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Effects of population density and body size on disease ecology of the European lobster in a temperate marine conservation zone

Charlotte E. Davies[1]*, Andrew F. Johnson[2], Emma C. Wootton[1], Spencer J. Greenwood[3], K. Fraser Clark[3], Claire L. Vogan[4], and Andrew F. Rowley[1]

[1]*Department of Biosciences, College of Science, Swansea University, Swansea SA2 8PP, UK*
[2]*Center for Marine Biodiversity and Conservation, Scripps Institution of Oceanography, 9500 Gilman Drive, La Jolla, CA 92093-0202, USA*
[3]*Department of Biomedical Sciences and AVC Lobster Science Centre, Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island, Canada C1A 4P3*
[4]*College of Medicine, Swansea University, Swansea SA2 8PP, UK*

**Corresponding author: tel: +44 7814 161356; e-mail: cedavies72@gmail.com*

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Marine conservation zones (MCZs) are a form of spatial marine management, increasingly popular since the move towards ecosystem-based fisheries management. Implementation, however, is somewhat contentious and as a result of their short history, their effects are still widely unknown and understudied. Here, we investigate the population and health of the European lobster (*Homarus gammarus*) in the Lundy Island Marine Conservation Zone, Bristol Channel, UK. Using the fished refuge zone (RZ) as a control area, catch per unit effort was calculated for both the no-take zone (NTZ) and RZ and binomial logistic regression models were used to examine the effects of site, sex, landing size, and loss of chelae on the probability of shell disease and injury presence in individuals. Lobsters were also tested for the causative agent of gaffkaemia, *Aerococcus viridans* var. *homari*, and white spot syndrome virus (WSSV). The analysis revealed a higher lobster density and larger lobsters in the NTZ compared with the RZ. Shell disease was present in 24% of lobsters and the probability of shell disease occurrence increased notably for individuals over the minimum landing size (MLS) of 90 mm carapace length. Shell disease was also more prevalent in lobsters displaying injury, and in males. Injury was present in 33% of lobsters sampled and prevalence was higher in lobsters in the NTZ compared with the RZ, and in lobsters >MLS. *Aerococcus viridans* var. *homari* was detected in <1% of individuals, but WSSV was absent from all sampled lobsters. Overall, the study demonstrates both positive and potentially negative effects of NTZs, methods for effective non-lethal sampling of disease agents, and highlights the need for more comprehensive, long-term monitoring within highly protected MCZs, both before and after implementation.

Keywords: gaffkaemia, health, Lundy Island, marine monitoring, MPA, no take zone, pathogen entry, shell disease, WSSV.

Introduction

Overfishing persists in many of the world's oceans. Fish and invertebrate stocks have been overexploited, leaving some species in dangerous decline (Kleisner *et al.*, 2013), and others in complete collapse (Myers *et al.*, 1997). To protect biodiversity, commercial stocks, and to aid in the recovery of declining populations, establishment of marine reserves (no-take zones, NTZs), or marine protected areas (MPAs) are seen as a new paradigm for spatial management (Kaiser, 2005). These areas of conservation are aimed at protecting habitats and species and in some cases have been of significant benefit to local fisheries worldwide (Rosenberg, 2003). The use of MPAs conforms to an ecosystem-based approach to management, as often, these reserves have benefits for multiple species and ecosystems (Botsford *et al.*, 2003; Gaines *et al.*, 2010).

While protecting habitats or species is often the primary objective of areas closed to fishing, it is of paramount importance to evaluate the success of MPA implementation (Pomeroy *et al.*, 2005). In general, surveys and monitoring are expensive, and as a result, the majority of commercial target species are currently inadequately assessed worldwide (Costello *et al.*, 2012). To provide a "fished" or "non-protected" comparison, it is necessary to establish a baseline before implementation as well as ongoing evaluations of the same measures (Pinnegar and Engelhard, 2007). There have been studies offering methods for evaluating the effectiveness of management of MPAs (Pomeroy *et al.*, 2005; Sciberras *et al.*, 2013). One example method is the before-after control-impact design, with data from replicated MPA and control sites both before and after MPA designation (Fenberg *et al.*, 2012). This has been considered the optimal way of assessing effects of protection (Moland *et al.*, 2013a). In cases where there is insufficient baseline information, it is difficult to ascertain when a healthy population reaches its threshold, both in terms of population density and individual species health (e.g. pathogens and disease). Subsequent MPA management measures may therefore be misguided and inappropriate (Hilborn *et al.*, 2004). Conflicting interests between stakeholder groups adds additional complexity and can exacerbate the problems associated with determining effective management strategies (Fox *et al.*, 2014).

Studies have shown that increased density in marine reserves may manifest in higher levels of disease (McCallum *et al.*, 2005; Wootton *et al.*, 2012; Wood *et al.*, 2013). Higher densities are also thought to increase injury due to more conspecific interaction (Debuse *et al.*, 2003; Wootton *et al.*, 2012), which may be linked to disease transmission (Vogan *et al.*, 1999; Whitten *et al.*, 2014). In addition, the number of diseases reported in marine organisms over the past three decades has risen substantially (Harvell *et al.*, 2004); therefore, health monitoring of conservation areas, such as MPAs, is vital to ensure the correct management measures are being assigned on a case by case basis (Agardy *et al.*, 2003; Pomeroy *et al.*, 2005).

A previous study by Wootton *et al.* (2012) of the Lundy Island NTZ, Bristol Channel, UK, demonstrated that the high densities of European lobster (*Homarus gammarus*) inside the NTZ (compared with the fished Refuge Zone; RZ) may be subjecting individuals to a higher risk of shell disease. As a consequence, it was concluded that a further survey of lobster disease ecology in the Lundy Island NTZ was warranted. The present study extends the original survey to include non-lethal diagnostic tests for the causative agent of gaffkaemia (a potentially fatal bacterial disease of lobsters), and white spot disease (WSD) caused by the white spot syndrome virus (WSSV). WSD is primarily associated with shrimp aquaculture and is rarely seen in the wild; however, it has been suggested that this may be due to limited monitoring of wild populations (Chapman *et al.*, 2004).

Shell disease syndrome, also known as enzootic (or "classical") shell disease, is a progressive condition of crustaceans whereby lesions develop on the cuticle and in extreme cases can totally erode through the carapace, exposing underlying soft tissues (Vogan *et al.*, 2008). Commonly observed among both European and American (*Homarus americanus*) lobsters (Sindermann, 1989), it must not be confused with the more severe epizootic shell disease, responsible for significant economic losses to American lobster fisheries (Castro *et al.* 2006; Wahle *et al.*, 2009).

Gaffkaemia is the disease resulting from an infection by the Gram-positive bacterium, *Aerococcus viridans* var. *homari* (Stewart, 1980). Also known as "red-tail" disease, it is primarily associated with American lobsters (*H. americanus*) in tidal pound holding facilities where the stress induced by high lobster density and increased temperature exacerbates the spread of the disease, resulting in mortalities (Snesizko and Taylor, 1947). It has also been detected in both holding facilities and in the wild in European lobsters (Wiik *et al.*, 1987; Stebbing *et al.*, 2012).

The current study examined the effect of population density on the presence of disease and injury by comparing sites both inside and outside the Lundy Island NTZ. We tested the hypotheses that density of *H. gammarus* was higher in the NTZ compared with the fished, RZ, and that individual lobsters from the NTZ had a higher probability of exhibiting signs of disease (shell disease, gaffkaemia, WSD) and injury. We also tested whether large individuals were more likely to be diseased and injured than small individuals and whether females had an increased likelihood of disease and injury than males (due to increased intermoult periods when older, or when holding eggs). Additionally, as a breached cuticle increases the potential risk of infection to disease (Davies *et al.*, 2014; Whitten *et al.*, 2014), the hypothesis that injured individuals were more likely to be diseased than non-injured individuals was also tested.

Materials and methods

Study area

Lundy Island, off the North Devon coast, was Britain's first MPA. The waters around the island were established as a voluntary Marine Nature Reserve (MNR) in 1971 and in 1986, it was designated as England's first and only statutory MNR. The MNR consists of an RZ, where pot fisheries (for crabs and lobsters) are authorized, but trawl and net fisheries prohibited, and in 2003, a statutory NTZ was implemented within the existing RZ under a byelaw from the local Sea Fisheries Committee (Devon and Severn IFCA). Within the NTZ, all fishing, including potting, and removal of wildlife is forbidden.

As a result of the 2009 Marine and Coastal Access Act, in 2010, the waters around Lundy Island also became England's first Marine Conservation Zone (MCZ). This new designation superseded the MNR designation and established the site as the foundation of a new network of MPAs. In November 2013, 27 sites were designated within the Defra marine area (UK Ministerial Orders, 2013) and Lundy Island's NTZ designation still remains.

Lobster collection and general observations

In May 2010 (2 d), July 2010 (3 d), and August 2011 (3 d), the *H. gammarus* population was surveyed across six sampling sites (4 in RZ and 2 in NTZ; Figure 1) similar to previous Lundy Island surveys (Hoskin *et al.*, 2011; Wootton *et al.*, 2012). One string of baited commercial parlour pots (35 pots with escape gaps closed) was deployed at each sampling site. Each string was immersed for 24 h, retrieved, emptied of all catch, rebaited, and redeployed. Animals were sexed and six additional measurements for each individual were taken (Table 1) before they were returned to the water. Recent, non-melanized breach of the cuticle (injuries), or claw loss (i.e. those sustained in the pot) were not recorded. Animals exhibiting exoskeletal abnormalities or severe shell disease were photographed.

Surveillance of *A. viridans* and WSSV

For all three surveys (May 2010, July 2010, and August 2011), haemolymph was drawn into 100% analytical grade ethanol using

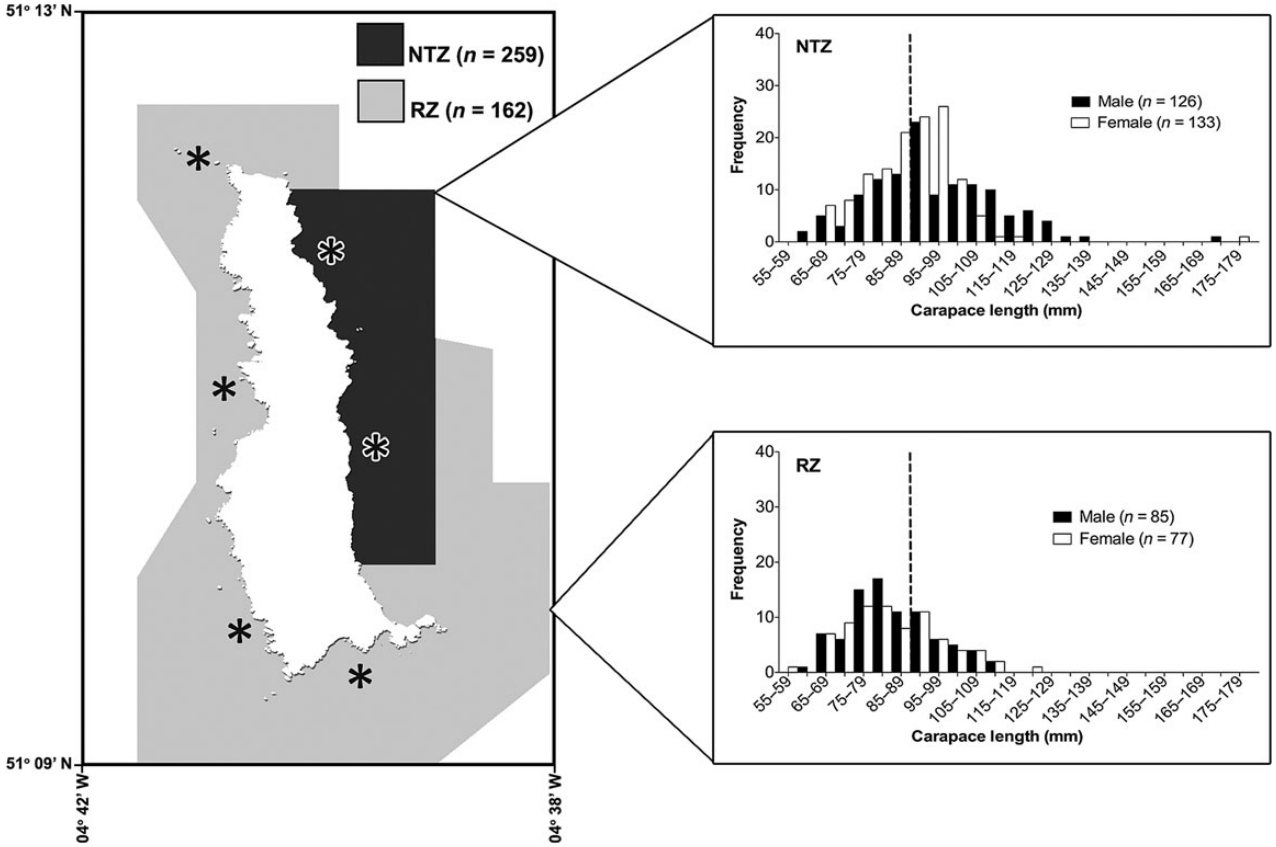

Figure 1. Map of Lundy Island MCZ, Bristol Channel, southwest UK (adapted from http://www.lundymcz.org.uk/). Latitude and longitude coordinates represent the MCZ boundary. Note the RZ where pot fisheries are authorized, but trawl and net fisheries are banned and the NTZ where removal of all wildlife is prohibited. Asterisks represent the sampling sites. Inset: size frequency distributions of male (black) and female (white) lobsters surveyed from the NTZ and RZ. Broken lines indicate MLS; CL >90 mm.

Table 1. Parameters recorded for each individual *H. gammarus* caught in the Lundy Island MCZ, used as predictor variables for subsequent models.

Measurement	Description	Measure
Carapace length (CL)	Length from rear of the eye socket to the rear of the carapace	Continuous measure (mm)
Minimum landing size (MLS)[a]	Whether carapace length was above the 90 mm MLS as designated by The Devon and Severn IFCA District shore Fisheries and Conservation Authority byelaw	Ordered binomial: 0 (no) or 1 (yes)
Berried	Presence of eggs attached to pleopods on the abdomen of females	Presence vs. absence (P vs. A)
Injury[a]	Wounds such as punctures and stress fractures to the cuticle as described in Wootton *et al.* (2012). Injuries inflicted during captivity within the pot (i.e. recent, non-melanized breach of the cuticle) were not recorded	(P vs. A)
Claw loss[a]	Missing cheliped (or dwarfed cheliped)	(P vs. A)
Shell disease presence and severity[a]	Presence of shell disease, as well as severity, was recorded (i.e. "high" or "low", as described in Wootton *et al.*, 2012)	(P vs. A) Ordered trinomial: 0 (none), 1 (low), 2 (high)
Other	Tagged individuals; those showing abnormalities	Numbered and photographic

[a]Parameters used for binomial logistic regression models.

23 G needles and 2 ml syringes, to assess the presence of *A. viridans* var. *homari*, and WSSV.

DNA extraction

DNA was extracted from haemolymph ($n_{total} = 508/1092$) using an adapted version of Ivanova *et al.* (2006) (see Supplementary materials) using 96-well filter plates (AcroPrep Advance 1 ml for DNA binding; Pall Life Sciences, Southampton, UK). DNA was eluted with water and stabilized with Tris-EDTA buffer (10×) then used as the template for subsequent polymerase chain reactions (PCR). Extraction was optimized to ensure detection of all pathogens using "spiked" haemolymph samples of both the "virulent" and "avirulent" strains of *A. viridans* var. *homari* (1×10^3, 1×10^4, 1×10^5, and 1×10^6 colony forming units (CFU) ml^{-1}; NVI 1032 and 88B; Table 2), and a positive control from WSSV-infected shrimp.

Polymerase chain reaction

All PCRs were carried out using MangoMix (Bioline Ltd, UK), primers synthesized by Eurofins MWG Operon (Ebersberg, Germany), and a Bio-Rad PTC-100 Peltier Thermal Cycler, before being

Table 2. *Aerococcus viridans* var. *homari*, causative agent of gaffkaemia, isolates from *H. gammarus* or *H. americanus* used to verify primers and positive template controls.

Isolate	Identification	Origin	Location, year of isolation	Virulence status
NCIMB1121[a]	*A. viridans* var. *homari*	*H. gammarus*	Harwich, England, UK, 1962	Avirulent
NCIMB1119, ATCC29838[a]	*A. viridans* var. *homari*	*H. gammarus*	Southampton, England, UK, 1962	Virulent
NVI 1032[bc]	*A. viridans* var. *homari*	*H. americanus*	NVI, Norway, 1977	Virulent
88B[c]	*A. viridans*-like coccus	*H. americanus*	Nova Scotia, Canada, 1962	Avirulent

[a]Isolate deposited in National Collection of Industrial and Marine Bacteria.
[b]National Veterinary Institute, Norway (infected lobsters imported from St Lawrence area, Canada, 1977).
[c] Strains supplied by Dr P. Stebbing, Cefas, Weymouth, UK.

visualized on a 1.5% agarose gel. Decapod-specific primers 143F 5′-TGCCTTATCAGCTNTCGATTGTAG-3′ and 145R 5′-TTCAG NTTTGCAACCATACTTCCC-3′ yielding an 848 bp amplicon (N represents G, A, T, or C) were used to verify the quality of the extracted DNA and the integrity of the PCR reaction (Lo, 2014). Cycling conditions were: 4 min at 94°C followed by 40 cycles of 1 min at 93°C, 1 min at 55°C, and 2 min at 72°C, followed by 5 min at 72°C.

Detection of *A. viridans* var. *homari*

Following preliminary evaluations of several candidate primer pairs developed from *A. viridans* var. *homari* GenBank ID: AY707775.1 (Greenwood *et al.*, 2005), the primers Av1F 5′-TCGGAAACGGG TGCTAATAC-3′and Av2R 5′-TAAGGTTCTTCGCGTTGCTT-3′ were chosen to detect all *A. viridans* var. *homari* (Primer 3 design for Av ATCC29838 16S rRNA, product 837 bp); verified with PCR of *A. viridans* colony boils (NCIMB1121, and NCIMB1119; Table 2). Cycling conditions were optimized to detect as little as 1×10^3 CFU ml^{-1}: 3 min at 95°C followed by 38 cycles of 30 s at 94°C, 1 min 30 s at 64°C (-0.5°C $cycle^{-1}$ for the first 33 cycles and 47.5°C thereafter) and 2 min at 72°C, followed by 5 min at 72°C. Positive samples were repeated and the PCR product was purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) and sequenced by Eurofins MWG Operon (Ebersberg, Germany). Contigs from sequences were created using the CAP3 sequence assembly programme (Huang and Madan, 1999) and matched to positive controls using NCBI BLAST search.

Detection of WSSV

Detection of WSSV was performed using an adaptation of methods from Clark *et al.* (2013), using forward primer F1- WSDvp28 5′-GTGACCAAGACCATCGAAAC-3′ and reverse primer R1-WSDvp28 5′-TGAAGTAGCCTGATCCAACC-3′ which are based around the VP28 gene of WSSV. Cycling conditions were: 5 min at 95°C followed by 40 cycles of 15 s at 95°C, 1 min at 60°C, followed by 5 min at 72°C. A positive control of DNA extracted from WSSV-infected shrimp was used to verify the integrity of the PCR reaction.

Statistical analysis

Population ecology

For population, shell disease and injury ecology, the 2011 data were analysed (see Wootton *et al.*, 2012, for 2010 data analyses). So that individuals were not double sampled (i.e. recaptured after day 1 and considered a unique individual), each was given an identifier based on the parameters in Table 1 and any individuals sharing the identifier were removed (a total of four individuals were removed before analysis). Population distributions between sexes and sites were visualized in GraphPad Prism 5.0. Differences in size frequency of populations were tested using a two-sample Kolmogorov–Smirnov test and Mann–Whitney *U*-test. To compare catch data between zones, *t*-tests were used (mean ± SEM); tests were two-tailed, used a significance level of 0.05, and tested to follow a Gaussian distribution (using the Kolmogorov–Smirnov test) before any further analysis. Catch per unit of fishing effort (CPUE) was calculated as the mean number of lobsters per pot.

Disease and injury ecology

To determine whether the measured parameters (Table 1) had a significant effect on the presence of shell disease, disease severity, and injury in the lobster population sampled, binomial logistic regression models were used (MASS library in R; R development Core Team, 2011). The information theoretical approach was employed for model selection and assessment of model performance (Richards, 2005) and initial models included all the binomial parameters highlighted in Table 1 ([a]). To select the best model among the entire set of initial models, i.e. the model that best described the presence of shell disease, shell disease severity, and injury, Akaike's information criterion (AIC) was used (Burnham and Anderson, 1998). The most complex models with full interaction terms between predictor variables were run first, followed sequentially by models with all combinations of predictor variables as full and partial interactions until a simple main effects model was reached. Model selections were based on the lowest AIC value (Table 3). Once selected, non-significant predictor variables were removed to produce final, reduced, and simpler models with increased predictive power (Zuur *et al.*, 2009).

Fitted probability plots were used to visualize the significant relationships inferred from the reduced models using carapace length (CL) as the independent variable. The probability of each of the tested response variables was calculated using the following equation:

$$\rho = \frac{1}{1 + exp^{-\beta x}}$$

where ρ is the probability of each response variable and βx the estimate (slope) for the predictor variable analysed (Table 1).

Results

General population ecology

Catch data from the 2011 survey revealed that significantly more lobsters were caught per string in the NTZ compared with the RZ ($p = 0.0105$, $t = 3.030$, d.f. $= 12$; NTZ $= 40.00 \pm 5.58$, RZ $= 20.25 \pm 3.81$, Figure 2). The CPUE was 2.13 times greater in the NTZ.

A two-sample KS test concluded that the size frequency distributions were different between the NTZ and RZ; and neither followed a

Table 3. Full models used to predict response variables of shell disease, shell disease severity, and injury before model reduction.

Model	Predictor variable	Estimate (slope)	*p*-value
Model 1: shell disease	Site	0.34	0.254
Shell disease ~ Site + sex + injury + landing size + claw loss	Sex	1.54	7.09e−08*
AIC: 363.37	Injury	1.07	9.50e−05*
d.f. = 396	Landing size	−1.40	1.72e−06*
	Claw loss	0.74	0.084
Model 2: shell disease severity	Site	0.02	0.977
Shell disease severity ~ Site + sex + injury + landing size + claw loss	Sex	−0.81	0.235
AIC: 119.22	Injury	−0.08	0.863
d.f. = 94	Landing size	0.88	0.219
	Claw loss	0.70	0.339
Model 3: injury	Site	1.03	3.21e−05*
Injury ~ site + sex + landing size + claw loss	Sex	0.35	0.12
AIC: 482.62	Landing size	−0.76	<0.001*
d.f. = 397	Claw loss	−0.03	0.93

*Significance ($\alpha = 0.05$)

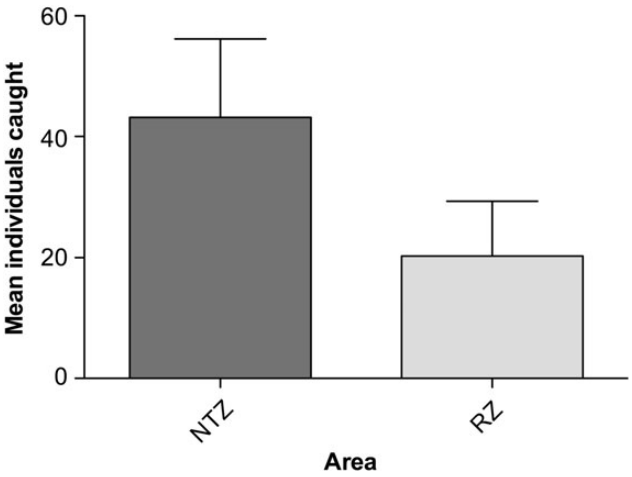

Figure 2. Mean numbers of individuals caught per string in the NTZ and RZ with 95% CI. Independent samples *t*-test, $p = 0.0105$, $t = 3.030$, d.f. = 12; NTZ = 40.00 ± 5.58, RZ = 20.25 ± 3.81 (mean ± SEM).

normal distribution ($p < 0.001$ for NTZ; $p = 0.003$ for RZ). In terms of population curve shapes, the skewness values were similar (NTZ = 1.50, RZ = 1.29), but the kurtosis values very different (NTZ = 2.00, RZ = 0.45). The difference between the two populations was therefore caused by the shape (kurtosis) of the two population distributions rather than the median values ($p = 0.215$). These results highlight that the NTZ population had a higher number of individuals around the median size compared with the RZ, but the spread from small to large individuals was similar between the two populations. Lobsters caught in the NTZ were significantly larger than those in the RZ (93.3 ± 1.0 vs. 85.0 ± 1.0 mm, respectively; $p < 0.001$). Separating by sex, the mean size of males in the NTZ was larger than in the RZ (96.6 ± 1.6 vs. 84.9 ± 1.2 mm; $p < 0.001$) and females were also larger in the NTZ than the RZ (89.8 ± 1.3 vs. 85.2 mm ± 1.5; $p = 0.012$). The NTZ population comprised 60.8% "large" commercially viable lobsters (those over 90 mm minimum landing size; MLS) and the RZ only 32.1%.

It was not possible to test whether there were more ovigerous ("berried") females being caught in the NTZ compared with the RZ, as the number was too small ($n = 3$). In total, 0.75% of females caught were berried (all from the NTZ).

Shell disease and injury ecology

In the Lundy MCZ as a whole, 24% of lobsters sampled had shell disease. In the NTZ, 28% had shell disease and in the RZ, 17%. Of the predictor variable combinations tested, the regression model that resulted in the best *AIC* was the main effects model (Model 1; Table 3). This model showed that there was no significant effect of site (inside NTZ vs. RZ) or claw loss (absence or presence of missing chelae) on the presence of shell disease in the lobsters sampled (Table 3). NTZ lobsters were therefore no more likely to be affected by shell disease than those caught in the RZ. Removing the non-significant predictors of site and claw loss on shell disease presence from the final main effects model produced a reduced model (Model 4; Table 4). Sex, injury, and landing size all had a significant effect on the presence of shell disease. Sex had the most significant effect on explaining the probability of shell disease presence, followed by landing size and then injury (Table 4). Probability calculations demonstrated that if a caught lobster was male it was 83% more likely to have shell disease than a female. If the lobster was injured, it was 76% more likely to have shell disease and finally, if the lobster caught was over the MLS of 90 mm, then it was 83% more likely to have shell disease.

Table 4. Binomial logistic regression Model 4, reduced from the full, main effects model (Model 1) used to explain the effects of variables; sex, landing size, and injury on the presence of shell disease.

Model	Predictor variable	Estimate (slope)	± Standard error	*p*-value
Shell disease ~ sex + injury + landing size	Sex (male)	1.50	0.28	1.18e−07*
d.f. = 398	Landing size (<90 mm)	−1.46	0.29	3.50e−07*
AIC: 363.47	Injury (Yes)	1.11	0.27	3.59e−05*

*Significance ($\alpha = 0.05$).

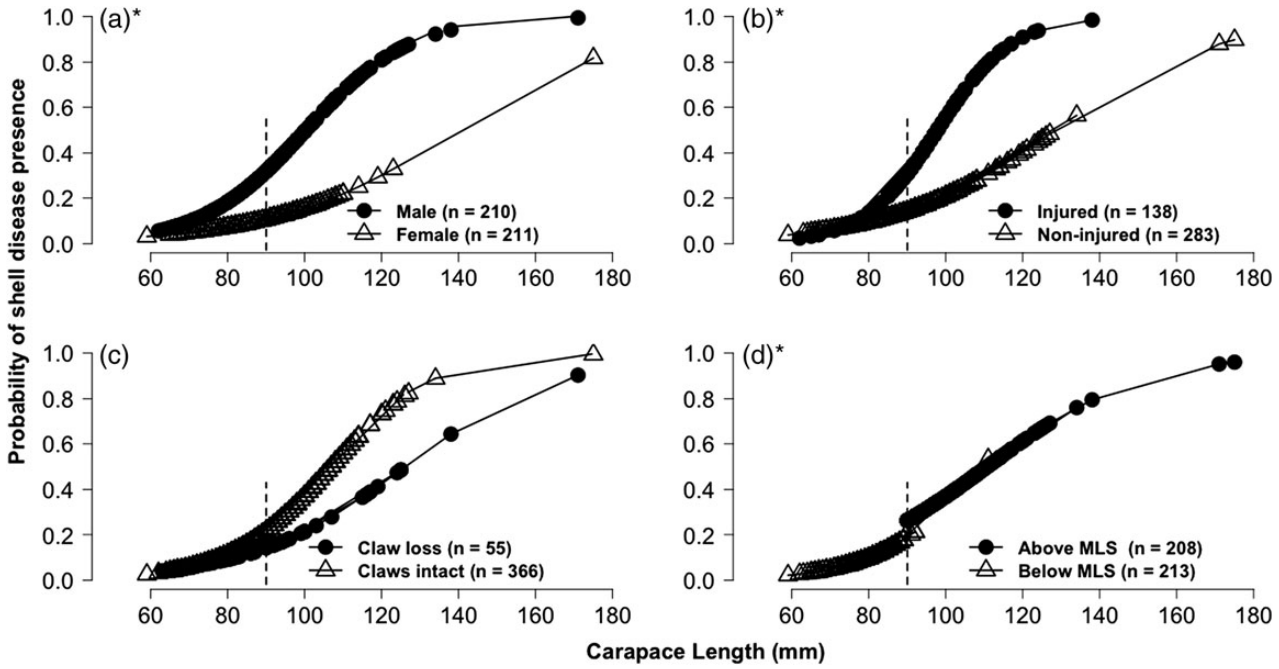

Figure 3. Fitted probability plots of shell disease presence against CL, separated by sex (a), injury (b), claw loss (c), and MLS (d). Asterisks denote significance of the predictor variable in the original full, main effects model and reduced model. The broken line in each plot represents the legal MLS of 90 mm CL.

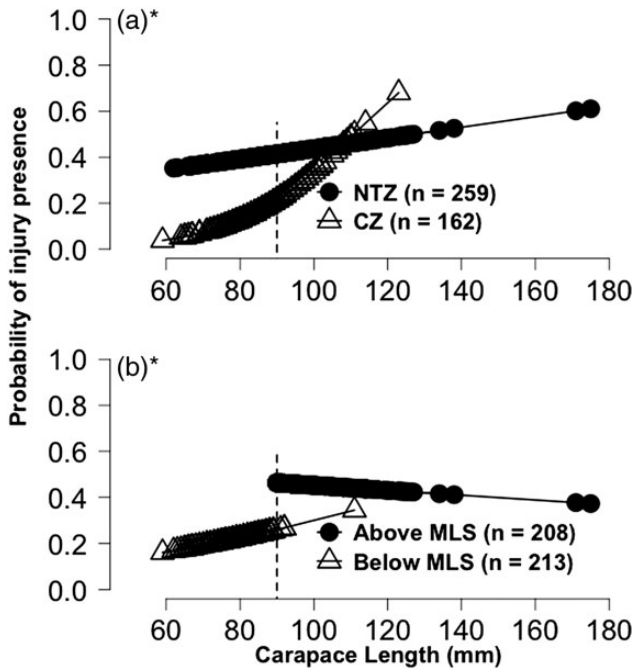

Figure 4. Fitted probability plots of injury presence against CL, separated by site (a) and MLS (b). Asterisks denote significance of the predictor variable in the original full, main effects model and reduced model. The broken line in each plot represents the legal MLS of 90 mm CL.

Fitted probability plots, using the model (Disease ~ CL), separated by the significant predictor variables sex, injury, claw loss, and above vs. below MLS allowed an in-depth examination of the relationship between shell disease presence, size, and each of these predictor variables (Figure 3). Separation between the two lines plotted in each graph indicated differences in the probability of disease between the categories for each predictor variable (i.e. male vs. female, injured vs. non-injured, claw loss vs. intact claws, and above vs. below MLS). Separations of the predicted probability lines for each predictor variable categories occurred at CLs of ~78, 79, and 95 mm for sex, injury, and claw loss respectively. It is noteworthy that caution should be taken when reading probabilities towards the larger sizes as few individuals were caught over 135 mm CL. Outlying individuals do, however, follow the general sigmoid probability trend well within each plot (Figure 3 and 4).

To test whether the predictor variables had a significant effect on the severity of shell disease in infected individuals, Model 2 was run, substituting presence vs. absence for shell disease severity (high vs. low) as the response variable (Model 2; Table 3). However, none of the predictor variables were significant in predicting the severity (high vs. low) of shell disease within individuals (Table 3; $p > 0.05$).

Overall, 33% of lobsters sampled were injured. In the NTZ, 41% were injured and in the RZ, 19%. To analyse the presence of injury in individuals, we used binomial logistic regression to examine injury as the response variable (presence vs. absence), which was tested against the roles of claw loss, landing size, sex, and site in injury presence (Model 3, Table 3). This model showed that there was no significant effect of sex or claw loss on the presence of injury in the lobsters sampled (Table 3). Therefore, the loss of claws or the sex of the lobster was not significant in predicting injury. Removing these non-significant predictors produced a reduced model (Model 5, Table 5) in which landing size and site both had a significant effect on the presence of injury. Landing size showed the highest significance followed by site. This indicates that lobsters over MLS, and those found in the NTZ were more likely to be injured than

Table 5. Binomial logistic regression injury Model 5, reduced from the full, main effects model (Model 3) used to explain the effects of variables; site and landing size on the presence of injury.

Model	Predictor variable	Estimate (slope)	± Standard error	*p*-value
Injury ~ site + landing size	Site (NTZ)	1.02	0.25	3.43e−05*
d.f. = 399	Landing size (<90 mm)	−0.78	0.23	<0.001*

*Significance ($\alpha = 0.05$).

those under the MLS and those in the RZ (Figure 4). If a lobster was caught inside the NTZ, it was 71% more likely to be injured than if it was caught in the RZ; similarly, if a lobster caught was over the MLS of 90 mm, then it was 71% more likely to be injured.

Detection of *A. viridans* and WSSV

Out of the 508 haemolymph samples analysed over the three surveys, one was positive for *A. viridans* var. *homari*. The closest BLAST match (% identity and % coverage) being 99 and 98%, respectively, was to *A. viridans* strain 1030 16S rRNA gene, partial sequence (GenBank ID: AY707775.1; Greenwood *et al.*, 2005). The positive individual was found in the NTZ during the August 2011 survey, thus the total gaffkaemia coverage in the Lundy NTZ (2011) tested, was 1.05% and in the total Lundy MCZ (2011) tested, was 0.52%. All samples tested from 2010 were negative, and all samples tested for WSSV were negative, regardless of year.

Discussion

The lack of fishing for over 8 years has resulted in an increase in the number of *H. gammarus* in the Lundy Island NTZ compared with the RZ; the higher CPUE in the NTZ was, therefore, to be expected. This is supported by previous studies which also found a higher CPUE in the Lundy Island NTZ (Hoskin *et al.*, 2011; Wootton *et al.*, 2012). However, in the current study, the CPUE was only 2.13 times higher in the NTZ than the RZ, compared with fivefold higher in 2008 (Hoskin *et al.*, 2011) and 7.7 times higher in 2010 (Wootton *et al.*, 2012). It is well known that there is generally an initial population expansion after fishery closure followed by movement out of the high-density territory, which is a suggestion for the decrease in CPUE experienced in this NTZ (e.g. Abesamis and Russ, 2005; Goñi *et al.*, 2006, 2010; Halpern *et al.*, 2010). The lobsters in the Lundy Island NTZ were found to be significantly larger than those in the RZ, a common occurrence in other studies of MPAs (Hoskin *et al.*, 2011; Wootton *et al.*, 2012; Moland *et al.*, 2013a, b). These findings of more abundant and larger individuals in the Lundy NTZ are likely to benefit the reproductive potential within the MPAs and possibly neighbouring lobster fisheries in terms of increased mating success and larval supply (Jennings, 2000; Díaz *et al.*, 2011; Moland *et al.*, 2013b).

The probability of shell disease, or shell disease severity, was not dependent upon zone (NTZ vs. RZ), contrasting with the previous study by Wootton *et al.* (2012), which found significantly more shell disease in the Lundy NTZ, compared with the RZ, particularly in large male lobsters. This may be explained by a number of possible factors. The area surveyed was relatively small (~8 km^2), and studies using tagging systems and acoustic telemetry have shown that *H. gammarus* migrate to find food and shelter, especially when an area is highly populated (Steneck, 2006). Mark–recapture studies have revealed that *H. gammarus* move several kilometres a year (e.g. Smith *et al.*, 2001; Agnalt *et al.*, 2007) and new studies using ultrasonic tracking technology have shown some individuals have home ranges of 20 km^2 or more from their burrows (Moland *et al.*, 2011a), with increased activity during the summer (Moland *et al.*, 2011b) such as those surveyed here. It must be noted that the current survey only provide a "snap-shot" of the population of interest and dynamic environmental conditions drastically affect the behaviour of organisms; hence, capture of lobsters will widely fluctuate on a temporal basis, resulting in variable data collection. Therefore, lobsters sampled by Wootton *et al.* (2012), and indeed Hoskin *et al.* (2011), may have migrated elsewhere by August 2011, possibly from the NTZ into the RZ and *vice versa*. Huserbråten *et al.* (2013) support this suggestion. They found that a significant portion of *H. gammarus* migrated to fishing grounds next to the reserves in which they were originally tagged. This spillover phenomenon has also been demonstrated by studies observing species such as the European spiny lobster, *Palinurus elephas* (Goñi *et al.*, 2006, 2010), and the squat lobster, *Pleuroncodes monodon* (Roa and Bahamonde, 1993). It must also be noted that the current study undertakes a binomial logistic regression approach to data analysis, not utilized by Wootton *et al.* (2012). This is advantageous in terms of determining detailed population ecology by examining interactive forces and parameters at a more complex level and is likely to more realistically reflect the status of the lobster population sampled as it accounts for multiple factors that may determine disease.

The fact that those lobsters over the MLS were more likely to be shell diseased was to be expected. Studies have shown that larger lobsters have longer moult increments (therefore moult less often) than smaller, younger lobsters (Castro and Angell, 2000), giving more time for shell disease and lesion progression to manifest (Smolowitz *et al.*, 1992; Glenn and Pugh, 2006). Shell disease levels are also usually higher in females, due to the increased moult increments when holding eggs (Glenn and Pugh, 2006). Our result, that there was a higher probability of shell disease in males, therefore was somewhat unexpected. This was perhaps because <1% of females sampled were ovigerous in this study. It could also be speculated that males are more likely to take part in conspecific interaction than females, due to intrasexual selection and mate protection (Debuse *et al.*, 2003), therefore more likely to be injured, which in turn may develop into shell disease. Injury presence was indeed a significant factor in predicting shell disease in the current study. In order for chitinolythic bacteria to enter and progress the development of shell disease, a breached cuticle is required so that the chitin containing layers beneath the outermost epicuticle may be reached (Smolowitz *et al.*, 1992; Vogan *et al.*, 1999, Davies *et al.*, 2014, Whitten *et al.*, 2014). Injury can therefore facilitate the initiation and progression of shell disease.

Although non-significant, results suggested that lobsters with claw loss were less likely to have shell disease than those with both claws intact. This may be explained by studies that have shown that decapod crustaceans experiencing claw loss moult more frequently in an attempt to regenerate the lost appendages (Skinner and Graham, 1972). This frequent moulting will in turn temporarily rid a lobster of shell disease (Smolowitz *et al.*, 1992; Glenn and Pugh, 2006).

Injury presence was found to be significantly higher in those lobsters over the MLS. Species of homarid lobsters are known to be solitary, highly territorial, and establish hierarchical ranks within

populations (Karnofsky *et al.*, 1989; Childress and Jury, 2006; Skog, 2009); they therefore may suffer from combative injuries when faced with intrasexual selection and shelter choice (O'Neill and Cobb, 1979; Debuse *et al.*, 2003; Skog, 2009). This will be especially prevalent when they are larger and sexually mature (Karnofsky and Price, 1989), such as those found above the MLS. The finding that the probability of injured lobsters in the NTZ was higher than the RZ may be explained by the aforementioned higher density of lobsters in the NTZ. Some studies have shown that when a population reaches high densities, there is an increase in conspecific interaction (Lizaso *et al.*, 2000), competition, and therefore fighting and injury (Castro *et al.*, 2012). This, in conjunction with the more abundant, and larger lobsters found in the NTZ, means that conflict would be especially increased, leading to injury.

The increase in injured lobsters in the NTZ may increase the risk of infection from other pathogens such as *A. viridans* var. *homari*. This bacterium lacks invasive mechanisms and therefore is likely to enter the lobster only through a damaged carapace, causing septicaemia that eventually results in death (Stewart, 1980). The low prevalence of *A. viridans* var. *homari* detected in the present study is comparable with other wild population studies, which found gaffkaemia in $<1\%$ of *H. gammarus* from UK (Stebbing *et al.*, 2012) and Norwegian (Wiik *et al.*, 1987) waters. It has been noted, however, that levels of disease in wild populations are probably underestimated as infected lobsters are lethargic and therefore less likely to enter traps (Lavallée *et al.*, 2001). It is also important to note that there are "virulent" and "avirulent" strains of *A. viridans* var. *homari* (Stewart *et al.*, 2004); therefore, perhaps the causative agent may lie dormant in sediment until the optimum temperature is reached (Stewart *et al.*, 1969).

Current diagnostics for identification of gaffkaemia are based on time-consuming methods (see Stebbing *et al.*, 2012) whereby haemolymph must be examined or cultured immediately. The technique developed in this study, utilizing PCR-based methods, whereby as little as 1×10^3 CFU ml^{-1} could be detected, has potential to be more effective since haemolymph may be stored in ethanol for an extended period before DNA extraction and screening.

Although there were no WSSV-infected lobsters found in this study, the European Union EC Directive 2006/88/EC states that all decapod crustaceans are susceptible to this viral infection (EC Directive, 2006). Recent studies have shown that both *H. gammarus* (Bateman *et al.*, 2012) and *H. americanus* (Clark *et al.*, 2013) may be susceptible to this disease. The phenomenon of over-population and disease is common in aquaculture systems where diseases must be monitored diligently (Pillay and Kutty, 2005), since populations grow rapidly and unlike "wild" ocean scenarios, the diseased individuals cannot migrate elsewhere or be "fished out" (Wood *et al.*, 2010). Although originating in Asia where waters are warmer, WSSV is not a tropical disease and studies have shown WSSV infection in water temperatures as low as 15°C (Guan *et al.*, 2003), with WSSV replication possible at 10°C (Du *et al.*, 2008). WSSV-infected shrimp have also been found in European shrimp farms (Stentiford and Lightner, 2011), and given the mean summer temperature of UK waters can be as high as 17.9°C (Cefas, 2014), it is viable that WSSV may enter and reside in UK waters. Thus, threat from this disease is plausible and reinforces the need for monitoring, especially in an area such as the Lundy Island NTZ, where population density is thought to have increased the risk of injury, apertures for pathogen entry (current study), and prevalence of shell disease (Wootton *et al.*, 2012).

Appropriately managed MPAs have been shown to increase low population numbers, satisfying both fishing industry and environmental stakeholders, especially those implemented and monitored over longer periods. For example, Goñi *et al.* (2010) showed density spillover of European spiny lobster (*P. elephas*) into adjacent fisheries using a decade of tag re-capture data, Aburto-Oropeza *et al.* (2011) recorded a 463% increase in fish biomass over a decade of no take protection, while in Norway, designation of an MPA increased *H. gammarus* CPUE by 245% over 4 years, compared with just 87% in control areas (Moland *et al.*, 2013a).

Of late, the subject of implementation of MPAs in the UK has caused much controversy (Jones, 2012; Rees *et al.*, 2013), and often their efficacy is up for debate worldwide (Roberts *et al.*, 2001; Caveen *et al.*, 2013). The Lundy Island MCZ was created to meet unspecified conservation benefits rather than verifiable management objectives (Hoskin *et al.*, 2009). The lack of pre-designation data from this MCZ prevents a true historical assessment of changes in population sizes or the spread and evolution of shell disease since the fishery closure, therefore, promotes a call for further, long-term monitoring studies to take place. This study highlights the necessity to monitor MCZs both before and after implementation, with a requirement for regular, standardized sampling protocols to discern and monitor the health status of species within NTZs and other types of MPA. Data collected can be used to make predictions for future management scenarios, and ultimately better manage the area under protection. This means that under changing environmental conditions, managers are better equipped at predicting resultant effects on the reserve and nearby fisheries, in some cases mitigating negative impacts such as injury, and potential increases in disease.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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