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2

3 **What do the terms resistance, tolerance, and resilience mean in the case of *Ostrea edulis* infected**  
4 **by the haplosporidian parasite *Bonamia ostreae***

5

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20

21 **Abstract**

22

23 The decline of the European flat oyster *Ostrea edulis* represents a loss to European coastal economies  
24 both in terms of food security and by affecting the Good Environmental Status of the marine  
25 environment as set out by the European Council's Marine Strategy Framework Directive  
26 (2008/56/EC). Restoration of *O. edulis* habitat is being widely discussed across Europe, addressing  
27 key challenges such as the devastating impact of the haplosporidian parasite *Bonamia ostreae*. The  
28 use of resistant, tolerant, or resilient oysters as restoration broodstock has been proposed by  
29 restoration practitioners, but the definitions and implications of these superficially familiar terms have  
30 yet to be defined and agreed by all stakeholders. This opinion piece considers the challenges of  
31 differentiating *Bonamia* resistance, tolerance, and resilience; challenges which impede the adoption of  
32 robust definitions. We argue that, disease-*resistance* is reduced susceptibility to infection by the  
33 parasite, or active suppression of the parasites ability to multiply and proliferate. Disease-*tolerance* is  
34 the retention of fitness and an ability to neutralise the virulence of the parasite. Disease-*resilience* is  
35 the ability to recover from illness and, at population level, tolerance could be interpreted as resilience.  
36 We concede that further work is required to resolve practical uncertainty in applying these definitions,  
37 and argue for a collaboration of experts to achieve consensus. Failure to act now might result in the  
38 future dispersal of this disease into new locations and populations, because robust definitions are  
39 important components of regulatory mechanisms that underpin marine management.

40

41 The European flat oyster *Ostrea edulis* (Linnaeus, 1758) naturally occurs in Atlantic Europe and  
42 North Africa, from Norway to Morocco, as well as the Mediterranean Sea extending into the Black  
43 Sea, and was intentionally introduced into North America and South Africa for culture (Cano et al,  
44 1997; Zaitsev and Alenxandrov, 1998; Airoldi and Beck, 2007; Lallias et al., 2007). *O. edulis* has also  
45 self-introduced to Albany Harbour, Western Australia, probably historically via shipping (Morton *et*  
46 *al.*, 2003). At one time the species was amongst the most commercially-important marine resources in  
47 European waters (Orton, 1937) and stocks in France, Spain, Ireland, Croatia, Holland and the UK are  
48 still exploited commercially (Smith et al., 2006; Kamphausen et al., 2011). However, populations of  
49 *O. edulis* have been in decline since before the 1970s, and now this species is listed by the Convention  
50 for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) (Haelters and  
51 Kerckhof, 2009) as ‘threatened’ or ‘declining’. The decline of *O. edulis* represents a loss to European  
52 coastal economies not only in terms of food security, but also by affecting the Good Environmental  
53 Status of the marine environment as set out by the European Council’s Marine Strategy Framework  
54 Directive (2008/56/EC). Oysters are a keystone species that contribute to the control of eutrophication  
55 in marine ecosystems (Newell, 1965; Ward and Shumway, 2004; Fulford et al., 2010). They play a  
56 major role in dissolved nutrient cycling by removing phytoplankton, suspended solids and organic  
57 particles from the water column, and support the development biodiverse ecosystems.

58 In Europe *Ostrea edulis* was designated a named species in the EU Biodiversity Action Plan in the  
59 mid-2000s, as part of a commitment to the International Convention on Biodiversity. Since this time,  
60 attempts have been made to restore *O. edulis* populations in Scotland (Donnan, 2007; Shelmerdine  
61 and Leslie, 2009), Ireland (Tully and Clarke, 2012; *CuanBeo*, 2020), the Dutch section of the North  
62 Sea (Smaal et al., 2015), Northern Ireland (*Department of Agriculture, Food and the Marine*,  
63 [www.agriculture.gov.ie](http://www.agriculture.gov.ie)) and England (Allison et al., 2019). Restoration of *Ostrea edulis* habitat is  
64 now being widely discussed across Europe and pilot schemes and trials have been conducted (Pogoda  
65 *et al.*, 2019, in press) and other potential restoration locations have been identified (Fariñas-Franco et  
66 al., 2018). However, one key challenge for *Ostrea* spp. restoration lies in the impact of parasites such  
67 as the paramyxean *Marteilia refringens* (marteliosis) and the haplosporidian *Bonamia* spp. (including  
68 *B. ostreae*, *B. exitiosa*, *B. perspora*, and *B. roughleyi*) (bonamiosis) that have caused mass mortality  
69 of oysters worldwide (Culloty and Mulcahy, 2007). Whilst *Bonamia* spp. have not yet reached all  
70 countries hoping to restore flat oysters, their distribution across Europe within the last 40 years pose a  
71 serious and imminent threat to the restoration of oyster habitat and commercial oyster beds.

72 In the absence of any means to eradicate these pathogens, disease control in Europe is achieved by  
73 means of biosecurity, with European Directives (EC Council Directive 2006/88/EC) underpinning  
74 national restrictions on the movement of diseased stocks. These restrictions attempt to control the  
75 spread of disease through the prohibition of movement of parasite-positive oysters from areas of  
76 existing disease into new areas with no oysters, or with oysters that are disease free. Although  
77 effective, the recent spread of *B. ostreae* to New Zealand in the absence of a host introduction (Lane  
78 *et al.*, 2016; Lane and Jones, 2020) suggests that efforts to limit the spread of bonamiosis are unlikely  
79 to provide absolute and permanent protection.

80

81 **The arguments for moving diseased stocks**

82 Whilst necessary to protect disease-free populations from the introduction of pathogens, movement  
83 restrictions have been considered as a bottleneck to the development of restoration projects across  
84 Europe. Organisations have reflected differently on the challenges presented by the moratorium on  
85 oyster movements. Some restoration practitioners have proposed benefits in translocating parasite-  
86 positive but otherwise healthy stocks, based on the premise that these stocks are likely to reflect the  
87 presence of resistant or tolerant oysters (e.g. Smaal et al., 2015; Pogoda et al., 2019). In support of this  
88 position, there are reports that oysters from *Bonamia*-exposed populations might demonstrate  
89 increased tolerance, or even resistance to *Bonamia ostreae*, when compared to naïve oysters (Martin  
90 et al., 1993; Naciri-Graven et al., 1998). However, strong counter-arguments that naïve wild stocks do  
91 not develop resistance and that attempts to reseed wild beds with resistant or resilient oysters may not  
92 work have also been proposed (Ross *et al.*, 2017). Other proponents for the movement of parasite-  
93 positive stocks have argued that the enforced isolation of naïve stocks may result in long term  
94 reductions in the genetic diversity of isolated stocks; although this has yet to be demonstrated for this  
95 oyster species (Launey et al., 2001; Bentsen and Olesen, 2002; Lapègue et al., 2006).  
96 Using *Bonamia*-exposed oysters for restoration may present a tractable proposition to address the  
97 lack of disease-free broodstock, although translocating these oysters into new environmental  
98 conditions could alter the host-parasite interaction; we still do not know what the ecological  
99 consequences may be for other local species previously naïve to the parasite (Culloty et al., 2001).  
100 Whatever argument is proposed, the implication of the intentional movement of parasite-positive  
101 organisms is extreme, as once released into a new wild environment, there will be no possibility for its  
102 later eradication from that environment.

103  
104 In order to clarify a position regarding the movement of *O. edulis* stocks for habitat restoration, the  
105 definition, and the implications, of concepts such as resistance and tolerance need to be agreed by all  
106 stakeholders. In the past this has proven to be a contentious issue, in part due to the differential  
107 understanding of key definitions being used in discussion. Although disease-resistance, -tolerance and  
108 -resilience are superficially familiar terms used to describe the various levels of response shown by an  
109 infected individual (Culloty et al., 2004; Bonanno et al., 2015; Gervais et al., 2016; Pardo et al.,  
110 2016), and have some formal definition in medical fields, absolute definitions of what these terms  
111 mean in *O. edulis* individuals and populations are yet to be agreed. Indeed, the immune mechanisms  
112 by which resistance might be acquired and retained by molluscs is still very poorly understood  
113 (argued in Ross et al., 2017). With the recent initiation of multiple international collaborations to  
114 produce best practice in flat oyster (*Ostrea spp.*) restoration (Native Oyster Restoration Alliance  
115 NORA [<https://nora-europe.eu>], Native Oyster Network NON [<https://nativeoysternetwork.org>]), there  
116 is an urgent impediment to standardise these concepts such that international discussion and  
117 collaboration can move forward coherently.

118  
119 This opinion paper aims to identify the challenge presented by the different interpretation of these key  
120 definitions, similar to that offered by the SER Primer (2004), and in so doing promote a wider  
121 discussion of an agreed framework. Framed only within the context of *Ostrea spp.* immune response  
122 to infection by *Bonamia spp.*, this paper aims to resolve some basic setbacks in communication  
123 amongst scientists and practitioners all reaching for the same goal of *Ostrea spp.* restoration.

124

### 125 **Existing reports of differential susceptibility to *Bonamia ostreae* in ostreid bivalves**

126 Individuals from *Bonamia*-exposed *O. edulis* populations have demonstrated a reduced susceptibility  
127 to the parasite than those from *Bonamia*-naïve populations (Elston et al., 1987; Naciri-Graven et al.,  
128 1998; Culloty et al., 2001, 2004; Lynch et al., 2014). Morga et al. (2017) compared the immune  
129 response of selected oysters from a selective breeding programme for *Bonamia*-resistance with wild  
130 type oysters from a *Bonamia*-exposed area after being injected with *B. ostreae*. Morga et al. (2017)  
131 identified a reduced parasite burden in the selected oysters as a form of resistance to the parasite,  
132 mediated through changes in immune performance. Selected oysters demonstrated inhibited  
133 phagocytotic activity that served to reduce the spread of parasites to wider tissues, whilst the  
134 expression of apoptosis-related genes was upregulated. Indeed, molecular responses are likely to be  
135 shaped by previous exposure to parasites. Many contemporary studies have started to explore the  
136 expression of proteins, genes, and micro-RNAs associated with phagocytosis, respiratory burst, and  
137 apoptosis and have compared *Bonamia*-naïve and *Bonamia*-exposed oysters to identify underlying  
138 mechanisms that might support a differential phenotype (Morga et al., 2012, 2017; Martín-Gómez et  
139 al., 2014; Gervais et al., 2016; Pardo et al., 2016; De La Ballina et al., 2018; Ronza et al., 2018; Vera  
140 et al., 2019). Whilst the details are still being unravelled, it is clear that mechanisms exist that might  
141 underpin, and indeed require, formal definitions of resistance, tolerance or resilience.

142

### 143 **Conceptual challenges in progressing agreed definitions of resistance, tolerance and resilience** 144 **that support a precautionary approach to oyster restoration**

145 It is important to recognise that different definitions may be required depending on discipline or  
146 working practice (Ferrandon, 2009). Moreover, it is also crucial to note that host-parasite interactions  
147 generally represent the current state-of-the-art in an arms race that has driven the evolution of host and  
148 parasite over many generations. Although it is possible to assign terminology to this state, i.e.  
149 tolerance or resistance, what we view at a single time point is part of a continuum of effect, which  
150 does not always have a precisely defined endpoint. A comprehensive appreciation of each mechanism  
151 might lead to more efficient control measures and a heightened understanding of host-parasite  
152 interaction (Schneider and Ayres, 2008). However, as will be discussed in detail later, infection status  
153 is an interaction between host, parasite and environment, and whatever the status of a population or  
154 individual, environmental perturbation may in fact completely alter that status; something which is  
155 particularly important in sessile, aquatic animals. Whilst an agreed definition remains constant, it  
156 must be appreciated that an individual or population may change status depending on the temporal  
157 development of the host-pathogen interaction, and the environment in which they find themselves.

158

159 Disease *resistance* is preferable to the host. The parasite is either not able to infect the host, or is able  
160 to infect the host but unable to multiply, reproduce and proliferate i.e. self-sustain within the host  
161 tissues (Fig. 1). Resistant individuals may also demonstrate the ability to actively reduce parasite  
162 burden and neutralise its virulence (Råberg et al., 2007; Schneider and Ayres, 2008; Ferrandon, 2009;  
163 Lynch et al., 2014; Louie et al., 2016; Morga et al., 2017). Disease *tolerance* is the ability to survive  
164 whilst maintaining a parasite burden. *Tolerance* can be beneficial for both host and parasite, in that  
165 host fitness is not greatly affected by the presence of the parasite, regardless of its successful

166 proliferation in host tissues (Schneider and Ayres, 2008; Råberg et al., 2009). Cao et al. (2009)  
167 reported tolerance in their *O. edulis* population in Ría de Ortigueira, which they identified as low  
168 disease prevalence and low mortality. Tolerance may be the result of a parasitic virulence mechanism  
169 whereby the parasite is able to neutralise the host immune reaction to the infection (Mauel, 1984;  
170 Cheng, 1987; Ferrandon, 2009; Råberg et al., 2009). Råberg et al., (2007) have conceptualized the  
171 differences between resistance and tolerance using the slope of a linear relationship between host  
172 health and pathogen load (Fig. 2). Although this concept does not necessarily consider the complex  
173 response of an individual infected with more than one disease (Louie et al., 2016), these response  
174 curves (later adapted by Schneider and Ayres, 2008) highlight that individuals can be both resistant  
175 and tolerant at the same time, and that tolerance can take on many forms depending on the pathogen  
176 strain (Ferrandon, 2009).

177

178 While resistance can be measured by monitoring fluctuations in parasite burden over time, features of  
179 tolerance do not offer the same ease of analysis. Even if an individual is displaying no sign of immune  
180 response to the presence of the parasite, there may be a tolerance threshold depending on parasite  
181 burden and sensitivity of immune system, which cannot be separated from environmental influence  
182 (Louie et al., 2016). This could mean a rapid change in observed disease-susceptibility of an  
183 individual oyster from one day to the next. The same tolerance threshold may be affected by stressors  
184 associated with handling during experiments, which likely plays a role in many studies (Culloty et al.  
185 2003) or through seasonal changes in the environment. These issues render the concept of disease-  
186 tolerance rather useless to the current conversation concerning broodstock origin for *Ostrea*  
187 restoration purposes, as - unlike mechanisms associated with resistance - there is no clear  
188 measurement of the mechanisms involved in disease-tolerance.

189

190 Disease *resilience* is a form of resistance which can be defined as the ability to recover from illness,  
191 regardless of the time or physiological requirement, which allows for manageable observation of  
192 individuals through illness and back to health (Gundersonet al., 2010; Schneider, 2011; Louie et al.,  
193 2016; Richardson, 2016) (Fig. 1). This definition of resilience is in accordance with the SER Primer  
194 (2004) explanation of resilience as a response to naturally fluctuating environmental pressures. The  
195 status of disease-resilience has been applied to ecosystems (Kelly et al., 2011) and even the  
196 aquaculture industry (Fernández Robledo et al., 2018) post disease exposure. However, whilst the  
197 concept of resilience demonstrates utility at a broad scale, it has perhaps limited validity when  
198 considering the risk of relocating and relaying individuals of unknown status. A previously resilient  
199 population is not necessarily protected against repeated disease challenges, and post-challenge  
200 outcome is likely to change.

201

202 When considering the manifestation of disease at a population level rather than an individual level,  
203 the definitions of resistance, tolerance and resilience remain the same. However, the population level  
204 outcome can be described in a different way. For example, a tolerant population formed of resistant  
205 and tolerant individuals, or a resistant population which has either completely overcome the parasite,  
206 or is completely resistant to infection. Further analysis of population levels of resistance would  
207 require long-term repeat studies to observe how the oysters dealt with parasite burden, and monitor

208 general physiology such as reproductive output to fully understand the process involved. Culloty and  
209 Mulcahy (2007) identified the *Bonamia*-exposed Brittany oyster populations as developing a tolerance  
210 to the parasite, due to a reduction in disease outbreaks and continued productivity post *Bonamia*  
211 introduction. Lallias et al. (2009) named one of their experimental oyster populations “non-  
212 susceptible to the disease” due to no detection of *Bonamia* parasite during a 6-month trial. This would  
213 be termed resistant under the above suggested definition. However, Lallias et al. (2009) only assessed  
214 disease progression over a short time frame, which may miss some of the overall picture due to the  
215 varied and unpredictable development of bonamiosis (Montes, 1991; Naciri-Graven et al., 1998;  
216 Montes et al., 2003; Lynch et al., 2005). Louie et al. (2016) suggest that there is a natural crossover  
217 between resilience and tolerance that can be understood to determine how a population may react to  
218 an infection through the study of its individuals. A resilient population may recover from a disease  
219 outbreak over time and indeed this would imply that some form of resistance has occurred. However,  
220 there are currently no clear examples of populations that have been impacted by *Bonamia* species in  
221 returning to their original population levels, suggesting a complex interplay of factors (Carnegie et al,  
222 2016).

223

224 Problems arise when applying the terms resistance, tolerance and resilience in real-time. Firstly,  
225 bonamiosis demonstrates unpredictable development, which makes it difficult to determine the level  
226 of infection and pressure on the individual or population. Secondly, as previously discussed, the host-  
227 pathogen relationship is a dynamic strongly influenced by environmental factors, which renders it  
228 site- and season- specific. Thirdly, there are shortcomings in definitive protocols for disease  
229 diagnostics and analysis, often limiting analysis to pathogen presence or absence. The challenge is  
230 allocating an appropriate term that can help direct policy decisions of whether *Bonamia*-exposed  
231 oysters are appropriate for restoration purposes, and moreover, to manage the risk of any associated  
232 relocations.

233

#### 234 **Complicating factors for the above definitions – non-lethal parasite detection.**

235 Although combined cellular and molecular approaches might, in time, provide mechanistic evidence  
236 to support definitions of resistance or tolerance, these do not necessarily represent tractable  
237 approaches for restoration practitioners working in the field. All bivalves shield any visible  
238 symptoms associated with soft tissue disease behind their calcareous shell. Symptoms reported in  
239 *Bonamia*-infected *Ostrea* spp. include black, emarginated & frayed gills (Dinamani et al., 1987;  
240 Kroeck and Montes, 2005), and a change in circulating haemocyte ratios. Cochenec-Laureau et al.  
241 (2003) found a higher number of large agranular haemocytes (hyalinocytes) in infected oysters, and  
242 lower number of granulocytes in *Bonamia*-susceptible oysters; data supported by da Silva et al. (2008)  
243 and Comesaña et al. (2012). If the disease has developed to a later stage, oysters will exhibit shell  
244 gaping, which is often a prelude to death. Earlier detection of bonamiosis is currently only accessible  
245 via sacrifice or by intrusive testing such as clipping the side of the shell to expose flesh or relaxing the  
246 oyster in anaesthetic to open the valves before removing gill tissue or haemolymph for histological or  
247 molecular analysis. These techniques are time consuming and expensive, and therefore undesirable in  
248 a commercial setting, and farmers are often limited to mortality statistics of their stocks. As a result of  
249 the difficulty in diagnosing levels of bonamiosis-tolerance in individuals, Louie et al. (2016) argued



250 that *tolerance* is a concept only applicable to populations. Nonetheless, we believe that tolerance to *B.*  
251 *ostreae* is an important and useful phenotypic concept that should be considered in all sections of  
252 study, even if that consideration simply involves noting its existence and difference to resistance.

253

### 254 **The challenge of identifying tolerance or resistance from cell based observations alone**

255 Many studies of the oyster/*Bonamia* spp. system have utilized cellular observations to understand *O.*  
256 *edulis* immune response. Broadly speaking, as immunological concepts, it is clear that there is a  
257 greater confidence in identifying cellular mechanisms of resistance, but less so for mechanisms of  
258 tolerance (Matzinger, 2002; Schneider and Ayres, 2008; Sun, 2008). In oysters, phagocytosis,  
259 respiratory burst and apoptosis are three of several cellular mechanisms that have been associated with  
260 disease-resistance (Cheng, 1981; da Silva et al., 2009; Comesaña et al., 2012; Martín-Gómez et al.,  
261 2012; Morga et al., 2012), but phagocytosis also represents the mechanism by which the parasite  
262 *Bonamia* spp. gains entry to the haemocytes to facilitate the infection cycle. As such, oyster  
263 haemocytes are both the target tissue, and host defence against *B. ostreae* (Comesaña et al., 2012;  
264 Gervais et al., 2016). Indeed, past studies have concluded that *B. ostreae* can modulate the host  
265 immune response for its own benefit through the inhibition of the respiratory burst and phagocytosis  
266 (Morga et al., 2009, 2011; Comesaña et al., 2012), which might observationally be interpreted as  
267 tolerance, or even a mechanism of parasite virulence. In short, based on our current state of the art, it  
268 is not possible to definitively discriminate the difference between resistance and tolerance from  
269 cellular observations in isolation (Table 1). Further research will be necessary, at a cellular and  
270 molecular level of both the host and the parasite, to disentangle these intimate interactions for  
271 different populations of oysters which might show a differential susceptibility in the field.

272

### 273 **Complicating factors for the above definitions - disease progression**

274 Successful proliferation of *Bonamia ostreae* within an infected host individual leads to the  
275 development of disease bonamiosis, which can result in high mortality rates (> 80 %) (Robert et al.,  
276 1991; da Silva et al., 2005; Laing et al., 2005; Lallias et al., 2010). *B. ostreae* swiftly infiltrate their  
277 target tissue and have been observed in haemocytes within 30 minutes to 1 h post infection (Mourton  
278 et al., 1992; Morga et al., 2011). However, proliferation of *B. ostreae* and progression of the disease  
279 bonamiosis is more unpredictable (Montes, 1991; Naciri-Graven et al., 1998; Montes et al., 2003;  
280 Lynch et al., 2005). Varying levels of bonamiosis intensity have previously been identified on the  
281 basis of parameters such as parasite abundance (heart smears for grading by levels 0-4, al., 1982;  
282 Culloty et al., 1999; Lynch et al., 2008; parasite separation by centrifuge before haemocytometer  
283 count, Gervais et al., 2016), parasite number per haemocyte (histology, Bachere et al., 1982; Gervais  
284 et al., 2016), or parasite spread rate (histology, da Silva and Villalba, 2004 and references therein).  
285 Lynch et al. (2014) observed that although *B. ostreae* was detected in a tolerant Irish stock 30 years  
286 after first introduction to that stock (<20% prevalence in 4+ oysters and <10% prevalence in 10+  
287 oysters by PCR, and <10% prevalence in 5+ oysters by heart smear screening), it occurred at low  
288 intensities of infection (Class 1 and Class 2) and no mortalities were observed. Moreover, it has been  
289 argued that *B. ostreae* may modulate its own metabolism to lie dormant within *O. edulis* haemocytes  
290 in a “latent stage” (Culloty et al., 2003) as seen by malaria *Plasmodium* spp. (Richter et al., 2016) and  
291 *Toxoplasma gondii* (Sullivan and Jeffers, 2012; Gervais et al., 2018). This could allow the parasite to

292 persist without using its own energy resources (Sullivan and Jeffers, 2012). *B. ostreae* has also been  
293 found outside the host (*O. edulis*); present but not infectious in zooplankton and other cohabiting  
294 macroinvertebrates (Lynch et al., 2006). In addition, *B. ostreae* has the ability to spread through *O.*  
295 *edulis* both horizontally and vertically (Arzul et al., 2011). This poses a threat to larval individuals and  
296 to populations of *Bonamia*-naïve populations in close proximity to infected brooding stock due to the  
297 pelagic nature and physical dissemination of *O. edulis* larvae.

298

299 From the above, it is clear that identifying definitive states of resistance or tolerance in individuals  
300 infested with parasites such as *B. ostreae* is a much more difficult proposition than for lethal viral or  
301 bacterial infections, which tend to achieve a more rapid infection outcome.

302

### 303 **Complicating factors for definitions - the importance of evolution and inheritance**

304 The eventual population-scale outcome of exposure to *B. ostreae* is that of mortalities, and survivors,  
305 of which some may be completely resistant, some tolerant and some resilient. Animals that survive to  
306 breed have the ability to pass their germ line to the next generation, and if these animals are  
307 genetically less susceptible to the parasite then the next generation of animals are likely to have  
308 inherited this favourable genotype. Indeed, selective breeding programmes have identified that that  
309 survival is in fact a heritable trait and that notable differences can be established over a single  
310 generation of selection (Martin et al., 1993; Nell et al., 2000; Culloty et al., 2004). One study showed  
311 reductions in parasite prevalence of 54 % after 3 generations of selection (Naciri-Graven et al., 1998),  
312 suggesting significant cumulative gains. Yet none of these studies have considered the differential  
313 phenotypes of biological resistance or tolerance in individuals or population, most likely due to the  
314 lack of clear definition of these traits. Including these as definitive phenotypes within genetics studies  
315 would likely add resolution to the data, and this may allow geneticists to quantify the number of  
316 generations it would take before uninfected but susceptible larvae individuals could be considered  
317 usefully tolerant or resistant. Broad markers of resistance can be found in previously exposed  
318 populations by identifying significant selective sweeps in the genome of a population (Vera et al.,  
319 2019). However, these same phenomenon can make it difficult to characterise the specific biological  
320 mechanisms. In addition, a natural system is likely to experience the dilution of resistance genes as  
321 unexposed (susceptible) stock can often contribute to progeny of exposed areas, for example through  
322 the movement of gametes or larvae through the water column (Culloty et al., 2001; Flannery et al.,  
323 2014), and by the sale and consumption of resistant oysters before they contribute genes to future  
324 generations (Lauckner, 1983). Therefore, when analysing either the genetic architecture of disease  
325 resistance, or the biology of disease progression, it is often preferable to work with populations of  
326 oyster that have a highly structured population, have not been exposed to the parasite and can be  
327 challenged in a controlled manner (e.g. Hervio et al., 1995). In doing so, variables associated with  
328 previous exposure (such as immune priming and selective sweeps) are accounted for (Vera et al.,  
329 2019).

330

### 331 **Complicating factors for the above definition applied to parasite diseases of ostreids - the** 332 **importance of considering the environment**

333 Unfortunately, we cannot rely solely upon host genotype alone to predict whether an individual will  
334 produce a defined immune response, be it disease-tolerant, -resistant or -resilient (Viney et al., 2005;  
335 Schneider, 2011), as no host parasite interaction explanation is complete without an environmental  
336 context. The disease triumvirate (Snieszko, 1974) simply portrays the very integrated and complex  
337 relationship between host, pathogen and environment that has implications for our understanding of  
338 tolerance, resistance and resilience. The relationship between host and parasite (disease and defence)  
339 is fluid, and pathogen strain or burden will have different virulence in different environments  
340 (Schneider and Ayres, 2008; Romero et al., 2012; Louie et al., 2016). Pathogen impact is influenced  
341 by individual fitness and immediate environmental pressures.

342  
343 Abiotic factors such as temperature and salinity can play a major role in the metabolism of both host  
344 and pathogen, which can effectively decide the fate of either player. Flannery et al. (2014) observed  
345 that *O. edulis* beds near a freshwater source at two culture sites had much lower prevalence (%) of *B.*  
346 *ostreae*. This has been observed with other haplosporidians such as *Haplosporidium nelsoni* in  
347 *Crassostrea virginica* in North America (Corbeil and Berthe, 2009) and with *Mercenaria mercenaria*-  
348 like protists in cockles in Ireland (Albuxech-Martí et al., *in press*). *B. ostreae* has been reported to  
349 survive and proliferate at lower temperatures of between 4 and 10 °C (Cochennec and Auffret, 2002;  
350 Culloty and Mulcahy, 2007; Arzul et al., 2009; Feng et al., 2013) and at higher salinities of >35  
351 (Arzul et al., 2009; Engelsma et al., 2010, 2014). These optimum temperatures and salinities are at  
352 ranges that are likely to put pressure on the oysters immunological response, resulting in higher  
353 mortality (Hauton et al., 2000; Mydlarz et al., 2006; Laing et al., 2014) such that what favours one,  
354 may impact the other.

355  
356 A further complication concerns the issue of multiple simultaneous infections. Hine (2002) observed  
357 that *B. exitiosa* was more virulent in oysters also infected with apicomplexan zoites. da Silva *et al.*  
358 (2011) similarly found oysters with disseminated neoplasia to be more susceptible to the disease  
359 bonamiosis. *Bonamia ostreae* and *B. exitiosa* can co-infect but the outcome of co-infections on the  
360 host survival is unknown (Abollo *et al.*, 2008; Ramilo *et al.*, 2014). Similarly, Nell and Perkins (2006)  
361 have reported that resistance to infection may be bred for a single pathogen, but not necessarily  
362 multiple pathogens. In the Sydney Rock oyster *Saccostrea glomerata* selective breeding could  
363 produce resistance to *Marteilia sydenyi* or *Bonamia roughleyi*, but not to both parasites  
364 simultaneously (Nell and Perkins, 2006). The potential complication of moving asymptomatic but  
365 pathogen positive oysters to new locations with pre-existing unique pathogenic microfauna adds  
366 considerable uncertainty in predicting the potential disease risk of relocation for restoration.

367

### 368 **Conclusion - Implications for restoration and future of this species**

369 We have used the example of *Bonamia* spp. as a focus for this review, but these definitions of  
370 resistance, tolerance, and resilience could be applied for other pathogens affecting ostreids.  
371 Herein, we argue that, currently, we simply do not have sufficient understanding of this host-parasite  
372 system to support clear definitions of resistance or tolerance as applied to *B. ostreae* and *O. edulis*.  
373 Without clear and accepted definitions, discussions about amending legislation to support the  
374 movement of disease positive stocks cannot be prosecuted. Maintaining control of disease in

375 culturally and commercially valuable marine organisms like *O. edulis* will require appreciating the  
376 nuance and integrity of scientific language used and eliminating vague terminology. Priorities  
377 imposed by laws and regulations can vary from country to country, but ultimately the goal is the  
378 same: in order to restore *O. edulis* populations, we must focus our attentions on what is best to sustain  
379 these restored populations in the future. We conclude that there is a very urgent need to establish a  
380 panel of experts to consider the implication of European restoration using parasite-positive broodstock  
381 and to develop a definitive road map for sustainable restoration; one that is fully endorsed by all  
382 stakeholders in this venture and supports the development of policy and regulation.  
383 The production of this roadmap will have to be supported by current and future initiatives to dissect  
384 the intricacy of this complex an intimate host parasite association to underpin the development of  
385 robust definition and, thereafter, management practice and advice. If we, as a community, fail to agree  
386 robust definitions that underpin effective management, we risk dispersing this destructive disease into  
387 new locations, with no prospect of reversing this outcome for future generations.

388

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399

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**Table 1.** Possible interpretations of cellular phenomena.

<b>Cellular observation</b>	<b>Possible interpretation</b>
<i>Bonamia</i> parasites fail to enter the oyster haemocytes by phagocytosis	Resistance (Morga et al., 2009, 2012)
<i>Bonamia</i> enters haemocytes, but phagolysosome formation is prevented, parasite survives intracellularly in parasitophorous vacuole	Parasite virulence, or tolerance (Mauel, 1984). At a population scale, tolerance may be interpreted as resilience.
<i>Bonamia</i> enters haemocytes, but phagolysosome formation is prevented, parasite survives intracellularly and infection develops	Parasite virulence (Cheng, 1987)
<i>Bonamia</i> enters haemocytes, phagolysosome formation occurs and parasite is broken down intracellularly	Resistance (Cheng, 1983; Chagot et al., 1992)
<i>Bonamia</i> enters haemocytes, cell undergoes apoptosis and apoptotic bodies are phagocytosed by other haemocytes	Resistance, or tolerance? (Gervais et al., 2016)

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723 **Figure legends**

724

725 Figure 1. A schematic to demonstrate the progression and spread of *B. ostreae* over time in oysters  
726 showing resistance, resilience, tolerance and susceptibility at the level of the individual.

727

728 Figure 2. Schematic adapted from Råberg, Sim and Read (2007) to show response of two genotypes  
729 to disease severity at different infection intensities. (a) Pink is more resistant than blue and  
730 therefore maintains a higher health status and a lower parasite burden. Arrows represent the  
731 fluctuations of resilience. (b) Red is less tolerant than blue. Therefore if exposed to a similar  
732 parasite burden, red has a more rapid decline in health. (c) Pink is more resistant than the  
733 tolerant blue, which results in a similar health status due to differing levels of parasite exposure.  
734 (d) two genotypes with no difference in exposure or resistance/tolerance, only a difference in  
735 health status ("general vigour").

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