SIZE-MEDIATED, DENSITY-DEPENDENT CANNIBALISM IN THE SIGNAL CRAYFISH *PACIFASTACUS LENIUSCULUS* (DANA, 1852) (DECAPODA, ASTACIDEA), AN INVASIVE CRAYFISH IN BRITAIN

BY

R. J. HOUGHTON[1]), C. WOOD and X. LAMBIN

School of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen, AB24 2TZ, U.K.

ABSTRACT

The role of cannibalism in crayfish populations is not well understood, despite being a potentially key density-dependent process underpinning population dynamics. We studied the incidence of cannibalism in an introduced signal crayfish *Pacifastacus leniusculus* population in a Scottish lowland river in September 2014. Animals were sampled using six different sampling techniques simultaneously, revealing variable densities and size distributions across the site. Cannibalism prevalence was estimated by analysing the gut contents of crayfish >20 mm CL for the presence of crayfish fragments, which was found to be 20% of dissected individuals. When seeking evidence of relationships between the sizes of cannibals and 'prey', the density of conspecifics <56% the size of a dissected individual yielded the best fit. The relationship between cannibalism probability and crayfish size and density was equally well described by three different metrics of crayfish density. Cannibalism increased with crayfish size and density but did not vary according to sex. These results suggest that large *P. leniusculus* frequently cannibalize smaller (prey) conspecifics, and that the probability of cannibalism is dependent upon the relative size of cannibal-to-prey and the density of the smaller crayfish. We suggest that removing large individuals, as targeted by many traditional removal techniques, may lead to reduced cannibalism and therefore a compensatory increase in juvenile survival.

[1]) Corresponding author; e-mail: rupert.houghton@abdn.ac.uk

of a dissected individual yielded the best fit. The relationship between cannibalism probability and crayfish size and density was equally well described by three different metrics of crayfish density. Cannibalism increased with crayfish size and density but did not vary according to sex. These results suggest that large *P. leniusculus* frequently cannibalize smaller (prey) conspecifics, and that the probability of cannibalism is dependent upon the relative size of cannibal-to-prey and the density of the smaller crayfish. We suggest that removing large individuals, as targeted by many traditional removal techniques, may lead to reduced cannibalism and therefore a compensatory increase in juvenile survival.

INTRODUCTION

Density-dependent processes play crucial roles in the population dynamics of many species (Vollestad & Jonsson, 1988; Hixon & Carr, 1997). The strength, shape and target of the density-dependence determines the extent to which mortality through harvest or culling is additive or compensatory to other causes of mortality. Knowledge of density-dependence is therefore essential both for predicting fisheries yields (Gardmark et al., 2006) and making informed and effective wildlife management decisions (Guthery & Shaw, 2013).

Cannibalism is common in populations that are structured by age or size and is a classical process responsible for negative density-dependence (Post et al., 1999) that may contribute to the regulation of populations (Polis, 1981). Typically, larger conspecifics prey upon the smaller, more vulnerable age classes (e.g., Lovrich & Sainte-Marie, 1997; Baras & Jobling, 2002; Persson et al., 2004). The size difference between cannibals and their conspecific prey define predator-to-prey size ratios within which cannibalism occurs (Polis, 1981). In some fish species, gape-size constrains the size of conspecifics that can be ingested and defines the cannibalism predator-to-prey size ratio (Baras & Jobling, 2002). However not all cannibal species have morphological features that can predict predator-prey size ratio so reliably. Crustacean claws, for example, are multi-functional organs and therefore their size may not be useful indicators of cannibalistic tendencies (Lee, 1995). Furthermore, crustaceans may be vulnerable to cannibalism irrespective of their size when they moult (Farhadi et al., 2014).

If cannibalism probability increases with density of predatory classes, then a range of complex destabilising population consequences can occur whereas if cannibalism probability increases with the density of prey classes, this is a compensatory, stabilising influence on population dynamics, as any increase in juvenile density will be compensated for by adult cannibalism (Moksnes, 2004). Predicting the impact of management interventions that disproportionly affect different classes with regards to population dynamics, thus requires knowledge of the nature of density-dependence that arises from cannibalism (Zipkin et al., 2009).

Non-native invasive crayfish species (NICS) are impacting freshwater ecosystems globally and considerable research seeks to establish management protocols

to control them (Freeman et al., 2010; Twardochleb et al., 2013). Management of NICS populations is difficult because widely applied removal techniques such as trapping, disproportionately affect classes composed of large individuals, which constitute only a small fraction of the population (Rabeni et al., 1997). While it is expected that density-dependent compensatory processes contribute to the resilience of NICS to size-selective harvest, the contribution of cannibalism is not well understood. Baited traps have also been reported to exhibit a bias towards catching males (Price & Welch, 2009). If cannibalism rates vary between the sexes, differentially removing either sex through such management interventions could influence cannibalism rates and impact population dynamics.

Despite claims in the literature that crayfish generally exhibit size-mediated, density-dependent cannibalism (Abrahamsson, 1966; Jones & Coulson, 2006; Sadykova et al., 2009), empirical evidence is scant, making even qualitative predictions about the response of crayfish populations to different management strategies difficult. Guan and Wiles (1998) found that cannibalism incidence increased with crayfish size (as measured by carapace length, CL), with the stomachs of small (20-33 mm), medium (33-45 mm) and large (>45 mm) crayfish containing 2, 6 and 38%, respectively, in a lowland river *Pacifastacus leniusculus* (Dana, 1852) population. Using similar methods, Alcorlo et al. (2004), observed higher frequency of cannibalism in *Procambarus clarkii* (Girard, 1852) in rice fields when compared to natural marshland and suggested this could be due to higher observed crayfish densities in the rice fields. Intra-class density-dependence in survival of juvenile crayfish is routinely observed in aquaculture research and often attributed to cannibalism, as the behaviour can occasionally be observed (e.g., Savolainen et al., 2004; Ulikowski et al., 2006; Farhadi & Jensen, 2015). However, given the artificially high densities and low habitat complexity in aquaculture, the relevance of inference on cannibalism rates from such studies to wild populations is uncertain. Habitat features such as substrate composition and water depth may play an important role in cannibalism as they are key drivers of crayfish size distribution and density in streams (Wooster et al., 2012).

One particularly problematic NICS is the North American signal crayfish, *P. leniusculus*, that is now common throughout Europe. Baited traps are a frequently employed control method for such NICS, a technique known for being biased towards catching large males (Somers & Stechey, 1986; Distefano et al., 2003; Price & Welch, 2009). If management practices differentially remove cannibalistic demographic classes from a population, then this could lead to full compensation. Thus this study aimed to investigate the prevalence of cannibalism in a feral *P. leniusculus* population and establish if the sex or size of an individual influenced the probability of cannibalism. Additionally we evaluated whether cannibalism was density-dependent and tested whether a predator-to-prey size ratio within which cannibalism occurs could be identified.

MATERIAL AND METHODS

Study site

The study was conducted in the Geddes burn, a lowland tributary of the River Nairn, Scotland, that flows through a mixture of arable and pastoral farmland with occasional woody margins. The habitat predominantly consists of shallow run with a low surface flow rate and infrequent pools but a few short lengths of riffle. Substrate composition was estimated by eye over every four metre length of stream and was dominated by silt but gravel and cobble occurrence increased at the upstream end. Stream width and midstream depth were measured every four metres. The mean midstream depth and width were 24.2 cm (SD = 8.8) and 185.1 cm (SD = 46.3), respectively, but mean section depth decreased steadily from 26.8 (SD = 7.7) in section A to 18.1 cm (SD = 7.0). It was assumed that environmental conditions would remain constant over the short sampling period, therefore water temperatures were taken daily in three locations: above, in the middle of and below the sampling site. Signal crayfish are the only crayfish species present, distributed in approximately 9 km of watercourse (Sinclair, 2010).

Sampling protocol

One kilometre of the Geddes burn (downstream grid reference NH 87720 53393 and upstream NH 88371 52982) was divided into eight 120-m sections (A-H) and each section was further divided into six 20-m sub-sections. Each sub-section was randomly allocated one of six different sampling techniques, such that each technique was replicated eight times. The six sampling techniques were: (i) baited minnow traps (as used in Acosta & Perry, 2000), (ii) baited cylindrical net traps (as used in Policar & Kozák, 2005), (iii) refuge traps (similar to those used in Dana et al., 2010), (iv) microhabitat traps (as used in Fjälling, 2011), (v) electro-fishing (backpack direct current continuous 100-200 V, similar to that used in Price & Welch, 2009) and (vi) kick sampling (as used in Gladman et al., 2010). These sampling techniques were known to have different size biases (Rabeni et al., 1997; Dorn et al., 2005; Price & Welch, 2009), such that the mixture of techniques ought to sample a wide size range of crayfish. Effort was standardised in each section: for all trap types, ten traps were placed evenly along the 20-m stream length at sufficient depth to allow crayfish access; for electrofishing and kick sampling, a constant number of anode sweeps or kicks respectively were employed every 4 m with the same number of samplers over a constant distance. Each section was sampled with the same set of randomly allocated techniques and we assumed the numbers of crayfish caught in each section are equally representative of prevailing abundances and size distributions. Mortality and movement were

assumed negligible over the short sampling period (18-21 September 2014) and therefore the population density was considered constant. Therefore, the total catch number per section was used as an approximation for section-level population density ($n/120$ m, henceforth referred to as total crayfish density). Once captured, sampled crayfish were stored on ice in a cool box prior to freezing. As part of another study, crayfish in this stream length were sampled with the same protocol for eight days prior to the sampling period of the present study, but were marked (sub-section specific uropod puncture combinations) and replaced. Locations of recaptured individuals will validate the assumption that movement was negligible.

Every individual of *Pacifastacus leniusculus* was sexed and its CL measured from the rostrum tip to the terminal thorax portion with digital Vernier callipers to 0.01 mm. For analysis, animals <30 mm CL were assigned to the "small size class" whilst animals >30 mm CL were assigned to the "large size class". This designation was used because 30 mm CL approximately bisects the range of sizes present in the population and also roughly coincides with the smallest maturing females, determined by the presence of glair glands. The frequency of animals in each size class was used as an approximation for density in each 120-m stream section (henceforth referred to as small crayfish density and large crayfish density respectively).

Analysis of gut contents

In order to detect instances of cannibalism, individuals >20 mm CL were dissected and their foreguts removed using the protocol of Guan & Wiles (1998). Smaller individuals were not considered because it was too difficult to reliably extract their digestive tracts. Once removed, foreguts were washed out with 70% ethanol. The contents were inspected under a binocular light microscope ($20\times$ magnification), and solid fragments were picked out with tweezers for further analysis. Once isolated, the solid fragments were assessed according to the following four questions to ascertain if they were indeed crayfish fragments: (1) Does the fragment stain with the use of Lugol's iodine, and does it stain to a similar degree as a known piece of crayfish exoskeleton? (2) Does the fragment show the rigidity and hardness of a crayfish exoskeleton? (3) Does the fragment show the layered cuticle structure of a crayfish exoskeleton? (4) Does the fragment show any discernible textures or structures that can identify it as a crayfish fragment? If the fragments could satisfy at least three out of four of the criteria, then the dissected individual was considered to have ingested crayfish hard parts and was deemed a recent cannibal.

Crayfish are known to eat their own exuviae after moulting in order to resorb calcium (Buřič et al., 2016) and this might lead to an incorrectly inflated estimate

of cannibalism rate. They may also prey upon recently moulted individuals, for which the detection of hardened carapace may be difficult. Field sampling was conducted at the end of the growth season, when moulting was infrequent. To quantify moulting incidence, prior to freezing, every individual was categorised as being pre-moult, moulting, post-moult or inter-moult using the method outlined by Scalici & Gibertini (2009).

Statistical analysis

In order to establish whether a predator-to-prey size ratio can predict the prevalence of cannibalism, we used Generalised Linear Mixed Models (GLMM) with the presence/absence of fragments of exoskeleton in the gut of dissected crayfish as the binary response variable and the density of crayfish in a section that were $<X\%$ of the size of the focal individual as the explanatory variable ('smaller prey density'). We evaluated multiple models with the value of X varying incrementally between $<30\%$ and $<100\%$ in order to establish what value yielded the best fitting model using a profile likelihood procedure. The lower size threshold of 30% was chosen because there were few individuals between 0-30% the size of the potential cannibals. Cannibalism incidence was further modelled as a function of sex, CL, total crayfish density, small crayfish density, large crayfish density and smaller prey density (using the X value defined as above) using GLMM. Both ln-transformed and untransformed crayfish densities were evaluated. Candidate covariates were retained using backward model selection based on the model Akaike Information Criterion (AIC). Akaike model weights were calculated following the methods of Wagenmakers & Farrell (2004). In all models, the section of origin of focal individuals was fitted as a random effect in order to account for the common environmental variation within section. Models were fitted using the 'lme4' package (Bates et al., 2014) in R 3.0.2 (R Core Team, 2013). The proportions of marginal and conditional variance explained by models were estimated using the 'MuMIn' package (Barton, 2014).

RESULTS

A total of 322 individual *Pacifastacus leniusculus* were caught in the eight sections, of which 95 were female, 98 were male and 129 were considered too small to reliably sex (and too small to dissect). The mean carapace length for the catch was 21.62 mm (SE 0.62). Individuals <16 mm CL dominated the size distribution, accounting for 40% of all crayfish (fig. 1A). The smallest female exhibiting glair glands was 33.99 mm CL. The density of small (<30 mm CL) but not large individuals increased along the stream from the downstream (A)
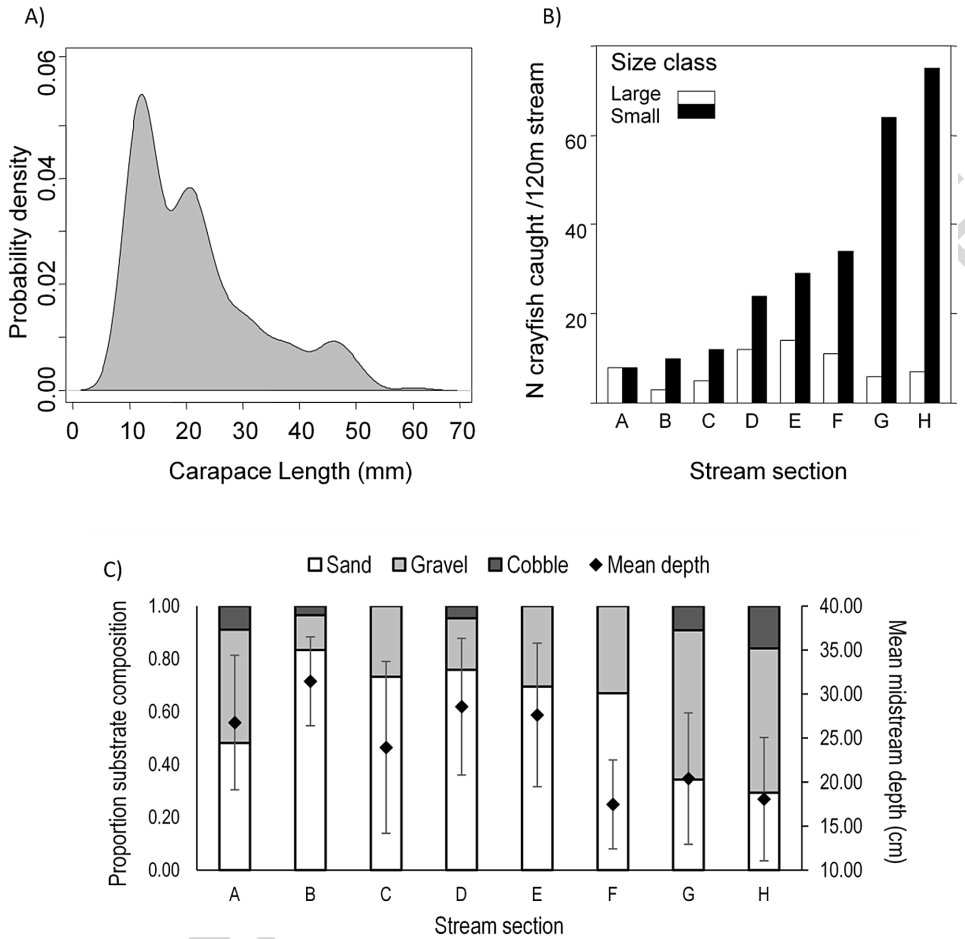
Fig. 1. A, size frequency distribution of carapace length (mm) for all crayfish captured from the Geddes Burn site; B, the catch number of small (<30 mm CL) and large (>30 mm CL) crayfish from downstream (section A) to upstream (section H); C, the proportion of substrate composition and mean midstream depths of each section from A-H.

to upstream (H) section (fig. 1B). Microhabitat traps, electrofishing and kick sampling selected for small crayfish whilst minnow traps, cylindrical net traps and refuge traps selected for large crayfish. The catch numbers in each section (A-H) were positively correlated between the sampling methods biased towards small and large crayfish respectively (fig. A1 in Appendix A). Mean midstream depth decreased and proportion of gravel coverage increased in an upstream direction (fig. 1C). Mean site water temperature ranged from 12.3 to 13.0°C during the sampling period. Whilst temperature is an important determinant of crayfish catch size, it is unlikely to have significantly influenced catches over this range (Somers & Stechey, 1986). Of the 43 marked crayfish that were recaptured from
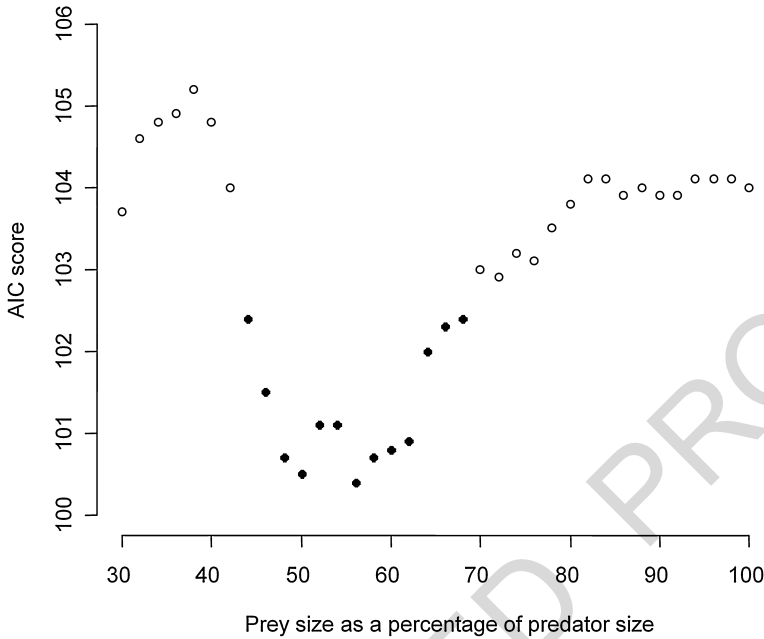
Fig. 2. Likelihood profile of prey density with varying predator to prey size relationships. The black plots indicate AIC scores within two units of the lowest AIC score.

the previous eight-day sampling period associated with another study, 41 (95.3%) were recaptured in the same sub-section in which they were originally caught. This suggests that crayfish movement between sub-sections was limited over the sampling period. The mean size of crayfish captured by all sampling methods increased with increasing water depth (fig. A2 in Appendix A). The sizes of the dissected crayfish ranged from 20.18 mm to 50.72 mm CL. Out of the 100 dissected crayfish, 21 contained potential crayfish fragments, of which 20 met at least three of the four cannibalism criteria previously outlined.

The size ratio between cannibals and the density of their putative prey that best predicted the probability of cannibalism was 56% (approximate 85%, CI 44-68%) of the size of the potentially cannibalistic dissected individual (fig. 2). The density of animals <56% the size of the dissected individual was therefore used to define the variable 'smaller prey density' used in subsequent model selection.

The most supported models consistently supported the notion that the probability of a crayfish having cannibalised a conspecific increased with density of conspecifics (table I: models 1-6; fig. 3A, C, E and F) and CL (table I: models 2-5; fig. 3B and D) but was not affected by sex. For example removing CL from model 2 substantially worsened the model parsimony (ΔAIC = 8.9). However the density of large crayfish did not have an effect on cannibalism probability as its removal consistently decreased the relevant models (models 2 + 4) AIC scores.

TABLE I

Comparison of the different competing models with their remaining significant factors displayed

Model name	Explanatory variable	Estimate	SE	z-value	p-value	Δ(AIC)	w(AIC)	Marginal R^2
Model 1	Log(smaller prey density)	1.34	0.48	2.78	<0.01	0.0	0.33	0.27
Model 2	Carapace length	0.10	0.03	3.05	<0.01	0.7	0.23	0.25
	Log(small crayfish density)	1.38	0.62	2.24	<0.05			
Model 3	Carapace length	0.10	0.03	2.97	<0.01	1.7	0.14	0.24
	Log(total crayfish density)	1.58	0.80	1.99	<0.05			
Model 4	Carapace length	0.10	0.03	3.05	<0.01	1.7	0.14	0.22
	Small crayfish density	0.04	0.02	2.09	<0.05			
Model 5	Carapace length	0.10	0.03	3.00	<0.01	2.1	0.12	0.21
	Total crayfish density	0.04	0.02	1.96	0.05			
Model 6	Smaller prey density	0.05	0.02	2.44	<0.05	4.7	0.03	0.13

Parameter estimates of logged variables have not been back transformed. Smaller prey density is defined as the number of crayfish in the same section as the dissected individual that are <56% the size of that individual. Small crayfish density is the number of crayfish in the section that are <30 mm CL, whereas total crayfish density is the total catch in the section.

The relationship between cannibalism probability and density were indeed broadly equally well described by five linear models (AIC scores separated by less than two units) with different density metrics as covariates (table I: models 1-5), each hinting at different but related biological process that might underpin the probability of crayfish cannibalism.

The model with log transformed prey density explained cannibalism incidence no better than that with log-transformed small crayfish density (table I: ΔAIC 0.7) but marginally better than the model using log transformed total crayfish density (table I: ΔAIC 1.7). The most supported model (1) had a 0.59 and 0.70 probability of being preferred over the next best models (2 + 3 respectively) with regard to the Kullback-Liebler discrepancy (Wagenmakers & Farrell, 2004). We suggest that density of smaller prey crayfish (i.e. crayfish 56% the size of the dissected crayfish) is therefore the most relevant descriptor of crayfish density with regards to cannibalism probability.

Comparing pairs of models with the same untransformed or log transformed density measures (table I: models 1 vs 6; 2 vs 4; 3 vs 5) also suggests that the true relationship is likely to be log-linear on the logit scale, even though model discrimination is constrained by the paucity of sections with very high conspecific density. Therefore the rate of increase in cannibalism probability declines as prey density becomes larger. For example at smaller prey densities, an increase from 20 to 30 smaller crayfish/120 m yields a predicted increase in cannibalism probability from 0.24 to 0.35, whereas an increase from 60 to 70 smaller crayfish/120 m is predicted to only increase cannibalism probability from 0.58 to 0.62 (fig. 3A). The
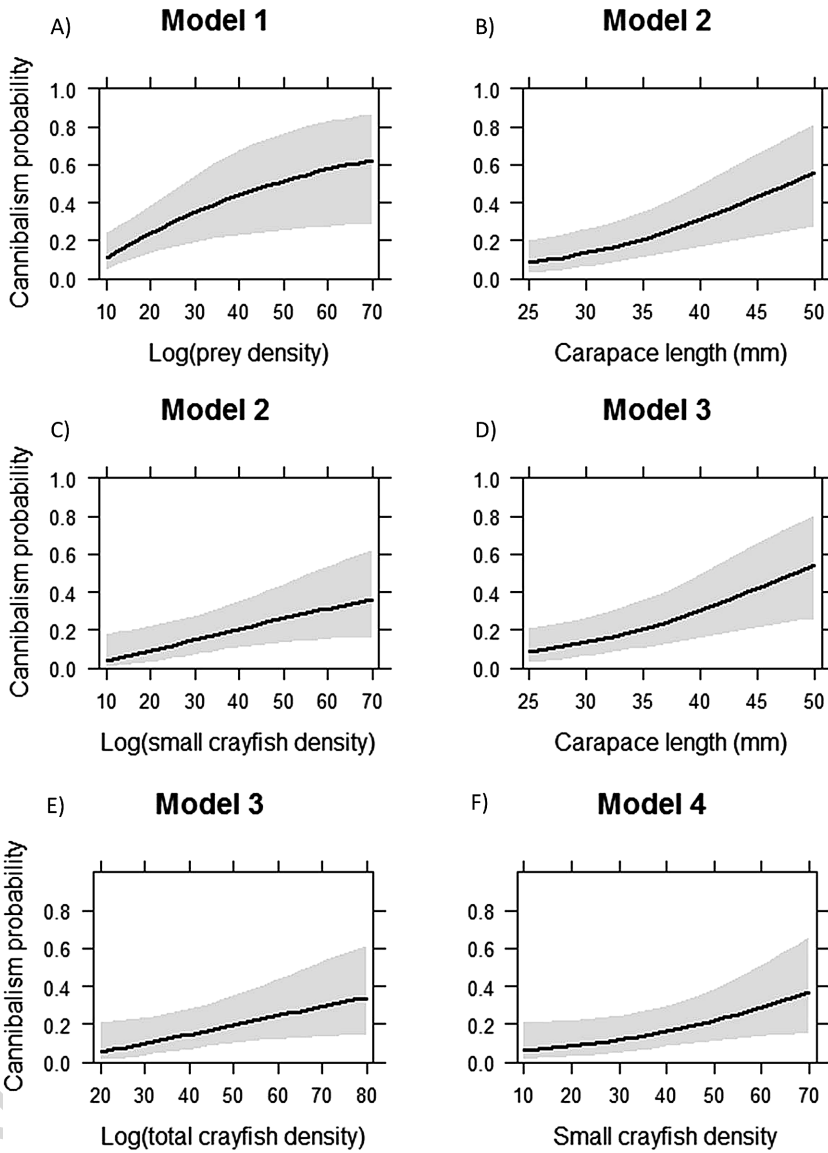
Fig. 3. A, the back-transformed effect of log(smaller prey density) on cannibalism probability taken from model 1; B, the effect of individual carapace length on cannibalism probability taken from model 2; C, the back-transformed effect of log(small crayfish density) on cannibalism probability taken from model 2; D, the effect of individual carapace length on cannibalism probability taken from model 3; E, the back-transformed effect of log(total crayfish density) on cannibalism probability taken from model 3; F, the effect of small crayfish density on cannibalism probability taken from model 4. The shaded area indicates 95% confidence intervals.

proportion variance explained by the random effect of section ranged from 7-9% of the total variance explained by models 1-5.

DISCUSSION

The observations made in this study suggest that the probability of an individual cannibalizing is not only affected by its size but also by the density of conspecifics within the immediate area. Furthermore, we found evidence that the density of crayfish that are less than 56% the size a potential predator to be the most relevant measure of density with respect to cannibalism probability.

Although there are issues with using crayfish trap catch numbers as a substitute for density (Acosta & Perry, 2000), the variation in catch across the stream sections was positively correlated between the different sampling methods (fig. A1A-F in Appendix A). We interpret the approximate agreement in catch number between sampling methods in different sections as validation that the total catch from the six methods in each section is a suitable approximation of crayfish density.

This study, as with previous studies (Guan & Wiles, 1998; Alcorlo et al., 2004), assumes that crayfish fragments observed in the guts of conspecifics are primarily due to cannibalism as opposed to scavenging dead individuals. Cannibalism events have been witnessed in aquaculture studies (e.g., Savolainen et al., 2004) and we therefore assume that the behaviour exists in wild population. It is not known, however, what proportion of carapace fragments in wild crayfish stomachs cannibalism will account for when compared to scavenging (Nyström, 2002). While it is conceivable that the size- and density-dependent nature of the observed crayfish fragments in stomachs could result in part from higher non-predatory mortality of smaller crayfish and, therefore, greater availability to larger, scavenging crayfish, it is more likely that scavenging would have masked rather than created the relationship we uncovered and we attribute to cannibalism. It has been argued in marine systems, that predation is the predominant source of natural mortality, leading to a low scavenging incidence (Britton & Morton, 1994). Further research will be required to fully validate observational evidence such as that presented in this study. False inference of cannibalism could also arise if fragments in the stomach were actually the individuals' previously moulted exoskeleton. In the present study only six percent of all sampled crayfish were soft and post-moult (i.e. recently moulted) based on the criteria of Scalici & Gibertini (2009). Accordingly, consumption of exuviae may lead to a small overestimation in overall cannibalism prevalence but is unlikely to create the relationship between density and cannibalism observed in the data (table BI in Appendix B).

Wutz & Geist (2013) found that habitat preference in *Pacifastacus leniusculus* was linked to individual size; smaller animals selecting shallower, gravel dominated habitat and larger animals selecting deeper water with substrate of a finer grain size. This was reflected in the variation of smaller crayfish density in the Geddes Burn and the mean size of crayfish being consistently smaller in shallower water regardless of the sampling method (fig. A2A-F in Appendix A). We interpret the conservation of this pattern across all sampling methods as evidence to suggest that each method provided a representative sample of the crayfish size distribution in the habitat. The observed increase in cannibalism in sections with many small crayfish may have been due to an effect of habitat as opposed to density. This seems unlikely given that large crayfish are known to avoid shallow water in order to minimise predation risk (Reynolds, 2011). Additionally, gravel substrate has been shown experimentally to increase the survival probability of small crayfish, in part because they can hide from potential cannibals (Savolainen et al., 2003). Therefore you might expect that any effect of habitat on cannibalism would mask the patterns observed.

Our findings are broadly consistent with previous studies with respect to cannibalism incidence and its relationship with crayfish size and sex. A total of 20% of crayfish >20 mm CL had cannibalised a conspecific in our lowland stream population of *P. leniusculus*. This is comparable to the 17-24% frequency of occurrence of conspecific fragments reported by Guan & Wiles (1998) in another British lowland river *P. leniusculus* population. In our study male and female crayfish had a similar propensity to engage in cannibalism, consistent with the findings of Gutierrez-Yurrita et al. (1998) in *Procambarus clarkii*. Probability of cannibalism increased with crayfish size. This has been observed in multiple studies (Capelli, 1980; Guan & Wiles, 1998; Bondar et al., 2005), although it is not always the case (Reynolds & O'Keeffe, 2005). The novel contribution of this study was to establish that the probability of cannibalism increased with crayfish density as well as to estimate the predator-prey size ratio in which cannibalism occurred. By iteratively varying the predator-prey size ratio, we identified a putative ratio that best predicted variation in cannibalism prevalence in crayfish of different sizes. The density of crayfish that were <56% the size of the potential cannibal was the best predictor of cannibalism. Our estimate of confidence intervals for the predator-prey size ratio encompasses 44-68% the size of any potential predator but our estimate only applies to cannibals >20 mm CL. While our inference is based on natural rather than experimentally induced variation in crayfish density within our free ranging population, it makes optimal use of both the gradient in crayfish density evident within the study site as well as size variation within sampling units. Cannibalism in *Orconectes propinquus* (Girard, 1852) was considered dependent on the density of juveniles because peak

cannibalism occurrence was observed shortly after the annual birth pulse (Capelli, 1980). Given the opportunistic foraging behaviour of crayfish (Rahel & Stein, 1988), larger crayfish might be expected to switch to a newly abundant food source which Capelli (1980) anecdotally validated by the frequent identification of young-of-year in crayfish stomachs after the birth pulse. Crucially, we believe the present study provides evidence describing the same phenomena but with prey density varying spatially rather than temporally, hence providing more compelling support for the invoked process.

There was evidence for a log-linear relationship on the logit scale, implying that the rate of increase in the probability of being a cannibal decreased at high density of potential prey. The scarcity of very large crayfish in stream sections of high densities of small prey precludes elucidating with any certainty the shape of the relationship, although clearly cannibalism probability increased steeply, from approximately 10 to 60%, over the observed range of variation in prey density.

It is generally expected that the impact of size- and density-dependent cannibalism will regulate recruitment and stabilise population dynamics (Moksnes, 2004), however population models incorporating our understanding of crayfish cannibalism will be required to formalise predictions. Therefore, quantification of the contribution that cannibalism makes to the mortality of individuals of different classes, as well as how this varies with density, is necessary. This consideration could be fundamental to future population control efforts. Our results would suggest that targeting large crayfish in a population may lead to a compensatory reduction in the mortality of small crayfish. Therefore, management interventions that target smaller crayfish or a wider range of size classes should be considered. For example, manipulation of crayfish catch size distribution was achieved by the modification of baited trap mesh size (as in Johnsen et al., 2014). Other removal methods should be considered such as netting, electrofishing (Price & Welch, 2009) and microhabitat traps (Parkyn et al., 2011), that have also been shown to select for smaller crayfish compared to baited traps.

APPENDIX

This Appendix is provided in order to provide evidence in support of a number of assumptions that we made in the paper in order to reach our conclusion. Appendix A is concerned with assumptions made regarding the field sampling methodologies of *Pacifastacus leniusculus* specimens in the Geddes Burn during 10-21 September 2014. Appendix B is concerned with the assumption that by working after the growth season, the likelihood of encountering moulting individuals was reduced sufficiently so that the potential presence of exuviae in the crayfish stomachs would not impact our conclusions.
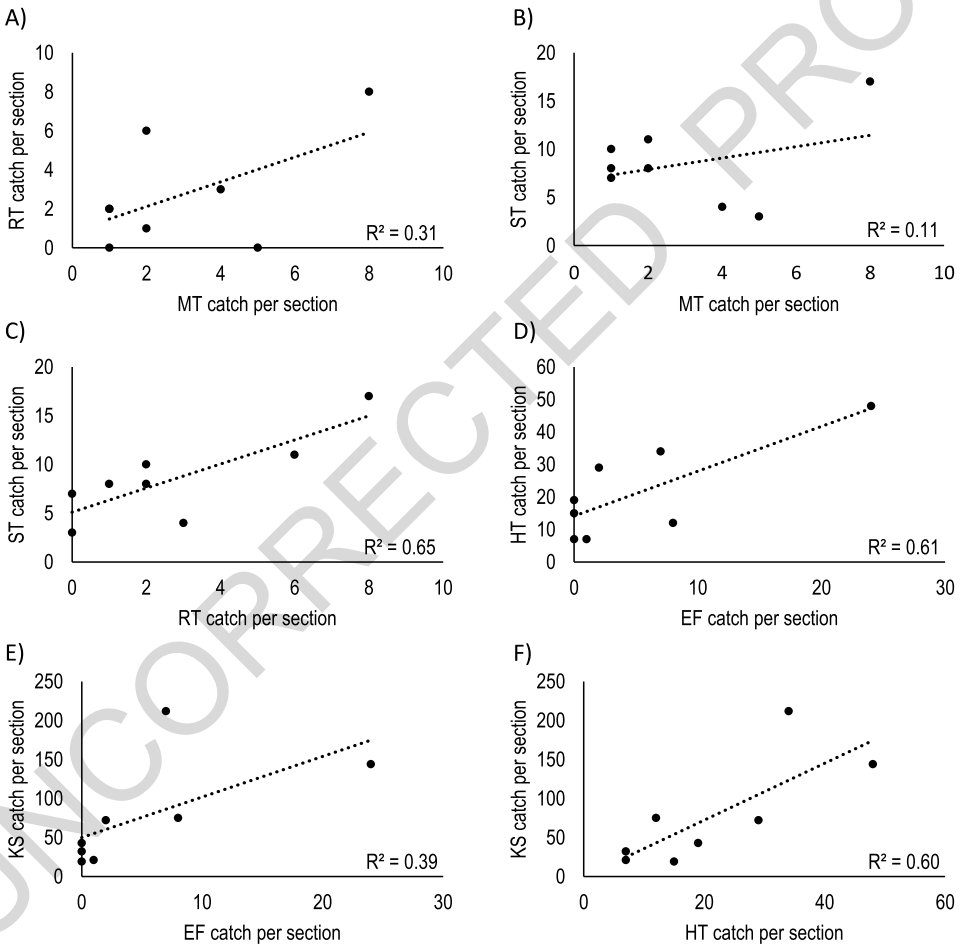

Fig. A1. A, Correlation between minnow trap and refuge trap catch number; B, correlation between minnow trap and cylindrical net trap catch number; C, correlation between refuge trap and cylindrical net trap catch number; D, correlation between electrofishing and microhabitat trap catch number; E, correlation between electrofishing and kick sampling catch number; F, correlation between microhabitat trap and kick sampling catch number.

Appendix A

The assumption was made that the total crayfish catch from each of the six sample methods in a section was a reasonable approximation of crayfish density. This was partially validated by correlating the catch numbers in each section of all the sampling methods in order to see to what extent they agree with one another (fig. A1).

A positive correlation between the catches in sections A-H of two different sampling methods would support an approximate reflection of true crayfish density. The catch numbers presented in fig. A1 are comprised of the crayfish caught during the 18-21 September 2014 capture period but also from two previous capture periods (10-13 and 14-17 September 2014 respectively). Thus each
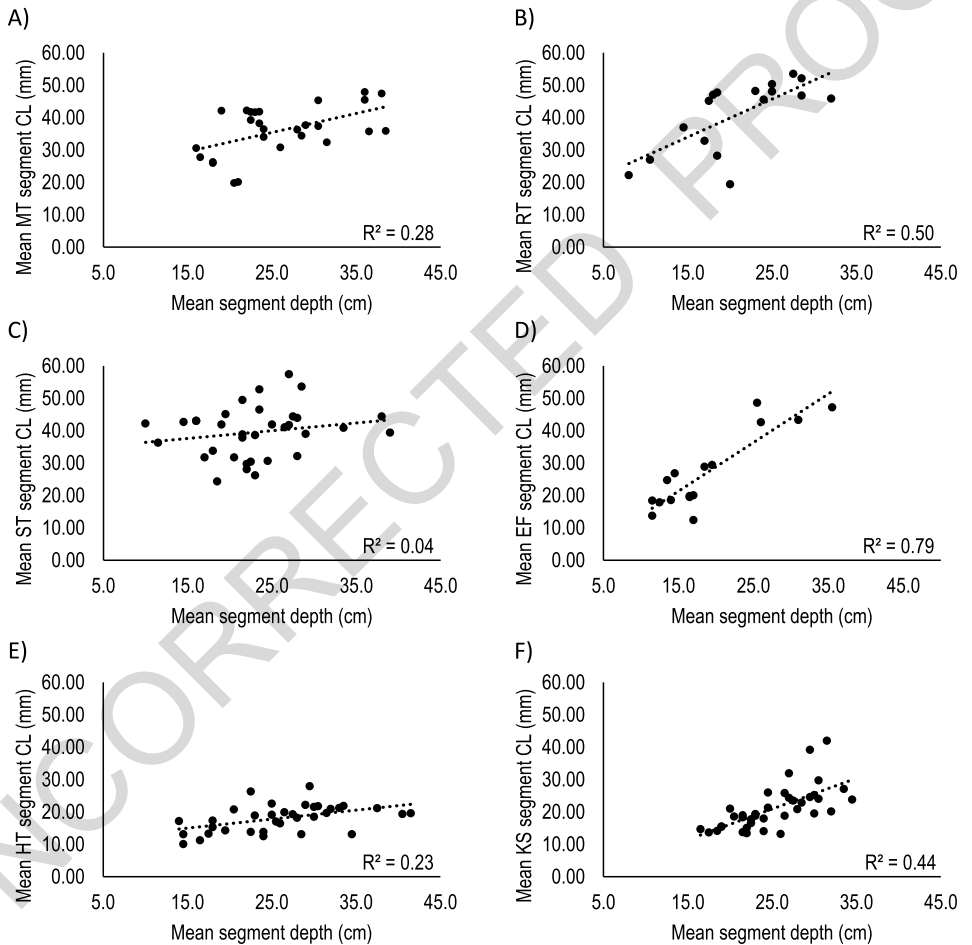

Fig. A2. A, Correlation between mean minnow trap captured crayfish size (CL) and water depth; B, correlation between mean refuge trap captured crayfish size (CL) and water depth; C, correlation between mean cylindrical net trap captured crayfish size (CL) and water depth; D, correlation between mean electrofishing captured crayfish size (CL) and water depth; E, correlation between mean kick sampling captured crayfish size (CL) and water depth; F, correlation between mean habitat trap captured crayfish size (CL) and water depth.

TABLE B1

Comparison of the different competing models run only using individuals that have hard carapace, can be considered between moults and are, therefore, unlikely to contain fragments of their own carapace ($N = 77$)

Model name	Explanatory variable	Estimate	SE	z-value	p-value	AIC	Δ(AIC)
Model 1	Log(smaller prey density)	1.25	0.53	2.40	<0.05	83.4	0.0
Model 2	Carapace length	0.09	0.04	2.59	<0.01	84.3	0.9
	Log(small crayfish density)	1.24	0.63	1.98	<0.05		
Model 3	Carapace length	0.10	0.03	2.97	<0.05	85.3	1.9
	Log(total crayfish density)	1.58	0.80	1.99	<0.08		
	Small crayfish density	0.04	0.02	2.09	<0.05		

Parameter estimates of logged variables have not been back transformed. Smaller prey density is defined as the number of crayfish in the same section as the dissected individual that are <56% the size of that individual. Small crayfish density is the number of crayfish in the section that are <30 mm CL, whereas total crayfish density is the total catch in the section.

section was sampled three times over 12 days. The number of large crayfish caught per section from the three sampling methods that were biased towards the capture of large crayfish; minnow traps (MT), cylindrical net traps (ST) and refuge traps (RT), were positively correlated with one another (fig. A1A, B, C). The number of small crayfish caught per section from the three sampling methods that were biased towards the capture of small crayfish; microhabitat traps (HT), electrofishing (EF) and kick sampling (KS), were positively correlated with one another (fig. A1D, E, F).

Similarly an assumption was made that each sampling method would not interact differently with different habitat types. Given that stream depth is a key determinant of stream habitat features and crayfish distribution we checked to see if the relationship between the mean size (CL) of crayfish and midstream depth was conserved across all sampling methods. There was a positive correlation between increasing crayfish CL and increasing water depth in all sampling methods, although the gradient and R^2 varied (fig. A2). Smaller crayfish are known to select shallower water and this pattern is conserved across sampling methods. We therefore suggest that generally each sampling method, whilst still exhibiting size selectivity, interacts in a similar fashion with the variation in habitat throughout the stream. The plotted points in this figure are the mean sizes of crayfish caught per four metre section of stream (termed segment in the plot) and are comprised of all catches from each of the three visits to each sub-section over the 12-day sampling period (10-21 September 2014).

Appendix B

The same order of support (quantified by AIC) is demonstrated for the top three competing models when run using only individuals that could be considered between-moult, as models run with the full data set and range of moult states (table BI). These between-moult individuals were hard-shelled and considered unlikely to contain fragments of their own carapace contain fragments of their own carapace as they will not have moulted recently.

REFERENCES

ABRAHAMSSON, S. A. A., 1966. Dynamics of an isolated population of the crayfish *Astacus astacus* Linné. Oikos, **17**: 96-107.

ACOSTA, C. A. & S. A. PERRY, 2000. Effective sampling area: a quantitative method for sampling crayfish populations in freshwater marshes. Crustaceana, **73**: 425-431.

ALCORLO, P., W. GEIGER & M. OTERO, 2004. Feeding preferences and food selection of the red swamp crayfish, procambarus clarkii, in habitats differing in food item diversity. Crustaceana, **77**: 435-453.

BARAS, E. & M. JOBLING, 2002. Dynamics of intracohort cannibalism in cultured fish. Aquaculture Res., **33**: 461-479.

BARTON, K., 2014. MuMIn: Multi-Model Inference. Published by the author.

BATES, D., M. MAECHLER, B. BOLKER, S. WALKER, R. CHRISTENSEN, H. SINGMANN, B. DAI, G. GROTHENDIECK & P. GREEN, 2014. lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-7. (R Foundation for Statistical Computing, Vienna).

BONDAR, C. A., K. BOTTRIELL, K. ZERON & J. S. RICHARDSON, 2005. Does trophic position of the omnivorous signal crayfish (*Pacifastacus leniusculus*) in a stream food web vary with life history stage or density? Can. J. Fish. Aquat. Sci., **62**: 2632-2639.

BRITTON, J. C. & B. MORTON, 1994. Marine carrion and scavengers. Oceanogr. Mar. Biol. Annu. Rev., **32**: 369-434.

CAPELLI, G. M., 1980. Seasonal variation in the food habits of the crayfish *Orconectes propinquus* (Girard) in Trout Lake, Vilas County, Wisconsin, U.S.A. (Decapoda, Astacidea, Cambaridae). Crustaceana, **38**: 82-86.

DANA, E. D., J. LÓPEZ-SANTIAGO, J. GARCÍA-DE-LOMAS, D. M. GARCÍA-OCAÑA, V. GÁMEZ & F. ORTEGA, 2010. Long-term management of the invasive *Pacifastacus leniusculus* (Dana, 1852) in a small mountain stream. Aquat. Invasions, **5**: 317-322.

DISTEFANO, A. R. J., C. M. GALE, B. A. WAGNER & R. D. ZWEIFEL, 2003. A sampling method to assess lotic crayfish communities. J. Crustacean Biol., **23**: 678-690.

DORN, N. J., R. URGELLES & J. C. TREXLER, 2005. Evaluating active and passive sampling methods to quantify crayfish density in a freshwater wetland. J. North Am. Benthological Soc., **24**: 346-356.

FARHADI, A., C. GARDNER & P. KOCHANIAN, 2014. Reducing cannibalism of narrow clawed crayfish *Astacus leptodactylus* Eschscholtz 1823 through management of photoperiod and stocking density. Asian Fish. Sci., **27**: 286-296.

FARHADI, A. & M. A. JENSEN, 2015. Effects of photoperiod and stocking density on survival, growth and physiological responses of narrow clawed crayfish (*Astacus leptodactylus*). Aquaculture Res., **47**: 2518-2527.

FJÄLLING, A. B., 2011. The enclosure trap, a new tool for sampling juvenile crayfish. Knowl. Manag. Aquat. Ecosyst., **401**: 9.

FREEMAN, M. A., J. F. TURNBULL, W. E. YEOMANS & C. W. BEAN, 2010. Prospects for management strategies of invasive crayfish populations with an emphasis on biological control. Aquat. Conserv. Mar. Freshw. Ecosyst., **20**: 211-223.

GARDMARK, A., N. JONZEN & M. MANGEL, 2006. Density-dependent body growth reduces the potential of marine reserves to enhance yields. J Appl. Ecol., **43**: 61-69.

GLADMAN, Z. F., W. E. YEOMANS, C. E. ADAMS, C. W. BEAN, D. McCOLL, J. P. OLSZEWSKA, C. W. McGILLIVRAY & R. McCLUSKEY, 2010. Detecting North American signal crayfish (*Pacifastacus leniusculus*) in riffles. Aquat. Conserv. Mar. Freshw. Ecosyst., **20**: 588-594.

GUAN, R. Z. & P. R. WILES, 1998. Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in a British lowland river. Aquaculture, **169**: 177-193.

GUTHERY, F. S. & J. H. SHAW, 2013. Density dependence: applications in wildlife management. J. Wildl. Manage., **77**: 33-38.

GUTIERREZ-YURRITA, P. J., G. SANCHO, M. Á. BRAVO, Á. BALTANÁS & C. MONTES, 1998. Diet of the red swamp crayfish *Procambarus clarkii* in natural ecosystems of the Donana National Park temporary fresh-water marsh (Spain). J. Crustacean Biol., **18**: 120-127.

HIXON, M. A. & M. H. CARR, 1997. Synergistic predation, density dependence, and population regulation in marine fish. Science, 277: 946-949.

JOHNSEN, S. I., J. SKURDAL, T. TAUGBOL & E. GARNAS, 2014. Effect of mesh size on baited trap catch composition for noble crayfish (*Astacus astacus*). Knowl. Manag. Aquat. Ecosyst., 413: 6.

JONES, J. P. G. & T. COULSON, 2006. Population regulation and demography in a harvested freshwater crayfish from Madagascar. Oikos, 112: 602-611.

LEE, S. Y., 1995. Cheliped size and structure: the evolution of a multi-functional decapod organ. J. Exp. Mar. Biol. Ecol., 193: 161-176.

LOVRICH, G. A. & B. SAINTE-MARIE, 1997. Cannibalism in the snow crab, *Chionoecetes opilio* (O. Fabricius) (Brachyura: Majidae), and its potential importance to recruitment. J. Exp. Mar. Biol. Ecol., 211: 225-245.

MOKSNES, P. O., 2004. Self-regulating mechanisms in cannibalistic populations of juvenile shore crabs *Carcinus maenas*. Ecology, 85: 1343-1354.

NYSTRÖM, P., 2002. Ecology. In: D. M. HOLDICH (ed.), Biology of freshwater crayfish: 192-235. (MPG Books, Bodmin).

PARKYN, S. M., R. J. DISTEFANO & E. M. IMHOFF, 2011. Comparison of constructed microhabitat and baited traps in Table Rock reservoir, Missouri, U.S.A. Freshw. Crayfish, 18: 69-74.

PERSSON, L., D. CLAESSEN, A. M. DE ROOS, P. BYSTRÖM, S. SJÖGREN, R. SVANBÄCK, E. WAHLSTRÖM & E. WESTMAN, 2004. Cannibalism in a size-structured population: energy extraction and control. Ecol. Monogr., 74: 135-157.

POLICAR, T. & P. KOZÁK, 2005. Comparison of trap and baited stick catch efficiency for noble crayfish (*Astacus astacus* L.) in the course of the growing season. Bull. Fr. Pêche Piscic., 376-377: 675-686.

POLIS, G. A., 1981. The evolution and dynamics of intraspecific predation. Annual. Rev. Ecol. Syst., 12: 225-251.

POST, J. R., E. A. PARKINSON & N. T. JOHNSTON, 1999. Density-dependent processes in structured fish populations: interaction strengths in whole-lake experiments. Ecol. Monogr., 69: 155-175.

PRICE, J. E. & S. M. WELCH, 2009. Semi-quantitative methods for crayfish sampling: sex, size, and habitat bias. J. Crustacean Biol., 29: 208-216.

R CORE TEAM, 2013. R: a language and environment for statistical computing. (R Foundation for Statistical Computing, Vienna).

RABENI, C. F., K. J. COLLIER, S. M. PARKYN & B. J. HICKS, 1997. Evaluating techniques for sampling stream crayfish (*Paranephrops planifrons*). N. Z. J. Mar. Freshw. Res., 31: 693-700.

RAHEL, F. J. & R. A. STEIN, 1988. Complex predator-prey interactions and predator intimidation among crayfish, piscivorous fish, and small benthic fish. Oecologia, 75: 94-98.

REYNOLDS, J. D., 2011. A review of ecological interactions between crayfish and fish, indigenous and introduced. Knowl. Manag. Aquat. Ecosyst., 401: 10.

REYNOLDS, J. D. & C. O'KEEFFE, 2005. Dietary patterns in stream- and lake-dwelling populations of *Austropotamobius pallipes*. Bull. Fr. Pêche Piscic., 376-377: 715-730.

SADYKOVA, D., J. SKURDAL, A. SADYKOV, T. TAUGBOL & D. O. HESSEN, 2009. Modelling crayfish population dynamics using catch data: a size-structured model. Ecol. Model, 220: 2727-2733.

SAVOLAINEN, R., K. RUOHONEN & E. RAILO, 2004. Effect of stocking density on growth, survival and cheliped injuries of stage 2 juvenile signal crayfish *Pasifastacus leniusculus* Dana. Aquaculture, 231: 237-248.

SAVOLAINEN, R., K. RUOHONEN & J. TULONEN, 2003. Effects of bottom substrate and presence of shelter in experimental tanks on growth and survival of signal crayfish *Pasifastacus leniusculus* (Dana) juveniles. Aquaculture Res., 34: 289-297.

SCALICI, M. & G. GIBERTINI, 2009. Molt and gastroliths in *Austropotamobius pallipes* (Lereboul-let, 1858). Knowl. Managt. Aquat. Ecosyst., **394-395**: 14.

SINCLAIR, C. A., 2010. Fine scale mapping of signal crayfish distribution in Scotland, Scottish Natural Heritage Commissioned Report, Project 26686. (Scottish Natural Heritage, Inverness).

SOMERS, K. M. & D. P. M. STECHEY, 1986. Variable trappability of crayfish associated with bait type, water temperature and lunar phase. Am. Midl. Nat., **116**: 36-44.

TWARDOCHLEB, L. A., J. D. OLDEN & E. R. LARSON, 2013. A global meta-analysis of the ecological impacts of nonnative crayfish. Freshw. Sci., **32**: 1367-1382.

ULIKOWSKI, D., T. KRZYWOSZ & P. ŚMIETANA, 2006. A comparison of survival and growth in juvenile *Astacus leptodactylus* (Esch.) and *Pacifastacus leniusculus* (Dana) under controlled conditions. Bull. Fr. Pêche Piscic., **380-381**: 1245-1253.

VOLLESTAD, L. A. & B. JONSSON, 1988. A 13-year study of the population dynamics and growth of the European eel *Anguilla anguilla* in a Norwegian river: evidence for density-dependent mortality, and development of a model for predicting yield. J. Anim. Ecol., **57**: 983-997.

WAGENMAKERS, E. J. & S. FARRELL, 2004. AIC model selection using Akaike weights. Psychon. Bull. Rev., **11**: 192-196.

WOOSTER, D., J. L. SYNDER & A. MADSEN, 2012. Environmental correlates of signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), density and size at two spatial scales in its native range. J. Crustacean Biol., **32**: 741-752.

WUTZ, S. & J. GEIST, 2013. Sex- and size-specific migration patterns and habitat preference of invasive signal crayfish (*Pacifastacus leniusculus* Dana). Limnologica, **43**: 59-66.

ZIPKIN, E. F., C. E. CRAFT, E. G. COOCH & P. J. SULLIVAN, 2009. When can efforts to control nuisance and invasive species backfire? Ecol. Appl., **19**: 1585-1595.