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Foraging Efficacy of Non-native Crayfish Under Nitrate Pollution

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Abstract

Life history traits such as predation and foraging are key behavioural mechanisms through which invasive, non-native species can have a detrimental effect on the resident flora and fauna of recipient ecosystems. Environmental change may alter these traits in ways that are currently poorly understood. In aquatic ecosystems, changes to the environment such as nitrate pollution can have chronic, unforeseen consequences for both native and invasive species. As keystone species and ecosystem engineers, non-native crayfish species can be highly destructive through predating on native species from multiple trophic levels. Here, we assessed the effect of nitrate concentration on the foraging behaviour of two invasive crayfish species from the UK (*Pacifastacus leniusculus* and *Orconectes virilis*), particularly focussing on whether there were any species-specific effects of nitrate. During experimental trials, elevated nitrate concentration had a similar impact on the foraging efficacy of both *P. leniusculus* and *O. virilis*, slowing both their initial reaction and latency to feed. Nitrate also reduced the ability of both crayfish species to catch live prey as well as the total number of prey consumed. Though both species were similarly influenced by nitrate, we highlight that even at levels deemed safe for drinking water (50 mg NO₃⁻/L), nitrate concentration can significantly impact the behavioural traits of aquatic species. In the case of invasive species, fluctuations in environmental nitrate concentration could therefore have implications for invader success and impacts on the wider ecosystem.

Keywords: eutrophication; nitrate; foraging; invasive non-native species (INNS); behavioural traits; Water Framework Directive

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Introduction

Invasive, non-native species are a leading cause of biodiversity loss worldwide (Mack et al. 2000; Sala et al. 2000), but only a small percentage of non-native species that are introduced subsequently become invasive (Williamson and Fitter 1996). The success of introduced species is often associated with particular life history traits, such as high reproductive output, disease resistance and/or tolerance to pollution (Colautti et al. 2004; Maceda-Veiga et al. 2013; Vilcinskas et al. 2015), as well as behavioural traits such as aggression and foraging (Holway and Suarez 1999; Viana et al. 2016). Aggressive, voracious invaders are often competitively dominant over other species when competing for resources (Vorbürger and Ribi 1999; Nakata and Goshima 2003; Strayer 2010). However, traits associated with invasive animals may change due to altered environmental conditions and increasing pressure from anthropogenic disturbances (Guan 1994; Holway and Suarez 1999; Wong and Candolin 2015). Ultimately, this could result in invaders losing their competitive advantage under differing environmental regimes and it is important to understand how predicted future global changes may influence the traits that make non-native species successful.

Nutrient pollution is one of the most pernicious forms of global change in aquatic ecosystems, resulting in cultural eutrophication (Camargo and Alonso 2006; Smith and Schindler 2009). Increased levels of inorganic nitrogen such as ammonia (NH_3), nitrite (NO_2^-) and nitrate (NO_3^-) can enhance ecosystem productivity, but can also have devastating effects on freshwater organisms (Camargo and Alonso 2006; Hickey and Martin 2009). The toxicity of nitrate is poorly studied compared to ammonia and nitrite, but growing evidence indicates that chronic nitrate exposure can profoundly alter the life-history traits of fish (Smallbone et al. 2006; Scott and Sloman 2009), amphibians and invertebrates (Maceda-Veiga et al. 2015; Guilette and Edwards 2005; Camargo et al. 2006). Behavioural studies can link physiological and ecological processes and may identify the subtle effects of chemical toxicity in aquatic ecosystems (Scott and Sloman 2004). One of the best indicators of toxic effects on aquatic organisms is reduced foraging efficiency (Colin et al. 2016), as reported in *Daphnia magna* under chronic nitrate exposure (Maceda-Veiga et al. 2015). Pollutants can alter the perception of visual and chemical cues (Halfwerk and Slabbekoorn 2015), with ramifications for the detection of food but also conspecifics and predators (Troyer and Turner 2015). However, the effect of nitrate on the sensory capacity of aquatic organisms has not yet been investigated (but see Camargo et al. 2005), and it is of primary interest considering nitrate levels are expected to increase (Galloway et al. 2008).

Invasion of freshwater ecosystems is non-random both in terms of taxon and the biological traits of invaders, with efficient predatory decapods, molluscs and fish being particularly prevalent (Strayer 2010). Crayfish are extremely successful decapod invaders, which are keystone species and ecosystem engineers that have a significant impact on freshwater ecosystems through profoundly modifying their habitat and influencing all levels of the food web through predation and herbivory (see Holdich et al. 2009; Williams et al. 2010; James et al. 2014). Despite this, research on the effects of nitrate enrichment on invasive crayfish performance is limited. The survival, feeding and escape response of the threatened European white-clawed crayfish (*Austropotamobius italicus*) was negatively affected by ecologically relevant nitrate concentrations (Benítez-Mora et al. 2014). In contrast, the presence of invasive red-swamp crayfish (*Procambarus clarkii*) seems to be related to nutrient enriched waters in Mediterranean rivers (Maceda-Veiga et al. 2013). Nonetheless, there is a distinct lack of experimental or observational evidence of nitrate toxicity to other crayfish species living in nitrate vulnerable zones.

The current experimental study tested for the effects of ecologically relevant nitrate concentrations on feeding efficacy of two invasive crayfish, the signal (*Pacifastacus leniusculus*) and virile (*Orconectes virilis*) crayfish in the River Lea, London, UK. The virile

crayfish was introduced comparatively recently (first detected in 2004), whilst the signal crayfish has been established in this catchment for over three decades (Ahern et al. 2008). Virile crayfish are competitively dominant over signal crayfish, which is leading to an apparent displacement of the signal crayfish (James et al. 2015) in this designated nitrate vulnerable zone (Environment Agency 2015). However, this competitive advantage may be altered depending on nitrate conditions. As crayfish feed on benthic invertebrates with key roles in ecosystem processes (e.g. filter feeders, shredders; Graça 2001), alterations in the number and type of prey ingested due to nitrate may influence predator metabolism with ramifications for ecosystem function.

Materials and Methods

Collection of study animals

Signal (*Pacifastacus leniusculus*) and virile (*Orconectes virilis*) crayfish were trapped from allopatric sites in the River Lee, London, UK (Signal crayfish - NGR TL 38414 07906; Virile crayfish - NGR: TL 36834 02891) during October 2014 using standardised trapping protocols ('trappy traps' baited with tinned fish and checked daily for two days). Between 2008 and 2012, data obtained from the Environment Agency during water quality monitoring on the River Lee at a site within 10 km of the trapping points, showed that nitrate concentration ranged between 33 and 72 mg NO₃⁻/l, with an average of 46.4 mg NO₃⁻/l. Water samples were collected monthly, and nitrate concentration determined using a standard method (Standing Committee of Analysts, 1987). Crayfish were subsequently transported back to Cardiff University in plastic tanks with source water and air pumps.

Animal maintenance

Upon arrival in the lab, crayfish were housed individually in 15 L tanks containing either <10 or 50mg NO₃⁻/L under a 16h:8h light/dark regime at 14±1°C for 14 days. Each holding tank contained gravel substrate (2 cm) and a refuge (plastic pot). The two nitrate concentrations represented clean dechlorinated tap water (<10mg NO₃⁻/L), and the higher nitrate level corresponds to the safe threshold for nitrate concentrations for drinking water in Europe (50 mg NO₃⁻/L; Council of the European Union 1998). Nitrate solutions were hand-made using potassium nitrate (KNO₃) as a nitrate source before we fully renewed water in each container every other day. To ensure the experimental conditions remained stable throughout the experiment, chemical water properties were measured using Sera® colourimetric test kits (previously used in our laboratory, Maceda-Veiga *et al.*, 2015; Smallbone *et al.*, 2016), where pH=7, carbonate water hardness (dKH) = 5, [ammonia] < 0.5 mg L⁻¹, [nitrite] < 0.5 mg L⁻¹ and [nitrate] < 10 or 50 mg L⁻¹. All animals were measured (range 38-67 mm carapace length) and sexed. Any crayfish with missing chela were omitted from the study. Crayfish were fed every other day with Tetra Crusta crayfish food pellets and frozen peas 20 min prior to the water change to maintain precise nitrate concentrations. Upon termination of experiments, all animals were humanely destroyed by freezing at -20°C, as under Section 14 of the Wildlife and Countryside Act 1981 it is prohibited to release or maintain invasive species in the long term.

Foraging behaviour

The foraging behaviour of individual signal and virile crayfish were assessed over two days after the fortnight acclimatisation period, under the same nitrate concentrations: <10 and 50mg NO₃⁻/L (bloodworm trials: N = 17, 27; gammarid trials N = 17, 31). Crayfish were individually transferred to one end of an experimental arena (L60 cm x W30 cm x D30 cm) and foraging trials were repeated using different prey types, either; 5 live *Gammarus pulex* (referred to as gammarids) or 20 defrosted *Tubifex* worms (referred to as bloodworm), with half of the crayfish being fed gammarids and half being fed bloodworm first, and then fed the alternative

prey item on the second day. Both crayfish and prey were initially restrained within opaque glass cylinders that were simultaneously lifted at the start of the experiment, thus ensuring that crayfish were equidistant from the prey items across trials. For each trial we recorded; latency to feed (first reaction), time to first prey capture, and total number of prey consumed within 30 min. Crayfish which did not feed on bloodworm within the 30 min observation period were recorded as non-responders and excluded from further analysis. In this study, gammarids were used as an example of a live prey item; however, since many crayfish failed to catch gammarids during the trials, these data were converted into a ‘fed/did not feed’ response.

Statistical analyses

All statistical analyses were performed using R statistical software (R Core Team, 2012). General Linear Models (GLMs) were used to determine the effects of nitrate concentration on feeding responses. Tukey’s HSD post-hoc comparisons of significant results were then applied for pairwise comparisons, using the ‘lsmeans package’ (Lenth 2015). All models also included crayfish sex, species and carapace length, as well as an interaction term between nitrate and species to investigate species-specific effects of nitrate concentration (Table 1). A large number of crayfish (N = 34) failed to feed on gammarids in 30 min, leading to a low sample size, therefore the data were converted to a yes/no feeding response variable (i.e. whether the crayfish fed on gammarids in 30 min or not). Error family and link functions (Table 1) were chosen to give the best fit and meet model assumptions (Crawley, 2007) and models were refined by stepwise deletion, removing the least significant term and re-running the model until only significant ($P < 0.05$) terms remained (Crawley, 2007). Residual diagnostic plots from the models were used to verify the assumptions of normality and homoscedasticity (Zuur et al. 2010).

Results

At 50 mg NO_3^-/L , both signal and virile crayfish were slower to initially react to bloodworm than conspecifics at <10 mg NO_3^-/L (Tukey HSD, $P=0.019$; Fig. 1). Overall, virile crayfish were slower to react to bloodworm than signal crayfish (Tukey HSD, $P=0.009$; Table 1; Fig. 1).

Table 1 - Model output from GLM (General Linear Model) stepwise refinement and post-hoc pairwise comparisons (TukeyHSD) of crayfish reaction.

| Dependent variable | Prey item | Model family, link function | Fixed terms | F value§ | df | p-value | Post-hoc contrasts | p-value | Estimate | |
|--------------------------|-------------|-----------------------------|-------------------|-------------------------|-------|------------------|-------------------------------|------------------|--------------|-------|
| Time to first reaction | Bloodworm* | Gaussian, identity | Species | 6.904 | 1, 41 | 0.012 | Signal v. virile <10 v. 50 | 0.009 | -0.806 | |
| | | | Nitrate | 5.512 | 1, 41 | 0.024 | | | -0.740 | |
| | | | Sex | 0.622 | 1, 40 | 0.435 | | | | |
| | | | CL | 0.120 | 1, 38 | 0.731 | | | | |
| | Gammarids* | Gaussian, identity | Species:Nitrate | 0.228 | 1, 39 | 0.636 | | | | |
| | | | Species | 0.036 | 1, 43 | 0.852 | | | | |
| | | | Nitrate | 3.196 | 1, 46 | 0.081 | | | | |
| | | | Sex | 1.429 | 1, 44 | 0.238 | | | | |
| | | | CL | 1.935 | 1, 45 | 0.171 | | | | |
| | | | Species:Nitrate | 0.069 | 1, 42 | 0.794 | | | | |
| Time to first feed | Bloodworm** | Gamma, identity | Species | 0.849 | 1, 41 | 0.362 | <10 v. 50 | <0.001 | -6.885 | |
| | | | Nitrate | 11.201 | 1, 42 | 0.002 | | | | |
| | | | Sex | 4.044 | 1, 39 | 0.494 | | | | |
| | | | CL | 0.522 | 1, 38 | 0.474 | | | | |
| | Fed or not | Gammarids | Binomial, cloglog | Species:Nitrate | 3.889 | 1, 40 | 0.056 | <10 v. 50 | 0.009 | 1.145 |
| | | | | Species | 0.003 | 1, 43 | 0.955 | | | |
| | | | | Nitrate | 6.626 | 1, 46 | 0.010 | | | |
| | | | | Sex | 1.626 | 1, 44 | 0.202 | | | |
| | | | | CL | 3.748 | 1, 45 | 0.053 | | | |
| | | | | Species:Nitrate | - | - | - | | | |
| Prey consumed | Bloodworm | Quasipoisson, sqrt | Species | 1.274 | 1, 41 | 0.266 | Signal v. virile <10 v. 50 | <0.001 | 0.293 | |
| | | | Nitrate | 1.080 | 1, 40 | 0.305 | | | | |
| | | | Sex | 0.560 | 1, 38 | 0.459 | | | | |
| | | | CL | 0.428 | 1, 39 | 0.517 | | | | |
| | Gammarids | Quasipoisson, sqrt (+1) | Species:Nitrate | 0.699 | 1, 40 | 0.408 | | | | |
| | | | Species | 12.365 | 1, 44 | 0.001 | | | | |
| | | | Nitrate | 15.627 | 1, 44 | <0.001 | | | | |
| | | | Sex | 1.393 | 1, 43 | 0.244 | | | | |
| | | | CL | 8.353 | 1, 44 | 0.006 | | | | |
| | | | Species:Nitrate | 1.211 | 1, 42 | 0.278 | | | | |
| *log transformed | | | | §LRT for binomial model | | | | | | |
| **squareroot transformed | | | | | | | | | | |

Fig. 1 – Nitrate concentration affects the initial reaction of crayfish to food. Initial reaction (s) of signal (*Pacifastacus leniusculus*; circles) and virile (*Orconectes virilis*; squares) crayfish to bloodworm prey at <10 and 50 mg NO₃⁻/L (log transformed). Points with shared letters denote non-significant differences (P > 0.05)

For both crayfish species, the time taken for them to subsequently feed on bloodworm was also significantly slower at 50 mg NO₃⁻/L (Tukey HSD, P=0.001; Fig. 2).

Fig. 2 – Nitrate concentration influences the feeding response of crayfish. First feeding reaction (s) of signal (*Pacifastacus leniusculus*; circles) and virile (*Orconectes virilis*; squares) crayfish to bloodworm at <10 and 50 mg NO₃⁻/L (square-root transformed). Points with shared letters denote non-significant differences (P > 0.05)

The total number of bloodworm eaten was not influenced by nitrate concentration, crayfish species, carapace length nor sex.

The initial reaction of crayfish to gammarids was not significantly influenced by nitrate, sex, species or carapace length of crayfish (Table 1). Overall however, crayfish were less likely to catch and feed on gammarids at 50 mg NO₃⁻/L compared to <10 mg NO₃⁻/L ($t_{1,46} = -2.535$, P = 0.015). The total number of gammarids eaten was also significantly influenced by nitrate concentration, species (Fig. 3) and carapace length (Table 1). Crayfish at 50 mg NO₃⁻/L ate fewer gammarids than those at <10 mg NO₃⁻/L (Tukey HSD, P<0.001), virile crayfish ate less than virile crayfish (Tukey HSD, P<0.001), and larger crayfish ate fewer gammarids overall.

Fig. 3 – Number of live gammarid prey consumed. Number of gammarids consumed by signal (*Pacifastacus leniusculus*) and virile (*Orconectes virilis*) crayfish at <10 and 50 mg NO₃⁻/L. Points with shared letters denote non-significant differences (P > 0.05)

Discussion

This experimental study has shown that nitrate, even at levels deemed safe for use as drinking water (Council of the European Union 1998), can reduce the foraging efficacy of aquatic non-native species in the UK. The effects of high nitrate are generally not lethal to crayfish; in fact, they appear to be relatively tolerant compared to other aquatic organisms (Jensen 1996; Benítez-Mora et al. 2014). However, the current study has shown that elevated nitrate may alter the predatory impact of crayfish on other organisms, a key trait that makes non-native crayfish particularly troublesome invaders. Altered behavioural responses that affect consumption can significantly alter food web structure (Wong and Candolin 2015), which may be particularly true in terms of keystone, invasive species that exert a disproportionately high impact on ecosystems.

Previous studies have shown that the chemical environment influences the perception and sensory performance of aquatic organisms (Troyer and Turner 2015; Halfwerk and Slabbekoorn 2015). In the current study, we show that elevated nitrate concentration slows the reaction time and foraging efficacy of non-native crayfish species. A previous study Benítez-Mora et al. (2014) showed a similar effect of nitrate on native European crayfish (*A. pallipes*) foraging efficacy. Crayfish have chemosensory hairs within their ambulatory feet (Fedotov 2009) and slower reactions to prey at higher nitrate concentrations suggests that nitrate can interfere with the sensitivity of crayfish to chemical cues from prey. Although the behaviour of the gammarids themselves in the current study may have been directly affected by elevated nitrate, a previous study suggested that nitrate concentrations of up to 128 mg NO₃⁻/L had little effect on *Gammarus pseudolimneaus* (see Stelzer and Joachim 2010).

Crayfish have wide-ranging impacts on ecosystems, largely through their omnivorous foraging nature (Lodge et al. 2000; Geiger et al. 2005; Bobeldyk and Lamberti 2008; Jackson et al. 2014) and a reduction in their foraging efficacy may alter the impacts of crayfish in high-nitrate areas. The two non-native species of crayfish in the present study, the signal and virile crayfish, are of particular interest in the UK due to the widespread invasion by signal crayfish since the 1980s, the recent introduction and continued invasion of the virile crayfish (Ahern et al. 2008), and the potential competitive dominance of the virile crayfish, apparently displacing the established signal crayfish in the River Lea catchment (James et al. 2015). Whilst the virile crayfish reacted slower to bloodworm and ate fewer gammarids than signal crayfish, both species were similarly affected by elevated nitrate, suggesting that nitrate conditions are unlikely to alter the competitive relationship between these two species. A key finding however, is that both crayfish may be less successful at foraging in high-nitrate areas than those present in low-nitrate areas.

The current study has shown that elevated nitrate at ecologically relevant levels can lead to significant changes to behavioural traits associated with invasive species in aquatic environments. Sub-optimal foraging conditions for non-native species that are introduced to new, high-nitrate environments may have implications for the “three-tens” rule of establishment of a non-native species (Williamson and Fitter 1996), where high-nitrate areas could be slightly more ‘resistant’ to invasion. In this case, aquatic non-native species may be at a relative disadvantage in disturbed, urban areas of high-nitrate, and may be even more successful when introduced to ecosystems less affected by excess nitrate.

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