

## Short Communication

## Distribution of amphipod communities in the Middle to Upper Rhine and five of its tributaries

Wei Chen, David Bierbach, Martin Plath, Bruno Streit and Sebastian Klaus\*

Dept. of Ecology and Evolution, Johann Wolfgang Goethe-Universität, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany

E-mail: [chenwei.bio@gmail.com](mailto:chenwei.bio@gmail.com) (WC), [david.bierbach@gmx.de](mailto:david.bierbach@gmx.de) (DB), [mplath@bio.uni-frankfurt.de](mailto:mplath@bio.uni-frankfurt.de) (MP), [streit@bio.uni-frankfurt.de](mailto:streit@bio.uni-frankfurt.de) (BS), [klaus@bio.uni-frankfurt.de](mailto:klaus@bio.uni-frankfurt.de) (SK)

\*Corresponding author

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### Abstract

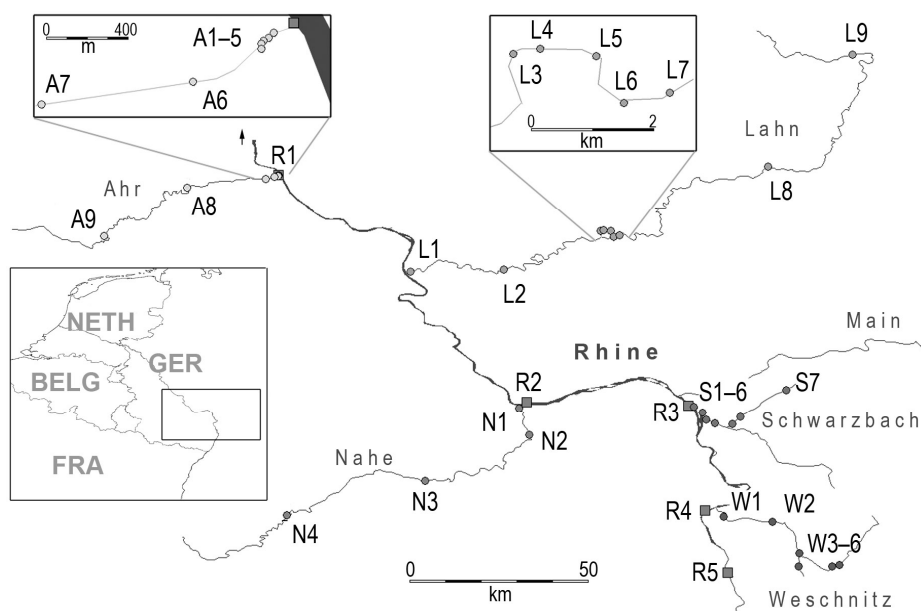
Ponto-Caspian amphipods have continuously been invading the Rhine system at least since the last quarter of the 20th century. This pilot study attempts to identify current regional invasion fronts of invasive amphipods for future continuous monitoring. A standardized kick and sweep sampling technique was used to collect amphipods between July 2011 and May 2012 at five sites in the Northern Upper and Middle Rhine and 35 sampling sites within the following tributaries: Ahr, Lahn, Nahe, Schwarzbach and Weschnitz. Invasive *Dikerogammarus villosus* dominated sites in the Middle Rhine, and *Echinogammarus ischnus* in the Upper Rhine. *Gammarus roeseli* was dominant in the middle and upper reaches of the tributaries, co-occurring with *Gammarus pulex* in the lower reaches, while *G. fossarum* was found exclusively in head stream regions. The invasive amphipod species, however, did not invade the majority of the tributaries. *D. villosus* was only found in the River Lahn, and *Chelicorophium robustum*, another invasive species, at the confluence of the Nahe and the Rhine. Thus, we identified two kinds of invasion fronts: (1) invasion fronts at the tributaries' river mouths, with a distinct change in faunal compositions over short distances and invasive species occurring only in the most downstream portion of the tributaries. These fronts appear to have been stable over the last decades despite dramatic species turnover within the Rhine itself. (2) A second kind of invasion front was in the middle to upper reaches of River Lahn, where *D. villosus* co-occurs with native species. The latter river is the only navigable tributary considered in this study, and the fact that invasive *D. villosus* occur just up to the point where shipping activities stop suggests shipping as an important factor promoting their occurrence.

**Key words:** *Dikerogammarus villosus*, *Echinogammarus ischnus*, *Echinogammarus trichiatus*, *Chelicorophium robustum*, *Chelicorophium curvispinum*

### Introduction

In terms of macro-invertebrates the Rhine ranks among the most dynamic and most heavily invaded freshwater ecosystems worldwide (Bij de Vaate et al. 2002; Galil et al. 2007; Leuven et al. 2009; Panov et al. 2009). The construction of canals between rivers that originally were part of independent drainages in Central and Eastern Europe starting effectively in the 18th century and culminating with the Rhine-Main-Danube canal, which was completed in 1992-opened invasion corridors that resulted in a massive influx of Ponto-Caspian amphipod species into Central and Western Europe (Bij de Vaate and Klink 1995; Kinzelbach 1995; Tittizer 1996; Van der Velde et al. 2000; Bij de Vaate et al. 2002). One of the most successful amphipod invaders that entered the Rhine in 1994 is *Dikero-*

*gammarus villosus* Sowinsky, 1894 (Van der Velde et al. 2000), a species that is characterized by active predation on other gammarids and thus occupies a similar trophic level as much of the Rhine ichthyofauna (Dick and Platvoet 2000; Bij de Vaate et al. 2002; MacNeil and Platvoet 2005; Buřič 2009; Kinzler et al. 2009). Local *Gammarus pulex* Linnaeus, 1758 and formerly invasive *Gammarus tigrinus* Sexton, 1939 continuously decreased in abundance after the arrival of *D. villosus* and *Echinogammarus ischnus* Stebbing, 1899 (= *Chaetogammarus ischnus*) (Leuven et al. 2002). Several other invasive amphipod species were affected by the invasion of *D. villosus*, like *Chelicorophium curvispinum* Sars, 1895, and *Dikerogammarus haemobaphes* Eichwald, 1841 (Van der Velde et al. 2000). *Echinogammarus trichiatus* Martynov, 1932 (= *Chaetogammarus trichiatus*) is the most recent



**Figure 1.** Sampling sites along the Rhine and five of its tributaries (see inset at lower left). Insets: dense sampling at sites with presumed short-distance faunal turnover at the confluence of river Rhine and Ahr (upper left) and up- and downstream of the northern limit of navigability in the River Lahn (right). For details see Appendices 1-2.

invader of River Rhine; it was first recorded in 2000 (Bij de Vaate et al. 2002). Thus, these invasive amphipods altered both indigenous and invasive community structure and species composition (Dick and Platvoet 2000; Dick et al. 2002; Kinzler and Maier 2003; Dick and Platvoet 2000), but also led to population declines of other macroinvertebrates, thereby influencing the structure of the entire river biocenosis (Van der Velde et al. 2000; Bruijs et al. 2001; Wijnhoven et al. 2003; Van Riel et al. 2006).

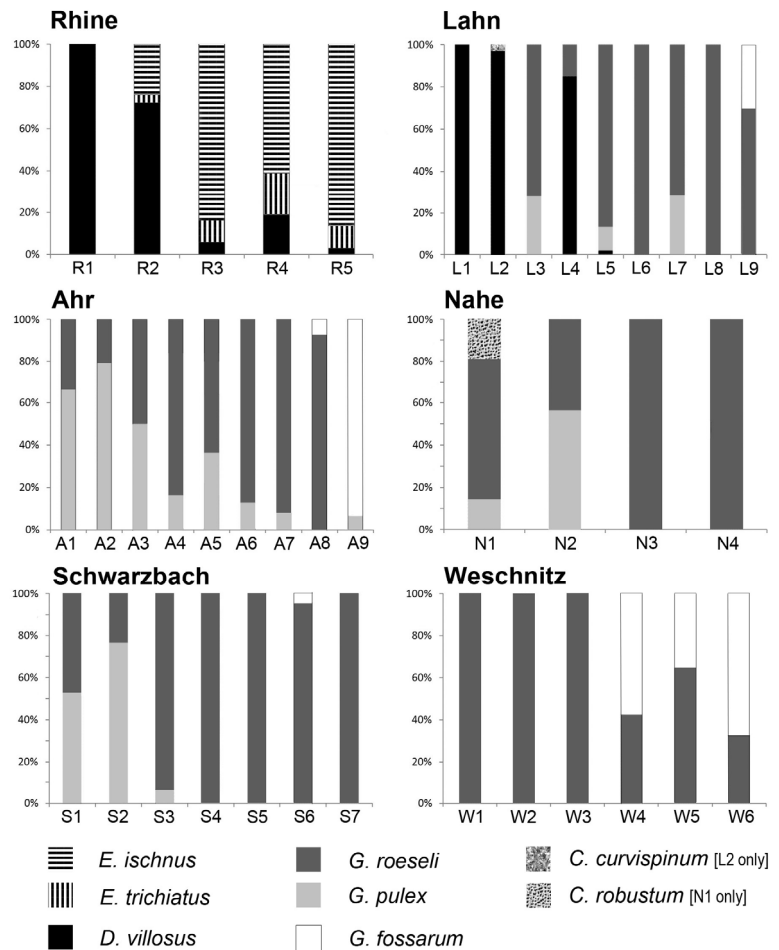
The continuous replacement of one or few temporarily dominant amphipod species by newly invading species is a preeminent phenomenon in the Rhine and some of its tributaries. To understand the underlying processes and resulting patterns, it is important to analyze the temporal and spatial dynamics at the invasion fronts, i.e. how community structures change over time. Here we report on amphipod species compositions in the Northern Upper and Lower Middle Rhine and five of its tributaries, to (1) provide a baseline for future long-term studies, (2) to investigate how strong the tributaries of river Rhine are affected by invasive species, and (3) to identify current invasion fronts.

## Materials and methods

We collected amphipods at five sampling sites along a 250 km stretch of the Upper and Middle Rhine at 10 to 50 km river distance, and in five of its tributaries (35 sites) between July 2011 and May 2012 (Figure 1; Appendix 1, 2). The Rhine samples were taken at a time of extraordinary water level low-stands in November 2011. Nine sampling sites were situated along river Ahr, nine along river Lahn, four along river Nahe, seven along river Schwarzbach, and another six along river Weschnitz (Figure 1). The sampling sites are not evenly distributed along the tributaries. In Ahr and Lahn dense sampling (sites were within 100–1000 m river distance) was conducted at the front between amphipod communities dominated by invasive and native species, i.e., at the confluence of the Ahr and Rhine, and up- and downstream of the limit of navigability in river Lahn. From Schwarzbach and Weschnitz there were no reports thus far of invasive amphipods.

We used a standardized kick and sweep sampling technique (Barbour et al. 1999) using a pond net (opening: 25×25 cm, depth: 60 cm,

**Figure 2.** Distribution patterns of amphipod communities at different sites along river Rhine and five of its tributaries.



mesh width: 500  $\mu\text{m}$ ). On pebbly and rocky ground, we turned stones by hand and wiped animals from the stone surface into the net. In addition, we carefully sampled roots or aquatic plants that might serve as hiding places for amphipods. These sampling procedures were repeated until no more animals were obtained from the target site (10  $\text{m}^2$ ). Species identification followed the keys provided by Eggers and Martens (2001, 2004) and Eiseler (2010).

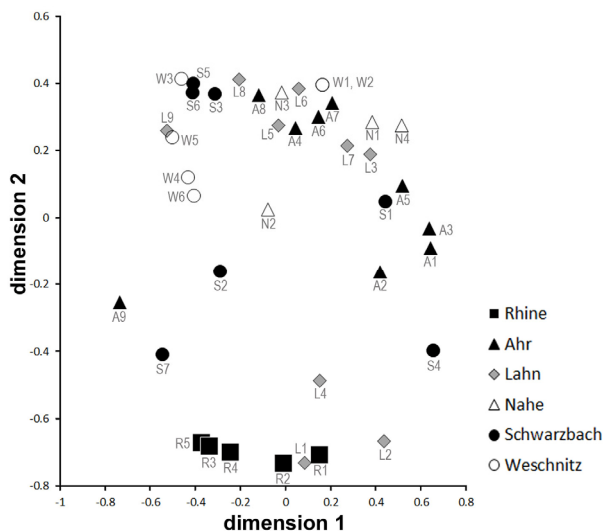
Abiotic parameters (pH, conductivity, dissolved oxygen and temperature) were measured with a Multi-Parameter Meter (HQ40d Portable Meter, HACH, Loveland, USA) at each sampling site (Appendix 2)

To investigate similarity and evenness of faunal composition between sites, we calculated pairwise Bray-Curtis dissimilarities (Bray and Curtis 1957) with the R-package ‘ecodist 1.2.7’

(Goslee and Urban 2012; R Development Core Team 2008), and used these for non-metric multidimensional scaling (NMDS function in ecodist). Additionally, we used chi-square tests to compare species compositions between sampling sites within river Rhine and between the Rhine and the five tributaries.

## Results

We sampled a total of 3047 amphipod specimens and identified eight species, two of which are native to the region (*Gammarus fossarum* Koch, 1835 and *G. pulex*), one that reached the Rhine system at least before 1835 (*Gammarus roeseli* Gervais, 1835; see Jażdżewski 1980), while five species are invasive (*Dikerogammarus villosus*, *Echinogammarus ischnus*, *E. trichiatus*, *Chelicerophium curvispinum*, *C. robustum*). 740 specimens



**Figure 3.** Non-metric multidimensional scaling of Bray-Curtis dissimilarities between amphipod communities at the River Rhine and five of its tributaries.

stemmed from the Rhine, 219 from the Ahr, 156 from Nahe, 566 from Lahn, 640 from Weschnitz and 726 specimens came from Schwarzbach (Appendix 2).

In the Rhine three dominant species were detected: *E. ischnus* was the most abundant species at sites R4 (61%), R3 (84%) and R5 (87%), followed by the congener *E. trichiatus* (R3: 11%; R4: 20%; R5: 10%) and *D. villosus* (R3: 6%; R4: 19%; R5: 3%; Figure 2). This pattern was different at site R2 where *D. villosus* dominated (72%), while *E. ischnus* (24%) and *E. trichiatus* (4 %) were considerably rarer. At the northernmost sampling site (R1) only *D. villosus* was found (Figure 2). Differences in species composition between sites along river Rhine were highly significant ( $X^2=329.51$ ,  $df=8$ ,  $P<0.001$ )

The composition of amphipod communities was significantly different between river Rhine and the investigated tributaries ( $X^2 = 179.43$ ,  $df = 7$ ,  $P < 0.001$ ; Figure 2). The tributaries were dominated by native gammarid species, with *Gammarus roeseli* being the most abundant (Ahr: 72%, Lahn: 59%, Nahe: 65%, Schwarzbach: 60%, Weschnitz 68%). It co-occurred with *G. pulex* at downstream sites, where the latter usually dominated (*G. pulex* was not found in the Weschnitz; Figure 2), and co-occurred with *G. fossarum* at the most upstream sites (not found in the Nahe and Schwarzbach).

Within the tributaries, the invasive species *D. villosus* was only found in the Lahn, at site L1 (100%), which is situated near the confluence with the Rhine, and at sites in the middle reaches (L3, L4, L5). There was a sharp drop in abundance of *D. villosus* between sites L4 and L5 (85 vs. 2%). Invasive *Chelicorophium robustum* was found in considerable numbers at the confluence of the Nahe and Rhine (N1: 19%), and *C. curvispinum* was occasionally collected in the River Lahn at L3 (3%).

Non-metric multidimensional scaling of Bray-Curtis dissimilarities illustrates the degree of faunal differentiation between River Rhine and its tributaries (Figure 3). Especially those sampling sites within the Lahn that were dominated by *D. villosus* clustered close to the Rhine sites. Species compositions of up-stream sampling sites loosely cluster together (A8, L9, W6 and S6), as do the sampling sites at the confluence regions of River Rhine and tributaries (S1, N1, W1 and A1). Sampling sites in the River Schwarzbach, River Ahr, and River Lahn are dispersed in a wide geographical range, and there was no obvious structure among the sites of above three rivers, even though faunal composition in the upper reaches appears to be more similar among tributaries than faunal compositions of downstream sites (Figure 3).

## Discussion

Amphipod communities changed profoundly after the incursion of Ponto-Caspian species, which occurred in several waves, facilitated especially by the opening of the Rhine-Main-Danube canal in 1992 (Tittizer 1995). Following the increase of *D. villosus* and other neozoans in the upper Rhine, the earlier invaders *C. curvispinum* and *G. tigrinus* have been decreasing since the early 1990s (IKSR 2002; Haas et al. 2002).

Our results confirm previous findings reporting on the Middle Rhine and the Northern part of the Upper Rhine being exclusively inhabited by invasive species, especially *D. villosus* and *E. ischnus* (Bernauer and Jansen 2006; Leuven et al. 2009). However, at present *D. villosus* is not the dominant species in the Upper Rhine, contrasting with earlier descriptions that proposed complete dominance of *D. villosus* in all parts of the Rhine (Bernauer and Jansen 2006). Leuven et al. (2009) found a recent decline of *E. ischnus* in the river Rhine

tributaries in the Netherlands between 1999 and 2003, which contrasts with the high numbers we found at sites R3 to R5 (belonging to the state of Hesse, Germany).

Different causes have been proposed for the complete replacement of the autochthonous amphipod fauna in central European rivers, such as predation (MacNeil and Platvoet 2005), competitive exclusion (Dick and Platvoet 2000), divergent life-history traits (Kley and Maier 2003, 2006; Pöckl et al. 2003; Pöckl 2007), broader ecological niches (Devin et al. 2003), and higher tolerance against pollutants (Kuhn and Streit 1994). For instance, *G. tigrinus* exhibited a higher tolerance against organophosphate agrochemicals, which have been accidentally released in large quantities into the Rhine in 1986 and which affected endemic *Gammarus* species more than the invasive *G. tigrinus* (Streit and Kuhn 1994). At a later stage, former invaders like *G. tigrinus*, *Orchestia cavimana* Heller, 1865, *D. haemobaphes* and *Obesogammarus obesus* have been replaced or marginalized by successive amphipod invaders, especially *D. villosus* (Leuven et al. 2009) and were not found in the Rhine in the course of our present study. However, we found reasonable numbers of *C. robustum* in the lower reaches of the Nahe (N1: 19%) and few *C. curvispinum* in the middle reaches of the Lahn (L3: 3%). These sites were sampled in spring (April/May), thus we cannot rule out an annual shift in species composition, as discussed already by Janetzky (1994).

The change in faunal composition (native vs. invasive) between the Rhine and its tributaries is abrupt, as exemplified by the small scale sampling at the confluence of river Rhine (R1) and Ahr (A1-A5), and Rhine (R4) and Schwarzbach (S1-S4), where we could not find any invasive species dispersing into the tributaries. The NMDS of Bray-Curtis dissimilarities illustrates this general pattern of species composition among sites: There is a clear separation between River Lahn (L1, L3, and L4) and Rhine on the one side, and the other tributaries on the other (Figure 3). All tributaries except River Lahn show similar faunal compositions from down- to up-stream. Thus, most invasion fronts are likely to be rather stable over time. This might reflect a general pattern, as Piscart et al. (2010) found that non-native species were dominant in the River Loire (France), but failed to colonise the tributaries. Mostly abiotic factors like temperature (Pöckl

2007) or substrate type (MacNeil and Platvoet 2005) were proposed to potentially restrain the further range expansion of invasive amphipod species. Abiotic conditions might also influence biotic interactions. Lower water temperature, characterising the habitat of *Gammarus fossarum*, was found to inhibit predation of *Dikergammarus villosus* on *G. fossarum* (see Van der Velde et al. 2009). A similar relation was found between *G. pulex* and *G. tigrinus* with respect to conductivity (Dick and Platvoet 1996). Also environmental heterogeneity, especially the microhabitat zonation, may influence invader-invader interactions and lead to species co-existence (Platvoet et al. 2009). Grabowski et al. (2009) investigated the correlation between salinity and distribution of non-native amphipods, and found that the invasives inhabit the larger rivers with higher-conductivity, whereas the native amphipods dwelled in smaller streams with lower conductivity. No such relation could be observed in the present data (Appendix 2).

The factors discussed above, however, fail to explain the patterns seen in river Lahn, where invasive *D. villosus* can be found. Within the Lahn the invasion front of *D. villosus* is between sampling sites L4 and L6, where shipping activities stop. It appears likely that a direct causal link between navigability and the occurrence of *D. villosus* in this tributary exists: ships might not only serve as vectors for invasive species (Nehring 2005; Leuven et al. 2009), but it was reported that unlike other amphipods, *D. villosus* is rather resistant to ship-induced waves (Gabel et al. 2011).

Although the dynamics of amphipod community turnover in the river Rhine itself was profound during the last decades, the native gammarid communities in most of the tributaries seem to be intact, and the invasion fronts appear to be rather stable over time. Thus, the success of invasive amphipods in river Rhine cannot be explained only by inherent capacities of the invaders themselves (e.g., dispersal capabilities, life history traits and predatory behavior), but is most likely-and possibly foremost-driven by abiotic factors. Our study represents just a snapshot in time and space of amphipod faunal composition and turnover in the Middle and Upper Rhine catchment. A full understanding of annual shifts and species dynamics, both at 'cold' and 'hot' invasion fronts can only be achieved through mid- to long-term monitoring at such sites; such studies are currently underway.

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**Appendix 1.** Records of native and non-native amphipods in the Northern Upper and Middle Rhine (R1–R5) and their tributaries Ahr (A1–A9), Lahn (L1–L9), Nahe (N1–N4), Schwarzbach (S1–S6) and Weschnitz (W1–W6), Germany in 2011–2012. Non-native species: D.v. – *Dikerogammarus villosus*, E.i. – *Echinogammarus ischnus*, C.c. – *Chelicorophium curvispinum*, E.t. – *Echinogammarus trichiatus*, C.r. – *Chelicorophium robustum*. Native species: G.r. – *Gammarus roeseli*, G.p. – *Gammarus pulex*, and G.f. – *Gammarus fossarum*.

Site	Location name	Amphipod species							
		D. v.	E. i.	E.t.	C.c.	C. r.	G. r.	G. p.	G. f.
A1	Remagen, Germany						2	4	
A2	Remagen, Germany						5	19	
A3	Remagen, Germany						3	3	
A4	Remagen, Germany						37	7	
A5	Remagen, Germany						7	4	
A6	Remagen, Germany						28	4	
A7	Sinzig, Germany						23	2	
A8	Dernau, Germany						52		4
A9	Antweiler, Germany							1	14
L1	Lahnstein, Germany	4							
L2	Obernhof, Germany	96			3				
L3	Dehrn, Germany						13	5	
L4	Dehrn, Germany	45					8		
L5	Steeden, Germany	1					46	6	
L6	Runkel, Germany						33		
L7	Runkel, Germany						18	7	
L8	Wetzlar-Naunheim, Germany						74		
L9	Göbfelden, Germany						144	63	
N1	Bingen, Germany					4	3	14	
N2	Gensingen, Germany						48	37	
N3	Bad Sobernheim, Germany							41	
N4	Enzweiler, Germany							9	
R1	Kripp, Germany	54							
R2	Rüdesheim, Germany	6	167	20					
R3	Mainz-Laubenheim, Germany	96	32	5					
R4	Worms, Germany	9	134	17					
R5	Sandhofen, Germany	38	122	40					
S1	Ginsheim-Gustavsburg, Germany						8	9	
S2	Trebur, Germany						39	126	
S3	Astheim, Germany						110	7	
S4	Trebur, Germany						1		
S5	Trebur, Germany						142		
S6	Nauheim, Germany						134		6
S7	Walldorf, Germany						144		
W1	Biblis, Germany						25		
W2	Einhausen, Germany						25		
W3	Hemsbach, Germany						177		
W4	Weinheim, Germany						54		73
W5	Weinheim, Germany						119		63
W6	Birkenau, Germany						34		70



Distribution of amphipod communities in the Rhine

**Appendix 2.** Abiotic parameters (pH, temperature, dissolved oxygen concentration, water conductivity, substrate) and date of sampling (site codes correspond to Appendix 1 and Figure 1). The substrate classification follows the Wentworth grain size chart (United States Geological Survey Open-File Report 2006–1195): boulders/cobbles (B/C); pebbles (P); sand (S); silt/clay (S/C). Additionally the presence of vegetation was noted.

Site	Coordinates		Date	pH	T (°C)	O <sub>2</sub> (mg/L)	Con. (µS/cm)	Substrate
	Lat, N	Long, E						
A1	50.55945	7.27720	31-AUG-11	8.16	17.10	10.67	607.00	P, S, S/C, plants
A2	50.55872	7.27645	12-OCT-11	7.96	15.40	8.89	616.00	P, S, S/C, plants
A3	50.55807	7.27577	12-OCT-11	7.95	15.40	8.52	613.00	P, S, S/C, plants
A4	50.55790	7.27545	12-OCT-11	7.94	15.35	8.51	609.50	B/C, P, S/C, plants
A5	50.55743	7.27552	12-OCT-11	7.95	15.40	8.56	606.50	B/C, P, S/C, plants
A6	50.55397	7.26578	31-AUG-11	7.95	16.80	10.76	629.00	B/C, P, S, S/C, plants
A7	50.54998	7.24488	31-AUG-11	7.86	18.30	12.50	544.00	B/C, P, S, S/C, plants
A8	50.52785	7.04262	4-MAY-12	8.64	12.40	11.09	386.00	B/C, P, S, plants
A9	50.40162	6.83167	4-MAY-12	7.99	10.80	10.86	398.00	B/C, P, S, plants
L1	50.30838	7.59805	31-AUG-11	7.74	20.70	8.03	399.00	P, S, S/C, plants
L2	50.31662	7.85183	4-MAY-12	8.34	17.40	9.79	415.00	B/C, P, S, S/C, plants
L3	50.41625	8.09838	31-AUG-11	8.04	20.10	9.44	388.00	B/C, P, S, S/C, plants
L4	50.41773	8.10692	31-AUG-11	8.01	20.40	8.86	389.00	P, S, S/C, plants
L5	50.41522	8.12457	31-AUG-11	8.09	20.30	9.36	390.00	P, S, S/C, plants
L6	50.40105	8.13283	12-OCT-11	8.10	13.90	9.59	501.00	B/C, P, S, S/C, plants
L7	50.40427	8.14718	31-AUG-11	8.03	19.90	9.04	385.00	B/C, P, S, plants
L8	50.58037	8.52707	4-MAY-12	8.00	17.00	7.39	368.00	P, S, S/C, plants
L9	50.86485	8.73898	4-MAY-12	7.80	14.20	98.83	259.00	P, S, plants
N1	49.96380	7.89143	2-MAY-12	8.13	16.40	8.61	389.00	B/C, P, S, S/C, plants
N2	49.89453	7.91643	2-MAY-12	8.24	17.10	9.02	368.00	P, S/C, plants
N3	49.77735	7.65033	2-MAY-12	8.26	15.20	9.85	249.00	P, plants
N4	49.69008	7.29838	2-MAY-12	8.10	14.00	9.86	169.60	P, plants
R1	50.55877	7.27770	24-NOV-11	8.22	8.30	11.03	777.00	B/C, P, S, S/C
R2	49.97932	7.91111	12-OCT-11	8.26	8.40	11.34	563.00	B/C, P, S, S/C
R3	49.96930	8.32387	12-OCT-11	7.95	8.30	10.85	686.00	B/C, P, S, S/C
R4	49.70268	8.36605	12-OCT-11	8.17	9.10	11.07	704.00	B/C, P, S, S/C
R5	49.54353	8.42427	12-OCT-11	8.10	18.70	10.98	602.00	B/C, P, S, S/C
S1	49.96673	8.33662	9-JUL-11	7.69	18.70	6.54	623.00	P, S, S/C, plants
S2	49.95287	8.35857	9-JUL-11	7.68	18.90	6.68	637.00	P, S, S/C, plants
S3	49.93363	8.36773	9-JUL-11	7.60	18.60	4.96	642.00	P, S, S/C, plants
S4	49.92425	8.39132	9-JUL-11	7.51	18.10	5.26	637.00	P, S, S/C, plants
S5	49.92318	8.43525	9-JUL-11	7.59	17.70	7.55	573.00	S, S/C, plants
S6	49.94182	8.45642	9-JUL-11	7.57	17.10	8.14	589.00	S, S/C, plants
S7	50.01123	8.57198	3-MAY-12	8.51	14.90	9.67	1340.00	S, plants
W1	49.68655	8.41330	22-SEP-11	8.53	20.40	11.13	771.00	B/C, P, S, S/C, plants
W2	49.67397	8.53782	22-SEP-11	8.31	20.30	9.63	905.00	B/C, P, S, S/C, plants
W3	49.56157	8.60462	22-SEP-11	7.62	18.80	11.01	1212.00	S/C, plants
W4	49.59495	8.60527	22-SEP-11	8.13	15.10	9.87	493.00	B/C, P, S, S/C, plants
W5	49.56085	8.69050	22-SEP-11	8.08	17.00	10.18	457.00	B/C, P, S, plants
W6	49.56487	8.70813	22-SEP-11	7.61	15.80	9.68	459.00	B/C, P, S, plants