North-American Signal Crayfish *(Pacifastacus leniusculus)*

1. Introduction

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In Central Europe, there are 3 native crayfish species: the noble crayfish (*Astacus astacus*), the stone crayfish (*Austropotamobius torrentium*) and the white-clawed crayfish (*Austropotamobius pallipes*), the former being the most prominent. Unfortunately, the local crayfish today are only rarely found in the wild. In contrast, there are numerous non-native species, many of which are more or less invasive. The number of these foreign crayfish species is impossible to determine since it is constantly increasing - mostly due to deliberate release as potential food sources in the past, but more and more often as surplus exotics from the aquarium today. Among the invasive species the North American signal crayfish (*Pacifastacus leniusculus*) stands out in particular.

In many countries of Europe, the invasive signal crayfish has become a severe problem for endemic crayfish species (Schulz et al. 2008, Momot 1988, Troschel & Dehus 1993, Dehus et al. 1999) as a vector of the crayfish plague (*Aphanomyces astaci*), a water mold that eradicates whole populations of European species when introduced into a water body – with no effect on the invaders (Unestam

1969, 1972). The European noble crayfish (*Astacus astacus*) once had big populations in Germany (in the 19th century, about 600 tons of crayfish were exported annually) which collapsed in the early 20th century due to crayfish plague infections. Other species including the signal crayfish have been introduced as a substitute. The crayfish plague is 100% lethal in native crayfish (Unestam 1969, 1972), which in many regions resulted in the total destruction of the affected populations in a short time. 20% of signal crayfish analyzed by Filipova et al. (2013) in France were infected with crayfish plague - and more than half of all the populations examined. Local prevalence showed fluctuations between 0 and 80 % with broad confidence intervals which suggests that the number of infected populations is even higher.

In principle, however, there is a potential for conflict with native species even without a crayfish plague infection. Invasive species that tend to proliferate rapidly, colonize the same habitats and use the same sources of food as native species can endanger them solely by means of ecological competition. In addition, not only other crayfish, but the general ecology of a water body, as well as the fish occurring therein, may be adversely affected by a dense population of these large omnivores. The last remaining populations of the native noble crayfish today are found in isolated waters, such as small lakes, storage ponds, dams, water-filled gravel pits and headwaters of streams (Blanke & Schulz 2002, Schulz 2000) while the lower reaches of larger waterways in particular are already populated with other non-native crayfish (Momot 1988, Troschel & Dehus 1993, Dehus et al 1999). The signal crayfish is considered particularly problematic among invaders. In comparison to other invasive crayfish, the species grows particularly large, is more aggressive and more fertile than native crayfish and grows faster, so that even a plague-free population is able to subdue a native one (Söderbäck 1991, Westman et al 2002). There are other crayfish species that have the potential for similar negative effects but they are far less widespread (Holdich et al. 2014).

In view of the catastrophic effects of invasive signal crayfish on native crayfish, further effects on the entire biocenosis often fade into the background. As signal crayfish continue to spread widely in Germany, as elsewhere in the world, there is an acute need for research. If the large omnivore affects not only other crayfish, but entire ecosystems including the fish population, not only ecological but also economic damages are to be expected. For example, it is estimated that measures that will help control the signal crayfish and repair the damage cost the UK over £ 2 million a year, which will increase as stocks increase (Holdich et al. 2014).

The aim of this thesis was to investigate the influence of invasive signal crayfish on domestic fish species - economically important species like trout (*Salmo trutta*) and salmon (*Salmo salar*) in particular - and the ecosystem in general. Since crayfish mainly interact with benthic fish species such as bullheads (*Cottus gobio*) and loaches (*Barbatula barbatula*), their influence on pelagic fish so far has been little investigated. To do this, both long-term effects of invasive signal crayfish populations in the field as well as direct behavioral effects on fish in the laboratory were monitored. The findings gained could lead to a better protection of the remaining water bodies in which the signal crayfish has not yet immigrated.

1.2 General Signal Crayfish Ecology (habitat, feeding behavior)

Wutz & Geist (2013) found the minimum population density of signal crayfish at 0.4/m² and a sex ratio of 1.18 males to 1 female. The habitat preference of the crayfish varies with size. Smaller individuals prefer flat areas with a lot of gravel, large animals deeper pools with a lot of mud. Migratory behavior is strongly dependent on sex and size with large males showing the strongest migrating tendency with up to 300 meters in 7 days. It is very important to keep in mind that local characteristics of populations - especially density, size and sex ratio - can have a strong influence on how fast they will spread.

Studies of the intestinal content of signal crayfish in England showed mainly vascular detritus, algae of the genus cladophora, crayfish fragments, chironomidae and ephemeroptera as main dietary components throughout the year. Direct predation of fish and cannibalism occurred, especially in adult animals and increased in summer and autumn. Furthermore, it was found that *Pacifastacus leniusculus* mainly feeds at night. The estimated day-to-day ratios varied between 0.22 and 6.02 % of crayfish body weight with greater rations in adults than juveniles, and in summer and autumn rather than other seasons (Guan & Wiles 1998). And even so they are generally omnivorous but preferentially predatory animals, if macrozoobenthos is not available in sufficient quantities, they can consume great amounts of less attractive food like macrophytes. These are an important piece of littoral habitats, functioning as spawning grounds and hiding places for juvenile fish and their prey (Roth et al. 2006). Carreira et al. (2014) found that an invasive crayfish (*Procambarus clarkii*) removed its preferred plant species from the community one after the other – a behavior that is likely to be expected in signal crayfish as well.

Average population densities of signal crayfish have been determined in a UK river at 10 to 15 animals with a biomass of 53 to 61 g per m². The density could however be signifivantly higher in optimal sections of the habitat - areas with the highest number of large stones and associated hiding opportunities (Guan & Wiles 1996). On the basis of these studies, it can be assumed that a signal crayfish consumes an average of 3.12 % of its body weight per day, which in a 4 m wide and 100 m long river section with an average crayfish mass of 57 g / m² would result in a loss of approximately 260 kg of biomass per year in the form of zoobenthos and macrophytes. These numbers could still greatly increase depending on the conditions found by the crayfish (hiding places, temperature). It is obvious that this can have an effect on the overall ecology of a water body.

However, the influence of signal crayfish on the ecosystem depends on various factors, which make it necessary to document it in different natural situations. For example, population density and size of the crayfish influence their effects (Fritschie & Olden 2016), but also the structure of the habitat itself, since crayfish need a heterogeneous habitat with hiding places for different ages, if they are to proliferate (Reynolds et al. 2013).

Of course, crayfish themselves are also the target of predators. Depending on body size, they are predated by eels, perches and even barbels (Basic et al. 2015). However, predators in clear water had significantly less success catching crayfish, since the latter could see them coming (Blake & Hart 1995). Since in contrast to many other non-native crayfish, signal crayfish can tolerate lower water temperatures, they pose a particular threat to the last indigenous crayfish populations, which are still mainly found in the upper reaches of the rivers, where at the same time the threat of predators is reduced because of the clear water conditions.

1.3 Crayfish effects on ecosystems

So how does biocenosis react to the presence of those preferably predatory omnivores?

Dorn and Wojdak (2004) found decreasing numbers in young-of-the-year residential fish after crayfish had been introduced to a pond in which they fed extensively on fish spawn. As a result, zoo plankton biomass increased while O_2 concentrations decreased, apparently mediated by unfavorable ratios of respiration and primary production. Filamentous green algae disappeared quickly while blue algae of the genus *Gleotrichia* (a less coveted food source) finally dominated the community. *Chara vulgaris* and vascular macrophytes, which covered 34 % of the area in control ponds, could not develop. Periphyton-consuming polliwogs and gastropods were significantly reduced or could not be found at all (Dorn & Wojdak 2004).

A decrease of 70 % of benthic invertebrates and 90 % of periphyton biomass could already be observed at a crayfish density of only 1.8 per m^2 (Phillips et al. 2009).

A study conducted in Swedish ponds revealed a decrease of biomass, vegetated ground area and diversity of macrophytes with increasing density of invasive signal crayfish. The composition of plant species was influenced as well. Apart from that, decreasing abundance in herbi- and detritivorous invertebrates could be examined, while predatory invertebrates only decreased in ponds with low pH. The invertebrate community was increasingly dominated by sediment-dwelling species. In addition to that, the organic portion of the sediment decreased (Nyström et al. 1996).

Similar results were obtained using in-situ cages in a pond populated by signal crayfish. Natural signal crayfish densities had a significantly negative impact on predatory invertebrates and a very strong one on aquatic snails. The snails' decrease led to an increase of periphyton biomass due to reduced grazing. Herbivorous tadpoles slightly increased, but the percentage of surviving frogs was smaller in crayfish cages than in controls – probably due to predation of injured tadpoles, which often suffered tail injuries in crayfish cages. Macrophyte cover decreased by consumption as well as mere dissection (Nyström et al. 2001).

In Swedish experiments over a period of one month, gut contents of surviving crayfish were examined after the time of exposure. Two different crayfish densities were kept in cages with twenty young trouts (*Salmo trutta*). Detritus and animal constituents proved to be the main food sources of crayfish in these experiments. Algae and macrophytes only played a subordinate role. Crayfish did not have any influence on the survival rate of trout, which was positively related to streaming velocity instead. However, negative effects on biomass and diversity of invertebrates (especially predatory species) were found again. Epilithic algae increased with crayfish density – probably due to improved conditions of lighting and nutrition since active crayfish resuspend and/or remove detritus and aging algae cells during periods of low flow velocities. The researchers predict a decrease of macroinvertebrate diversity in invaded communities as well as elimination of susceptible predatory invertebrates. In streams that carry huge amounts of sediment or organic material, high crayfish densities will increase benthic algae production by bioturbation (Stenroth & Nyström 2003).

Crawford et al. (2006) examined the effect of a newly introduced signal crayfish population on the invertebrate community in the River Clyde (Scotland). River sections populated by crayfish were compared to similar sections without colonization. Reduction of invertebrates could be verified in this study. Their population density in crayfish sections proved to be only 60 % of the density in non populated sections. Biodiversity also decreased in areas with crayfish population (Crawford et al. 2006).

The littoral food web of a marsh was taken into focus in a cage experiment in Japan. In addition, effects of differently sized crayfish were examined. Big crayfish (> 30 mm carapax length) quickly eliminated aquatic macrophytes by mechanic destruction while similar effects of smaller crayfish were only noticeable after a longer period of time. Biomass of benthic algae was reduced in the presence of big crayfish but only marginally influenced by small ones which leads to the conclusion that big crayfish act as bioturbators. In this study, diversity of invertebrates was almost halved in the presence of big crayfish, which is probably due to the reduction of rare taxa. According to the Japanese study (Usio et al. 2009), possible influences of crayfish on invertebrates are:

- predation of big susceptible taxa like caddis flies and predatory invertebrates
- mechanic destruction of macrophytes and associated reduction of invertebrate's microhabitats
- increased emigration of invertebrates due to bioturbation and/or
- relief in predation and competition for small invertebrates as a result of the reduction of their

enemies/competitors.

The functional roles of signal crayfish in an ecosystem stayed the same during their ontogenetic development but the magnitude and rate of their influences intensified with growing size (Usio et al. 2009).

Mathers et al. (2016) found the strongest influence of signal crayfish on the benthic community in autumn, but still noticeable in spring. They registered mainly the decline of omnipresent species of leeches, snails, may- and caddis flies – always with differences between regions and seasons. Changes in the community structure were permanent without any signs of a recovery.

However, not only a decline in macrobenthic species with increasing crayfish density was observed, it also worked the other way around. Moorhouse et al. (2014) concluded that the removal of signal crayfish by trapping in rivers of the United Kingdom led to an increase in the number and diversity of the macroinvertebrate society. They removed 6181 crayfish in 4 trapping sessions, leading to a reduction in density towards the center of the river section. In contrast, the crayfish density in control sections (crayfish caught and put back) increased towards the center. Macroinvertebrate numbers and species richness were found to be inversely correlated with crayfish densities. Kats et al. (2013) registered increasing proliferation of newts with decreasing numbers of invasive crayfish (in this case *Procambarus clarkii*).

In addition to direct predation and competition, signal crayfish can provoke physical changes in an ecosystem solely by their behavior. In a river densely populated by signal crayfish, Rice et al. (2013) observed daily strong fluctuations of water turbidity. Measurements of the water level showed that this was not responsible for the nightly turbidity – but that it was due to nocturnal crayfish. Signal crayfish accelerate the mobilization of fine sediment and promote its fluctuation. They can therefore have a significant impact on the sediment dynamics of a waterbody. Dorn and Wojdak (2004) as well suspected effects on phytoplankton due to bioturbation.

Ruokonen et al (2014) found that macroinvertebrate species diversity and composition were reduced / altered in shore areas of lakes with stony underground, while this effect could not be observed in areas with vegetation or in deeper water. They suggested that therefore crayfish influences should be checked over various habitats. *Procambarus clarkii* too had different effects in two neighboring streams, which differed in structure (Klose & Cooper 2012). It is even possible that personality of the invaders is a factor for the course of biological invasions (Juette et al. 2014).

Non-native crayfish species can change the structure of benthic communities, but with different functional effects, often triggered over trophic cascades. For example, the presence of *Procambarus clarkii* produced an increase in benthic algae because the crayfish reduced snails while their reduction of invertebrate schredders had less effects since the crayfish themselves (*Procambarus clarkii* and *Pacifastacus leniusculus*) took over their activity of breaking up dead leaves. Benthic invertebrate predator abundance was significantly reduced by sympatric red swamp and Turkish crayfish (*Astacus leptodactylus*) but not independently when in allopatry, indicating an amplified effect overall when in sympatry. The researchers noticed the lack of a general pattern in the effects (Jackson et al. 2014). Also, Magoulick & Piercey (2016) stated that ecological redundancy of a species (two sympatric species feeding on the same sources) can lead to post-invasive more limited effects of the species on the ecosystem. Ecological processes are complex and members of one community can benefit from foreign species in ways that are not passable in other systems. After removing invasive *Orconectes rusticus* from a lake (-99% in 8 years with no significant increase over the following 4 years), Hansen et al. (2013) found increasing populations of native *Orconectes virilis* as well as Sunfish (*Lepmis spp.*) and plant growth. However, their effects on macroinvertebrates varied as fish would predate them more strongly with decreasing crayfish density. A decrease of Ephemeroptera, Odonata and Amphipoda in consequence of crayfish removal suggested that crayfish have an indirect positive effect on these groups. The researchers point out that indirect effects are very important when evaluating invasions, and that effects can be reversed relatively quickly.

All of these different results in different systems point toward the complexity of crayfish effects on ecosystems and that it can't be generalized from one system to another. Therefore we found it necessary to examine the situation in different types of headwaters in Germany, where the conflict between signal crayfish and native crayfish as well as trout and salmon is the biggest.

1.4 Differences between effects of native and invasive crayfish

Foreign crayfish species introduced to ecosystems that are not laid out for their presence can become invasive. They show different reproduction rates, behavior and feeding habits than residential species and might have a considerably stronger impact on the system (Söderbäck 1991, Westman et al. 2002, Lewis 2002, Pasini 2008, Dunn et al. 2009). However, it is not easily predictable, if and how strongly non-native crayfish may impact a complex system, since that impact is influenced by many factors.

For example, signal crayfish from invasive populations are more aggressive towards congeners in allopatric areas, more voracious and active in the search for food, and more courageous towards enemies, compared to populations in the home area and invaded areas where they are sympatric with native crayfish. Sympatric populations did not differ in behavior. Analyses of water bodies revealed that invaded allopatric waters had significantly less biomass of prey animals than invaded sympatric waters. Allopatric invasive situations could force signal crayfish into a more aggressive and bolder behavior to build up a population (Pintor et al. 2008). This could mean that their more aggressive approach is part of an invasive biological program, as it would show in Germany and

Europe, for example, where they were artificially introduced into the streams be it by accident or deliberately. Sargent & Lodge (2014) also found indications that in case of small starting populations of invasive species, selection will favor increased reproduction rates. In this study, *Orconectes rusticus* from invasive populations showed significantly higher growth and survival rates than animals from the domestic area - especially in mesocosms where fish were present. This evolutive factor of invasive species in contrast to native populations of the same species has to be kept in mind, whenever such a species' population development is examined.

In Sweden, Nyström and Strand (1996) compared grazing behavior of native noble crayfish and invasive signal crayfish on seedlings and adult macrophytes. Seedlings and adult plants of tule (*Scirpus lacustris*) and broad-leaved pondweed (*Potamogeton natans*), as well as *Chara vulgaris* were offered to both species. *Chara vulgaris* was preferred over other plant species. Signal crayfish consumed significantly more *Chara* than noble crayfish – especially at higher temperatures. Results indicate that signal crayfish are the more voracious grazers with a larger negative impact. *Chara* seems to be particularly vulnerable since it is preferred by crayfish and the genus contains a large number of rare species. The occurrence of signal crayfish thus harbors a higher risk of reduction or even extinction of submersed plants than that of the native and less voracious noble crayfish (Nyström & Strand 1996).

Three years later Nyström et al. compared effects of both crayfish species on a complete benthic food web. They imitated a pond shore habitat in large plastic basins filled with natural densities of macrophytes, invertebrates and either signal or noble crayfish or as crayfish-free controls. Results were evaluated after two summer months. With regard to the overall impact on the ecosystem, similar findings as in previous research could be found. Crayfish

- took in most of their carbon from invertebrates and less from primary producers
- had no effect on biomass of predatory invertebrates which mainly consisted of active swimmers
- had a strong impact on grazers
- had an indirect positive impact on periphyton on the substrate, probably due to the reduction of grazing snails
- grazed selectively on macrophytes and reduced the biomass of Chara while Elodea was less affected.

Again, the overall impact of the exotic signal crayfish proved to be greater than that of the native noble crayfish (Nyström et al. 1999). Since consumption rates are higher in signal crayfish, it is to be expected that this species will have a stronger impact on an ecosystem.

Dunoyer et al. (2014) as well found massive effects of invasive crayfish, when they replaced native crayfish. Crayfish had a strong impact on macrobenthos and leaf litter decay. The researchers came to the conclusion that in contrast to another far spread invasive species *Orconectes limosus* (only minor changes in the system), the larger and more aggressive *Pacifastacus leniusculus* will probably have a strong impact on invaded systems when replacing the noble crayfish. Overall, no functional redundancy between the species was observed in this study and the effects of an invasion were species-specific.

Twardochleb et al. (2013) also found strong but variable negative effects of non-native crayfish. In an experimental setup, the crayfish generally influenced all components of the fresh water food chain, reducing aquatic plants as basal food sources, invertebrates (snails, caddis flies) as well as the number and growth of amphibians and fish. Though only sometimes they would lead to an increase in algae biomass. Non-native crayfish in this study tended to have a greater negative impact on fish and amphibians and a greater positive effect on algae growth than native crayfish, but the magnitude of those effects varied widely. The species of the non-native crayfish seemed to be less important than the characteristics of the invaded ecosystem.

James et al. (2015) additionally showed that invasive crayfish's effects on decomposition and primary production were significantly stronger than that of the native species. However, they did not have the same effect on invertebrate density, mass, and diversity. McCarthy et al. (2006) found that total zoobenthos densities (primarily gastropoda and diptera) were significantly lower in treatments containing crayfish relative to controls; a result that was significant for non-native crayfish but not for crayfish in their native range. They suspected that this might be due to a small sample size. Most crayfish prefer similar plants and invertebrates, but it is possible that the introduced species have a stronger impact on their biomass due to higher consumption and reproduction rates. Furthermore, introduced species seem to have different adaptive possibilities than native species. In some cases, they could even populate habitats that are unsuitable for native species (Nyström 1999).

Ercoli et al. (2014) came to the conclusion that - on the species level - the nutritional-ecological niche of the signal crayfish is significantly larger than that of the noble crayfish but populations in a lake showed no differences in niche-size. Isotopic niches of both species overlapped strongly. Estimated amounts of food needed varied widely between different populations but the two species did not differ consistently. The researchers therefore believe that the substitution of the noble crayfish by signal crayfish populations might not significantly alter the structure of the littoral food web in northern lakes. The number of invertebrates was not significantly altered, but lakes with crayfish had fewer species (especially molluscs) than lakes without them. However, there were no differences in species richness and composition between lakes with signal and noble crayfish. This indicates that the two species have the same ecological effects on the invertebrate society of northern lakes (Ercoli et al. 2015a). But while the sublitoral invertebrate community in crayfish-free and noble-crayfish-populated northern lakes was found to be the same it was discovered that the density of caddis- and mayflies, as well as the density and diversity of chironomids, and the total density and diversity of invertebrates were decreasing if the signal crayfish which reaches into greater depths was present. In the sublitoral habitat, signal crayfish had a more negative effect on invertebrates than noble crayfish. However, the results show that ecological differences between crayfish species can not be generalized across all habitats (Ercoli et al. 2015b).

Lagrue et al. (2014) found invertebrate shredders in mesocosm-experiments to be equally decimated by signal as well as noble crayfish. They reduced the rate of decomposition of dead leaves indirectly by reducing the shredders without compensating directly for this. Reduced decomposition of dead leaves as an effect of crayfish presence was also observed in the field. The overall density of invertebrates was not affected, the effects were species-specific (eg. caddis fly density reduced) and occurred equally strong with both crayfish species. Both crayfish had a strong, but species-specific influence on benthic organisms and an indirect effect on the decomposition of leaves in the waterbody. Therefore, the researchers claim that from the management perspective, it is better to have invasive crayfish in a waterbody rather than no crayfish at all, if their absence would lead to habitats that are unsuitable for native species. Magoulick (2014) also found no differences between a native and a non-native crayfish in the impact on leaf litter decay (in this case two *Orconectes* species) but pointed out that even the smallest difference in the environmental impacts over the food chain can form cascades and must be investigated more closely.

It is to be expected that successful invaders usually have broader ecological niches and are more flexible than non-invasive species. In another study, the signal crayfish showed double the niche width of the noble crayfish on the species level. However, niche widths of individual populations of the two species were comparable in size. It can be concluded that the signal crayfish has a higher adaptability with regard to habitat use and nutrition. With larger quantities and species richness of benthic invertebrates, the niche range of both crayfish species also broadened, which shows that they are both strongly dependent on animal food sources. The researchers suggest that the effects of the two species are similar within a brook, but that the signal crayfish is more successful on a regional scale, as its flexibility makes it possible to inhabit more streams (Olsson et al.. 2009).

The ecological differences between non-native and native species can also be brought along by other factors, like predators and parasites. For example, infection with microphallus resulted in reduced hiding-time and increased risk-tolerance in crayfish, but the intensity of individual species' behavioral responses differed strongly. This can lead to increased predation of infected crayfish and bring about selection advantages or disadvantages when two species occur in parallel (Reisinger et al. 2015). It was indeed observed by Mather & Stein (1993) that different types of crayfish are differently influenced by the presence of predators, benefitting the less influenced species. They claimed that even small differences in size between species can cause one of them being preferred by predators. The presence of predators can therefore accelerate the displacement of the native species if it is the disadvantaged one.

Differences of crayfish species' effects especially on fish are summarized in chapter 1.5 “effects on fish” below.

1.5 Effects on fish

In the following chapters, a distinction is made between long-term studies and short-term studies on the effect of invasive crayfish on fish. While the former study type mostly try to give a statement on the general ecology and population development of fish, the latter are dealing with behavioral changes, as they occur in fish even after just a short time in crayfish presence.

1.5.1 Long-term field studies

So far, three studies dealt with the long-term impacts of invasive crayfish on fish, only two of which with the main focus on fish – and they all came to different results.

The first long-term field study (4 years), which exclusively focused on the reactions of fish stock on signal crayfish, was conducted in Sweden by Degerman et al. (2007). Examination of streams showed no negative effects of signal crayfish on fish. Comparisons of fish densities within stream sites in years with and without crayfish presence revealed no significant impact. Population density of crayfish had no effect either (Degerman et al. 2007).

Wilson et al. (2004) examined effects of a rusty crayfish (*Orconectes rusticus*) invasion on an entire ecosystem in a lake in the USA, lasting 19 years at the time of examination, during which the crayfish had spread along the entire littoral zone. In contrast to Degerman et al., they found a decrease in fish species that shared prey with the crayfish, while piscivorous fish showed no such reactions. Those selective effects can be easily explained by changes in the ecosystem. Snails were partially reduced from > 1,000 to only < 5 animals per square meter. Average numbers of Odonata, Trichoptera and Amphipoda decreased significantly. Native crayfish disappeared almost completely, although overall crayfish occurrence increased due to the high density of rusty crayfish. Diversity of submerged macrophytes decreased up to 80 % in some places. This long-term study showed a different result using the same approach at least with regard to fish species in direct food competition with the crayfish (Wilson et al. 2004).

The third long-term study addressed the impact of signal crayfish on fish of the water column. Since the increasing spread of the signal crayfish in England causes concern for the native trout (*Salmo trutta*) and salmon (*Salmo salar*), Peay et al. examined the head water of a Yorkshire stream in which native white-clawed crayfish (*Austropotamobius pallipes*) were gradually displaced by

Pacifastacus leniusculus. Densities of fish and both crayfish species were compared over a period of two years. The study revealed a significantly negative correlation between fish and signal crayfish densities. Sample areas with white-clawed crayfish (1-2 crayfish caught per night) had numerous young trout (> 47 / 100 m²). Signal crayfish in contrast, did not only reach higher densities (4-8 crayfish caught per night), the populated areas also had less fish (0-18.8 / 100 m²) (Peay et al. 2009).

1.5.2 Shorter studies regarding direct competitive behavior

Results of shorter studies draw a clear picture of negative invasive crayfish effects on fish. Competition for shelter and food can be identified as the main reasons for fish decline.

Changes in behaviour / competition for shelter

Competition for shelter seems to play a particularly important role between fish and crayfish, apart from food competition and direct predation by crayfish.

Guan & Wiles (1997) investigated competition for shelter and predation by crayfish. They focused on interactions between signal crayfish and benthic fish in a British river and discovered a negative correlation between crayfish density and densities of the two most abundant fish species – bullhead (*Cottus gobio*) and stone loach (*Noemacheilus barbatulus*). Population density of benthic fish was lowest in the riffle closest to the original crayfish stocking site and gradually increased up- and downstream with decreasing crayfish density. The hypothesis that crayfish and benthic fish compete for shelter and the fish are predated by crayfish was tested in a flume containing artificial shelters. Fish of either one of the species were kept alone or with crayfish in 3-day cycles for a total of 12 days. Results showed crayfish to be superior to both fish species in shelter occupation. Direct predation was examined by keeping 24 fish of each species either alone or with 36 crayfish in the flume for 10 days at a time. Mortality rates of both fish species were significantly higher when crayfish were present. Crayfish guts contained remains of some lost fish and they were observed to catch fish of both species. In the river, crayfish reached high densities (more than 20 individuals / m² in riffles) and the population was still continuing to spread. A strong reduction, and even local extinctions, of benthic fish might be the outcome (Guan & Wiles 1997).

In California, signal crayfish have been associated with reduced growth rates and gut content of Paiute sculpin (*Cottus beldingi*). Light (2005) tried to determine their effects on behavior and habitat use of the sculpin. These reduced their use of shelters and pools, switched to microhabitats with higher flow velocities and spent more time on flight behavior if crayfish were present. Crayfish on the other hand, used shelters, pools and low-flow-velocity habitats more often than sculpin. Both species were mainly active at night. Detailed field studies in the lower reaches of the creek revealed that potential shelters (single exposed rocks) were closely related to total numbers of sculpin and crayfish which leads to the suspicion that the abundance of shelters can have a limiting effect under natural conditions. Therefore, crayfish might increase the predation risk on sculpin by displacing them from shelters and pools and increasing their activity rate. Behavioral changes of sculpins seemed to be at least partially responsible for their reduced growth rate in the presence of crayfish (Light 2005).

Bubb et al. (2009) were able to demonstrate that competition for shelter occurs more strongly with signal crayfish than with native crayfish. They examined behavioral interactions and competition for shelter between native sculpins and white-clawed crayfish as well as invasive signal crayfish. Although both crayfish species proved dominant to sculpin (sculpin evaded approaching crayfish, left shelters if they were entered by them and rarely swam into shelters occupied by them), signal crayfish were significantly more aggressive than white-clawed crayfish. If sculpins were kept alone, they spent most of the day in shelters (averagely 96 %) which slightly relaxed at night (averagely 60 %). While both species of crayfish reduced the shelter use of sculpins, the fish would share shelters with white-clawed crayfish more often than with signal crayfish. But higher fertility and population densities of the species in comparison to native crayfish might ultimately be even more important than behavioral differences (Bubb et al. 2009).

However, competition for shelter with signal crayfish not only has negative effects on benthic fish but also on fish of the water column. Griffiths et al. (2004) showed that signal crayfish displaced juvenile salmon from shelters. The experiments were conducted in winter since salmon become nocturnal if water temperatures drop below 10 °C, increasing competition with the generally nocturnal crayfish. The percentage of sheltering Atlantic salmon was significantly lower if crayfish were present. The percentage of sheltering signal crayfish on the other hand, was not influenced by the presence of salmon. If salmon instead of crayfish density was increased, the percentage of sheltering salmon was significantly higher in intraspecific trials than in interspecific ones. Apparently, fish were able to compromise with their own better than with crayfish. It is to be expected that salmon which do not shelter during winter days are highly vulnerable to predation. Therefore, competition for shelter with crayfish could lead to negative effects on the salmon population (Griffiths et al. 2004).

Additionally, Matsuzaki et al. (2012) found out that both the absence of hiding places and the presence of *Procambarus clarkii* increased weight loss in goldfish. The growth of the fish could be improved by presence of hiding places, unless there were crayfish present. In this case, offering a lot of shelters could not improve the negative effect of the crayfish on fish growth (interactive effect). This suggests that crayfish can alter the growth of fish by chemical influence as well as the occupancy of hiding places. In contrast, availability of shelters was the main factor for the weight loss of a protected species of loach. However, the growth rate of these fish was not affected by crayfish presence. The change in growth rate due to lack of shelter was greater in the loach than in goldfish. The researchers come to the conclusion that both decreased shelter availability as well as the presence of invasive crayfish can have significant indirect negative effects on fish, but whether these effects are interactive may vary from species to species.

Crayfish size can be decisive for the takeover of a shelter – at least among the crayfish themselves. When shelters become scarce and the crayfish compete, the heaviest crayfish has the best chance to take over a cave, whereby females are particularly successful (in their case even smaller weight differences are decisive). Also, within the population the crayfish which have originally owned a shelter have better chances of keeping and defending it (Ranta & Lindström 1993). If the same holds true for competitive situations with fish, is not necessarily sure, but at least has to be kept in mind when performing laboratory experiments, since crayfish might be even more successful defending and regaining a shelter in the field, where they have been present for months and years, than in an experimental setup where they have to start invading shelters from scratch.

Competition for food

Effects of food competition between fish and crayfish were studied in the laboratory by Carpenter (2005). Experiments were conducted with the aggressive omnivorous crayfish *Orconectes virilis*, which had invaded the previously crayfish-free Colorado River Basin, and two native fish species – the gila chub (*Gila intermedia*) and the flannelmouth sucker (*Catostomus latipinnis*). Population density of the species were varied in the experiments. Each fish species was tested in separate trials. While growth rates of the Gila chub were mostly affected by intraspecific competition, growth of the flannelmouth sucker was more strongly affected by crayfish presence. In Contrast, growth rates of crayfish were not significantly influenced by the presence of either one of the two fish species. Carpenter thus found a species-specific influence of food competition (Carpenter 2005).

In contrast to this, Nilsson et al. (2012) found that the number of benthic invertebrates decreased with increasing relative crayfish density (rusty crayfish) but feeding habits of most pelagic fish were not influenced by the crayfish. These could obviously compensate the loss of the macrozoobenthos.

1.6 Conclusions, hypotheses and study goals

Crayfish definitely have the potential to influence ecosystems – mainly by consumption of macroinvertebrates and plants. Invasive species like the signal crayfish reach higher population densities, have higher consumption rates and spread at a higher pace, which is the main reason for their – in many situations - even stronger negative effects. Even at low population densities, they can lead to decreasing numbers and diversity in benthic invertebrates and macrophytes as well as a shift in species composition. However, this is not the case in every situation and the magnitude of their impact might also vary depending on even smallest differences in the invaded systems. Results on their impact on natural fish populations were inconclusive in long-term studies, showing negative, selectively negative or no impact on fish. Short term studies however were mainly pointing towards a negative effect on fish by competition for shelter and food. But here too, exceptions could be found. Figure 1 shows an overview of possible crayfish effects and the possible influence of the invaded ecosystem on the severety of these effects.

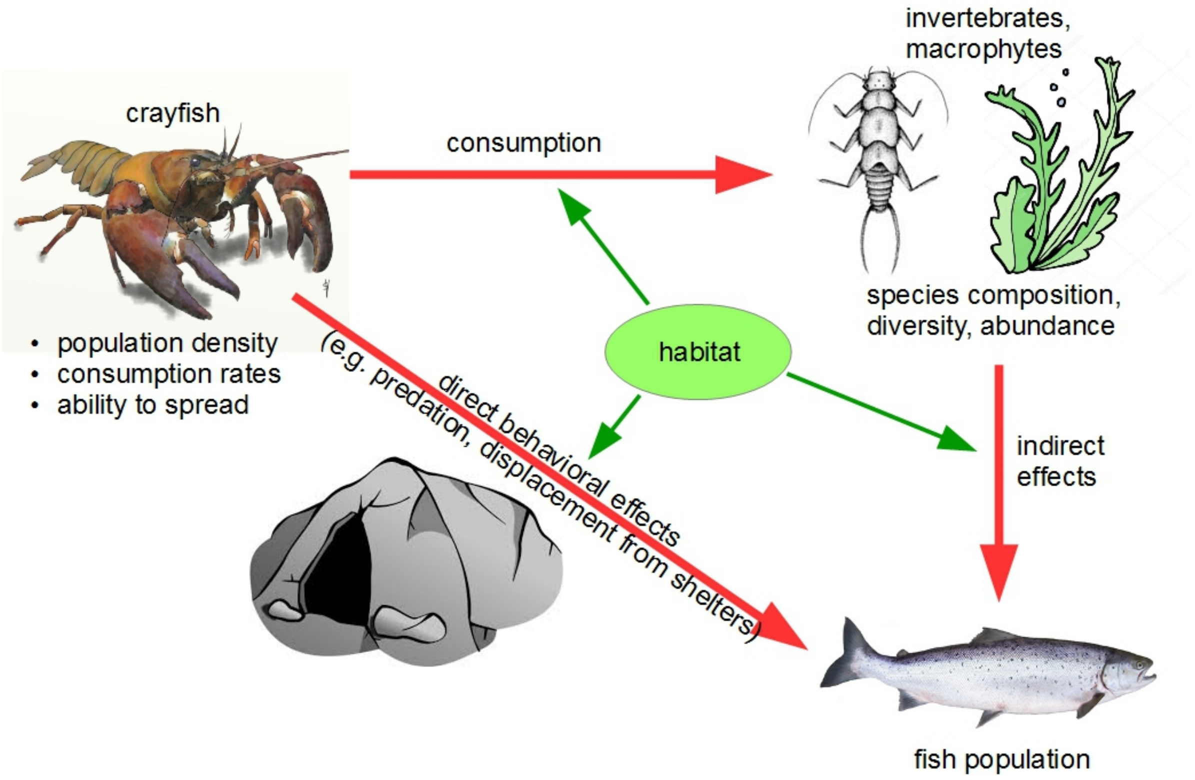


Figure 1: possible direct and indirect effects of signal crayfish on fish and the influence of the individual ecosystem

Since the overall image that was drawn by previous studies showed that crayfish effects vary over habitats, species and many other factors, it's necessary to examine crayfish effects under natural as well as laboratory conditions, over a long time period and in different but typical invaded habitats. It has become clear that it won't be possible to transfer effect patterns from one habitat to the other, so one should concentrate on typical field situations to be able to give a prognosis at least for these. Therefore, the spread of two different signal crayfish populations and the responses of macroinvertebrate and fish fauna was monitored closely in two different headwaters to follow their impact under completely unaltered, natural conditions. At the same time, behavioral responses of fish to crayfish should be examined under laboratory but also long term conditions, since it is necessary to give the animals time to get used to the presence of one another like it would be possible in the field. It has been considered crucial to compare signal and noble crayfish effects on fish behavior only after a suitable habituation time.

The overall objective of this study is to find out more about the effects of the invaders by closely watching certain areas of different streams while they are being populated. The field studies were mainly focused on the indirect effects of crayfish on fish, mediated by the crayfish's habitat use and consumption of invertebrates and macrophytes. The field setup was designed designed to examine two hypotheses:

1. Invasive Signal crayfish populations have a negative effect on the overall ecology of a water body by reducing species diversity and biomass of invertebrates and macrophytes.
2. Via these negative effects on the system as a whole, crayfish will indirectly affect the fish population in a negative way.

Direct behavioral long-term effects of signal crayfish (*Pacifastacus leniusculus*) and the native noble crayfish (*Astacus astacus*) on fish were recorded and compared under laboratory conditions. The long-term aspect should be adressed in particular during this study since this is mostly disregarded under experimental conditions. 3 more hypotheses were checked in the laboratory:

1. Signal crayfish affect fish directly negative by repression and predation.
2. This negative effect increases with crayfish population density.
3. Signal crayfish effects are generally stronger than those of the noble crayfish.

2. Material and methods

The work of this project was split into two sections. The first and more extensive section covered indirect effects of signal crayfish on fish and consisted of fieldwork and the examination of effects under natural conditions in different invaded streams. Its aim was the exploration of crayfish induced changes in the invertebrate and macrophyte communities and the impact of these changes on fish. In contrast to previous studies, distribution limits of selected crayfish populations were determined and their continuing spread was monitored closely over a period of three years. This way, the growing crayfish density in selected stretches of river and their impacts could be directly documented.

The second section of research was conducted under laboratory conditions and examined the direct impact of crayfish on fish - especially via stress, predation and competitive behavior. Since the negative impacts of signal crayfish on sculpins were already known, open water fish species (*Salmo salar, Salmo trutta*) were used in these caging experiments. In addition, the native noble crayfish was examined in the same experimental set-up, to compare its influence with that of the signal crayfish. Observation of behavioral responses was accomplished by video surveillance using underwater cameras.

2.1 Section one: indirect signal crayfish effects on fish under natural conditions

The field studies were designed to examine two hypotheses:

1. Invasive Signal crayfish populations have a negative effect on the overall ecology of a water body by reducing species diversity and biomass of invertebrates and macrophytes.
2. Via these negative effects on the system as a whole, crayfish will indirectly affect the fish population in a negative way.

Four sample sites in each of two rivers were strategically selected to monitor areas with strong signal crayfish occurrence, areas on the edge of the population and yet uninfested areas. Since the crayfish populations of the selected rivers spread as quickly as in the rest of the world, this practice allowed accurate tracking and observing of the populations' distribution limits and their associated ecological effects at the respective sample site over the period of 3 years. An approach like this has not been practiced in any other study so far. It made it possible to accurately observe certain water sections and the ecological reactions therein from the beginning of colonization to increasing population densities.

2.1.1 Identification of signal crayfish populations and their distribution limits prior to long-term monitoring of ecosystem effects

To evaluate the impacts of a spreading signal crayfish invasion, it is crucial to monitor selected spreading populations over a long period of time and document changes in the ecosystem that can not be explained by other factors. To do this, it was necessary to find these populations and determine their exact distribution boundaries to follow their movements as the invasion continued. Two signal crayfish populations in two different watercourses were selected for the study.

The first population is situated in the river Ahr in German Rhineland-Palatinate. It is a siliceous low-mountain-range river containing fine as well as coarse substrates (Type 9 based on the standardization of watercourse types according to the Water Framework Directive of the European Union). The second population colonized the river Inde close to Aachen in North Rhine-Westphalia. The Inde is characterized as a type 7: a calcareous low-mountain-range stream dominated by coarse substrates.

The current distribution limits of both populations were determined using crayfish traps of the Scandinavian model "Pirat" (Fig. 2) in a systematic monitoring during the late summer and early autumn months of 2012. Fresh rainbow trout (*Oncorhynchus mykiss*) was used as bait. The use of fykes was preferred over hand search to avoid deviations due to daily changes of the examiner's form and concentration.

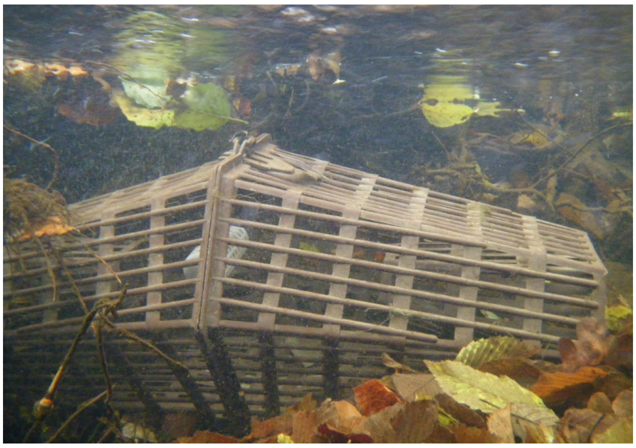


Figure 2: Crayfish trap "Pirat"
from „Erhaltung autochthoner Populationen bedrohter Krebs- und Fischarten - Abschlussbericht Zeitraum: Juni 2009 – Dezember 2010"

Since the Ahr is much bigger than the Inde, extending over 85 km from the source to the mouth, a full exploitation by mere trapping was impossible. Instead, a systematic survey of tenants was performed, asking them for any knowledge of signal crayfish inside their respective river sections. The results drew a rough picture of where the distribution limits were to be expected. Consecutively, trapping was carried out between areas with previously known crayfish distribution and areas without evidence of their presence. This resulted in a questionable section of 4 kilometers of river (Fig. 3).

Figure 3: Trapping limits Ahr (total length 4 km, 1 trap / 100 m)

Kartierungsgrenzen = mapping boundaries

Figure 4: More intensively examined stretch of the Inde in Aachen Kornelimünster

Within these boundaries, one trap was placed on every 100 m (40 traps total). In order to adapt this approach to local conditions and achieve best trapping results, the traps were deployed in groups of 5 every 500 m and distributed in a way that would allow particularly attractive areas (slow-flowing sections, potholes, large overhanging banks) to be examined with special attention. At the Ahr, the upstream boundary of the signal crayfish population was chosen to be followed further, since the downstream boundary could not be determined for sure. It is possible that the crayfish invaded the Ahr directly from its mouth to the Rhine, moving upstream. Therefore areas downstream of the current population maximum might have been already overrun by them, leaving a benthic community that might not represent original conditions.

At the smaller stream Inde near Aachen, the situation was more difficult, since the complete stream was leased by a single tenant who was not well-informed about the signal crayfish situation. As a result, the stream had to be examined on a length of about 20 km. Due to the huge structural diversity and large crayfish dissemination gaps, a far greater number of traps had to be placed. Ultimately, 178 traps were set in the Inde, examining each pothole on a stretch of 6 km where signal crayfish had been detected (Fig. 4). A stretch of 520 meters within Kornelimünster had to be investigated by hand search due to high risk of theft for the traps. During the search, recognizable crayfish caves were palpated and larger stone blocks turned over. In addition, the stream Iter which is mouthing into the Inde was examined on a total length of 2.34 km using the same trapping scheme, since this stream was the origin of the signal crayfish invasion before they migrated into the Inde. A total of 61 traps were set in the Iter. The origin of the signal crayfish spread was known to be upstream in the Belgian parts of this stream, where the animals must have been released illegally. The population spread preferably downstream from there, probably due to the better migration prerequisites, not having to migrate against the current. According to findings of Bubb et al., the spread of the population is to be expected faster downstream, since the crayfish will move greater distances in that direction (Bubb et al. 2004). Therefore, after defining the population boundaries, the downstream boundary was examined at the Inde.

In both streams traps remained in the water for a period of one night (12 hours) and were lifted the following morning after sunrise. Depending on accessibility and terrain of the trapping sites 10-20 traps were set or raised per day.

After the population boundaries had been determined and the sampling sites established in the vicinity, crayfish population development was monitored by follow-up fykings in the chosen sampling sites. 5 fykes were used on each of the 30 meter long defined areas then. The first control trapping took place in winter 2012/13, initially with the intention to repeat this every season. After the next control fykings in spring and summer 2013 however, it became apparent that the best fishing results were obtained in summer, when the animals are most active due to water temperature, having the highest nutritional requirements and thus are more attracted by the bait. The catches in winter and spring on the other hand fell far short of the summer numbers, which lead to waiving the 2013 autumn sample. Instead, in 2014, two trappings were carried out - one in early and one in late summer - which led to more meaningful results. Bubb et al. (2004) also found midsummer to be the time with maximum crayfish movement and temperature as a major influencing factor. The last control fyking took place in summer 2015. Since the study ended, only one fyking was done then.

The captured animals were measured and their gender determined. In the first study year, total as well as the carapace length of the crayfish were measured. Thereafter, only carapace length was taken into account since it is easier to measure. Crayfish often writhe against the benchmark during the measurement and thereby distort the outcome of total length. Usually, the total length is approximately equal to twice the carapace length. Injuries and other features such as egg-bearing females and missing limbs were noted as well. The carapace length (CL) of crayfish, was measured from the rostral apex to the posterior median edge of the cephalothorax to the nearest 0.1 mm using a ruler that was pressed on the upper site of the carapace. An assessment of the actual exact population density was waived because estimates of this type proved to be unreliable. This was underlined by varying catch results as well. Apart from that, the traps are only suitable only for crayfish above a certain size. Crayfish, which are smaller than 5 cm, usually will not go into traps anyway, even when the mesh size is very small (Hogger 86, Holdich & Domaniewski 95, Shimizu & Goldman, 1983). Therefore, one obtains an indication of the density of adult animals at best. Since the main aim was the observation of population development with time and not the absolute number of crayfish at a certain point, it was not necessary to catch all sizes of crayfish. The detection of adult crayfish in formerly unpopulated areas was sufficient.

2.1.2 Selection of sample sites along the boundaries of crayfish distribution and macrobenthos monitoring

Following the identification of population boundaries was the selection of the sampling sites in each stream that were to be monitored during the rest of the study. Most important selection criterion was comparability, meaning highest possible physical and ecological similarity of the sites. It is impossible of course to accomplish 100 % comparability of sites in a field study. But the sites were matched as closely as it was practicable by selecting and matching as many ecological, physical and chemical factors as possible. The grain size of the substrate played a particularly important role, since it is not only crucial for the composition of the invertebrate community, but also for the number of possible fish and crayfish shelters within the section. Apart from this, width, depth, flow velocity, degree of shading and chemical water parameters were adjusted. The use and vegetation of the banks (meadow, forest, urban development, buildings, streets etc.) was aligned as well. Table 1 shows the selection criteria that were used for aligning the sampling sites.

Table 1: characteristics that had to be aligned in chosen sample sites

Selection criteria for highest possible comparability of sample sites
• four main substrate types / grain sizes • width • depth • flow velocity • vegetation, use and possible reinforcement of banks • degree of shading • chemical water parameters

As a preferred habitat for fish and crayfish, areas with lower flow velocities (potholes) were favored in the selection, making sure that at the same time these were still flat enough to map and sample the substrate for macrobenthos. The length of each sample site amounted to 30 m. 4 sample sites were selected in each stream: one in the still uninhabited area, one at the point of crayfish distribution limit and two in the already densely populated areas. The reason for the double occupancy within the crayfish population was chosen to make sure electrofishing would not have negative impacts on the community. Only one of these two sites was examined by that method in the course of the study.

The selected sampling sites were captured on score sheets according to the principle of multi habitat sampling of the Water Framework Directive (WFD; Fig. 9). The score sheets were updated on each sampling (3 times per year). In addition to that, shore structure (vegetation, reinforcement), flow velocity / depth, occurrence and composition of submerged macrophyte species were recorded and the sites were documented photographically.

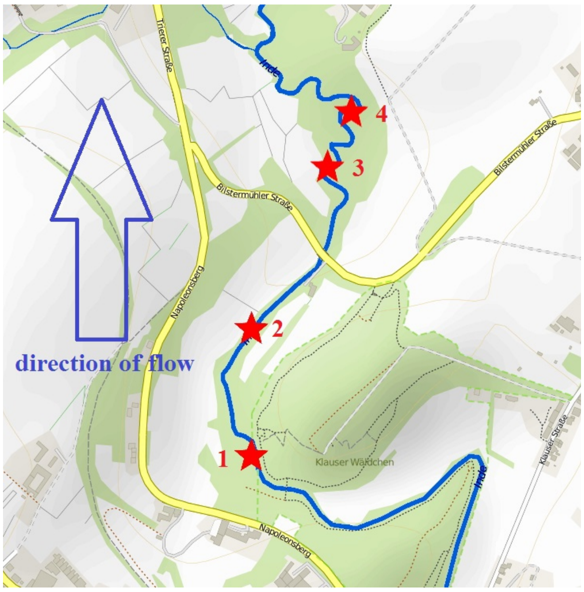


Figure 5: sample sites at the Inde: signal crayfish distribution boundary at site # 2, high densities at #1, #2

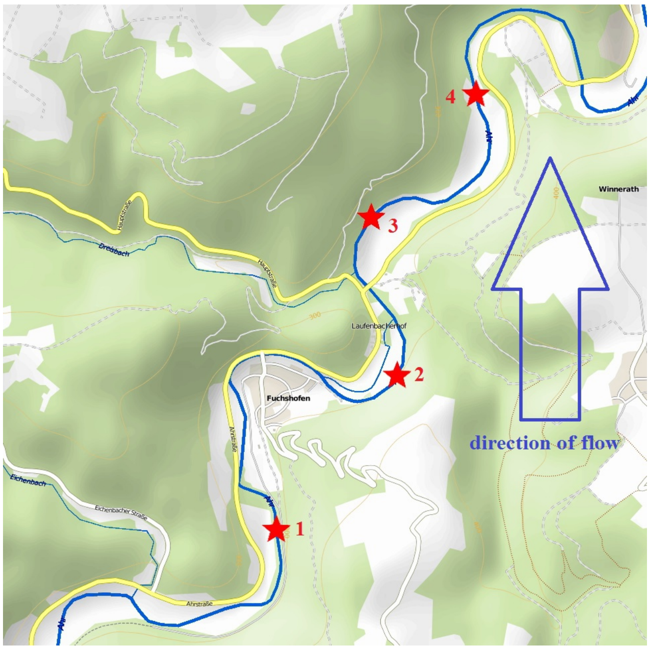


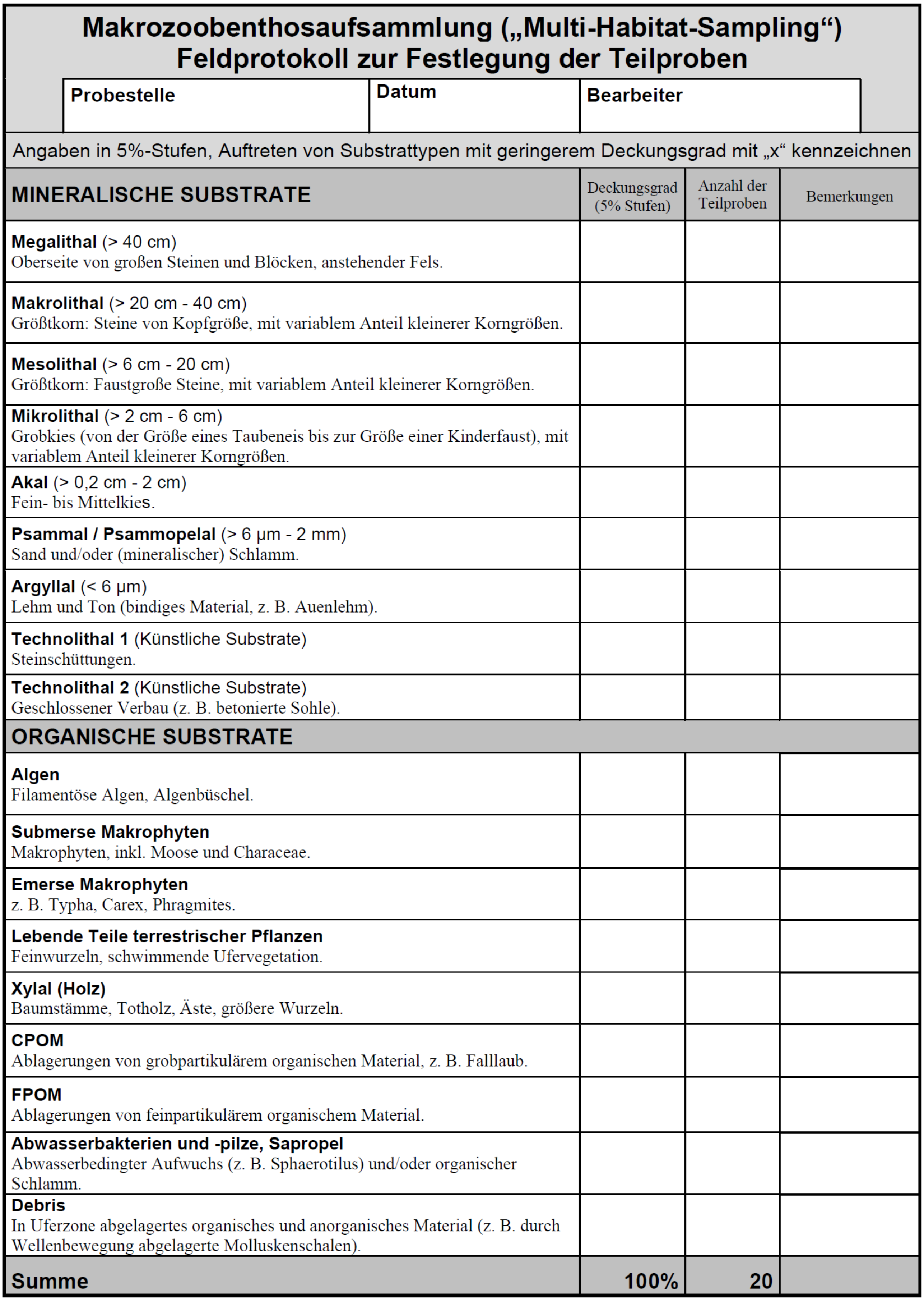
Figure 6: sample sites at the Ahr: signal crayfish distribution boundary at site #2, high densities at #3, #4

Figures 5 and 6 show the chosen sample sites in each of the two streams. At the Inde, 4 sample sites with an average depth of 45 cm, an average width of 3 m and a moderate flow rate were selected. At the sample locations 1, 2 (densely populated with signal crayfish) and 4 (no colonization) rate of shading was 100 %, while at sample point 3 (limit of the population) it reached only 75% due to a tree felled by beavers. On all sites, the banks consisted in floodplains with black alders (*Alnus glutinosa*) and shrub vegetation on one side and a row of poplars (*Populus*) followed by meadows with no livestock but regular mowing on the other. All banks were unfortified. In the area where the study was conducted, the Inde is located in a nature reserve with prohibited access and is throughout populated with beavers and muskrats, which did have some influence on the banks during the course of the study.

At the Ahr all sample sites had an average depth of about 55 cm and a width of averagely 5 m with a low flow velocity. Banks showed mixed deciduous forest on one side and meadows on the other, that were in parts used for temporary cattle grazing. They were divided from the stream by shrub vegetation with single trees on the banks. Due to the steep slopes in the valley, shading varied between the sample points. If the flat valley floor was on the south side of the sample point, more light could reach the water, than if there was the escarpment was on this side. Due to the strong meandering, this effect could not be avoided. Sampling site 1 had a shading degree of 100%, site 3 75%, while site 2 and 4 only had 50%. Figures 7 and 8 show typical sampling sites in both streams.

Figure 7: sample site at the Ahr

Figure 8: sample site at the Inde



Makrozoobenthosaufsammlung („Multi-Habitat-Sampling“)
Feldprotokoll zur Festlegung der Teilproben

Probestelle	**Datum**	**Bearbeiter**

Angaben in 5%-Stufen, Auftreten von Substrattypen mit geringerem Deckungsgrad mit „x“ kennzeichnen

MINERALISCHE SUBSTRATE	Deckungsgrad (5% Stufen)	Anzahl der Teilproben	Bemerkungen
Megalithal (> 40 cm) Oberseite von großen Steinen und Blöcken, anstehender Fels.			
Makrolithal (> 20 cm - 40 cm) Größtkorn: Steine von Kopfgröße, mit variablem Anteil kleinerer Korngrößen.			
Mesolithal (> 6 cm - 20 cm) Größtkorn: Faustgroße Steine, mit variablem Anteil kleinerer Korngrößen.			
Mikrolithal (> 2 cm - 6 cm) Grobkies (von der Größe eines Taubeneis bis zur Größe einer Kinderfaust), mit variablem Anteil kleinerer Korngrößen.			
Akal (> 0,2 cm - 2 cm) Fein- bis Mittelkies.			
Psammal / Psammopelal (> 6 µm - 2 mm) Sand und/oder (mineralischer) Schlamm.			
Argyllal (< 6 µm) Lehm und Ton (bindiges Material, z. B. Auenlehm).			
Technolithal 1 (Künstliche Substrate) Steinschüttungen.			
Technolithal 2 (Künstliche Substrate) Geschlossener Verbau (z. B. betonierte Sohle).			
ORGANISCHE SUBSTRATE			
Algen Filamentöse Algen, Algenbüschel.			
Submerse Makrophyten Makrophyten, inkl. Moose und Characeae.			
Emerse Makrophyten z. B. Typha, Carex, Phragmites.			
Lebende Teile terrestrischer Pflanzen Feinwurzeln, schwimmende Ufervegetation.			
Xylal (Holz) Baumstämme, Totholz, Äste, größere Wurzeln.			
CPOM Ablagerungen von grobpartikulärem organischen Material, z. B. Falllaub.			
FPOM Ablagerungen von feinpartikulärem organischem Material.			
Abwasserbakterien und -pilze, Sapropel Abwasserbedingter Aufwuchs (z. B. Sphaerotilus) und/oder organischer Schlamm.			
Debris In Uferzone abgelagertes organisches und anorganisches Material (z. B. durch Wellenbewegung abgelagerte Molluskenschalen).			
Summe	**100%**	**20**	

Figure 9: Multi habitat sampling score sheet according to the Water Framework Directive (Meier et al. 2006)

After signal crayfish population assessment and selection of comparable sampling sites in both streams in autumn 2012, regular multi habitat sampling (Meier et al. 2006) could start. Since crayfish may have different effects due to seasonal dietary habits (Guan & Wiles 1998), samples on each location were taken three times a year in spring, summer and autumn. According to Meier et al. the sampling should be carried out between February and August. In this case, the spring and summer samples were taken between March and August, the autumn sample outside the recommended sample period between September and November (except in 2015 when it was waived). It was of the essence to take as many seasonal samples as possible in all sites, which is why this additional sample at least could not hurt, since there were still enough samples within the regular time frame to compare it to. Percentual proportions of occurring substrate types in the location had been estimated in 5% steps prior to the sampling as part of the site selection. These proportions were checked with every sample to make sure no significant changes had occurred in between. Each sampling was carried out using a long-handled landing net with a 25 x 25 cm frame, a mesh size of 500 microns and a depth of about 70 cm (Meier et al. 2006). Sampling was always carried out against the flow starting at the downstream end of the sample site. The substrate in front of the net opening was swirled up with the current on an area of 25x25 cm (projected frame dimensions of the landing net) using a long bristled brush so that particles and organisms were washed into the net, which was placed perpendicular to the stream bed. In accordance with the WFD, one of these samplings was to be carried out for every 5% fraction of the respective substrate inside the chosen sampling area leading to 20 sub-samples per site. This method is usually used to evaluate general ecologic quality of a sampling site (Meier et al. 2006). Since this wasn't the main aim of this study, sampling was slightly adjusted to reach maximum comparability of the chosen sites with regard to signal crayfish density and especially quantitative effects on macrobenthos. Therefore, the four main substrate types in a site were chosen and the 20 sub-samples were distributed in a 8-6-4-2-pattern taking 8 samples of the most prominent most frequent substrate and 2 of the rarest. This alteration of method increased the comparability of the sites and allowed to choose sites that were indeed similar with respect to main substrate types. An exact similarity of different stretches of river is impossible to find in a natural situation, but it was possible to find stretches that were similar with respect to the four main substrate types and the other physical characteristics like depth, width, bank vegetation etc. (Tab. 1). Subsamples were dispersed over the sampling area as evenly as possible. In addition, organic substrates were not sampled, contrary to the WFD approach because their share strongly altered throughout the year (e. g. foliage in fall, dead plant material in late summer, etc.). Also, the substrate was not moved on 2 to 5 cm depth, but only the surface sampled with the brush as the kick-sampling-method described by Meier et al., which was tried out in another water body beforehand, often led to unintentional variations in depth depending on the substrate grain. For example, large stones that were stuck in finer substrates led to a deeper agitation of the substrate below, when they were moved. To avoid resulting variances, the brush sample was chosen as a less forceful method which was possible on all substrate grainsizes. Per subsample, 12 brush strokes towards the net-opening on the 25x25 cm base area were carried out. The samples were taken with the following principles considered (Meier et al. 2006):

- Subsamples of substrates with very high coverage were taken both in the shore areas as well as in the central areas of the stream bed.
- At least two to three subsamples covered the immediate shore area.
- If a frequent substrate type was present in riffles as well as in pools, the subsamples were taken in both, roughly in accordance with the frequency of the substrate type in these two areas.
- If possible, the assessment of substrate types was done from the shore using polarized glasses to achieve the best possible view of the stream bed. If this method was insufficient, the water was entered and left prior to sampling only on one strategically optimal point to avoid disturbances of the substrate.

At the Inde, mesolithal (fist-sized stones with a variable proportion of smaller sizes from 6 cm to 20 cm in diameter) was the predominant substrate, followed by makrolithal (stones up to head size between 20 and 40 cm). Third most substrate was mikrolithal (coarse gravel with a grain size between a pigeon's egg and the size of a child's fist – so between 2 and 6 cm) and fourth most large stones and blocks over 40 cm in size (megalithal).

The average substrate grain size was significantly bigger in the Ahr. At all selected sample sites the main grain size consisted in makrolithal (head size and smaller), followed by megalithal (larger stones, blocks, rock), mesolithal (stones up to fist-size) and lastly mikrolithal (2-6 cm in diameter).

Samples were distributed in the following pattern (Tab. 2):

Table 2: Substrate ratio and according number of sub-samples per macrobenthos examination

Water body	Substrate type (portion of substrate)	Number of sub-samples
Inde	mesolithal (40%)	8
	makrolithal (30%)	6
	mikrolithal (20%)	4
	megalithal (10%)	2
Ahr	makrolithal (40%)	8
	megalithal (30%)	6
	mesolithal (20%)	4
	mikrolithal (10%)	2

Before the sub-samples were taken, a rough sketch of the location and its distribution of substrates was prepared and the site was divided into four sections in each of which 5 sub samples were taken. This made sure that the samples were distributed over the site as evenly as possible.

The substrate contained in the sub samples was placed in a bucket with 1 liter of 96% vol. Ethanol. The usual WFD sorting of live organisms, in which they are fixed in 70% vol. Ethanol in the field was not carried out due to the necessity to pick up all samples in one day or at least on two following days. Reasons for this were not only organisational matters but also the fact that species composition and abundance changed within the streams in very short periods of time. The short time frame in which the samples were taken made them as comparable as possible with regard to possible temporal changes in the system. But this also resulted in many samples taken in just one or two days, not allowing for long sorting times of each one of them. The laboratory sorting also ensured that all organisms could be included, avoiding the death and decomposition of very sensitive and oxygen-loving species during time consuming live sorting under warm and low-oxygen conditions.

The sorting of organisms and transfer to 70% ethanol was then carried out in the laboratory. The sample material was transferred into white bowls and treated in portions. During the first months of the study, the samples were sorted completely, as it would have been common procedure for the WFD live sorting. Sorting in the laboratory would usually include the separation of a sub-sample (at least 1/6 of the original sample) to reduce sorting time (compare Meier 2006). This was ignored to make sure rare species that might only occur in small numbers weren't overlooked. It was only in the last year of the study that this method was discarded for a 25% sorting of each sample – mainly to reduce the immense sorting time. To accomplish this, the sample material was distributed in a large white bowl with a base of 32 x 26 cm and divided into four quadrants. The one quadrant to be counted was chosen randomly. The material was sorted by tablespoon portions in a small layer of water in shallow white bowls using a 10x magnifying glass. The organisms were sorted into their systematic orders and counted. A further determination down to species level was not carried out since crayfish effects can be expected to be most visible on quantitative levels, rather than qualitative. Certain groups and orders might be affected more than others but it is very unlikely that crayfish will show feeding preferences on certain species within an order. Animals in the pupal stage and terrestrial imagos were not counted.

In addition to the benthos samples, important water parameters were tested on each sampling day to identify chemical changes that might lead to reactions in the benthic community and distinguish them from crayfish effects. Tested parameters were O_2-, NO_3-, NO_2-, NH_4-, PO_4-, Cu-, Fe-levels as well as pH, temperature, conductivity, carbonate and general hardness. Most parameters were checked using chemical drip tests made by Filterking. pH was measured with indicator paper and conductivity with a digital conductivity meter with a measuring range of 0 to 2999 µS, an sensitivity of 1 µS and an aberration of approximately 2%.

The results were subjected to a correlation test according to Pearson. To further elucidate possible relationships between invertebrate and crayfish densities, the data were also analyzed calculating a detrended correspondence analysis. Then a PCA was calculated with default settings and actual crayfish density as an overlay variable.

2.1.3 Monitoring the fish population

The quantitative and qualitative evaluation of the fish population was accomplished by electric fishing once a year in mid to late summer according to the guidelines detailed in Peter and Erb (1996). Electric fishing was carried out in only three of the four sites. One of the two densely populated sites was not examined to ensure that the method itself would not affect crayfish and macrobenthos in a negative way. Possible negative effects would have shown in the comparison of the communities of both sites over the course of the study. In this method, an electric current is passed through the water using an anode and a cathode. Fish that swim into the circuit, are drawn to the anode where they can be collected. This response to electrical stimulation results from the stimulation of the nervous system, which triggers a series of involuntary muscle twitches, which simultaneously trigger the positioning of the body and the involuntary swimming in a particular direction (depending on the strength of the stimulation; Hartley 1980). This allows for a quick and gentle capture and examination of the fish and assessment of the population. Since the sites had to be selected shallow enough to enable macrobenthos sampling, all sites were also suitable for electric fishing, where one is dependent on a certain visibility. Beforehand, the fixed 30-meter sample sites in the Ahr were extended by 35 meters each upstream and downstream to achieve a total length of 100 meters. At the Inde, stretches of 70 meters in total were sufficient due to the smaller scale and more homogeneous structure of the stream. Here, an up- and downstream extension of 20 meters each was carried out. Since fish are a lot more mobile than benthic invertebrates, the elongation of the sites ensured a more realistic image of the population. In each of both water bodies, two electric fishing devices type EFGI 650 by "Jürgen Bretschneider special electronics" were used employing direkt (cDC) or pulse current (pDC) with 115 volts and an amperage of 25-30% (~ 1.4 A, adapted to the respective conditions). Compared to cDC, pDC differs as follows:

1. Galvanotaxis is weaker than with cDC.
2. Tetany (muscle spasms) occurs even at very low voltage gradients (0.16 V cm^{-1}). Therefore, the fish are already stunned at a relatively large distance from the anode and show no proper anodic reaction. These facts reduce the catching probability and might affect the fishing

results.

By different combinations of pulse frequencies and lengths it is attempted to reduce the negative features of pulse current. Despite some drawbacks the use of pDC can not always be dispensed with. Battery-powered, pDC delivering fishing gear is light, highly mobile and the circuit can quickly be shut off and on by pushing a button. Also, today fish-friendly pDC devices are in use (Peter & Erb 1996). Only if mobility could not be dispensed with, pDC was used, as was the case at the Ahr due to very long periods of using the devices. They were used in parallel throughout the day for a salmon fry monitoring with very long sample sections. But since the carriers of the anodes were very experienced, an optimal catch result could still be achieved.

The Inde on the other hand, could be examined using constant DC (cDC). The advantages are:

1. High catch probability (no other power type exceeds cDC)
2. Risk of injury: cDC is the gentlest current type for fish (Peter & Erb 1996).

Low flow conditions with good visibility often occur in late summer and early fall. At low drain, flow velocities are reduced and the water surface calmed. This has a positive effect on the catch. Also, information regarding the importance of natural spawning can be obtained in late summer and autumn. The 0+ fish (young of year) have grown large enough at this time and can be detected with the electrical gear without any problems. For these reasons, the time between the end of August and beginning of October proves ideal for electrical fishing (Peter & Erb 1996). This period was also observed in this study. Correspondingly, fishings took place only at usual to low water levels and not after floods with resulting dampening.

The aim of the electrofishing was not the estimation of a population size. Since the distribution limit of the signal crayfish in both water bodies was restricted to a small scale area and only few kilometers lay between the sampling sites, it could not be assumed that population sizes for each of the 100 m were determinable. It certainly would be unrealistic to assume a fixed population size within individual sample sites. It can rather be assumed that an exchange of individuals took place between the sites. The aim was to estimate how many fish were staying in the area examined at the time of the sample and to work out differences with respect to signal crayfish densities. Qualitative fishing methods are suitable to quickly gain an overview of the occurring fish species in a watercourse. In a small creek, usually several representative stretches are fished, all sections are examined with the same intensity and all habitat types are considered. The identification of the fish (species, length or size class) is done either directly in the landing net or in a kettle. Small fish are usually divided into two size categories, for large species three size classes suffice: 0+, fish up to 20 cm in length and fish bigger than 20 cm (or 0+, juvenile, adult). The note taker runs a tally for every species and size class. It is also possible to dispense with the division into size classes and measure the fish accurately instead. However, this would greatly increase the expenditure of time (Peter &

Erb 1996). As a compromise solution, in this study the fish were divided in 5 cm stages and a corresponding tally conducted. According to Serchuk (1978), in some situations population size can be estimated by direct count of the entire population or part of it. This includes visual counting in clear water, which was possible in both watercourses. To get most reliable figures with respect to fish contained in a sample site, not only the caught fish were counted, but also those that were seen escaping. The size of these fish was estimated in 5 cm steps and announced to the note taker as well.

Both examined streams are small, which is why a high effectiveness of the current was to be expected. To ensure that as few animals as possible were overlooked in the fishing, the sample sites were worked with two electrodes in parallel. In this way, the effected area can be enlarged. The size of the fishable radius depends on the current intensity, the conductivity and temperature of the water and the type of current used (cDC vs. pDC). Fortunately, conductivity and temperature values were constant in both streams throughout all sample sites. Variations of these external parameters might have lead to variances in efficicacy of cDC. An appropriately adapted pulse current as used in the Ahr has almost constant effectivity (Hartley 1980).

Fishing was performed in teams of five: two anode-carriers, two to three catchers using nets and one note-taker (Fig. 10). The number of catchers was determined by the width of the sample sites as well as the structure of the river bed. The sample sections were systemically scoured upstream. After noting their size, caught fish were immediately released behind the row of catchers and anode-carriers to make sure that no fish was caught twice. The upstream movement of the catching team made sure that the paralyzed fish were swept further down behind them. Additional notes were taken on the condition of the fish including injuries caused by crayfish, nutritional status, parasites or other signs of illness. Electrofishing for this study has been conducted free of charge in both rivers by specially trained personnel (Ahr: office for ecological fish and aquatic studies in Frankfurt, Dr. Jörg Schneider - Inde: Heinz-Josef Jochims, Fish Conservancy Rur). The problem of invasive signal crayfish was already known in both facilities and the project was met with great support.

Figure 10: electric fishing with two anode carriers and three catchers in the Ahr (Foto: Heinz Stetzuhn)

2.2. Behavioral effects of signal crayfish on native fish and differences between signal and noble crayfish

Direct behavioral long-term effects of signal crayfish (*Pacifastacus leniusculus*) and the native noble crayfish (*Astacus astacus*) on fish were recorded and compared under laboratory conditions. The long-term aspect should be adressed in particular during this study since this is mostly disregarded under experimental conditions. 3 more hypotheses were checked in the laboratory:

1. Signal crayfish affect fish directly negative by repression and predation.
2. This negative effect increases with crayfish population density.
3. Signal crayfish effects are generally stronger than those of noble crayfish's.

The experimental caging was conducted on the premises of the State Fisheries Association Westphalia and Lippe e. V. in Münster. The experiments started in autumn 2012. Two plastic basins with a bottom area of 2x2 m were centrally divided by a steel mesh and an adequately dimensioned filtration system installed. In the resulting 4 compartments, 4 different experimental setups could be run simultaneously. Each of the four test chambers (2x1 m ground area each after subdivision) was set up equally. Since a consistently good water quality is essential for salmonids, a natural gravel substrate was renounced for hygienic reasons. Instead, stainless steel grids were used to install the shelters on them. This design facilitated the easy removal of the whole construction for cleaning it as well as the bare basins. One tripond beadfilter 500 was used per divided tank with two chambers. Beadfilters are closed pressure filters. They are filled with a special plastic granulate. These small filter balls have an extremely big surface area and filter both mechanically and biologically. The beads float in a tight container very close together (mechanical filtration). The water is pumped from the bottom up through the plastic balls. Dirt particles get stuck in between. If the filter remains in use for longer periods of time, the biofilm on the plastic balls grows thicker and therefore also retains even small dirt particles. The plastic balls have a surface area of about 1,600 m^2 per m^3. The biofilm on them provides a large biologically active filter area for bacteria which catalyze aerobic and anaerobic degradation of pollutants (biological filtration). In addition, the basins were equipped with UV systems (TMC ProClear / 25 watts) to keep the water clear and free of germs. Shelters consisted in plastic tubes with a diameter of 7.5 and a length of 15 cm. 6 shelters were installed in each compartment (finished structure: Figure 11). Industrial trout food (Biomar Efico Enviro 920) was provided by automatic feeders in the same quantity and quality in all four basins. The crayfish did not receive any additional food, since they are omnivorous and willingly took the trout food as well.

Figure 11: Still image of the video surveillance system showing the 4 compartments

The basins were stocked according to the three study phases listed below.

- Phase 1: Influence of signal crayfish on trout
- Phase 2: Influence of signal crayfish on salmon

- Phase 3: Differences in behavior and biological influence between signal and noble crayfish.

In phases 1 and 2, the four basins were stocked with differing numbers of signal crayfish, namely 5, 10 and 15 animals, while one basin was used as a control setup, where fish were kept alone. It was taken care that a similar size and sex ratio of the crayfish was achieved in the respective basins.

Since the negative impact of crayfish on bullheads and other benthic fish was already known, it was essential in this study to fathom their influence on fish of the water column that are not necessarily in direct contact with the crayfish. The selected open water species brown trout (*Salmo trutta fario*) and Atlantic salmon (*Salmo salar*) were investigated in separate study phases (phases 1 and 2). The fish were introduced to the basins in groups of six each. A third study phase was aimed at the behavioral differences between signal and native noble crayfish. Here, stocking of the basins was carried out in the same manner as in the earlier phases using the more affected fish species in combination with noble instead of signal crayfish in the same density as well as size/age structure. All fish used in the experiments had a body length between 12 and 15 centimeters on the day they were placed in the basins. The exact stocking scheme of all study phases can be seen in table 3. The keeping of fish and crayfish was approved and monitored by the Veterinary Office of the city of Münster.

Table 3: Distribution of fish and crayfish in the experimental setups throughout the three study phases

Phase	Stocking	Basin 1	Basin 2	Basin 3	Basin 4
1	Signal crayfish	0	5	10	15
	Brown trout	6	6	6	6
2	Signal crayfish	0	5	10	15
	Atlantic salmon	6	6	6	6
3	Noble crayfish	0	5	10	15
	Most affected fish species from before	6	6	6	6

Main focus of the experimental long-term surveillance was on the behavioral analysis of fish-crayfish interactions – particularly with regard to competition for shelter, predation and changes in activity. This was monitored and analyzed using four infrared-sensitive water proof cameras which were installed under the water surface (Lupus LE138, Resolution 752x582 pixels, about 92 ° perspective 1/3" (8.5mm) Sharp sensor (color)). Video surveillance of the complete trials would have been possible but evaluation of such a huge amount of data would have been unrealistic within a reasonable time frame. Hence, recording time was limited to one week per trial of which 10 minutes per day during mid day time were analyzed each day.

The explicit aim of this study was to give the species time to get used to one another before the actual recording of their behavior started. Under natural conditions, invasive crayfish do not immerse in large quantities overnight but the population slowly evolves. This was to be mimicked as closely as possible which can not be done if animals are just kept together for a few hours. In some studies, experimental fish groups were taken from watercourses, which were already invaded by signal crayfish (Guan & Wiles 1997, Light 2005) in other cases the fish had no signal crayfish experience (Bubb et al. 2009, Griffiths et al. 2004). In only one study, the fish were accustomed to crayfish for a period of 24 hours before their behavior was observed (Light 2005). The observation phase in all these studies lasted one to three days. In this study, the observation/recording week started after fish and signal crayfish had been kept together for four weeks, making sure that the species had gotten used to each other as they would under natural conditions. The trout and salmon had no previous experience with signal crayfish and crayfish in general. When the experimental setups were repeated with noble crayfish in phase 3, the adjusting time was skipped due to pressure of time. However, these trout were kept together with noble crayfish at the breeding facility so it could be expected that they were already used to them.

Monthly weight checks were performed on fish as well as crayfish during phase 1, which lasted five months. This long period of time was chosen especially to detect differences in fish nutrition that might have been caused by higher crayfish densities. The duration of the experiments was shortened after phase 1, since the fish's weight gain proved to be too irregular to be evaluated against crayfish presence. Figure 11 shows a still image of the installed video system.

The results of the laboratory tests were also subjected to a Pearson correlation analysis and checked for significance.

3. Results and discussion

The study revealed that signal crayfish spread and multiplied in both streams, but the speed of the spread and the population maxima varied depending on the individual system. Once an area is reached and populated, a population maximum is established, which varies according to the encountered conditions – probably also depending on whether or not the population is regularly harvested by anglers. The macrozoobenthos did not always respond negatively to the presence of the crayfish. Groups that had a high proportion in the total benthos count were decimated the most. This effect could vary greatly depending on the seasons. Other factors also played into the strength of influence. There was evidence that decimation of one group by the crayfish resulted in increased proliferation in other groups. Some groups did not show any reaction to crayfish at all. In any case, species composition changed due to these different reactions. The crayfish had a negative impact on total macrozoobenthos numbers, but this effect only became significant if the crayfish population reached a certain level. Population densities that corresponded to those of the native noble crayfish, could apparently be coped with better by the invertebrate community. The examination of the fish population in the field showed that ground-dwelling, nocturnal fish species with benthic invertebrates as their main food source, like sculpins and loaches were most negatively affected by signal crayfish. Fish species of the water column like trout and minnow were less affected but still showed negative tendencies. Brown trout in both streams tended to be only slightly negatively correlated to signal crayfish density. In the laboratory, displacement from shelter by signal crayfish could be observed in both examined open water fish species. Brown trout were more strongly affected, as these fish generally used shelters more often than salmon.

3.1. Progress of signal crayfish colonization during the course of the study

During the first year of control trapping at the Inde, it became apparent that spring and winter did not yield significant catch results. After the initial trappings to find the edge of the population in late summer 2012, there was no crayfish to be found in the edge-sample-site I3 in winter as well as in spring 2013, even though the crayfish had been detected there just a few months earlier. During the summer months 2013 on the other hand, 19 crayfish could be caught in the boundary site, and even 3 in the I1 site that had not been colonized in summer 2012. In addition to that, especially the females become very secretive in spring because they carry the newly hatched juveniles, hardly feed, and thus are not easy to lure into traps. As a reaction to these findings, crayfish trapping was only done during the summer months from this time on. Also, spring and winter results were not compared to summer results since their meaningfulness is highly doubtful at most. There were not less crayfish in the sites during spring and winter of course, they just did not feed as intensely and were less active due to lower temperatures (compare also Bubb et al. 2004).

Table 4: Results of the control trappings in the Inde (densely populated areas in grey, I3 population boundary, 14 initially unpopulated site).

Sampling date	Sampling site	Number of crayfish	Average carapace length [cm]	Sex ratio (m/f)	Injured individuals	Injury quotient*
Winter 2012/13	I1	8	4.7	5/3	-	-
	I2	3	5.2	2/1	-	-
	I3	0	-	-	-	-
	I4	0	-	-	-	-
Spring 2013	I1	7	4.2	6/1	-	-
	I2	9	4.6	5/4	-	-
	I3	0	-	-	-	-
	I4	0	-	-	-	-
Summer 2013	I1	56	5.2	30/26	4	0.07
	I2	68	5.5	19/49	13	0.19
	I3	19	5.6	11/8	3	0.16
	I4	3	5.7	1/2	0	0.00
Summer 2014-1	I1	38	5.3	15/23	5	0.13
	I2	31	5.2	16/15	2	0.06
	I3	8	6.3	8/0	1	0.13
	I4	2	7.1	1/1	0	0.00
Summer 2014-2	I1	46	5.2	22/24	5	0.11
	I2	62	5.4	21/41	2	0.03
	I3	23	5.8	15/8	1	0.04
	I4	5	5.9	3/2	0	0.00
Summer 2015	I1	62	5.0	21/41	8	0.13
	I2	62	5.2	17/45	13	0.21
	I3	47	5.7	21/26	3	0.06
	I4	30	5.8	23/7	2	0.07

*number of injured crayfish divided by total number of crayfish in the catch

A striking factor was that the average carapace length of the trapped animals, which increased with declining crayfish density in the sampling sites. This trend remained constant throughout all three years of the investigation. Since its colonization with few individuals in summer 2013, the previously uninhabited site always showed the lowest crayfish density, but the animals here were bigger than the ones in the sites with higher population (Tab. 4). The average size of the animals at the edge of the population lay exactly between the individual size in the initially unpopulated and densely populated areas. This result being reproducible every year shows that the migration is forced particularly by full-grown adults. Two previous studies of Budd et al. in 2004 and 2006 had shown no influence of crayfish size on migration behavior. In these studies however, radiotagged crayfish were followed, while in this case, a fixed checkpoints / sample sites within the system were watched for crayfish to turn up. Hence, the sample was bigger, basically consisting in the whole crayfish population.

Injuries of signal crayfish could not be associated with their densities (Tab. 4). The sex ratio varied from year to year and does not seem to be associated with crayfish density in a sample site. Only in summer 2015 more females than males were caught in all sites - with the exception of the previously uninhabited site I4 with now 30 animals in the traps. In this site, the sex ratio was strikingly reversed - with three times more males than females. This trend however was not visible in previous years, which shows that the migratory behavior in the Inde was not dependent on gender. This is in accordance with the findings of Bubb et al. 2004 and 2006.

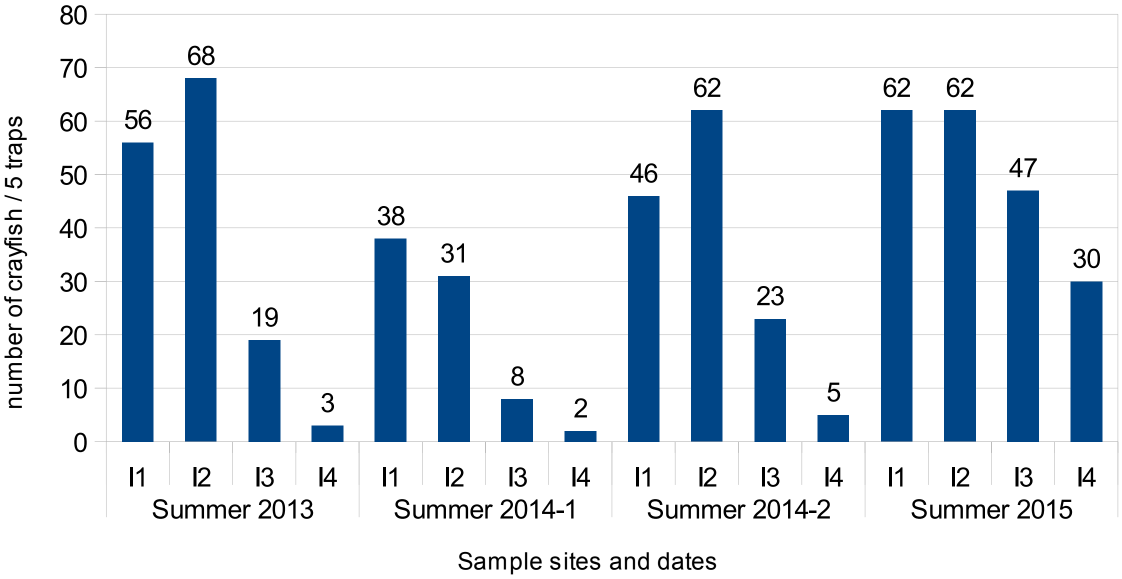


Figure 12: Signal crayfish trapping results in the Inde (I1 and I2: densely populated, I3: population border, I4: not populated at beginning of study

As becomes visible in figure 12, the signal crayfish population of the Inde remained consistently high in the densely populated locations I1 and I2 with an average of 60 individuals in 5 traps per 12h trapping. Only in the early summer trapping of 2014, the catch numbers decreased slightly, but reached their former scope again in late summer. This slight deviation can be explained by the relatively cool temperatures in June 2014. The fact that in the densely populated areas no further increase in density could be registered within the three years of the study shows that the population has reached its maximum there which will lead to emigration pressure. According to that, the development in the I3 site at the previous border of the population and in the previously unpopulated I4 turned out differently. Here, a significant population increase of crayfish could be witnessed. In the boundary region the total catch rose from 19 to 47 animals per trapping. In the initially unpopulated area (I4) 30 animals were caught in the last trapping 2015.

These results show a significant spread of the signal crayfish in the Inde. The examined population border was located on the downstream end of the populated area in this case, therefore the animals can migrate with the flow which accelerates their spread as was already observed by Bubb et al. 2004. They covered 200 meters between sites I3 (boundary) and I4 (unpopulated in less than a year) – and probably went a lot further downstream. Bubb et al. (2004) had observed a spread of 2.4 km per year in an established population in an upland river – and a significantly slower rate within a population in the initial stages of establishment. The signal crayfish population of the Inde has been established for several years.

Table 5: Results of the control fykings in the Ahr (densely populated areas in grey, A2 population boundary, A1 initially unpopulated site)

Sampling date	Sampling site	Number of crayfish	Average carapace length [cm]	Sex ratio (m/f)	Injured individuals	Injury quotient*
Autumn 2012	A1	0	-	-	-	-
	A2	2	6.9	2/0	-	-
	A3	32	6.3	25/7	-	-
	A4	11	5.6	9/2	-	-
Summer 2013	A1	1	7.0	1/0	0	0.00
	A2	1	5.2	1/0	0	0.00
	A3	28	6.2	13/15	4	0.14
	A4	6	5.0	4/2	1	0.17
Summer 2014-1	A1	0	-	-	0	-
	A2	0	-	-	0	-
	A3	25	5.7	15/10	5	0.20
	A4	4	5.2	3/1	0	0.00
Summer 2014-2	A1	0	-	-	0	-
	A2	4	6.4	2/2	1	0.25
	A3	22	5.5	15/7	1	0.05
	A4	23	5.5	16/7	3	0.13
Summer 2015	A1	1	7.0	0/1	0	0.00
	A2	7	4.8	5/2	0	0.00
	A3	25	5.2	17/8	6	0.24
	A4	5	5.4	1/4	2	0.40

*number of injured crayfish divided by total number of crayfish in the catch

At the Ahr, the first control trapping after the determination of the distribution limits was carried out in early fall 2012. In spring 2013, trapping had to be cancelled due to flooding. After the results in the Inde showed that spring and winter trappings are not useful, trapping was done in the Ahr only during the summer months as well. Due to high temperatures in September 2012, the fishing results of this trapping are still usable. There even were a few more animals caught than in the following summer trappings. In contrast to the Inde, signal crayfish are caught intensively by local anglers in the Ahr. In 2014, more than 4000 animals were caught and removed in the two densely populated sampling sites solely by the responsible tenant of this stretch. How many crayfish have been removed by residents and illicit anglers could not be determined. But it is known that removals were made from this side as well. This is also reflected in the declining average carapax length of the crayfish and the significant overage of males in the catch. Anglers often remove specifically the large males, because they contain more meat in their bigger claws and body. Their respective residential areas of these males can then be taken over by smaller ones, of which several fit into the niche that was previously occupied by one big male. This results in growing numbers of males combined with declining average size and in many cases a growth of individual numbers, as was observed in a project to subdue a signal crayfish population in the Iter, an orbituary of the Inde (M. Zocher, personal communication). This underlines once more the impossibility to reduce an established signal crayfish population within a stream by simple trapping. In addition to that, it has to be taken into account that the previously unpopulated sample site A1 had to be moved due to flooding in spring 2013. A lot of sediment had been washed into the site which led to a collapse of invertebrate and fish populations which would have massively distorted the results of the study. The move was carried out at the beginning of 2014. Due to the structure of the water body, the next comparable sample site was located 1.3 km upstream – 2 km above the current distribution limit of the crayfish. Since crayfish had to travel against the current and surmount several very deep areas with many possibilities to hide and proliferate, it seemed very unlikely that this new sample site would be invaded within the study time. Nevertheless, a single crayfish could be found at the site in 2015 already, even so the downstream population had not grown to higher densities which would have led to emigration pressure. This demonstrates the enormous potential of the signal crayfish to spread rapidly. Just as in the Inde, no association between signal crayfish densities and injuries of individuals could be found in the Ahr (Tab. 5).

Overall, signal crayfish densities remained relatively constant within the existing population of the Ahr (Fig. 13). The utter downstream sample site A4 which has been populated the longest almost always contained less animals than the second densely populated site A3 closer to the population boundary. Only the late summer trapping in 2014 showed an elevated density with an even higher number of crayfish in A4 than in the previously denser populated A3. It allows the assumption that the true crayfish density is not always proportionally represented in every trapping. In the population boundary sample site A2, 2 crayfish were caught in the beginning of the study, 7 at the last trapping two and a half years later. In the meantime, results varied between 0 and 4 animals. A weak upward trend is recognizable. In the former unpopulated site A1, a single animal was caught in 2013 already. After the flood-induced shift of the site 1.3 km upstream, expectedly no crayfish were caught in both trappings 2014. Surprisingly though, the first signal crayfish was caught in this new site approximately 2 km upstream of the population boundary in summer 2015 already. Considering the traveling direction against the current and the many very deep and diversely structured river stretches within these two kilometers, this migrating performance is remarkable. The crayfish had several possibilities for hiding and populating on this stretch, yet they migrated even further than necessary. Obviously, single individuals travel several kilometers against the current without any pressure from maximum densities – which is in accordance with the findings of Bubb et al. (2004).

In both first finds of signal crayfish – in the first as well as the shifted unpopulated A1 site – the respective animal was a very big adult with a carapax length of 7 cm and a total length of 14 cm. This continues the trend that was already recognizable in the Inde: It appears that only the adult animals migrate while the subadults are not taking part in the spread of the population. The declining size of the crayfish within the densely populated areas has already been explained above.

As in the Inde and in accordance with studies named above, no connection between migrating behavior and gender could be found. The single crayfish that were caught first in the previously unpopulated sites were once male (2015) and once female (2013). Though there was a male overage in the population boundary site A2, this was also the case in the densely populated areas and therefore does not show a higher migration potential of the males.

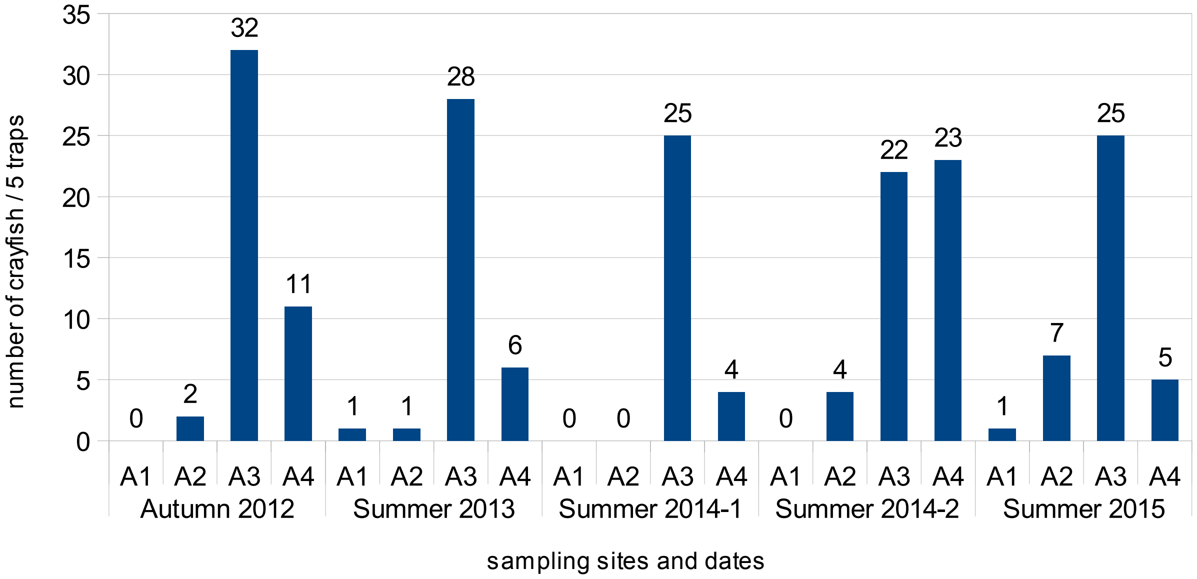


Figure 13: Signal crayfish trapping results in the Ahr (A3 and A4: densely populated, A2: population border, A1: not populated at beginning of the study)

In summary, it became clear that the signal crayfish population is not rising beyond a certain density once it is established in an area. The reachable density seems to be dependent on the structure – especially the resulting food supply and the number of shelters – of a water body, while the scale of a river or stream did not influence densities. Human exploitation might play a role in keeping the population density slightly lower than it would be without interference, but did not show realistic potential to exterminate it to a degree that it would prove lesser of a thread for the environment. Significantly higher densities were reached in the Inde even so the stream is smaller than the Ahr. The colonization at the population boundaries was also slower at the Ahr. However, single animals travelled longer distances in short periods of time, leading to a rapid and irreversible spread, since it is impossible to completely remove these animals from the stream. The migration was independent from gender but especially the biggest adults were most likely to migrate.

3.2. Signal crayfish effects on macrobenthos

3.2.1 General effects in the Inde

In the following general quantitative discussion, only invertebrate groups were considered that reached at least two-digit numbers. Rare species that were only caught sporadically are very unlikely to be considerably influenced by the crayfish, since encounters are unlikely and crayfish will not actively seek out certain rare groups. Also, Annelida were completely dismissed in the discussion. Due to their soft body tissue, they tend to be destroyed in the samples, while these are transported and handled. The resulting pieces were impossible to count properly – especially if the individuals were very small. It would have been necessary to only count the head pieces but the head was not distinguishable from the tail end from certain sizes downwards.

Sample site	Crayfish	Mollusca	Crustacea	Ephemeroptera	Plecoptera	Coleoptera	Megaloptera	Trichoptera	Diptera	Total
I-k-2013	3	77	3448	459	76	722	4	191	1035	6012
I-m-2013	19	236	2822	273	106	381	8	320	1342	5488
I-d-2013	56	61	1061	349	30	144	3	150	325	2123
I-d-2013	68	167	1088	373	60	105	8	233	444	2478
	r	**-0,03**	***-0,99***	**-0,23**	**-0,67**	***-0,95***	**0,15**	**-0,26**	**-0,85**	***-0,97***

Figure 14: Pearson correlation of signal crayfish and macrobenthos density 2013 (significant correlations in italics, sample site code: i = Inde, k = crayfish free site, m = boundary site, d = densely populated site)

Within the three first year samples (spring, summer, autumn) in 2013, a reduction of macrobenthos by 59 % up to 65 % in the heavily crayfish populated areas, became visible already (Fig. 14). For all samples, a Pearson correlation was calculated, so that every year the correlation between macrozoobenthos numbers and the actual number of crayfish in the sample sites became visible. In 2013, the effect of crayfish on macrozoobenthos total numbers was already significantly negative (r=-0,97, Fig. 14). While in the areas at the population boundary (3 crayfish in the 2013 catch) and in the initially crayfish free site (19 crayfish in the 2013 catch), the samples contained 5480 to 6008 invertebrates, the numbers dropped to 2470 and 2120 animals in the already densely populated areas (56-68 crayfish in the 2013 catch). The overall impression in 2013 was that the ecosystem can handle crayfish up to a certain density. Only if this density is exceeded, the invertebrate community is breaking down.

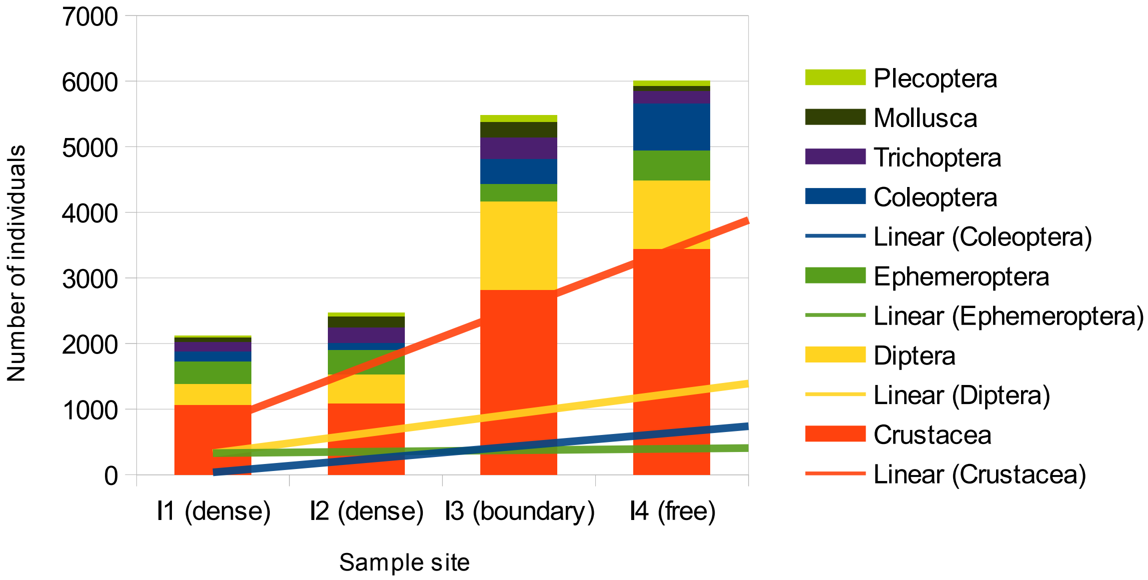


Figure 15: Macrobenthos in the Inde 2013 (crayfish densities in brackets)

As becomes evident in figure 15, the most common groups were most affected. At the Inde, these were Crustacea, Diptera and Coleoptera. The more frequent a taxon, the stronger the crayfish influence appeared to be. Trending lines were inserted for the three most common groups to allow the comparison. The higher the individual numbers, the steeper was the slope of the line. This effect can be considered typical for groups that are directly predated by the crayfish. Since crayfish will browse for food, randomly searching through the substrate with their claws, they will inevitably feed on the most common groups since they are the most likely to get caught by this method. Crustacea, as the most common group lost 18 % in the boundary site and 68-69% in the longer invaded sites I1 and I2. A Pearson correlation, which was calculated with the actual crayfish catch numbers, revealed a value of r=-0,99 - a highly significant negative correlation. The negative response of Coleoptera was also highly significant (r=-0,95), while the negative response of Diptera did not reach a siginficant value but was still high at r=-0,85. However, it can be assumed that the negative effect is also significant here. Every summer the Diptera population collapsed completely, falling out of the crayfish's diet, which can lead to fluctuations in the evaluation of the results. Less common groups that only occurred in small numbers like Trichoptera (r=-0,26), Ephemeroptera (r=-0,23) and Mollusca (r=-0,03) were not significantly influenced by crayfish presence and/or density, as expected. However, Plecoptera – though a small proportion group – were slightly negatively influenced (r=-0,67). They were able to cope with small crayfish numbers but declined strongly (-34-67 %) at high densities.

Seasonal Macrobenthos Sample Results 2013 (Fig. 16)

Crayfish effects varied strongly dependent on time of year. Negative effects were very visible in Spring and Summer and a little less pronounced in autumn. The highest number of invertebrates was still found in the signal crayfish free site I4 though. Invertebrate numbers reached their peak in summer and were diminishing again in autumn. This might be one reason for the less pronounced crayfish effects in the last sample of the year.

Also, the species' composition within the community changed with the seasons. In spring, Diptera were the most common and therefore most strongly impacted group, suffering a loss of 82 % to 91 % compared to the uninvaded or just recently populated areas. They were followed by Crustacea (-25% in the recently populated site with 19 crayfish in the 2013 summer catch, averagely -55 % in the densely populated areas), Coleoptera (-42% in the recently populated area, averagely -80 % in the densely populated ones) and Ephemeroptera (only -2 % in the population boundary sample site, but averagely -62 % in the densely populated areas). Trichoptera, Plecoptera and Mollusca played only a subordinate role and therefore were not significantly influenced.

During the summer months, invertebrate densities rose and Crustacea proliferated to become the dominating taxon – expectedly even more strongly affected by signal crayfish densities than they were in spring. While low crayfish densities in the population boundary sample site I3 (19 crayfish) did not influence them, their population went down by 72 % to even -84 % in the site that was invaded the longest (56-68 crayfish in the summer catch 2013). Coleoptera rose to second place in total density – and so did the negative effects of the crayfish on them. They lost -52 % in the population boundary site already and 92 % to 93 % in the areas that were populated with high crayfish densities. They were not that strongly affected in spring, when their overall densities had been lower due to the time of year. Diptera on the other hand broke down completely every summer, thereby falling out of the crayfish's diet and not showing any negative affects anymore. Trichoptera diminished proportionally as well, surprisingly showing heavily negative crayfish affects (-3 % in the crayfish population boundary site, -83 % in both densely populated areas) during the summer catch. Plecoptera and Mollusca remained in their subordinate and uninfluenced position, while Ephemeroptera gained in proportion and surprisingly showed a slightly positive reaction to signal crayfish densities. Their numbers decreased in the boundary site I3 by -43% but increased by 21% in the densely populated site I2 and still by 2 % in the densely populated site I1 that had been invaded for a longer period of time. Over the year, however, this group did not seem to be responsive to crayfish (r = -0.23 in the 2013 results), so this reaction may be more likely to be considered a low-grade fluctuation.

In the autumn 2013 sample, Crustacea remained the most common and most negatively affected group, even so their numbers underwent a certain seasonal decrease. Interestingly, even so their numbers were highest in the crayfish free site I4, their losses diminished deeper into the signal crayfish population, showing a loss of 71 % at the crayfish population boundary, 66 % in the first densely populated site and only 46 % in the site deepest into the crayfish populated area. This variation is most likely due to the overall seasonal decrease of the group, which was significantly negatively influenced throughout the year based on the actual number of crayfish (r = -0.99). Diptera and Mollusca did not show visible reactions to crayfish densities, even so their numbers varied within the sample sites, but not in any correlation to crayfish. Although Diptera were negatively correlated over the whole year, they did not show this response after the annual summer population decline. Trichoptera – though a group of small proportion and only little reaction to crayfish overall – were decimated to a mere sixth (17 %) of their former numbers in autumn 2013. Coleoptera and Ephemeroptera on the other hand, showed negative reactions, even so they were only present in small numbers and therefore were not influenced as strongly as Crustacea. Coleoptera seemed negatively influenced regardless of their densities and seasonal fluctuations therof. They diminished by 37 % in the boundary site and by averagely 53 % in the densely populated sites. Their percentage loss in comparison to spring numbers decreased with their overall numbers just as it did in Crustacea. Ephemeroptera lost 62 % in the boundary site and averagely 67 % in the densely populated areas – their positive reaction during summer turning into a negative one. Mollusca remained unaffected by crayfish.

In 2014, negative crayfish effects were still visible but less pronounced than they had been in 2013 (Fig. 18) but overall, their negative effect on the total number of macrozoobenthos remained significantly negative (r = -0.95, compared to the previous year r = -0.97, Fig. 17). Macrozoobenthos total numbers in the samples increased from 16,078 in 2013 to 27,041 in 2014 by 68 % - mainly due to massive Diptera proliferation in the spring sample. Crayfish densities in the boundary site varied between 8 in the first and 23 in the second summer catch, showing an average catch of 15.5 animals, which is slightly less than in 2013 with a result of 19 crayfish in that site. In the densely populated areas, crayfish numbers had slightly dropped to 38-46 in the inner site I1 (56

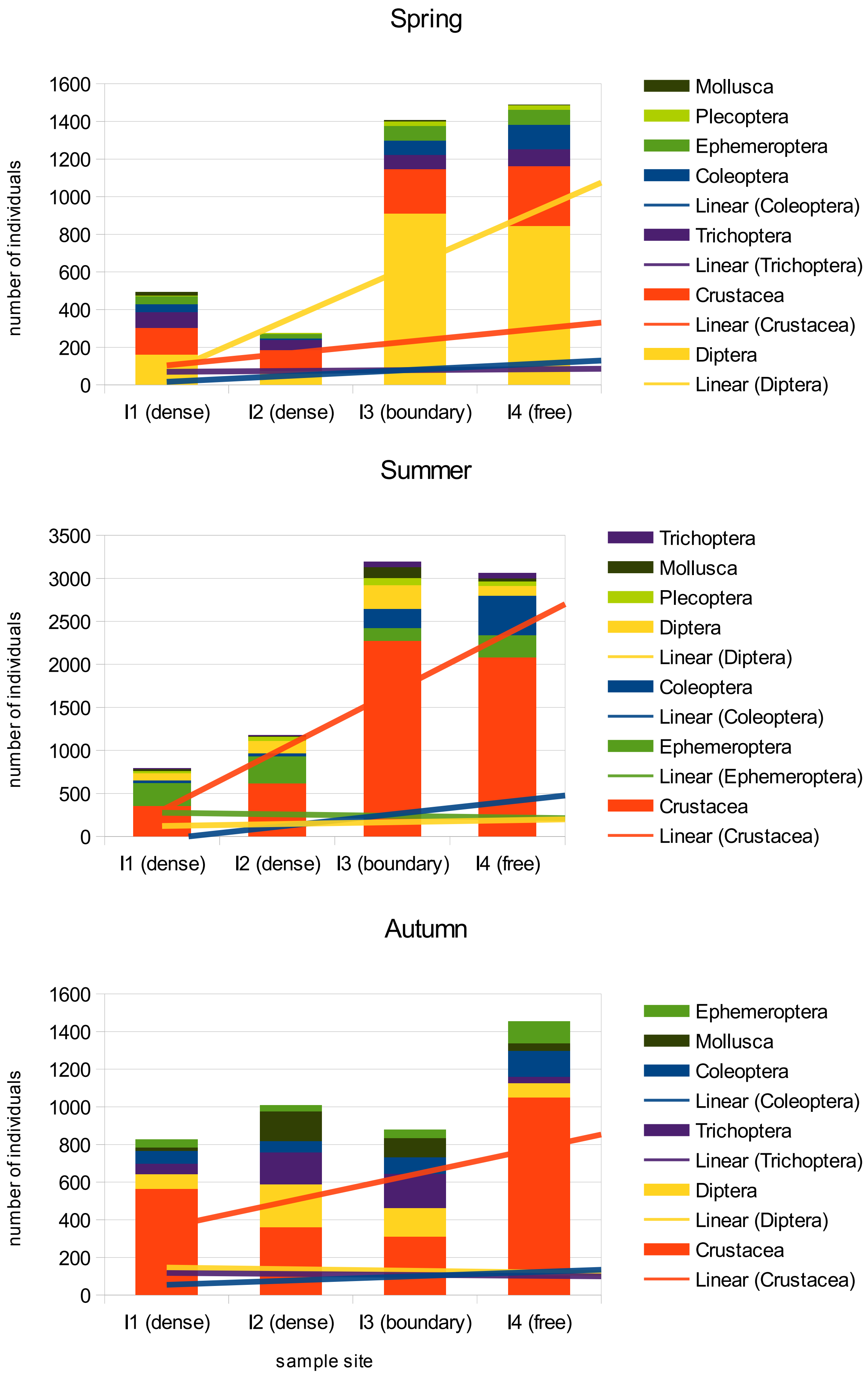


Figure 16: Macrobenthos in the Inde over the three seasonal samples 2013

in 2013) and 31-62 in the utter site I2 (68 in 2013). Since catch results may vary daily due to temperature, water level, flooding and other disturbances, the slight decrease in results does not necessarily mean that the crayfish population itself decreased. But it seems safe to assume that they did not proliferate to a great deal. For the Pearson correlation, the most meaningful trapping results of the respective sample site were selected. It would appear that at least Diptera have been able to cope with the signal crayfish – probably indirectly affected by the decline of other invertebrate predators.

Sample site	Crayfish	Mollusca	Crustacea	Ephemeroptera	Plecoptera	Coleoptera	Megaloptera	Trichoptera	Diptera	Total
I-k-2013	3	77	3448	459	76	722	4	191	1035	6012
I-m-2013	19	236	2822	273	106	381	8	320	1342	5488
I-d-2013	56	61	1061	349	30	144	3	150	325	2123
I-d-2013	68	167	1088	373	60	105	8	233	444	2478
	r	**-0,03**	***-0,99***	**-0,23**	**-0,67**	***-0,95***	**0,15**	**-0,26**	**-0,85**	***-0,97***
I-k-2014	5	168	2969	3401	55	1033	5	241	1046	8918
I-m-2014	23	400	2949	1446	58	619	9	238	1321	7040
I-d-2014	46	272	867	1167	9	308	0	220	2692	5535
I-d-2014	62	417	1895	608	31	205	10	312	2019	5497
	r	**0,64**	**-0,73**	**-0,91**	**-0,73**	***-0,98***	**0,11**	**0,58**	**0,79**	***-0,95***

Figure 17: Pearson correlation of signal crayfish and macrobenthos density 2013 to 2014 (significant correlations in italics, sample site code: i = Inde, k = crayfish free site, m = boundary site, d = densely populated site)

Diptera more than doubled in comparison to 2013 and suddenly showed a positive tendency with rising signal crayfish densities. Over the year, they showed highest numbers and highest proportion in comparison to the other groups in the densely and longest populated area I1. They lost 25 % in the other densely populated site I2, 51 % in the boundary site I3 and had 61 % less individuals in the initially crayfish free site I4. Instead of the clearly negative response to crayfish in 2013 (although not yet statistically significant), in 2014 they appeared rather positively influenced (r=0.79, Fig. 17). It is very likely that the Diptera's annual population dips in the summer will prevent a reliable assessment of signal crayfish effects, as the group was formally completely eliminated from the food spectrum of the crayfish after this slump. However, they showed the positive reaction to crayfish during the spring sample as well – when their numbers were generally high. It may be a reaction to the disappearance of other groups, most of which were negatively affected by the signal crayfish.

Other common groups continued to show negative responses to rising crayfish densities. This holds true for Crustacea, Ephemeroptera and Coleoptera. While Crustacea did not increase in overall densities, Ephemeroptera showed 4.5 times higher numbers than in 2013 and Coleoptera 1.5 times higher. Both groups thus achieved a higher percentage in the macrozoobenthos community, while Crustacea slightly lost in proportion. According to this development, the still strongly negative influence on Crustacea decreased slightly (r = -0.73 from -0.99 in 2013) loosing statistic significance but still remaining negative. While Crustacea densities in the boundary and initially crayfish free sites I3 and I4 did not show much difference, they dropped inside the dense crayfish population by 36 % in the utter site I2 and even 71 % in the inner, longer invaded site I1. In contrast to this, the two proportionally increasing groups were even more negatively affected than in the previous year. Ephemeroptera rose to be the third most common group in 2014 after playing only a subordinate role in 2013. They lost 57 % in the boundary site and between 66 % and 82 % in the densely populated areas and their r-value dropped from just -0.23 in the previous year to -0.91 in 2014 meaning that the negative influence of crayfish on this group increased. Coleoptera lost 40 % in the boundary site and between 70 % and 80 % in the densely populated areas. Compared to 2013, when the negative influence of the crayfish was already at r = -0.95 and thus of statistical significance, in 2013 it even became larger with an r value of -0.98. This supports the theory that a group is affected most negatively if it has a particularly high proportion of the total benthos - because the crayfish is more likely to encounter them in its foraging than the rarer groups.

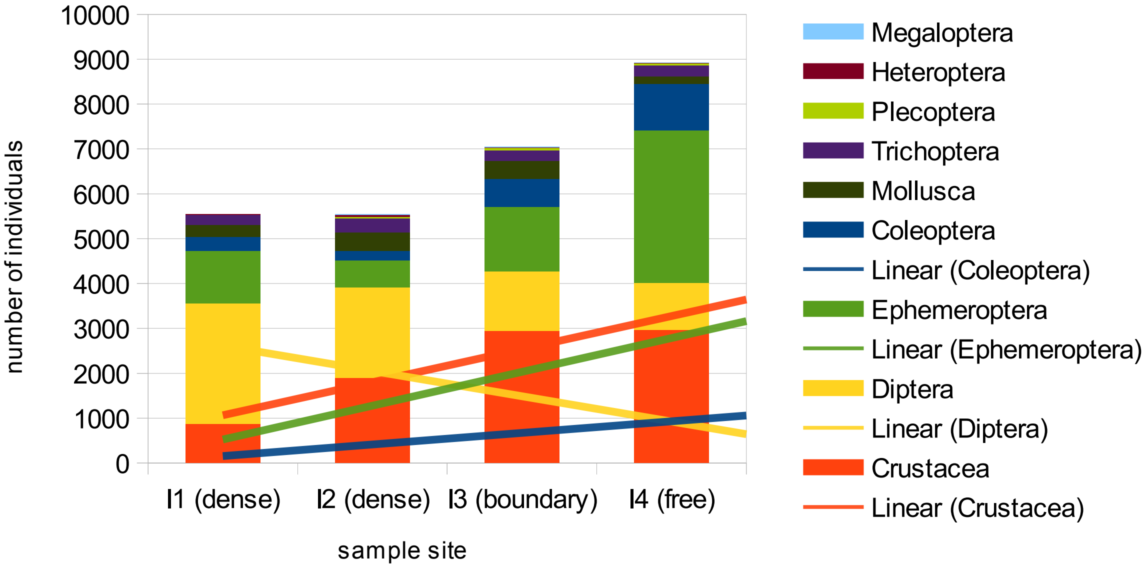


Figure 18: Macrobenthos in the Inde 2014 (crayfish densities in brackets)

Groups of small proportion showing no obvious crayfish influence were Heteroptera, Megaloptera, Mollusca and Trichoptera. If these groups reached densities that were included in the Pearson correlation, they either showed little correlation to signal crayfish density (eg. Megaloptera: 2013 r = 0.15, 2014 r = 0.11, Fig. 17), or the r values fluctuated strongly (Mollusca: r = -0.03 in 2013, r = 0.64 in 2014, Trichoptera: r = -0.26 in 2013, r = 0.58 2014, Fig. 17). Plecoptera were the only rare group that was still negatively affected by crayfish. Low crayfish densities in the initially unpopulated and boundary sites did not affect them, but their densities declined within the densely populated areas by 45 % to 84 %. This is in accordance with the 2013 results, but the effect grew bigger even so the group showed a general decline of 44% compared to the 2013 numbers which should take the nutritional focus of the crayfish off them. In 2013, Plecoptera's negative correlation to signal crayfish had already reached a value of r = -0.67, which dropped further to -0.73 in 2014.

Since they are a rare group, it is likely that they are not only predated, but influenced by other crayfish induced factors.

Seasonal Macrobenthos Sample Results 2014 (Fig. 19)

Seasonal variations in the results remained as strong as in the year before. In spring, massive proliferation of Diptera (by the factor of 2.6 compared to spring 2013) led to a huge quantitative rise in the site I1 that was populated by signal crayfish the longest. In all years, Diptera were by far the most frequent taxon and showing their highest numbers in spring. But after appearing negatively influenced in 2013, they showed positive reactions to signal crayfish presence in 2014, with more than four times more individuals in the longest densely populated site than in the crayfish free one. As stated above though, this might not be a direct reaction to crayfish but rather a reaction to the crayfish induced decline of other groups. This decline was most prominent in Ephemeroptera and Coleoptera. Ephemeroptera lost 66 % in the boundary and averagely 93 % in the densely populated sites. Their overall spring numbers increased by the factor 7.5, but the magnitude of negative crayfish affects heightened as well. Coleoptera lost 85 % in the boundary site already and up to 95% in the densely populated sites. Crustacea showed their lowest proportion in spring as they did every year. Compared to spring 2013, their overall numbers increased by 37%. They lost 8 % in the boundary site and averagely 52 % in the densely populated stretches, which basically is in accordance with their reactions in 2013.

In summer, the former biggest group of Diptera broke down to only 12% of its proportion in the spring sample and the formerly positive correlation to signal crayfish densities disappeared. With the decline of the Diptera, Macrobenthos numbers in general fell back to their former magnitude. Numbers in summer 2014 were even slightly lower than in 2013.

Also, after the decline of Diptera, quantitative differences between the sample sites went back to their former proportion – showing the lowest count in the densely crayfish populated areas. Apart from the numbers being approximately 40% lower than in summer 2013, the results were even very similar. Crustacea lost some of their dominance to Ephemeroptera which came to be the most common group in summer 2014. Both groups were negatively influenced by the signal crayfish, loosing 59-71 % (Ephemeroptera) and 49-70 % (Crustacea) at high crayfish densities. Coleoptera remained as negatively influenced as they were in the samples before. Reaching slightly lower numbers than in spring, their losses in the densely populated sites still amounted up to 78 %, staying within range of their reactions to crayfish troughout the whole study. Plecoptera, Diptera and Mollusca as subordinate groups did not show significant reactions to crayfish densities, while Trichoptera were negatively influenced even so they were only reaching small proportions, they lost up to 59% of their numbers in the crayfish free site I4.

In autumn 2014, invertebrate numbers increased again – showing Crustacea as the most common group and Ephemeroptera in second place. Both groups showed a significant increase compared to the summer sample as well as the autumn sample of the year before. Crayfish effects stayed negative, leaving the highest macrobenthos numbers in the unpopulated site, with a -28 % decrease in the boundary and -34 % and -56 % in the densely populated sites with the highest loss in the area that had been populated the longest. Throughout the first two years of the study, macrobenthos numbers could vary, rising and falling probably due to outside factors like climatic changes, floods and low water conditions as well as water parameters. The effect of crayfish densities remained the same though – with the one exception of massively proliferating Diptera in spring 2014 mainly in the longest populated site I1, which can be explained by the decrease of other groups most prominent in this site.

Crustacea as the most common group were negatively influenced. They lost 4 % in the boundary site, 32 % in the utter densely populated area and 75 % further into the crayfish population. Ephemeroptera as the second most dominant group lost 72 % in the boundary site already, 77 % in the utter densely populated site but only 51 % in the inner densely populated site, where the crayfish had been present the longest and all other groups were affected most. This might be a sign for the same phenomenon that was experienced in Diptera in spring 2014, where the decline of other groups lead to proliferation of another one. Ephemeroptera were to keep this tendency towards 2015, but in autumn 2014 they were still definitely negatively affected by crayfish. The other groups were only present in small numbers and no certain statements about their reactions could be made. Heteroptera only occurred in areas with dense crayfish population. But they appeared in such small numbers that it was questionable whether this was coincidence or a reaction to lower numbers of other groups. Trichoptera showed their highest numbers in areas with dense crayfish population – but also in small proportions. This trend being the other way around in summer (also at very low proportions), it would appear likely that this was just a coincidence. Mollusca and Diptera did not show visible reactions to crayfish densities.

In 2015, the numbers of the macrobenthos community were surprisingly even, except for one densely populated crayfish site. It appeared that the community would finally cope with the spreading signal crayfish population (Fig. 21). This was also visible in the r values of the Pearson correlation (Fig. 20). Compared to the previous year ($r = -0.95$), the negative correlation of macrobenthos numbers to crayfish only amounted to $r = -0.51$ and was thus no longer statistically significant (Fig. 20). It should be noted that due to the missing autumn sample 2015, the overall

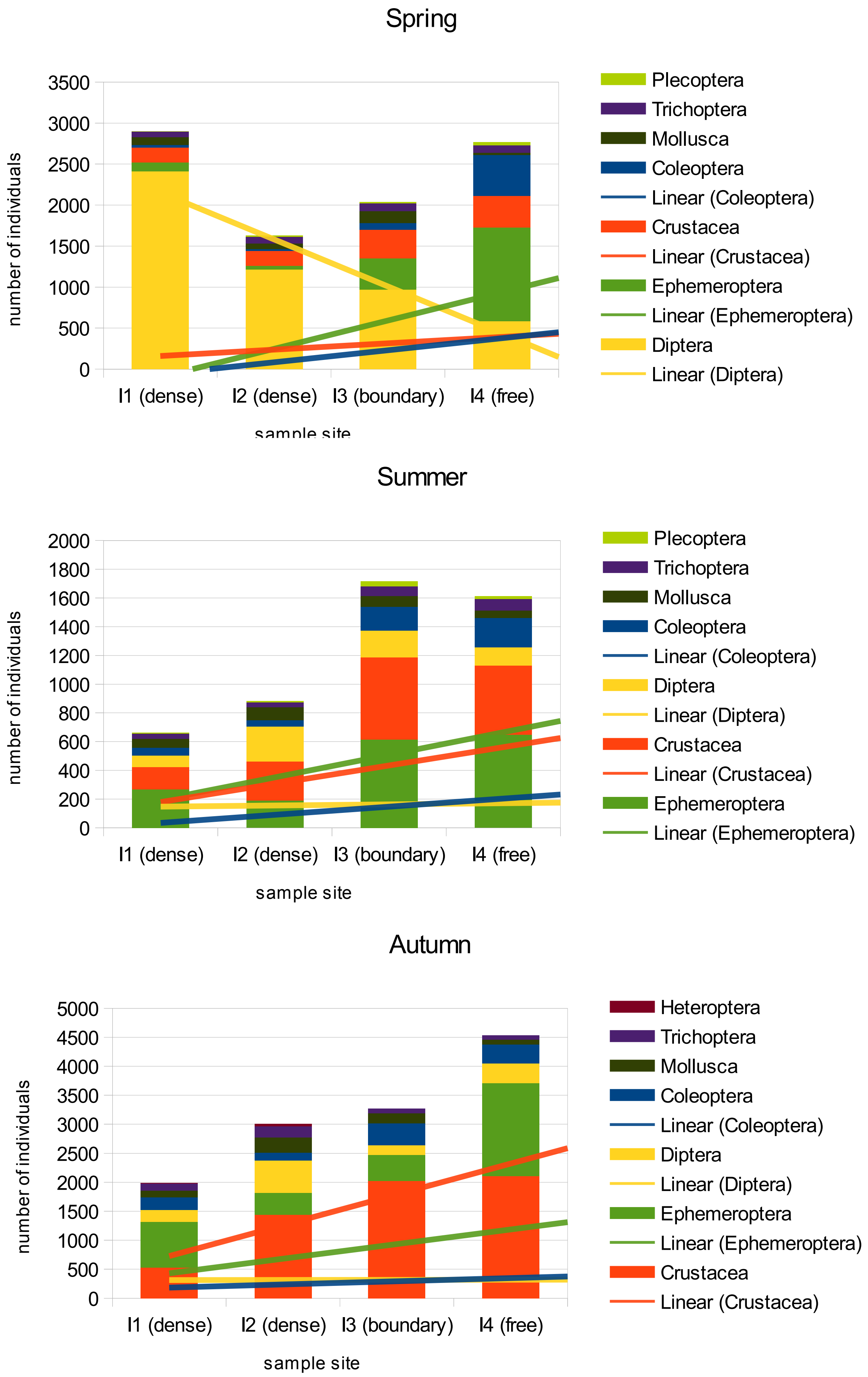


Figure 19: Macrobenthos in the Inde over the three seasonal samples 2014

result from the spring and summer samples was extrapolated (X/2x3) in order to make the sample volume comparable to previous years. This may influence the correlation values of groups which usually appeared in particularly large numbers in autumn.

Sample site	Crayfish	Mollusca	Crustacea	Ephemeroptera	Plecoptera	Coleoptera	Megaloptera	Trichoptera	Diptera	Total
I-k-2013	3	77	3448	459	76	722	4	191	1035	6012
I-m-2013	19	236	2822	273	106	381	8	320	1342	5488
I-d-2013	56	61	1061	349	30	144	3	150	325	2123
I-d-2013	68	167	1088	373	60	105	8	233	444	2478
	r	**-0,03**	***-0,99***	**-0,23**	**-0,67**	***-0,95***	**0,15**	**-0,26**	**-0,85**	***-0,97***
I-k-2014	5	168	2969	3401	55	1033	5	241	1046	8918
I-m-2014	23	400	2949	1446	58	619	9	238	1321	7040
I-d-2014	46	272	867	1167	9	308	0	220	2692	5535
I-d-2014	62	417	1895	608	31	205	10	312	2019	5497
	r	**0,64**	**-0,73**	**-0,91**	**-0,73**	***-0,98***	**0,11**	**0,58**	**0,79**	***-0,95***
I-k-2015	30	186	5220	1170	90	1314	12	258	2208	10458
I-m-2015	47	582	5766	1062	66	942	12	480	1530	10440
I-d-2015	62	738	3306	588	102	420	18	360	2850	8382
I-d-2015	62	390	4242	1578	102	654	18	378	3108	10470
	r	**0,7**	**-0,72**	**-0,09**	**0,46**	***-0,97***	**0,89**	**0,45**	**0,63**	**-0,51**

Figure 20: Pearson correlation of signal crayfish and macrobenthos density 2013 to 2015 (significant correlations in italics, sample site code: i = Inde, k = crayfish free site, m = boundary site, d = densely populated site)

Since no autumn sample was taken in 2015, the results from the first two samples (spring, summer) were extrapolated (X/2x3) to make them comparable with the samples from previous years.

Crustacea as the biggest group remained negatively influenced, as did Coleoptera which showed this reaction throughout the whole study in every sample and with statistical significance which fluctuated only very little (r = -0,95 to -0,98, Fig 20). Crustacea showed their highest numbers in the crayfish free as well as the boundary site, in which 47 crayfish could be caught in the 2015 trapping. The initially crayfish free site had 30 crayfish in this trapping, while the longest populated sites both contained 62 animals.

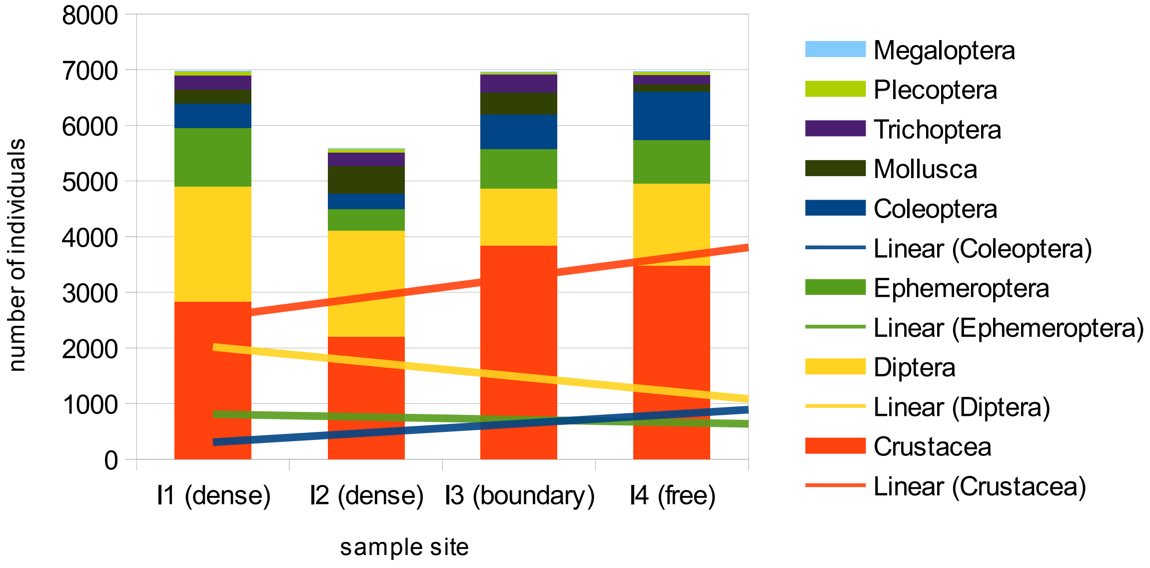


Figure 21: Macrobenthos in the Inde in 2015 (crayfish densities in brackets)

The fact that crayfish densities did not significantly rise in the densely populated areas, due to an obvious population maximum, but did so in the formerly un- or merely thin populated areas, allows for another explanation of the balanced macrobenthos numbers. They might be due to progressively leveling crayfish numbers rather than a recovery of benthic communities. However, the overall numbers of benthic invertebrates rose throughout the study. Starting at 16,078 individuals in 2013, 27,041 in 2014 and 26,500 in 2015, where the autumn sample had not been taken, so approximately another half of that number would have to be added to get the true 2015 numbers (For the Pearson correlation in Fig. 20, 39,750 individuals were assumed.).

Crustacea and Coleoptera still showed a negative reaction to rising signal crayfish densities. Coleoptera lost 28 % in the boundary site and averagely 59 % in the densely populated sites (r = -0,97). Crustacea were most frequent in the boundary site and the initially free site (I4) – the sites in which crayfish densities had not reached a maximum yet, loosing between 23 % and 40 % in the densely populated area. Their negative correlation to the crayfish flattened during the course of the study. While the correlation was significantly negative in 2013 (r = -0.99), it decelerated to r = -0.73 in 2014 and r = -0.72 in 2015. However, this group's response to signal crayfish density remained negative during the whole study.

Diptera on the other hand showed a converse reaction. After loosing 31 % in the boundary site, they gained 29-41% in the densely populated areas. The population of Diptera generally seems to be subject to strong fluctuations. While they were still heavily negatively impacted in 2013 (r = -0.85), this turned into a positive reaction in 2014 (r = 0.79), which remained in 2015 in a slightly weaker form (r = 0.63). Overall, therefore, the influence of signal crayfish on this group must be considered questionable, but a positive trend is obvious – be it mediated by the crayfish itself or by the corresponding decimation of other groups.

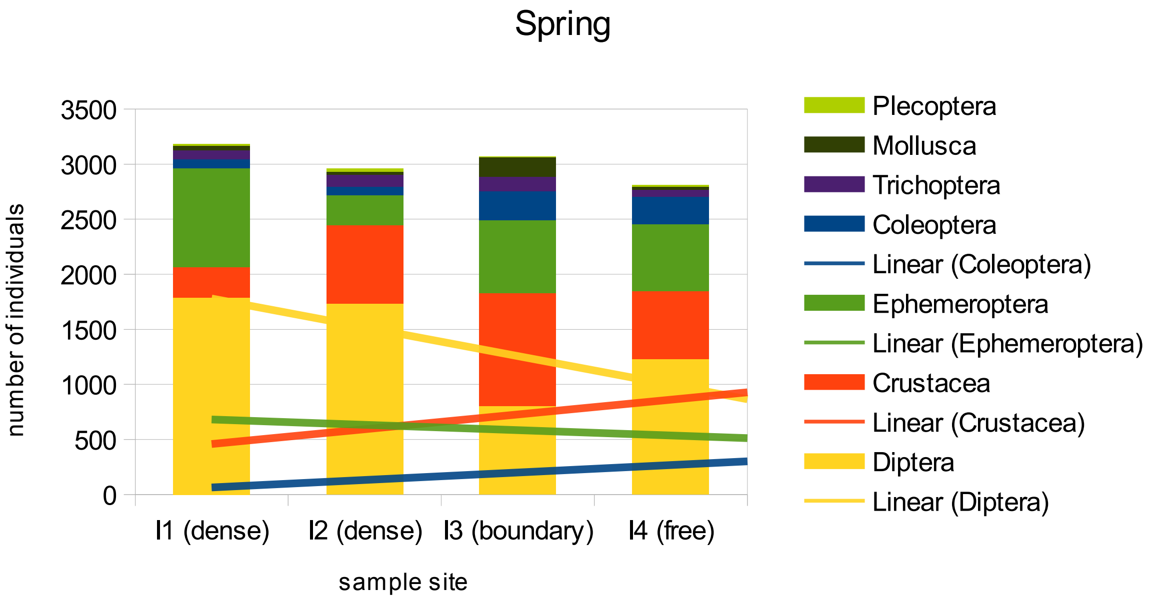
Ephemeroptera showed no significant negative reaction in 2015. They had their lowest count in one of the densely populated areas, but their highest in the other with a negligible correlation value of r = -0.09. In previous years, they were unaffected (2013) or negatively affected below the level of significance, so it is fair to assume that they are not likely to be affected by the signal crayfish overall. The other groups (Megaloptera, Plecoptera, Mollusca, Trichoptera) were only present in small numbers. Their reactions showed a positive tendency. It can be assumed that the increasing positive trend of the megaloptera (r = 0,89), in 2015 is due to the extrapolation of the sample size (no autumn sample in 2015) and small numbers of the group. In the previous years, they had shown no correlation with signal crayfish density (R = 0.11 to r = 0.15) due to their very low numbers. Mollusca (r = 0,70) showed a positive tendency, which is within range of their previous reactions to crayfish (r = 0.64 in 2014). In 2013, no correlation could be established (r = -0.03). However, a positive correlation is quite possible. As long as the snails are not predated by crayfish, the latter can promote algae growth by resuspending sediment, which in turn can lead to better conditions for grazers. Plecoptera (r = 0.46) and Trichoptera (r = 0.45) 2015 showed about the same, slightly positive influence. This is surprising for Plecoptera, since they had previously been negatively affected (r = -0.67 in 2013 and r = -0.73 in 2014). Again, it can be assumed that the influence of crayfish has not fundamentally changed, but was distorted by the extrapolation of the sample volume. Trichoptera already fluctuated strongly before (between r = -0.26 in 2013 and r = 0.58 in 2014). Here, it can be assumed that the signal crayfish does not signifivantly affect the group.

Seasonal Macrobenthos Sample Results 2015 (Fig. 22)

In spring 2015, macrobenthos stocks were almost completely even in all four sites. Just like in the year before, Diptera were turning up in massive numbers appearing to be positively related to crayfish densities, reaching their highest numbers in the densely populated sites, loosing 55 % in the boundary and 31 % in the initially free site. Ephemeroptera showed no significant reactions, while Crustacea and Coleoptera again seemed negatively affected. However, negative effects were not as strong as they had been in the beginning of the study. Crustacea showed averagely 40 % less individuals in the high crayfish density sites than in the sites with lower densities. Coleoptera on the other hand lost averagely 69 % in the high density sites – just like they did from the beginning. Both groups proliferated throughout the study. The other less common groups like Plecoptera, Mollusca and Trichoptera were not visibly influenced.

The summer sample of 2015 revealed quite similar results as the spring sample. Differences between sample sites were mostly due to quantitative variations in Crustacea – the by far most common group. They broke down in the utter densely populated site by 48 % compared to the initially free site I4. In the inner dense population site as well as the boundary site, their losses only amounted to 11 % and 2 % respectively, showing that their negative reaction became a lot more flat than it was in the beginning. However, one has to keep in mind that with spreading signal crayfish, their differences in density between the sites became a lot more flat as well. Coleoptera remained negatively influenced, loosing 41 % in the boundary and averagely 55 % in the densely populated sites. Just like Crustacea, they had their population minimum in the utter densely populated site I2. Interestingly, Mollusca had their maximum in this site which points to an interaction between these groups. Them and all other less common groups (Megaloptera, Plecoptera, Trichoptera, Diptera, Ephemeroptera) showed no significant crayfish reaction. Diptera broke down again in the summer months, like they did every year, loosing any positive or negative relation to crayfish. Apart from the Crustacea numbers, gerneral invertebrate numbers were strikingly even. The utter densely populated site saw a decrease of many groups that was filled up again by Mollusca, which seemed to profit from the retreat of other groups.

Overall, visible negative influence of signal crayfish density on benthic invertebrates diminished during the course of the study. Since crayfish density rose in the formerly uninhabited areas, approaching the density in the longer invaded areas, this is not surprising. However, invertebrate numbers rose throughout the study, gaining 68% in 2014. In 2015, the numbers from the year before were almost reached by just two of the usual three yearly samples, which means that an overall quantitative growth of approximately 90% is to be expected for the whole study. Crayfish densities did only rise in the formerly unpopulated or low populated sites, but not in areas where the population had already reached its maximum. These facts lead to the conclusion, that crayfish affect macrobenthos in a negative way when they hit a stretch of river but do not lead to decreasing numbers on the long run since individual numbers of other invertebrates did not diminish but rise with time. The composition of these communities changed though, since some groups were affected more than others.



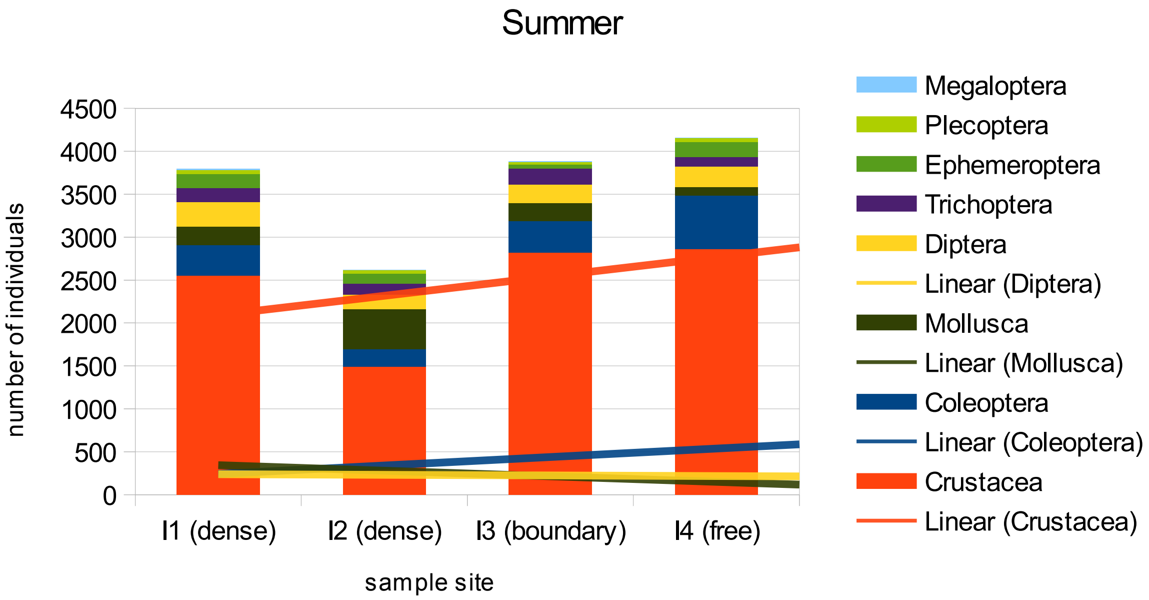


Figure 22: Macrobenthos in the Inde over the two seasonal samples 2015

Statistical analysis of the macrozoobenthos results of the Inde

To further elucidate possible relationships between invertebrate and crayfish densities, the data were analyzed further. First, a detrended correspondence analysis was calculated. The length of gradient was determined at <2, so that a linear analysis was appropriate. Then a PCA was applied with default settings and actual crayfish density as an overlay variable. The analysis yielded two ordinal axes: X and Y with inherent values of 0.572 and 0.259 respectively, the Y-axis not being very meaningful. Figure 23 shows the resulting ordination chart.

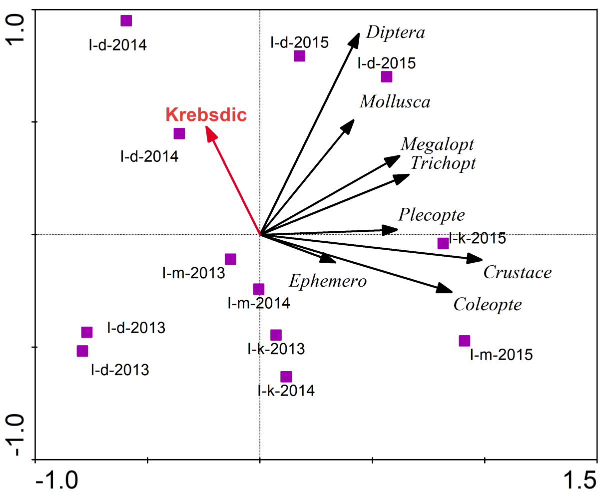


Figure 23: PCA Ordination chart for the Inde (crayfish desity as overlay variable)

Crayfish density appeared to be most negatively correlated with densities of Ephemeroptera and Coleoptera, hence crayfish seem to have the strongest negative effect on these groups. This had already been visible with respect to Coleoptera, which have been signifivantly negatively effected even at low densities. Ephemeroptera on the other hand, as stated above, appeared rather uninfluenced as long as they existed in only smaller proportions. The analysis shows that the negative influence on them as soon as they reached higher numbers within the general macrobenthos population might be going deeper than becomes visible at first sight. The negative effect of crayfish on Crustacea – even so diminishing within the course of the study - was also backed up by the analysis.

The small numbers of Plecoptera that seemed negatively affected throughout the study but showed a tendency towards a more positive development in the last study year, were also corroborated as rather negatively influenced. Trichoptera, Megaloptera and Mollusca, which were claimed to be uninfluenced, showed no positive or negative relation to crayfish density in the analysis as well.

The seemingly positive reaction of Diptera to crayfish presence was also underlined, showing an indeed positive reaction of this group to growing crayfish densities.

3.2.2 General effects in the Ahr

In contrast to the Inde, the spread of the signal crayfish in the Ahr was observed on the upstream end of the population, moving from sample site A1 (inner densely populated site) against the current towards A4 (initially crayfish free). Therefore, the spreading rate should be slower than in the Inde, where floods and less required migration energy downstream could lead to faster diffusion of the crayfish population. Also, it has to be taken into account that the initially crayfish free site A1 had to be moved in the beginning of 2014, suffering massive sediment deposition due to flooding in spring 2013. Since the next comparable site was only to be found several kilometers upstream, it was to be expected that the new A1 site would not be populated by crayfish during this study.

Sample site	**Crayfish**	**Mollusca**	**Crustacea**	**Ephemeroptera**	**Plecoptera**	**Coleoptera**	**Megaloptera**	**Trichoptera**	**Diptera**	**Total**
A-k-2013	1	73	3	359	3	72	19	905	4549	5983
A-m-2013	1	860	0	317	7	60	1	4022	2683	7950
A-d-2013	6	22	28	1141	8	58	0	336	858	2451
A-d-2013	28	24	5	505	1	37	5	1761	1262	3600
	r	**-0,45**	**-0,03**	**0,05**	**-0,67**	**-0,94**	**-0,19**	**-0,11**	**-0,56**	**-0,53**

Figure 24: Pearson correlation of signal crayfish and macrobenthos density 2013 (sample site code: a = Ahr, k = crayfish free site, m = boundary site, d = densely populated site)

Throughout 2013, crayfish impact on macrobenthos in the Ahr appeared to be negative, however, the negative influence was not as strong as at the Inde (r = -0.53) and no group reached statistically significant values within the negative impact (Fig. 24), when correlated to the actual signal crayfish count. Figure 25 shows the overall macrobenthos sample results of 2013. It suggests that signal crayfish have had a very strong negative impact on the most common groups Diptera and Trichoptera. It should be noted, however, that fewer crayfish were actually caught in sample site A4 this year than in A3 (see Fig. 24). Diptera lost 41 % in the population boundary site A2 (1 crayfish caught in summer 2013), 72 % in the utter densely populated site A3 (28 crayfish caught in summer 2013) and even 81 % in the inner densely populated site A4 (6 crayfish caught in summer 2013). In comparison to the actual crayfish count within the sites, the correlation value only reached r = -0.56, which is negative but far from significant. Trichoptera as the second most common group seemed to be influenced not only by the crayfish but also by the sediment deposition in sample site A1. They turned up in their highest numbers in the boundary sample site A2, with small crayfish numbers and no sedimentation. Compared to this, they lost 56 % in the utter and 92 % in the inner densely populated site and 77 % in the crayfish free A1 site with a thick fine sediment cover on the river bed. Here, too, the smaller number of crayfish within the inner densely populated area must be taken into account. The actual correlation value of this group is only r = -0.11, so there is no correlation with the actual crayfish densities at all (Fig. 24). The third most common group of Ephemeroptera on the other hand, had their highest numbers within the dense crayfish population. Compared to the boundary and crayfish free sites, they gained 49 % in the utter densely populated site and more than tripled (factor 3.4) in the inner and longest crayfish populated site. This development was probably due to the decrease of other groups in this site. However, the Pearson correlation showed that their numbers were not related to the actual crayfish density in any way (r = 0.05, Fig. 24). Mollusca played a subordinate role in almost all sites, but could develop very well in the boundary site A2, which showed the highest macrobenthos numbers in general. Since the crayfish free site A1 was covered in sediment, not allowing for any algae growth that could have nourished the dominating snails, it appears very likely that Mollusca can be seen as negatively influenced by crayfish as well (their correlation value only showed an insignificant, but still negative r = -0.45). Sample site A1 was not suitable for them with or without crayfish. Megaloptera only played a subordinate role. They were found clearly in their highest numbers in the crayfish free sample site – showing 19 individiuals, but only 1 in the boundary site and 5 or 0 respectively in the densely populated areas. However, their density showed no correlation to the actual crayfish density (r = -0.19). Coleoptera in the Ahr appear not influenced by crayfish densities in Fig. 25, but actually had a correlation value of r = -0.94 with respect to actual crayfish numbers (Fig. 24). Crustacea only turned up in considerable numbers in the longest crayfish populated site A4 (28 individuals versus 0 to 3 in the other sites) and expectedly, did not show any correlation to signal crayfish density (r = -0.03).

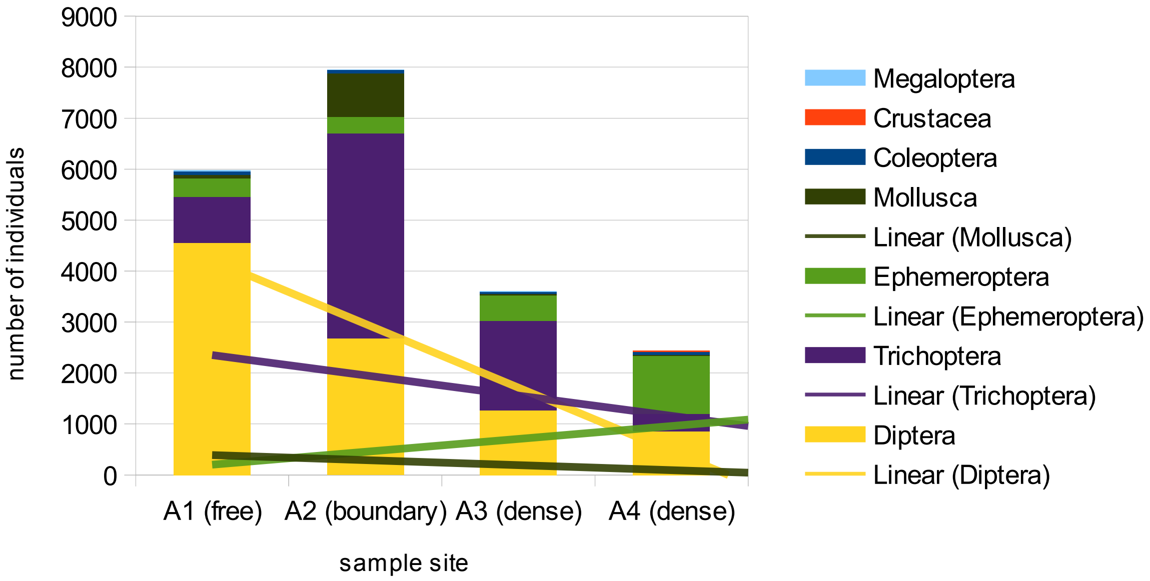


Figure 25: Macrobenthos in the Ahr 2013 (crayfish densities in brackets)

Seasonal Macrobenthos Sample Results 2013 (Fig. 26)

Compared to the Inde, seasonal fluctuations of macrobenthos numbers in the Ahr were only minor. In spring, Diptera were the by far most common group and as such clearly negatively influenced by crayfish. They also seemed to thrive in the sediment affected crayfish free site A1, which is not surprising since many of the species contained in this group were free swimming, not being directly influenced by sediment covered substrates. Compared to the A1 site, they showed losses of 74 % in the boundary site and averagely 67 % in the densely populated areas with the biggest loss (-79 %) in the site that had been invaded the longest. This leads to the suspicion that the signal crayfish is a direct predator to Diptera in the Ahr. Compared to Diptera, all other groups played only very subordinate roles. Trichoptera however still showed negative tendencies with respect to crayfish, loosing 12% in the boundary site and averagely 56 % in the densely populated ones. Coleoptera reacted very similar with 33 animals in the crayfish free site but only 6 to 9 in the boundary and densely populated sites. Ephemeroptera showed no reaction that could be related to crayfish colonization. Since crayfish densities in the boundary site appeared fairly low in 2013 but many invertebrate groups still showed considerable losses in this site, it has to be taken into consideration that invertebrates rather reacted to the sedimentation in the A1 site than to signal crayfish.

During the summer months, Diptera remained the dominating group and they remained negatively impacted. Compared to the spring samples, their numbers increased in all sample sites and recovered in the boundary site, where only a loss of 26 % could be registered (74 % in spring). However, losses of averagely 84 % in the densely populated sites now pointed towards a clear negative effect of high crayfish densities. Sedimentation had slightly subsided in the A1 site and was not an issue on the population boundary site A2 so the decline could not be explained by any other factor than the crayfish. Trichoptera showed a negative impact as well as Mollusca, which were still hardly present in the crayfish free A1 site due to lack of algae growth as well as in the densely populated sites, but proliferated in the boundary site with low crayfish numbers and no sedimentation. Coleoptera were negatively influenced in the Ahr just like they were in the Inde. They only occurred in very small numbers showing 15 animals in the crayfish free site, 20 in the boundary site (which did not yield more crayfish than the initially free site in the summer trapping 2013 - namely 1 individual) and between 4 and 5 in the densely populated sites. Since this resembles the result of the spring sample, it appears to be a fixed tendency nonetheless. Megaloptera, which were not found at all in the spring sample, turned up in small numbers and negatively affected by crayfish (18 in the free, 1 in the boundary and 0 to 2 in the densely populated sites). Interestingly, the second most common group – Ephemeroptera – showed a positive correlation to crayfish densitites. They turned up in the sample with 155 individuals in the free, 208 in the boundary and 286 in the utter densely populated site. In the inner, longest populated site, they showed an increase by the factor 4.3 (665 individuals) compared to the free site.

Autumn 2013 saw a breakdown of the formerly dominating Diptera and their replacement by Trichoptera, which rose to be the by far most frequent group. They could not develop in the crayfish free site with the flood induced sedimentation but reached their highest numbers (3892 individuals in the sample) in the boundary site, with just as little crayfish colonization and normal substrate conditions. Their population decreased by 54 % in the utter densely populated site and even 93% in the densely populated and longest invaded inner densely populated site. The crayfish free but sediment damaged site A1 had 79 % less Trichoptera in it than the boundary site without this damage. This tendency was already visible in the summer, while in spring – immediately after the sediment had been washed into the A1 site – Trichoptera had still been most frequent in this site, followed by the boundary site A2. This leads to the conclusion that sedimentation had a great impact on Trichoptera. Compared to their density peak during the summer months, Diptera broke down to only 9 % of their former numbers remaining slightly negatively influenced by crayfish densities. The breakdown of this group in autumn appeared in both autumn samples – 2013 and 2014 – and seems to be a normal seasonal effect. Ephemeroptera remained the second most common group even with some space to the leading Trichoptera and the remained in positive correlation to crayfish densities. Since they held this trend throughout the year, it appears to be constant.

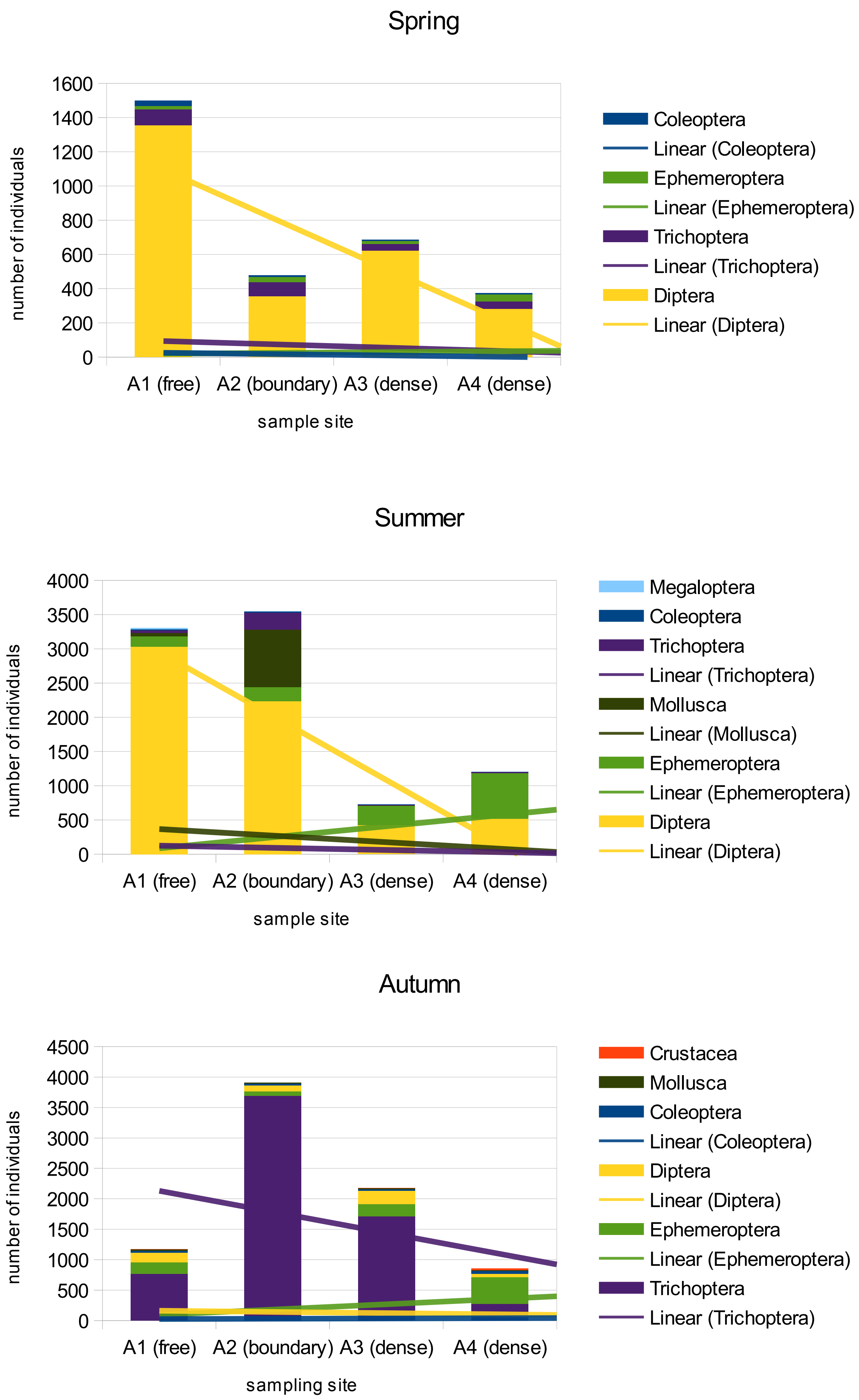


Figure 26: Macrobenthos in the Ahr over the three seasonal samples 2013

In the beginning of 2014, the heavily sedimented crayfish free site A1 was moved further upstream to reach comparability with the other sites again. It also meant that the site was moved further away from the current crayfish population boundary, making it improbable for the site to get invaded within the study. The new A1 site was physically comparable to the other sites in the Ahr, but had the highest degree of shading compared to the other sites. This was due to the geographical makeup of the surrounding valley and the fact that it had no treeline with ensuing meadow on one site, like it was found in all three other sites, but an actual strip of forest comparable to up to three treelines in a row, before the meadow ensued.

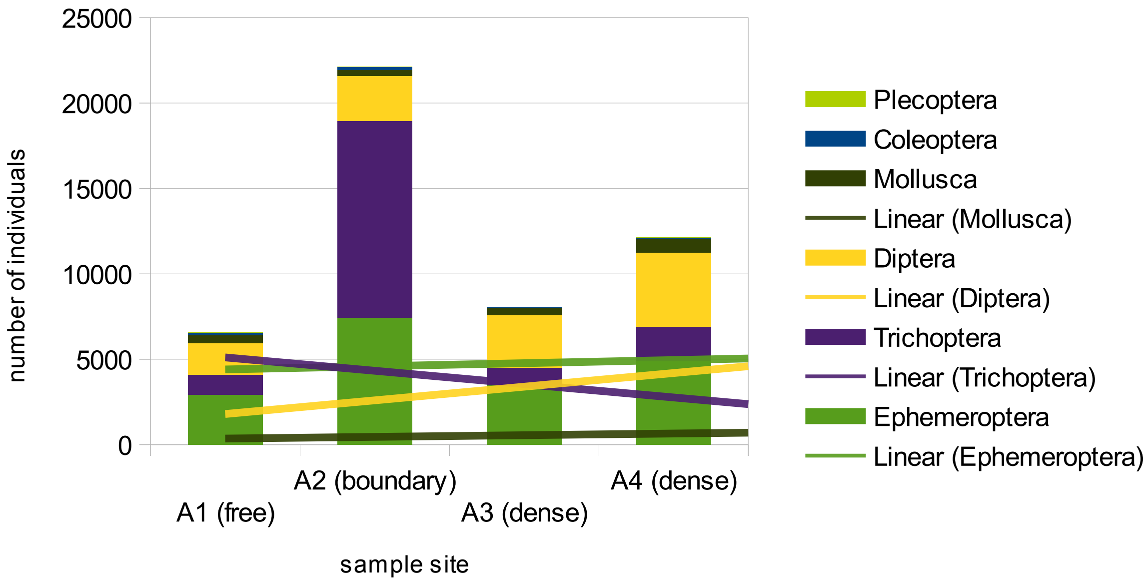


Figure 27: Macrobenthos in the Ahr in 2014 (crayfish densities in brackets)

As figure 27 shows, the new location showed deviations from the other three sites and had the lowest invertebrate count. The boundary site with the most light falling on it during mid day time, always had the most individuals in it. Nonetheless, the site was kept in place, since there was no suitable alternative in the closer vicinity.

Sample site	Crayfish	Mollusca	Crustacea	Ephemeroptera	Plecoptera	Coleoptera	Megaloptera	Trichoptera	Diptera	Total
A-k-2013	1	73	3	359	3	72	19	905	4549	5983
A-m-2013	1	860	0	317	7	60	1	4022	2683	7950
A-d-2013	6	22	28	1141	8	58	0	336	858	2451
A-d-2013	28	24	5	505	1	37	5	1761	1262	3600
	r	**-0,45**	**-0,03**	**0,05**	**-0,67**	**-0,94**	**-0,19**	**-0,11**	**-0,56**	**-0,53**
A-k-2014	0	457	1	2937	11	155	0	1154	1849	6564
A-m-2014	4	350	0	7430	13	190	1	11522	2640	22146
A-d-2014	22	423	9	3485	6	47	5	1003	3104	8082
A-d-2014	23	772	1	4863	13	68	7	2047	4353	12124
	r	**0,59**	**0,57**	**-0,15**	**-0,37**	**-0,93**	***0,98***	**-0,42**	**0,87**	**-0,21**

Figure 28: Pearson correlation of signal crayfish and macrobenthos density 2013 to 2014 (significant correlations in italics, sample site code: A = Ahr, k = crayfish free site, m = boundary site, d = densely populated site)

In contrast to 2013, Diptera appeared slightly positively influenced by crayfish in 2014 – a trend that remained in all four sample sites and could not be related to the move of the A1 site. It was also confirmed by the Pearson correlation (Fig. 28). The negative correlation to the actual crayfish density detected this year was statistically not yet significant, but positive (r = 0.87). They had their highest frequency in the inner densely populated site, diminishing upstream towards the crayfish free site A1, where only 42 % of their original densities remained. Mollusca played only a minor role in the community but showed slightly positive correlations to signal crayfish as well with their highest numbers in the inner densely populated site. However, their second highest frequency could be found in the unpopulated site, while the boundary site with only small crayfish numbers had the lowest Mollusca count, even so it had the least shadowing and therefore best prerequisites for algae growth and a high snail population. A fact that was verified in 2013, when this site had by far the highest Mollusca population of all four sites. The Pearson correlation showed a slightly positive correlation to actual crayfish density (r = 0.59) after a slightly negative correlation was observed in 2013 (r = -0.45). The massive variations of this group lead to the conclusion that they might be influenced by several factors that can not be identified without a doubt. Ephemeroptera remained uninfluenced by crayfish (r = 0.05 in 2013, r = -0.15 in 2014). Trichoptera were only slightly negatively affected (r = -0,42), showing their highest numbers in the boundary site A2, where in the 2014 summer trappings 0-4 crayfish were caught. Compared to this, their numbers in the densely populated sites were reduced by 82 to 91 %, showing a stronger decrease than in the year before. Plecoptera and Coleoptera were groups that only represented a very small part of the community as a whole. Nonetheless, Coleoptera remained strongly negatively influenced by crayfish. The impact in 2014 (r = -0.93) remained about as strong as in 2013 (r = -0.94). Compared to their highest frequency in the boundary site, they lost averagely 70 % in the densely populated sites. In the crayfish free site, their counts were still 18 % lower than in the boundary site which might be due to the higher degree of shading which seemed to influence several invertebrate groups. Plecoptera showed no visible relation to crayfish densities (r = -0.37). The only group whose influence by the crayfish reached statistical significance in 2014 was the Megaloptera (Fig. 28), which occurred in such small quantities that the apparent positive correlation (r = 0.98) was probably due to chance. This is also supported by the fact that in 2013 – also in very small amounts - they were not correlated with crayfish density (r = -0.19). Overall, the negative impact of signal crayfish on macrozoobenthos in 2014 (r = -0.21) was significantly lower than in 2013 (r = -0.53). The negative correlation was so small that it can be considered void.

Seasonal Macrobenthos Sample Results 2014 (Fig. 29)

Seasonal variations turned out to be very strong in 2014. The spring sample still showed a negative influence of the signal crayfish on the invertebrate community – except the crayfish free site A1 which yielded the smallest numbers of all sites. In contrast to spring 2013, Diptera did not dominate the community as much as they did then. They and Ephemeroptera shared the top rank of the most frequent groups. Even so the reactions of Ephemeroptera to signal crayfish invasion appeared to be positive overall, a closer look at the results for each sample site showed that they were rarest in the crayfish free site (228 individuals) but most frequent in the boundary site (1988 individuals), from where they diminished with crayfish density and increasing timespan in which the sites had been invaded (1600 in the utter and 924 in the inner and longest densely populated site). In the crayfish free site, it was the massive breakdown of

Ephemeroptera that lead to the smaller macrobenthos numbers in the first place. They only showed up with 11 % of their boundary-site-densities. Since the boundary site had only 0-4 crayfish in a catch in 2014, it appears unlikely that crayfish densities lead to the decline. Therefore it can be expected that something else triggered the breakdown. If the crayfish free site is left out of the analysis, Ephemeroptera seem to be rather negatively affected by crayfish densities, even so they showed positive reactions in the former samples. Diptera showed negative reactions as well, but they were less extreme than in the year before (averagely -32 % in the densely populated sites compared to the boundary site). Just like Ephemeroptera, they showed their main decrease (-36 %) in the crayfish free site A1. But since the site had not much less crayfish than the boundary site, it appears improbable that crayfish densities caused the decline. The proportion of Trichoptera was a lot smaller and their reaction to crayfish inconclusive. They showed their highest count in the boundary site, their lowest in the crayfish free site. The densely populated sites lay in between. Mollusca as a group of small proportion were negatively influenced throughout all 4 sites with their highest count in the unpopulated site A1 (304 individuals) and only 33 % of their original density (100 individuals) in the boundary site, followed by 14 % (44 individuals) to 11 % (32 individuals) into the denser signal crayfish population. The very small numbers of Coleoptera showed a negative crayfish reaction as well – with the same deviation in the crayfish free A1 site with the highest degree of shading that was visible in Ephemeroptera and Trichoptera. Overall, crayfish effects in spring 2014 appeared to be negative, but a high degree of shading in the transferred A1 site seemed to play an even bigger role with regards to benthic communities than crayfish densities.

Summer saw an almost complete reversal of results with the highest invertebrate counts in the longest densely populated crayfish site A4 – deepest into the population – and the lowest count as in spring within the crayfish free site A1. As often, the summer sample revealed the highest invertebrate counts in 2014.

The high invertebrate numbers in the densely populated sites were mostly due to a massive proliferation of Diptera with 1851 to 3080 animals in the densely crayfish populated sites and only 600 to 651 (averagely 20 % of the highest frequency in the A4 site) animals in the boundary and unpopulated sites. The fact that the breakdown took place in the boundary site as well as the unpopulated one, leads to the conclusion that degree of shading was not the decisive factor in this case. Diptera had shown the opposite reaction before – a clear negative effect of signal crayfish independent of sedimentation or other possible factors. After these negative effects had been less extreme in spring 2014, they turned around completely in summer. Astonishingly, Mollusca showed the same reaction. Having been mostly negatively influenced by crayfish in the samples before (even so in much smaller numbers than Diptera), they now showed a positive response independent from shading effects etc. 699 snails were found in the site deepest into the crayfish population, but only 43 % of that count in the utter densely populated site, 27 % on the boundary and only 15 % in the crayfish free site. Again, degree of shading and the connected algae growth could not be a decisive factor since the lowest degree of shading was found in sites A4 and A2. If shading would have had an influence, the difference between both sites would not have been this big. The main difference between both was the number of crayfish inhabiting them. The number of Trichoptera in the sample grew to higher proportions in summer. Crayfish effects did not become visible. Instead, Trichoptera seemed to be related to degree of shading, showing their highest numbers in the sites with the most light, namely the boundary site A2 (1965 animals) as well as the densely and longest populated site A4 (1436 animals). They had their lowest count in the crayfish free A1 site with the highest degree of shading (309 animals). This trend has been visible to a lower degree in spring 2014 as well, but not back in 2013, when crayfish seemed to be the major factor influencing their densities. Ephemeroptera showed a similar pattern with their highest numbers in the areas with the lowest degree of shading (2192 animals in the boundary site A2, 1906 animals in the densely and longest populated site A4). However, in the areas with more shading and less light, they were more common in the site with no crayfish (site A1, 1448 animals) than in the site with a little more light but high crayfish densities (site A3, 813 animals). Coleoptera and Plecoptera where only showing up in very small numbers, as usual. And while Plecoptera did not show any relation to crayfish densities at all, Coleoptera were negatively influenced, loosing 24 % in the boundary site and 76-80 % in the densely populated sites, showing their usual reaction from the former samples.

Autumn saw the usual seasonal decline of the invertebrate community but at the same time a huge proliferation of Trichoptera in the boundary site A2. The proliferation of this group during the autumn months occurred in every year, but this time differences between the sites were massive. 9101 Trichoptera were caught in the A2 boundary sample site alone, showing an almost five-fold increase, while the second highest number was found in the crayfish free site A1 with only 649 animals. Their numbers had still more than doubled compared to the summer months. The crayfish populated sites A3 and A4 contained the smallest numbers of Trichoptera with 237 and 279 individuals respectively, and their numbers did not follow the seasonal proliferation pattern and went down instead and showed only half of their summer count in A3 and a fifth in A4. The second main part of the invertebrate community were Ephemeroptera which also turned up in the boundary site in their highest numbers (3250 animals). They seemed to be more dependent on good lighting conditions, since the areas with the most light had the most animals of this group, but not related to crayfish densities. Other groups only appeared in small numbers. Coleoptera remained negatively influenced with their highest population in the boundary site A2 (69 animals), closely followed by the crayfish free site A1 (52 animals), loosing averagely 55 % of their original numbers in the densely populated sites A3 (20 animals) and A4 (34 animals). Mollusca were not visibly influenced by crayfish densities. Diptera showed a slightly negative reaction, also with their highest densities in the boundary site and the lowest in the crayfish population sites A3 and A4, but the differences were minor and since their densities were rapidly dropping towards autumn, variations might be due to the seasonal overall decline of this group.

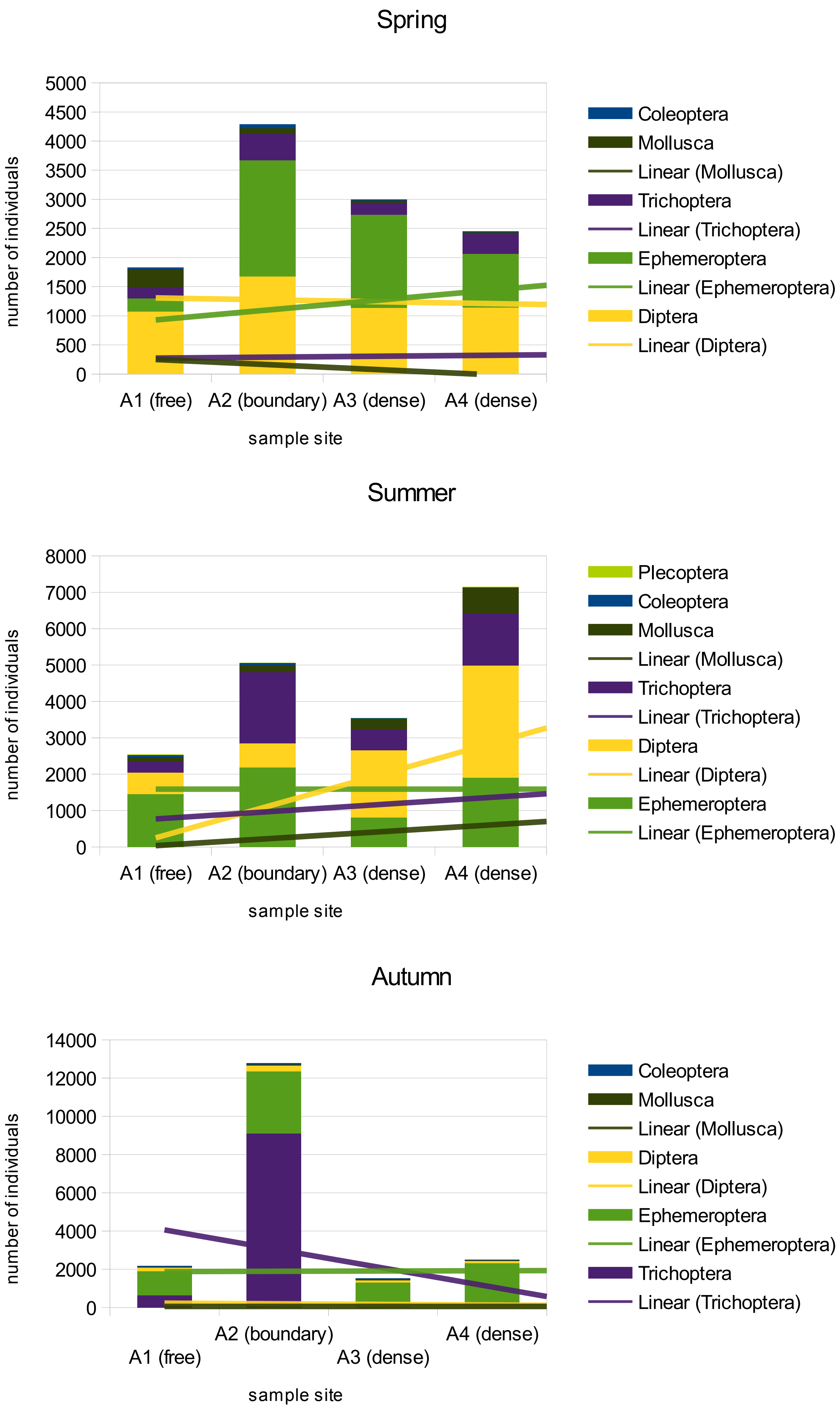


Figure 29: Macrobenthos in the Ahr over the three seasonal samples 2014

Figure 30 shows the overall results of the Ahr benthos samples in 2015. Note that this overview was created from only 2 samples instead of the usual 3. Only the spring and summer sample were taken before the project came to an end. Hence, Trichoptera play only a minor role, since they always reached their highest numbers in autumn, which is missing from this overview.

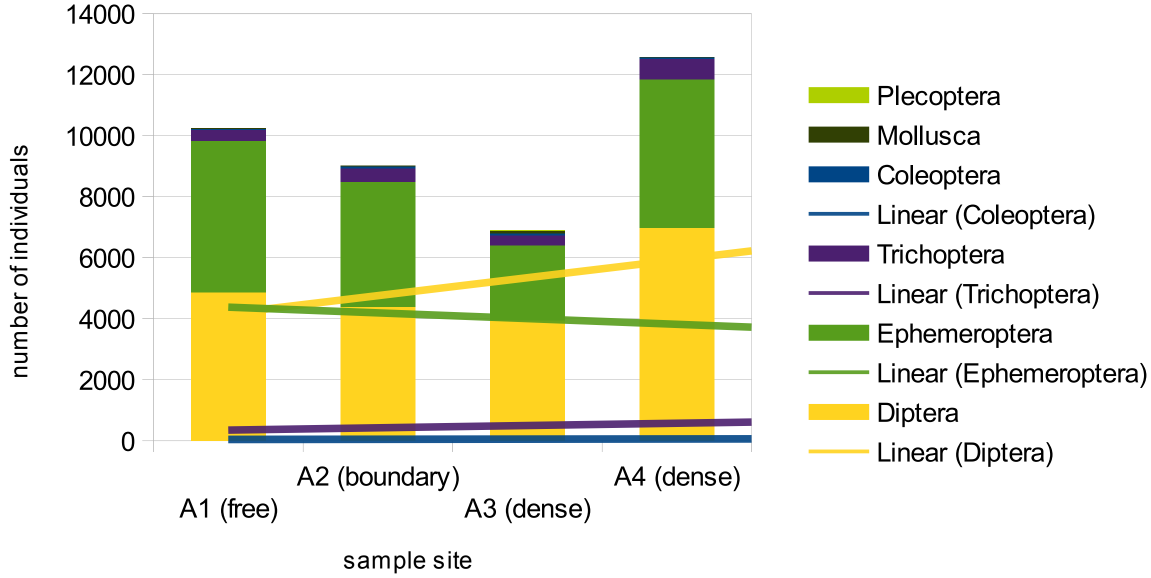


Figure 30: Macrobenthos in the Ahr 2015 (crayfish densities in brackets)

In Figure 31, on the other hand, the numerical values were extrapolated (X/2x3) to compensate for the missing autumn sample.

Sample site	Crayfish	Mollusca	Crustacea	Ephemeroptera	Plecoptera	Coleoptera	Megaloptera	Trichoptera	Diptera	Total
A-k-2013	1	73	3	359	3	72	19	905	4549	5983
A-m-2013	1	860	0	317	7	60	1	4022	2683	7950
A-d-2013	6	22	28	1141	8	58	0	336	858	2451
A-d-2013	28	24	5	505	1	37	5	1761	1262	3600
	r	**-0,45**	**-0,03**	**0,05**	**-0,67**	**-0,94**	**-0,19**	**-0,11**	**-0,56**	**-0,53**
A-k-2014	0	457	1	2937	11	155	0	1154	1849	6564
A-m-2014	4	350	0	7430	13	190	1	11522	2640	22146
A-d-2014	22	423	9	3485	6	47	5	1003	3104	8082
A-d-2014	23	772	1	4863	13	68	7	2047	4353	12124
	r	**0,59**	**0,57**	**-0,15**	**-0,37**	**-0,93**	***0,98***	**-0,42**	**0,87**	**-0,21**
A-k-2015	1	30	0	7440	0	30	0	564	7296	15360
A-d-2015	5	18	0	7320	0	54	0	1008	10458	18858
A-m-2015	7	30	0	6120	0	96	0	684	6594	13524
A-d-2015	25	138	0	3696	36	96	0	474	5916	10356
	r	***0,96***		***-0,98***	***0,97***	**0,72**		**-0,5**	**-0,53**	**-0,78**

Figure 31: Pearson correlation of signal crayfish and macrobenthos density 2013 to 2015 (significant correlations in italics, sample site code: A = Ahr, k = crayfish free site, m = boundary site, d = densely populated site)

With these results, the tendency of the boundary site A2 to always have the most invertebrates in the yearly overview was broken for the first time, after already slightly weakening in 2014. For the first time, this year more crayfish (7) were caught in this site than in the densely populated A4 sample site (5 crayfish – also note the different sequence of sites in the Pearson correlation, Fig. 31). The location with the most crayfish (25 animals caught in A3) clearly had the lowest number of invertebrates and the negative influence of the crayfish in the Ahr reached its maximum value of r = -0.78 in 2015. Although statistically this value is still not significant, it is lower and thus more pronounced than in previous years. Within the the 3 upstream sample sites, invertebrate numbers decreased with rising crayfish densities. A reason why crayfish would prefer the upstream "densely populated" site A3 over A4 could not be identified without a doubt, but the fact that a few thousand crayfish were extracted by local anglers from the A3 site directly above without a visible population decline in the sample trappings might be a reason for lower densities downstream since the animals would move upstream towards the vacant territories. A single crayfish was found in the initially crayfish free site – even so the colonization of this site appeared extremely unlikely due to its distance to the crayfish population and the structure of the water body in between. It would appear that the mostly low crayfish count – due to whatever reason – allowed the invertebrate community to redevelop stable numbers in 2014 and keep them in 2015. While the site hardly contained 2,500 invertebrates in 2013, it constantly had around 12,000 in 2014 and even over 18,000 (extrapolated) in 2015. An upstream movement of the crayfish density peak would offer an explanation for the development within the sites.

Diptera had the highest proportion in 2015 and they were mostly responsible for the overall quantitative picture of the invertebrate community. They followed the actual crayfish densities, showing their second highest numbers in the the unpopulated site (4864), followed by the boundary site (4396) and their lowest in the utter densely populated site (3944). Their highest number (6972) was then displayed in the inner densely populated site which was the one deepest into the crayfish population but did not yield high crayfish numbers since 2014. The tendency of their reaction seems to be negative, but reached only an insignificant correlation value of r = 0.53. Their slightly negative reaction in 2013 and the strongly positive one in 2014, make it doubtful that there even is any connection of Diptera densities to crayfish densities.

In contrast, Ephemeroptera were significantly negatively affected by crayfish densities in 2015 (r = -0.98), after appearing completely unaffected in 2013 (r = 0.05) and 2014 (r = -0.15). Their highest numbers 2015 were found inside the crayfish free A1 site, though closely followed by the inner "densely populated" A4. Taking the current crayfish distribution into account, Ephemeroptera followed exactly the actual crayfish numbers and showed a clearly negative reaction. However, their reactions in the previous years were just as inconstant as those of Diptera.

Trichoptera only played a subordinate role due to the missing autumn sample that would usually yield the highest Trichoptera densities. Just like Diptera, they had their highest densities in the inner densely populated site A4 (672), followed by the boundary site (456). Even at smaller numbers due to the missing sample, the relation between sites remained. The most animals were found in the sites with the best lighting conditions. Among the sites with a higher degree of shading, namely the crayfish free site A1 and the utter densely populated site A3, A1 reached the highest densities (376 – 316 in the densely populated A3). Trichoptera therefore appeared to be influenced by crayfish densities only in second place. Lighting conditions seemed to play the major role. The Pearson correlation showed a value of r = -0.5. The negative correlation with crayfish was therefore still negligible, although it reached its lowest value in 2015 compared to previous years.

Coleoptera only occurred in small numbers and were slightly positively influenced by crayfish densities (r = 0.72). Their numbers were lowest in the crayfish free site A1, which had always been the one with the highest Coleoptera count before. They were clearly negatively affected by crayfish in the years before (r = -0.93 to -0.94). This might be a sign for an actual change within the invertebrate community.

Mollusca displayed a siginificantly positive reaction (r = 0.96), occuring in their highest numbers in the densely populated A3. Their counts as well have always been inconstant pointing towards a slightly negative reaction in 2013 (r = -0.45) and slightly positive (r = 0.59) in 2014. Plecoptera were only found in the site A3 with the currently highest crayfish density, therefore too displaying a significantly positive correlation value (r = 0.97), after showing decreasing negative reactions in previous years. Megaloptera and Crustacea could no longer be detected in 2015.

Seasonal Macrobenthos Sample Results 2015 (Fig. 32)

Spring 2015 showed an obvious difference of the inner densely populated site A4 in comparison to all other sites. Only 5 crayfish were caught in this site during the summer. Invertebrate numbers were significantly higher – mostly due to high numbers of Diptera (4,692) – as was reflected in the overall results of the year 2015. The other densely populated site (25 crayfish) showed a significantly lower Diptera count which was still the second highest of all sample sites (2,576). In the boundary site A2 (1,976) and the crayfish free site A1 (2,136) their numbers were slightly lower. Their significant increase in the A4 site could be connected to a decrease of the local signal crayfish stocks. Overall, in 2015, they had shown a rather negative correlation with actual crayfish numbers. Ephemeroptera were negatively influenced in the three upstream sites with their highest density in the unpopulated site (2,336), follwed by the boundary site (1,964) and the utter densely populated site A3 (1,360). However, just as Diptera, they slightly topped their count in the inner densely populated site A4 with 2,572 animals. Their numbers correspond almost exactly to the current crayfish distribution with the now reduced density in the A4 site – a significant negative impact that persisted over the full-year results. In the spring samples of the previous years however, they did not show a connection to crayfish but only to lighting conditions. Trichoptera – even so in much lower numbers – followed the same pattern with 324 animals in the unpopulated, 308 in the boundary and 240 in the utter densely populated site followed by their highest numbers (432) in the inner densely populated site. This group was showing an overall negative reaction to crayfish densities though with decreasing extent and in the full year results, they were rather uninfluenced. Coleoptera and Mollusca showed the exact opposite effect at much lower numbers. Their densities rose from the unpopulated A1 site to the densely populated A3 site just to drop again in the inner densely populated site with the currently smaller crayfish count. Mollusca were even significantly positively influenced throughout the year. Crustracea were only found in the area with the highest crayfish density (A3; 16 crayfish in the summer catch).

In the summer sample of 2015, it became obvious that the relatively high numbers in the inner densely populated site A4 were not just a variation but could be reobtained in summer. The decrease of benthic invertebrates with increasing crayfish density in the other three sites however became more prominent as well. Overall, the summer 2015 macrobenthos numbers reflect the current crayfish densities in the sample sites with the highest numbers in the free site (1 crayfish) followed by the A4 site which was considered densely populated but in fact had the second lowest crayfish count (5), followed by the boundary site (7 crayfish) and the by far lowest invertebrate stock in the densely populated A3 (25 crayfish).

Ephemeroptera and Diptera as the most common groups followed exactly the same pattern, while the much rarer Trichoptera seemed to be mostly influenced by lighting conditions again (highest numbers in sites A4 and A2 with the lowest degree of shading). Mollusca and Coleoptera as the rarest groups did not show significant reactions to crayfish densities.

Overall, results of the Ahr suggest that signal crayfish do have a negative influence on benthic invertebrates but the extent of this varies depending on the season, certain groups of invertebrates and other physical factors that can not be ruled out completely. The macrozoobenthos showed a negative correlation to the actual crayfish density within the sites throughout the 3 study-years, but unlike at the Inde it never reached statistical significance. The development of the negative influence also fluctuated. It was the strongest in 2015 (r = -0.78), but had previously shown declining tendencies. Throughout the study, the utter densely populated site A3 had the highest crayfish density, varying between 32 and 22 animals per trapping. It also had low counts of invertebrates throughout the study. The A4 site, which was located deeper into the crayfish distribution range and considered densely populated had much lower crayfish catches, mostly between 11 and 4 animals with a sinking tendency and the exception of summer 2014, where 23 animals could be caught in the second trapping only. It is possible that crayfish were drifted away from the upstream site by flooding, since the first trapping of this year as well as all the others did not yield such high numbers. Accordingly, this site had the lowest invertebrate count of all in 2013, recovering in 2014, when it had the second highest count and reached the highest count of all in 2014. At the same time, crayfish numbers decreased from 11 in the beginning to 4 to 5 in the end. Only a slight decrease, but a general sign that there might have been a higher crayfish population in the first place that was already diminishing when the study began, allowing the invertebrate community to recover. The absolute invertebrate numbers in this site did not rise but even decrease, which is due to the time the samples were taken in the specific year. Since the samples were at most a few days apart, total numbers can be disregarded since lower numbers would also occur in the other three sites, hence the ratio was the decisive factor. Except for the 2015 samples, the boundary site A2 always had the highest number of invertebrates. It combined low crayfish densities growing from 1 in 2013 (2 in the presample in autum 2012) to 7 in 2015 with a low degree of shading. In this year for the first time, invertebrate numbers mirrored the crayfish distribution, lying between a higher number in the A1 site with lower crayfish densities and the lowest number in the A3 site with the highest crayfish density. This might mean that crayfish only affect benthic invertebrates from a certain density upwards, which was reached in this site in 2015. It would also account for the higher numbers in the previously higher populated area A4 in which crayfish numbers sank but invertebrate numbers increased, taking over the former leading role of the A2 site. The crayfish free site A1 could not be assessed consistently, since it had to be moved between 2013 and 2014. Contrary to the assumptions that it would contain the highest numbers of invertebrates, the results varied greatly. In 2013 the old A1 site, compromised by flood induced sedimentation, yielded the second highest number of invertebrates, which was within expectations. When moved further upstream however, this site had the lowest count of invertebrates of all. Surprisingly, in 2015, it had

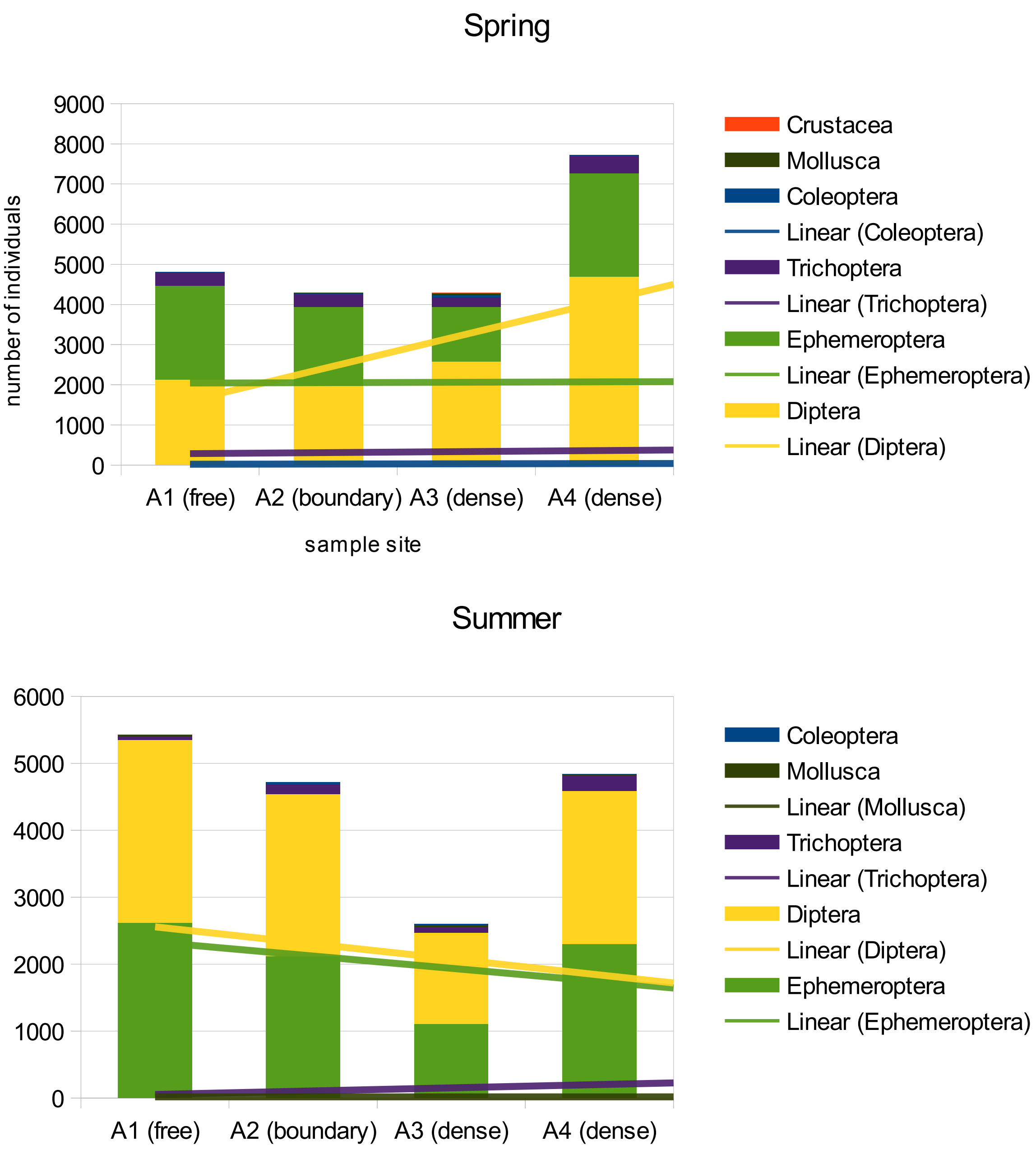


Figure 32: Macrobenthos in the Ahr over the two seasonal samples 2015

the second highest count lying perfectly within expectations, especially during the summer months when it even reached the highest numbers of invertebrates of all sites. The current crayfish distribution within a small area seems to play a bigger role than the overall distribution of crayfish seen on a bigger scale. Not the location within the populated area is decisive, but the population density within a small area – no matter where it is located in the population of crayfish. Apart from that, strong variations show that not all other factors can be ruled out under field conditions and that a single sample will never yield a finished picture of the true conditions and interactions but that a long term surveillance is necessary. Overall, the situation at the Ahr seemed much more complex and dependent on various other factors.

Statistical analysis of the macrozoobenthos results of the Ahr

Just as for the Inde (see chapter 3.2.1), a PCA was also calculated for the Ahr (default settings) with crayfish density as an overlay variable. Figure 33 shows the results.

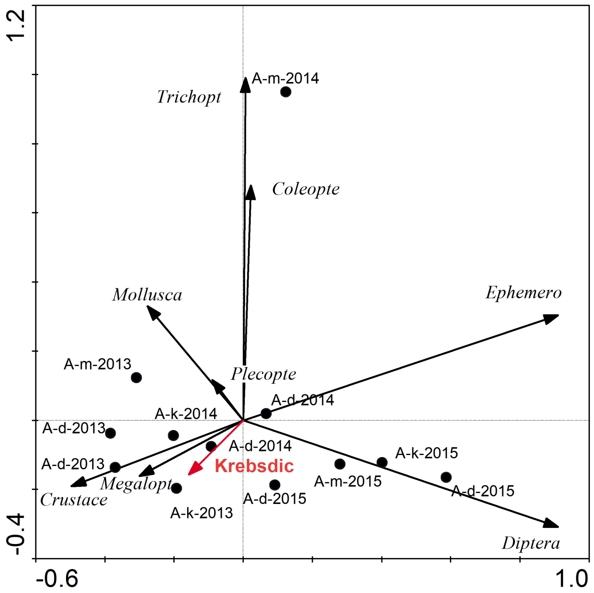


Figure 33: PCA ordination chart for the Ahr (crayfish desity as overlay variable)

The analysis points to a positive correlation of crayfish densities to Megaloptera and Crustacea and a negative correlation to Ephemeroptera. The first two groups only occurred in very small numbers that either varied throughout the study (Megaloptera) or were indeed positively correlated to crayfish (Crustacea only showed up in 2013 in the site that was populated by crayfish the longest). Due to their very low counts, it is unlikely that the results are meaningful.

Ephemeroptera so far did not show a clear relation to crayfish densities. They appeared in high numbers, which seemed to be positively influenced by crayfish presence in 2013, in 2014, they were most rare in the crayfish free site but negatively correlated to crayfish in the populated sites and significantly negatively influenced in 2015. The analysis reinforces the assumption that the population collapse in the uninhabited area in 2014 was due to factors other than the lack of crayfish colonization and that other factors were responsible for the decline of the Ephemeroptera population in 2013 as well. In the group of Ephemeroptera, it can be assumed that the overall effect is negative.

Furthermore, the analysis shows that the fluctuating reaction of Diptera, which seemed to be more influenced by a reduction of Ephemeroptera than by the signal crayfish population, is rather negatively than positively associated with the signal crayfish density after all.

The analysis showed no influence of the signal crayfish population on Trichoptera (slightly negatively correlated each year), Coleoptera (strongly negative in the first two years, positively influenced in the last) and Mollusca (fluctuating between negative and significantly positive), which is in accordance to the previous estimates. The groups showed either insignificant or greatly varying responses to the crayfish.

3.2.3 Crayfish affects on the most common taxa

Throughout the study it became clear that invertebrate numbers were varying greatly in between seasons and years. It therefore appears useful to look at the different groups of invertebrates and their individual reactions to crayfish presence and possible interactions between certain groups. It is important to consider the complete development instead of individual sample results. A single sample does not represent the overall situation. Even with the full year overviews, fluctuations may still occur which have nothing to do with the signal crayfish density. However, the statistical power should be greater than of the seasonal results.

The correlation values given above were calculated over the full year results and therefore do not take into account the seasonal variations. They showed that at the Inde the macrozoobenthos numbers were actually significantly negatively affected by the presence of the crayfish - in the first two of the three years. In 2015, the negative correlation was no longer as strong, probably also due to the more rapidly adjusting signal crayfish numbers within the sample sites, which rose sharply in the initially uninhabited places,

At the Ahr as well, the overall influence remained negative, but fluctuated and reached no statistical significance. Here, the spread of the signal crayfish took place far slower. The numbers remained relatively constant over the three years - probably due to the intensive crayfish trapping and the upstream spread.

Most common taxa of the Inde

In the Inde, Crustacea were the most common taxon and influenced negatively. This negative influence however became less and less pronounced due to the differences in signal crayfish densities, which also adjusted throughout the sample sites – staying the same in the densely populated sites and growing in the little to non populated areas (Fig. 34). They always reached their highest proportion in summer and autumn. Crayfish effects stayed the same throughout the study as well as the seasons (with only small variations) but their scale decreased in 2015 which was also the year when the crayfish population in all sample sites progressively aligned (Fig. 12). All in all, Crustacea as the main group of the Inde can be considered negatively influenced by invading signal crayfish. Their correlation values (r = -0.99 in 2013 to -0.72 in 2015) were so clear that their decrease can safely be attributed to the crayfish densities. This decreasing trend was also reflected in the PCA analysis of the Inde samples shown above, in which a negative effect of the signal crayfish on the group of Crustacea was recognizable but not entirely clear - presumably due to the gradual alignment of the signal crayfish and gammarid population.

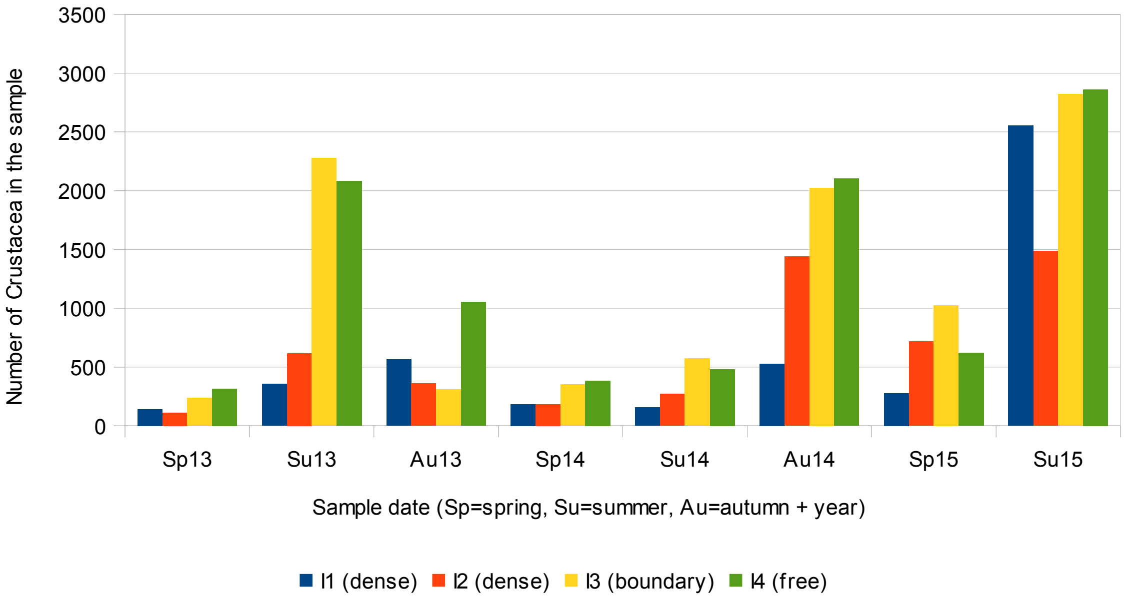


Figure 34: Crustacea population within the different sample sites (crayfish densities in brackets) throughout the study

Diptera were the second most common group and reached their highest numbers in spring when Crustacea were at their lowest proportion (Fig. 35). While they showed a clearly negative response to crayfish presence in 2013 (r = -0.85), they showed the exact opposite reaction in 2014 (r = 0.79). Their then positive development with rising crayfish densities diminished in 2015 but stayed recognizable nonetheless (r = 0,63). They only showed a negative crayfish reaction in spring 2014, the two following spring samples showed a positive correlation to crayfish densities. At lower numbers in the summer and autumn samples, their reactions were inconclusive. All in all, they seemed to react positive to crayfish. The weakening of this response in 2015 may also be attributed to the equalizing crayfish densities between sites, as the weakening responses of negatively affected groups. Here too, the result was supported by the PCA analysis (see above), which showed that Diptera might be positively influenced by increasing signal crayfish densities (Fig. 23).

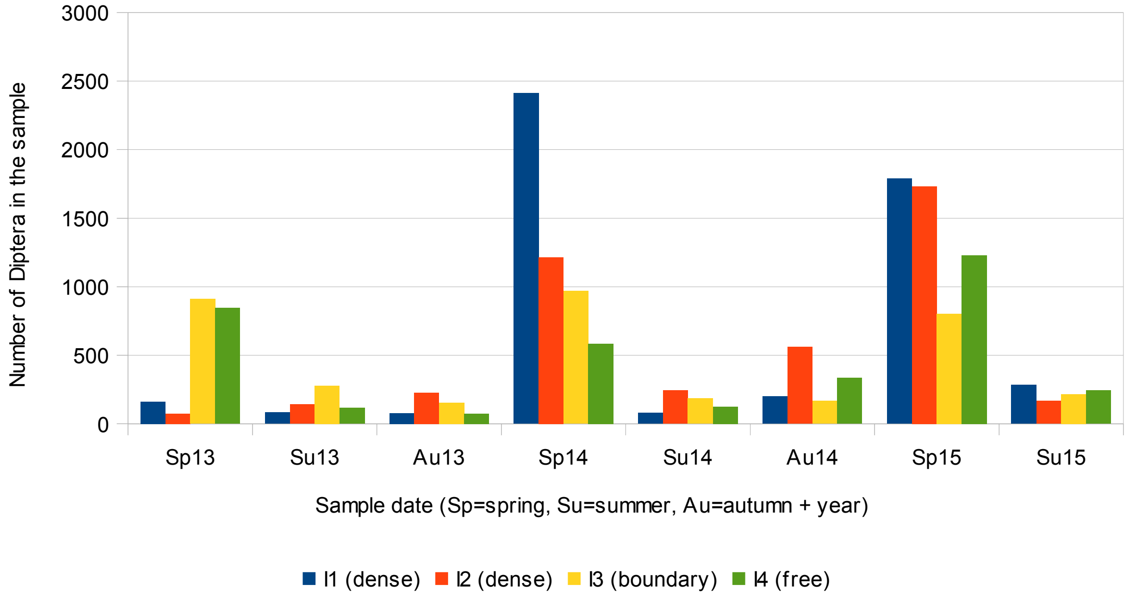


Figure 35: Diptera population within the different sample sites (crayfish densities in brackets) throughout the study

However, this might be an indirect crayfish effect, since the formerly rare Ephemeroptera were able to reach higher proportions from spring 2014 onwards – especially in sites with no to low crayfish densities. Throughout 2014 at higher proportions, their numbers were negatively correlated to crayfish density (Fig. 36) which was confirmed by the PCA analysis (Fig. 23) that showed a clearly negative correlation of the group to crayfish density. In spring 2015, this tendency slightly flattened before their population broke down in summer 2015 and Coleoptera took over. Since the rise of the Ephemeroptera population in the low to no crayfish sites marked the decrease of Diptera in the same area, a predator-prey relationship between the two groups can be suspected. Some species of Ephemeroptera, for example from the family Ephemeridae are predators, feeding of Diptera larvae. These provided the second most common family within the Inde following the Baetidae. As this family consists of burrowing species, creating U-shaped burrows, their signal crayfish related decline is understandable. The crayfish sort through the soil substrate and will thereby naturally capture many Ephemeridae.

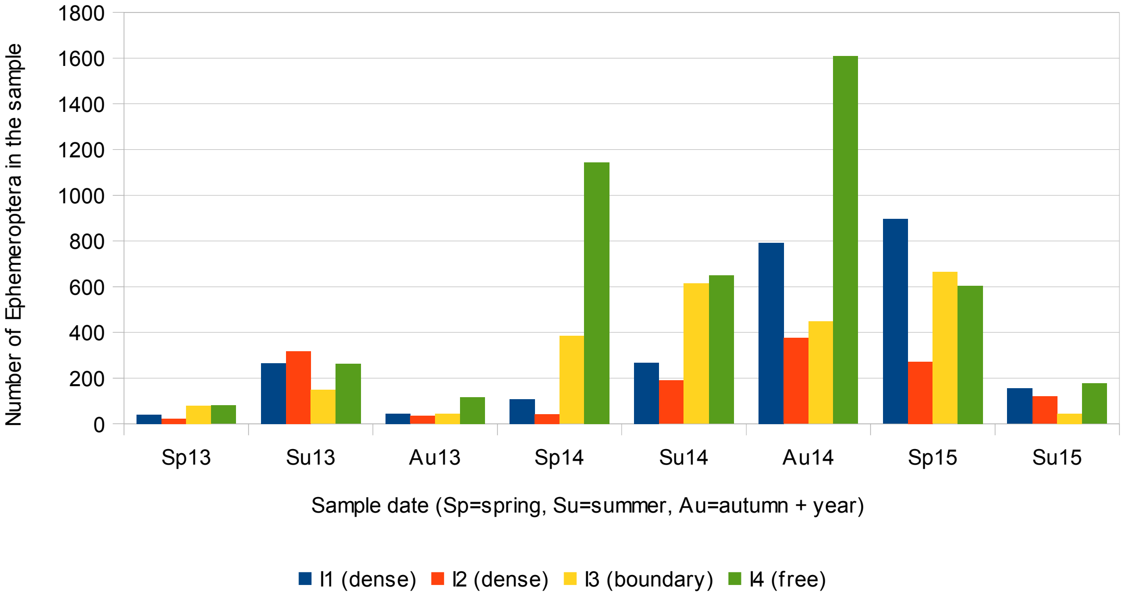


Figure 36: Ephemeroptera population within the different sample sites (crayfish densities in brackets) throughout the study

Coleoptera always constituted only a small proportion of the total benthic invertebrates, which remained stable over all samples. Nonetheless they were negatively influenced throughout the study (Fig. 37, Fig 23, r = -0,95 to -0,98), an effect which did not even diminish in the numerical adjustment of signal crayfish numbers between sites in 2015. This correlation was also clearly underlined by the PCA analysis (Fig. 23). It can therefore be assumed that the signal crayfish is a clearly correlable factor for Coleoptera distribution of the Inde.

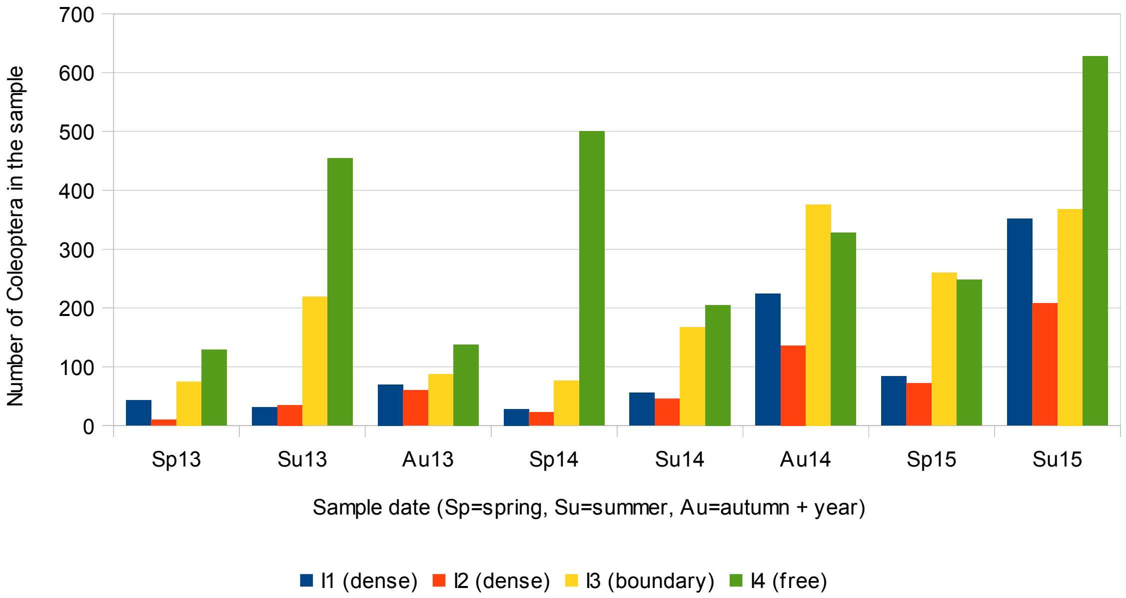


Abbildung 37: Coleoptera population within the different sample sites (crayfish densities in brackets) throughout the study

Overall, in the Inde the most common order of Crustacea, as well as the less common order of Coleoptera, were unambiguously negatively affected by signal crayfish. The same applied for Ephemeroptera, as long as they reached higher proportions within the community. Diptera however seem to be dependent on other factors as well and seem to be influenced by other groups – mainly Ephemeroptera. It became clear that a single sample does not allow a clear statement on crayfish effects, but that their impact changed within the community over time. Overall however, the signal crayfish had a significantly negative impact on the macrozoobenthos numbers of the Inde, which only decreased with population adjustment across all sample sites (increase in the previously sparsely populated areas, stagnation in the densely populated ones).

<u>Most common taxa of the Ahr</u>

In contrast to the Inde, the signal crayfish population in the Ahr remained stable. There was no significant increase in either one of the sample sites. This was partly a result of intensive exploitation and the fact that the population was spreading upstream here and hence was slowed down by the more strenuous movement against the current. It was notable in the Ahr, that the signal crayfish density in the innermost densely populated point A4 - located closest to the center of the population - always had significantly lower crayfish densities than the more peripheral location A3. This only changed during a single sample in summer 2014. A previous sample in this summer, as well as the following sample in 2015, again showed the regular density ratio like the other samples - with significantly fewer crayfish in the A4 sample point. This imbalance in the densely populated sites must be considered in the following interpretation of the data, because the A4-site declared as densely populated in fact only contained a small crayfish population over most of the time, which was comparable to the border point A2. However, the Pearson correlation, which took into account the actual crayfish numbers, showed that not only the unbalanced distribution of crayfish between sites was responsible for less impressive results. Although the r-values were negative in all years, they fluctuated between negligible r = -0.21 and stronger r = -0.78. The values did not reach statistical significance in any year, as it was the case in the Inde. It should be noted that the PCA analysis (see above) was calculated on the absolute numbers of crayfish as well and therefore is also not affected by der unpassenden Verteilung der Krebse.

The most common group in the Ahr were Diptera. However, they only occurred in considerable densities in spring and summer. They appeared to be weakly negatively influenced in 2013 (r = -0.56), positively influenced in 2014 (r = 0.87) and slightly negatively influenced again in 2015 (r = -0.53) showing a significant increase in the A4 sample site. The turning from negative to positive took place in spring 2014, when the former negative correlation to signal crayfish densities became rather inconclusive, turning to a positive correlation in summer of that year. This tendency was maintained in spring 2015 and slightly turned again in summer towards a slightly negative response (Fig. 38). It was noticeable that the higher the proportion of Diptera in the overall macrobenthos numbers were, the more pronounced the negative crayfish effects on this group became. This was particularly the case in the 2013 spring and summer samples. In spring 2014, Ephemeroptera took over as the most common group – showing the same interconnection as in the Inde. While Ephemeroptera were negatively influenced by crayfish and developed strongest in the sites with no to low crayfish population, Diptera disappeared most strongly in exactly these areas. This tendency had become visible already in summer 2013, even so at much lower Ephemeroptera densities. In summer 2014, Coleoptera also proliferated, and Diptera decreased – loosing a lot of their former proportion to Coleoptera and Ephemeroptera. The fact that this occurred at both streams suggests that it is not a random fluctuation, but Diptera rather react negatively to Ephemeroptera than positively to signal crayfish. In addition, the PCA analysis found a rather negative correlation of Diptera to the actual signal crayfish density.

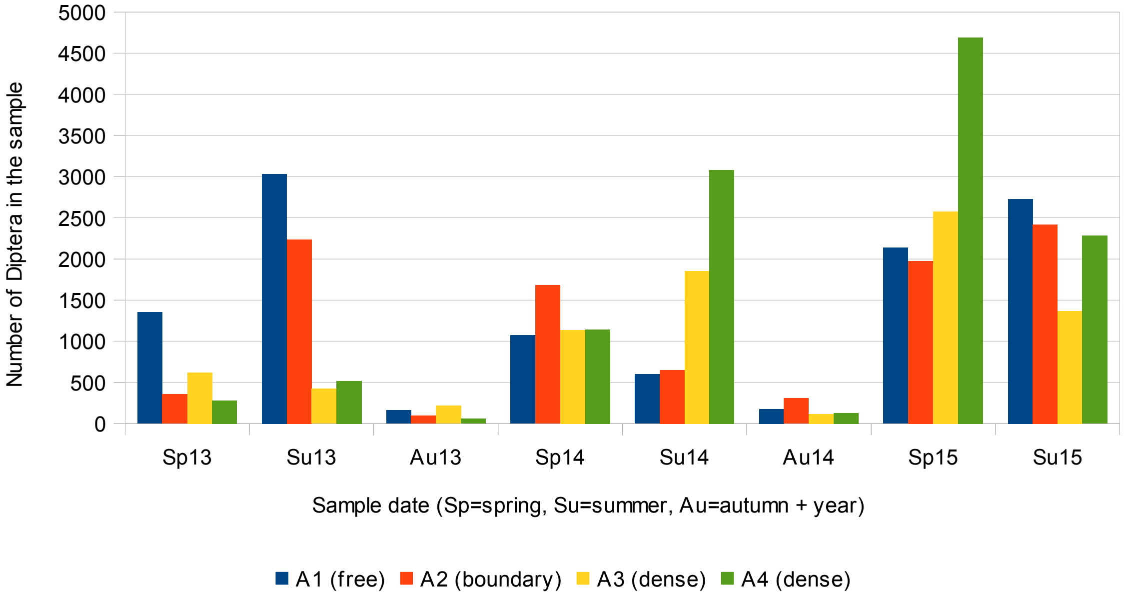


Figure 38: Diptera population within the different sample sites (crayfish densities in brackets) throughout the study

Ephemeroptera were only represented in low densities in 2013. Their occurrence did not show any seasonal variation. During this year they reached their highest density in the two “densely populated” sites A3 and A4, however, without any correlation to actual crayfish numbers (r = 0.05). In 2014 their proportion strongly increased but remained indifferent with regard to crayfis densities (r = -0.15) - with the highest Ephemeroptera population in the sparsely crayfish populated boundary site A2. In spring it was followed by the high-crayfish-density sites, while the lowest Ephemeroptera population occurred in the crayfish-free A1 site. Both in summer and autumn, however, the crayfish free site A1 and the sparsely populated A4 within the crayfish population occupied second place, which points to rather low Ephemeroptera densities in combination with high signal crayfish population. The overall proportion of mayflies continued to rise in 2015 (Fig. 39). With increasing proportion of the group, a clear negative correlation to signal crayfish emerged, which even became statistically significant (r = -0.99). Compared with the current crayfish catch numbers, mayflies were most abundant in spring as well as in summer, the lower the signal crayfish catch numbers in the respective site were. It seems as if only a certain critical proportion of a group within the total invertebrate community leads to negative reactions to signal crayfish occurrence. The PCA analysis also found a clearly negative correlation between Ephemeroptera and absolute signal crayfish density. This suggests that the group is indeed a preferred foodsource for the crayfish, but becomes uninteresting with decreasing density as soon as representatives of other groups are found more frequently during its search for food.

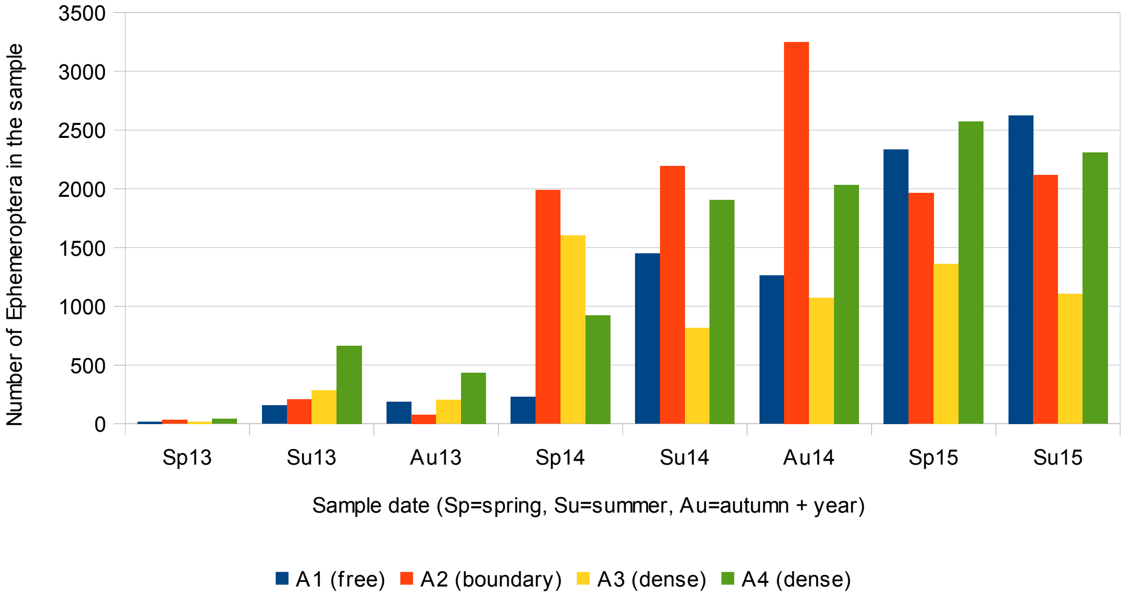


Figure 39: Ephemeroptera population within the different sample sites (crayfish densities in brackets) throughout the study

Caddisflies played only a minor role for most of the year, but represented by far the main component of the benthic invertebrate community in autumn. Consequently, the results from 2015 are of little relevance, since the autumn sample was left out in this year. In the spring and summer samples with negligible caddisfly numbers no clear trends became apparent. The two autumn samples 2013 and 2014 showed the by far highest numbers in the boundary site A2, where also the lowest degree of shading was observed (Fig. 40). 2013, the caddisfly population sank from there, the deeper one penetrated into the area populated with signal crayfish. In addition, fewer caddisflies were found in the flood-damaged crayfish free sample point A1. In autumn 2014, their population broke down almost completely in the two "densely crayfish populated" sites A3 and A4 and remained high only in the boundary sampling site, while in the now translocated sample point A1 (high degree of shading, but not crayfish occurrence) a small number of caddisflies remained as well. A negative connection of this group to degree of shading could already be observed in summer, when the sample was taken relatively late and the seasonal increase in caddisflies had already started. The increase took place mostly in the boundary site A2 as well as the sparsely crayfish populated site A4 within the signal crayfish distribution area - the latter also having a low degree of shading. Basically, this group seems to benefit from a high incidence of light and the associated strong growth of algae. Signal crayfish occurrence acted as a second factor, doch deren negativer Einfluss erreichte keinen nennenswerten Werte (r values developing from -0.11 in 2013 over -0.42 in 2014 to -0.50 in 2015). In addition, an increase in the mayfly population seemed to negatively affect caddisflies - similar to the Diptera. The PCA analysis found no association between Trichoptera density and actual signal crayfish counts. It ist possible that the negative influence of the crayfish would become significant once the Diptera reached a certain proportion within the community, as it was the case with Ephemeroptera.

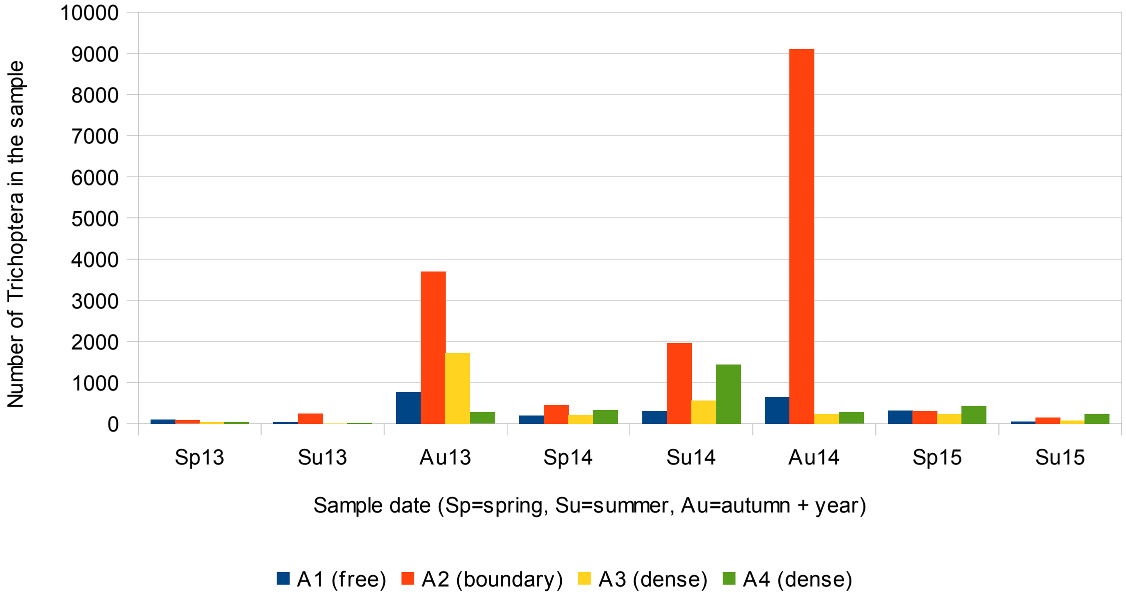


Figure 40: Trichoptera population within the different sample sites (crayfish densities in brackets) throughout the study

Mollusca were still relatively common in the boundary site A2 in 2013. In the uninhabited, but flood-damaged A1 there was no algae growth to maintain a snail population. In the areas more densely populated by signal crayfish, snails were rare and only appeared in low numbers in A4 with a low degree of shading. 2014 their proportion in the overall benthos community decreased further. They then reached their highest numbers in the uninhabited site A1, and in the sparsely populated and only slightly shaded A4. 2015 their share of the total stock became so low that it no longer had any significance (Fig. 41). Overall, a clear tendency for this order was barely detectable. If at all, they reached significant proportions within the Community only in spring and summer. In summer 2013 they appeared with increasing numbers in the boundary site A2, small numbers were also found in the uninhabited, but flood damaged A1. Although in 2014 their total share decreased, most snails were found in the newly selected unpopulated and undamaged but highly shaded A1 and gradually decreased in the direction of the signal crayfish population center. Both sample results indicate a negative crayfish effect. However, in summer of 2014, the trend completely reversed - with most snails in the innermost densely populated point A4 and the fewest in the unpopulated A1. Subsequently, the snail population broke down in its proportion to the other groups so massively that it lost any significance. Because snails are a recognized food source for crayfish and not very mobile, it can be assumed that they will be predated. The variations within the snail population, however, were so great and sudden that in this study no clear trend could be detected. The PCA analysis also found no association between mollusc density and actual signal crayfish population and the Pearson correlation revealed negligible negative (r = -0.45 in 2013) as well as positive (r = 0.59 in 2014) correlations, the latter even reaching significant proportions (r = 0.96) in 2015. Aspart from the last sample being extrapolated to make up for the missing autum-sample, snails might actuelly profit from crayfish to a low degree, since their foraging behavior can resuspend sediment, providing more surface for algae growth.

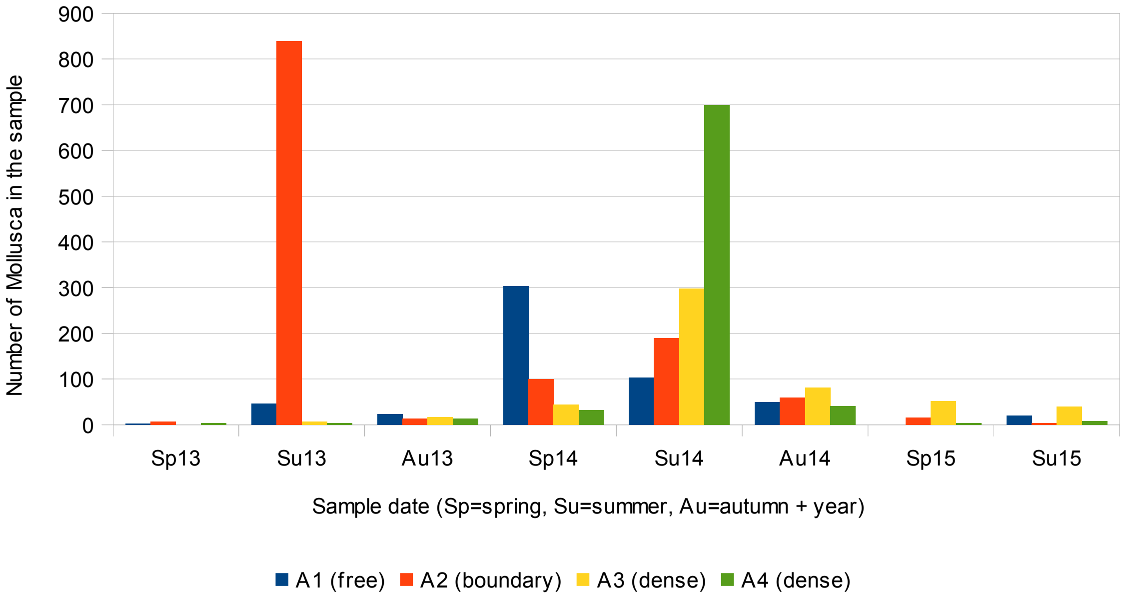


Figure 41: Mollusca population within the different sample sites (crayfish densities in brackets) throughout the study

3.2.4 Conclusions

There were clear indications that a group is more strongly affected by signal crayfish, the higher their proportion in the total benthic community is. This makes sense, since they will be encountered by foraging crayfish most often, thereby also being predated the most. With regard to competition as well, it is likely that the most common group will show the first reaction to the presence of crayfish. In the Inde this role was taken by the crustaceans, which consisted almost solely of gammarids. Their negative correlation to signal crayfish densities was immense and stable throughout the study. They live at the streambed in the thickets of plant debris, feed on small worms, insect larvae and dead organic material (Kriska & Tittizer 2009) and thereby not only inhabit the same habitat and have the same diet like signal crayfish but at the same time are prey for the much larger species. Thus, competition and predation can take their toll on the gammarids in parallel. With adjusting the signal crayfish population throughout the individual sample sites, the negative correlation of the crustacea was slightly attenuated, but was still clearly in the negative range (r = -0.73 to -0.72, at the beginning of colonization r = -0.99).

Diptera were the most common group in the Ahr and the second most common one in the Inde. In both streams they were negatively affected by signal crayfish in 2013, which changed to a seemingly positive influence in 2014. The total macrobenthos numbers at the Inde remained negatively correlated with signal crayfish densities though, only the proportion of Diptera within it now increased in the more densely crayfish populated areas. At the Ahr as well, an increasing proportion of Diptera within the macrobenthos and a slight positive correlation to crayfish could be observed in 2014, which turned oppositely again in 2015 (slightly negatively correlated to crayfish). At the Ahr, the correlation of Invertebrates to crayfish in gerneral was rather unsteady. Diptera might profit from the decline of other groups negatively affected by crayfish. In the Inde, which yielded more stable results, the decline of the Diptera population - and their positive correlation to signal crayfish densities - occurred contemporaneous to a proliferation of Ephemeroptera especially in the sites low- or unpopulated by crayfish i. e. negatively correlated. Dipterans were positively correlated with the signal crayfish only if ephemeroptera were negatively affected and vice versa. The strength of the correlation also depended on the strength of the opposite correlation of the other group. The only exception was the study year 2015 at the Ahr, when both groups were negatively correlated, which may also be due to the general fluctuation of the invertebrate population there. In the Inde, the positive correlation of Diptera was retained in that year, even so Ephemeroptera showed no clear correlation to crayfish in then. Changes in frequency of certain species may result in strong and unexpected reactions from other species as they try to compensate for the changes in the associated group. So this could be a competitive situation, which is only indirectly linked to signal crayfish. The inconclusive results of the last study year might be due to the alignment of the crafish population.

In both streams several Diptera families occurred, but they were by far dominated by the midges (Chironomidae). Chironomid species live freely in the water, in the substrate on the ground or in the growth on stones and plants. Few of them are creating burrows from sediment grains. They swirl food particles towards them by wriggling movements of their bodies and play a major role in the cycles of materials and energy of water bodies. They counteract eutrophication by withdrawing their environment considerable amounts of biomass during mass emergence. Also because of their sometimes massive occurrence, they play an essential role as a nutritional basis for predatory arthropods (crustaceans, water mites and aquatic insects) as well as fish. Aquatic larvae of midges live mostly of by-products in the water and algae. They accomplish this by grazing on the substrate (i. e. Diatoms) or using their weave as a net to catch suspended matter. Some species feed on filamentous algae of the genus Spirogyra, live as leaf miners in the floating leaves of Potamogeton species or on the surface of various aquatic plants. Some even live as predators and hunt other insect larvae (Kriska & Tittizer 2009). With this ecological diversity, it is understandable that over a hundred chironomid species may occur (as larvae) next to one another in a creek. Due to that it is difficult to determine on which points Ephemeroptera and Diptera might compete with each other or where Diptera might profit from crayfish. It is possible that the proliferation of mayflies resulted in a local decrease of Diptera in the low crayfish-populated sites, as well as that crayfish supported them through their subversive activity by making organic churned material available to the swirling open water Diptera species - or a combination of both. In any case, the same pattern of development of the Diptera and Ephemeroptera population in both streams shows that it is most unlikely to be a mere coincidence.

In the Inde, the mayfly population consisted of the families Baetidae, Ephemeridae and Ecdyonuridae. In the Ahr Baetidae represented the majority, Ecdyonuridae were much less frequent. In the Inde, Baetidae were followed by Ephemeridae. Nymphs of the Baetidae are strong swimmers and feed mainly on algae and detritus (Edmunds 1978, Edmunds et al. 1976). Ecdyonuridae larvae nestle tight to the rocks, feeding on their algae growth. However, they might use a wide range of food sources with herbivorous, scavenging, and predatory species known. Still, they are primarily scrapers (Flowers 1975). Ephemeridae usually require a layer of silt as the nymphs have strong legs which are adapted for burrowing („burrowing mayflies“). Their nymphs are largely carnivorous and collect their food either through predation or scavenging (McGavin 2005). Animals like these can therefore use dipteran larvae as a source of food.

Trichoptera, which were the third most common group in the Ahr but only occurred in significant numbers in autumn, displayed another phenomenon. In spring and summer, when their proportion within the Community was small, they were only slightly influenced by the signal crayfish. In the autumn samples, when they reached their population maximum building the by far major component of the invertebrate community, their population broke down almost completely in the sample sites with high crayfish densities. In addition, the amount of sunlight in the sample site seemed to play a role, since a high degree of shading resulted in decreasing caddisfly occurrence. This effect became evident even at lower densities, such as in summer of 2014, when the caddisfly population started to increase the most in the only slightly shaded sites A2 (crayfish population boundary) and "densely populated" A4 (in fact with only minor crayfish numbers). In autumn however, high Trichoptera numbers could only persist when a low degree of shading was combined with low crayfish density. Trichoptera larvae can be divided into predators (small insects and their larvae), crushers (larger living and dead plant parts) and grazers (algae growth) (Kriska & Tittizer 2009). In the Ahr, numerous families occurred but the Sericostomatidae were most strongly represented due to mass emergence in the autumn samples. The fact that caddisflies did not yield the majority of the overall macrobenthos community and only occurred in the Ahr (generellay lower crayfish counts) in noticeable numbers, already suggests that this group might be strongly influenced by signal crayfish presence. Since their species are not very mobile due to their body shape and locomotion compared to mayflies and dipterans, it can be assumed that they represent an easy prey for the signal crayfish and are very likely consumed in large amounts. Another sign of the direct predation by crayfish is the correlated collapse of Trichoptera only above a certain density when the group becomes relevant as a food source. Since crayfish randomly search the ground for food rather than "hunt" on sight, only prey animals from a certain population density upwards are of interest to them, because they will rarely find individuals that only occur in low numbers. In this study however, the impact of crayfish on Trichoptera was inconclusive.

Coleoptera only occurred in considerable densities in the Inde, where they constituted the fourth most common group. Their correlation to crayfish was consistently negative. Unlike many other groups whose frequency varied over time, the connection to signal crayfish occurrence even stayed statistically significant with aligning crayfish numbers over all sites. At the Inde, the by far largest part of the beetle population was formed by the rather immobile hook beetles (Elmidae) and their larvae, which graze on the algae growth on stones and are exposed there to direct predation by the crayfish just as the caddisflies (Elliott 2008).

Mollusca only appeared in significant quantities at the beginning of the study in the Ahr. There they were the fourth most common group in 2013 and 2014. However, their proportion had already strongly declined in 2014. 2015 their presence was no longer significant. A clear connection to signal crayfish occurrence could not be determined or rather varied so strongly that no clear trend became evident.

Overall, it became evident that signal crayfish density plays a crucial role. The density was significantly higher at the Inde. Accordingly, crayfish impact was clearly negative and stable here, while larger fluctuations appeared at the Ahr and crayfish could not be identified as a negative influence on other invertebrates without a doubt. Overall, correlational values of the macrobenthos remained negative at the Ahr but fluctuated strongly ($r = -0.21$ to -0.78), while at Inde, they were almost consistently negative ($r = -0.95$ to -0.97). Only during the last year of the study with almost balanced signal crayfish levels in all sites, the correlation flattened ($r = -0.51$). Only high crayfish populations, as found in the Inde, thus lead to a clearly negative influence on aquatic invertebrates. The ability of this species to achieve higher densities than the native noble crayfish, might therefore be even more crucial for the overall system, than the higher aggressiveness or higher dietary intake of individuals. Up to a certain population density, these properties appear to be manageable for the system. Also, it became evident that crayfish effects can only be monitored over longer periods of time. Single samples within this study would have drawn a completely different picture. Only recurring samples within repetitive time frames were able to show certain seasonal and long term trends.

3.3 Influence of signal crayfish on fish

The results of the electric fishings of the Inde are shown in Figure 42. As with the macrozoobenthos results, it must be noted that the signal crayfish population in the different locations progressively aligned in the course of the three years of the study, increasing in the previously un- or only sparsely populated sites but remaining the same in the densely populated one, so that an attenuation of effects can be expected. As described in material and methods, only three sites were examined, since the second densely populated site was left out to identify negative effects of this method on crustaceans and benthic invertebrates, if they should occur. Only fish species which occured regularly were considered in the evaluation. Single finds (i. e. stickleback) were not counted.

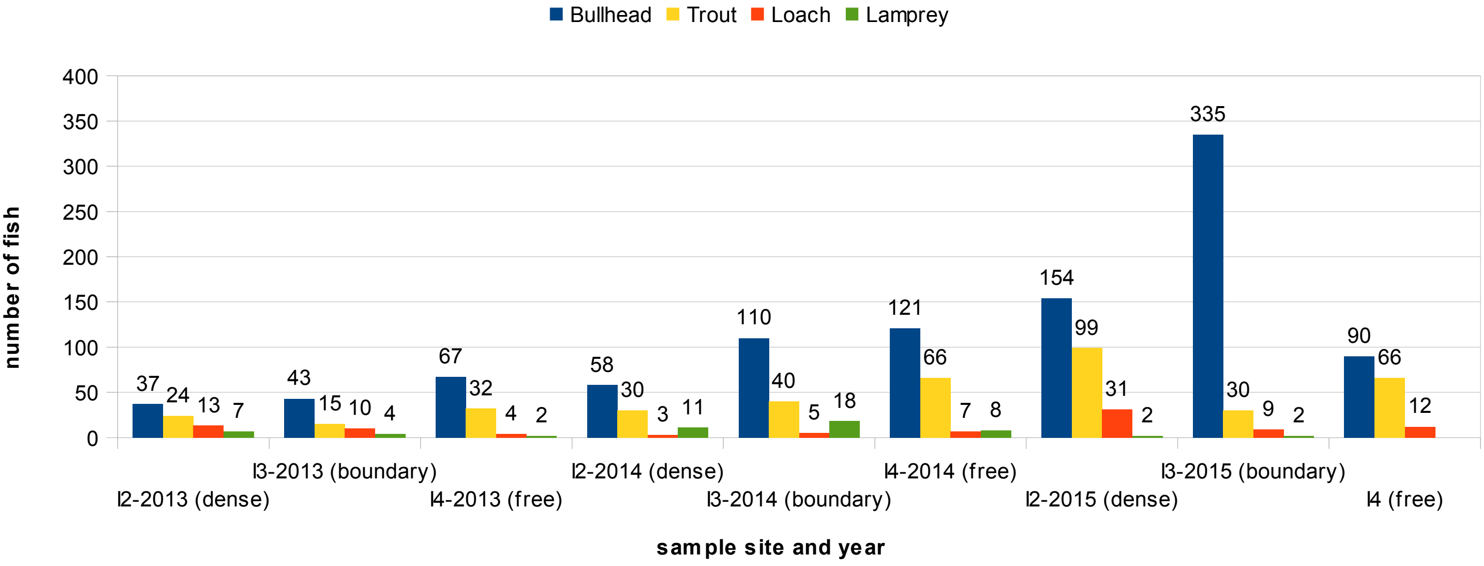


Figure 42: Electric fishing results in the Inde

In the Inde, the most abundant species was the bullhead (*Cottus gobio*). It is a nocturnal benthic fish with a regressed swim bladder, growing about 12 to 16 cm long. The fish are poor swimmers, usually skipping over the riverbed with spread pectoral fins to save energy. They are well camouflaged on stony ground when they do not move and stay hidden during the day. The bullhead lives in trout to grayling regions of rivers up to altitudes of about 2000 m above sea level as well as in summer cool lakes. It is very demanding on water quality, requires a high concentration of oxygen, low water temperatures and can be found mainly on stony ground. Because river bank stabilization and pollution can endanger populations of this species, the bullhead has been included in Annex II of the Habitats Directive. Since they are only moderate swimmers, even small obstacles are insurmountable for them. The bullhead feeds on small benthic invertebrates such as insect larvae and gammaridae. During the spawning period, males build a pit under rocks, in which the females spawn. The male then guards the nest until the fry hatch after four to six weeks (Gerstmeier & Romig 1998, Waterstraat 1992). The bullhead is thus endangered by the signal crayfish on several levels simultaneously. It has the same activity phase during the night, as a poor swimmer is at risk through direct predation or at least injuries, needs the same hiding places during daytime that are occupied by crayfish and also subsists on benthic invertebrates. In addition, their spawn in the nests between rocks is threatened by crayfish.

Sample site	**Crayfish**	**Bullhead**	**Loach**	**Trout**	**Lamprey**
I4-2013 (free)	3	67	4	32	2
I3-2013 (boundary)	19	43	10	15	4
I2-2013 (dense)	68	37	13	24	7
	r	**-0,81**	**0,89**	**-0,2**	***0,99***
I4-2014 (free)	5	121	7	66	8
I3-2014 (boundary)	23	110	5	40	18
I2-2014 (dense)	62	58	3	30	11
	r	***-0,99***	**-0,98**	**-0,9**	**0,09**
I4-2015 (free)	30	90	12	66	0
I3-2015 (boundary)	47	335	9	30	2
I2-2015 (dense)	62	154	31	99	2
	r	**0,29**	**0,68**	**0,45**	**0,89**

Figure 43: Pearson correlation of signal crayfish and fish density in the Inde throughout the study (significant correlations in italics)

As expected, the bullhead population of the Inde decreased with increasing signal crayfish density. Overall however, the number of fish increased throughout the study. The effect of crayfish on this group was strongly negative in the first two years of the study and became statistically significant in the second study year, whereas in the third year with adjusting signal cryfish densities in all sites no clear effect was recognizable anymore (Fig. 43). However, the bullhead population seemed to increase over time. Although the species was negatively linked to signal crayfish, more individuals were caught each year.

As Figure 44 shows though, the average individual size of fish decreased at the same time, so increasing number of individuals was accompanied by smaller specimens of bullhead. This was also reflected in between the different sample sites. 2013, the largest sculpin were caught in the boundary (I3) or crayfish free site (I4). In 2014 and 2015, the crayfish free site I4 always had the largest bullhead specimens. In the first two years, the most bullheads were always caught inside the crayfish-free site I4 and the least in the densely populated site I2. 2015, this trend disappeared with an increasingly balanced signal crayfish density over all sites. Then the most sculpins were caught in the sample site at the former boundary of the signal crayfish population (I3), where it generally came to a massive increase of bullheads during this year, while the fewest fish were caught in the signal crayfish free site I4.

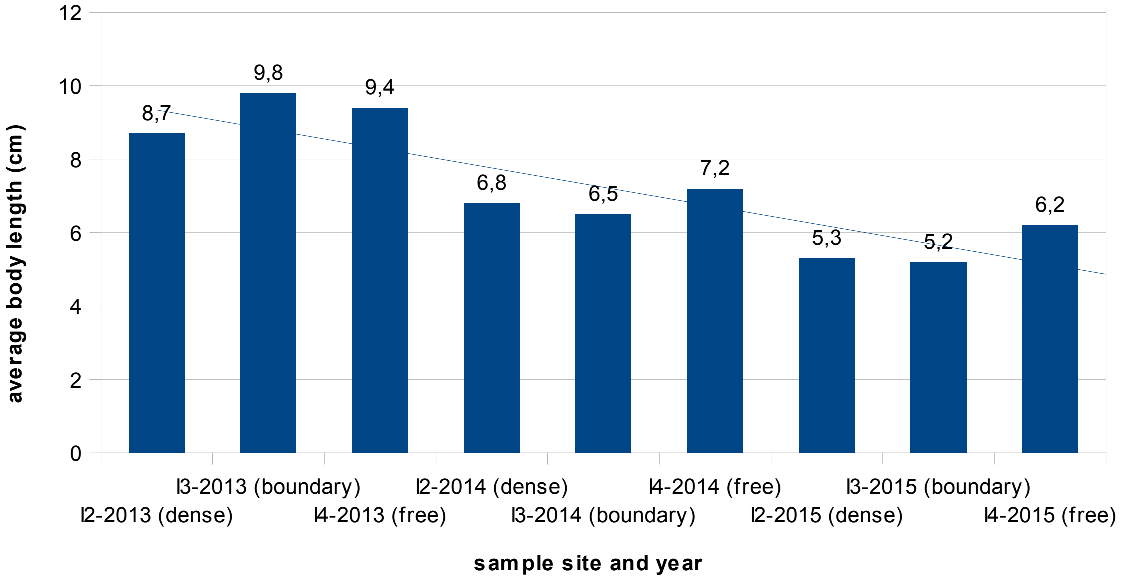


Figure 44: Average body length of bullhead (Cottus gobio) in the Inde

As long as the differences in signal crayfish density between sample sites were still huge (2013, 2014) a negative effect on the sculpin population appeared, which is not surprising due to the multi-level competition listed above. The bullhead numbers as well as their average body length decreased with increasing and adjusting crayfish density over all sites. The very striking and sudden increase of bullheads in summer 2015 in the former boundary area (I3) combined with the lowest average body length throughout the study points to an increased incidence of juvenile fish at this time. In fact, the catch contained 314 young fish of the same year and only 21 older specimen. The same number of older fish was found in the unpopulated site (I4) (with only 96 young of year), while in the densely populated I1 only 8 older fish and 146 juveniles were caught. Considering only the development of the adult bullhead stock (Fig. 45), their numbers have been declining during the study, while the percentage of young fish and their absolute numbers greatly increased. Regarding adult bullheads, a negative correlation to signal crayfish remained visible throughout all samples, while the occurrence of juvenile fish revealed no clear connection to crayfish density. It is therefore quite possible that the competitive situation only occurs above a certain body size of the fish. Older bullheads can escape the signal crayfish by migration to other stretches of water, while the few remaining animals may produce more offspring due to diminishing stocks or the offsping might gradually move to the sites with less adult fish territories. Since the stock of two year old bullheads continues to decrease nevertheless, it can be assumed that most juveniles do not survive their first year - especially when signal crayfish occur in great densities.

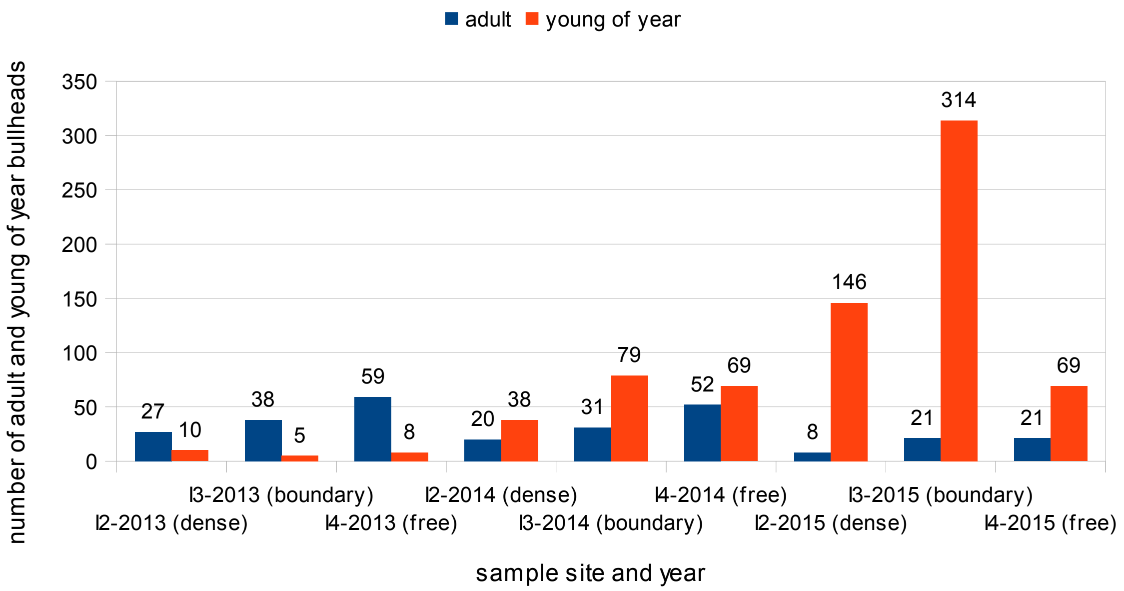


Figure 45: Adult fish and young of year bullheads (Cottus gobio) in the Inde

Brown trout (*Salmo trutta fario*) as the second most common fish species of the Inde, showed a slightly increasing number of individuals during the course of the study as well. In 2013, no clear connection to signal crayfish occurrence was recognizable yet, but the largest number of trout appeared in the crayfish-free site and the average individual size of fish decreased with increasing crayfish population, which already points to a certain competition for food with crayfish. 2014, no clear trend in relation to body length was detectable anymore (there were still the smallest fish in the densely populated site though), but the numbers of individuals were now clearly negatively correlated with crayfish density (r=-0,9; Fig. 43). 2015 most trout were caught in the densely populated sample site I2, which was the only one with still considerable decrease of benthic invertebrates compared to low or densely populated sites. The average body length of brown trout decreased over the course of the study, just like it did with bullheads. During the two years in which the crayfish in the sites not considered densely populated in fact only reached low densities, the average body length of fish in both years was still bigger than in the densely populated site. It was only with increase and equalization of crayfish densities in these sites in 2015 that the average body length of fish equalized – just like the macrobenthos population did. However, the size differences were not as pronounced as in bullheads. Overall, the negative influence of the signal crayfish on trout seems to be less pronounced. Brown trout are very faithful to a habitat, leaving their individual space only to reproduce and usually return to their traditional places even after disturbances. Adult trouts claim their own territory. They stay hidden in the shadow of the banks during the day, standing with the head against the current. Depending on their size and habitat they mainly feed of insects and aquatic insect larvae, small fish like the bullhead, smaller crustaceans and snails and other aquatic animals. Even cannibalism is often observed in brown trout. They are fast hunters, but mostly take on drifting by prey in rivers and streams (Gerstmeier & Romig 1998). Due to their better swimming abilities (as opposed to bullheads) which allow them to ascend in the water column, effectively escaping the signal crayfish, they are already better protected against attacks. Their search for food is more compatible with the crayfish as well, since trout do not prefer ground-dwelling invertebrates like sculpins and crayfish do, but also hunt in the water column. However, it can be assumed that crayfish from a certain size onward will compete with them for hiding spaces. A displacement of fish from shelters could lead to an increased exposure to other predators.

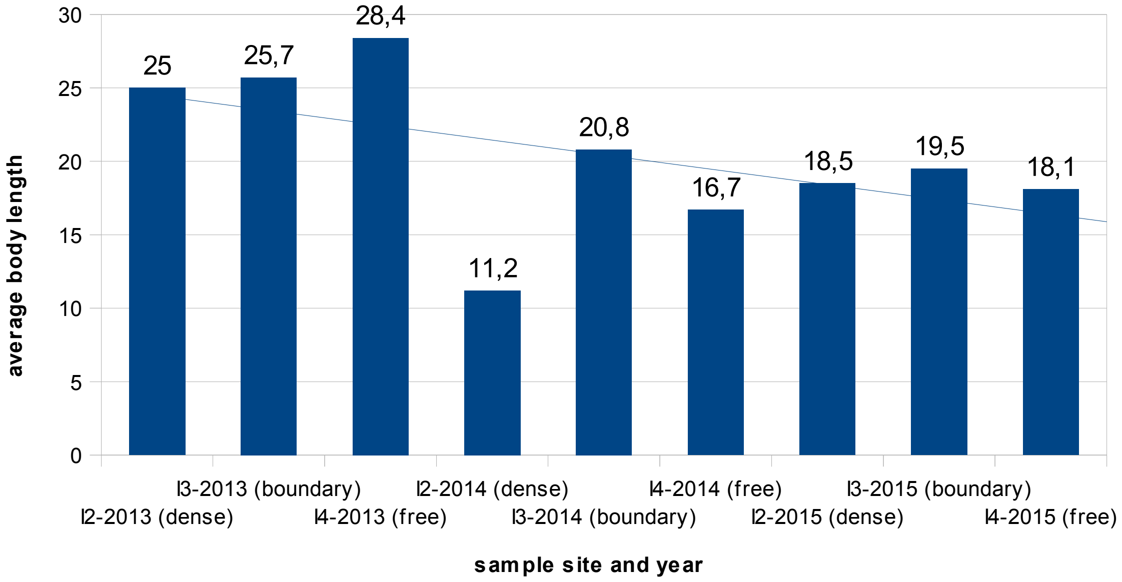


Figure 46: Average body length of brown trout (Salmo trutta fario) in the Inde

Although the adverse effect by crayfish on trout remained somewhat inconclusive in comparison of the individual sample points, the decrease in average body length over the three study years and in all sites points to a competitive situation. Again, however, there were variations. Figure 46 shows that in contrast to the bullhead, the decrease in average body length was not due to an increase in juvenile trout. These represented only a small part of the trout population and correlations to signal crayfish occurrence were not recognizable.

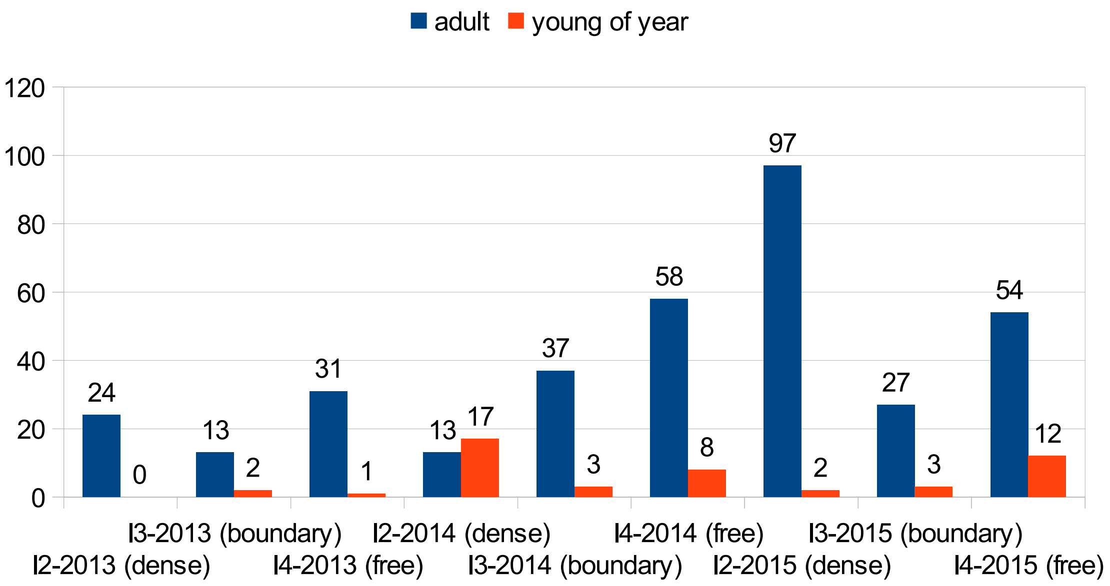


Figure 47: Adult fish and young of year brown trout (Salmo trutta fario) in the Inde

Stone loach (*Barbatula barbatula*) and brook lamprey (*Lampetra planeri*) only occured in very small numbers. Therefore, signal crayfish influence was not easily determinable. In lampreys, a significantly positive correlation with signal crayfish was observed in 2013 (r = 0.99, Fig. 43), then no correlation at all in the second year (r = 0.02) and again a positive reaction in 2015 (r = 0.89). This year, however, only four lampreys were caught altogether. It can be assumed that lamprey catch results are random in no way associated with the signal crayfish population. The apparently sinking lamprey occurrence towards the end of the study also could not be associated unambiguously with signal crayfish, as the catch of lampreys only succeeded when the anode was held onto sandbanks for an extended period of time to pull the fish out of hiding (They stay hidden in detritus during the day.). However, this method requires a departure from the normal procedure and was therefore not considered to be statistically valid. It was also only applied by the anode carriers, where the opportunity arose for it, so not all sand and mud banks could be examined. The loaches gave a similar picture. These were positively influenced in 2013 (r = 0.89) and 2015 (r = 0.68), but strongly negative in 2014 (r = -0.98). Here, too, it can be assumed that the influence of signal crayfish did not fluctuate that much, but rather that the low catch numbers of loaches were responsible for the results.

Sample site	Crayfish	Bullhead	Loach	Trout	Minnow
A1-2013 (free)	1	3	1	0	8
A2-2013 (boundary)	1	206	145	26	113
A3-2013 (dense)	28	80	16	6	166
r		**-0,14**	**-0,42**	**-0,3**	**0,76**
A1-2014 (free)	0	225	55	63	205
A2-2014 (boundary)	4	290	65	44	240
A3-2014 (dense)	22	141	20	46	176
r		**-0,81**	**-0,93**	**-0,56**	**-0,73**
A1-2015 (free)	1	333	32	38	62
A2-2015 (boundary)	7	351	51	57	48
A3-2015 (dense)	25	55	14	36	38
r		**-0,96**	**-0,71**	**-0,36**	**-0,93**

Figure 48: Pearson correlation of signal crayfish and fish density in the Ahr throughout the study

At the Ahr, the bullhead was the most abundant fish as well. Figure 49 suggests that the bullhead population of the Ahr gradually increased. However, this is not the case. Only 50 m were fished in 2013 which was doubled to 100 m in 2014 to achieve a better statistical validity. Thus, catch figures from 2013 would have to be basically doubled to be comparable with the results of the other two years. Just as in the Inde, a negative correlation of bullhead and signal crayfish was evident in the Ahr which even became more apparent during the course of the study. It should be noted that in the Ahr in contrast to the Inde, the signal crayfish population of the sparsely populated and completely crayfish free site did not significantly increase and the differences in signal crayfish density between locations therefore remained more or less stable. 2013 represented an exception, since the signal crayfish free sample site A1 was damaged by a massive flood induced sediment glut and almost no fish were caught there. However, between the sparsely populated site A2 and densely populated A3 a significantly negative correlation between crayfish and bullhead occurrences was observed in 2013 already. In the densely populated site, only 39% of the bullhead population in the uninhabited A2 could be found. 2014 and 2015 the highest bullhead numbers were always registered in the sparsely populated location A2, where the highest invertebrate numbers existed. In the crayfish-free sample site A1, which contained less invertebrates due to a higher degree of shading, the bullhead population was only slightly lower (-22% in 2014, -5% in 2015). In the densely crayfish populated site, bullhead occurrence has always been the lowest, which was to be expected due to the lower invertebrate density there (-51% in 2014, -84% in 2015). This impression was also confirmed by the Pearson correlation (Fig. 48) for the Ahr, which demonstrated an increasing negative effect of signal crayfish on bullheads (r = -0.14 in 2013, r = -0.81 in 2014, r = -0.96 in 2015, see Fig. 48).

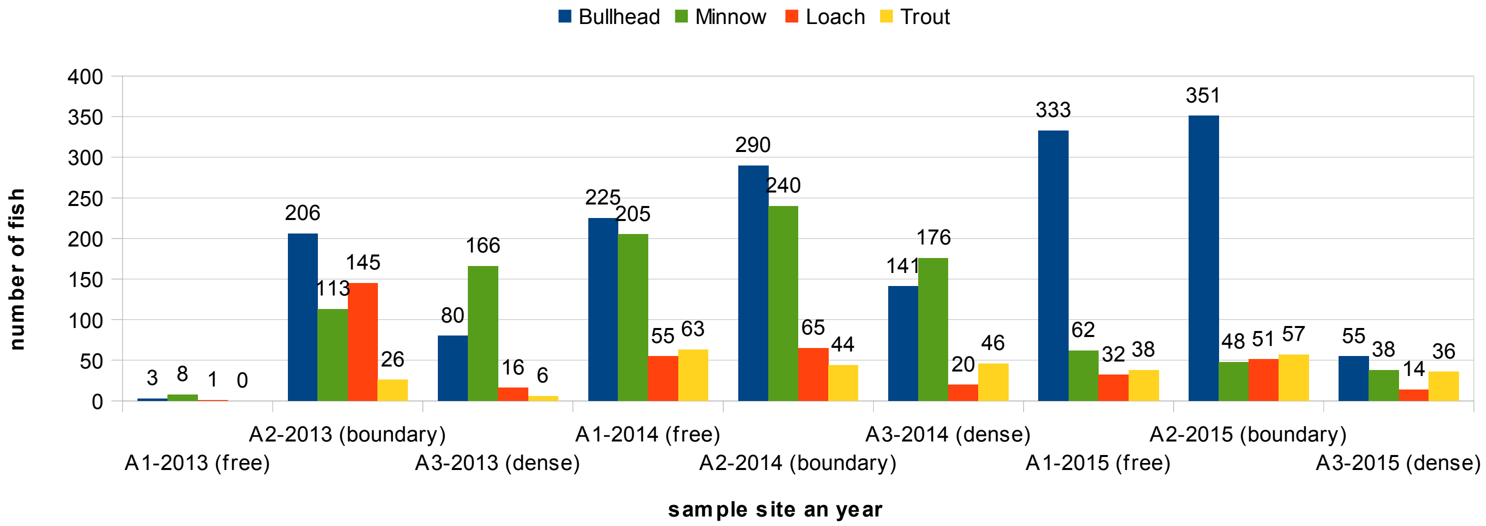


Figure 49: Electric fishing results in the Ahr

Figure 50 shows that the average body length of bullheads in the Ahr remained the same during the course of the study, yet the biggest fish were always caught in the densely crayfish populated sample point A3, which means that there were fewer but larger individuals. This was especially due to the decimation of juvenile fish by the signal crayfish, as becomes apparent from figure 51.

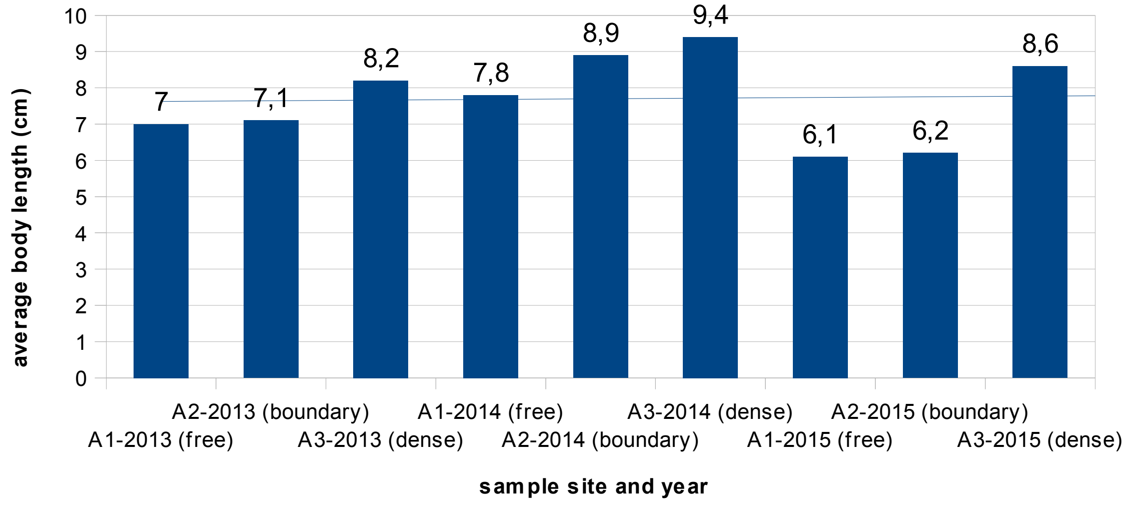


Figure 50: Average body length of bullhead (Cottus gobio) in the Ahr

Figure 51 further shows, that the surplus of young of year bullheads in the Ahr was less pronounced than in the Inde. But here as well, a slight decrease of fish older than one year and a strong increase of young of year juveniles became apparent, in particular in 2015. Considering the fact that the signal crayfish population of the Ahr did not fundamentally change while the population of the Inde rose sharply, the reaction is relatively the same as in the Inde. It shows an increase of juvenile fish and a decrease of fish older than one year - particularly in the crayfish free or less populated places. Here too, the bullhead population responded to crayfish presence with an increased proliferation rate, but increasingly less juvenile fish surviving the first year of life. The fact that the number of juvenile bullheads is decimated in the densely crayfish populated site in comparison to the sites with only a few crayfish is a very clear sign for a strong bullhead predation by crayfish.

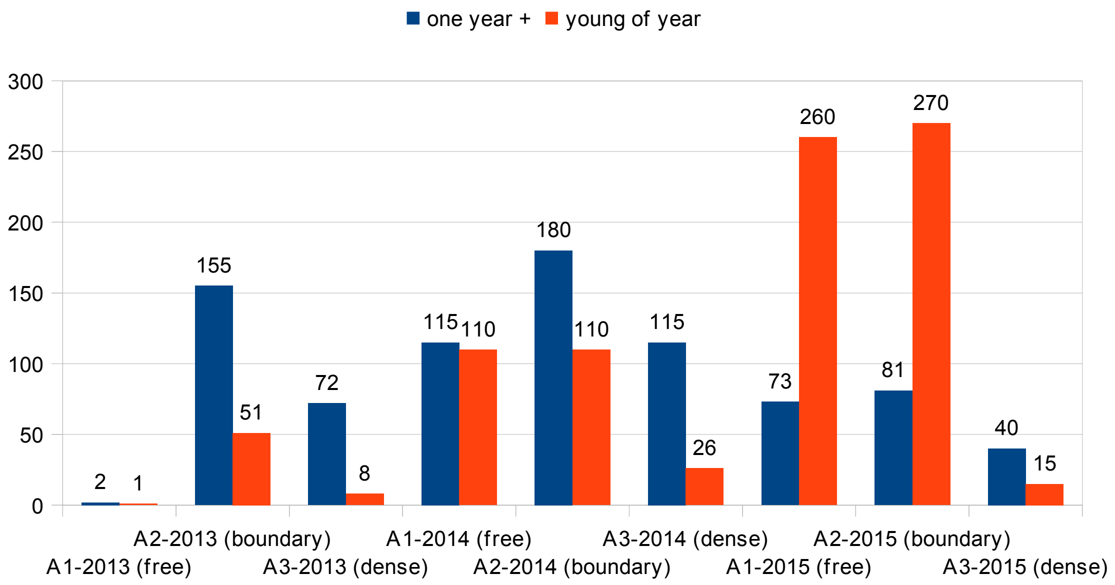


Figure 51: One year + and young of year bullhead (Cottus gobio) in the Ahr

Contrary to the situation in the Inde, the minnow (*Phoxinus phoxinus*) was the second most abundant species in the Ahr. As can be seen in figure 49, except for the first year of the study the least fish were always found in the densely populated site A3. In 2015 in general, far fewer minnows were captured. In the first year the crayfish free site was strongly affected by the sediment glut and the trout population in the densely populated site was decimated. This may be an advantage for the minnow as a bait fish of trout. This year they seemed positively affected by signal crayfish (r = 0.76, Fig. 48), but in fact they may have been influenced more by the decimation of brown trout. In the following years minnows were negatively correlated with signal crayfish and the proportion of the influence was only slightly more moderate than the one on the ground-dwelling sculpins (r=-0,73 in 2014, r=-0,93 in 2015, Fig. 48). The minnow is a small and lively schooling fish. It needs clean, clear and oxygenated water and is therefore highly vulnerable to water pollution and obstruction of rivers. Their favorite habitat are the upper water layers where young fish withstand up to 30 °C near the bank. Their diet consists of insect larvae, fish eggs, small crustaceans, algae, drifting particles and juvenile fish. It grows very slowly and is sexually mature at the end of the first to second year of life. The spawning season stretches from April to June, when they lay their eggs into fine gravel or coarse sand. They are typical swarm spawners (Gerstmeier & Romig 1998). As inhabitants of the upper water layers they are thus relatively well protected from direct predation by crayfish and more likely to be decimated by the local brown trout. The fish also has numerous alternatives of food sources if the crayfish decimates macrozoobenthos due to its broad nutritional spectrum.

One can hardly make a statement regarding the proportion of juvenile fish within the minnow population, because it is difficult in this small and slow-growing species to distinguish young of year juveniles from older fish. The differences are minimal. Also, no clear tendency with regard to body length could be ascertained. This varied widely between the sample sites and during the course of the examination.

The third most common fish species in the Ahr is the stone loach (*Barbatula barbatula*). It is a fish of the river bed and inhabits shallow, fast-flowing streams and rivers preferably with gravelly to sandy beds. They inhabit territories where they are staying hidden during the day under stones or roots. It is more sensitive than other small fish species and therefore also occurs even in water bodies of pollution grade 2-3. With heavier water-pollution the loach is soon gone - but it is also one of the first fish that populate the water body again if the water quality improves. In this case, their population can spread about 1.5 km per year. The nocturnal loach searches the gravel or sand for anything edible. It feeds predominantly on organic waste, insect larvae, small crustaceans, leeches and snails. Fish spawn will not be disdained as well (Freyhof 2013, Gerstmeier & Romig 1998). Thus, they are very similar to the bullhead (and signal crayfish) in their living and feeding habits, but more robust with regard to water quality and less strictly dependent on benthic invertebrates.

Figure 49 shows that the loach population is generally declining in the Ahr and reacted negatively to the signal crayfish. In 2013, 145 animals were still detected in the crayfish population boundary site (crayfish free site damaged by sediment glut). 2014 there were only 65 at the boundary and 55 at the crayfish-free site. In 2015, 51 fish were caught in the boundary site, and 32 in the unpopulated one. In the densely populated site on the other hand, figures were significantly lower in each year with 16 fish in 2013, 20 in 2014 and 14 in 2015. This effect was slightly attenuated in the first year of the study (r=-0.42), presumably due to the flooding and destruction of the crayfish free sample site, but it remained constant in the following two years (r=-0.93 in 2014, r=-0.71 in 2015; Fig. 48). Therefore, despite its robustness the loach shows an overall negative correlation to signal crayfish occurrence as well.

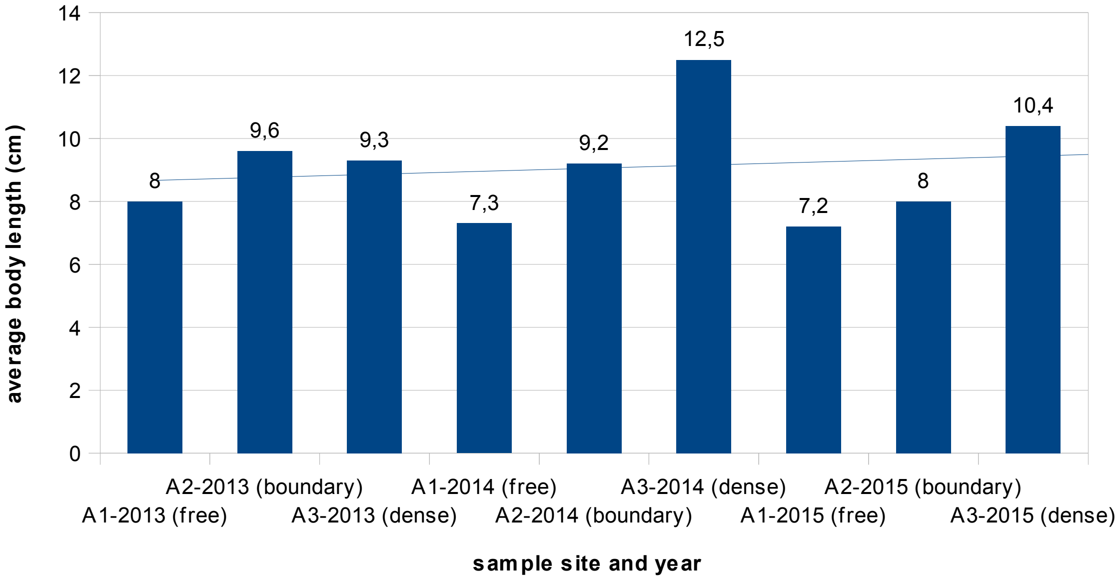


Figure 52: Average body length of stone loach (Barbatulus barbatulus) in the Ahr

It was striking that the body length of loaches increased with decreasing population density (i. e. with increasing signal crayfish density), similar to the observation in bullheads. So with reduced numbers came bigger fish within the densely crayfish populated site (Fig. 52). In this site it could also be noticed that loaches only occurred within the plants in a riffle, while none were to be found on the stream bed or in pools. Overall, the loach appears to be clearly negatively affected by the signal crayfish just like the bullhead, which is little surprising with regard to their similar lifestyles and needs.

The brown trout (*Salmo trutta fario*) took place 4 in the frequency ranking in the Ahr. They only accounted for a relatively small proportion of the fish population and the influence by signal crayfish on them was limited. Although there was a tendency to find less fish in the densely populated site, the differences were very small in most of the samples (Fig. 49) as were the correlative values which fluctuated between r=-0.3 and r=-0.56 (Fig. 48). Only in the first study year, the difference between the boundary site with 26 fish and the densely populated site with 6 fish was far more significant (crayfish free site damaged by sediment glut, which wasn't considered in the Pearson correlation). The average body length of trout increased minimally with increasing signal crayfish density and slightly declining fish density (Fig. 53). Juvenile trouts hardly played a role in that. In each site, there were only up to 4 juveniles of the same year in the samples - and even these low figures varied greatly. Overall, no threatening influence by the signal crayfish on the trout population could be found in the Ahr. This suggests that the Atlantic salmon (*Salmo salar*), which currently spreads upstream in the Ahr, will not be significantly affected, because it is quite similar to the trout in terms of lifestyle and diet. However, the trout of the Ahr migrate into small tributaries to spawn, while the salmon build their spawning pits within the main stream. It remains to be verified whether the reproduction of salmon is disturbed due to crayfish feeding on their spawn. A spawning pit that was visible during a helicopter-overflight within the region populated by signal crayfish (Dr. Jörg Schneider, oral communication) did not lead to a discovery of young fish in this area during the electric fishing in summer.

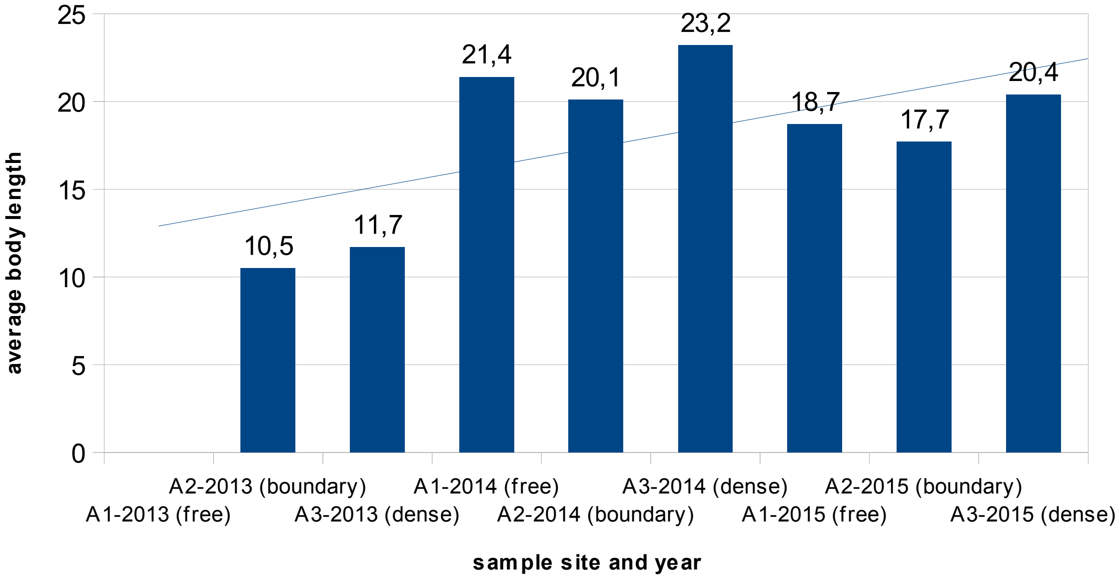


Figure 53: Average body length of brown trout (Salmo trutta fario) in the Ahr

Overall, it was found that the bullhead as a ground-dwelling, nocturnal fish with benthic invertebrates as the preferred main diet was most negatively affected by signal crayfish. The more signal crayfish occurred at a sampling site, the fewer sculpins were found there. During the course of the signal crayfish invasion, an overall increase of juvenile bullheads was observed with decreasing numbers of adults. The continuing decrease of older fish however showed that the juveniles usually did not survive their first year to rebuild the population. This suggests that crayfish not only compete with the fish at several levels, but also predate their offspring. The also ground-dwelling but much less demanding stone loach (only occurring in considerable numbers within the Ahr) was also negatively correlated with the signal crayfish and showed a general decline throughout the study. The increasing average body length in parallel to declining density shows that the survival of juveniles is obviously insufficient.

Fish species of the water column were less affected. Brown trout in both streams only tended to be slightly negatively correlated to signal crayfish density. The average body length decreased at the Inde with increasing total population, indicating a sufficiently high reproduction rate. However, juvenile trout were added by anglers in the area of study, so that this finding is not one hundred percent valid. At the Ahr on the other hand, the fish grew rather large during the study. The minnows as inhabitants of the upper water layers still showed a negative reaction to crayfish, though. Their negative response just did not reach the same intensity as the one of the bullheads. As substrate spawners in the shore area of the main stream, their spwan is probably most endangered by the crayfish, while brown trout at least migrate to the side streams to spawn creating at least some distance between their spawn and the crayfish.

Thus, as expected, fish species of the water column appear less strongly, but certainly still slightly negatively affected.

3.3 Direct behavioral effects of signal crayfish on brown trout (*Salmo trutta fario*) and atlantic salmon (*Salmo salar*)

The analysis of video recordings under laboratory conditions in which brown trout and salmon were exposed to different densities of signal crayfish, led to the results shown in Table 6 below.

Table 6: Use of hiding opportunities by trout at different densities of signal crayfish

Pool (number of signal crayfish)	Use of shelters by brown trout (min / max daily data) 2013
1 (0)	60% (47-67%)
2 (5)	26% (16-39%)
3 (10)	2% (0-13%)
4 (15)	34% (17-50%)

The long-term laboratory examination with brown trout in 2013 showed a shelter use of 60% of the examined time frame, if they were kept on their own without crayfish. Hiding time varied over the week between 47 to 67 %. With a signal crayfish stock of only 5 animals, shelter use by fish already declined by more than half to an average of 26% fluctuating between 16 to 39 %. At 10 crayfish present, the trout were almost continuously active - shelter use by fish went down to only 2% on average, varying between 0 and 13 %. The fact that the hiding time of fish increased again to 34% with a maximum crayfish stocking of 15 individuals, appears unexpected at first glance, but the video images clearly showed the reason for the now improved conditions for fish. The crayfish started to show an obvious migrating behavior, probably due to the high density of their own population. Crayfish wandered around in the basin rather than hide in the provided shelters, climbed up to the separation grids or simply sat in the corners. Thus, the caves were released for the fish to use. Figure 54 shows a still image in which the high number of crayfish sitting freely in basin 4 can be seen. At least 10 of the 15 animals are located outside the tubes. In contrast, one can see that all 10 crayfish are hidden in the pipes in basin 3. Thus, the situation of the fish in basin 4 is more similar to the one in basin 2 (5 crayfish that consistently accepted the hiding pipes), which is also reflected in similar percentages in shelter use (Tab. 6). The fish generally showed a strong change in behavior as soon as crayfish - even in small numbers - were in the pool. Trout became so hectic at the presence of 5 crayfish already that the evaluation of the video results was no longer possible with a single sighting of the selected time frame. Since the fish very often tried to take shelter in the tubes and were usually unsuccessfull and left again quickly due to the occupying crayfish, each tube had to be evaluated individually, so that the time frame had to be replayed 6 times (once for every single tube in the basin). The unhidden fish swam around frantically as well, instead of standing in the shelters quietly like the fish that were kept alone in basin 1. In this basin, there was generally very little movement of the fish. Therefore, it can be assumed that brown trout are not only exposed to the significant displacement of shelters and related increased pressure by predators, but also use significantly more energy when signal crayfish are present. Of course, in the field the migration behavior of crayfish would not lead to a relaxation of the situation, as was the case in laboratory setting, since the crayfish can actually migrate there and their population would thus stabilize at their tolerable maximum. Assuming that the density was still tolerable for them in basin 3 (10 crayfish), it is to be expected that this density would be established under natural conditions as a worst case scenario for fish.

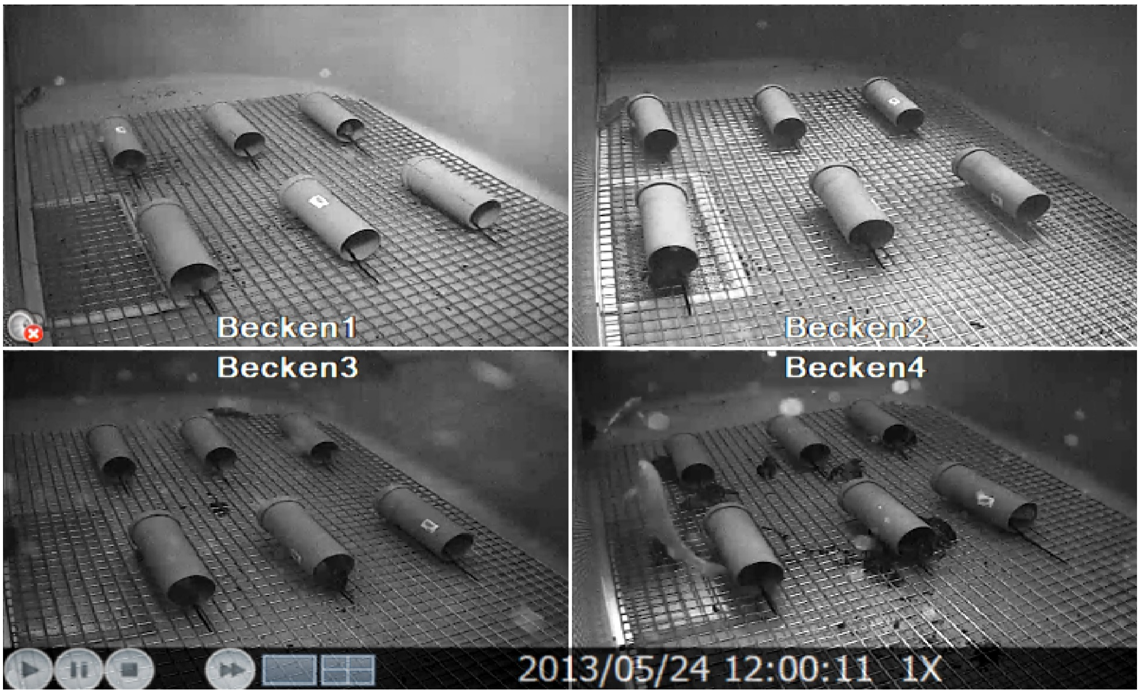


Figure 54: Still image from the video surveillance of the laboratory setup - In Basin 4, migration behavior of crayfish is visible.

In the first test series (trout / signal crayfish), weight of the fish was regularly measured in addition to the behavioral study (Table 7).

Table 7: Fish weight and growth rates in the laboratory setup

	Fish total weight [g] at date...		
Basin (number of crayfish)**	26.2.13	11.4.13	2.5.13
1 (0)	213,0	355,0 (+66,66 %*)	383,0 (+7,89 %*)
2 (5)	351,0	387,0 (+10,26 %*)	388,5 (+0,26 %*)
3 (10)	352,0	407,0 (+15,63 %*)	412,0 (+1,23 %*)
4 (15)	236,5	431,0 (+82,24 %*)	441,0 (+2,32 %*)

*Percentages relate to the growth rate in relation to the last measurement
**Basins on the same water circuit are highlighted equally

The growth rates of brown trout did not allow any conclusions regarding crayfish influence. In March, the weighing of the fish had to be suspended due to ice on the basins. Water heaters were installed as a consequence to keep the pools free of ice. This led to slightly different temperatures in the two water circuits. It was expected that this would affect fish growth. When evaluating the results, however, it became apparent that within both circuits, one group of fish grew quickly and the other one rather slowly - thus the temperature seemed to have no major impact on the growth rates. From mid April onward, nights became frost-free again and the water heaters were turned off. Henceforth the temperature in both circuits prevailed at a constant temperature of 15 °C. The measurement in early May showed continued weight gain of 7.89 % of the trout in the crayfish-free pool. In pools with crayfish stocking the fish grew more slowly than in the basin without crayfish, however, their growth rate was positively correlated with the number of crayfish - a phenomenon that could be observed in April already. In part, the differences in growth rates could be explained by the differing weights of the fish groups when the basins were first stocked. The groups with the lowest starting weight (basins 1 & 4) grew the fastest. When comparing the weight gain, one has to consider that under laboratory conditions and with the use of automatic feeders an oversupply of food was available. Animal protection laws alone would have made any other approach impossible. A recognizable competition for food between the two species, as it would have occurred in the field, could not be provoked under these conditions though.

Furthermore, it could be already deduced from the weekly cleaning work that crayfish have a positive effect under laboratory conditions in a way that would not work in a natural ecosystem. In the basins 1 and 2 (cycle 1) with 5 crayfish for example, significantly more feces and uneaten food had to be siphoned off than in basins 3 and 4 (cycle 2) with a total of 25 crayfish. Apparently, the crayfish supported the filter system by their permanent foraging and the connected resuspension of solid particles on the basin floor, thereby cleaning the basin of dirt. This was also reflected in water quality, which always was a bit better in cycle 2 than in cycle 1.

So overall, apart from the undeniable significant displacement of brown trout from shelters, the laboratory setup showed quite a positive impact of crayfish as well. This only proves however, that one can never create completely natural conditions in an experiment. Neither will high crayfish densities lead to a vacancy of shelters, nor will they improve water quality by their constant rummaging in the field. It can therefore be regarded as established that signal crayfish have a very strong negative impact on the supposedly uncorrelated fish species of the water column by competition for shelter. Apparently this also applies if the species are kept together for several weeks to get used to each other (which had not been practiced in previous studies). Species that are even more bound to the stream bed and thus are in contact with crayfish more frequently, such as the bullhead, will be even more severely affected by competition for shelter - which already showed in the electrofishing results in the field.

2014 the next experimental setup - study part 2 "Influence of signal crayfish on salmon" – was run. The fish had kindly been provided by the salmon center Hasper Dam. In Germany today, the salmon is found sporadically in the Rhine and its tributaries, as well as in the Elbe. It will still take decades before there will be stable self-replicating salmon populations in Germany again though. At the moment, hundreds of thousands of smolts are released each year, of which naturally only a fraction recurs (Froehlich-Schmitt, 2004). For freshwater habitats, the salmon has been granted legal protection under the Habitats Directive (Behrens et al. 2009). In the Ahr - one of the two rivers examined in this study - natural offspring could also be detected during contol electrofishings in 2015. Salmon and signal crayfish are therefore definitely in contact, because the salmon ultimately travels up the rivers just as the crayfish does. A displacement from shelters might result in a decisive disadvantage for the rare fish and their successful spread.

The salmon in the experimental setup showed significantly lesser hiding behavior than trout. This varied greatly, but averaged only at about 20 % of time used for hiding. It made no difference whether no or only a small number (5) of crayfish were in the basin. Only at a higher crayfish density of at least 10 animals the salmon ceased their hiding behavior completely and consequently remained in the water column (Tab. 8). Thus, a displacement of salmon from shelter by signal crayfish is also detectable, but as these animals generally had a smaller tendency to use shelters, the effects in relation are not as extreme as in brown trout. In the salmon experiment as well, crayfish showed migration behavior at the highest density of 15 animals in basin 4, but this brought no improvement of the shelter situation for the fish, given that they stayed in the upper water layers from a certain crayfish density of 10 animals onward and no sheltering attempts took place anymore. It should be noted that the observations took place in spring at constant water temperatures of about 15 °C. Salmon become increasingly nocturnal at low temperatures (Fraser et al. 1993, 1995, Heggenes et al. 1993). Crayfish stay hidden more frequently during the winter months as well, because they take up little food and move less and much slower due to low temperatures. So it is quite conceivable that the situation for salmon in winter could become much more dramatic when they can not find fitting daytime shelters and are therefore increasingly vulnerable to predators such as cormorants.

Table 8: Use of hiding opportunities by salmon at different densities of signal crayfish

Pool (number of signal crayfish)	**Shelter use by Atlantic salmon (min / max daily data) 2014**
1 (0)	18% (9-33%)
2 (5)	19% (0-46%)
3 (10)	0% (0-1%)
4 (15)	0% (0-1%)

All in all, displacement from shelter by signal crayfish could be observed in both examined open water fish species. Brown trout were more strongly affected, as these fish generally used shelters more often than salmon. The latter on the other hand, avoided any conflict with the crayfish and did no longer attempt to even use any shelters from a certain crayfish density (which is also reached under natural conditions) upward. Even several weeks habituation of the fish and their tenlegged competitors resulted in no improvement of the situation. Even if the laboratory setup can not imitate a natural situation one to one, the results were clear enough to be able to assume that in the natural ecosystem a competition for shelter will occur between salmonids and signal crayfish. Ground-dwelling fish will naturally be affected far more strongly. This is probably one of the reasons why bullheads and loaches already appeared massively negatively affected by crayfish in the wild.

3.3.1 Influence of different densities of native noble crayfish (*Astacus astacus*) on brown trout (*Salmo trutta fario*)

Table 9: Use of hiding opportunities by brown trout at different densities of noble crayfish

Pool (number of noble crayfish)	**Shelter use by brown trout (min / max daily data) 2015**
1 (0)	10% (0-29%)
2 (5)	3% (0-17%)
3 (10)	0% (0-1%)
4 (15)	1% (0-6%)

Since trout generally showed a stronger urge for hiding than salmon, 2015's crayfish comparison experiment was conducted with brown trout. It was aimed to show whether the European noble crayfish would actually cooperate better with fish than the invasive species.

A similar trend as in the 2013 signal crayfish trial became visible: The fish were hidden most often when no noble crayfish were present and their shelter use decreased with increasing crayfish density (Tab. 9). Brown trout in this trial however showed a generally reduced hiding behavior – even if no crayfish were present in the basin. Whether this might be due to seasonal factors or the use of another group of individuals, could not be clarified. Because of time pressure in the last study year the fish and crayfish also could not be allowed a habituation period quite as long as the ones in the previous trials, but the fish were already accustomed to the presence of noble crayfish from the breeding facility. But it is possible that they were not sufficiently accustomed to the proffered hiding form of tubes yet, which did not exist in the nursery. This might have been the reason why they did not accept the tubes as well as in the first test series.

The generally reduced hiding behavior of the fish can not be finally explained and was so low that it hardly allows meaningful conclusions. But tendencially, the same trend as in the signal crayfish trials occurred. Based on the results, one must assume that the higher reproductional rates and higher attainable densities of signal crayfish play a more decisive role in their invasive and competitive success in the ecosystem than their higher aggression. However, one must note that these are some of the undeniable differences between the two types of crayfish. Signal crayfish reach much higher densities in the field than noble crayfish and multiply much faster (Söderbäck 1991, Westman et al. 2002, Lewis 2002, Pasini 2008, Dunn et al. 2009). This seems to be the key difference between the two species, which triggers the negative effects in the field. Up to a certain density, the ecosystem can supply for large omnivores. However, European ecosystems are not able to support the high densities of the signal crayfish. This reaction was also noticeable in the field studies on indirect effects of signal crayfish through the food chain. At low densities the entire ecosystem showed little adverse reaction, only in high densities the crayfish affected it to the negative. This seems to apply to both the overall system and for the direct interaction with fish.

The additional higher aggressiveness of signal crayfish can still also play an albeit subordinate role. It could not be observed that trout and noble crayfish shared a tube, which would have led to the assumption that native species can tolerate each other better. However, with noble crayfish in contrast to signal crayfish no direct attacks of crayfish on fish have been observed in the trials. In contrast, signal crayfish regularly caused injuries to the fish, which sometimes led to the death of the individuals affected (Fig. 55).

Figure 55: Injured brown trout in the experimental setup with signal crayfish

4. Conclusion

The study revealed that signal crayfish spread and multiplied in both streams, but the speed of the spread and the population maxima varied depending on the individual system. Once an area is reached and populated, a population maximum is established, which varies according to the encountered conditions – probably also depending on whether or not the population is regularly harvested by anglers. Where the invasion travelled downstream (Inde), the crayfish spread much faster than where they spread against the current (Ahr). However, here too, individual animals could be detected at a great distance from the main population, which had traveled long distances against the current within a short amount of time.

The macrozoobenthos did not always respond negatively to the presence of the crayfish. Groups that had a high proportion in the total benthos count were decimated the most. This effect could vary greatly depending on the seasons. Other factors also played into the strength of influence. There was evidence that decimation of one group by the crayfish resulted in increased proliferation in other groups. Some groups did not show any reaction to crayfish at all. In any case, species composition changed due to these different reactions. The crayfish had a negative impact on total macrozoobenthos numbers, but this effect only became significant if the crayfish population reached a certain level. Population densities that corresponded to those of the native noble crayfish, could apparently be coped with better by the invertebrate community.

The examination of the fish population in the field showed that ground-dwelling, nocturnal fish species with benthic invertebrates as their main food source, like bullheads and loaches were most negatively affected by signal crayfish. Fish species of the water column like trout and minnow were less affected but still showed negative tendencies. Brown trout in both streams tended to be only slightly negatively correlated to signal crayfish density. In the laboratory, displacement from shelter by signal crayfish could be observed in both examined open water fish species. Brown trout were more strongly affected, as these fish generally used shelters more often than salmon.

Since many negative effects only occurred in conjunction with high signal crayfish densities, it can be expected that the ability of this species to reach higher densities in less time than native crayfish is ultimately more crucial to the ecosystem than higher food consumption / aggression of the single animal. Signal crayfish were well sustained by ecosystems to a certain extent - just as well as noble crayfish. The large differences in effects between the two streams farther show that the signal crayfish effects on an ecosystem can not be predicted but are dependent on many factors and local conditions. The crayfish can have different effects in any system. Therefore, studies on the reproductive and migrant/invasive behavior of different crayfish species seem to be particularly important for the future.

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