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# Attempts to re-colonise water insects in German brooks

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Attempts were made to reintroduce water insects (five stonefly species and one mayfly species) into several third order streams in Rhineland-Palatine and Hessen, Germany. All these streams had been strongly affected by waste water and rubbish and had lost most of their macroinvertebrates. As a consequence of the installation of several purification plants in the past three decades, water quality has improved. Since no stoneflies returned, 700–1300 eggs of three different species (*Isoperla goertzi*, *I. oxylepis*, and *I. grammatica*) were exposed in the Selz brook (1997). In February 1998 a few larvae and in 1999 about 10,000 eggs of *Perla marginata* were added in the Walluf brook, and in 1998, 500 larvae of *Oligoneuriella rhenana* each in the Ruwer, Alf and Elz brooks. After 10 years, I found only one single larva of *Perla marginata* in the Walluf brook. In the laboratory, the total number of eggs produced per female could be determined by rearing. On that basis coupled with the assumption of about 3% survival chances for the embryonic and larval development and of about 50% survival chances for the adult females, it was calculated that at least two females of *I. goertzi*, eight females of *I. oxylepis* and seven females of *I. grammatica* were required to found a new and sustainable population.

**Keywords:** egg production; egg number for re-colonisation; *Isoperla*; *Perla*; *Oligoneuriella*

## Introduction

In the past decades many streams in Central Europe were polluted to such an extent that most of the natural fauna was lost. Since this process developed on a broader scale changing the environment radically, the original diversity of fauna is neither likely to return by its proper impulse nor to regenerate from neighbouring waters. It is true that in recent years considerable attempts have been undertaken to purify domestic and industrial waste water. This resulted in a reduction of the organic charge in the so-called main drainage ditches. The improvement can also be confirmed by the return of ubiquists. However, there is still a high demand in little charged source water which almost without exception is absorbed by municipal water plants. Furthermore, water from purification plants is not free from charges, neither from non-degradable substances such as pesticides (Seel, Knepper, Gabriel, Weber and Haberer 1994, 1996) or heavy metals, nor form substances like $NH_4$, $PO_4$ etc. which in middle-size purification plants can only be discharged to a certain

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degree (for $NH_4$ e.g. a degree of 10 mg/l is considered a sufficient standard). For these reasons it is difficult to determine if species can be recolonised in these partly purified waters which seem to be suitable for re-implantation from their size, morphological structure, and oxygen content.

Lake, Bond and Reich (2007) focused on the theoretical implications of re-establishing (fish) populations or Woolsey et al. (2007) on the assessment of river restoration success, whereas other scientists (Handschin 1995; Tittizer, Fey, Sommerhäuser, Málnás and Andrikovics 2008) have adopted an experimental approach. In the present study, my own recolonisation experiments of different stonefly and mayfly species undertaken in the years 1997–1999 are described and their outcome is evaluated.

## Materials and methods

For the experiment, brooks in Rhineland-Palatine and Hessen, Germany were selected which in the past had been highly polluted. It must be pointed out that there is no historical evidence for the occurrence of stoneflies in the selected brooks (Selz/Walluf), but it can be assumed by their occurrence in neighbouring brooks. I am convinced that the exposed species had been absent in the Selz and Walluf brooks over the past three decades. In recent years, the oxygen content of these brooks has significantly increased, and some macroinvertebrates have returned. In the Selz brook, one of the most polluted brooks, the water was permanently controlled from October 1994 through August 1997 by means of a WAS® equipment (MDS II). The following data were recorded: discharge, temperature, content of $O_2$ (WTW Oxi 96 resp. 323), conductivity (WTW LF 96), pH (WTW pH 96), and occasionally the redox potential (Ingold-Electrode Pt 4805-S7/120). To ensure that the different electronic instruments did not influence each other, they were uncoupled by means of additional module separators. From 1 February 1995 through 30 September 1996, additionally water samples were analysed monthly by using an IC of Dionex 100 as to the following parameters: the cations Na, K, $NH_4$, Mg, Ca, and the anions Cl, $NO_3$, $NO_2$, $PO_4$, and $SO_4$. For 31 out of 50 samples, the content of boron was determined photometrically (Merkoquant Nr. 1.14839), and the number of microbial germs was established by using standardised coli tests (bacto-strip of Retorte, $n = 34$).

From streams in remote areas (Palatinate Forest, Eifel, Taunus), either larval or adult specimens of *Isoperla goertzi*, *I. oxylepis*, and *I. grammatica* (short and long type) were taken and kept in the laboratory for rearing and mating. The males were kept in 15 ml snap cap bottles with a drop of water, the females in Erlenmeyer flasks of 250 ml with water, a culm of *Phalaris arundinacea*, and a snap cap of milk powder (Milupa®). The species were initially determined by identifying their drumming signals according to Rupprecht (1969, 1984). Then one male and one female were put together in a box for mating, and the animals were separated only after having copulated and separated by their own instinct (average time for copulation procedure: 1 hour; temperature in laboratory: 21–22°C). In the evening the eggs were collected and stored on wet paper at 15°C. After two to three days they were put in different places of the stream. To ensure that the exposed eggs were indeed fertilised and suited for larval development, a number of eggs were kept in rearing water in snap-cap bottles covered with gauze and put in an aerated aquarium (standard Taunus water with an average ion concentration of 18 streams; conductivity of 200 $\mu$S/cm, Rupprecht 1991). The cultivation was performed in a Memmert incubator (type: BKE 500) the temperature of which was kept oscillating between 13°C and 15°C (higher temperature during the day; manual regulation). All samples were kept individually in separate, numbered glasses; starting time: mid-June to end of July 1997.

### *Donor streams and their species*

Aubach (tributary of the Weilbach, Taunus Mountains, 50°17′ N, 8°28′ E): *Isoperla oxylepis* (Despax, 1936) and *I. grammatica*, long type.

Elz (tributary of Mosella, near Pillig, 50°14′ N, 7°17′ E): *Isoperla grammatica*, short type.

Hermannsborn (Taunus Mountains, 50°30′ N, 8°28′ E): *I. goertzi* Illies, 1952.

Sauerbach (near Hirschfeld /Palatinate, 49°3′ N, 7°45′ E): *I. grammatica*, short type.

Weilbach (Taunus Mountains, 50°17′ N, 8°28′ E): *I. grammatica*, long type.

Wisper (at the mouth of the Ernstbach, 50°5′ N, 7°55′ E resp. Hüttentalbach, 50°4′ N, 7°52′ E): *I. grammatica*, short type.

So far, the species within the *I. grammatica* complex can only be distinguished by their drumming signals (see Rupprecht 1969; Berthélemy 1979). The populations of *Isoperla grammatica s.l.* long and short type that were used for reintroduction do not belong to the species *I. grammatica* Poda (1761) sensu stricto (see Rupprecht 1984)! For further details on the drumming signals see Rupprecht (1983).

For the field experiment an unpolluted tributary belonging to the hypocrenal with a saprobic level of 1.5 (according to the 1998 evaluation of the Rhineland-Palatinate Board of Waterways in Mainz) was chosen. It has a discharge of 3–5 l/s and a conductivity of 7–800 $\mu$S/cm. 1100 eggs of four females of *I. goertzi* were released there. About 200 m downstream another 1300 eggs of *I. oxylepis* and again 1300 eggs of *I. grammatica* (long type) were stocked. This arrangement was adopted to make sure that the species best fitted for the local habitat was able to establish itself (for a saprobic ranking at the used species see Table 1).

In the Selz, a stream whose saprobic level ranged between 2.0 and 2.7 (according to an evaluation by the Rhineland-Palatine Board of Waterways in 1998), a total of 2270 eggs of *I. grammatica* (short type) were deposited at sex different places (Figure 1).

In most cases only some eggs of the last egg clusters which the females produced after 8–10 days were used for the control experiments, while the first and big clusters

Table 1. Saprobic ranking of the used species, according to various literature.

| Saprobic index SI & Valency V of | DEV, (first outline of 1989) | | Mauch et al. (1985) | | Moog (1995) | |
|---|---|---|---|---|---|---|
| | SI | V | SI | V | SI | V |
| - *Isoperla grammatica* | 1.7 | 4 | 2.0 | – | 1.6 | 1 |
| - *I. oxylepis* | 1.3 | 8 | 1.0 | – | 1.0 | 2 |
| - *I. goertzi* | 1.1 | 16 | 1.0 | 5 | 0.6 | 2 |

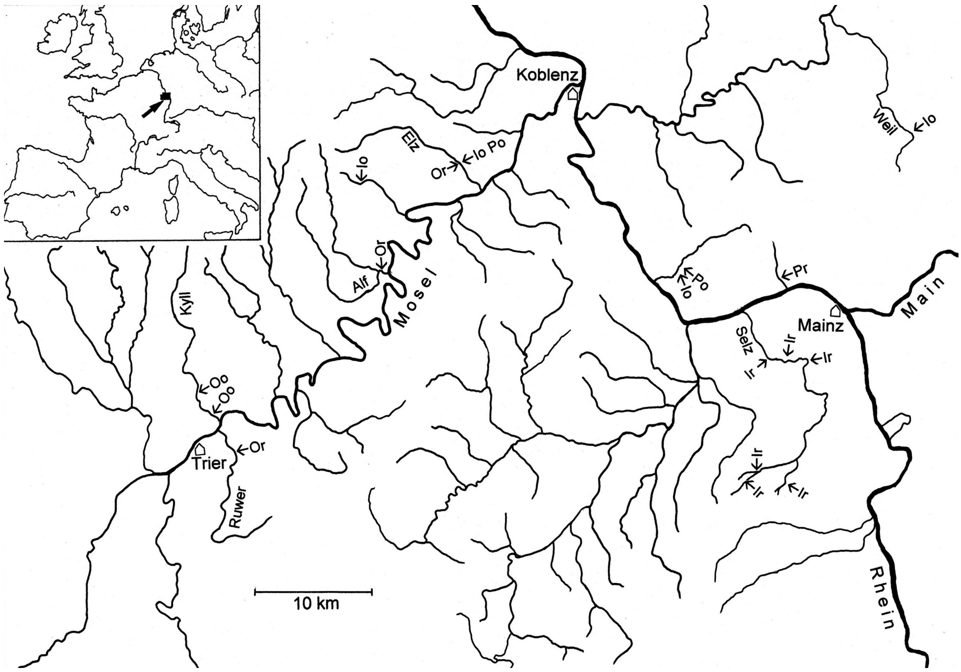


Figure 1. Map showing the localities where animals were put in (index r) or taken out (index o for a donor brook). I = *Isoperla*, P = *Perla* und O = *Oligoneuriella*.

were put into the stream. All in all 27 parts of egg clusters with four up to 42 eggs (an average of 21.4 eggs per rearing sample) were used for control experiments in the laboratory. Of each of the three species, eggs developed up to the eye-dot phase, the earliest after 40 days, i.e. the dark, red-brown eyes shimmered through the chorion.

Only a few free first larvae of *I. grammatica* (short type) could be found (controls were affected at larger intervals of 2–5 days), but increasing numbers of empty chorions were found. That means that first larvae could either escape through the gauze, or they expired relatively early and were dissolved.

Donor brooks for *Perla marginata*: in 1998, 10 3-year-old larvae taken from the Kiedrich brook (55° 30′/8° 4′) were exposed, and in 1999 about 10,000 eggs taken from animals living in the Elz brook (a tributary of the Mosel river, near Pillig), the Endert brook (55°63′/7° 3′), and the Wisper (a tributary of the Rhine, near the mouth of the Ernst brook [55° 49′/7° 56′]). Species were determined on the basis of their drumming signals. Copulation took place in the laboratory. Development of the eggs of *Perla marignata* was not controlled. Extensive controls of the success of the re-settlement were done in 2000, 2002 and 2008.

The donor brook for *Oligoneuriella rhenana* was the Kyll brook near Trier-Ehrang (55° 20′/6° 41′) and Kordel (55° 24′/6° 38′). In July 1998, 500 larvae were exposed each in Ruwer downstream of Waldrach (55° 14′/6° 43′), in the Alf upstream of Alf (55°47′/7° 5′), and in the Elz near Pillig. On several evenings by the end of July 2001, about 10,000 eggs of *Oligoneuriella rhenana* taken from the Kyll were exposed in the Elz near Pillig. The females were caught in the air. When brought into contact with water – which was offered in small basins interspersed with stones – the animals discharged their eggs. Since the eggs quickly stick to the substrate, the content of the basins (water plus stones) were dripped into the Elz. As to *O. rhenana*, sample controls of the development were done on egg clusters reared in the laboratory. Extensive controls of the success of the re-settlement were done in 1999, 2003 and 2008.

## Results

### *Resettlement of Isoperla in the Selz brook*

#### *Physical and chemical characterisation of the water*

The water of the target stream is characterised by tertiary soil layers and as such by a relatively high conductivity. In the hypocrenal of the tributary the conductivity was 770 $\mu$S/cm (reference temperature: 20°C), at the permanent measuring station it was about 1400
$\mu$S/cm (20°C). The conductivity decreases considerably after rain fall (minima of 700 $\mu$S/cm, maxima up to 1600 $\mu$S/cm). The temperature oscillates between 0°C in winter and 25°C in summer (the average summer temperature is 20°C). In the hypocrenal zone the temperature amplitude is lower and ranges between 2 and 15°C (only occasional measurements).

The following data refer to the permanent measurement station: the discharge level varies between 0.2 and 1.0 m, this corresponds to 0.15 $m^3$/s (normal low water) and 2.0 $m^3$/s at high water (maximum). The $O_2$ content varies between 20 and 100%, the average being at 65% saturation. Depending on the temperature, this corresponds to an average of 6.6 mg/l (minimum: 1.9 mg/l at 20°C, maximum 9–10 mg/l at 16–20°C). The redox potential was measured sporadically between May 2, 1995 and September 30, 1996: it was ($n = 3$) Ø = 472 $\pm$ 17 mV (SWE) in free water at a temperature of 12–15°C, and at the surface of the substrate it was ($n = 9$) Ø = 367 $\pm$ 67 mV ($t$ = 5–18°C) with a minimum of about 230 mV (at $t$ = 12.6°C: 240 and at $t$ = 18.6°C: 220 mV). The pH value was constantly above 7 in winter, and in summer it varied between 7 and 9 depending on the consumption of oxygen by assimilating algae, with an average value of about 7.5. On 15 out of 36 measuring days, two samples (on one day three samples) were taken at an interval of 1–2 hours. The chemical results of the twin samples were practically the same, only $NH_4^+$ and $PO_4^{3-}$ occasionally showed remarkable deviations (between 0 and 4, respectively, 0 and 7 mg/l).

Occasional chemical controls (average of 50 samples) showed the following results for ion concentration (numbers show average, standard deviation and maxima) of the anions: $Na^+$ 86.2 $\pm$ 17.5 mg/l (141), $K^+$ 21.4 $\pm$ 3.9 mg/l (30.6), $NH_4^+$ 4.3 $\pm$ 2.7 mg/l (14.2), $Ca^{2+}$ 124 $\pm$ 19.4 mg/l (157), $Mg^{2+}$ 68 $\pm$ 15.2 mg/l (98), and for the cations it was $Cl^-$ 185 $\pm$ 35.5 mg/l (291), $Fl^-$ 0.98 $\pm$ 0.92 (4.2), $NO_3^-$ 48 $\pm$ 14.6 mg/l (88), $NO_2^-$ (always below limit of evidence) of $<$ 1.0 mg/l, $PO_4^{3-}$ 3.96 $\pm$ 4.7 mg/l (17.5), and $SO_4^{2-}$ 173 $\pm$ 35.5 mg/l (242). The content of boron of 30 samples was 0.34 $\pm$ 0.12 mg/l (0.68), and the number of coliforms was ($n = 34$) at 61.2 $\pm$ 48 colonies/l (160).

The conductivity and thus the ion concentration of the donor streams the animals were taken from were mostly considerably lower than that of the target stream. However, there are only few measurements available for the different streams: for the Aubach it is 125 $\mu$S/cm (average of measurements per month during one year, according to Rupprecht 1991), for the Weilbach 190 $\mu$S/cm (Rupprecht 1991), for the Wisper 250 $\mu$S/cm (Rupprecht 1991), for the Hermannsborn 60 $\mu$S/cm

(Rupprecht 1991); for the Elz only two measurements are available (344 $\mu$S/cm for May 1997, and 460 $\mu$S/cm for August 1998). For *I. goertzi* (taken from the Hermannsborn for this experiment), another population is known in the Taunus Mountains in a little stream with a considerably higher conductivity of 410 $\mu$S/cm. The $O_2$ saturation of the donor streams is between 80 and 100% (only a few random measurements at day time).

*Present waste water charge (2006)*

Meanwhile all villages on the Selz – with the exception of some isolated farms – have been connected to a purification plant (I am indebted to Mr E. Bayer of the country government of Alzey for oral information). The saprobic level of the stream has remained practically unchanged since 1993, and the data collected from permanent registration and from individual analysis were still valid at the time of the experiment in 1997.

According to my own unpublished investigations since 1993, the saprobic level had an average of 2.6 on a scale of 1–4, varying between 2.4 in Elsheim and 2.7 near Nieder-Olm. These findings agree with those of the recent water quality map of Rhineland-Palatinate of 2004. Everything seemed to indicate chances for survival of the species to be implanted. For re-colonisation, places were chosen with an average current of 0.2–0.4 m/s and rough substrate (gravel and scree), largely corresponding to the conditions in the donor streams.

*Remarks to the biology and crossing*

Taken from a single locality, two males and four females of *Isoperla goertzi* were mated among themselves for various reasons: difficulties in collecting suitable samples, shortness of time available, difficulties in keeping the samples, and observation of behaviour (recording of drumming signals, control of mating).

The same was done with *I. oxylepis* and *I. grammatica* (long type); the latter ones coming from two different rivulets, one of which is a tributary to the other. The eggs of *I. oxylepis* originated from nine different females fertilised by only two males. Additional males were not successful in mating. On average, the females of *I. oxylepis* answered at an age of 6.5 $\pm$ 1.8 days to the drumming signals of the males. They began depositing eggs at an age of 8.8 $\pm$ 2.1 days and continued doing so in intervals during one to five days. On average, the life span of adult females ($n = 9$) was 23 $\pm$ 11.6 days. The eggs of *I. grammatica* (long type) come from eight different females fertilised by five different males after 5.5 $\pm$ 2.3 days. Deposition of eggs began Ø 3.0 $\pm$ 1.9 days later and continued at intervals for 1–6 days. The Ø life span of the females was 24.5 $\pm$ 6.9 days. A larger number of *I. grammatica* (short type) could be disposed of. This meant that not only animals of the same locality were mated (11 females), but five additional females were crossed with males of different streams (females of Sauerbach x males of Elz, Elz x Sauerbach, Wisper x Elz). Mating was effective after Ø 8.7 $\pm$ 1.3 days, a first deposition of eggs was carried out after 2.9 $\pm$ 1.2 days and continued in intervals for 2–12 days. The Ø age of females ($n = 14$) was 33.6 $\pm$ 12.7 days (with a maximum of 65 days).

A first control in loco naturae was carried out in November 1997, followed by further controls in April and May 1998. Additional and careful controls were undertaken in May 2001 and May 2005. Nowhere, not even in the best locality (saprobic level 1.8 [Rupprecht, unpublished results] respectively, 1.5 according to the evaluation of the Rhineland-Palatinate Board of Waterways in Mainz), neither larvae nor adults nor exuviae could be found ashore.

### *Resettlement of Perla marginata in the Walluf brook*

In February 1998, about 8–10 larvae of *Perla marginata* taken from the Kiedrich brook were exposed near the monastery of Tiefenthal, and in the summer of 1999 about 10,000 eggs of this species were added. Controls in 2000 and 2002 did not give any evidence of the presence of this species. However, a male larva was found on March 12, 2007. This means that the species has survived up to the third generation. Controls in the summer of 2007 and 2008 did not produce any more findings. However, numerous crayfish (Astacidae) were encountered. They hide preferably under stones of 10–25 cm diameter. These stones are also the preferred living place of large larvae of *Perla*. The species of *Pacifastacus* cf. *leniusculus* (immature) is new in the Walluf brook. It was not encountered either in previous studies done in the 1980s and 1990s nor in controls undertaken in 2000 and 2002. The species might have emigrated from the Rhine or have been introduced by stoke. In any case it represents a dangerous predator for the larvae of *Perla*.

Only occasional water controls were done of the Walluff brook. They showed a very good oxygen condition. Furthermore, the presence of some oxygen sensitive genera like *Epeorus* and *Baetis* proves that the recovery is permanent.

### *Resettlement of Oligoneuriella rhenana in tributaries of the Mosel*

Several tributaries of the Mosel (Ruwer, Alf and Elz) have significantly improved in their water quality over the past 20 years. They show a broad spectrum of species except for *Oligoneuriella*, which however was proved by Schoenemund (1930) to have lived in the Alf in the past century. In spite of improved water quality, no individuals of *O. rhenana* could be recorded.

## Discussion

### *Physical and chemical patterns of the Selz*

Comparisons with the donor streams show that only at one place the content of ions reaches a similar concentration, whereas it is two to three times superior at the remaining places. Only *I. rivulorum* which was not used in the present experiment is also known to exist on chalky substrate (Küry 1994).

At the place where *I. goertzi* was re-colonised, the annual amplitude of temperature of 2–15°C corresponds relatively well to the general conditions under which the animals lived in the Breitenbach: 1–13°C (Schwarz 1967). According to this author, higher winter temperatures accelerate the development of the larvae. The temperatures encountered in the Selz are likewise considered sufficient when compared to the Wiesbach.

#### *The importance of oxygen content within interstitice*

Larvae of stoneflies develop only in running water (or on agitated lake shores) since they have no gills. Organs previously considered as gills have proved to be places of the exchange of ions (Wichard and Komnick 1974; Wichard and Eisenbeis 1979). No species of *Isoperla* has outer filaments that could function as gills. They are dependent on running water, and as yet they have only been found in running water. For this reason breaking a stream into sections of running and standing water, the latter with muddy substrate, always implies a considerable impediment to the distribution. Since the size of the substrate depends on the charge and the velocity of the stream, these parameters are also very important. Whenever attempts for re-colonisation were made, care was taken that the new substrate was similar to that of the donor stream.

The availability of oxygen in the water is a much more sensitive matter. One has to remember that oxygen minima are always reached during the night. Thus correct and reliable data on the content of oxygen in a stream can only be collected if it is registered permanently. This, however, is not done as a rule. However, the fauna encountered in the donor stream allows conclusion as to the amount of oxygen in the water. Especially in the interstitial substrate, it is superior to that of the receiver stream where at different places sewer gases evade when you dig in the substrate. The low oxygen content is confirmed by the low redox potential. While the donor streams have no or only a low charge of rotting organic matter, four of the five places of recolonisation are still highly polluted. Furthermore, it cannot be excluded that following old habits, local residents still use the Selz as a waste water channel to discharge their superfluous pesticides, thus obstructing the re-appearance and distribution of insects. The experiments undertaken with *Isoperla* can or must confirm the result described by Beichle (1988) about the unsuccessful re-colonisation of the bird black grouse (*Lyrurus tetrix*). According to him, success in suboptimal habitats is considerably lower than in optimal habitats, especially for the starting phase.

### *Historical and actual distribution*

Unfortunately, there is no way of finding out if or which species of *Isoperla* ever existed in the Selz. During the 1960s and 1970s, the diversity of invertebrates in the Selz rivulet was most certainly at a very low level (Dittewig 1978; Witteman 1978, unpublished studies at the University of Mainz, and own observations). Only in some insignificant but likewise highly polluted tributaries few macroinvertebrates (Tubificidae and Chironomidae) had been able to survive. The rivulet was characterised by waste water ciliates as well as straightened and canalised shores originally deemed necessary to avoid a new secular inundation (Krause 1970). This resulted in the lowering of the stream bed, structural monotony and steep shores.

The closest neighbouring rivulets (Wiesbach and Pfrimm) do not contain any stoneflies of the genus *Isoperla*. The linear distance between these rivulets and the Selz is 6 km and 9 km, respectively. In 1982 (Achenbach 1984), one larva of *Isoperla* sp. was found at least in the neighbouring Wiesbach which in many respects (size, substrate) is similar to the Selz. Further thorough investigations done by myself at Achenbach's location and additional locations further upwards of the stream did not gain new evidence of *Isoperla*. We have to assume that meanwhile this relatively nearby population has disappeared and that a natural re-colonisation cannot be expected. The nearest evidence of a small population of *Isoperla* in the whole area is 16 km away at a linear distance (data provided by the Rhineland-Palatinate Board of Waterways in Mainz, information system biology, data bank 1998). This minimal distance, however, does not mean that at this specific spot the Selz is suited for re-colonisation.

The linear distances between the donor rivulets and the Selz are as follows: Elz (Eifel): 80 km, Sauerbach (Hirschfeld) 85 km, Wisper 20 km plus a mountain chain of 400 m difference in altitude, and Aubach about 48 km plus a mountain chain of 500 m difference in altitude.

*Flight performance*

Own observations show that, compared to other aquatic insect orders such as Ephemeroptera and Odonata, the flight of central European species of *Isoperla* and *Perla* is slow and clumsy, covering approximately only 1–2 m/s. Wesenberg-Lund (1943) characterises their flight as "a helpless beating of wings, and most often the animals prefer creeping to flying". *Isoperla* cannot overcome a distance of several kilometres by their own means. On average, individual flights as observed in nature do not cover more than 30–80 m. Even with favourable winds, animals of strong constitution do not go beyond a few hundred metres. By marking *I. goertzi*, Schwarz (1970) could prove that in intervals and within several weeks, this species flies stream upwards up to 1.5 km. Roos (1957) observed similar flights stream upwards for *I. grammatica*, depending on the direction of the wind. There is so far no evidence that this species moves sidewards away from the rivulet, or that they can fly without orientating themselves on a stream. In disturbances or storms the animals drop to the ground and hide in the vegetation. Schwarz (1970) also mentions "the very low readiness to fly" when winds are strong. All this means for the Selz that no recolonisation can be expected from neighbouring areas.

After a temporary drying out of inhabited water systems, the return of stoneflies could be proved by own observations for certain species in rivulets such as the Ernstbach and the Aubach in the Taunus mountains in Germany (Rupprecht 1991) and in a similar stream in England (Wood and Petts 1994). It seems that this is not possible in rivulets that for many years or even decades were heavily effected by waste water and as such especially by demand of oxygen within the interstitial substrate. A new and good proof for the low distribution velocity of stoneflies was given by Hughes, Mather, Sheldon and Allendorf (1999). They showed that stonefly populations from mountain streams have low dispersal abilities because the genetic distance between two populations within one stream is distinctly lower than that between two neighbouring streams of 2–5 km distance. The genetic distance between two populations from streams separated by 25 km is much greater. This leads to the conclusion that recolonisation is advisable only when the conditions of impoverished streams have improved. Küry (1994) reports of attempts to re-colonise the stonefly *Dinocras cephalotes* in the Rhine near Basel. However, since larvae need 3–4 years to develop, nothing is known as yet about the outcome of the experiment.

Adults of *Oligoneuriella rhenana* are strong flyers, but as my own observation confirms their flight is directed upstream. Even if some females happen to fly downstream, they will halt when coming to the broad main stream, the Mosel. Furthermore, the fact that even the closest nearby brooks have not been resettled shows that a lateral distribution is practically impossible due to the deep valleys and the wooded heights. Originally, *O. rhenana* was distributed in the Rhine from Basel to Cologne as well as in the Mosel (Eaton 1883; Mauch 1963 according to Haybach 2006). In the case of rivers uninterrupted by barriers such as the Neckar (Jansen, Kappus, Böhmer and Albrecht 2000) and Elbe (Schöll 1998), a natural expansion is quite possible. In the case of the Mosel, the main river which originally was connected to a whole system of tributaries nowadays makes a further expansion impossible, since numerous barriers block the flowing water. Temporary drying up (Ledger and Hildrew 2001) or pollution (Fenoglio, Agosta, Bo and Cucco 2002) do not prevent a resettlement of macro-invertebrates from the ground water or from downstream sections.

### *Importance of the population size*

When assessing the chances of success of a recolonisation, physical and chemical cues as well as auto-ecological demands as to food, habitat, individual development and finally the size of the population have to be taken into consideration. Chironomids are the preferred food of predating insects in running water; they are available in sufficient number. The substrate of the Selz resembles that of the Wiesbach, in which Achenbach (1984) proved the last representative of this genus in 1982.

Few cues as to the size of the population can be found in the literature. Natscher (1979) referred to a survival rate of only 2% for the phytophagous mayfly *Epeorus assilimis*. Enders and Wagner (1996) reported for the grazer *Apatania fimbriata* (Trichoptera) that 8% of this species perish during the embryological development, 11.3% during the larval development, and 80% as adults before depositing eggs. These observations on single species are supplemented by studies of Lavandier (1982) on the carnivore species *Isoperla viridinervis* (Pictet) 1865 encountered in the Pyrenees: at high altitudes, the survival rate is only 1%, compared to 6% at lower altitudes.

On the basis of a survival chance of 5% until hatching and of 50% for adult females all populations have the potential of doubling their number; that means they are able to survive. Assuming a survival chance of only 1% as found out by Lavandier (1982) under strong alpine conditions, but which are also likely to be realistic for highly polluted rivulets, one must conclude the survival of such a small number of eggs that all species disappear in the first year, except *I. goertzi* which can survive in the second year. Since indeed there is no evidence of survival, different factors are likely to contribute to this result and should be re-examined.

The water quality does not satisfy the demands of the specific species. In this context, the relatively high conductivity of the downstream Selz of Ø = 1500 $\mu$s/cm is noteworthy, while the hypocrenal upstream with 770 $\mu$S/cm corresponds exactly to

Table 2. Theoretical development of the population at 5% resp. 1% chance of survival.

| Species | Ne per ♀ | Ne Σ/a | survival chances at 5% | survival chances 1% | remaining females at 5% | remaining females 1% | Ne (5%) Ne (1%) in the following year | changes in year 2 5% | changes in year 2 1% |
|---|---|---|---|---|---|---|---|---|---|
| *I. goertzi* | 275 | 1100 | 55 | 11 | 13 | 2.7 | =3575 742 | 3x | 0.5x, † |
| *I. oxylepis* | 143 | 1295 | 64 | 13 | 16 | 3 | =2288 429 | 2x | 0.3x, † |
| *I. gram.*(l-t) | 166 | 1330 | 66 | 13 | 16 | 3 | =2656 498 | 2x | 0.3x, † |
| *I. gram.* (s-t) | 162 | 567 | 28 | 5,6 | 7 | 1 | =1134 162 | 2x | † |

† means that the population disappears; Ne = number of eggs/female, Σ/a number of eggs introduced in 1997.

the values of the Wiesbach where Achenbach (1984) found the last larva of *Isoperla*. This rivulet also had a conductivity of 700 $\mu$S/cm (one single measurement), an oxygen content of 90–115% (three measurements during daytime) and a pH value of 8.0.

The assumed basis for the saprobic index as well as for the chances for survival of the species were too optimistic, at least when applied to the local conditions. According to Hovestadt, Roeser and Mühlenberg (1991), small populations are especially threatened by disappearance. Table 2 also shows that a recolonisation based on the assumption of an accidental displacement by the wind of a single female is not possible (without regard to the low genetic variability).

Knowing the chances of survival of a species and the number of eggs produced by one female, one could make calculations as to the number of females required in an accidental displacement to ensure the re-establishment of a new independent population in a neighbouring rivulet. For such an assumed accidental displacement over a number of kilometres, only such females are suitable who are relatively light, i.e. whose abdomen is at most only half-filled with eggs. If, furthermore, we assume for the new rivulet a halfway realistic survival chance of 3% (instead of 1 or 5%), two females of *I. goertzi* have to be carried away to the same spot at the same time in order to found a population with a chance of survival. Under similar conditions, eight females of *I. oxylepis* or seven females each of *I. grammatica* (short and long type) again have to be blown away to the same place at the same time. Since females of *Isoperla* do not form swarms but fly around alone, a natural re-colonisation must be denied under the given conditions and distances. With this background, the apparently ''artificial'' re-colonisation of stoneflies seems to be the only method to re-establish these species in an interval comparable to that of its extermination, i.e. within several decades.

Our present knowledge leads us to make four factors responsible for the present failure in re-colonising stoneflies, and these factors should be given close attention in future experiments: the presence of carnivorous species, the content of oxygen, the concentration of ions, and – if measurable – the occasional charge of pesticides.

Independently of my studies, important experiments of re-colonisation of *Dinocras megacephala* were executed in the Rhine downstream of Basel (Handschin 1995 and oral information by Handschin 2008). In spite of considerable efforts, they were unsuccessful.


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