FIRST RECORD OF THE CALICO CRAYFISH *FAXONIUS IMMUNIS* IN THE NETHERLANDS (CRUSTACEA: DECAPODA)

Pim Lemmers, Jelle Wissink, Ronald Gylstra, Sander Heeman, Paul van Hoof & Bram Koese

In April 2025, an aberrant crayfish chela was found in a floodplain of the river Rhine in the Netherlands near the German border. Based on morphology and DNA, it was confirmed that it concerned a calico crayfish *Faxonius immunis*. Sampling using crayfish traps and dip nets in April 2025 did not yield any individuals. However, sampling using eDNA in October revealed that there was DNA present at two out of three sampled locations, suggesting the presence of a nearby population. We expect the calico crayfish to establish in the Netherlands, and expand in the floodplains of the river Rhine and interconnected waterways. Biodiversity and socio-economic impacts can be expected since this crayfish can establish high densities and is a good burrower.

INTRODUCTION

Invasive alien crayfish species can pose significant threats to the functioning of aquatic ecosystems (Angeler et al. 2001). The number of records and the abundance of alien crayfish in the Netherlands increased strongly over the last decade (Koese & Soes 2011, Koese 2021). Seven non-native crayfish species have established populations in the Netherlands, of which red swamp crayfish *Procambarus clarkii* (Girard, 1852), virile crayfish *Faxonius virilis* (Hagen, 1870), white river crayfish *P. acutus* (Girard, 1852), and signal crayfish *Pacifastacus leniusculus* (Dana, 1852) are associated with adverse impacts on biodiversity,

Figure 1. Chela of the calico crayfish *Faxonius immunis* found on 4 April 2025 in the Klompenwaard, a side channel of the Rhine in the Netherlands near the German border. The colouring of the chela appeared to have faded from exposure to sunlight. Genetic analysis confirmed the species identification. Photos Pim Lemmers.
Figuur 1. De schaar van de calicotrivierkreeft *Faxonius immunis*, gevonden op 4 april 2025 in de Klompenwaard, een nevengeul van de Rijn in Nederland, vlakbij de Duitse grens. De kleur van de schaar leek enigszins verbleekt door blootstelling aan zonlicht. Genetische analyse bevestigde de soortidentificatie. Foto's Pim Lemmers.

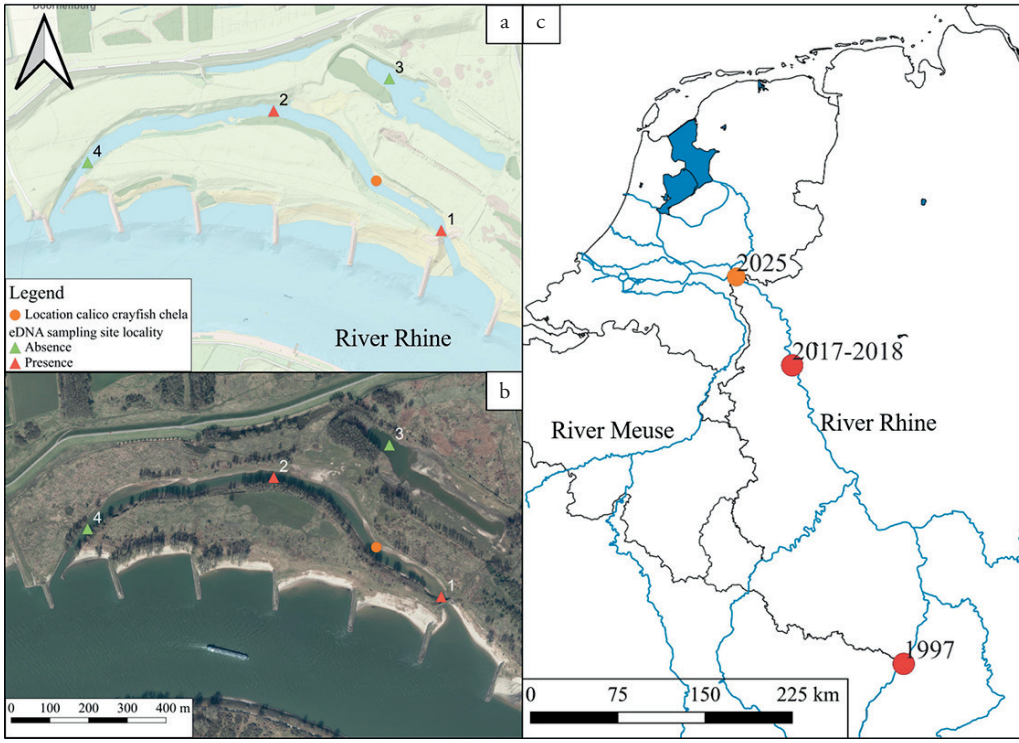


Figure 2. Geographical information, a. area 'Klompenwaard', the location of side channel of the Rhine in the Netherlands and the location where the calico crayfish *Faxonius immunis* chela was found in April 2025. The positions of eDNA sampling points (October 2025) are also shown (green indicates absence, red presence). The flow direction of the Rhine is from east to west, b. aerial image of the location in a, c. locations of calico crayfish records in Europe, based on Dehus et al. (1999), Vermiert (2020), and LANUK (2025).
Figuur 2. Geografische informatie, a. de Klompenwaard de locatie van de nevengeul van de Rijn in Nederland en de plaats waar in april 2025 de schaar van de calicotrivierkreeft *Faxonius immunis* werd aangetroffen. Ook de locaties van de eDNA-bemonsteringspunten (oktober 2025) zijn aangegeven (groen betekent afwezigheid, rood aanwezigheid). De stromingsrichting van de Rijn is van oost naar west, b. luchtfoto van de locatie in a, c. locaties waar de calicotrivierkreeft is aangetroffen in Europa, gebaseerd op Dehus et al. (1999), Vermiert (2020) en LANUK (2025).

ecosystem functioning, and water safety risks by instability of dikes and erosion of banks (Angeler et al. 2001, Koese & Soes 2011, Van der Wal et al. 2013, Lemmers et al. 2021a, Van Veenhuisen et al. 2026). The spiny-cheek crayfish *F. limosus* (Rafinesque, 1817) is the most widespread crayfish species in the Netherlands but is not associated with adverse impacts, primarily since the species does not burrow (Koese 2021, Lemmers et al. 2021a). Similarly, the narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823) does not seem to have an ecological impact, and this species has a very limited distribution. Finally, the marbled crayfish *Procambarus virginalis* (Lyko, 2017) has a limited distribution but the species is rapidly expanding and new populations are found annually (Lemmers et al. 2021b). Ecological impacts of this species have not yet been established, although they are expected (Lemmers et al. 2024). The narrow-clawed crayfish is the only alien species with a European origin; the other species originate from North-America. The likelihood of establishment appears to be a result of high organic load, deteriorated habitat quality due to anthropogenic

pressure, which has also led to a decline in crayfish predators (Lemmers et al. 2021a, Kanters et al. 2025).

A major dispersal corridor for alien species in the Netherlands is the vast network of interconnected waterways such as rivers, canals and small watercourses (Leuven et al. 2009). This also applies to the calico crayfish *F. immunis* (Hagen, 1870). This species is native to the North American continent. It has a wide natural range, spanning from Wyoming to Maine in the United States, and extending into Canada near Montreal. The first calico crayfish in Europe was recorded in 1997, in a small canal near the river Rhine, in the vicinity of the German city Karlsruhe (Dehus et al. 1999). Since then, the species has spread from the Stuttgart area in the river Rhine basin in a northern (downstream) and southern (upstream) direction (Herrmann et al. 2018, Tricarico & Lucy 2020). In 2017, a chela was found in the river Rhine near Düsseldorf (Germany), and a year later, a live specimen was found in the river Düssel (Vermiert 2020, LANUK 2025). This is approximately 100 km from the Netherlands. Given the rapid expansion of the calico crayfish in Germany, it had been anticipated that it would reach the Netherlands via the river Rhine. In 2019, this species was reported from the river Rhine (Amerongse Bovenpolder, Nederrijn) near Wageningen (Ottburg et al. 2019), but it was later concluded that the observation concerned aberrant virile crayfish. In this paper we present the finding of a calico crayfish chela in a side channel of the river Rhine east of Nijmegen (fig. 1). Molecular analysis confirmed the provisional identification of calico crayfish. In this article, we present information on species identification and the results of conducted

Figure 3. The side channel of the river Rhine in the Klompenwaard where the chela of the calico crayfish *Faxonius immunis* was found on 4 April 2025 during an extremely low water level in the river. At that time, the side channel was no longer flowing with the river Rhine. In October 2025 a positive detection of eDNA was established here. Photo Pim Lemmers.
Figuur 3. De nevengeul van de Rijn in de Klompenwaard waar op 4 april 2025 tijdens een extreem laag waterpeil in de rivier de schaar van de calicotrivierkreeft *Faxonius immunis* werd gevonden. Op dat moment stroomde de nevengeul niet meer mee met de Rijn. In oktober 2025 werd hier een positieve detectie met eDNA vastgesteld. Foto Pim Lemmers.

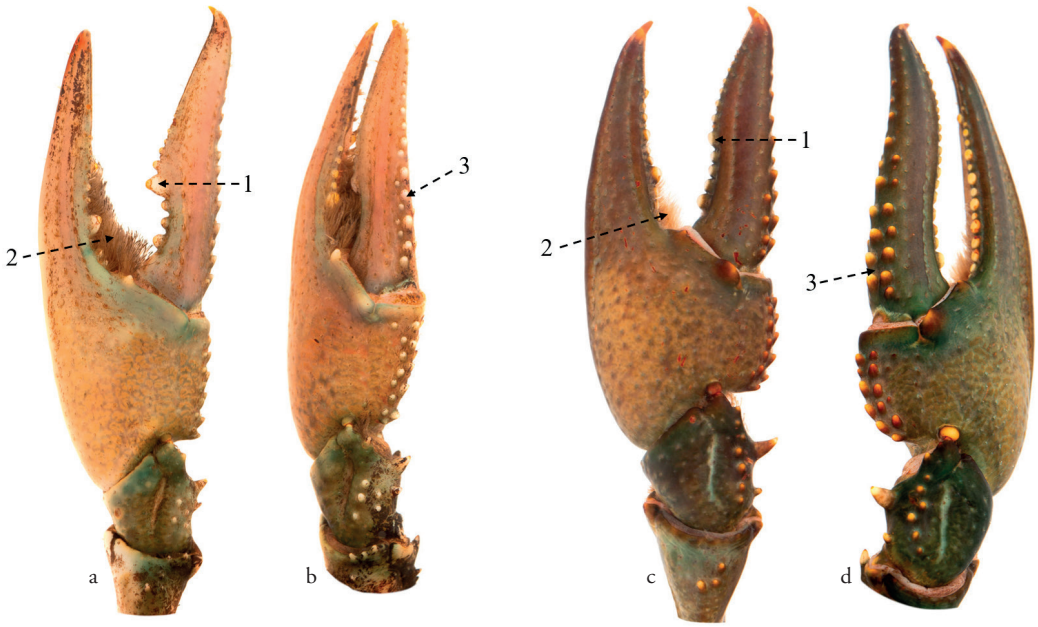
surveys using crayfish traps, dipnets and eDNA. Finally, a future perspective is briefly discussed.

KLOMPENWAARD

The study area 'Klompenwaard' is located in the Rhine floodplains, close to where the river flows from Germany into the Netherlands (fig. 2). At the end of the 1990s, the area was developed from agricultural land into a nature reserve to provide more space for the river Rhine during high water events. A side channel was developed, which until 2016 only flowed with the main channel during high water events. Since November 2016, an inlet structure has been constructed so that the side channel now always flows with the river Rhine (except during extremely low water levels).

FIRST DUTCH RECORD

The chela was found on the 4th of April 2025, during a period when the water level in the river



Faxonius immunis *Faxonius virilis*

Figure 4. Differences between chelae of a male calico crayfish *Faxonius immunis* (left) and male virile crayfish *Faxonius virilis* (right), a. lateral view of a calico crayfish chela with a pronounced tubercle in the middle of the dactylus (movable finger) that resembles a hook (1) and many setae (hair) on the inside of the chela (2), b. dorsal view of a calico crayfish chela with a single row of large tubercles on the dactylus (movable finger) of the chela (3), c. lateral view of a virile crayfish chela with no pronounced tubercle in the middle of the dactylus (1) and few setae on the inside of the chela (2), d. dorsal view of a virile crayfish chela with two rows of tubercles on the dactylus (3). Photos Paul van Hoof.

Figuur 4. Verschillen tussen de scharen van een mannelijke calicotrivierkreeft *Faxonius immunis* (links) en een mannelijke geknobbelde Amerikaanse rivierkreeft *Faxonius virilis* (rechts), a. zijaanzicht van een schaar van een calicotrivierkreeft met een uitgesproken knobbel in het midden van de dactylus (beweegbare vinger) die lijkt op een haak (1) en veel beharing aan de binnenkant van de schaar (2), b. bovenaanzicht van een schaar van een calicotrivierkreeft met een enkele rij grote knobbels op de dactylus (beweegbare vinger) van de schaar (3), c. zijaanzicht van een schaar van een geknobbelde Amerikaanse rivierkreeft met geen uitgesproken knobbel in het midden van de dactylus (1) en minimale beharing aan de binnenkant van de schaar (2), d. bovenaanzicht van een schaar van geknobbelde Amerikaanse rivierkreeft met twee rijen grote knobbels op de dactylus (3). Foto's Paul van Hoof.

Rhine was extremely low (fig. 3). At that time, the side channel was no longer flowing with the river Rhine. To determine whether there were any live calico crayfish present, 15 baited crayfish traps were placed in the side channel and monitored daily during the following week. This did not result in any catches of the calico crayfish, but several alien monkey gobies *Neogobius fluviatilis* (Pallas, 1814) were caught. Attempts to catch crayfish with dip nets on the 18th of April were unsuccessful. Several individuals of European chub *Squalius cephalus* (Linnaeus, 1758), a river lamprey *Lampetra fluviatilis* larva (Linnaeus, 1758), and several individuals of the alien monkey goby, round goby *N. melanostomus* (Pallas, 1814), Chinese mitten crab *Eriocheir sinensis* (Milne-Edwards, 1853), and the freshwater shrimp *Katamysis warpachowskyi* (Sars, 1893) were caught.

EDNA

Environmental DNA (eDNA) samples were collected on 22 October 2025 at three localities in the side channel: location 1 at the inflow entry point of the side channel (east), location 2 in the middle of the side channel near the location where the chela was found, and location 3 an isolated waterbody in the floodplains. The outflow point of the side channel back into the river Rhine (locality 4) was dry at the time of sampling. Hence, absence was assumed. The samples were analysed by AQUON for the presence of six invasive alien crayfish species of North American origin (calico crayfish, virile crayfish, spiny-cheeked crayfish, marbled crayfish, red swamp crayfish, and white river crayfish) using a species-specific primer set for each individual crayfish species.

Two of the three eDNA samples tested positive for the presence of calico crayfish. No other crayfish species was detected. Most eDNA was measured at location 2 showed (2.15 DNA copies * ml^{-1}), a positive but non-quantifiable amount (DNA found in very low concentrations, making quantification impossible) was measured at location 1. The results of the eDNA analysis indicate the presence of a calico crayfish population in the side channel of the Dutch river Rhine at the time of sampling. Since the side channel was not flowing at both the times of the chela discovery and the eDNA sampling, it seems unlikely that dead crayfish or their DNA were washed in. Given the considerable time interval between the discovery of the chela and the subsequent eDNA sampling (201 days, including the summer period), the likelihood that the detected eDNA originated from residual material of the same individual is assumed to be very low. DNA can persist in freshwater for approximately one month, but this period is significantly reduced by relatively warm conditions (Dejean et al. 2011).

MORPHOLOGICAL IDENTIFICATION

The calico crayfish looks similar to its close relative, the virile crayfish, which also occurs in the Rhine delta. Both species typically have a mottled brown abdomen. The most appropriate distinguishing features between these two species can be found on the chelae (chelipeds) and the second pair of legs, or pereopods (fig. 4-5). Chelae of the calico crayfish have a typical tubercle (lump) and inward curvature from the middle of the dactylus (movable finger), resembling a hook. Setae (hairs) are more prominent in the chela of calico crayfish than virile crayfish. The calico crayfish also has a single row of tubercles on top of the dactylus, while the virile crayfish has two rows of tubercles. In addition, the calico crayfish has very prominent setae on the pereopods, which are also much more prominent in males than in females. The virile crayfish has very few setae on the second pair of legs. Furthermore, the rostrum of the calico crayfish is more pointed than that of the virile crayfish (fig. 5).

GENETIC IDENTIFICATION

The genetic analysis for the purpose of species identification was performed by AQUON. A small part of the discovered chela (fig. 1) was pulverised and dissolved in a preservative buffer with protei-

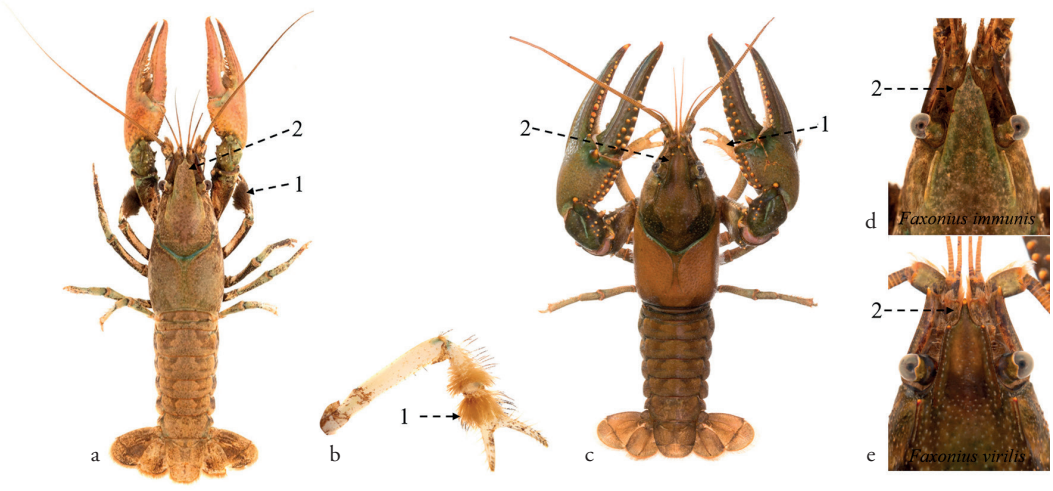


Figure 5. Differences between a male calico crayfish *Faxonius immunis* (left), and male virile crayfish *Faxonius virilis* (right), a. calico crayfish with pronounced setae (hairs) on the pereopods (second pair of legs) (1) and the pointed rostrum (2), b. close-up of the setae on a calico crayfish pereopod (1), c. virile crayfish with very few setae on the pereopods (1) and a relatively wide rostrum (2), d. close-up of the rostrum of calico crayfish (2), e. virile crayfish (2). Photos Paul van Hoof.

Figuur 5. Verschillen tussen een man calicotrivierkreeft *Faxonius immunis* (links) en een man geknobbelde Amerikaanse rivierkreeft *Faxonius virilis* (rechts), a. calicotrivierkreeft met uitgesproken beharing op de pereopoden (tweede pootpaar) (1) en puntig rostrum (2), b. close-up van de beharing op een pereopode van een calicotrivierkreeft (1), c. geknobbelde Amerikaanse rivierkreeft met zeer minimale beharing op de pereopoden (1), een relatief breed uitlopend rostrum (1), d. close-up van het rostrum van calicotrivierkreeft (2), e. geknobbelde Amerikaanse rivierkreeft (2). Foto's Paul van Hoof.

nase K. The DNA was then extracted using the DNeasy Blood & Tissue Kit.

For the genetic analysis of both the extracted chela DNA and the subsequent eDNA analysis, a qPCR analysis specifically targeting the calico crayfish was developed. The species-specific qPCR analysis for the calico crayfish targeted the mitochondrial cytochrome oxidase subunit I (COI) gene. The full COI DNA sequence of the target species was identified and retrieved from GenBank. To ensure specificity against common local species, reference COI sequences from potentially co-occurring and closely related species found within the Netherlands and surrounding regions were collected from GenBank.

The retrieved sequences were aligned, and a phylogenetic tree was constructed to determine the evolutionary relationships among the species (fig. 6). The three genetically most closely related species were specifically identified from this analysis to guide the subsequent primer design process, focusing on regions of maximal sequence divergence.

Primers were designed to target a specific region within the COI gene that was unique to the calico crayfish and highly divergent from the sequences of the three genetically most closely related species identified in the phylogenetic analysis. The specificity of the designed primer pair was rigorously evaluated in silico using the Nucleotide Basic Local Alignment Search Tool (BLASTn) against the comprehensive nucleotide database (nt) hosted by the National Center for Biotechnology Information (NCBI). The primers were confirmed to be 100 % specific to the target spe-

cies, showing no predicted cross-reactivity with the genetic material of other species known to be present in the Netherlands and surrounding areas.

The optimal reaction conditions for the qPCR assay were determined. This included defining the final concentrations of primers, the DNA polymerase master mix, template DNA concentration, and the thermal cycling parameters (e.g., denaturation temperature/time, annealing temperature/time, extension temperature/time, and melt curve analysis protocol). A standard curve was generated using serial dilutions of known target DNA concentrations to evaluate the assay's efficiency (E), sensitivity and linear dynamic range. The synthetic DNA of the two genetically most closely related species were used to determine the specificity of the qPCR assay.

The optimal and species-specific qPCR assay for the calico crayfish was applied to confirm the species identity of the discovered chela (fig. 1). The COI sequence obtained from the discovered chela showed a similarity of 96-99 % to known calico crayfish COI sequences, while exhibiting a maximum similarity of 89 % to the closely related species mena crayfish *Faxonius menae* (Creaser, 1933).

Following successful amplification and verification that the chela originated from calico crayfish, the same qPCR analysis was subsequently employed for the described environmental DNA (eDNA) analysis of water samples collected from the side channel of the river Rhine.

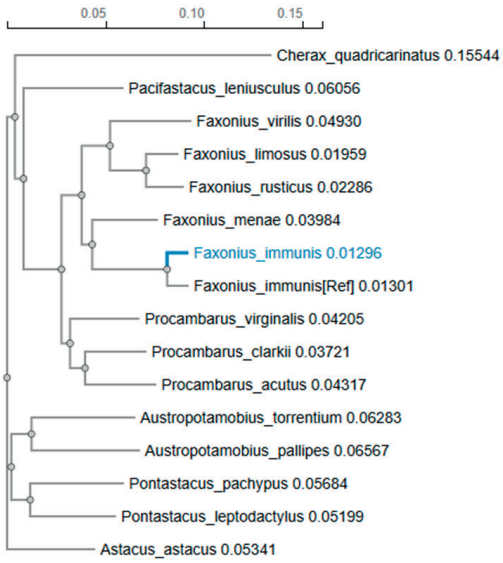


Figure 6. Phylogenetic tree of the COI sequences of calico crayfish *Faxonius immunis* and related species. The individual that was found on 4 April 2025 in the Netherlands is indicated in blue. The numbers in the scale bar indicate genetic distance, where a higher number indicates a higher genetic divergence.
Figuur 6. Fylogenetische stamboom van de COI-sequenties van de calicotrivierkreeft *Faxonius immunis* en verwante soorten. Het exemplaar dat op 4 april 2025 in Nederland werd gevonden, is blauw weergegeven. De cijfers in de schaalbalk geven de genetische afstand aan, waarbij een hogere waarde duidt op een grotere mate van genetische divergentie.

FUTURE PERSPECTIVE

DNA analysis provided the first confirmed record of a calico crayfish in the Netherlands, in April 2025, in the floodplains of the river Rhine near the German border (fig. 1-2). At that moment, the water level in the river was very low, which caused the side channel to stop flowing for some time. Sampling with baited traps and dip nets did not yield any live specimens. Therefore, it was unclear whether it was a dead calico crayfish that had drifted via the river Rhine from Germany, a crayfish that had died on the spot or been preyed upon due to the drought, or whether a population had already established. The presence of the species is difficult to determine at low densities (Tricarico & Lucy 2020). However, analysis of eDNA revealed a positive signal in the middle section of this side channel of the river Rhine, suggesting that one or more specimens of the species have established here. The record of this invasive alien species, which has been listed on the List of Invasive Alien Species of Union concern (EU Regulation 1143/2014) since August 2025, has also been reported to the European Alien Species

Information Network - Notification System (Notsys).

The calico crayfish has the capacity to spread over land (Herrmann et al. 2018), covering at least several hundred metres (pers. comm. Alexander Herrmann). This occurs almost year-round during relatively humid and warm conditions. The calico crayfish can establish in most, stagnant to slow-flowing waters and become numerous, particularly in riparian zones such as the location where the presence was established in the Netherlands (fig. 3). Due to its high reproduction rate, the species is able to build up very high densities in a relatively short period of time, i.e., several years (Chucholl 2011). The type of bank sediment appears to be an important factor in the establishment possibility; the species was found to be able to dig well in clay/silt-like sediment, which makes up the banks of larger rivers, while it was unable to burrow in sand and gravel (Herrmann & Martens 2024). The species prefers relatively shallow waters (Jansen et al. 2009).

Both through predation and the effects of its burrowing behaviour, the calico crayfish can have adverse effects on aquatic plants, amphibians, macrofauna and water quality (Chucholl 2011, Tricarico & Lucy 2020, Herrmann et al. 2022). The species is a strong burrower and is therefore capable of negatively affecting the morphology of stream and river banks (Tricarico & Lucy 2020). As a result of burrowing crayfish, banks erode faster, and dredging may be required more often (Gylstra et al. 2016). Because of the adverse effects, the calico crayfish was listed in August 2025 as an invasive alien species of European Union concern, following a risk assessment (Tricarico & Lucy 2020).

At present, mainly waters in the Rhine and IJssel river basins are considered to pose a risk to the spread and establishment of the species in the Netherlands (Lemmers et al. 2026). The river Rhine basin is characterised by clayey floodplains, with side channels and isolated water bodies (pools or ponds). These waters are optimal habitat for the calico crayfish, and it seems highly probable that this invasive alien crayfish will establish and expand in the near future. The establishment of the calico crayfish may also depend on the presence of other crayfish species (Jansen et al. 2009). It has previously been shown that the calico crayfish has the potential to replace the spiny-cheek crayfish due to aggressive interactions and competition for shelter between the two species (Chucholl et al. 2008). However, other species appear to be capable of replacing the calico crayfish, such as the virile crayfish (Bovbjerg 1970) or the marbled crayfish (Hossain et al. 2020). The present record of the calico crayfish is a cause for concern for biodiversity in isolated waterbodies of floodplains, as the calico crayfish can reproduce rapidly and, in large numbers, can cause aquatic plants, invertebrates and amphibians to disappear. In Germany, several measures have been tested to prevent the further spread and establishment of the calico crayfish in isolated waters (Herrmann & Martens 2024). It is advisable to look into these measures now and to take preventive action in some areas of high natural value. It is unlikely that the calico crayfish can or will be removed from the Netherlands due to the high degree of connectivity of waters.

ACKNOWLEDGEMENTS

We would like to thank Nika van den Meiracker of the State Forestry Department (Staatsbosbeheer) for granting access to the research area Klompenwaard. We are also grateful to Frans Komen senior and Frans Komen junior for providing permission that crayfish traps could be placed and a survey with dip nets could be conducted. Water Board Rivierenland financed the genetic analysis of the chela and the sampling and analyses of eDNA samples by AQUON.

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SAMENVATTING

Eerste waarneming van de calicotrivierkreeft *Faxonius immunis* in Nederland (Crustacea: Decapoda)

In april 2025 werd een opmerkelijke rivierkreeftschaar gevonden in een meestromende nevengeul van de Rijn in Nederland, vlakbij de Duitse grens. Op basis van de verdeling van de knobbeltjes op de schaar werd vermoed dat het om een calicotrivierkreeft *Faxonius immunis* (Hagen, 1870) ging. DNA-analyse bevestigde de soortidentificatie. Dit betrof de eerste bevestigde waarneming van de calicotrivierkreeft in Nederland, die is opgenomen op de Unielijst van invasieve uitheemse soorten die voor de Europese Unie van belang zijn. Bij bemonstering met rivierkreeftenvallen en schepnetten in april 2025 werden geen exemplaren gevonden. Uit eDNA-bemonstering in oktober bleek echter dat er op twee van de drie bemonsterde locaties DNA van calicotrivierkreeft aanwezig was, hetgeen suggereert dat zich hier een populatie heeft gevestigd. Verwacht wordt dat de soort zich verder zal uitbreiden, in eerste instantie in de uiterwaarden van de Rijn en mogelijk verder via de onderling verbonden waterwegen. Er zijn gevolgen voor de biodiversiteit en sociaaleconomische effecten te verwachten, omdat de soort hoge dichtheden kan bereiken en een goede graver is. Aangezien de calicotrivierkreeft enkele honderden meters over land kan lopen, lopen ook geïsoleerde amfibieënwateren met hoge natuurwaarden in de uiterwaarden het risico te worden gekoloniseerd.

P. Lemmers
Radboud University, Radboud Institute for Biological and Environmental Sciences, Nijmegen
Netherlands Centre of Expertise for Exotic Species (NEC-E), Nijmegen
p.lemmers@science.ru.nl

J. Wissink
Radboud University, Radboud Institute for Biological and Environmental Sciences, Nijmegen

R. Gylstra
Water Board Rivierenland, Tiel

S. Heeman
AQUON, Houten

P.H. van Hoof
Paul van Hoof Nature Photography

B. Koese
EIS Kenniscentrum Insecten, Leiden