Factors contributing to the disease ecology of brown crab (*Cancer pagurus*) in a temperate marine protected area.

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ABSTRACT

Marine ecosystems are affected by multiple, well-known stressors like fishing and climate change, but a less documented concern is disease. Marine reserves have been successful in replenishing stocks and aiding recruitment but studies have shown that high population abundances in marine reserves may lead to unwanted secondary effects such as increase in predators and competition, altering trophic webs, and disease. Here, we investigate factors contributing to disease prevalence in a brown crab (*Cancer pagurus*) population around Lundy Island (the UK's first MPA) after 7 years of no-take protection. Population parameters (size, sex, and abundance), disease (shell disease, *Hematodinium* spp. infection) and injury presence (a known precursor to some disease conditions) were assessed over two years in both fished and unfished areas of the MPA. We found no significant difference in prevalence between the disease prevalence in fished and unfished areas, however overall, the number of injured crabs increased significantly over the two years (12%), as did the prevalence of shell disease (15%). The probability of crabs having shell disease increased significantly in male crabs, and in those missing limbs. The probability of crabs being injured increased significantly in crabs below the minimum landing size. In terms of population parameters, crabs were more prevalent in the fished area compared to the unfished area, thought to be a result of an increase in the predatory European lobster. The findings of the present study highlight potential secondary community changes as a result of MPA implementation. Therefore, surveillance for such changes, as part of MPA management, would provide useful information on the health and overall function of the protected ecosystem.

Key Words:

Crustacean fisheries, disease ecology, endoparasites, marine conservation zone, marine management, monitoring, no take zone, population density, dinoflagellates

INTRODUCTION

Numerous commercial fish stocks have been overexploited and many continue to be fished at unsustainable levels (Jackson et al. 2001, Steadman et al. 2014). This is leading to both a loss of biodiversity and significant concerns for global food security (Pauly et al. 2005, Worm et al. 2009). Conservation areas in marine ecosystems, such as marine protected areas (MPAs) are one management tool that can be used to help reduce fished species decline, aid in stock replenishment and conserve habitats of special interest (Halpern & Warner, 2002, Aburto-Oropeza et al. 2011).

Closure of areas to fishing may, however, result in secondary effects such as overpopulation of a species, in turn altering natural local community compositions (Leo & Micheli 2015, Wood & Lafferty 2015). In some protected areas, changes in species assemblages and population abundance have been shown to have negative effects due to overcrowding such as disease increase, reduction in habitats and change in food web structure due to changes in competition or predation rates (Lebarbenchon et al. 2006, Wootton et al. 2012, Wood et al. 2013, Christianen et al. 2014). With increased abundance comes an increase in both conspecific (within species) and heterospecific (between species) interactions, which can result in injury and limb loss in individuals, contributing to disease in some species (Davies et al. 2015). Disease can influence community composition, age distributions, trophic interactions and biotic structure within a population (Harvell et al. 2002). In some cases, disease is also thought to be exacerbated by the emerging threat of climate change (Burge et al. 2014, Maynard et al. 2016) and can be a useful indicator of ecosystem health (Harvell et al. 1999). The prevalence and distribution of pathogens and disease in marine ecosystems is growing globally (Ward & Lafferty 2004), has been reported across a wide range of taxa over the past three decades (Harvell et al. 2004) and is seen as an often neglected, but emerging field (Lafferty & Hofmann 2016).

Cancer pagurus, the edible or brown crab, is an important European fisheries species, with global landings of over 32,000 tonnes in 2014. This species is, however, susceptible to a range of pathogens (Stentiford 2008), the most documented of which, that have been known to cause significant economic and population losses, are pink crab disease and 'black spot' or shell disease. Pink crab disease, in some species referred to as bitter crab disease, is caused by the endoparasitic dinoflagellate, *Hematodinium* spp., named as such because of the hyperpigmentation and bitter taste exhibited by some heavily infected species (Wilhelm & Mialhe 1996, Stentiford et al. 2002, Ryazanova 2008). Chatton & Poisson (1931) first reported the disease in France in both harbour *Liocarcinus depurator* and shore crabs *Carcinus maenas*. It has since been found to infect over 40 species of decapod crustaceans worldwide, and because infected animals become unmarketable due to poor muscle quality, *Hematodinium* spp. infections have had huge economic impacts on commercial fisheries (Field et al. 1992, Wilhelm & Mialhe 1996, Shields et al. 2005, Stentiford & Shields 2005).

'Black spot' or 'Burn Spot' disease, herein referred to as shell disease, is characterised by melanised lesions that can progress to erode through the carapace, exposing underlying soft tissues in the infected individual (Vogan et al. 2008). Mortality occurs due to secondary infection by opportunistic bacteria (Baross et al. 1978, Vogan & Rowley 2002), or during a moult if old and new shells adhere at the lesion site (Fisher et al. 1978). Shell disease has become prevalent in many UK *C. pagurus* and European lobster *Homarus gammarus* fisheries since it was first reported by Pearson (1908). The Isle of Man (UK) fishery displayed shell disease in almost 25% of *C. pagurus* sampled during the summer of 2012 (King et al. 2014), and in a South Wales (UK) fishery, over 50% of *C. pagurus* were affected in 1997-1998 (Vogan et al. 1999). Between 1985-1987, the West coast of Scotland fishery displayed shell disease in almost 100% of individuals, significantly higher than in other crab species analysed in the same survey (Comely & Ansell 1989). Ayres and Edwards (1982)

reported 5-7% of affected animals were rejected from a South West Irish fishery and suggested that the incidence of shell disease was higher in lightly fished populations than in established fisheries, where intensive exploitation results in the removal of larger, older crabs from the stock.

The aim of the present study was to examine factors contributing towards these two diseases in a population of *C. pagurus* in both a fished and un-fished area of the Lundy Island MPA in the Celtic Sea, UK. In the past, this MPA has been reported to have higher levels of disease in the unfished area due to increased population density, or overcrowding (Wootton et al. 2012, Davies et al. 2015). Therefore, it was first hypothesized that the population abundance of *C. pagurus* would be higher in the un-fished area of the MPA compared to the fished area, as observed in a previous study (Hoskin et al. 2011). The second hypothesis was that the crabs from the un-fished area would have an increased probability of injury, limb loss and therefore disease than individuals from the fished area due to overcrowding. Individuals missing one or more limbs, or with open wounds are expected to have a higher probability of disease than those with limbs intact, as limb loss creates a large wound, which can act as an aperture for pathogen entry. Our third hypothesis was that, as experienced in previous studies, the unfished area would have a higher prevalence of large individuals (herein classified as individuals over the minimum landing size; MLS ≥ 160 mm carapace width for males and ≥ 140mm for females) which would in turn exhibit an increased probability of disease and injury than smaller individuals (those under MLS) as experienced for some infections by Bateman et al. (2011). In larger animals, longer inter-moult periods give disease more time to manifest and competition brought on by sexual maturity has also been shown to increase in older, larger crustacean species (Edwards 1966).

MATERIALS AND METHODS

123

124 **Study area**

125 The study took place around Lundy Island, 12 miles off the coast of North Devon,
126 England, UK (Fig. 1), which was Britain's first MPA. Lundy first consisted of a Refuge Zone
127 (RZ), established in 1986 when it was designated as Britain's first and only Marine Nature
128 Reserve. Here, pot fisheries were authorised, but trawl and net fisheries were prohibited. In
129 2003, A No-Take Zone (NTZ) was incorporated, where all fishing, including potting, and
130 removal of wildlife is forbidden. Lundy Island became Britain's first Marine Conservation
131 Zone in 2010.

132

133 **Population sampling**

134 The *C. pagurus* population around Lundy Island was sampled in May and July 2010,
135 and August 2011. One string of baited commercial parlour pots (35 pots with escape gaps
136 closed) was deployed at a total of 6 sampling sites (4 in the fished RZ and 2 in the unfished
137 NTZ, Fig. 1). In total, 30 strings were deployed in 2010 and 18 in 2011 with a total of 397
138 crabs sampled (213 in 2010 and 184 in 2011). Each string was similarly baited, immersed for
139 24h, retrieved and emptied of all catch. Seven measurements were recorded for each *C.*
140 *pagurus* caught (Table 1). In order to assess the presence of *Hematodinium* spp. using
141 molecular methods, *ca.* 500-700 µl of haemolymph was drawn into 1 ml of 100% analytical
142 grade ethanol using 23 g needles and 2 ml syringes. Crabs exhibiting exoskeletal
143 abnormalities or severe shell disease were photographed (see Figure 2 for examples of these
144 conditions). All individuals were measured, traps were re-baited, re-deployed and all catch
145 returned to the water.

146

147 **Surveillance of haemolymph pathogen communities**

148 ***DNA extraction***

149 DNA was extracted from haemolymph using a modified version of Ivanova et al.
150 (2006, see Section S1, Supplementary Materials). DNA was eluted with water, stabilized with
151 Tris-EDTA buffer (10 X) and used as the template for polymerase chain reactions (PCR).
152 Haemolymph DNA extraction was optimized to ensure detection of *Hematodinium* spp. by
153 using known, positive controls initially derived from *C. pagurus*, confirmed by sequencing.

154

155 ***PCR conditions***

156 All PCR were carried out using primers synthesized by Eurofins MWG Operon
157 (Ebersberg, Germany) and performed on a Bio-Rad PTC-100 Peltier Thermal Cycler before
158 being visualized on a 1.5% agarose gel. First, decapod-specific primers were used to verify
159 the quality of the extracted DNA and the integrity of the PCR reaction (Section S2,
160 Supplementary Materials). DNA was then amplified using *Hematodinium* spp. specific
161 primers optimized by Hamilton et al. (2009) in order to test haemolymph DNA for the
162 presence of *Hematodinium* spp. infection (Section S3, Supplementary Materials).
163 *Hematodinium* spp. positive samples were repeated, the PCR product cleaned up using the
164 Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA) and sequenced by
165 Eurofins MWG Operon (Ebersberg, Germany). Contigs from sequences were created using
166 the CAP3 sequence assembly programme (Huang & Madan 1999) and identity confirmed
167 using matched positive controls via NCBI BLAST.

168

169 **Statistical Analysis**

170 ***Dataset Determination***

171 Data from May and July 2010 were pooled so that one coherent year was used to
172 compare with the 2011 catch data (see Table S1, Supplementary Materials). To minimize the

173 possibility that individuals from May and July sampling trips were not double sampled (i.e.
174 released in May, recaptured in July) each individual in the database was given a unique
175 identifier based on the predictor variables in Table 1 and any individuals sharing the identifier
176 were noted. The number of potential recaptures was 11.61%. In order that individuals were
177 not double sampled on consecutive sample days within each month (i.e. recaptured after day
178 one of a survey and considered a unique individual), the same method was used and 0.36% of
179 individuals were classified as potential recaptures and removed.
180
181 ***Population Ecology***
182 Population distributions of males and females were visualised in GraphPad Prism 5.0, plotted
183 per site and year. In order to compare catch and size-frequency data between the NTZ and RZ,
184 data were first tested to follow a normal distribution (using two-sample Kolmogorov-
185 Smirnov tests) followed by either an independent T test (if data was normal) or Mann-
186 Whitney test (if data did not follow a normal distribution). Tests were two-tailed and used a
187 significance level of 0.05. Catch Per Unit of fishing Effort (CPUE) was calculated as the
188 mean number of animals per pot. A linear regression was used to examine the
189 relationship between the CPUE of *C. pagurus* and European lobster, *H. gammarus*, the
190 two commercially viable species found in the pots.
191
192 ***Disease and Injury Ecology***
193 Binomial logistic regression models with Logit link functions (following Bernoulli
194 distributions) were used (MASS library, R Development Core Team 2014) to determine
195 whether specific predictor variables (Table 1) had a significant effect on the presence of shell
196 disease, *Hematodinium* spp. infection and injury presence in the crab population sampled.
197 The information theoretic approach was used for model selection and assessment of

198 performance (Richards 2005). To begin, a suite of models ranging from fully additive to
199 models combining all possible combinations of single, interactive terms were generated using
200 the predictor variables highlighted in Table 1. The best models from each suite were selected
201 based on Akaike's Information Criterion (AIC) which measures model "quality" based on the
202 goodness of fit and parsimony of the model: the lower the AIC, the better the model
203 (Burnham & Anderson 1998, Zuur et al. 2009). Selected initial models are herein referred to
204 as the *full models*. Once selected, each non-significant predictor variable from the full models
205 was sequentially removed using the drop1 function (in R) to produce final models with
206 increased predictive power, herein referred to as the *reduced models*. The drop1 function
207 compares the initial full model with the same model, minus the least significant predictor
208 variable. If the reduced model is significantly different from the initial full model (in the case
209 of binomial response variables, a Chi-squared test is used to compare the residual sum of
210 squares of both the models), then the removed predictor variable is kept out of the new,
211 reduced model. This process continues hierarchically until a final reduced model is produced
212 (Zuur et al. 2009). Fitted probability plots were used to visualize the significant relationships
213 inferred from the reduced models using carapace width (CW) as the independent variable.
214 The probability of a crab having shell disease, *Hematodinium* spp. infection or being injured,
215 was calculated using the following equation:
216
217 *Equation 1:* $\rho = 1/(1 + exp^{-\beta x})$
218
219 Where ρ is the probability of shell disease, *Hematodinium* spp. infection or injury presence
220 and βx is the estimate for the predictor variable analysed (Table 1).
221
222 **RESULTS**

223

224

225 **General population ecology**

226 Comparative analysis between the two sites (fished; RZ vs. un-fished; NTZ) revealed
227 that there were significantly more crabs caught per string in the RZ than in the NTZ in both
228 the 2010 and 2011 surveys (2010: P = 0.003, t = 3.62, NTZ = 6 ± 1.86, RZ = 38 ± 9.82;
229 2011: P = 0.002, t = 2.97, NTZ = 0.83 ± 0.83, RZ = 22.38 ± 6.19 [mean values ± SEM], Fig.
230 3). The CPUE was 6.3 times greater in the RZ than in the NTZ in 2010 and 26.9 times greater
231 in 2011.

232 There was no significant difference in the number of crabs above the MLS caught
233 between the NTZ and RZ in either 2010 or 2011(P > 0.05, see Table 2 and Fig. 5A-D). Not
234 accounting for the sex of crabs, the size frequency distributions were significantly different
235 between the NTZ and RZ in 2010 (P = 0.005) and 2011 (P < 0.001). There was, however, no
236 significant difference between the size of crabs in the NTZ and the RZ in 2010 (P = 0.205) or
237 2011 (P = 0.077), even when separating crabs by sex in 2010 (males P = 0.249, females P =
238 0.488, Fig. 5A-D). Due to the low abundance of crabs caught in the NTZ in 2011 (n male = 4,
239 n female, = 1), size differences between zones could not be tested. No ovigerous ('berried')
240 females were caught in either year from either zone.

241

242 **Shell Disease**

243 Comparative analysis between the two sites (fished; RZ vs. un-fished; NTZ) revealed
244 that there was no significant change in shell disease prevalence between sites. However,
245 when combining data from both sites, analysis highlighted that the percentage of shell
246 diseased crabs increased significantly by *ca*.15% between 2010 and 2011 (P = 0.002, Table
247 2). There was no significant effect of site, limb loss, landing size, injury, sex or

Hematodinium spp. infection in explaining shell disease presence in 2010 (Table S2, Model S1). Sex, limb loss, and the interaction between *Hematodinium* spp. and sex (infected male crabs), all had a significant effect on the presence of shell disease in 2011 (Table 3). If a crab was male, or a crab was missing a limb, the probability of it having shell disease increased by 87% and 74% respectively (Fig. 6). Crabs which were male and harboured *Hematodinium* spp. were 2% **less** likely to exhibit shell disease.

Presence of *Hematodinium* spp

Comparative analysis between the two sites (fished; RZ vs. un-fished; NTZ) revealed that there was no significant change in *Hematodinium* spp. prevalence between sites. However, only 19 out of 397 individuals tested positively for *Hematodinium* spp. (Table 2). None of the predictor variables were significant in predicting the presence of *Hematodinium* spp. in 2010 (Table S2, Model S3). Sex had a significant effect in determining *Hematodinium* spp. infection in 2011 (Table S2, Model S4) but this proved marginally non-significant following model reduction (Table 4).

Injury

Comparative analysis between the two sites (fished; RZ vs. un-fished; NTZ) revealed that there was no significant change in injury prevalence between sites. However, when combining data from both sites, analysis highlighted that the percentage of injured crabs (examples of which can be seen in Fig. 2E-G) increased significantly by *ca*. 12% between 2010 and 2011 ($P < 0.001$, Table 2).

In 2010, landing size, site and sex were not significant in predicting the presence of injury (Table S2, Model 5), even after model reduction, and therefore no reduced model was produced. In 2011, although none of the predictor variables significantly predicted the

273 presence of injury in the full model (Table S2, Model S6) after model reduction, landing size
274 was significant in predicting injury presence (Table 5). Crabs below the MLS in 2011 had an
275 11% higher probability of being injured than those above the MLS (Fig. 7).
276
277 **DISCUSSION**
278 Overall, more crabs were caught in the RZ compared with the NTZ in both years,
279 rejecting our first hypothesis. Our second hypothesis was also rejected (crabs from the un-
280 fished NTZ would have an increased probability of injury and therefore disease than
281 individuals from the fished RZ). Shell disease increased from 2010 to 2011 in line with injury.
282 However, only if a crab was male or missing a limb, did the probability of it having shell
283 disease increase in 2011. Crabs below the MLS in 2011 had a higher probability of being
284 injured than those above the MLS, rejecting the third hypothesis that larger animals would
285 have higher disease/injury.
286 **Hypothesis 1.** The population ecology observed in this study contrasted to that
287 of a previous survey of *C. pagurus* around Lundy Island MPA. In a 4-year survey from 2003-
288 2007, Hoskin et al. (2011) described higher abundances, and larger *C. pagurus* within the
289 NTZ than the RZ. This contrasts to the current results from 2010 and 2011 in which we found
290 higher abundances of *C. pagurus* in the RZ. There are various possible explanations for this
291 finding. *C. pagurus* migrate up to 345 m day^{-1} in order to avoid predators, competition from
292 conspecifics, and to mate or find brooding sites (Ungfors et al. 2007, Hunter et al. 2013), it is
293 therefore plausible that the low crab abundance described in 2011 is due to natural movement
294 of populations out of the NTZ into adjacent areas. However, in addition, density dependent
295 habitat selection whereby an increased abundance of one species may change the dynamics of
296 another has been described in detail by many authors (e.g. Breen & Mann 1976, Morris 2003,
297 Acheson & Gardener 2014). European lobsters, *H. gammarus*, were also caught in the pots

298 sampled during this study (further discussed in Davies et al. 2015) and it is noteworthy that as
299 the abundance of lobsters per string increased, the abundance of crabs decreased significantly
300 (2010: $R^2 = 0.537$, $F_{1,\ 14} = 16.22$, $P = 0.0012$, 2011: $R^2 = 0.437$, $F_{1,12} = 9.320$, $P = 0.01$; Fig.
301 4). Although studies have shown that if a larger predator is present in a pot, other animals
302 will not enter as willingly (Lovewell et al. 1988, Miller & Addison 1995), there is also
303 evidence to that low *C. pagurus* abundances may be driven by increased abundances of the
304 European lobster, *Homarus gammarus,* found in the NTZ (Wootton et al 2012, Davies et al.
305 2015). This phenomenon was also observed by Howarth et al. (2016) who noted that in a
306 fully protected MPA in Scotland, the greater densities of large adult lobsters appeared to be
307 predating and/or competitively displacing juvenile lobsters, *C. pagurus*, and velvet swimming
308 crabs (*Necora puber*) from the area. This highlights community changes as a result of MPA
309 implementation which may not always be positive, and that recovery is not straightforward,
310 since the recovery of some species can have knock-on effects on others. As one species
311 benefits from implementation (in this case, the apex predator, *H. gammarus*), others (i.e. *C.*
312 *pagaurus*) can be detrimentally affected, altering overall ecosystem function.

313 **Hypothesis 2.** Crabs were no more likely to be found with injury, limb loss or disease
314 in the un-fished NTZ compared to the fished RZ as has been found in previous studies. This
315 is likely due to higher population density in the RZ and interactions with other species. The
316 higher levels of shell disease found in males compared to females (in both sites combined) is
317 likely caused by increased male-male interaction and resultant injuries during the mating
318 season (the time of our study). Male crabs engage in agonistic behaviour with conspecifics
319 due to their territorial and competitive nature, increasing the risk of injury (Schöne 1968,
320 Vogan et al. 1999) limb loss and therefore disease (Vogan et al. 2008). Lost limbs create a
321 large aperture for haemolymph loss, tissue exposure and pathogen entry, in addition to an
322 increased risk of shell disease lesion initiation stemming from the fracture site. This may

323 therefore account for shell disease presence being higher in those crabs that had lost one or
324 more limbs.

325 **Hypothesis 3.** The unfished NTZ in Lundy was not found to harbour larger crabs than
326 in the fished, RZ as previously noted by Hoskin et al. (2011). Similarly, larger crabs (>MLS)
327 were not found to exhibit more injury and disease as previously found in some crustacean
328 species (Bateman et al. 2011, Wootton et al. 2012, Davies et al. 2015). In contrast, the
329 probability of injury increased significantly in both male and female crabs **below** the MLS. *C.*
330 *pagurus* above the MLS are sexually mature and potential injury above this size in theory is
331 likely to increase due to conflict from protection and display during mating (Edwards 1966).
332 However, one explanation for the increased injury in individuals below the MLS found in the
333 present study, could be due to competitive interactions with the significantly more abundant,
334 larger and more aggressive predator, the lobster, *H. gammarus*. Another explanation could be
335 injury from species on which *C. pagurus* feeds. *C. pagurus* is an active predator that
336 consumes a variety of crustaceans including the green shore crab *Carcinus maenas*, which is
337 an aggressive and territorial species (Kaiser et al. 1990). *C. maenas* has been shown to
338 contribute to injury in its predators especially if the predators are smaller and inexperienced
339 hunters (Rossong et al. 2006), and this could include small *C. pagurus* (below the MLS).

340 Overall, there were very few crabs caught infected with *Hematodinium* spp. over both
341 years sampled. Similarly, low numbers have been described in other studies of wild *C.*
342 *pagurus* populations (Chualáin et al. 2009) and may be explained by the observation that
343 *Hematodinium* spp. in *C. pagurus* is mainly found in smaller or juvenile crabs which live
344 closer to the shoreline than the areas sampled in this study (Stentiford 2008, Chualáin et al.
345 2009). Therefore, most crabs surveyed in the current study were adults (even though escape
346 gaps on sampling pots were closed). Due to the low numbers of *Hematodinium* spp. infected
347 individuals found in this study, it would be beneficial in future studies to survey closer to

348 shorelines and modify traps to restrict larger crab and lobster entry. This would allow for a
349 better census of juveniles, who are more likely to display the disease.

350 The current study only provides a 'snap shot' of population abundance and disease
351 ecology. It does, however, reiterate the importance of disease monitoring within both fished
352 and protected populations, and especially those that have shown significant increases in local
353 abundance. Long-term monitoring studies of MPAs (those observed over a number of years)
354 have revealed strong patterns, including spillover into adjacent fisheries and significant
355 increases in abundance (Abesamis & Russ 2005, Stobart et al. 2009, Aburto-Oropeza et al.
356 2011) that may not be possible to detect during small-scale studies such as this. It would
357 therefore be beneficial to monitor MPAs such as Lundy for longer periods, accounting for
358 natural variability driven by external factors such as adjacent fisheries, natural competition
359 and climate anomalies. At present, disease monitoring does not appear to be a priority in the
360 implementation and management of protected areas. By monitoring disease, managers can be
361 better prepared to deal with any unwanted consequences of fisheries closures such as
362 potential increases in disease and consequent population decline. Better monitoring will allow
363 pre-emptive management measures to be taken. An example of this could be the re-opening
364 of closed areas for limited fishing of certain species, before levels of abundance become high
365 enough to contribute to disease. This would allow real-scale tests of 'fishing out' disease, or
366 help strike a balance between allowing enough fishing to keep populations healthy without
367 interfering with explicit conservation aims (McCallum et al. 2005, Wood et al. 2010).

368 To effectively and sustainably manage, exploit and conserve marine populations, it is
369 imperative to monitor both the prevalence and geographical range of important marine
370 pathogens, especially those affecting keystone and species of commercial interest (McCallum
371 & Dobson 1995, Lamb et al. 2016). This is particularly pertinent for protected areas, where
372 detrimental secondary community effects have been shown to occur. Robust monitoring

373 programmes in such areas, covering a range of species and variables, would assist in
374 achieving conservation aims and allow management strategies to be adjusted according to
375 local ecological change.
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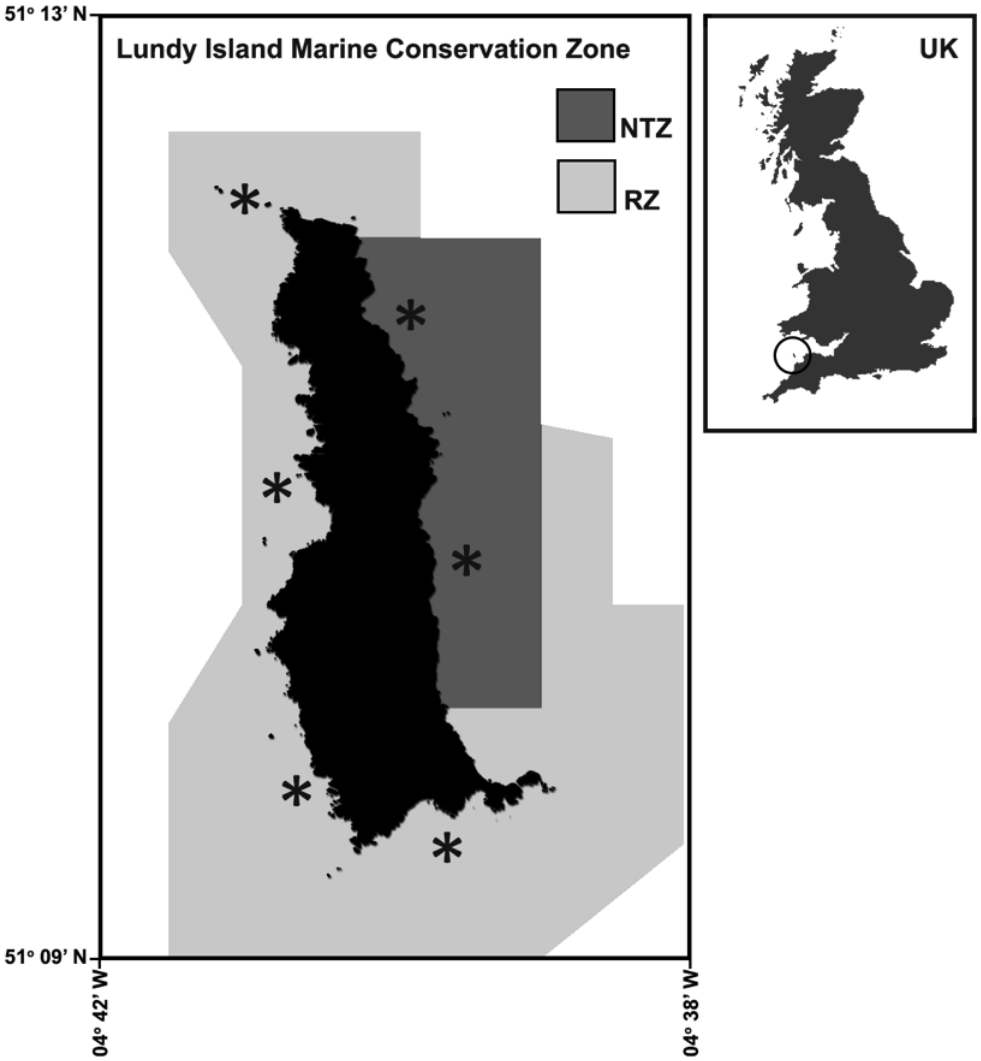


615

616 **Figure 1.** Map showing the Lundy Island MPA Marine Conservation Zone and sampling
617 sites (*) at which strings of pots were deployed within the NTZ and RZ. Lundy's position
618 relative to the UK is circled in the smaller map. Adapted from Davies *et al.* (2015).

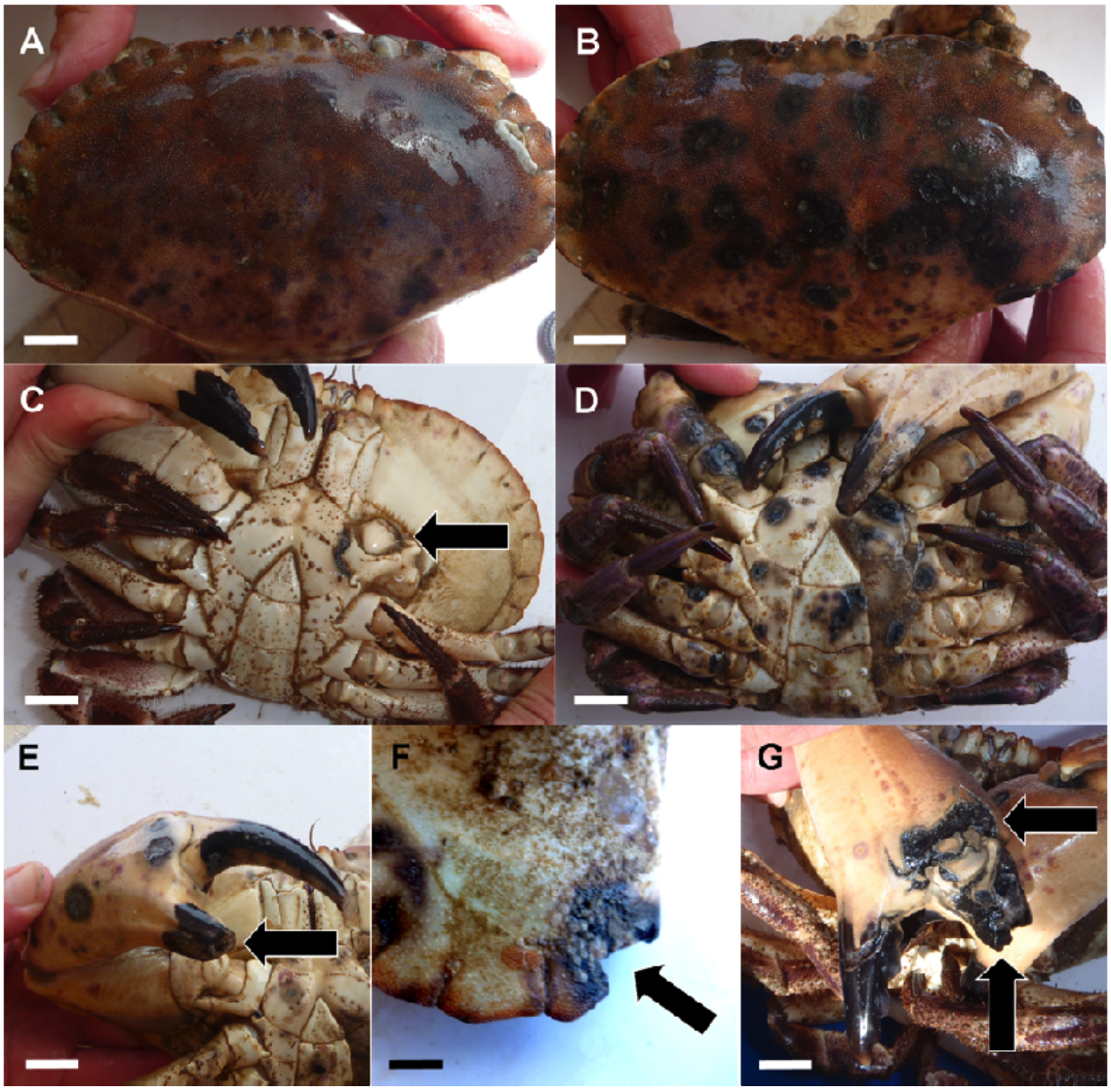
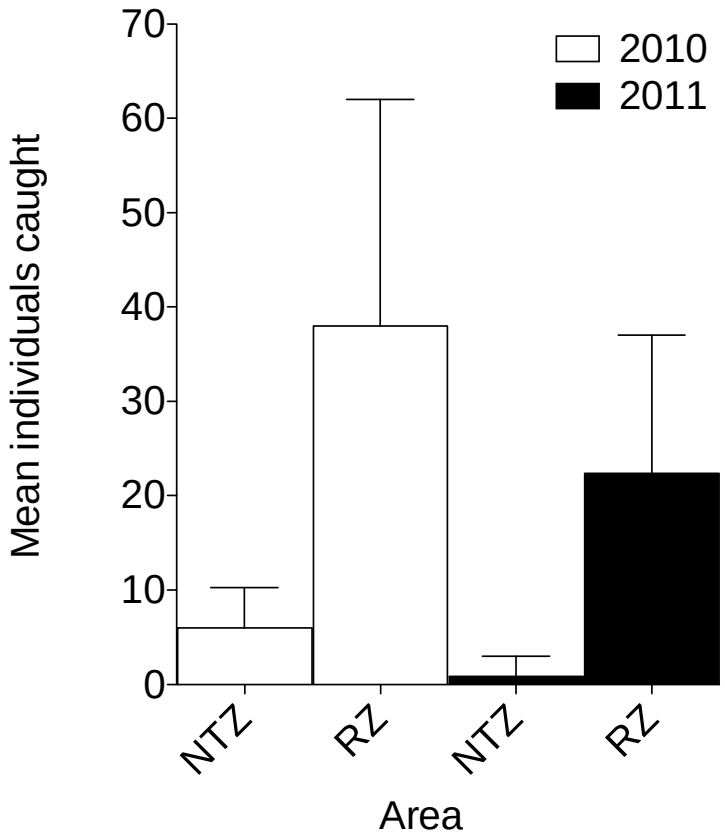


Figure 2. Examples of *C. pagurus* displaying a healthy (A) and shell diseased (B) dorsal carapace, a healthy (C) ventral carapace with limb loss (black arrow), a shell diseased ventral carapace (D) and (E-G) examples of injury at various sites (black arrows): (E) the propodus region of a chela, missing, (F) edge of carapace and, (G) the propodus region of a chela. Scale bars represent 2 cm.



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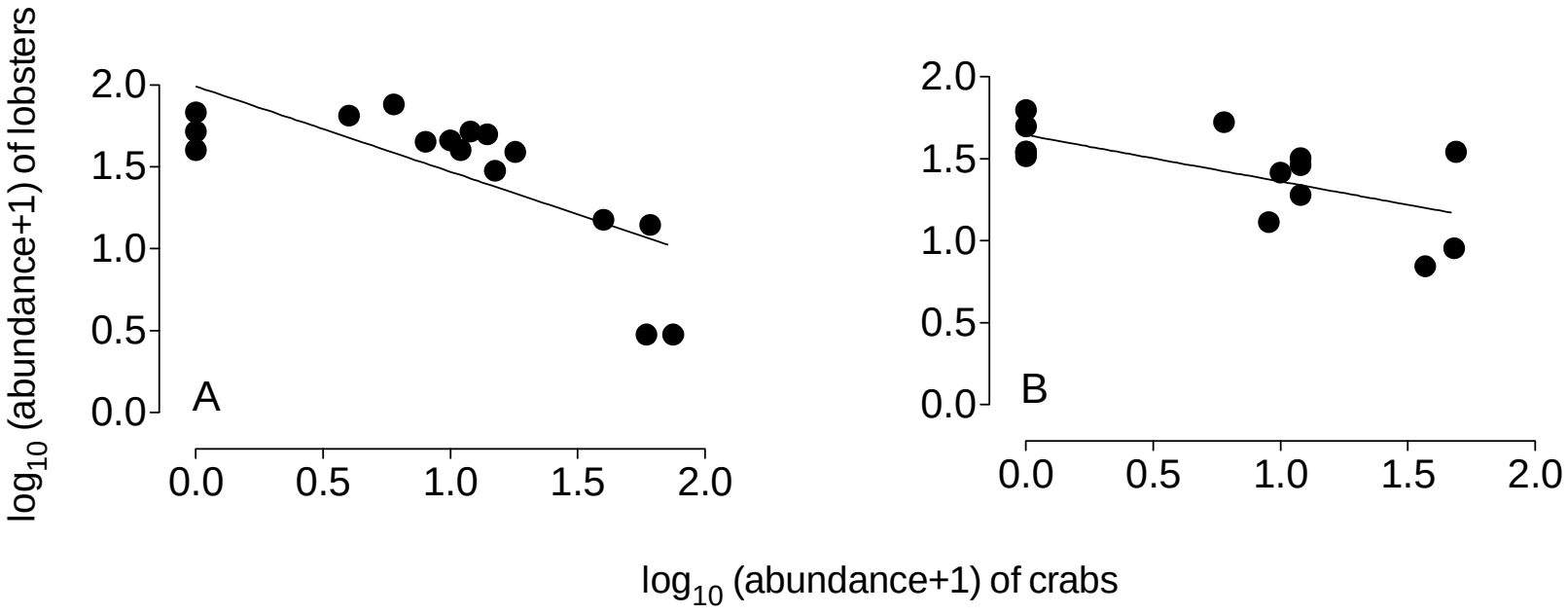
627

628 **Figure 3.** Mean number of edible crabs (with 95% CI) caught per 35-pot string in the No-

629 Take Zone (NTZ) and Refuge Zone (RZ) in 2010 and 2011.

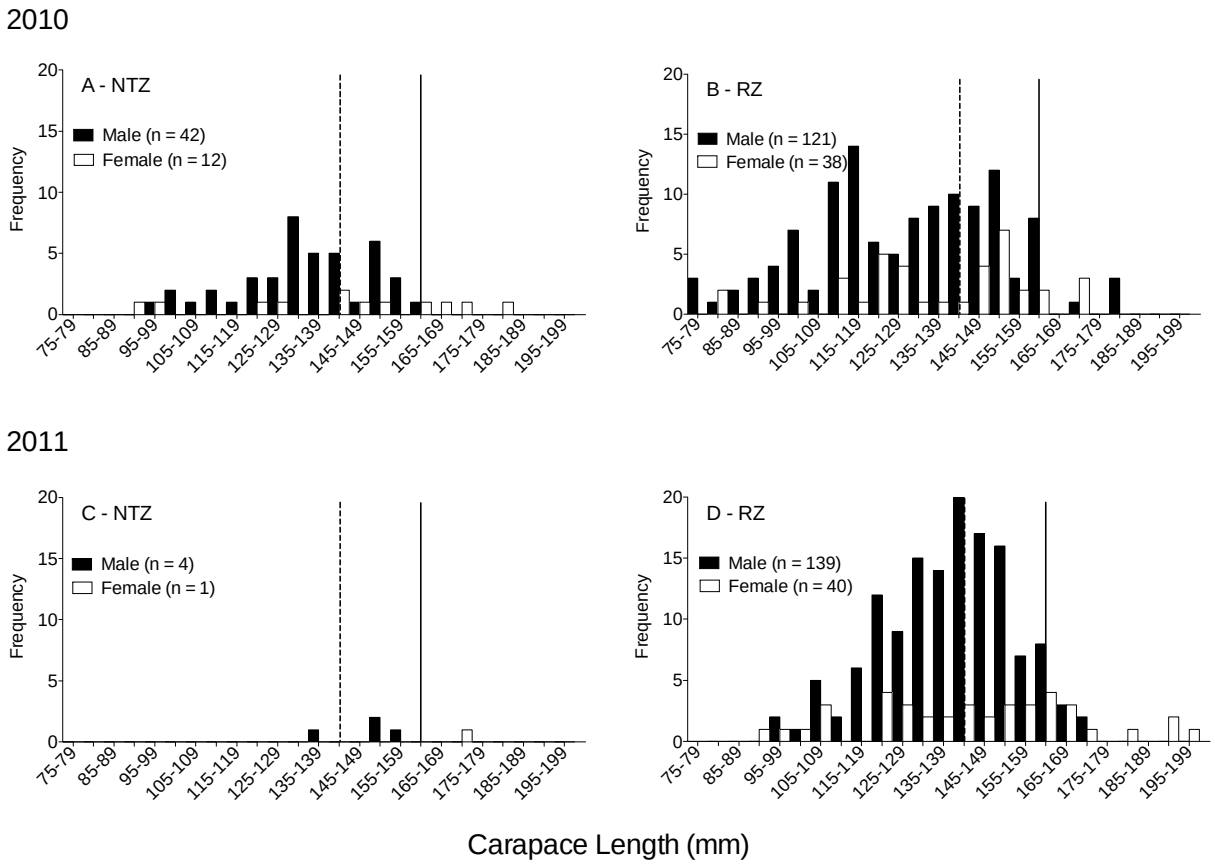
630



631

632 **Figure 4.** Linear regression showing the abundance of lobsters plotted against the abundance of crabs per string in (A) 2010 and (B) 2011. Each

633 point represents one string of 35 parlour pots.

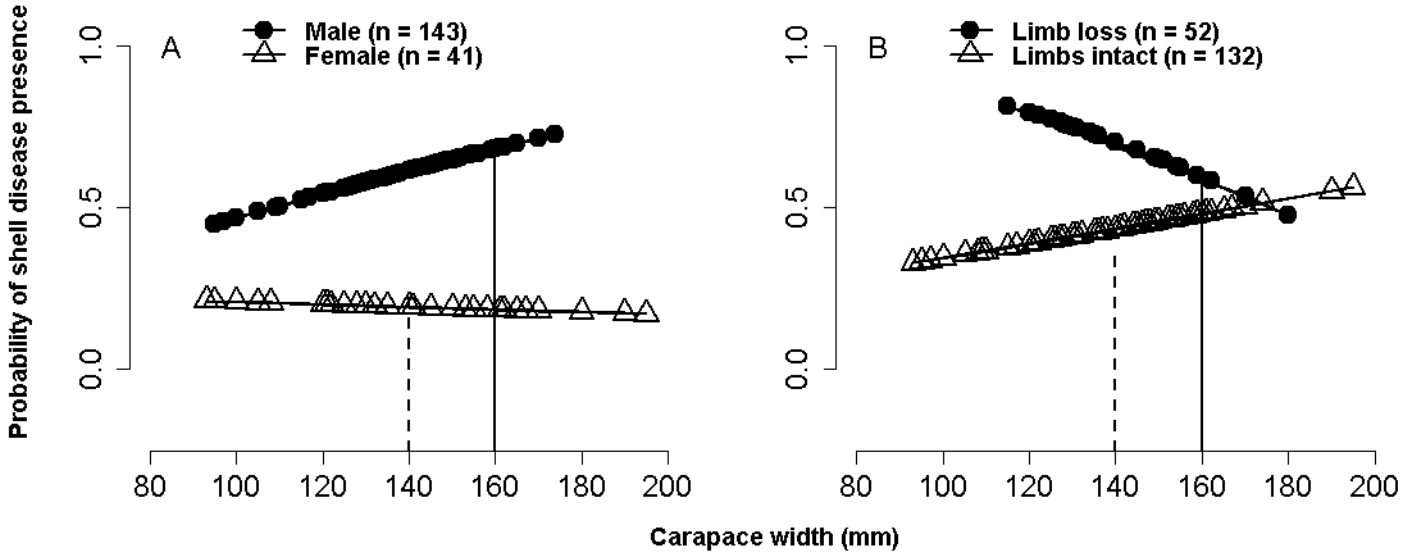


634

635

636 **Figure 5.** Size-frequency distributions of male and female crabs surveyed from 2010 (A, B) and 2011 (C, D). Broken lines indicate minimum

637 landing size (MLS) for females (carapace width ≥140 mm) and solid lines indicate MLS for males (carapace width ≥ 160 mm).



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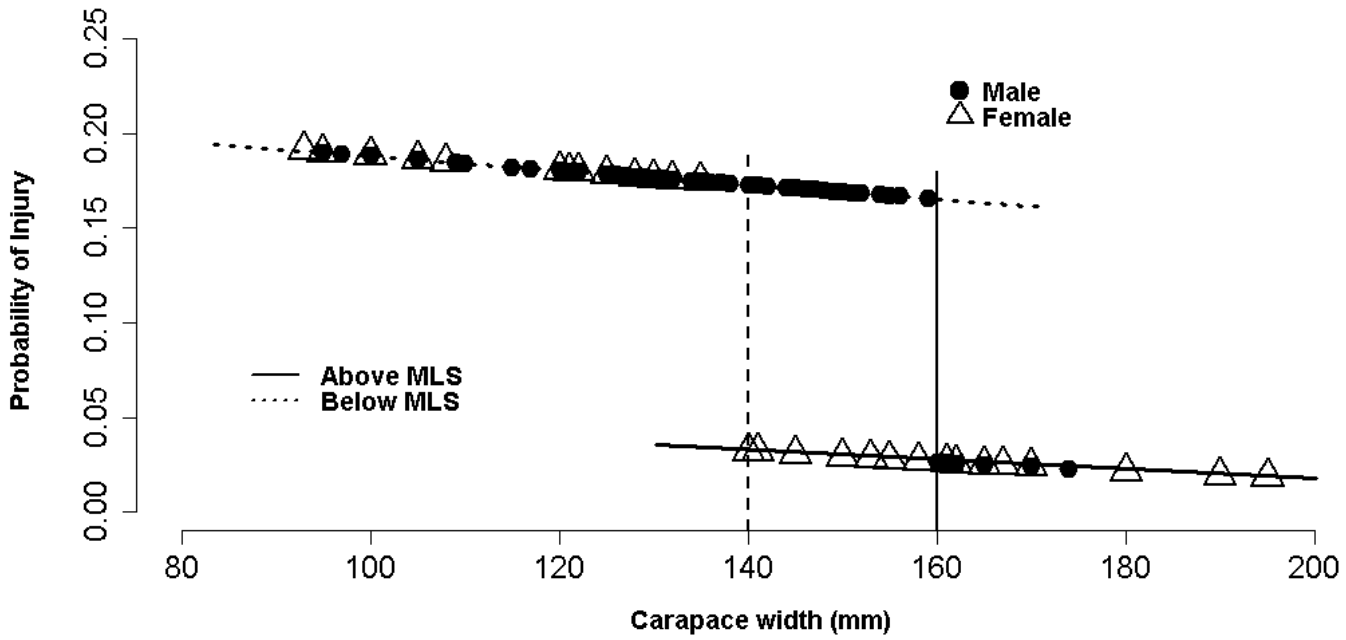
640

641 **Figure 6.** Fitted probability plots of shell disease presence in 2011 against carapace width, separated by significant predictor variables (A) sex

642 and (B) limb loss (Table 3). The broken line in each plot indicates minimum landing size (MLS) for females (carapace width ≥140 mm) and the

643 solid line indicates MLS for males (carapace width ≥ 160 mm).



644

645 **Figure 7.** Fitted probability plot of injury presence against carapace width, separated by the significant predictor variable of above *vs.* below
646 minimum landing size (MLS) (Table 5). The broken line indicates minimum landing size for females (carapace width ≥140 mm) and the solid
647 line indicates MLS for males (carapace width ≥ 160 mm).

648

649 **Table 1.** Parameters recorded for each individual *C. pagurus* caught. Parameters used as predictor variables in binomial logistic regression

650 models are denoted as: *for shell disease, †for *Hematodinium* spp. and ‡for injury.

Measurement	Description	Measure
Berried	Presence of eggs attached to underside of abdomen of females	Presence *vs*. Absence (P *vs.* A)
Carapace width (CW)	Width across the dorsal carapace at widest point	Continuous measure (mm)
Injury *,†	Wounds such as punctures and stress fractures breaching the cuticle. Injuries inflicted during captivity within the pot (i.e. recent, non-melanised breach of the cuticle) were not recorded	P *vs.* A
Limb loss *,†	Missing cheliped or walking leg	P *vs.* A
Minimum landing size (MLS) *,†,‡	MLS (width of carapace) as designated by The Devon and Severn IFCA District shore Fisheries and Conservation Authority byelaw (≥140mm for females, ≥160mm for males)	0 (below MLS) or 1 (above MLS)
Sex *,†,‡	Gender of crab (male or female)	M *vs.* F
Shell disease presence	Shell disease (photographic examples in Figure 2)	P *vs.* A

651

652 **Table 2.** Percentages of crabs according to predictor variable used in models.

	2010 (%)			2011 (%)		
	NTZ	**RZ**	**Total**	**NTZ**	**RZ**	**Total**
Shell disease	33	36.5	35.7	80	50.3	51.1
***Hematodinium* spp.**	7.4	4.4	5.2	0	4.5	4.3
Injury	1.9	3.1	2.8	20	14.5	14.7
Above MLS	16.7	18.9	18.3	20	20.1	20.1

653

654 **Table 3.** Binomial logistic regression Model 1, reduced from the full model (Table S2, Model S2), testing the effects of sex, *Hematodinium* spp.

655 presence and limb loss on the presence of shell disease in 2011.

656

Model	Predictor variable	Estimate (slope)	± Standard Error	P value
Shell Disease ~ Sex * Hematodinium* spp. *+ Limb Loss	***Sex (Male)***	1.8977	0.4866	9.63e-05 *
	Hematodinium* spp. *(Yes)	1.7533	1.0978	0.1102
df = 179	***Limb Loss (Yes)***	1.0307	0.3843	0.0073 *
AIC: 230.89	***Sex (Male) :Hematodinium* spp.**	-3.8199	1.6371	0.0196 *

657 ***** Asterisk denotes significance ($\alpha \leq 0.05$).

658 **Table 4.** Binomial logistic regression Model 2, reduced from the full, main effects model
659 (Table S2, Model S4), used to explain the effects of sex on the presence of *Hematodinium*
660 spp. in 2011.
661

Model	Predictor variable	Estimate (slope)	± Standard Error	P value
Hematodinium spp. ~ Sex				
df = 182	***Sex (Male)***	-1.3236	0.7309	0.0702
AIC: 66.714				

662

663 **Table 5.** Binomial logistic regression Model 3, reduced from the full, main effects model
664 (Table S2, Model S6), used to explain the effects of landing size on the presence of injury in
665 2011.
666

Model	Predictor variable	Estimate (slope)	± Standard Error	P value
Injury ~ Landing Size				
df = 182	***Landing size (>MLS)***	-2.0458	1.0366	0.0484 *
AIC: 150.38				

667 * Asterisk denotes significance ($\alpha \leq 0.05$).