, Harmanli | 41.933071 | 25.930415 | 69 | R12 | 27.8.2011 | V. Tyufekchieva | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | MM |
| 48 | Aegean Sea | yes | Maritsa River, Svilengrad | 41.76829 | 26.19217 | 54 | R12 | 26.7.2011 | Y. Vidinova | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | MM |
| 49 | Aegean Sea | no | Maritsa River, Svilengrad | 41.77275 | 26.19336 | 51 | R12 | 24.6.2016 | N. Simov | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | MM |
| 50 | Aegean Sea | no | Maritsa River, Svilengrad | 41.774475 | 26.192409 | 52 | R12 | 27.9.2006 | Zneprovski | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 2 | MM |
| 51 | Danube | yes | Nishava River, bridge before Kalotina Vill. | 42.99125 | 22.86325 | 495 | R3 | 2.8.2015 | D. Stoianova | 0 | 3 | 0 | 1 | 1 | 0 | 0 | 5 | MM |
| 52 | Danube | yes | Nishava River, bridge before Kalotina Vill. | 42.99120 | 22.86316 | 498 | R3 | 1.7.2012 | N. Simov, Pingping Chen | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | MM |
| 53 | Danube | yes | Ogosta River, Hurlets Vill. | 43.70999 | 23.83930 | 33 | R7 | 2.8.2015 | Y. Vidinova | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 3 | MM |
| 54 | Danube | yes | Ogosta River, Kobilyak Vill. | 43.51896 | 23.44371 | 81 | R7 | 2.7.2015 | R. Soufi | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | MM |
| 55 | Danube | no | Ogosta River, Marchevo Vill. | 43.48841 | 23.35663 | 95 | R7 | 27.6.2014 | T. Trichkova, L. Kenderov, A. Cardeccia | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | MM |
| 56 | Danube | yes | Ogosta River, outfall in Danube River | 43.74078 | 23.87708 | 33 | R7 | 25.8.2009 | V. Tyufekchieva | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | MM |
| 57 | Danube | yes | Osam River, Cherkvitsa Vill. | 43.62909 | 24.84998 | 28 | R7 | 17.9.2014 | M. Kerakova, R. Soufi | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | MM |
| 58 | Danube | yes | Osam River, Varana Vill. | 43.35209 | 25.18513 | 59 | R7 | 27.8.2015 | L. Kenderov | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | MM |
| 59 | Black Sea | no | Rezovska River, Slivarovo Vill. | 41.96058 | 27.65919 | 223 | R5 | 1.11.2014 | N. Simov, T. Stefanov | 0 | 0 | 1 | 0 | 1 | 3 | 2 | 7 | MM |
| 60 | Danube | yes | Ribine River, before infusion into Ogosta River (bridge to Beli Breg Vill.) | 43.53603 | 23.56700 | 63 | R8 | 16.7.2009 | Y. Vidinova | 0 | 49 | 1 | 1 | 10 | 3 | 2 | 66 | MM |
| 61 | Danube | yes | Ribine River, before infusion into Ogosta River (bridge to Beli Breg Vill.) | 43.53667 | 23.56741 | 66 | R8 | 4.8.2015 | R. Soufi | 11 | 2 | 0 | 0 | 0 | 8 | 3 | 24 | MM |
| 62 | Danube | yes | Rositsa River, bridge beween Polikraishte Vill. and Kutsina Vill. | 43.21666 | 25.63865 | 53 | R7 | 11.8.2015 | R. Soufi | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | MM |

**Table 1.** Continuation.

| CS | Drainage basin | Used for modelling | Locality | Latitude (N) | Longitude (E) | Alt. (m) | WT | Date | Leg. | Number of specimens | | | | | | | | Method |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | juveniles | | | | | adults | | | |
| | | | | | | | | | | I | II | III | IV | V | ♀ | ♂ | T | |
| 63 | Danube | yes | Shavarna River before it flows into Osam River | 43.34050 | 25.11975 | 65 | R8 | 27.8.2009 | V. Tyufekchieva | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | MM |
| 64 | Danube | yes | Tsibritsa River, Dolni Tsibar Vill. | 43.81373 | 23.52269 | 70 | R8 | 1.8.2015 | R. Soufi | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | MM |
| 65 | Danube | yes | Tsibritsa River, Valchidram Vill. | 43.69198 | 23.45198 | 77 | R8 | 1.8.2015 | R. Soufi | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | MM |
| 66 | Aegean Sea | yes | Tundzha River, before Binkos | 42.63450 | 26.07786 | 300 | R5 | 21.7.2011 | Y. Vidinova | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 3 | MM |
| 67 | Black Sea | no | Veleka River | no data | no data | no data | R10 | 22.8.2011 | T. Stefanov | 1 | 2 | 0 | 1 | 0 | 1 | 0 | 5 | MM |
| 68 | Black Sea | no | Veleka River, after Kosti Vill. | 42.08139 | 27.85994 | 23 | R10 | 10.8.2011 | M. Kerakova | 0 | 0 | 3 | 0 | 0 | 5 | 0 | 8 | MM |
| 69 | Black Sea | yes | Veleka River, Brodilovo Vill. | 42.08139 | 27.85994 | 23 | R10 | 18.6.2013 | M. Kerakova | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 6 | MM |
| 70 | Black Sea | no | Veleka River, Brodilovo Vill. | 42.08139 | 27.85994 | 23 | R10 | 8.10.2011 | M. Kerakova | 1 | 1 | 2 | 4 | 0 | 2 | 0 | 10 | MM |
| 71 | Black Sea | yes | Veleka River, Brodilovo Vill. | 42.08148 | 27.86023 | 11 | R10 | 14.8.2011 | T. Stefanov | 1 | 14 | 10 | 0 | 0 | 3 | 4 | 32 | MM |
| 72 | Black Sea | yes | Veleka River, Kosti Vill. | 42.05617 | 27.76447 | 30 | R10 | 16.7.2013 | M. Kerakova | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | MM |
| 73 | Black Sea | yes | Veleka River, Kosti Vill. | 42.05617 | 27.76447 | 30 | R10 | 13.10.2012 | M. Kerakova | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | MM |
| 74 | Black Sea | no | Veleka River, Kosti Vill. | 42.06051 | 27.77081 | 27 | R10 | 13.7.2014 | B. Velkov | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | MM |
| 75 | Black Sea | yes | Veleka River, Kosti Vill. | 42.06050 | 27.77189 | 27 | R10 | 22.8.2014 | D. Stoianova | 0 | 0 | 0 | 1 | 0 | 2 | 3 | 6 | MM |
| 76 | Black Sea | yes | Veleka River, Kosti Vill. | 42.06019 | 27.77114 | 30 | R10 | 26.9.2014 | N. Simov | 14 | 1 | 2 | 4 | 1 | 0 | 0 | 22 | MM |
| 77 | Danube | yes | Vidbol River, Dunavtsi | 43.90302 | 22.80533 | 40 | R8 | 21.7.2014 | R. Soufi | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | MM |
| 78 | Danube | no | Vidbol River, Sinagovtsi Vill. | 43.90302 | 22.80533 | 40 | R8 | 5.7.2015 | R. Soufi | 2 | 0 | 2 | 2 | 14 | 5 | 3 | 28 | MM |
| 79 | Danube | yes | Vit River, Bezhanovo Vill. | 43.23195 | 24.41165 | 120 | R4 | 10.11.2011 | M. Kerakova | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | MM |
| 80 | Danube | yes | Vit River, Gulyantsi Vill. | 43.63356 | 24.69761 | 26 | R7 | 1.5.2011 | M. Kerakova | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 2 | MM |
| 81 | Danube | no | Vit River, Gulyantsi Vill. | 43.63356 | 24.69761 | 26 | R7 | 10.11.2011 | M. Kerakova | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | MM |
| 82 | Danube | yes | Vit River, Gulyantsi Vill. | 43.63410 | 24.69868 | 26 | R7 | 16.9.2014 | M. Kerakova, R. Soufi | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | MM |
| 83 | Danube | yes | Vidbol River, Dunavtsi Vill. | 43.90302 | 22.80533 | 40 | R8 | 12.11.2010 | T. Stefanov, T. Trichkova | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | MM |
| 84 | Danube | yes | Yantra River, between Karantsi and Polski Trambesh | 43.37904 | 25.66774 | 36 | R7 | 20.9.2014 | M. Kerakova, R. Soufi | 1 | 1 | 0 | 1 | 0 | 2 | 1 | 6 | MM |
| 85 | Danube | no | Yantra River, bridge beween Karantsi Vill. and Polski Trambesh Town | 43.37904 | 25.66774 | 36 | R7 | 13.8.2015 | R. Soufi | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 5 | MM |

compared visually with a water courses map available through the GIS Module on the web site of the Freshwater Ecosystem Services Mapping and Assessment in Bulgaria (link in the references). We then removed from the layer all polygons of catchments that according to the cited above map did not have water course on their territory. For each of the six agricultural land cover classes a land cover variable was calculated for each catchment (for description see Table 2).

We tested for correlation among the predictors using the built-in function cor in R (R Core Team 2017). None of the variables had r higher than 0.7 (threshold used in Kuemmerlen et al. 2016 following Dormann et al. 2013), therefore we used all of them for modelling.

The preparation of the input data (for building the models) was performed in QGIS Desktop 2.16.2 with GRASS 7.0.4.

### Habitat suitability modelling and validation

We modelled the probability of habitat suitability for *A. aestivalis* on catchment scale in Ecoregion 12 in Bulgaria using Maxent version 3.4.1 (Philips et al. [Internet]).

Maxent software uses a machine learning algorithm applied for predicting the distribution of suitable habitats for a species within a given area (Phillips et al. 2006). It requires input of environmental data for all locations (here scaled to catchment) in the studied area. The Maxent models are produced based on the principle of maximum entropy (Phillips et al. 2006). Our choice for applying this algorithm was based on its good performance when used for presence-only models (Elith et al. 2006, 2011, Phillips et al. 2004, 2006, Phillips & Dudik 2008).

The background locations used for building habitat suitability models with Maxent should represent the full environmental range of the species and exclude the areas (in our case catchments) where it has not been searched for (Elith et al. 2011). We excluded the catchments with minimal altitude higher than 500 m from the input background data. It was done for the following reasons: (1) on the Balkan Peninsula the species is known to inhabit only lowland rivers up to 500 m (Živić et al. 2007); (2) there were no localities in catchments with higher minimum altitude in the occurrence data for the studied area. The catchments with Strahler order 2 or less (Strahler 1952) were also excluded from the in-

**Table 2.** Variables used for building habitat suitability model for *Aphelocheirus aestivalis* within Ecoregion 12 in Bulgaria.

| Predictors | Description | Source of data |
|---|---|---|
| Predictors representing physical characteristics of catchments or river segments | | |
| 1. Catchment area (AREA_KM2) | Area of the primary catchment (in square kilometers) | CCM2 River and Catchment database for Europe (CCM2) –Geodatabase, containing shape files with polygons and data for the river segments and catchments within Danube River Basin district and Black Sea basin district part of the: ohttp://ccm.jrc.ec.europa.eu/php/index.php?action=view&id=24;<br>We use the definitions as given in Vogt et al. (2007). |
| 2. Mean slope (SLOPE_MEAN) | Mean slope in the primary catchment (in percent) | |
| 3. Cumulative length (CUM_LKM) | Cumulative length of the upstream flow network, including the river segment itself (in kilometers) | |
| Predictors representing point and diffusepollution sources | | |
| Agricultural areas (diffuse pollution sources):<br>4. Non-irrigated arable land (Cum211);<br>5. Vineyards (Cum221);<br>6. Fruit trees and berry plantations (Cum222);<br>7.Pastures (Cum231);<br>8. Complex cultivation patterns (Cum242);<br>9. Land principally occupied by agriculture, with significant areas of natural vegetation (Cum243); | Land cover variables representing the percentage of the cumulative area (area of the relevant catchment plus area of its tributaries' catchments and/or that of immediately upstream catchment) covered by each of 6 agricultural land cover class. Similar approach has been used in Kuemmerlen et al. (2014, 2016). | Vector polygon layer with Corine Land Cover 2012: http://eea.government.bg/wp/publicregister/PWSRegisters.aspx;<br>We used the nomenclature of the Corine land cover classes as described in CORINE Land cover - Part 2: Nomenclature (available at: https://www.eea.europa.eu/publications/COR0-part2). |
| 9. Discharge (Disch_Qy) (point pollution sources) | Total yearly flow rate of all the discharges of wastewater within every catchment (in cubic meters). | Register of issued permits for discharge of waste water into surface water bodies in Bulgaria: http://eea.government.bg/wp/publicregister/PWSRegisters.aspx. |

put background for two reasons: (1) streams with Strahler order 2 or less are characterised by shallow depth and in other studies on the Balkan Peninsula the species has been registered only in deep water courses (Živić et al. 2007); (2) there were no localities of *A. aestivalis* in catchments with order lower than 3 in the occurrence data for the studied area.

In our dataset, most of the occurrences of *A. aestivalis* were in the Danube River Basin District (DRBD), while only few (three localities) were in the Black Sea Basin District (BSBD). Therefore, for models' training and testing, we used as background and occurrence only data for catchments in the DRBD.

For modelling of the habitat suitability, we followed the method described in Elith et al. (2011). The input was in samples-with-data format: spreadsheet-like summaries of environmental variables in two comma separated value (CSV) files. Both files contained the values of the candidate predictors: the first for 1158 catchments in the DRBD; the second for 41 catchments with occurrence of *A. aestivalis* in the DRBD. As input file for projection, we used a CSV file containing the values of the candidate predictors for 1558 catchments in both DRBD and BSBD.

All environmental variables were continuous. Following Elith et al. (2011), the default settings for features and regularisation were used and the output was set to be logistic. For obtaining out-of-sample estimates of predictive performance and estimates of uncertainty around fitted functions ten-fold cross-validation was used. The maximum number of background points was set to 1158 (the number of our background catchments) instead of the default 10 000.

We first obtained ten "full models" as a result of ten replicate runs using all ten predictors in our dataset. For the final variable selection, we used the averaged results over these ten replicate runs provided as output by Maxent. In these results, we looked at the jackknife analysis of the AUC area under the receiver operating characteristic (see Hanley & McNeil 1982). According to Elith et al. (2010), AUC could be used for assessing the ability of the predictions to discriminate presence from background locations (catchments in our case). Based on this AUC jackknife analysis, in the "final models" we included only the predictors, which when omitted decreased the AUC as compared to AUC when all predictors were used. The output from Maxent contains also response curves. Each curve represents a model based on one of the variables and showing not only the dependence of the predicted suitability on each variable but also dependencies induced by correlations between the selected variable and other variables (Merow et al. 2013).

The Moran's I autocorrelation coefficient was used to evaluate the spatial autocorrelation of the average logistic output of the final models.

The probability of habitat suitability predicted by the final models was mapped: we joined the average logistic output to the respective polygons of the catchment layer (for description of the layer see section "Environmental dataset").

We used maximum test sensitivity plus specificity threshold for discrimination between catchments predicted as suitable vs those predicted as unsuitable. This was done in order to display and compare the range of the predictor variables for: background catchments; catchments with occurrences predicted to be suitable; catchments with occurrences predicted to be unsuitable. The distribution of the predictors' values for each of the three groups was displayed with the use of the functions ggplot and geom_boxplot in the R (R Core Team 2017) package ggplot2 (Wickham 2016).

# Results

### Phenology observations

Both adults and nymphs were present throughout the collection period (summer, autumn and spring). Nymphs of 1st and 2nd instars comprised on average 40% of the specimens in the samples collected in the summer months (June, July and August). In the samples collected in spring and autumn (April, May, September, October and November), nymphs of 1st and 2nd instars constituted only 26% of the collected specimens.

### Localities of *A. aestivalis*

In the present study, we reported 65 localities of *A. aestivalis* in 27 rivers in Bulgaria: 25 rivers within Ecoregion 12 and two rivers within Ecoregion 7. Of those, 23 are in the DRBD, two flow directly into the Black Sea and two are part of the Aegean Sea watershed (Table 1).

We found *A. aestivalis* in water bodies of seven types, determined in accordance with the typology adopted in Bulgaria: medium and small rivers in the DRBD (ten rivers); large tributaries of the Danube River (ten rivers); large Black Sea rivers (Veleka River); semi-mountainous rivers with gravel bottoms in Ecoregion 12 (Malak Iskar River, Rezovska River and Vit River); mountainous rivers (Nishava River); large floodplain rivers in Ecoregion 7 (Maritsa River) and semi-mountainous rivers in Ecoregion 7 (Tundzha River). During the present study, 11 lo-

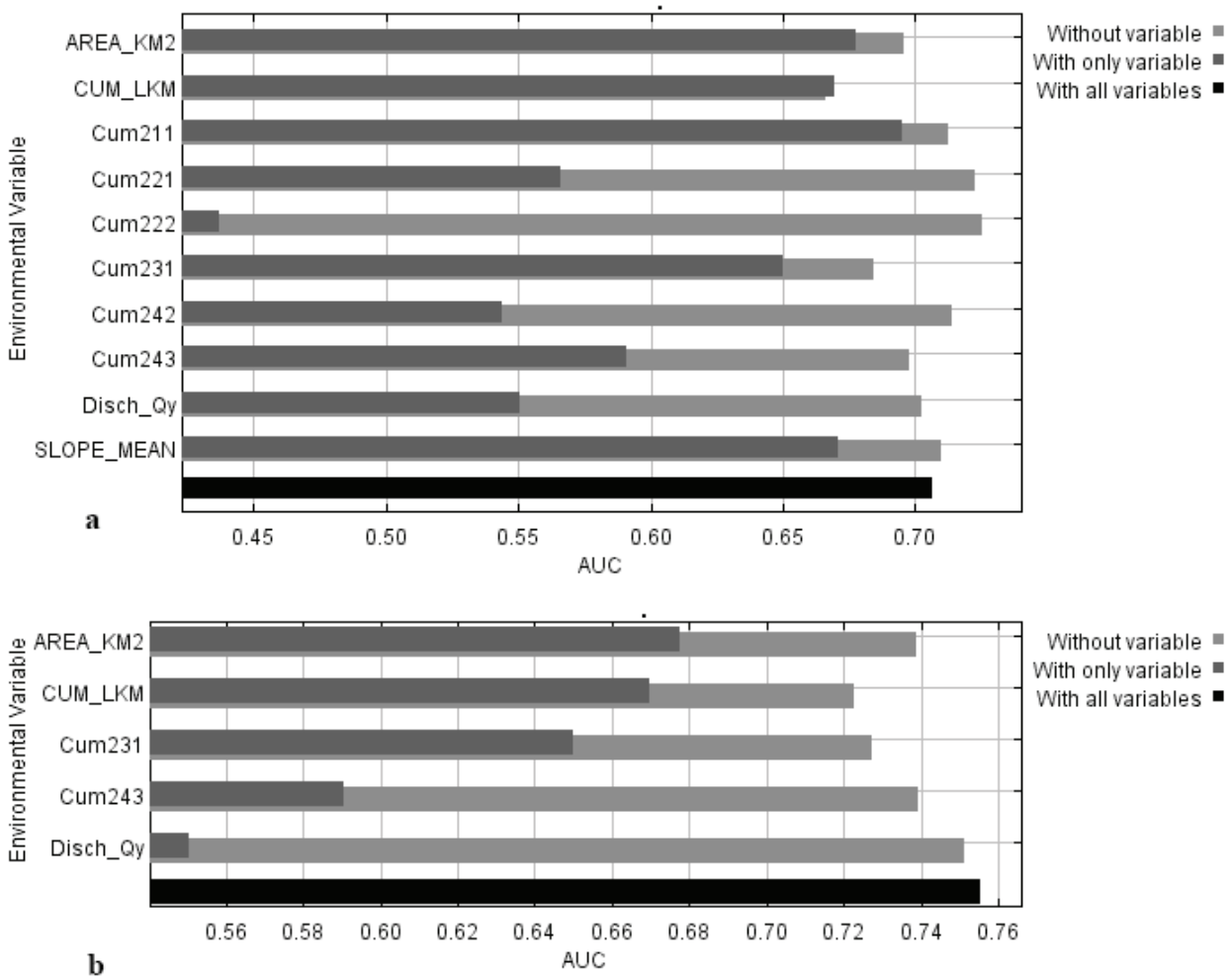


**Fig. 1.** Average results of jackknife test based on AUC for test data: a) calculated for the ten full models; b) calculated for the ten final models.

calities of the species in the Danube River were also registered. Overall, the species has been found exclusively in habitats belonging to the EUNIS type permanent non-tidal, smooth-flowing watercourses (C 2.3).

**Models of habitat suitability**

Based on the results of the AUC jackknife analysis for the full models (Fig. 1a), we omitted half of the predictors from the subsequent modelling. The final models were built using the following five predictors: catchment area; cumulative length; pastures; land principally occupied by agriculture, with significant areas of natural vegetation and discharge.

The full models were built only as a step in the predictor selection process; therefore, from here on we shall comment only the predictions of the final models. The mean AUC test for the replicate final models was 0.76 (s. d. = 0.07). It showed relatively high probability (76%) of a random presence site to be ranked higher than a random background site.

Each of the five input predictors contributed to the model fit (Table 3). The cumulative length had the highest influence on the model, having a permutation importance of 31%. In the jackknife tests, removing this variable from the model resulted in the largest decrease in AUC showing it contained the most information that was not present in any of the other variables (Fig. 1b). The elimination of each of the other four variables from the model also caused drop in AUC, indicating some unique information was contained within each of them (Fig. 1b). The catchment area produced the highest gain in AUC when used in the model alone.

The plots of the response curves showed that the highest predicted suitability (above 0.7) occurred in catchments with area between 30 and 40 km$^2$ (Fig. 2a). The predicted suitability increased together with the cumulative area occupied by pastures (Fig. 2b) and rapidly decreased with the increase of the wastewater discharge (Fig. 2c). The catchments with about 10% of their cumulative area occupied by ag-

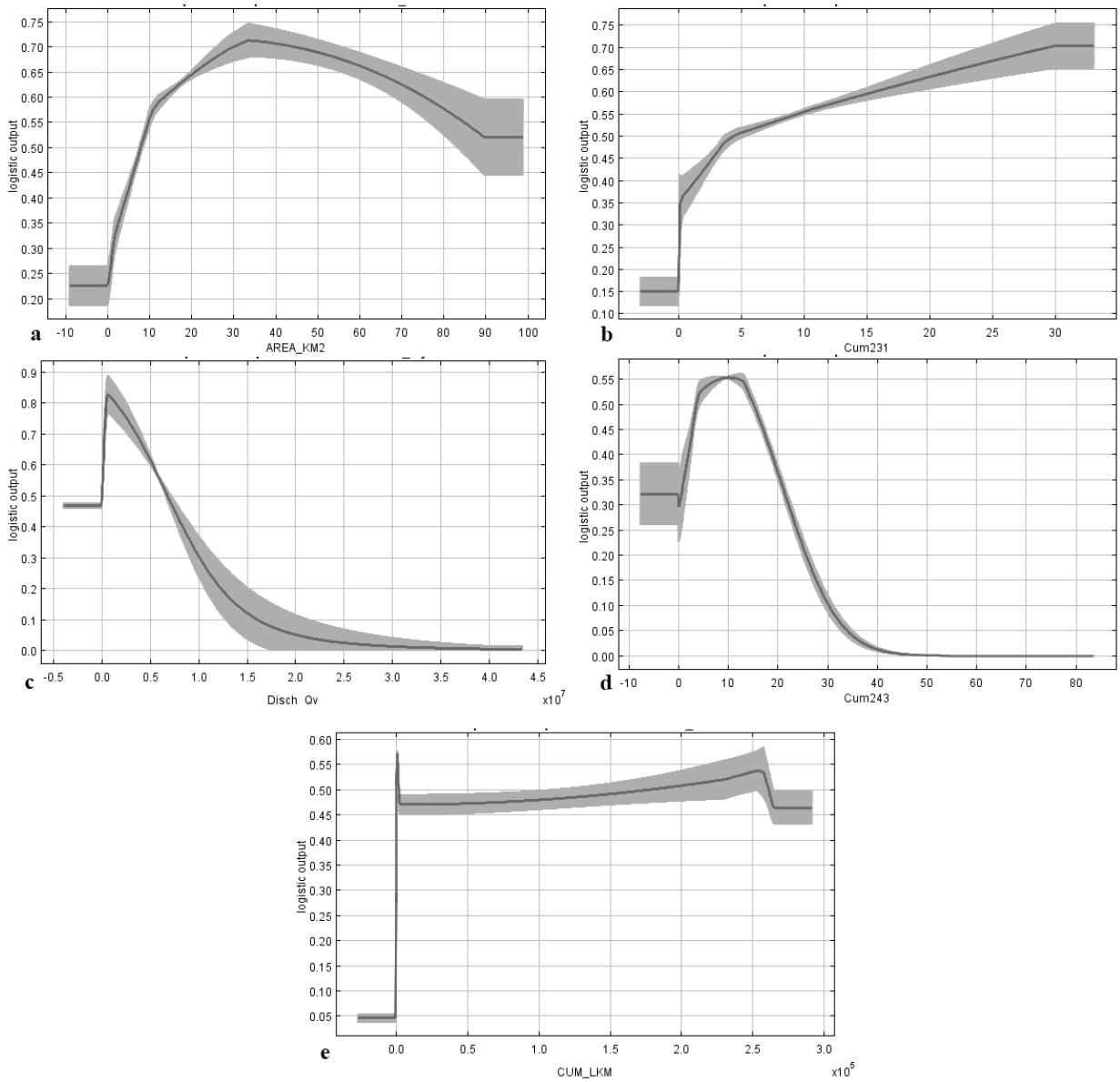


**Fig. 2.** Response curves averaged over the ten full models for each of the predictor variables: a) catchment area (AREA_KM2); b) pastures (Cum 231) – percentage of the cumulative area; c) total yearly discharge of wastewater within every catchment (Disch_Qy); d) land principally occupied by agriculture, with significant areas of natural vegetation (Cum243) – percentage of the cumulative area; e) cumulative length of the upstream flow network including the river segment itself (CUM_LKM).

riculture, with significant areas of natural vegetation (described in Table 2) had the highest predicted suitability (0.55, see Fig. 2d). The response curve for cumulative length (Fig. 2e) was bimodal: with one maximum of predicted suitability for values between 200 and 1500 km (data from the output of Maxent, not included; on the graph it is shown as close to 0); and one maximum for very high values of the variable ~ 260000 km. A bimodal response curve for this variable could be expected, because in the data used for the modelling we included occurrences both in river segments with extremely high (five records from the Danube River, average cumulative length ~250000 km) and river segments with relatively (to the previous group) low values of this variable (37 records from tributaries of the Danube River, average cumulative length ~600 km).

We used maximum test sensitivity plus specificity threshold (average 0.40) for discrimination between catchments predicted as suitable vs those predicted as unsuitable. Seven (or 17%) of the 41 catchments with occurrence of the species, used for

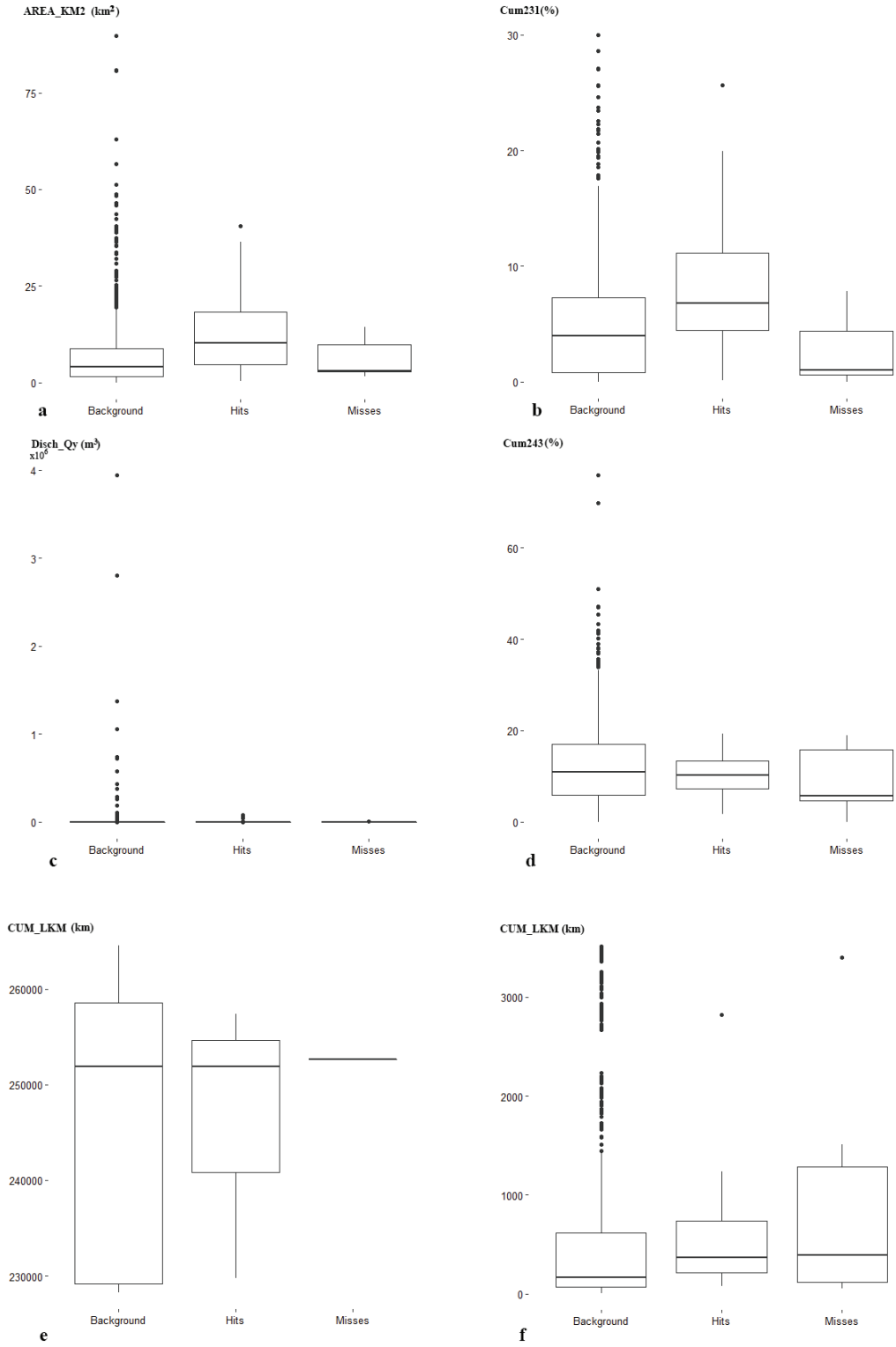


**Fig. 3.** Range of the predictor variables for: background catchments (Background); catchments with occurrences predicted to be suitable (Hits); catchments with occurrences predicted to be unsuitable (Misses). The range is displayed by Box-and-Whisker plots for each variable: a) catchment area (AREA_KM2); b) pastures (Cum 231) – percentage of the cumulative area; c) total yearly discharge of wastewater within every catchment (Disch_Qy) d) land principally occupied by agriculture, with significant areas of natural vegetation (Cum243) – percentage of the cumulative area; e) cumulative length of the upstream flow network including the river segment itself (CUM_LKM) for river segment belonging to the Danube River; f) cumulative length of the upstream flow network including the river segment itself (CUM_LKM) for river segment not belonging to the Danube River.

building the model, were categorised as unsuitable (misses) and 34 (or 83%) – as suitable (hits). The seven misses had smaller catchment area than the hits (Fig. 3a), and also lower percent of the cumulative area occupied by pastures (Fig. 3b). The box and whiskers plots for the other three variables (Fig. 3c, d, e, f) did not give us such valuable hints for possible differences between the hits and the misses.

The logistic probability of habitat suitability was above the chosen threshold also for 382 catchments without known localities of the species. Of these 298 contained segments of rivers flowing into the Danube River, 16 contained segments of the Danube River and 68 – segments of rivers flowing directly into the Black Sea (Fig. 4).

The logistic probability of habitat suitability showed a clustered pattern: Moran's autocorrelation index: 0.184526, z-score: 36.772080, p-value < 0.000001.

# Discussion

### Phenology

The subpopulations of *A. aestivalis* in the Lower Danube River (Bulgarian section) and the rest of the rivers in Bulgaria included in the present study have complex age structure. In each of the three seasons (spring, summer and autumn) all stages of development were present: from 1st instar nymphs to adults (eggs have not been found in the examined samples). This phenomenon could be explained by the continual spermatogenesis of males which enables them to repeat copulation during the year (Papáček & Soldán 2008).

Nymphs of 1st and 2nd instars were found mostly in the summer samples, indicating that eggs likely hatch mainly through that season. This finding is consistent with previous observations that females lay eggs during the spring and summer and nymphs hatch after a couple of weeks (Papáček 2012). The presence of both nymphs and adults in spring and autumn is in compliance with the reported by Saulich & Musolin (2007) that *A. aestivalis* can overwinter in every stage of its development. In the autumn and spring samples, mostly adults and nymphs of 3rd, 4th and 5th instars have been found. Possible explanation of such ratio could be overwintering of *A. aestivalis* in the rivers, included in the study mostly in these stages.

Remarkable downstream drift of nymphs and adults was observed in the Danube River during spring high water levels. Near Magarets Island (rkm 568) on 11 May 2013 eight specimens were caught using an ichthyoplankton net after only 10 minutes exposition at 6.6 m depth. This corresponds with observations on possibilities of spreading of *A. aestivalis* by drift in the Rhein River (Hoffman 2008).

### Notes on the distribution

The species is known from most of Europe (except for the Iberian Peninsula and the southern parts of Balkan Peninsula), Anatolia and Georgia (Kanyukova 1995, Rabitsch & Zettel 2000, Coulianos 2005, Živić et al. 2007), Asian Turkey (for references see Fent et al. 2011). In some European countries, *A. aestivalis* is considered as endangered species and is included in local and national Red Lists or Red Data Books (Papáček 2012). Nevertheless, *A. aestivalis* has been found in many new localities in Austria (Rabitsch 2007), the Czech Republic (Papacek et al. 2009) and Belgium (Vercauteren et al. 2002) during the last 15 years. The reasons for the high number of newly found localities of *A. aestivalis* in Europe are not clear. Some of the hypothesis point at the hydrobiological methods of sampling as better for searching of the species than the commonly used entomological hand net sampling (Papacek 2012). Another reason could be the improvement of the ecological state of some rivers in Europe (Vercauteren et al. 2002).

The same is the tendency for the territory of Bulgaria. Before this study *A. aestivalis* has been registered only in six rivers within Ecoregion 12: the Danube River (Josifov 1958, Russev 1966), Rusenski Lom (Russev et al. 1987, 1994), Vidbol (Russev et al. 1994), Vit, Batova and Veleka Rivers (data from the collection of the Natural History Museum, Sofia). In this paper, we present data for localities of *A. aestivalis* in 20 rivers with no previous records of the species in Ecoregion 12. The great number of new records from the recent years could be partly due to transposition of the European water legislation and requirements and implementation/application of intensive and periodic monitoring programs of the rivers in Bulgaria. On the other hand, after the economic collapse of the country in 1990s, many industrial productions ceased and in addition infrastructure for urban wastewater treatment was developed, which caused massive improvement of the water quality. As a result, some rivers that were practically deprived of life at a distance of 40-50 km after large cities or industrial centers, are now restoring their fauna (Rusev 1959, 1968, Kenderov & Apostolova 2008, Kenderov & Yaneva 2009, Uzunov et al. 2011, Kenderov et al. 2012). The reduced anthropogenic impact could have led to colonisation or re-colonisation of the recently registered localities by *A. aestivalis*. Even if the species has many localities in the studied area,

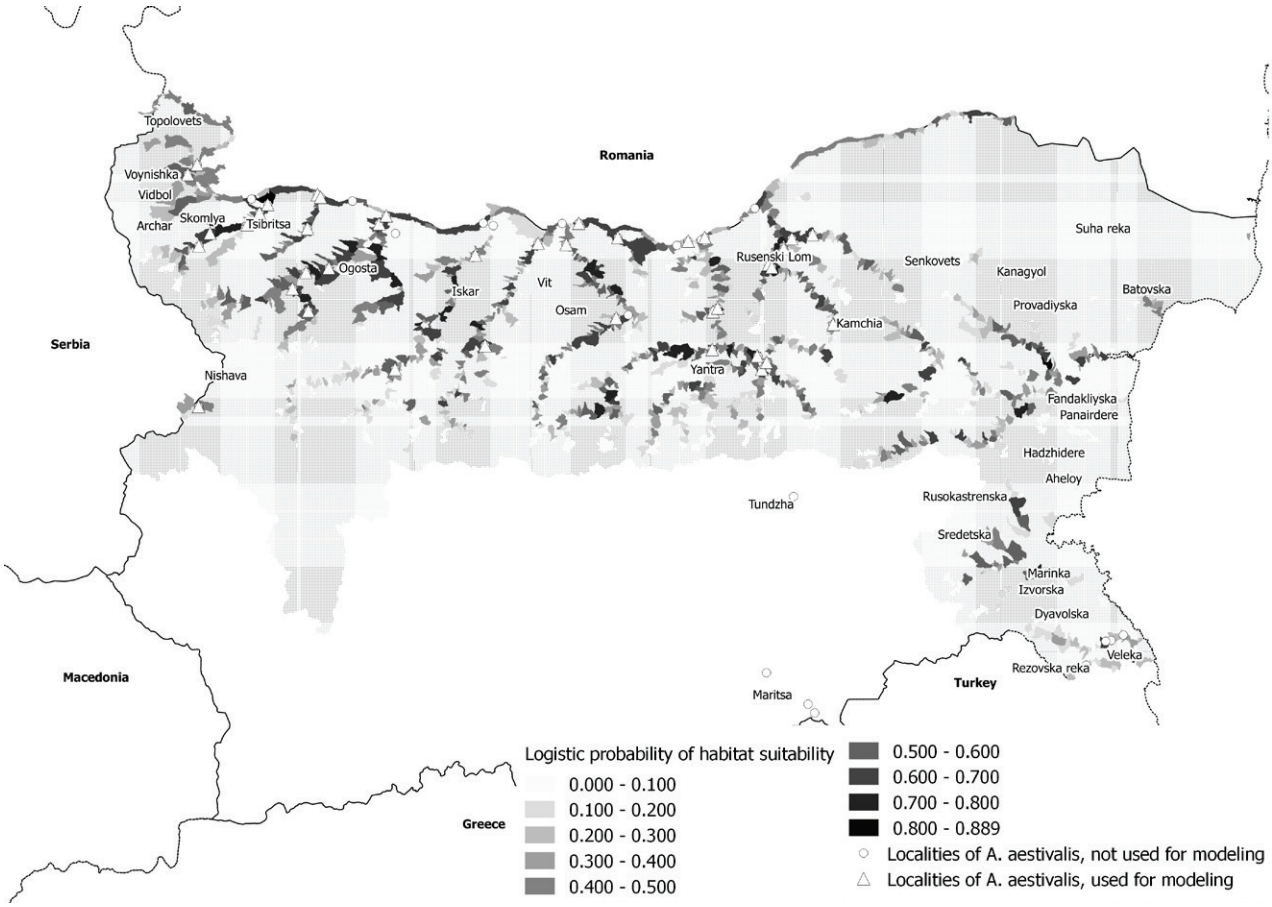


**Fig. 4.** Localities of *Aphelocheirus aestivalis* and probability of habitat suitability (averaged over the ten final models) for primary catchments within Ecoregion 12.

**Table 3.** Relative contributions and permutation impotence averaged over the ten final models.

| Predictor variable | Percent contribution | Permutation importance |
|---|---|---|
| CUM_LKM | 33.8 | 31.1 |
| AREA_KM2 | 25.6 | 24.3 |
| Cum231 | 24.2 | 25.6 |
| Cum243 | 9.7 | 15.9 |
| Disch_Qy | 6.7 | 3.1 |

its vulnerability to changes of its habitats' characteristics (Papáček 2012) should be kept in mind. The species could be especially sensitive to hydromorphological modifications as discussed in Papáček (2012): "watercourse clearance, stream regulation or construction of weirs and dams". Further, a recent study on both canalised and unmanaged stream cross-sections (without hydromorphological alterations) found *A. aestivalis* only in the unmanaged cross-sections (Wyżga 2014). The Aphelocheiridae has been also pointed as a family that is strongly influenced by fine sediment load as fine sediment may disrupt the respiratory functioning of these insect (Turley et al. 2016).

As there were many localities in tributaries of the Danube River but very few in rivers flowing directly into the Black Sea, we explored further the distribution of *A. aestivalis* in Ecoregion 12 (especially in the Black Sea Basin District) by building habitat suitability models. It should be mentioned that their average AUC was not very high. A possible reason could be the use of proxy variables instead of variables known to have direct effect on the habitat suitability for the species: flow velocity, water depth, substratum composition, stream size, pollution levels (Aukema 2002, Živić et al. 2007, Hoffmann 2008, Landeck 2009,Lock et al. 2013). We expect that models based on data for these variables could have higher accuracy but data for them are not available for the studied area on river segment/primary catchment scale. In a study using predictors' data on even smaller scale, higher AUC has been reached for a model of *A. aestivalis*' distribution within the Kinzig River's catchment in Germany (Kuemmerlen et al. 2016).

According to the response curves produced by Maxent in the present study, *A. aestivalis* is more likely to be found in the middle and downstream stretches (high values of cumulative length) with low levels of pollution from point (low quantity of wastewater discharge) and diffuse pollution (low percent of land occupied by agriculture) sources. These results are to a great extent in accordance with the published information about the species ecology from other European countries (Aukema 2002, Hoffmann 2008, Živić et al. 2007, Landeck 2009, Lock et al. 2013).

The final models tended to underpredict the probability of habitat suitability for catchments with smaller area and those with relatively low percent of the cumulative area occupied by pastures. It should be noticed also that the logistic probability of habitat suitability predicted by the model shows clustered pattern. Organisms with low dispersal ability have more clumped distributions than those with ability to move easily and fast among habitats (discussed in a review by Shurin et al. 2008). *Aphelocheirus aestivalis* seems to possess rather low dispersion ability: it has almost exclusive water dispersion (see introduction) and has been noticed mostly to crawl over the substratum and very rarely to swim (Beutler & Frutiger 1988). Therefore, we assume a habitat suitability model showing clustered pattern could be more useful for identifying catchments where new localities of *A. aestivalis* could be searched for in future.

We did not use any of the occurrence data for *A. aestivalis* in the Black Sea Basin District for model training or testing. This data was available for validation of the models' predictions. Catchments containing segments of two rivers in the Black Sea Basin District (Veleka and Batova Rivers) with occurrence of the species were assigned higher average probabilities of habitat suitability than the chosen threshold value for suitability. These results suggested that final models built here could be useful for identifying catchments with habitats potentially suitable for the species in the studied area. Interestingly the final models predicted high probability of habitat suitability for catchments in the basins of six rivers also flowing into the Black Sea, but without known localities of the species: Aytoska reka, Kamchia, Fakiyska reka, Provadiyska reka, Rusokastrenska reka and Sredetska reka Rivers (Fig. 4). Therefore, the final models fit the initial aim of the present study: to gain more information about potentially suitable habitats in rivers in the Black Sea Basin. Our next step in the study of *A. aestivalis* in the region is sampling of these river basins to test further the predictions of the models.

To our knowledge, the models built here for *A. aestivalis* represent the first attempt of modelling habitat suitability on river segment scale for aquatic Heteroptera (infraorder Nepomprha) species with Maxent.

Besides our study, habitat suitability/distribution model for *A. aestivalis* has been previously built in two recent studies (Domisch et al. 2013, Kuemmerlen et al. 2016). In Domisch et al. (2013), data about the distribution of *A. aestivalis* (together with other 224 riverine macroinvertebrate species) have been used in experiments for testing how different study areas and predictors affect species distribution models. Although the authors have included a map with the modelled habitat suitability for the species, they discussed the produced models only in the contexts of differences between the tested approaches. In Kuemmerlen et al. (2016), the distribution of almost 200 riverine macroinvertebrate taxa has been modelled within the Kinzig River's catchment in Germany, based on predictor's data on smaller scale than the one we have used here. When there is accurate data on such small scale and the study is aiming at the production of models for a great number of species, the approach in Kuemmerlen et al. (2016) would be more appropriate. The approach used in the present study was fitted to our purpose and the available data, but we assume it could be applied successfully for modelling the habitat suitability for *A. aestivalis* also within other areas (e. g. including multiple river basins), especially when there are only data about the species presence (occurrence) and environmental data are available at larger scale: river segment scale.

Although the aim of this paper was to contribute to the knowledge of the species' distribution in Ecoregion 12, we reported also a few localities of *A. aestivalis* in two rivers within Ecoregion 7: Maritsa River and Tundzha River. The locality in Maritsa River near Svilengrad (Russev 1967, own data) represents the southernmost record of *A. aestivalis* in Europe up to now. Possible findings downstream in Maritsa River on the territory of European Turkey and Greece are expected and the southernmost boundary could be extended. In the Late Pliocene, the Tundzha River was a part of the Black Sea watershed (Angelova 2003). In the lower part of the above-mentioned river, some freshwater fish species, such as *Petroleuciscus borysthenicus* (Kessler, 1859) and the Ponto-Caspian relict goby *Neogobius fluviatilis* (Pallas 1814) were also found (Drenski 1951, Stefanov et al. 2008). At the moment, we could only speculate of a possible Pontic origin of *A. aestivalis* in Tundzha and Maritsa Rivers.

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