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Impact of invasive crayfish on water quality and aquatic macrophytes in the Netherlands

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Editor's note:
This study was first presented at the Centre for Wetland Ecology (CWE) symposium (24 June 2016, Wageningen, the Netherlands) on the role of exotic species in aquatic ecosystems (https://www.wetland-ecology.nl/en/calendar/good-bad-or-bit-both-role-exotic-species-aquatic-ecosystems). This symposium provided a venue to unravel how exotic plants and animals impact ecosystem functioning, find out whether they coexist or compete with native species and discover their impact on native flora and fauna.

Abstract

Several species of invasive crayfish have become established in the Netherlands, the most recent addition being *Orconectes virilis*. Since crayfish are known to impact water quality and aquatic macrophytes in areas they invade, this study investigated whether this was also the case for this species under Dutch conditions and if so, whether a crayfish density producing "no effects" could be established. We focussed on the potential impact of *O. virilis* on water quality variables (pH, electrical conductivity, dissolved oxygen and turbidity), as well as their impact on submerged and emergent macrophytes. In a compartment experiment with different densities of *O. virilis*, statistically significant effects were observed at crayfish densities of 1.25 crayfish/m^2 on electrical conductivity, turbidity, submersed macrophyte biomass, and the emergent plant *Sagittaria sagittifolia*, due to crayfish actively severing plants and physically disturbing the sediment. No statistically significant differences with controls were observed at a density of 0.63 crayfish/m^2. Since densities of 0.03 to 5 crayfish/m^2 have been found in different water types in the Netherlands, this indicates that the water quality and macrophyte biomass in Dutch waters are being negatively impacted by invasive crayfish. As a consequence, attempts to reach a good ecological status as required in the Water Framework Directive will be frustrated by the presence of this invader.

Key words: freshwater, effect threshold, *Orconectes virilis*, *Procambarus clarkii*, good ecological status, Water Framework Directive

Introduction

Biological invasions are recognized as one of the most significant changes in the environment (Sala et al. 2000). They rank second after "changes in land use" as major causes of biodiversity loss (Lodge et al. 2000). Freshwater environments are particularly prone to invasions, as it is in these systems that more alien introductions occur (Lodge et al. 1998; Ricciardi 2001). Eventually this can lead to situations where invasive species dominate the aquatic community, as has happened with water hyacinth (*Eichhornia crassipes,* (Mart.) Solms, 1883) and the red swamp crayfish (*Procambarus clarki,* Girard, 1852) in Southern Europe (Holdich and Pöckl 2007; Souty-Grosset et al. 2016), or floating marsh-pennywort (*Hydrocotyle ranunculoides* L. f.), Chinese mitten

crab (*Eriocheir sinensis* Milne-Edwards, 1854), red swamp crayfish and musk rats (*Ondatra zibethicus* Linnaeus, 1766) in North-Western Europe (Bos and Ydenberg 2011; Paulissen and Verdonschot 2007). The economic damage caused by biological invasions is estimated to comprise approximately 12 billion euros a year in Europe alone (Kettunen et al. 2009).

Freshwater crayfish have been introduced to many water bodies as they have commercial value as food or fish bait. These introductions comprise only a handful of species (Gherardi 2010; Hobbs III et al. 1989) but their spread continues today. For instance, of the eight species introduced into the UK, five are now considered pest species with severe impact on their environment (Holdich and Pöckl 2007; Kouba et al. 2014). Crayfish can alter the structure of aquatic ecosystems via their behaviour and polytrophic omnivorous diet, one of the most dramatic impacts being that on aquatic macrophytes (Cronin et al. 2002; Feminella and Resh 1989; Lodge et al. 2012; Rodriguez et al. 2003; Rodriguez et al. 2005; Twarlochleb et al. 2013). In turn, such changes can lead to alterations in water quality and to increased turbidity and nutrient loads (Angler et al. 2001). Through these major effects, crayfish act as ecosystem engineers (Nyström et al. 1996).

In the Netherlands, several species of invasive crayfish have become established (Kouba et al. 2014; Soes and Koese 2010; Souty-Grosset et al. 2006; Tilmans et al. 2014), the most recent addition being *Orconectes virilis* (Hagen, 1870). Since these crayfish species inhabit smaller waterbodies and do so at high densities, their potential impact on water quality and aquatic macrophytes could be high (Soes and Koese 2010) and have serious consequences for the ecological status of these waters as specified in the Water Framework Directive. Therefore, a compartment study was performed to investigate at which density the crayfish *O. virilis* starts to cause significant effects on aquatic macrophytes and water quality variables such as pH, dissolved oxygen (DO), electrical conductivity (EC) and turbidity.

Methods

The compartment study was performed between July 24th and September 4th, 2009 at the "De Sinderhoeve" experimental station in Renkum, The Netherlands. The experiment was performed in an experimental ditch (40 m length, water depth 0.5 m; see also Drent and Kersting 1993). The ditch used was fully covered with aquatic vegetation, dominated by the submerged macrophytes *Elodea nuttallii* and *Chara* sp. but also harbouring, though in smaller amounts, *Myriophyllum spicatum* and the emergent macrophyte *Sagittaria sagittifolia*. The ditch was fenced off with high-density polyethylene (HDPE) boarding to prevent the crayfish from escaping over land. To avoid any bias through changing reproductive status of female crayfish, only male *O. virilis* were used in these experiments. In addition, by selecting males we ensured that any potential escapees could not reproduce at the test site and thus start a new population. The ditch was enclosed within a net cage to prevent interference by waterfowl and other predators.

A total of 18 compartments were set up. Each comprised a ditch section 2 m wide separated by curtains of synthetic rubber (EPDM; frequently used as pond liner). Additional sandbags were aligned carefully along the curtain edge to press the curtains to the ditch walls and bottom and seal off the compartments from each other. The size and weight of the bags left no room for the crayfish to penetrate and no tunnels were found at the end of the experiment, indicating that the compartments stayed isolated from each other. Compartments had a total accessible bottom surface area of 8.1 m^2, of which 3.2 m^2 comprised the sediment surface and the remainder comprised the sloping walls of the ditch (Drent and Kersting 1993). Since crayfish were never observed on the walls, we used the sediment surface in all further calculations. Crayfish used in the test, had a carapace length of 38.4 (± 4.2) mm, claw size 40.7 (± 7.1) mm and fresh weight 34.8 (± 8.2) g. Before weighing, the crayfish were blotted dry using a towel to remove any adherent water. After measurement crayfish were marked non-invasively by fastening a tie-wrap just behind their right or left claw. For the different treatments tie-wraps of different colours were used to identify potential escapees, i.e. crayfish that moved between compartments with different treatments. Either 0, 2, 4, 8, 16 or 32 male *O. virilis* crayfish were introduced per compartment (186 individuals in total), resulting in densities of 0, 0.63, 1.25, 2.5, 5 and 10 crayfish/m^2 (n = 3; for details see Figure 1). This range overlaps with the densities of 0.03 to 5 crayfish/m^2 observed in different water body types in the Netherlands (Van Emmerik 2010; Van Emmerik and De Laak 2008; Van Giels 2011). Shelters made of PVC tube and dark wide-mouth glass jars were provided such in each compartment included one more shelter than crayfish. Since fine particulate sediment matter became suspended during the installment of the compartments, aeration was installed using air pumps (AP-40; 4.5 L/min) to mitigate any major oxygen demands and create at least a small oxygenated zone for the crayfish when needed.

Values of pH, DO, EC, turbidity and temperature were measured in the morning on days 7, 14, 21, 28,

Compartment:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
No. of crayfish:	0	2	8	16	4	32	8	32	16	4	0	2	8	32	16	2	4	0

Figure 1. Impression of the compartment set-up during finalisation of the experiment and distribution of crayfish densities over the different compartments in the experimental ditch. Photo by Ivo Roessink.

35 and 42 after the start of the experiment. pH, DO, temperature and EC were measured in the middle of the compartment at mid-depth in the water column using a HACH HQ40d portable multimeter. Turbidity was measured by taking a water sample at the same location, which was subsequently analysed using an EUTECH TN-100 Turbidimeter. No measurements were performed at day 0 because effects of compartment instalment, e.g. suspended sediment, would bias the measurements. Aquatic macrophyte coverage (estimated as percentage coverage) and the number of dislodged or severed plants/stems were monitored by visual inspection and recorded. Floating plant material was not removed from the water surface. At the end of the experiment (day 42), the compartments were drained, after which the crayfish and plants were collected. The emergent *Sagittaria* plants were first collected by hand, then submerged macrophytes were collected using rakes and by hand. The plants were cut directly at sediment level and in case roots were still attached these were removed. Submerged macrophytes were not separated by species because *Elodea* dominated the community and the biomass of the remaining taxa was considered too low for a robust analysis. The remaining aboveground material was then dried for at least 48 h at 70 °C after which its dry weight biomass was measured. The remaining crayfish were collected by carefully entering the drained compartment and sieving through the sediment top-layer by hand.

Data analysis

In the compartment study, a stronger impact on aquatic vegetation and water quality variables was expected with increasing crayfish densities. When expecting such a monotonic function of a treatment, i.e. an increasing effect with increasing treatment level (i.e. increased number of crayfish per compartment), a Williams test can be used (Williams 1972). The Williams test will derive a so-called No Observed Effect Concentration (*NOEC*; the concentration at which no statistically significant effect compared to the control is observed) at the parameter level, which in this case will indicate the effect threshold for *O. virilis*, i.e. the crayfish density at which no statistically significant difference with the control treatment is observed. The analyses were performed with the Community Analysis computer programme (Hommen et al. 1994), resulting in an overview of *NOEC*s in each sampling event for the data analysed. The threshold level for *P* was 0.05 for all statistical analyses. It is always possible that on an individual sampling date an incidental statistical significant difference is observed. Occurring only in isolation, this response might not be related to the treatment (e.g., a Type I error or ecological artefact). Consequently, such isolated statically significant differences were not noted and responses were only considered ecologically relevant if they occurred on at least two consecutive sampling dates or when they were part of a (statistically non-significant) trend.

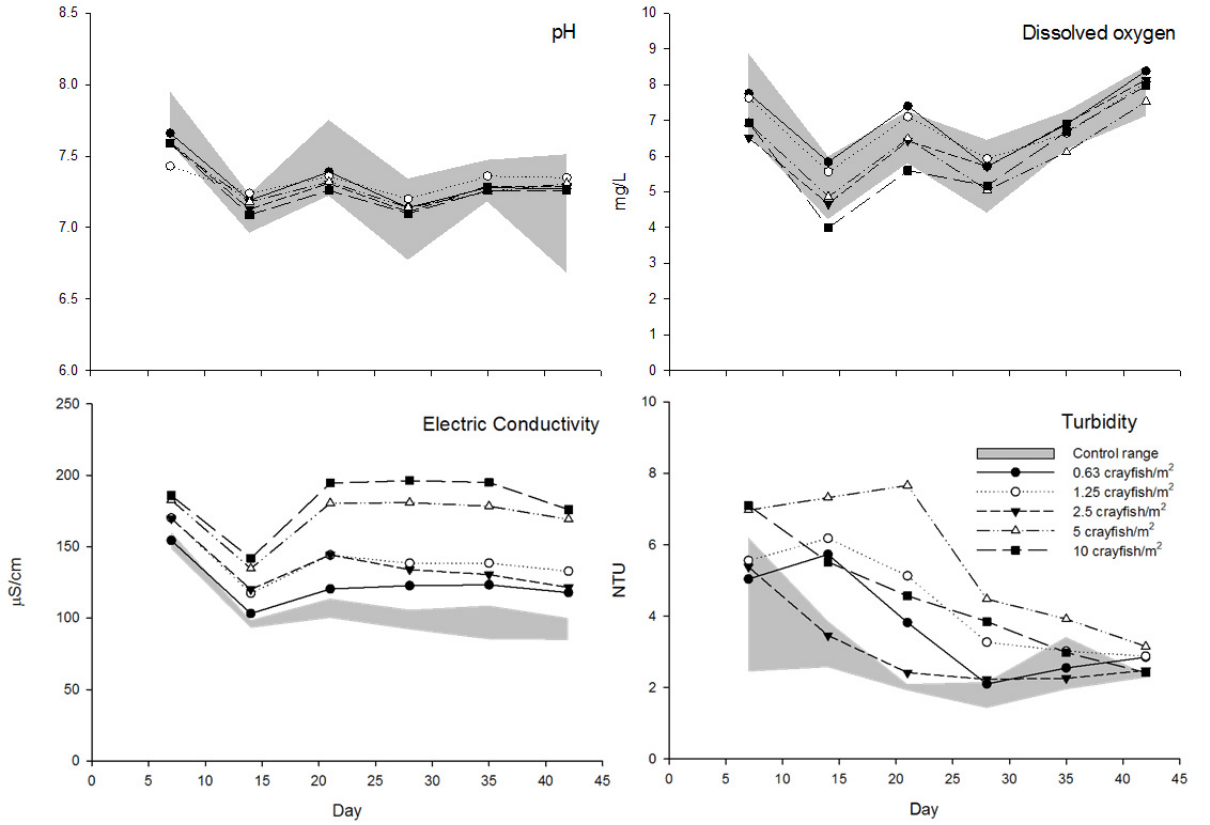

Figure 2. Dynamics of pH, dissolved oxygen, electric conductivity (EC) and turbidity in the compartments over time. The grey area represents the control range.

Table 1. No Observed Effect Concentration (NOEC) values in crayfish/m^2 for the different endpoints measured over time in the compartment study during the experimental phase. Treatments comprised crayfish densities of 0, 0.63, 1.25, 2.5, 5 and 10 individuals/m^2 (each density replicated three times).

Day	pH	DO (mg/L)	EC (µS/cm)	T (°C)	Turbidity (NTU)	Submerged macrophytes dry biomass (g)	*Sagittaria sagittifolia* dry biomass (g)	Floating macrophytes dry biomass (g)	*Sagittaria* floating (number of stems)
7	≥ 10	≥ 10	0.63	≥ 10	≥ 10	–	–	–	≥ 10
14	≥ 10	≥ 10	0.63	≥ 10	≥ 10	–	–	–	≥ 10
21	≥ 10	5	0.63	≥ 10	2.5	–	–	–	2.5
28	≥ 10	≥ 10	0.63	≥ 10	2.5	–	–	–	2.5
35	≥ 10	≥ 10	0.63	≥ 10	≥ 10	–	–	–	2.5
42	≥ 10	≥ 10	0.63	≥ 10	≥ 10	0.63	5	2.5	≥ 10

– indicates that this parameter was not measured at this date

Results

Of the 186 crayfish introduced in the compartment study, 142 were retrieved. Losses were rather evenly distributed with approximately 24% loss per treatment level, resulting in 1, 3, 5, 12, 23, 44 crayfish lost of the 6, 12, 24, 48, 96 individuals present in the 2, 4, 8, 16 and 32 crayfish per treatment (n = 3), respectively. Crayfish were not weighed after collection, but the fact that most individuals had lost their tie-wraps was considered a sign of moulting, and therefore of growth. Individuals still bearing their mark were retrieved from their respective compartments and no surplus individuals were found in any of the compartments.

During the experimental period, overall water temperatures ranged from 18.5–19.6, 21.6–21.9,

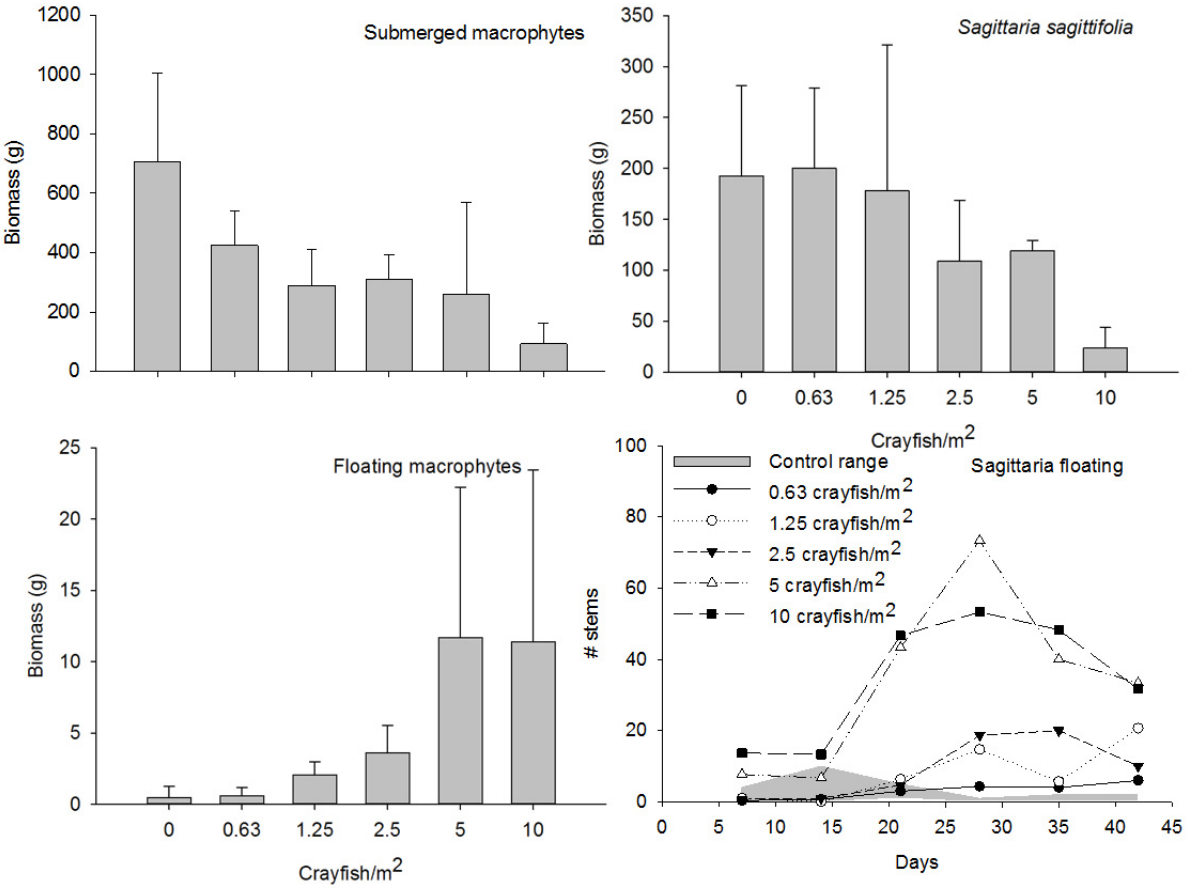

Figure 3. Macrophyte biomass (dry weight) at the end of the compartment study and dynamics over time of floating *Sagittaria sagittifolia* stems. The grey area in the last panel represents the control range.

17.1–17.6, 19.5–20.4, 17.7–18.6 and 13.8–14.3 °C on days 7, 14, 21, 28, 35 and 42, respectively. Furthermore, pH values did not show clear effects of crayfish presence (Figure 2, Table 1). As regards DO levels, a No Observed Effect Concentration of 5 individuals/m^2 was found (Table 1) indicating that a statistically significant difference between the highest crayfish density and controls was only observed on day 21 (Figure 2). Since this difference occurred only once, this was not regarded as a consistent response. Effects on EC, however, were consistent, and over the whole experimental period a NOEC of 0.63 crayfish/m^2 was found (Table 1). In addition, EC increased with increasing crayfish densities (Figure 2). Although turbidity in the compartments with more than 0.63 crayfish/m^2 was higher during the first 28 days of the experimental period (Figure 2), statistically significant differences were only observed on days 21 and 28 at the two highest densities of 5 and 10 crayfish/m^2, respectively.

Although *Chara* sp. was present at the start, it was replaced by *Elodea* over the duration of the experiment. Statistically significant decreases in submerged macrophytes compared to controls were observed from the 1.25 crayfish/m^2 density and higher, and their biomass decreased further as more crayfish were present (Figure 3). The biomass of the emergent macrophyte, *Sagittaria sagittifolia,* was rather variable in the controls and at the 0.63 and 1.25 crayfish/m^2 treatment levels (Figure 3). Although *Sagittaria* biomass decreased at the 2.5 and 5 crayfish/m^2 treatment levels, only the biomass at the 10 crayfish/m^2 level was significantly lower than that in the controls. Although a steady increase in floating macrophytes was observed from the 1.25 crayfish/m^2 treatment level upwards (Figure 3), this increase was only statistically significant at the 5 and 10 crayfish/m^2 treatment levels. This was corroborated by the observed impact over time on *Sagittaria*, where from day 21 onwards, a significantly higher number of *Sagittaria* stems

were found afloat in the 5 and 10 crayfish/m^2 compartments (Table 1). Although no longer statistically significantly different from controls on the last two sampling dates, the response depicted in the graph showed that the two highest treatment levels still deviated from the others (Figure 3).

Discussion

Clear effects on EC and submerged macrophytes were observed at densities of 1.25 crayfish/m^2 and higher. A negative impact of crayfish on macrophytes has been reported by other researchers (Baldridge and Lodge 2014; Carreira et al. 2014; Chambers et al. 1990; Chambers et al. 1991; Cronin et al. 2002; Feminella and Resh 1989; Rodriguez et al. 2003; Rodriguez et al. 2005; Souty-Grosset et al. 2016; Twarlochleb et al. 2013; van der Wal et al. 2013) but why the EC increased is less clear. A negative impact on macrophytes could have affected photosynthetic activity in the experimental ditch, which consequently would have impaired the uptake of HCO^{3-} ions from the water phase, resulting in a higher EC. However, this would very likely also have impacted upon pH, and to a lesser extent upon the DO, since these variables are all linked to photosynthetic activity (Brock et al. 1993; Van den Brink et al. 1995). Since no effects on pH and DO were observed, it is more likely that the EC was raised by crayfish activity. Although *O. virilis* does not seem to burrow extensively under normal circumstances (Aiken 1968; Bovbjerg 1970), its activity in the upper sediment layer might have released more ions into the water thereby increasing EC. Other activities of the animals could also be a factor. For instance, at higher crayfish densities the number of encounters would increase and therefore the frequency of fights or tail-flip escapes, which might also have disturbed the sediment and thus increased EC.

In addition, turbidity increased at the 5 and 10 crayfish/m^2 levels, but only 21 and 28 days after the start of the experiment. Apparently, *O. virilis* did not induce a permanent increase in turbidity in the compartments, in contrast to the findings of e.g. Angler et al. (2001) who did report a long-lasting increase in turbidity. However, although this study examined *P. clarkii* at densities of approximately 3 crayfish/m^2, the enclosures lacked submerged macrophytes, thus enabling a more direct impact on the sediment than in our compartments.

A clearer indication of crayfish activity was formed by the severed stems of the emergent macrophyte *Sagittaria sagittifolia* (Figure 3). From day 21 onwards, a significant increase in severed stems was observed at the 5 and 10 crayfish/m^2 levels. This is consistent with a semi-field experiment in which male *O. virilis* consumed the submerged macrophyte *Myriophyllum exalbescens*, while merely severing shoots and leaves of *Nuphar variegatum*, *Sparganium eurycarpum* and *Potamogeton richardsonii* and letting them float to the surface (Chambers et al. 1990; Chambers et al. 1991). Interestingly, *O. virilis* males and females have been found to have different impacts on both macrophytes and aquatic invertebrates (Chambers et al. 1990; Hanson et al. 1990). However, since the females in these experiments were berried (i.e. carrying eggs; Chambers et al. 1990) or had just released their young (which is usually followed by moulting; Hanson et al. 1990), they remained hidden for most of the day and were much less active than the males. This makes it likely that outside this phase, their behaviour and thereby their impact will probably change as well.

Of the 186 experimental individuals only 142 were retrieved at the end of the study and losses were rather evenly distributed amongst treatment levels. Since no escaped individuals were found, i.e. individuals with deviating colour tags in a compartment or more than the inserted individuals in the compartment, these losses were attributed to (i) mortality and to (ii) missed individuals during collection at the end of the study. Mortality could not be related to any clear external causes since DO levels were always above the critical threshold level (i.e. > 3 mg/L; Barbaresi and Gherardi 2000) and the other water quality variables did not show extreme values indicating sub-optimal conditions either. Mortality possibly occurred during the moulting process, which is a very vulnerable period for the animals and fatal problems leaving the old exoskeleton and/or lethal infections in the still soft new exoskeleton may occur (Hamr 2002; Huner 2002) resulting in considerable losses (Taugbol and Skurdal 1992; Chen et al. 1995). Furthermore, the crayfish had to be hand-collected by walking carefully through the drained compartment at the end of the study. Some animals may have buried themselves in the mud and were missed during collection, since burrowing under drought-stress is also observed in *Orconectes* species (Kouba et al. 2016).

We show that *O. virilis* negatively impacts emergent macrophyte vegetation under Dutch conditions at densities of 1.25 crayfish/m^2 and higher. Previous research has shown similar impacts for *P. clarkii* at densities of approximately 5 crayfish/m^2 under Dutch conditions (van der Wal et al. 2013). Our study found no statistically significant impacts on macrophytes and/or water quality at crayfish densities of 0.63 crayfish/m^2. Therefore, it is unlikely that negative effects of crayfish invasions on water quality and macrophytes will be detected in the field at densities

approximating this value. However, our threshold of 0.63 crayfish/m^2 was derived from a system with fully established aquatic vegetation already present. Such a system may have been more robust than an aquatic vegetation in its start-up phase, e.g. with emerging plants after winter hibernation. Crayfish densities ranging from 0.2 to 15 individuals/m^2 have been reported in rice fields, farm ponds and lakes (Gherardi and Acquistapace 2007; Maezono et al. 2005; Rhodes and Avault Jr 1986) and densities of 0.03 to 5 crayfish/m^2 have been found in different water types in the Netherlands (Van Emmerik 2010; Van Emmerik and De Laak 2008; Van Giels 2011). Based on our findings, this indicates that crayfish densities currently occurring in the field can exceed the effect threshold of 0.63 crayfish/m^2. This suggests that water quality and macrophyte biomass in Dutch waters are negatively impacted by invasive crayfish and that achieving the ecological status described in the Water Framework Directive will be frustrated by the presence of these invaders.

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