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

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

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Abstract

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

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

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

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

The arguments for moving diseased stocks

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Complicating factors for the above definitions - disease progression

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

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

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

Complicating factors for definitions - the importance of evolution and inheritance

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

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

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

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

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

Conclusion - Implications for restoration and future of this species

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

culturally and commercially valuable marine organisms like *O. edulis* will require appreciating the nuance and integrity of scientific language used and eliminating vague terminology. Priorities imposed by laws and regulations can vary from country to country, but ultimately the goal is the same: in order to restore *O. edulis* populations, we must focus our attentions on what is best to sustain these restored populations in the future. We conclude that there is a very urgent need to establish a panel of experts to consider the implication of European restoration using parasite-positive broodstock and to develop a definitive road map for sustainable restoration; one that is fully endorsed by all stakeholders in this venture and supports the development of policy and regulation.

The production of this roadmap will have to be supported by current and future initiatives to dissect the intricacy of this complex an intimate host parasite association to underpin the development of robust definition and, thereafter, management practice and advice. If we, as a community, fail to agree robust definitions that underpin effective management, we risk dispersing this destructive disease into new locations, with no prospect of reversing this outcome for future generations.

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

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Cellular observation	**Possible interpretation**
Bonamia parasites fail to enter the oyster haemocytes by phagocytosis	Resistance (Morga et al., 2009, 2012)
Bonamia 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.
Bonamia enters haemocytes, but phagolysosome formation is prevented, parasite survives intracellularly and infection develops	Parasite virulence (Cheng, 1987)
Bonamia enters haemocytes, phagolysosome formation occurs and parasite is broken down intracellularly	Resistance (Cheng, 1983; Chagot et al., 1992)
Bonamia enters haemocytes, cell undergoes apoptosis and apoptotic bodies are phagocytosed by other haemocytes	Resistance, or tolerance? (Gervais et al., 2016)

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Figure legends

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

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