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Management in Practice

Management of invasive, plague-carrying signal crayfish by physical exclusion barriers

Christoph Chucholl[1,*], Franziska Chucholl[2,3], Laura Saskia Epp[3] and Alexander Brinker[1]

[1]*Fisheries Research Station (LAZBW), Argenweg 50/1, 88085 Langenargen, Germany*
[2]*Wildlife Ecology and Management, University of Freiburg, Tennenbacher Str. 4, 79106 Freiburg, Germany*
[3]*Laboratory of Environmental Genomics, Limnological Institute, University of Konstanz, Mainaustr. 252, Germany*
**Corresponding author*
E-mail: *christoph.chucholl@lazbw.bwl.de*

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Abstract

Invasive, plague-carrying signal crayfish represent a significant threat to imperiled European crayfishes. In the absence of a feasible eradication technique, physical barriers that separate invasive from native crayfish populations have been suggested as management strategy. Here, we investigated the effectiveness of three serial barriers with different functionality (flow-based *vs*. waterfall-based) in a headwater stream in southwestern Germany on containment of signal crayfish and crayfish plague. Crayfish distribution was surveyed three and six years after the construction of the barriers using manual search, trapping, and eDNA detection, whereby a tributary stream without barriers served as a form of control for a "do-nothing" scenario. The efficacy of the barriers was also empirically assessed by stocking marked crayfish downstream of the barriers and tracking their nocturnal behavior. After six years, native crayfish were still present upstream of the barriers but went extinct in the control stream due to signal crayfish invasion. Following two years of extreme drought after the first survey, signal crayfish were able to overcome the flow-based barrier but were never detected upstream of the waterfall-based barriers. Overall, this case study provides evidence for the effectiveness of intentional stream fragmentation as management strategy against invasive aquatic species.

Key words: *Pacifastacus leniusculus*, *Austropotamobius torrentium*, *Astacus astacus*, containment, conservation, crayfish barriers

Introduction

Native freshwater crayfish (Crustacea: Decapoda: Astacidae) are among the most threatened animal taxa in Europe (Souty-Grosset et al. 2006; Baer et al. 2014; Chucholl and Brinker 2017). In Germany, the conservation status of the three native species noble crayfish (*Astacus astacus* (Linnaeus, 1758)), stone crayfish (*Austropotamobius torrentium* (Schrank, 1803)), and white-clawed crayfish (*Austropotamobius pallipes* s. str. (Lereboullet, 1858)) has worsened to "unfavourable-bad" (national report under Article 17 of the Habitats Directive, BfN 2019) and population numbers are reported to be falling (Chucholl and Schrimpf 2016; Chucholl 2016).

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The main causes of the long-lasting decline of native crayfish are known: ongoing spread of invasive North American crayfish species, which are natural reservoir hosts of a deadly disease, is the primary and most widespread driver of native crayfish species disappearance (Holdich et al. 2009; Kouba et al. 2014; Chucholl 2016; Mozsár et al. 2021). Habitat loss and deterioration represent further, secondary threats that mostly act on local to regional scales (Souty-Grosset et al. 2010; Chucholl and Schrimpf 2016).

Invasive North American crayfish species have been shown to be ecologically dominant over native crayfish, resulting in their gradual displacement through interference competition (Gherardi and Daniels 2004; Huber and Schubart 2005; Holdich et al. 2009). Especially the aggressive and large signal crayfish (*Pacifastacus leniusculus* (Dana, 1852)) has been repeatedly implicated in systematic large-scale displacement of native crayfish species due to its high rate of spread and its significant niche overlap with native crayfish species (Holdich et al. 2014; Chucholl 2016; Grandjean et al. 2017).

In addition, most populations of North American crayfish species in Europe are carriers of the parasitic oomycete *Aphanomyces astaci* Schikora, 1906, which inflicts devastating mass mortalities among native European crayfish, known as crayfish plague (Cammà et al. 2010; Svoboda et al. 2016; Grandjean et al. 2017). Crayfish plague outbreaks, caused by spill-over from invading or translocated non-native crayfish hosts, are widely considered as one of the most severe threats to the long-term survival of European crayfish species (Souty-Grosset et al. 2006; Chucholl 2016; Préau et al. 2020).

While habitat loss and deterioration can theoretically be turned off and have potentially reversible effects, invasive non-native crayfishes and crayfish plague may represent long-term threats that cannot be removed. Eradication of established non-native crayfish occurrences is generally considered a daunting task and relies on brute force methods (biocide treatments and habitat destruction) (Gherardi et al. 2011; Manfrin et al. 2019; Peay et al. 2019). These eradication methods are not widely applicable because of adverse effects on non-target organisms and the associated costs are often prohibitive (Peay et al. 2019; Krieg et al. 2020).

In the absence of a widely-applicable feasible eradication method, prevention and control of the further spread of invasive, plague-carrying crayfish remains the only viable management strategy (Chucholl 2014, 2016; Krieg et al. 2020). In particular, separation of already established non-native crayfish populations and native crayfish populations can be promoted by impassable natural or artificial physical barriers (Dana et al. 2011; Manenti et al. 2014; Krieg and Zenker 2020; Barnett and Adams 2021; Jones et al. 2021). This strategy of invasive species containment is mainly applicable to headwater reaches and low order tributary streams, where the vast number of remaining populations of native crayfish species is located (Chucholl and Schrimpf 2016; Chucholl 2016; Mozsár et al. 2021).

Design and construction of exclusion barriers for invasive crayfish has consequently received increasing attention by conservation biologists (Rosewarne et al. 2013; Chucholl 2014; Waldmann 2019; Krieg and Zenker 2020; Barnett and Adams 2021). However, being a rather novel and as yet rarely used conservation strategy, exclusion barriers have been implemented in only a few instances in Spain, southern Germany, Switzerland, Great Britain, Norway and the USA (Dana et al. 2011; Rahel 2013; Cowart et al. 2018; Krieg and Zenker 2020). Several of these barriers have not been reached by non-native crayfish yet and information on the effectiveness of exclusion barriers is overall scarce (but see Dana et al. 2011; Cowart et al. 2018; Krieg et al. 2021).

This paucity of information is unfortunate in that effective species management strongly relies on information about success or failure of management measures in practice (Catalano et al. 2019; Krieg et al. 2021). In the case of failure, it is important to understand the underlying reasons in order to be able to refine or eventually abandon management actions. This holds especially true for containment of invasive signal crayfish, which is a prerequisite for the long-term viability of native European crayfish species (Holdich et al. 2014; Chucholl 2016), as requested by the Habitats Directive (Council of the European Communities 1992), as well as a management goal arising from the EU regulation on invasive alien species (EU Regulation no. 1143/2014).

To advance our knowledge on containment of invasive signal crayfish, we investigated the effectiveness of three serial in-stream barriers with different functionality (flow-based *vs*. waterfall-based) in a small headwater stream in southwestern Germany under field conditions. For this, crayfish distribution was intensively surveyed three and six years after the construction of the barriers and the efficacy was also empirically assessed by stocking and tracking marked crayfish. The insights from this study can help decision-makers engaged in invasive species management or native crayfish conservation to set up effective exclusion barriers for non-native crayfish.

Materials and methods

Study catchment and barrier installation

Study area

The study area is located in southwestern Germany (federal state of Baden-Württemberg) and comprises the headwater reaches of the river Bottwar (mean elevation 387 m a.s.l., range: 232–535 m a.s.l.). The Bottwar is a carbonate low mountain stream of 18 km length and features a total catchment area of approx. 80 km^2. It is part of the Neckar catchment, which drains into the Rhine River.

The headwater system is composed of a 2nd order stream (Bottwar), which merges with a 3rd order stream (Kurzach) after approximately 5.5 km

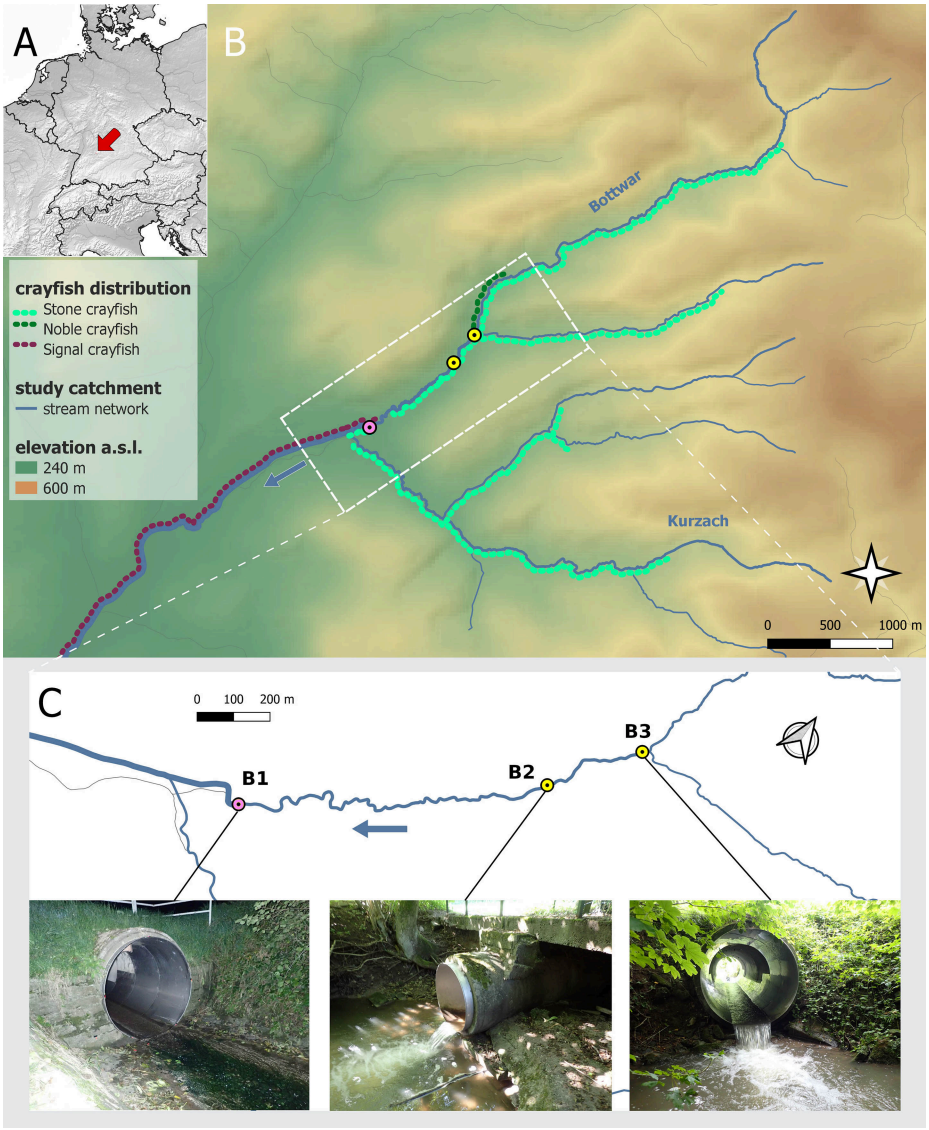

Figure 1. Overview of the study area and the known crayfish distribution in 2014 (A, B), and location and pictures of the three exclusion barriers (modified pipe culverts; panel C; please note the rotated map); fill color indicates barrier functionality with pink = flow-based and yellow = waterfall-based barriers. Blue arrows indicate the direction of stream flow. The red arrow in A denotes the study area.

(Figure 1). Both headwater reaches are in a near natural state and characterized by riffle-pool sequences, with a stream width between 0.5 and 2.0 m and an average depth of 0.05 to 0.40 m. The substrate is largely composed of stones, gravel and sand, and the riparian vegetation is dominated by deciduous trees. Bullhead (*Cottus gobio* Linnaeus, 1758) and brown trout (*Salmo trutta* Linnaeus, 1758) are the predominant fish species.

Crayfish fauna and crayfish plague status

Both headwater streams originally harbored an abundant stone crayfish population. The population within the Bottwar stream has been routinely monitored for reporting under the Habitats Directive (Council of the European Communities 1992) since 2011. Fish ponds along the Bottwar stream were

further inhabited by native noble crayfish, which co-occurred with stone crayfish in a short section of the stream (Figure 1).

In 2013, invasive non-native signal crayfish were reported for the first time near the confluence of the Bottwar and Kurzach streams, overlapping in distribution with stone crayfish for approximately 150 m. A subsequent crayfish survey in fall 2013 revealed that signal crayfish had already invaded a 5.5 km-stretch in the middle course of the river Bottwar (Figure 1). The circumstances of the initial signal crayfish introduction remain unknown.

During the fall survey, signal crayfish had been sampled from the Bottwar stream (N = 21) and subsequently tested for presence of the crayfish plague agent at the University of Landau (according to Vrålstad et al. 2009). *Aphanomyces astaci*-DNA was detected in four signal crayfish, which corresponds to a prevalence of infection of 0.19 (0.05–0.42 95% CI).

Installation of exclusion barriers

In the face of the imminent threat by invading signal crayfish and crayfish plague, a conservation project for stone crayfish was initiated by the Fisheries Research Station and the fishery agency (Chucholl 2014). To prevent the upstream invasion of signal crayfish and crayfish plague into the headwater section of the Bottwar stream, three pre-existing pipe culverts have been modified into exclusion barriers in summer 2014 (Figure 1).

For this, the most downstream pipe-culvert (diameter 1.5 m) was lined with smooth metal sheets throughout its whole length (18 m) to prevent upstream passage by signal crayfish (Figure S1A). Laboratory studies suggest that signal crayfish are not able to pass over smooth surfaces against water currents > 0.65 m/s (Frings et al. 2013; Krieg et al. 2021). The effectiveness of this barrier construction depended therefore on water flow. At the time of construction, water velocities above the smooth surface exceeded 1.3 m/s.

The downstream end of the culvert transitions into a concrete platform with a small waterfall at the end (Figure 1). The concrete platform and waterfall feature rich biofouling (algae growth) on top of a rough surface and were therefore deemed passable for crayfish (Krieg et al. 2021). The discovery of two signal crayfish immediately upstream of the pipe-culvert in late 2013, prior to the barrier construction, corroborated this notion.

The two pipe-culverts following upstream (diameter 1 m) were also lined with smooth metal sheets throughout their whole length (5.2 m) (Figure S1B). At the downstream outlets, both culverts were elongated by 0.15 m with the metal sheets to produce an overhanging free fall (Figure 1). At average water discharge, the vertical heights of the waterfalls were 0.15 and 0.20 m; total height differences between the outlets and stream bed surface were approx. 0.5 m. The primary barrier principle was to prevent invasive crayfish from climbing into the culverts through the vertical rupture of stream bed continuity and the overhanging smooth metal lids at the outlets (*cf.* Chucholl

2014; Krieg and Zenker 2020). As opposed to the 1st barrier (B1), functionality of the 2nd (B2) and 3rd barrier (B3) was therefore independent of water flow. All of the pipe-culverts are integrated into small dams (bridges) with a bank height ≥ 1.5 m and a width ≥ 5 m.

Due to a lack of similar pipe culverts or other suitable structures, no exclusion barriers have been implemented in the Kurzach stream. For the purpose of the present study, it served therefore as a form of control, representing a "do nothing" scenario.

Crayfish monitoring

Crayfish distribution was intensively surveyed three and six years after the construction of the barriers to examine their long-term efficacy. Surveys were conducted in summer months, when crayfish activity is generally high (17 to 19 July 2017 and 17/18 August 2020). Sampling effort was concentrated on the stretches between and upstream of the barriers for early detection of invading signal crayfish (Figure 1 and Figure S2).

The total number of sampling sites (20 to 80 m in length) varied between the two sampling occasions to account for updated knowledge on crayfish distribution in the control stream (Kurzach). In 2017, 19 sampling sites were surveyed for crayfish presence, of which 10 were situated in the Kurzach stream, and in 2020, 12 sampling sites were searched for crayfish, of which 4 were located in the Kurzach stream.

At each survey occasion, we used active and passive detection methods (see below) to ensure a high detection probability. To eliminate any risk of inadvertent transmission of crayfish plague with sampling equipment, all of the sampling sites within one stream were visited in the direction of water flow, and sampling equipment was completely changed and later thoroughly disinfected before entering a new stream (Chucholl and Schrimpf 2016).

Captured crayfish were identified and sexed, and either carefully returned to the sampling site (native crayfish) or removed (signal crayfish). All crayfish sampling and handling was done with permission from the fishery agency and the local fishing association.

Manual search

Manual search was the principal detection method that was used at all sampling sites. The method has been previously established for detection of crayfish in small headwater streams and is commonly used for routine monitoring (Peay 2004; Chucholl and Schrimpf 2016; Hilber et al. 2020). Within each sampling site, the best potential shelters (typically large stones) were carefully inspected for hiding crayfish using hand-held nets. Sampling effort averaged 18 and 19 inspected shelters per site in 2017 and 2020, respectively, with a range of 10 to 50 shelters per site. Shelter

occupation, expressed as average number of detected crayfish per inspected shelter, has been shown to correlate strongly with crayfish population density (crayfish × m^{-2}) and was therefore considered as a measure of relative abundance (Chucholl and Schrimpf 2016).

Visual nocturnal census

In addition to manual search during daylight, the stream sections between the barriers and one site downstream of B1 were searched for active crayfish using flashlights (Hilber et al. 2020), beginning one hour after sunset, *i.e.*, a period of crayfish peak activity (Holdich 2002; Schubert 2017). The site downstream of B1 has been colonized by signal crayfish since 2014 (Figure 1) and was included as control for crayfish activity. Nocturnal crayfish census was performed with an effort of three man-hours.

Trapping

Commercial crayfish traps ("Pirat" model, Rapu-Rosvo®, Finland) were set in deeper stream sections (depth > 0.25 m) between the barriers, and one known presence site downstream of B1 to control for crayfish trappability. Spacing between individual traps was at least 10 m, with a total of 13 and 10 traps used in 2017 and 2020, respectively. Traps were baited with cat food (Sheba®, Germany), lowered in the evening and recovered on the next day, allowing for an overnight exposure of at least 14 h. In 2020, an extra of eight traps was exposed in the Kurzach stream.

eDNA detection

In 2020, the conventional survey methods were complemented by species-specific environmental DNA (eDNA) detection of signal crayfish, stone crayfish and noble crayfish (Cowart et al. 2018; Manfrin et al. 2019; Chucholl et al. 2021). Species-specific primers from the Cytochrome *c* oxidase subunit I (COI) of *A. astacus* (Ast_COI_324F, Ast_COI_434R), *A. torrentium* (Torr_COI_333F, Torr_COI_514R) and *P. leniusculus* (Len_COI_320F, Len_COI_428R) were obtained from Chucholl et al. (2021). Water samples were collected from three sites in the Bottwar stream (upstream of B1 and B2, as well as within the downstream signal crayfish population) and one site in the control stream (Kurzach) using sterile standup Whirl-Pak® sampling bags (V = 2041 ml, Nasco). From each eDNA-sampling site, we took three replicate water samples, except for the Kurzach stream (one water sample). Per sample 1 to 1.5 l of water was filtered depending on how quickly the filter was clogged. The transport and filtration procedures of the water samples were carried out as described in Chucholl et al. (2021).

All extractions were carried out under an UV hood used solely for eDNA-extraction and located in a pre-PCR lab that is physically separated from the PCR and post-PCR lab to avoid back-contamination from PCR

products. Before and after every DNA-extraction the bench of the extraction hood was cleaned with DNA-ExitusPlus™ (PanReac AppliChem), wiped with ddH_2O and sterilized with UV-light for 15 min. Extractions were carried out using the DNeasy PowerWater Kit (Qiagen), following the protocol of the manufacturer. For every six samples, one extraction blank was included.

All water samples were subjected to qualitative endpoint PCR using a Flex Cycler (Analytik Jena). PCR assays were prepared in a prePCR lab under a UV hood reserved for PCR setup of eDNA extractions (environmental water samples) that was cleaned as outlined above for the UV hood used for DNA extraction. For each water sample three PCR replicates were undertaken including a positive control (tissue), an extraction blank and a negative template control (NTC) for every run of N = 22 samples. Prior to use, the PCR buffer, $MgSO_4$ and BSA solution were subjected to five minutes of UV irradiation at short distance from the light bulbs in a UVP crosslinker CL-1000 (see recommendations by Champlot et al. 2010). Each subsequent 25 µl reaction contained 15.05 µl water, 2.5 µl buffer (10x), 0.25 µl dNTPs (25 mM), 1 µl BSA (4 mg/ml), 1 µl $MgSO_4$ (50 mM), 1 µl of each Primer (10 µM), 0.2 µl polymerase (Platinum™ *Taq* DNA-Polymerase High Fidelity, Invitrogen™, ThermoFisher Scientific, 5U/ul) and 3 µl template DNA. For amplification the following cycling protocol was used: Initial denaturation at 94 °C for 4 min, 55 cycles of denaturation at 94 °C for 30 s, primer-specific annealing at 56 °C (*A. astacus*), 58 °C (*A. torrentium*), 60 °C (*P. leniusculus*) for 30 s, elongation at 72 °C for 20 s, and a final elongation step at 72 °C for 2 min. After amplification PCR products were visually checked for bands of the correct amplicon size using a 2% agarose gel electrophoresis stained with GelRed (Biotium, Hayward, CA). If only one technical PCR replicate was positive, at least one PCR product from this site was purified with ExoSAP-IT™ PCR Product Cleanup (ThermoFisher Scientific) and subjected to Sanger-Sequencing (performed by Microsynth Seqlab, Göttingen) to exclude the possibility of false positives.

Estimation of detection probability

To account for potentially imperfect detection, we used occupancy models to estimate the detection probability of the applied survey methods (manual search, trapping, and eDNA detection). For this, each inspected shelter, exposed trap or water sample was considered as a spatial replicate of the sampling sites (Dougherty et al. 2016; MacKenzie et al. 2017). Occupancy models estimate both site occupancy (probability of true species presence) and the detection process (probability of detection) and were constructed for each sampling occasion and method in the R package "unmarked" (Fiske and Chandler 2011). Full models included average water depth and the proportion of stone substrates (> 63 mm) as site covariates

for detection probability and occupancy. The full model for eDNA detection additionally included pH as site covariate for detection probability. Optimal models were derived by model selection based on the small-sample corrected Akaike Information Criterion (AICc). In case of competing models with a delta AICc < 2, the one with the highest AICc weight was chosen. The probability of detecting at least one crayfish given the species occupies the site (P_{det}) was then calculated for each sampling occasion and method from the estimated detection probability per sample (P_i) and the sampling effort (minimum number of samples per site, i) as $P_{det} = 1 - (1 - P_i)^i$ (Chucholl and Schrimpf 2016; MacKenzie et al. 2017).

Empirical assessment of barrier efficacy

To further assess the effectiveness of flow-based B1 and waterfall-based B2 *in situ*, we stocked marked crayfish directly downstream of the barriers and tracked their nocturnal behavior (Krieg et al. 2021). A total of three trials (two at B1, one at B2) were conducted from late September to mid-October 2017. This period coincided with the onset of the crayfish mating period, leading to elevated activity of males (Holdich 2002; Buřič et al. 2009), and low stream discharge, which challenged the functionality of B1.

Crayfish were marked with small glow sticks (DAM Megalite, 4.5 × 37 mm) that were attached to the dorsal surface of the carapace, and filmed by a camera (GoPro Hero 5) that was installed at a height of approximately 2 m above the downstream end of the barriers. Due to their small size and negligible weight, the glow sticks induce no behavioral changes in crayfish (Pollhammer 2014; Krieg et al. 2021).

The trials at B1 were performed with signal crayfish (N = 36 and N = 35 for the first and second trial, respectively), which have occurred downstream of the barrier since 2014. At B2, we used native noble crayfish (N = 27) as surrogate species to eliminate any risk of accidental crayfish escape or crayfish plague spread. Due to its rather high similarity to signal crayfish in terms of size and overall biology (Holdich 2002), the behavior of noble crayfish was considered as representative for signal crayfish (Krieg et al. 2021).

The experimental animals were captured on the same day by trapping, marked as described above, and subsequently left undisturbed in enclosures within the study stream. The size of the marked crayfish averaged 50.3 (± 8.5), 49.6 (± 7.0), and 47.9 (± 6.8) mm carapace length in the first, second and third trial, respectively (Table 1 and Figure S3), and all animals featured intact chelae and walking legs.

One hour after sunset, the marked crayfish were successively released directly downstream of the barriers. At B1, animals were placed at the margin of the concrete platform, while at B2 they were released into the pool underneath the waterfall (Figure 1). To prevent downstream migration of the marked crayfish, a barrier net was installed 2 m downstream of the

Table 1. Overview of the crayfish used for the *in-situ* assessment of barrier efficacy (trials 1–3). *CL* indicates the carapace length in mm and SD gives the standard deviation. See Figure 1 for location of the barriers and Figure S3 for size frequencies.

trial	barrier	crayfish species	*N*	sex ratio m:w	*CL* males mean (± SD)	*CL* females mean (± SD)
1	B1	signal crayfish	36	1.3	53.3 (± 9.3)	46.7 (± 5.8)
2	B1	signal crayfish	35	0.9	53.1 (± 7.8)	46.4 (± 4.2)
3	B2	noble crayfish	27	3.5	49.2 (± 6.9)	43.3 (± 4.2)

Table 2. Estimated detection probability per sample (P_i), sampling effort (minimum number of samples per site, i), and the probability of detecting at least one crayfish per site (P_{det}) across the different detection methods and sampling occasions. Estimates for P_i were derived from occupancy models; SE indicates the standard error.

survey method	year	P_i (± SE)	i	P_{det}
manual search	2017	0.36 (± 0.07)	10	0.99
	2020	0.70 (± 0.09)	10	1.00
trapping	2017	1 (± 0.01)	3	1.00
	2020	0.88 (± 0.12)	2	0.98
eDNA detection*	2020	0.83 (± 0.15)	3	0.99

* Species-specific detection of signal crayfish.

barriers. The nocturnal behavior of the marked crayfish was filmed for at least 4.5 h. After that time span, crayfish activity dropped markedly and the animals were removed.

The film sequences were subsequently analyzed in the software Tracker (v.4.11.0) (Pollhammer 2014). The camera images were corrected for perspective and calibrated based on real-world measurements. For each visible crayfish, individual movement tracks were created that recorded the movement pattern, walking distance and duration. Attempts to overcome flow-based B1 were defined as consistent movements in direction of the pipe-culvert across an upstream distance of a least 0.25 m. Attempts were considered as finished when the crayfish actively moved downstream or was displaced downstream by water current over the same distance.

During the trials, water velocities above the concrete platform directly downstream of B1 ranged between 0.76 and 0.84 m/s at an estimated discharge of 0.04 m^3/s. Water velocity at B2 was 1.63 m/s at an estimated discharge of 0.06 m^3/s. Water temperature dropped from 16 °C in late September to 13 °C in mid-October.

Results

Detection probability

The probability of detecting at least one crayfish (P_{det}) given the species occupies the site was consistently estimated at ≥ 0.98 for all survey occasions and methods (Table 2), which indicates a negligible probability of false negatives. The optimal occupancy models for the manual search method included depth and proportion of stones as site covariates for detection probability and occupancy, respectively (Table S1). The optimal models for trapping included either no covariates (survey in 2020) or depth as site covariate for detection probability and occupancy (survey in 2017). The

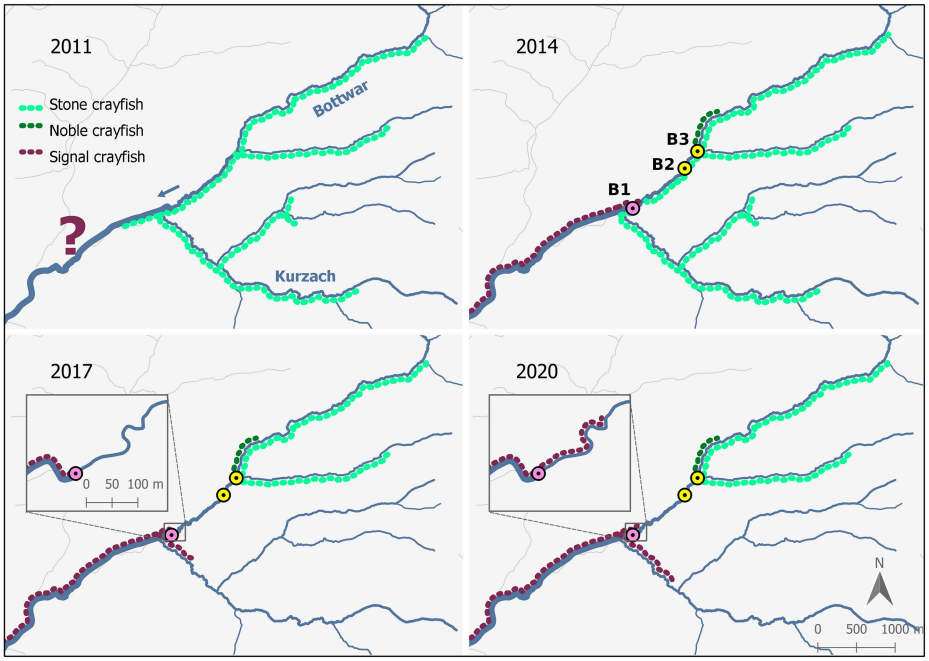

Figure 2. Distribution of native and invasive crayfish species in the study area from 2011 to 2020, as indicated by monitoring data of the Fisheries Research Station (2011–2014) and the intensive crayfish surveys in 2017 and 2020 (this study; Table S2 and Figure S2). The signal crayfish distribution prior to 2013 remains unknown, as indicated by the question mark in the top left panel. Dots represent the exclusion barriers implemented in 2014 (numbered in direction of upstream signal crayfish spread; fill color indicates barrier functionality with pink = flow-based and yellow = waterfall-based). Detail maps in the lower panels show the fine-scale signal crayfish distribution at B1.

optimal model for eDNA detection included no covariate for detection probability, while depth and proportion of stones were included as site covariates for occupancy. Furthermore, all of the detection methods were consistently able to detect crayfish at known presence sites, in particular downstream of B1.

Crayfish distribution in 2017 and 2020

The applied sampling methods provided congruous distribution information in both survey years (Table S2) and the emerging distribution pattern and trends are summarized in Figure 2. The most dramatic change in crayfish distribution occurred between the installation of the barriers in 2014 and the first survey in 2017. Between these years, native stone crayfish has apparently vanished from the control stream (Kurzach) and the stream sections between the barriers. The subsequent survey in 2020 also yielded no records of stone crayfish in these formerly populated stream sections. Most remarkably, we were not able to detect any stone crayfish eDNA in the Kurzach stream.

Upstream of B3, by contrast, native crayfish occurrences were confirmed in 2017 and 2020 (Figure 2). Occupancy of the inspected shelters averaged 0.5 crayfish/shelter in both years (range 0.2–0.9, N = 50 shelters across 5 sampling sites). eDNA of stone crayfish and noble crayfish was also detected

between B2 and B3; however, conventional sampling methods were not able to detect any crayfish in this section, which points to eDNA drift from the upstream populations (Chucholl et al. 2021).

Invasive signal crayfish was not recorded upstream of B1 in 2017. In 2020, by contrast, the species was present in an approximately 250 m-long stretch directly upstream of this barrier, but was still not recorded upstream of the remaining barriers (B2 and B3, Figure 2). Capture per unit effort (CPUE) of traps in the newly invaded stretch was 9 crayfish (N = 3 traps), compared to a CPUE of 19 crayfish at the old occurrence site downstream of the barrier (N = 2 traps). Likewise, shelter occupancy was lower at the newly invaded site (0.4 crayfish/shelter, N = 10 shelters) than at the old occurrence site (1.4 crayfish/shelter, N = 10 shelters). The crayfish captured from upstream of the barrier included small, juvenile crayfish (carapace length < 25 mm) as well as large, adult individuals (carapace length > 60 mm). Signal crayfish eDNA was detected at the old invasion site and the newly invaded site immediately upstream of B1 but not from further upstream, *i.e.,* between B2 and B3.

In the control stream (Kurzach), signal crayfish has gradually expanded its range in an upstream direction. By 2020, the species was recorded in an approximately 1140 m-long stretch, which corresponds to an average rate of upstream spread of 190 m per year since 2014. The CPUE of traps was lowest at the most upstream detection site (2.5 crayfish, N = 2 traps), *i.e.*, near the invasion front, and increased in downstream direction (7.5 crayfish, N = 2 traps). Likewise, shelter occupancy increased from 0.3 signal crayfish/shelter at the population edge (N = 20 shelters) to 1.4 crayfish/shelter at old invasion sites (N = 10 shelters).

Empirical assessment of barrier efficacy

None of the 71 tracked signal crayfish was able to pass through B1 during the two trials in late September 2017. However, 30 percent of the experimental animals succeeded to enter the pipe-culvert at one point (and were flushed downstream from within the culvert), while 32 percent moved upstream on the concrete platform but failed to climb into the culvert. The rest of the tracked animals (38 percent) moved directly downstream upon release. Overall, 62 percent of the animals showed an initial positive rheotaxis, which is more than to be expected by chance (chi-squared test, $p < 0.05$).

Animals that successfully entered the culvert also undertook more attempts (median = 3 attempts) to overcome the barrier than animals that moved upstream but failed to enter the culvert (median = 1 attempt; Mann-Whitney U test, N = 44, $p < 0.001$). The most attempts to overcome the barrier by a single crayfish were recorded for a larger male (carapace length ~ 55 mm); the animal made seven attempts to overcome the barrier within 32 min.

Average duration of the individual movement tracks was 15 min. After a maximum of 71 min, all of the tracked animals left the platform in downstream direction or were flushed downstream by water current. The movement tracks showed a strong propensity of the crayfish to remain at the shallow margins of the platform with water velocities < 0.2 m/s. Movements over land were only recorded for short distances (≤ 1 m) and time (≤ 2 min). None of the animals made an attempt to circumvent the barrier over land.

At B2, none of the 27 tracked noble crayfish attempted to leave the water and to climb into the pipe-culvert. The animals mostly roamed along the pool margins with water velocities < 0.2 m and avoided the turbulent water underneath the waterfall. After 5 h crayfish activity became very low and the trial was ended.

Discussion

Our study is among the first to investigate the effectiveness of crayfish exclusion barriers under field-conditions and across several years (Krieg and Zenker 2020; Krieg et al. 2021). Because crayfish are difficult to detect at low population densities (Peay 2004), we used multiple active and passive sampling methods, including highly sensitive eDNA detection (Cowart et al. 2018; Manfrin et al. 2019; Chucholl et al. 2021). Given the used sampling effort, the probability of detecting at least one crayfish given the species occupies the site was consistently estimated as very high and all detection methods arrived at congruent and plausible distribution information. We are therefore confident that the results reflect actual pattern and trends in crayfish distribution, even if species absence from a sampling site cannot be concluded from field surveys with absolute certainty.

A unique feature of this study was the inclusion of a control stream, in which no management action was applied. This offers the opportunity to contrast the effects of the implementation of the barriers in the Bottwar stream with a "do-nothing" scenario. Finally, we empirically assessed the functionality and potential weaknesses of the barriers *in situ*. Considering all of these elements combined, we believe that this case study is informative for future management actions on invasive crayfish.

Barrier effectiveness

This study provides evidence for the effectiveness of physical exclusion barriers to safeguard native crayfish populations from invasive, plague-carrying signal crayfish. This is best illustrated by the fact that native crayfish have vanished from the control stream without barriers but are still present in the Bottwar stream, where exclusion barriers have been set up. Native crayfish had initially faced a similar extinction risk in both streams due to imminent invasion of plague-carrying signal crayfish, which

strongly points to a causal link between barrier construction and native crayfish species survival in the Bottwar stream.

The apparent extinction of stone crayfish from the control stream (Kurzach) is most likely the result of an unrecorded crayfish plague outbreak between 2014 and 2017, caused by spillover of *A. astaci* spores from invading signal crayfish (*cf.* Kozubíková-Balcarová et al. 2014; Collas et al. 2016; Svoboda et al. 2016). Once the disease agent has been introduced into a European crayfish population, it is rapidly spreading up- and downstream by migration of infected native crayfish, resulting in elimination of the population within short periods of time (Kozubíková-Balcarová et al. 2014; Collas et al. 2016). This scenario is consistent to the detection of the crayfish plague agent in the invading signal crayfish population as well as the lack of any stone crayfish record from the Kurzach catchment since 2014. An alternative explanation, such as a pollution event, seems improbable, since other aquatic biota showed no obvious population changes during the study period and because stone crayfish vanished from the main Kurzach stream as well as a northern tributary; this points to a concurrent catastrophic event in both stream sections, consistent to a fatal disease outbreak.

The epidemic stone crayfish mortality had seemingly progressed upstream much faster than signal crayfish population spread, leaving a temporarily vacant niche in the middle and upper course of the Kurzach stream. At its current rate of upstream spread, the now vacant habitat will be taken over by invasive signal crayfish within the next decade, though. The pattern of crayfish plague spillover in lower stream sections and subsequent rapidly upstream-progressing mortality of native crayfish, followed by—slower—upstream spread of the invasive host population may well explain the repeatedly observed vacant "buffer zones" between invasive and native crayfish populations, with the latter often confined to distant headwater sections isolated by natural or artificial barriers (this study; Souty-Grosset et al. 2006; Kozubíková-Balcarová et al. 2014; Chucholl 2016).

The headwater catchment of the Bottwar stream, by contrast, still features viable native stone crayfish and noble crayfish populations because signal crayfish and *A. astaci* spread has been successfully controlled by the exclusion barriers. However, the 1st, flow-based barrier (B1) has been apparently overcome by signal crayfish between 2017 and 2020. This was an unexpected finding, since the *in-situ* assessment of barrier efficacy in 2017 as well as the survey in the same year indicated that this barrier had previously effectively stopped signal crayfish spread. However, the results of the empirical *in-situ* assessment of barrier efficacy are only indicative for the flow conditions encountered during the trials. Any conditions with stream flow lower than during the trials may have resulted in a failure of barrier functionality (Frings et al. 2013; Krieg et al. 2021).

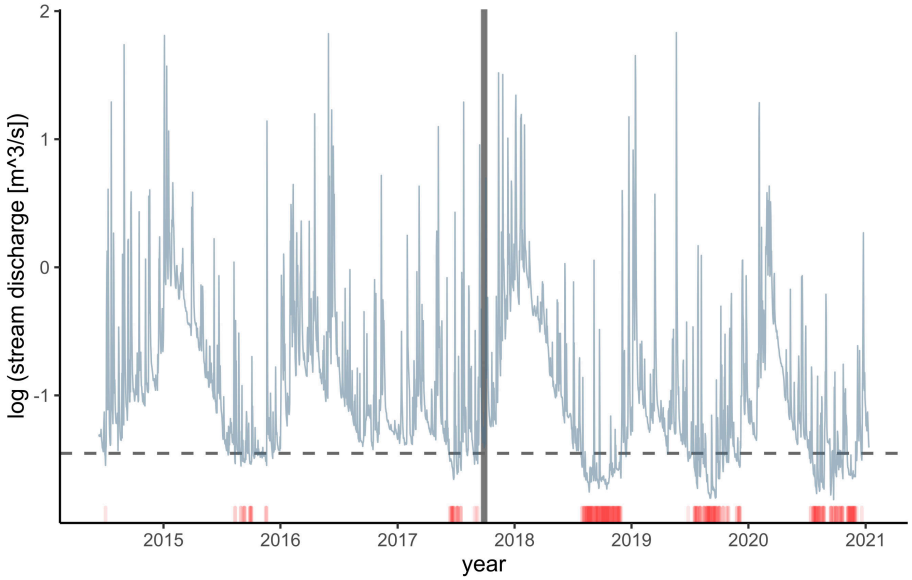

Figure 3. Discharge of the Bottwar stream (blue line) since construction of the barriers (daily means measured in the lower course of the stream, obtained from the "Umweltinformationssystem (UIS) der LUBW Landesanstalt für Umwelt Baden-Württemberg"). The horizontal hatched line corresponds to the stream discharge at which the 1st, flow-based barrier (B1) was effective to exclude signal crayfish during the *in-situ* assessment of barrier efficacy (date highlighted by vertical line). The red ticks beside the time axis indicate days with a stream discharge lower than 0.95 x this threshold, *i.e.,* conditions at which barrier functionality has been presumably compromised by low stream flow. Please note the marked increase in duration and intensity of extreme low-flow conditions since 2018.

Intriguingly, signal crayfish were observed upstream of the flow-based barrier following two years of extreme drought. Since 2018 there has been a marked increase in duration and intensity of extreme low-flow conditions in the Bottwar stream, which may have rendered the barrier repeatedly ineffective (Figure 3). In particular, stream discharge in autumn 2018 and 2019 (as well as in late 2020) was considerably lower than during the *in-situ* assessment of barrier efficacy. A breach of B1 by signal crayfish since 2018 would also be consistent with the signal crayfish population structure, comprising multiple age cohorts, and population extent, spanning a roughly 250 m-long stretch, upstream of the barrier in 2020. Although we cannot entirely rule out other means of signal crayfish spread across B1, such as intentional transport by humans or extended migration over land, a causal relationship between exceptional low-flow conditions since 2018 and barrier failure since 2018 seems likely. This deduction is also in line with recent work reporting facilitation of invasive signal crayfish spread by low-flow events (Mathers et al. 2020).

Because frequency and intensity of drought conditions are likely to increase further due to anthropogenic climate change (Forzieri et al. 2014; Spinoni et al. 2018), B1 will probably become increasingly ineffective. Based on this understanding, as well as the fact that flow-based barriers are inherently highly sensitive to any obstruction of water flow (Krieg et al. 2021), we advocate against the installation of further flow-based barriers in natural streams with a dynamic flow regime (Chucholl and Brinker 2017).

Functionality of the waterfall-based barriers, by contrast, is unlikely to be compromised by climate change effects; the *in-situ* assessment of barrier efficacy suggests further that barriers with an overhanging water fall are most effective at stopping invasive crayfish spread; specifically, we did not record any attempt of crayfish to enter the pipe-culvert at the 2nd barrier (B2). This finding coincides with related studies that position waterfall-based barriers, consisting of vertical walls with an overhanging lip above the water surface, among the most effective designs to exclude invasive crayfish (Ellis 2005; Dana et al. 2011; Krieg et al. 2021). A further requirement are smooth surfaces that prevent crayfish from climbing across the barrier (Ellis 2005; Chucholl and Brinker 2017; Krieg et al. 2021). In this study, this constraint has been met by the smooth metal sheets that protruded from the outlets of the modified pipe culverts.

The waterfall-based barriers were seemingly also able to halt the upstream spread of crayfish plague, as indicated by the disappearance of native crayfish upstream of the flow-based barrier (B1) but not upstream of the 2nd waterfall-based barrier (B3). Similar observations were reported from three streams in the Czech Republic and one stream in southwestern Germany, where physical dams (0.5–2 m in height) prevented upstream progression of crayfish plague mortalities (Kozubíková-Balcarová et al. 2014; *unpublished data*). The stone crayfish population upstream of B1 probably contracted the disease prior to or during the barrier installation in 2014 and the disease seemingly progressed past the 1st waterfall-based barrier (B2), until the chain of infection was eventually disrupted by B3. Since the barriers were installed in direction of stream flow to avoid any accidental upstream transport of *A. astaci* spores, B3 was the first barrier to be finished. By the time B2 was installed, the disease might have already spread past this barrier location. This seems conceivable, given that crayfish plague mortalities can progress upstream at a rate of more than one km per month during summer (Collas et al. 2016).

Overall, we are confident that the remaining waterfall-based barriers will continue to shield the native crayfish population and its habitat from invasive signal crayfish and crayfish plague. For this, barriers will be monitored and maintained on a regular basis (Chucholl and Brinker 2017; Krieg et al. 2021). The local fishing association, municipalities and district agencies are also well aware of the barriers and their functionality, which minimizes the risk of accidental crayfish plague or signal crayfish introduction by people. Although we cannot entirely rule out the risk of crayfish plague transmission through wild animals that move between streams, we deem this a low-probability event. Since *A. astaci* spores are highly sensitive to desiccation and unlikely to survive a passage through the digestive tract of warm-blooded predators, the only potential mode of *A. astaci* spore transmission through wild animals is on their moist surface (Svoboda et al.

2016). Because of the continuous production and anti-infectious characteristics of fish mucus, this mode of transmission is unlikely for fish but might occasionally occur in mammals and birds (Oidtmann et al. 2002; Svoboda et al. 2016). However, movements of water birds between water bodies struck by crayfish plague and nearby uninfected sites did not result in *A. astaci* transmission (Unestam 1973). Transmission of *A. astaci* spores on the surface of mammals and birds seems therefore rather unlikely, especially at spore doses relevant for successful infection of crayfish hosts (Svoboda et al. 2016). The primary mode of *A. astaci* dispersal is undoubtedly horizontal transmission among crayfish host individuals (Svoboda et al. 2016, and citations therein), which is effectively disrupted by impassable barriers (Kozubíková-Balcarová et al. 2014).

Outlook

Exclusion barriers are the only feasible method to prevent North American, plague-carrying signal crayfish from invading into upstream refuges of native European crayfish and ecosystems. This case study provides evidence for the effectiveness of this management strategy in practice and across several years. The failure of the flow-based barrier after three years of seeming functionality highlights the latent risk associated with this type of barrier design, in particular when accounting for the climate-change mediated increase of low-flow events, and stresses the importance of long-term function monitoring.

Based on our findings and in agreement with related research (Kozubíková-Balcarová et al. 2014; Krieg and Zenker 2020; Krieg et al. 2021), we recommend the implementation of waterfall-based barriers to halt the upstream spread of invasive crayfish and crayfish plague. To minimize the risk of failure and crayfish-plague transmission, at least two serial barriers are suggested. The section in between the barriers should be subjected to regular monitoring for early detection of and rapid response to barrier breach by invasive crayfish (Krieg et al. 2021). Furthermore, *in situ* experiments tracking crayfish behavior in front of barriers proved to be a suitable instrumental means to evaluate the functionality of site-specific barrier designs (this study; Rosewarne et al. 2013; Krieg et al. 2021).

Finally, upstream movements of co-occurring native fish and other aquatic taxa are likely to be also restricted by artificial waterfall-based barriers, which may result in undesired fragmentation of populations (Barnett and Adams 2021; Jones et al. 2021). In particular, upstream passage of hololimnic macroinvertebrate taxa and fish species with weak leaping and swimming abilities, such as bullhead, is likely to be hampered, whereas fish species with strong leaping and swimming capabilities, such as brown trout, may be able to force waterfall-based barriers (Utzinger et al. 1998; Holthe et al. 2005). The benefit of exclusion of invasive crayfish should therefore be

balanced on a case-by-case basis against potential disadvantageous effects of stream fragmentation. Exclusion barriers located in headwater streams represent the potentially best trade-off in this regard (Chucholl 2015; Chucholl and Brinker 2017).

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Authors' contribution

CC: research conceptualization, sampling design and methodology, investigation and data collection, data analysis and interpretation, and writing of original draft and revision. FC: investigation and data collection, data analysis and interpretation, and writing of original draft. LSE: contributed to funding provision, methodology, and writing the original draft. AB: contributed to research conceptualization, funding provision, and writing of original draft.

Ethics and permits

This work required no specific ethics and all crayfish sampling and handling was done with permission from the fishery agency and the local fishing association (please see Methods section).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Overview of the three best candidate occupancy models across the different detection methods and sampling occasions.
Table S2. Details of sampling sites and crayfish detection during the surveys in 2017 and 2020.
Figure S1. Simplified schematic drawings of the 1st (B1) and 2nd (B2) exclusion barrier for invasive signal crayfish in the Bottwar stream.
Figure S2. Map of the sampling sites during the crayfish surveys in 2017 and 2020.
Figure S3. Size frequency of the crayfish used for the *in-situ* assessment of barrier efficacy.

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