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Reviewing the physio-chemical parameters associated with in-situ Austropotamobius pallipes populations and its application to the establishment of ark sites

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Reviewing the physio-chemical parameters associated with in-situ *Austropotamobius pallipes* populations and its application to the establishment of ark sites

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A thesis submitted for the degree of

Master of Research

in the School of Natural Sciences



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Accompanying material

• Excel spreadsheet denoting a framework for stakeholders to assess the initial suitability of potential ark sites for *A. pallipes*. The framework asks users to collect data specified in the 'Data requirements' sheet. Inputting that data allows users to critically evaluate potential sites in regard to initial suitability, colonisation potential of invasive crayfish and water quality.

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Abstract

Current conservations methods for Austropotamobius pallipes persistence in the UK revolve around the establishment of ark sites into which populations are translocated to isolated sites where the risk of colonisation and subsequent plaque exposure is minimised. Characterisation of the physical habitat and water chemistry of A. pallipes populations have been discussed. However, little discussion exists on the role of water chemistry in explaining establishment success or failure of translocated populations. This study culminates physio-chemical parameters of waterbodies associated with in-situ A. Pallipes populations, comparing them to theoretical thresholds that may encourage/discourage population establishment and persistence. Calcium and pH seem to play vital factors in influencing population establishment, with 93% of populations occurring above the half saturation point of their calcium uptake mechanisms and 83% occurring above the suggested pH establishment threshold of 6.8. Furthermore, a discussion of optimal thresholds and supplementary factors that may be used as new guidelines for stakeholders evaluating the potential of untested ark sites. These findings provide new insight into the role water chemistry plans in successfully establishing a translocated population and provide the basis of a complementary framework which helps conservationists better assess the initial suitability of potential sites.

Literature Review

A. pallipes in Europe

The White Clawed Crayfish (*Austropotamobius pallipes*) is the only crayfish species indigenous to Great Britain (Holdich, 2003), their distribution has become increasingly restricted and fragmented (Trouilhe *et al.*, 2003). Such decline has been observed in many crayfish species, with up to 50% of all freshwater crayfish species becoming threatened with population decline or extinction (Taylor, 2002). Typically, such declines are associated with threats resulting from invasive species, over-harvesting of stocks and

degradation of suitable habitat (Edgerton *et al.*, 2004; Gherardi *et al.*, 2001).

Five crayfish species are native to Europe (Kouba *et al.*, 2014), all of which have been adversely affected by the introduction of non-native species. The most problematic of which is commonly attributed to the Signal Crayfish (*Pacifastacus leniusculus*). Endemic to Northern Africa, *P. leniusculus* has been introduced into over 20 European countries since the 1960's (Holdich, 2002). Attributed specifically as the main decline of *A. pallipes* in England (Alderman *et al.*, 1990), *P. leniusculus* possess a number of superior characteristics over *A. pallipes* including advantageous life history traits and wider environmental tolerances (Firkins, 1993; Sáez-Royuela *et al.*, 2006). Despite these ecological advantages, the biggest threat to *A. pallipes* persistence in Britain is the crayfish plague for which *P. leniusculus* acts as a vector (Chucholl and Schrimpf, 2016).

Competition with Non-Native Species

Interspecific interference competition often includes a dominant (usually larger) and a subordinate (usually smaller) competitor —which may involve direct aggression (Harrington *et al*, 2009) — and may lead to the death of the lesser competitor (Palomares and Caro, 1999). Documented effects of interspecific competition on the subordinate species include reduced access to limited resources including food, refuge and mates (Vorburger and Ribi, 1999). Such competition plays a pivotal role in determining species replacements (Butler and Stein, 1985; Hill and Lodge, 1999), in part underpinning the increasing and decreasing distributions of *P. leniusculus* and *A. pallipes* respectively (Kouba *et al.*, 2014).

Documented favourable life history traits of *P. leniusculus* (over *A. pallipes*) include: faster growth rates, higher fecundity and wider environmental tolerances (Firkins, 1993; Guan and Wiles, 1999; Sáez-Royuela *et al.*, 2006) which will be advantageous when competing for the same niches. Mature individuals of both species utilise similar refuge habitat (Bubb *et al.*,

2006) providing a driver of direct interspecific competition between the species. The larger size and increased aggressiveness of P. leniusculus (Bubb et al., 2009) result in them being the dominant species when determining access to refugia (Nakata and Goshima, 2003; Gherardi and Daniels, 2004), as access to shelter and aggressive dominance is determined by the larger individual (Vorburger and Ribi, 1999, Nakata and Goshima, 2003). Behavioural experiments found P. leniusculus were able to evict Bullhead's (Cottus gobio) from occupied refugia, undertaking significantly more aggressive approaches compared to A. pallipes. If sharing of a refuge did occur between a combination of the three study species, it was significantly less likely to include P. leniusculus (Bubb et al., 2009). P. leniusculus possess more favourable life history traits, achieve larger sizes, and are likely to outcompete A. pallipes for refugia (Garvey and Stein, 1993; Streissl and Hödl, 2002; Griffiths et al., 2004). Illustrating the mechanisms that likely underpin the widespread success P. leniusculus has experienced in successfully colonising many British waterbodies.

Crayfish Plague

The crayfish plague is caused by the water mould *A. astaci*, to which North American species are less susceptible and act as a vector of (Svoboda et al., 2017; Unestam and Weiss, 1970). Outbreaks of crayfish plague have been recorded in many European countries including the UK, Sweden, Spain and France (Alderman et al., 1990, Diéguez-Uribeondo et al., 1997; Bohman et al., 2006; Bramard et al., 2006). DNA analysis can be used to identify the species origin of plague outbreaks as there are multiple documented *A. astaci* genotypes (Caprioli et al., 2018). *A. astaci* originates from zoospores that are transmitted to new hosts (Unestam and Weiss, 1970) within freshwater environments. Thus potential hosts will inhabit freshwater habitats for at least part of their life cycles, criteria that is filled by a large number of crustacean species (Svoboda et al., 2017). *P. leniusculus* is not the only confirmed vector of the plague in the UK, the established Chinese Mitten Crab (*Eriocheir sinensis*) has also been

documented to be capable vector (Schrimpf, 2014, Svoboda et al., 2017). Both *P. leniusculus* and *E. sinensis* possess significant dispersal ability within freshwater systems (Bubb et al., 2006; Dittel and Epifanio, 2009) thus much of the UK is at risk of becoming exposed to the crayfish plague.

The decline of *A. pallipes* in the UK led to the concept of ark sites, referring to isolated waterbodies where new populations can be established whilst minimising the colonisation potential of invasive species. Ark sites are being considered the primary long term conservation strategy of *A. pallipes* in the UK (Nightingale *et al.*, 2017) in order to ensure breeding populations will persist in case of the total loss of native populations (Kindemba and Whitehouse, 2009). Souty-Grosset and Reynolds (2009) compiled 59 case studies of attempted ark sites across Europe, only 26 of these introductions were successful (44%). Insufficient information was suggested as the primary cause for failure when assessing potential ark sites, as in most cases no biotic data was collected whilst only occasionally testing for physio-chemical parameters.

Selection of Ark Sites for A.pallipes

Kemp *et al.* (2003) produced the first guidelines that aimed to assess the suitability of sites for translocation potential. The basis of which involved reintroducing *A. pallipes* back into waterbodies where they had been historically found. However, such sites were potentially prone to the threats associated with *P. leniusculus*. Subsequently building on the principles outlined by Kemp *et al.* (2003), Holdich *et al.* (2004) proposed isolated waterbodies that had never hosted *A. pallipes* were used as 'ark sites'. An "simple but flexible tool" was subsequently developed for stakeholders to assess the suitability of potential new ark sites (Peay, 2009). It was based around a series of criteria that could be grouped into measures relating to protection from invasion and suitability of habitat.

Peay's framework is somewhat limited by its age, as since its 2009 publication a variety of published literature that aims to increase the available information regarding to ark sites has been published (Rosewarne, Mortimer and Dunn, 2013, 2017; Haddaway et al., 2015; Nightingale et al., 2017; Beaune et al., 2018). GQA (General Quality Assessment) gradings were used to assess whether the quality of the waterbody is sufficient to successfully establish a translocated population. However, these gradings alone are insufficient to evaluate whether the ecological requirements of A. pallipes are met. Gradings are based on a variety of factors including chemical (dissolved oxygen, biochemical oxygen demand and ammonia); biological (macro-invertebrate bioindicators); nutrient status (phosphate and nitrate levels); and aesthetic quality (litter, foam, odour, colour). These indicators are used to assess water quality (REF) but fail to evaluate chemical parameters essential to A. pallipes survival such as pH and calcium concentrations (REF). The non-optimal success rate in establishing ark sites (44%, Souty-Grosset and Reynolds, 2009) could potentially be explained by a lack of specific evidence relating to aspects of water chemistry which can either encourage/discourage the persistence of the species. An understanding of the physio-chemical requirements required for A. pallipes may help differentiate sufficient & non-sufficient habitat (Favaro et al., 2010; Haddaway et al., 2015).

Haddaway *et al* (2015) published a review of the water chemistry associated with *A. pallipes* populations where the summary statistics were compared to European and global means in order to identify potential patterns. Utilising summary statistics may mask populations that exist at the extremes of an individual parameter's spectrum. Furthermore, there's no discussion of laboratory delineated thresholds that aim to investigate suitable levels of in-situ populations (Benítez-Mora *et al.*, 2014; Rosewarne *et al.*, 2014). As such, there's a gap within the literature that investigates the water chemistry of in-situ populations in order to compare them with

laboratory delineated thresholds and produce a set of guidelines that will encourage./discourage population establishment.

This study aims to update Peays (2009) framework by incorporating advancements in the literature that will help policy makers assess the suitability of ark sites for the long-term persistence of *A. pallipes* in the UK. An updated series of recommendations that relate to the role water chemistry plays in determining the suitability potential of ark sites is produced. Furthermore, advancements in our understanding of the dispersal ability for *P. leniusculus* is discussed to help determine suitable characteristics that influence an sites security to minimise the risk of colonisation and subsequent exposure to the crayfish plague.

Dissertation

1.1 Introduction

White-clawed crayfish (*Austropotamobius pallipes*) are widely distributed across Western Europe (Kouba *et al.*, 2014), however fluctuations both in population numbers and mortality rates throughout the mid 1800s – 1950s (Holdich and Reeve, 1991) have caused their distribution to become increasingly fragmented in many regions (Trouilhe *et al.*, 2003). Such declines are primarily a result of the invasive signal crayfish (*P. leniusculus*) and the associated crayfish plague for which they act as a vector (Svoboda *et al.*, 2017). Recent conservation strategies for *A. pallipes* include translocations of populations to isolated waterbodies coined 'ark sites' (Kindemba and Whitehouse, 2009; Haddaway *et al.*, 2015; Nightingale *et al.*, 2017; Rosewarne *et al.*, 2017), in order to ensure long term persistence of the species in the UK and Ireland (Nightingale *et al.*, 2017).

Attempts have been made to produce guidelines that will encourage the successful translocation of individuals to ark sites (Kemp, 2003, Peay, 2009). Such guidelines are out-of-date considering newly published literature, said literature includes laboratory-based tolerance experiments (Rosewarne, Mortimer and Dunn, 2013) and studies that aim to characterise the physio-chemical parameters of waterbodies inhabited by in-situ populations (Haddaway *et al.*, 2015; Beaune *et al.*, 2018)

Tolerance studies provide possible explanations for multiple instances of established populations disappearing (Lyons and Quinn, 2003). Whilst characterising their habitat will provides insight into suitable characteristics potential ark sites should possess, indicating how to best conserve the species (Rosewarne *et al.*, 2017). Previous attempts have not always been successful, of recorded case studies from across Europe only 44% (n = 59) of attempted ark sites were successful (Souty-Grosset and Reynolds, 2009). Insufficient physio-chemical analysis was suggested as the primary cause for failure, as testing only occurred occasionally. Thus, habitat data

will allow conservationists to make more informed decisions regarding the suitability of ark sites when translocating native crayfish populations.

Multiple studies have tried to characterise the physio-chemical parameters associated with the habitat of A. pallipes (Beaune et al., 2018; Benvenuto et al., 2008; Broquet et al., 2002; Favaro et al., 2010; Reyjol and Roqueplo, 2002; Smith et al., 1996; Troschel, 1997; Trouilhe et al., 2003, 2007; Trožić-Borovac, 2011). Haddaway et al. (2015) collated such parameters in a review which collected descriptive statistics and compared them to global and European means, attempting to identify potential patterns. Alternatively, this study focuses on literature which presents data on individual populations, as the utilisation of summary statistics may mask populations which exist at the extremes of a parameter's spectrum. Furthermore, focusing on individual populations will allow one to compare distributions of A. pallipes populations and assess the proportion that meet certain delineated thresholds, potentially explaining their distribution. While this approach reduces the overall sample size compared to the total sum of populations used in Haddaway et al (2015), the summary statistics within this study are likely to be more representative of the range of physiochemical conditions of waterbodies inhabited by A. pallipes. The advantages of this approach may be best illustrated by the large disparity in total suspended solids (herein TSS). Values ranging from 0.2 - 493.3mg.L⁻¹ (n = 4) offer little insight into the accuracy of the calculated summary statistics (mean minimum = 7.00, mean mean = 11.78 & mean maximum = 16.90) when these summary statistics were calculated from such extreme ranges with a very low sample size (Haddaway et al., 2015). The trade-off is that by using such an approach, studies that do not meet the inclusion criteria are not utilised - potentially not representing evidence of populations existing in fringe conditions. While there is some significant overlap in the utilised literature (Troschel, 1997; Broquet et al., 2002; Trouilhe et al., 2003; Scalici and Gibertini, 2005; Renai et al., 2006; Barbaresi et al., 2007; Trouilhé et al., 2007; Brusconi et al., 2008; Favaro

et al., 2010; Trožić-Borovac, 2011), the raw data for two of these studies was obtained directly from the authors and included (Broquet *et al.*, 2002; Favaro *et al.*, 2010). This study aims to compare the results of this new approach to previous similar literature and identify whether statistically significant differences occur. Furthermore, where available thresholds from tolerance experiments were used to determine the proportion of populations that occur above or below said thresholds to determine if such experiments are an accurate representation of in-situ tolerances. These findings hope to offer further insight into the role certain physio-chemical parameters play in increasing or decreasing the establishment success of translocated *A. pallipes* populations into newly established ark sites.

2.0 Methods

2.1 Literature Search

Web of Science was searched for literature relating to water chemistry associated with Α. pallipes populations. The search string "Austropotamobius pallipes OR white clawed crayfish AND physiochemical AND abiotic AND water chemistry AND habitat requirements" was used, returning 581 results. After initial title screening, 61 papers were shortlisted and had their abstracts scanned to assess for suitability. The criteria for shortlisting included literature that presented data for at least one variable relating to the water chemistry of waterbodies that host individual A. pallipes populations. Twenty-three of those papers presented data into at least one of the physio-chemical parameters focused on in this study. However, each study focused on a different set of parameters, thus some parameters have larger sample sizes in the final dataset than others (see table 2). Nine of those papers presented data that was representative of individual populations and subsequently extracted. In studies where only the summary statistics were presented, the authors were contacted to request a copy of the raw data. Data from another four studies were obtained this way and included. In total 13 studies were used in the complete dataset (Table 1).

Table 1. Summary table of the literature used in the culmination of the physio-chemical parameters associated with A. pallipes and A. italicus populations. COI refers to country of origin in which the study and consequently the populations were sampled. Extracted data was presented and subsequently extracted if it referred to individual populations rather than summary statistics, Requests for the raw data was made to authors which did not present data on individual populations and obtained. Studies from Favaro et al. and Tirelli et al. feature the same dataset.

Citation	Species	COI	<i>Data Obtained</i>
Trouilhe <i>et al</i> (2007)	Pallipes	France	Extracted
Haddaway <i>et al</i> (2015)	Pallipes	England	Extracted
Troschel (1997)	Pallipes	Germany	Extracted
Trožić-Borovac (2011)	Pallipes	Bosnia and Herzegovina	Extracted
Favaro <i>et al</i> (2010)	Pallipes	Italy	Request
Favaro <i>et al</i> (2011)	-	-	-
Tirelli <i>et al</i> (2011)	-	-	-
Renai <i>et al</i> (2006)	Itallicus	Italy	Extracted
Brusconi <i>et al</i> (2008)	Itallicus	Italy	Extracted
Barbaresi <i>et al</i> (2007)	Italicus	Italy	Extracted
Broquet et al (2002)	Pallipes	France	Request
Trouilhe <i>et al</i> (2003)	Pallipes	France	Extracted
Benvenuto <i>et al</i> (2008)	Pallipes	Italy	Request
Wendler <i>et al</i> (2015)	Pallipes	Germany	Request
Scalici and Gibertini (2005)	Italicus	Italy	Extracted

2.2 Sampling

Additional data was collected from *A. pallipes* populations in South-West England. Samples for nitrites, ammonia, pH, conductivity and phosphate were taken using portable Hanna probes (Appendix 1) in October 2020. Samples came from one population in the Candover River, New Arlesford a tributary of River Itchen (Appendix 2) and three others along the River Culm, Devon (Appendix 2). Multiple populations along the same river were considered separate if population mixing is unlikely to occur, as a result of either significant unoccupied habitat between them or sufficient barriers that prevent population mixing.

Map (Co-ords in appendix)

https://sci-hub.41610.org/

https://oa.mg/

3.0 Results & Discussion

3.1 Water Chemistry

The physio-chemical parameters of collective populations are summarised in Table 2. Values were summarised from 162 populations of *Austropotamobius pallipes and italicus*, originating from various countries across Europe including Italy (65%), Bosnia and Herzegovina (11%), England (12%), France (7%) and Germany (5%) (Table 1). The focus species in this study is *A. pallipes* (British Isles, France and northern Italy). However, three studies sampled *A.italicus* populations (Iberian Peninsula, Italy, Southern Alps, and Balkans). Both of which form a species complex with *Austropotamobius torrentium* (alpine regions) (Diéguez-Uribeondo *et al.*, 2008; Grandjean *et al.*, 2000). *A. Italicus* share significant phylogenetic similarities with *A. pallipes* and was relatively recently reclassified from *Austropotamobius pallipes italicus* (Grandjean *et al.*, 2002), thus were included in this study in-line with similar previous studies (Haddaway *et al.*, 2015) under the pretence that they probably occupy similar environments.

Data for 16 variables pertaining to water chemistry were collated, only variables that in sufficient excess/deficiency may explain the mediocre success rate (Souty-Grosset and Reynolds, 2009) of establishing ark sites are discussed below. The more general aspects of water chemistry in relation to *A. pallipes* have been discussed elsewhere (Haddaway *et al.*, 2015).

Table 2. Summary table of the physio-chemical parameters associated with A. pallipes and A. italicus populations, collated from the literature and samples collected in this study. Units; pH, Temperature (°C), Conductivity (μ S.cm⁻¹) & all other variables are displayed in mg/l. N indicates the total number of results collected for each parameter.

Variable	Min	Max	Mean	Median	N	SD±
Temperature	8.6	19	13	12.8	88	2.45
Conductivity	38	793	262	228	143	168.04
рН	5.97	9.0	7.69	7.81	124	0.68
Calcium	2.56	156	41.4	30.9	101	32.87
DO	2.12	19.4	8.37	8.29	170	2.55
TSS	10.2	34	15.7	13.2	9	7.09
Sodium	3.1	50.3	12.7	9.8	27	9.94
Nitrates	0.0	33.5	4.14	2.09	118	5.97
Nitrites	0.0	0.1	0.0462	0.05	52	0.03
Ammonium	0.001	2.07	0.250	0.05	94	0.38
Phosphate	0.002	1.68	0.139	0.0445	68	0.26
Magnesium	0	52.1	10	6	74	10.18
Chloride	0.013	65.8	15.3	12.4	76	13.65
Sulphates	0.88	356	37.9	12.4	27	69.56
Potassium	0.14	5.78	2.49	2.74	27	1.36
Silicates	5.6	17.2	10	11.4	17	3.7

Environmental Variables

3.1.1 Water Temperature

Temperatures associated with A. pallipes populations range between 8.6-19°C (Table 2) although temperature will vary with a variety of factors including water depth, lentic/lotic systems, season and time of day. While water temperatures in A. pallipes populations vary widely, higher temperatures have been shown to increase crayfish moult and growth rates in Austropotamobius torrentium and Paranephrops zealandicus (Hammond et al., 2006; Kozák et al., 2009). However, exceeding a species temperature tolerance can cause it to become a limiting factor to survival (Hammond et al., 2006). A. pallipes hatchlings raised in controlled conditions achieved the highest total length, weight, percent survival and growth rate when reared at 20.3°C, compared to 18.3 and 13.3°C on a diet of *Daphnia* species (Policar, 2010). Another study found that the mortality rate of *A. pallipes* increases at 24°C — whilst consuming a purely animal diet (earthworms) — but declines prematurely at 20°C on a suboptimum detritus diet (Paglianti and Gherardi, 2004). Detritus features a prominent proportion of A. pallipes diets - especially in juveniles - despite their consumption of detritus decreasing proportionally with age in favour of vegetal components (Scalici and Gibertini, 2007). This indicates that diets of in-situ juveniles resemble more closely the latter diet utilised in Paglianti and Gherardi (2004), as such, in-situ temperature induced mortality rates of juveniles may be initiated at ~20 °C .

While temperatures for ark sites cannot be narrowed down to an optimum range, waterbodies should be relatively cool and not subject to large fluctuations. It is possible that 20-24 °C may represent an upper limit for the thermal tolerance of *A. pallipes*, thus the potential of thermal extremes should be assessed when evaluating potential ark sites. Temperature recordings should be taken when thermal extremes are most likely e.g. low flow regimes in summer months.

3.1.2 Dissolved Oxygen

A. pallipes and A. Italicus kept in controlled aquaria for 12 days at dissolved oxygen (DO) concentrations above 5.5 mg.L⁻¹ showed no observable hypoxic stress (Demers et al., 2006). However both species exhibited evidence of hypoxic stress (disruptions to their ion exchange processes) under hypoxic conditions denoted by 3.0 mg.L⁻¹, but also demonstrated an aptitude to tolerate hypoxia for 12 days. (Demers et al., 2006). The observed tolerance of *A. pallipes* to hypoxic conditions may be a resulting adaptation of regular exposure to periods of uncharacteristic hypoxia, which can persist for up to 6 hours (Lyons and Kelly-Quinn, 2003). Periods of uncharacteristic hypoxia can be a temporary but regular occurrence in freshwater systems, explained by: flooding, flow regimes, eutrophication and daily/seasonal temporal changes (Demers et al., 2006; Guasch et al., 1998; Haddaway et al., 2015; Lyons and Kelly-Quinn, 2003; Pârvulescu et al., 2011; Whitney, 1942). Documented consequences of hypoxia to crayfish include; exclusion from deoxygenated zones (Kusabs et al., 2015), mortalities if DO becomes sufficiently limited (Landman et al., 2005) and forcing the need to surface for access to atmospheric oxygen (Wheatly and Taylor, 1981; Broughton et al., 2017) subsequently increasing their chance of being predated upon.

Only one waterbody (0.6%) in this study recorded DO concentrations below 3 (Table 2), providing complementary evidence that DO concentrations of 3.0-3.5 mg.L⁻¹ presents a lower limit that may explain *A. pallipes* absence (Haddaway *et al.*, 2015). While *A. pallipes* can tolerate extended periods of hypoxia in controlled systems (Demers *et al.*, 2006), in-situ populations are exposed to other factors that if significantly elevated can reduce oxygen availability/uptake such as temperature, nitrates and TSS (Camargo *et al.*, 2005; Rosewarne *et al.*, 2014).

As such, oxygen concentrations in ark sites should be sufficiently high so that during periods of uncharacteristically low DO (flooding, drought, low flow regimes) concentrations are unlikely to fall below \sim 3.5 mg.L⁻¹ for an

extended period. Recorded oxygen sags in previously occupied *A. pallipes* habitat vary between 5 & 7mg.L⁻¹ (Lyons and Kelly-Quinn, 2003). Thus, incorporating a 7mg.L⁻¹ buffer against a minimum of 3.0mg.L⁻¹ (10 mg.L⁻¹ total) may be indicative of 'optimum habitat'. Whilst DO concentrations and the extent of deficits will vary between systems, 10 mg.L⁻¹ may be representative of the best general guidelines this study can suggest without the use of long-term monitoring of specific sites.

3.1.3 Conductivity

It's been suggested that A. pallipes populations thrive in waters with conductivity ranges between 450-900 µS.cm⁻¹ (Alonso et al., 2000; García-Arberas and Rallo, 2000) with samples from inhabited waterbodies recording measurements of over 1600 µS.cm⁻¹ (Alonso et al., 2000; Rallo and Garcia-Arberas, 2000). Presumably because high concentrations of conductivity can be indicative of calcareous geology, with ranges between \sim 150 – 1000 µS.cm⁻¹ being associated with carbonate karsts (Krawczyk and Ford, 2006) which typically denote suitable A. pallipes habitat (Firkins, 1993; Gil-Sánchez and Alba-Tercedor, 2002; Peay et al., 2002; Holdich, 2003). The majority of waterbodies collated in this study report values under 400 μ S.cm⁻¹ (78%), potentially explained by the increasing fragmentation of A. pallipes habitat (Trouilhe et al., 2003) where populations are only surviving in non-invaded area's despite potentially not indicating historically optimal habitat. High conductivity can also indicate various anthropogenic inputs, with studies finding positive correlations with; biological pollutants, pharmaceutical contaminants, and heavy metal pollution (García-Criado et al., 1999; de Sousa et al., 2014). Thus ark sites with particularly high conductivity measurements should validate that it is not a result of anthropogenic inputs. If such concentrations can be explained by geology (based on the assumption that a high proportion of calcium carbonate is present), then it is likely that the waterbody will have favourable pH and calcium concentrations which is beneficial for ecdysis (Greenaway, 1974; Beaune et al., 2018).

Variables affecting Ecdysis

3.1.4 pH

Ecdysis refers to the shortest stage of the moulting process where the exoskeleton of crustaceans and other arthropods is exuviated to accommodate for growth (Philippen et al., 2000). The process is vital in their life cycle as if unsuccessful will result in the individual's death (Philippen et al., 2000). Low pH negatively impacts growth, survival and the mechanisms underpinning ecdysis in crayfish (including; calcification, moulting frequency, calcium uptake from the environment and cuticle thickness) (Haddaway et al., 2013; Beaune et al., 2018). With an pH of < 6.8 having been suggested as the threshold for preventing crayfish establishment (Beaune et al., 2018), an assumption supported by 85% of sampled populations, n = 105 (Table 3). Intermoult Rusty Crayfish (Orconectes Rusticus) individuals (LC₅₀ 3.5) exhibited higher pH resistance than post-moult individuals (LC₅₀ 2.5) (Malley, 1980; Morgan and McMahon, 1982; Zanotto and Wheatly, 1993). It is extremely likely postmoult A. pallipes share the same susceptibility to reduced pH. Increasing their sensitivity to low ambient pH and inhibiting regrowth of the exoskeleton, subsequently increasing their susceptibility to cannibalism or predation (Stein, 1977). Seventeen of the populations in this study inhabited waterbodies of pH below 6.8 (14%) (Table 1), of those, nine waterbodies were > 6.5 and none were < pH 5.97. All waterbodies bar one (0.81%), concur with the suggestion that populations of A. pallipes do not subsist at < pH 6.0 (Haddaway et al., 2015). This evidence bolsters recommendations that potential ark sites exhibit pH 6.5 - 9 (Peay et al., 2002; Holdich, 2003), although recommendations of > pH 7 have been suggested in the anticipation of future decreases under climate change scenarios (Beaune et al., 2018). Although, mortality of A. pallipes individuals positively scale with increasing pH. pH 8.6 resulted in significantly increased survival time compared to lower pH treatments of

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3.1.5 Calcium

Sufficient calcium concentrations are essential to crayfish survival and growth/calcification rates (Rukke, 2002; Hammond *et al.*, 2006). Sufficient calcium can be obtained via: consumption of gastroliths, ingested material and/or ions from the water (Malley, 1980). Different crayfish species have documented different minimum calcium requirements (Cairns and Yan, 2009; Edwards *et al.*, 2015). The calcium uptake mechanisms *of A. pallipes* require concentrations of 5.2 mg.L⁻¹ to become half saturated and 16 mg.L⁻¹ to become fully saturated (Greenaway, 1974). The half-saturation point likely influenced the commonly utilised calcium benchmark that conservationists use (5.0 mg.L⁻¹) to assess minimum calcium concentrations necessary for translocation to potential ark sites (Peay *et al.*, 2002; Holdich, 2003).

Post-moult crayfish lose ~80% of total body calcium during ecdysis (Cairns and Yan, 2009) with the replacement process starting rapidly (Greenaway, 1974). 15-30 minutes post-moult net calcium uptake peaks over the entire inter-moult period although uptake remains consistently high for the first 15 days, continuing for approximately 45 days (Greenaway, 1974). In calcium deficient waterbodies it's suggested that crayfish can obtain supplementary calcium from dietary sources (Peay, 2009). Individuals tend to remain inactive immediately post-moult (Reynolds, 2002), thus they are unlikely to seek out supplementary calcium from dietary sources, relying heavily on ambient calcium in the water (Cairns and Yan, 2009). Furthermore, crayfish become restricted to refugia, tending to remain inactive when at risk of fish predation (Stein, 1977; Garvey *et al.*, 1994; Nyström, 2005). Thus it's unlikely that dietary calcium contributes

significantly to the required heavy demand for calcium post-ecdysis. Highlighting the benefits of sufficient calcium being available as ions from the water. External calcium concentration influences the rate of net calcium uptake in early post-moult *A. pallipes* up to a certain point (Greenaway, 1974) thus calcium concentrations close to 16 mg.L⁻¹ will ensure crayfish achieve maximal remineralisation rates, reducing the risks associated with inter-/intraspecific encounters.

In relation to ark sites, minimal calcium concentrations should exceed 5.2 mg.L⁻¹ (threshold met by 93% of populations, n = 94) to ensure calcium uptake mechanisms are at least half saturated (Greenaway, 1974). Optimal waterbodies will possess calcium concentrations >16 mg.L⁻¹ (72% of populations, n = 73) to ensure calcium concentrations aren't a limiting factor during ecdysis (Greenaway, 1974).

Table 3. Percentage of collated populations that meet significant thresholds in relation to A. pallipes and A.italicus.

Variable	Threshold	%	Ν	Threshold	Reference(s)	
	Equal to or above 16 mg.L ⁻¹	72	73	Threshold to fully saturate calcium uptake mechanisms		
Calcium	Equal to or above 5.2 mg.L ⁻¹	93	21	Threshold to half saturate calcium uptake mechanisms	(Greenaway, 1974; Cairns and Yan, 2009)	
	Below 5.2 mg.L ⁻¹	7	7	Uptake mechanisms will not reach half saturation.		
pН	Equal to or above 6.8 pH	86	103	Suggested limiting factor for <i>A. pallipes</i>	(Beaune <i>et al.</i> , 2018)	
•	Below 6.8	14	17	establishment		
DO	Equal to or above 8.5 mg.L ⁻¹	46	78	Representative normoxic and hypoxic conditions, significant		
	Below 3.0 mg.L ⁻¹	0.6	1	differences in haemolytic sodium concentrations found between groups exposed to control and hypoxic conditions, indicating hypoxic stress in the latter.	(Demers <i>et al</i> , 2006)	
Nitratos	Equal to or above 68 mg.L ⁻¹	0	0	Recommended maximum threshold to minimico offecto	(Benítez-Mora <i>et</i>	
Mitales	Less than 68 mg.L ⁻¹	100	93	on juvenile A. italicus individuals	ai., 2014)	
TSS	Less than 65 mg.L ⁻¹	100	9	Recommend upper limit to minimise impact on gill function	(Rosewarne <i>et al.</i> , 2014)	

Simultaneously ensuring post-moult crayfish recalcify as quickly as possible whilst retaining their ability to hide when most vulnerable from predation/cannibalism. Depending on the intended longevity/security of the site, concentrations above the suggested thresholds may be beneficial as there is evidence of global calcium decline in freshwater systems (Weyhenmeyer *et al.*, 2019).

In relation to ark sites, minimal calcium concentrations should exceed 5.2 mg.L⁻¹ (threshold met by 93% of populations, n = 94) to ensure calcium uptake mechanisms are at least half saturated (Greenaway, 1974). Concentrations below 5.2mg.L⁻¹ should be considered sub-optimal, as the remineralisation of the exoskeleton may become compromised (Greenaway, 1985). Optimal waterbodies will contain >16 mg.L⁻¹ (72% of populations, n = 73) to ensure calcium concentrations aren't a limiting factor during ecdysis (Greenaway, 1974). Simultaneously ensuring postmoult crayfish recalcify as quickly as possible whilst retaining their ability to hide when most vulnerable from predation/cannibalism. Depending on the intended longevity/security of the site, concentrations above the suggested thresholds may be beneficial as there is evidence of global calcium decline in freshwater systems (Weyhenmeyer *et al.*, 2019).

Anthropogenic Inputs

3.1.6 Total Suspended Solids

It's commonly reported that *A. pallipes* live in clean, well-oxygenated water that do not contain high concentrations of sediment (Holdich *et al.*, 2006). However, case studies of well-established populations inhabiting 'muddy' waters (characterised by high concentrations of suspended sediment) do exist (Holdich *et al.*, 2006). Rosewarne *et al.* (2014) demonstrated that juvenile *A. pallipes* exposed to various time-averaged suspended solid concentrations (2.5, 42, 65 and 133mg.L⁻¹) for 45 days exhibited lower aerobic scopes across all treatments, in all individuals bar two (n = 30/32, 94%). While the effect of suspended solids (herein SS) on mortality was

also investigated, higher mortality than may be expected for aquarium based organisms were observed across all treatments and the control, so no correlation between elevated TSS and mortality could be substantiated (Rosewarne *et al.*, 2014).

Rosewarne *et al.* (2014) demonstrated that even low TSS concentrations reduced aerobic scope after 45 days when compared to day one. Although more drastic alterations to aerobic scope were observed in the higher SS treatments, reducing aerobic scope 17 and 28% relative to the control. Insitu populations are frequently exposed to oxygen sags (Lyons and Kelly-Quinn, 2003) and seem tolerant of hypoxic conditions for relatively long periods of time (Demers *et al.*, 2006). However, in habitats where TSS are continuously high, reduced aerobic scope could be an almost continuous state (Rosewarne *et al.*, 2014) that could negatively impact individual fitness. The effects of which are likely exasperated in waterbodies with low DO that reduces initial availability. Rosewarne *et al.* (2014) recommended TSS concentration < 65 mg.L⁻¹ in ark sites to minimise negative impacts on *A. pallipes*.

3.1.7 Inorganic Nitrogen (NH₄, NO₂, NO₃)

Elevated concentrations of nitrites, nitrates and ammonium are all indicative of anthropogenically polluted waters (Benítez-Mora *et al.*, 2014) which have been reported to threaten persistence of *A. pallipes* in the UK (Smith *et al.*, 1996). Historically *A. pallipes* have been regarded as inhabiting clean and unpolluted waterbodies (Firkins, 1993; Holdich *et al.*, 2006). However, an increasing amount of confounding evidence has been relating populations to moderately polluted waters (Rallo and Garcia-Arberas, 2000; Trouilhé *et al.*, 2007). No study investigating the toxicity of nitrites to the *Austropotamobius* complex could be found, although studies relating to ammonia and nitrate toxicity have been published (Firkins, 1993; Benítez-Mora *et al.*, 2014). As no specific evidence relating to the tolerance of *A. pallipes* to nitrates could be found, a study focusing on *A.* *italicus* was utilised due to their phylogenetic similarities (Grandjean *et al.*, 2002)

A.italicus juveniles demonstrate low susceptibility to nitrate toxicity, although mortality rates did increase while escape response and food consumption decreased with increasing nitrate concentrations and exposure times (Benítez-Mora *et al.*, 2014). Food consumption was the most sensitive variable to nitrate concentrations followed by escape response and finally mortality (Benítez-Mora *et al.*, 2014). Indicating that while high concentrations of nitrates may not initiate high mortality rates, sub-lethal effects are observed at lower concentrations. The no-observed effect threshold for the study was 100 mg NO₃ - N/L after 14 days, with the authors recommending an estimated safe concentration of 68 mg NO₃ - N/L (Benítez-Mora *et al.*, 2014).

100% (n = 93) of the waterbodies in this study reported values below 68 mg.L⁻¹ (Table 3), 86% recorded nitrate concentrations below 7.5 (n = 102, Figure 1) and even the maximum value (33.5 mg.L⁻¹) was less than half of the authors recommend safe upper threshold (Benítez-Mora *et al.*, 2014). Seemingly, natural populations have not been recorded to inhabit waterbodies that approach the upper limit of the suggested threshold (Benítez-Mora *et al.*, 2014).

Of the Inorganic nitrates (NH₄, NO₂, NO₃), ammonia is typically the most toxic to aquatic organisms followed by nitrites then nitrates (Romano and Zeng, 2013). Inorganic nitrogen enters aquatic ecosystems via agricultural practices, surface run off and sewage effluent (Camargo et al., 2005), thus elevated concentrations of one form are observed alongside elevated concentrations of the more toxic forms of inorganic nitrogen. This may explain the disparity between the reported high tolerance of *A. italicus* juveniles to nitrates in isolation and the drastically lower observed range at which populations are found. Over-/underestimating species tolerances based on laboratory studies in comparison to the realities of in-situ is a frequently reported limitation of laboratory-based studies (Brady *et al.*,

2013; De Lisle and Roberts, 1986; Melvin and Houlahan, 2012; Tagliarolo and McQuaid, 2016).

Ammonia naturally occurs in either unionised (NH₃) or ionised (NH₄) forms (Romano and Zeng, 2013), with the unionised form being more toxic (Evans and Cameron, 1986). Ammonia is particularly toxic to aquatic organisms and becomes increasing toxic with increasing pH to a variety of aquatic organisms including crustaceans, fish species and invertebrates (Tomasso et al., 1980; Schubaur-Berigan et al., 1995; Romano and Zeng, 2013). Frikins et al. (2013) demonstrated that a single severe pollution event has the capability to induce total mortality of a population. Juvenile A. pallipes were exposed to a 6 hour pulse of increased ammonia (10 mg.L⁻ ¹) (Firkins, 1993) which initiated 100% mortality, such evidence provides credibility to observations that populations crash rapidly when certain tolerance thresholds are exceeded (Fureder and Reynolds, 2003). While populations are not likely to be exposed to such high concentrations outside of single pollution events (~ 5 times the maximum record value of this study, Table 2), sub-lethal effects occur at lower concentrations in P. leniusculus (Edwards et al., 2018). This has important implications for populations in which A. pallipes and P. leniusculus are sympatric. P. leniusculus demonstrate a greater short-term resistance to ammonia (Firkins, 1993), pollution events that increases ammonia concentrations significantly may have greater detrimental impacts on A. pallipes individuals compared to P. leniusculus. Leaving a greater proportion of P. leniusculus individuals alive, individuals that have demonstrated the ability to recover after surviving the initial pulse (Firkins, 1993).

While the *Austropotamobius* complex seems tolerant to relatively high concentrations of inorganic nitrogen, optimal sites should exhibit minimal concentrations of inorganic nitrogen and be unlikely to be exposed to large pollution events. To quantify said concentrations in relation to *A. pallipes*, it is suggested that the individual upper quartiles for each nitrogen form represent target guidelines for potential ark sites (Table 4). However, it is

also likely that populations could persist in higher concentrations. Sites exhibiting inorganic nutrient concentrations that are not problematic but are continuously elevated are probably subject to small and constant inputs from an anthropogenic source. Such sites may be more at risk from problematic pollution events and should be investigated before translocation proceeds.

3.2 Physical Factors

3.2.1 Levels of isolation

Ideally ark sites should be fully enclosed with no watercourses leading in or out (Peay, 2009) to minimise the risk of colonisation. Risk factors affecting site security include inflow/outflow, proximity to nearest P. leniusculus population, angling/boating access, flow rate and the frequency of both flooding & low flow regimes (Peay, 2009; Frings et al., 2013; Mathers et al., 2020). Where possible, complete isolated sites will possess the highest level of security against threats from invasive crayfish. However, where not possible the direction of connecting systems have direct consequences to the security of a site. Sites with inflow are far more susceptible to becoming compromised, A. pallipes populations downstream of P. leniusculus are susceptible to plague exposure. Inflow is capable of carrying A. astaci spores into the waterbody, subsequently exposing the population to the plague (Frings et al., 2013). Sites with exclusively outflows make far more secure sites, as the lack of inflow vastly reduces the chances of contracting the plague. Outflows help exclude invasive crayfish from a site. Evidence suggests that P. leniusculus possess an significantly lower dispersal ability upstream when compared to downstream dispersal, potentially due to the influence of flow rate and gradient (Bubb et al., 2004). Sufficiently fast outflow allows the fitting of aquatic barriers to help prevent colonisation (Frings et al., 2013) whereas they cannot be effective in watercourses exhibiting inflow.

While P. leniusculus is the most commonly-attributed vector of the plague, non-isolated sites may be susceptible to other migratory crustaceans such as the invasive Chinese Mitten Crab (Eriocheir sinensis) whom also function as a vector of A. astaci (Schrimpf, 2014). Originating from marine larvae (Kobayashi and Matsuura, 1995), juveniles may migrate hundreds of kilometres up/downstream when entering freshwater systems (Herborg et al., 2003, Dan et al,. 1984 - Cited in Rudnick et al,. 2000). With annual average range expansions of up to 562 km^{year}, E. sinensis exhibit significantly further and faster transmission potential than P. leniusculus (Herborg et al., 2003; Bubb et al., 2004; Dittel and Epifanio, 2009; Svoboda et al., 2017). Fish are also capable of dispersing viable A. astaci forms, surviving passage through the gastrointestinal tract post ingestion (Oidtmann et al., 2002). This highlights the multiple manners in which transmission of the crayfish plaque can occur between systems. When evaluating the safety of a non-isolated site, all possible avenues in which the plaque could be introduced need to be evaluated. For example, freshwater systems connected to estuaries may become colonised from migrating E. sinensis (Herborg et al., 2003; Rudnick et al., 2005). Such systems may be at risk from plague transmission, especially in areas of sympatricity with P. leniusculus where the chances of transmission to migrating crabs may increase.

3.2.2 Terrestrial barriers

Invasive crayfish colonise new waterbodies via means of both active (terrestrial traversal) and passive dispersal (Lipták *et al.*, 2016). Typically, multiple individuals would need to successfully traverse overland, simultaneously avoiding desiccation and predation to successfully colonise a new waterbody (Thomas *et al.*, 2018). However, even singular *P. leniusculus* individuals that successfully traverse a terrestrial barrier to a new waterbody may endanger native crayfish populations.

The crayfish plague can be introduced from actively migrating crayfish, passive dispersal during flooding events or dispersal by waterbirds (Peay *et*

al., 2010; Águas *et al.*, 2014). Potential ark sites should be sufficiently isolated to minimise the risk of colonisation by *P. leniusculus* (Peay, 2009) particularly when barriers are separating sites from waterbodies that are either colonised, or at immediate risk from colonisation. The required level of isolation increases depending on the risk of colonisation via dispersal, affected by proximity to colonised waterbodies and the tendency/magnitude to which barriers may flood (Peay, 2009).

Delineating an appropriate size for terrestrial is difficult as only a few studies have investigated the capabilities of *P. leniusculus* for terrestrial dispersal (Marques *et al.*, 2015; Thomas *et al.*, 2018, 2019). Their ability to successfully traverse an terrestrial barrier will be limited by the risk of being predated upon or becoming desiccated during traversal (Thomas *et al.*, 2018). While avoiding predation is likely random chance, their tolerance to desiccation will vary based on climatic factors including humidity and temperature (Banha and Anastácio, 2014). Humidity and temperature becomes more favourable to *P. leniusculus* (higher and lower respectively) during the night when individuals are most active (Thomas *et al.*, 2016), benefiting from favourable conditions that decrease the chances of desiccation during their most active hours, when the tendency to migrate may be most likely.

Lethal time 90 (LT90 – time taken for 90% mortality of the test population) via desiccation for *P. leniusculus* — controlled conditions of 24°C and a Relative Humidity of 50% — is achieved at 21.5 hours, with first mortality occurring at 9 hours (Banha and Anastácio, 2014). 24°C was chosen to represent the maximal range of annual average maximum temperatures experienced on the Iberian Peninsula (Banha and Anastácio, 2014). However, significant proportions of *Austropotamobius* populations exist north of the Iberian peninsula (Kouba *et al.*, 2014) and will be exposed to lower average temperatures, indicating mortalities may occur later in-situ within the more northernly regions of their range.

Walking speed for *P. leniusculus* is reported to be approximately 0.6 ± 0.28 cm/s (n = 6, Thomas *et al.*, 2019), equating to 21.6m hour⁻¹. Utilising an LT90 of 21.5 hours (Banha and Anastácio, 2014) *P. leniusculus* could achieve an theoretical travel distance of 0.47km before their reported LT90. Based on the assumptions that they travelled at a constant speed in one direction (Banha and Anastácio, 2014). However, such assumptions are subject to limitations:

- Low sample size was used to calculate walking speed (n = 6)
- Walking speed and time to desiccation is positively correlated with size (in *Faxonius rusticus*) and will increase with increasing age or size of an individual (Claussen *et al.*, 2000)
- Positive correlation between carapace length and terrestrial migration tendency (Thomas *et al.*, 2018), thus larger individuals are more likely to migrate and possess greater dispersal capabilities
- Different climatic conditions experienced across the range of *A.* pallipes will incite different desiccation rates and thus different LT90's to those reported in Banha and Anastácio (2014)
- Increasing severity of desiccation reduces locomotive ability in crustaceans (Claussen *et al.*, 2000) thus the total distance achieved likely cannot be calculated via a linear approach.

There is no evidence *P. leniusculus* actively seek new waterbodies for colonisation. Marques *et al.* (2015) dehydrated test individuals for half an hour to ensure they would actively seek water to avoid desiccating, however when placed near a waterbody (~5m away), could not orientate themselves towards it. Thus active dispersal over 100 metres seems improbable if the barrier is sufficiently dry and segmented (Peay, 2009). Exposed barriers that offer little shelter may exhibit slightly higher temperatures and increased light intensities which *P. leniusculus* seem adverse to (Marques *et al.*, 2015; Thomas *et al.*, 2016), potentially helping to `steer' migrating crayfish away. Conversely, barriers prone to flooding are at greater risk of being actively or passively traversed, increasing

nearby waterbodies susceptibility to colonisation (Lipták *et al.*, 2016). Floods can carry invaders into new waterbodies/systems (Lipták *et al.*, 2016) or provide a partly flooded environment (Peay, 2009) that is wet enough to prevent desiccation during traversal. As such, terrestrial barriers that are prone to flooding are likely to aid the terrestrial dispersal of *P. leniusculus* to uninvaded waterbodies and should be ruled out prior to site selection. Due to the relatively understudied and understood tendencies of terrestrial dispersal in *P. leniusculus*, it is difficult to suggest an appropriate terrestrial barrier size. However, it's a reasonable assumption that with increasing size the chance of successful traversal to another waterbody will decrease (Peay, 2009).

3.2.3 Flow Velocity

It's been suggested that flow velocity is an important factor in influencing crayfish colonisation potential and distribution (Light, 2003; Bubb *et al.*, 2004; Kerby *et al.*, 2005; Pintor and Sih, 2011; Wendler *et al.*, 2015; Rosewarne *et al.*, 2017), with flow velocities of 0.6 and 0.65m/s having been suggested as the potential threshold for preventing colonisation in *A. pallipes* and *P. leniusculus* respectively (Frings *et al.*, 2013; Wendler *et al.*, 2015). Temporal lows in flow velocity have been suggested to greatly increase the chance of colonisation by *P. leniusculus* (Mathers *et al.*, 2020). With 80% of recorded invasions occurring during below average flow magnitudes years (n = 64), whereas only 1.6% occurred during high flow years (Mathers *et al.*, 2020).

Flow rate also plays an important role in ensuring aquatic barriers can effectively prevent breaching. Frings *et al.* (2013) reports that the chance of an adult crayfish breaching a constructed aquatic barrier in flume trials (25° slope and a flow velocity of >0.65 m/s) is approximately 1:100,000. Although, traditional barriers do not seem to be as effective at preventing upstream colonisation (Krieg and Zenker., 2020). Weir presence can reduce passage frequency by 45% compared to its absence, but did not prevent breaching (Rosewarne *et al.*, 2013). However, flow rates were far below

those necessary to prevent barrier breaching in Frings *et al.* (2013), with flow rates not exceeding 5.53 m³/s (over 17 months) and the observed average remaining drastically lower (3.24 ± 2.66 , Rosewarne *et al.*, 2013). Based on the downward gradient of the weir face, flow velocities > 0.7 m/s would be required to render the weir 'impassable' according to Frings *et al.* (2013) study (Rosewarne *et al.*, 2013). The evidence suggests flow velocity plays a vital role in determining the success of excluding *P. leniusculus* from uncolonized waterbodies.

3.2.4 Aquatic Barriers

Aquatic barriers including dams, weirs, waterfalls, fish-passable barriers, electric fencing and culverts can help prevent the upstream colonisation of invasive crayfish (Renz and Breithaupt, 2000; Rahel, 2007; Frings *et al.*, 2013; Rosewarne *et al.*, 2013; Benejam *et al.*, 2015; Manenti, 2016). Reports exist of *P. leniusculus* having breached a road-crossing culvert 69cm high (Ikeda *et al.*, 2019) which demonstrates the need of barrier to possess significantly favourable characteristics that encourage exclusion. It's been recommend that artificially constructed barriers possess; high and smooth walls with no cracks/seams for climbing purposes, wall extensions on either side of a barrier to prevent circumnavigation and a overhang comprised of continuous stainless steel that overhangs a minimum free fall of water two crayfish lengths in height (Krieg and Zenker, 2020). Such barriers are likely to provide the highest level of security, but may become problematic for migratory fish species (Wofford, *et al.*, 2005; Sheer and Steel, 2006).

Ideally, aquatic barriers should possess smooth slopes >25° and outflow velocity >0.65m/s as its a fair assumption that with increasing barrier slope & flow velocity the chance of a crayfish breaching will reduce (Frings *et al.*, 2013) Such safety margins may be especially important in-situ, as crayfish deploy swimming behaviour to overcome barriers in response to multiple failed attempts, behaviour that could not be initiated via anterior visual stimulus (Frings *et al.*, 2013). While resorting to swimming reduced

successful breaches from 44% (successful breaches via walking) to 16%, distances of at least 35cm were achieved which may help breaching in sufficiently low flow velocities (Frings *et al.*, 2013). However, the study only applies to experimental conditions. In-situ crayfish have an indefinite amount of time to attempt crossing (Frings *et al.*, 2013) and can terrestrially circumnavigate it, behaviour that's been observed in the Louisiana Crawfish (*Procambarus clarkii*) as a means to overcome obstacles (Kerby *et al.*, 2005).

As such, ark sites with outflows should have a year-round outflow velocity of >0.65 m/s and should be fitted with an appropriate aquatic barrier, characterised by >25° slope and an smooth surface (Frings *et al.*, 2013). However, the practicalities of in-situ conservation may render this a difficult assumption to meet. Flow rates are likely to show significant temporal variation, despite river flow rates in the UK being less influenced by seasonal contrasts (in rainfall & melt-water contributions) relative to most of the world (National River Flow Archive, 2015). Temporary drops in flow velocity may be combatted by electric barriers, which have demonstrated an aptitude for preventing upstream progression of invasive crayfish (Benejam *et al.*, 2015). Although such measures should only be used if invasive crayfish are at immediate risk of colonisation as practical challenges (including electricity supply/cost and the hazard they pose to humans and animals) limit the usefulness of electric barriers as long term solutions (Benejam *et al.*, 2015).

3.3 Further discussion points

3.3.1 Synergistic effects

Increasing amounts of literature are relating *A. pallipes* populations to moderately polluted waters (Trouilhé *et al.*, 2007; Haddaway *et al.*, 2015), bringing their use as 'bioindicators' of good water quality into debate (Fureder and Reynolds, 2003; Trouilhé *et al.*, 2007). However, *A. pallipes* do not possess the ability to terrestrial disperse away from unfavourable

conditions and are highly reliant on the waterbodies they inhabit (Fureder and Reynolds, 2003). As such they are subject to any alterations in habitat or environmental chemistry that may have fitness consequences, such as increased pollutant concentrations (Firkins, 1993; Benítez-Mora *et al.*, 2014). Crayfish of the *Austropotamobius* complex have been deemed tolerant of environmental stressors for short periods of time within laboratory settings (Demers *et al.*, 2006; Benítez-Mora *et al.*, 2014). Although observations indicate that wild populations persist up to certain thresholds, that when exceeded, cause rapid population decline/loss (Fureder and Reynolds, 2003; Lyons and Kelly-Quinn, 2003). Controlled systems have demonstrated that a single pollution event can result in complete mortality of *A. pallipes* populations (Firkins, 1993). Illustrating the consequences of not being able to migrate from adverse conditions, a trait that terrestrial animals, birds or other benthic macroinvertebrates (with ariel adult stages) possess (Neveu, 2009).

Studies typically investigate the effect of individual stressors on *A. pallipes* (Demers *et al.*, 2006; Haddaway *et al.*, 2013; Benítez-Mora *et al.*, 2014) so may not reflect true tolerances of in-situ populations, in part because of the disparity between delineated tolerance thresholds in laboratory and field conditions (De Lisle and Roberts, 1986; Melvin and Houlahan, 2012; Brady *et al.*, 2013; Tagliarolo and McQuaid, 2016). In-situ populations are exposed to a suite of chemicals which may have negative synergistic effects on individual/population fitness (Haddaway *et al.*, 2015), so said studies may not be able to fully evaluate the compounding effects of different environmental stressors upon *A. pallipes* populations. Thus, care needs to be taken in assumptions of tolerance based on laboratory settings and/or effect of individual stressors. Not critically evaluating the limitations of such studies may help explain the mediocre success rate (44%) experienced from establishing ark sites (Souty-Grosset and Reynolds, 2009).

3.3.2 Aerobic Scope

Species of the Austropotamobius genus are relatively tolerant of hypoxic conditions (Demers et al., 2006), however their ability to access sufficient oxygen is impeded by elevated levels of other factors including temperature and nitrates/TSS concentrations (Camargo et al., 2005; Rosewarne et al., 2014). Nitrate toxicity to freshwater invertebrates is attributed to the conversion of oxygen carrying pigments to forms that cannot carry oxygen (Camargo et al., 2005; Benítez-Mora et al., 2014), becoming increasingly toxic with increasing concentrations and exposure times (Tsai and Chen, 2002; Alonso and Camargo, 2003). Thus, the toxic effects of elevated nitrates likely become exacerbated in conditions of low oxygen availability. While A.italicus are reportedly tolerant of elevated nitrate concentrations, mean DO concentrations were 10.1 mg.L⁻¹ for the study duration (Benítez-Mora et al., 2014). Therefore, it is unlikely that DO would be a limiting factor to crayfish survival, potentially indicating a higher tolerance of nitrates than is realistic in-situ. In-situ populations can become exposed to temperature variations, high concentrations of TSS and anthropogenic inorganic nitrogen inputs which may occur simultaneously with temporal decreased oxygen availability (temporal oxygen sags, increased seasonal temperatures, low flow regimes). Consistent ambient pollution (e.g. surface run off upstream) and extended temperature extremes (e.g. Summer/Southernly Range of *A. pallipes*) may also be present, exposing populations to indefinite hypoxia related stressors. The tolerance of A. italicus to hypoxic stress may not be representative of in-situ Austropotamobius populations. Such results still provide useful information that can be used to help evaluate the suitability of sites. Conservationists should understand the limitations of such studies and be aware of potential negative synergistic interactions, allowing them to critically evaluate potential sites in a multi-dimensional manner.

3.3.3 Ecdysis

Low pH conditions and insufficient ambient calcium impede the ecdysis process (Rukke, 2002; Hammond et al., 2006; Beaune et al., 2018). Unsuitable concentrations of both initiate negative synergism that has been suggested as the cause of population decline in multiple Cambarus bartonii populations (Hadley et al., 2015). Despite pH concentrations normalising, it was suggested that insufficient ambient calcium prevented subsequent population recovery (Hadley et al., 2015). Juveniles are likely to be most affected by such synergism because they moult more frequently than mature individuals (Franke and Hoerstgen-Schwark, 2013) and are more susceptible to environmental stressors (Distefano et al., 1991; Hartman et al., 2010). Such synergism has the potential to supress population recruitment, as individuals become increasingly tolerant of pH with age (France, 1984). Similarly, individuals are likely to become less susceptible to calcium deficiencies with increasing age due to the decrease in moulting frequency. Mature individuals may subsist off concentrations that may not be adequate for juvenile ecdysis, creating a developmental 'calcium bottleneck' (Hessen et al., 2000). Whilst the term has been mainly used in regard to smaller crustaceans (Hessen et al., 2000; Rukke, 2002), pH and calcium can limit juvenile crayfish survival (France, 1984; Hammond et al., 2006). Emphasising the need to incorporate tolerances for species at all life stages to ensure steady and successful recruitment (Rukke, 2002), which should be considered carefully when evaluating ark sites suitability.

3.3.4 Juvenile Recruitment

Crayfish are particularly susceptible to mortalities during the early stages of life (González *et al.*, 2010), they are threatened by; cannibalism, exclusion from refugia, predation, more frequent moulting periods, and alteration of behaviour from both mature crayfish and non-predatory fish (Brewis and Bowler, 1982; Ranta and Lindström, 1992; Blake *et al.*, 1994; Blake and Hart, 1995). Sufficient recruitment rates are essential to ensuring proper population structure. There is a risk of creating a population with high adult density and low recruitment rates because of poor juvenile survival. This would lead to an mature dominant age class, which can further supress recruitment through consumption of juveniles or eggs (Polis, 1981), thus further increasing the chance of an unsustainable population structure developing.

Suitable pH in potential ark sites will be key to ensuring proper establishment of a translocated population and needs to be evaluated in conjunction with calcium concentrations. Haddaway *et al.* (2013) investigated the effect of pH on juvenile *A. pallipes* survival and growth rate, finding that survival was highest at pH 8.6 (94%) but became significantly compromised at pH 7.1 (34%). However calcium may have become a limiting factor as it was not controlled for, despite influencing crayfish survival (Hammond *et al.*, 2006; Hadley *et al.*, 2015). As such the observed survival rates may not be reflective of those observed in-situ. Although, the study does illustrate that increasing pH initiates higher proportional juvenile survival (Haddaway *et al.*, 2013). Indicating that translocated populations will experience superior recruitment in higher pH conditions compared to lower pH waterbodies with other factors being equal.

Potential synergistic interactions should be carefully evaluated to maximise establishment success. Both physical and physio-chemical properties of a waterbody need to be evaluated together, as insufficiencies in one area can be exacerbated by another. For example, the negative effects experienced by sub-circumneutral pH can be combatted or magnified through habitat complexity. High habitat complexity increases refugia density, which would encourage juvenile survival and continuous recruitment (Olsson and Nyström, 2009). Disputes over shelter is the main cause of agonistic intraspecific interactions (Cioni and Gherardi, 2004), with the losers of such interactions likely being smaller and less dominant individual (Vorburger and Ribi, 1999). Those crayfish excluded from refugia remain exposed and become more vulnerable to predation. Post-moult crayfish are likely to be even more vulnerable, being particularly susceptible to cannibalism (Holdich *et al.*, 1978) and the preferential prey of various fish species (Stein, 1977). Insufficient pH will increase the duration spent between post-moult and intermoult as it reduces many of the mechanisms underpinning ecdysis (Haddaway *et al.*, 2013; Beaune *et al.*, 2018), resulting in a dual vulnerability to inter- and intraspecific interactions. Excess refugia increases the likelihood that post-moult individuals find refuge during the period they are most vulnerable, especially relevant for post-moult juveniles.

4.0 Conclusions

This review offers the most comprehensive understanding of the physiochemical parameters associated with waterbodies inhabited by A. pallipes populations available in literature. Relating in-situ populations to thresholds established from field and laboratory observations helps increase our understanding of how appropriate physio-chemical parameters can maximise the chances of successfully establishing ark sites. This review offers target guidelines for water chemistry that indicate optimal ark sites to maximise population fitness and recruitment; a discussion of the role of physical habitat features in reducing the colonisation potential of invasive crayfish and further discussion of the potential for synergistic effects to compromise the successful establishment of translocated populations. It is vital that potential ark sites should ensure that water chemistry is suitable for A. pallipes juveniles to maximise recruitment potential. Key variables to success likely being sufficient calcium concentrations and high pH to minimise negative impacts on ecdysis. Complemented by sufficiently complex habitat to minimise there vulnerability to inter- and intraspecific agonistic encounters.

Successful ark sites are likely underpinned by suitability in three key area's; water chemistry, habitat structure (see Rosewarne *et al*, 2017) and comprehensive planning and assessment. All three of which must be underpinned by proper protective measures against the spread of invasive

crayfish and the plague. It is suggested that previously failed ark sites could be explained by failure to properly accommodate one or more of these areas. Proper planning considers long term suitability (e.g. year-round water sampling) and local knowledge (e.g. flooding frequency) to assess suitability. Upon meeting such assumptions, ark sites offer to be a promising conservation strategy for ensuring long term persistence of *A. pallipes* in the UK.

The efforts of future research may be best aimed at understanding and controlling the dispersal potential of *P. leniusculus*. Their unprecedented rate of spread and wide tolerance to a wide variety of environmental stressors has facilitated their invasion of much of the UK, forcing the need for conservation-based methods. Protecting isolated populations represents an 'band-aid' solution, ensuring population persistence for future restocking potential and genetic diversity. However, it seems unlikely *A. pallipes* will be widespread in UK rivers until effective control methods have been developed.

Appendices

Appendix 1

Probe Model	Variable	Reagent	Accuracy±
HI-98129	рН	-	0.05 pH
HI-98129	Conductivity	-	2 F.S.
HI-713	Phosphate	HI-713-25	0.04ppb
HI-707	Nitrite	HI-707-25	20 ppb
HI-700	Ammonia	HI-700-25	0.05ppm

Appendix 2

Population Ref	Site location	Latitude	Longitude
1	River Candover, New Arelesford	51.087736	-1.184072
2	Hunkin Wood, River Culm	50.914317	-3.305459
3	Culmstock, River Culm	50.914537	-3.3291291
4	Hemyock, River Culm	50.917696	-3.247822

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