NRC Research Press

ARTICLE

Modeling shell disease in American lobster (*Homarus americanus*) as individual-based health trajectories

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Abstract: The emergence of epizootic shell disease in American lobsters (*Homarus americanus*) has presented many new challenges to understanding the interface between disease and the management of the lobster fishery. While a variety of the potentially causative and correlative factors for shell disease have been explored, a clear etiological agent remains elusive. The recency of this disease and the lack of identifiable causal agents have hindered the development of conceptual models that can yield testable predictions. Here, a model originally developed for human–parasite interactions was applied to lobster shell disease as a means to unify the broad experimental and field observations. The model is a graphical means to understand the onset and severity of shell disease and is a function of the length of the molt cycle and the rate of the decrease of health both before and after lesion formation as a function of bacterial abundance and pathogenicity. The model also accounts for shell hardening and passive and active portals of entry for the bacteria. The timing for a conceptual understanding of the epidemiology of shell disease is critical because its prevalence is increasing in key fishing areas. Ideally, such a model will help researchers create hypothesis-driven predictive experiments from which we can further our understanding of an important disease to a critical member of the Gulf of Maine ecosystem.

Résumé : L'apparition de l'épizootie de la maladie de la carapace chez le homard (*Homarus americanus*) a posé de nombreux défis nouveaux en ce qui concerne la compréhension de l'interface entre la maladie et la gestion de la pêche au homard. Si divers facteurs potentiellement causatifs et corrélatifs ont été examinés, un agent étiologique bien défini n'a pas encore été cerné. Le court laps de temps écoulé depuis l'apparition de cette maladie et l'absence d'agents causaux identifiables ont limité l'élaboration de modèles conceptuels pouvant fournir des prédictions vérifiables. Nous avons appliqué un modèle élaboré à l'origine pour des interactions humains–parasites à la maladie de la carapace du homard afin d'intégrer les différentes observations expérimentales et de terrain. Le modèle est un outil graphique pour comprendre l'apparition et la gravité de la maladie de la carapace et est fonction de la longueur du cycle de mue, du taux de détérioration de l'état de santé avant et après la formation de lésions en fonction de l'abondance bactérienne et de la pathogénicité; il explique le durcissement de la carapace et les portails d'entrée passifs et actifs pour les bactéries. Il est d'importance capitale d'établir rapidement une compréhension conceptuelle de l'épidémiologie de la maladie de la carapace étant donné l'augmentation de sa prévalence dans des zones de pêche clés. Idéalement, un tel modèle aidera les chercheurs à concevoir des expériences prédictives reposant sur des hypothèses, qui permettront d'étayer la compréhension de cette importante maladie affectant un membre clé de l'écosystème du golfe du Maine. [Traduit par la Rédaction]

Introduction

Epizootic shell disease (ESD) is a bacteria-induced degeneration of the cuticle of the American lobster (*Homarus americanus*) that can lead to disfigurement, decreased health, and subsequent mortality (Castro et al. 2012; Tlusty et al. 2007). It has been most prevalent in the southern end of the range of the lobster (Glenn and Pugh 2006; Gomez-Chiarri and Cobb 2012). There is evidence suggesting that natural population mortality is positively associated with ESD (Howell 2012). While not typically associated with the productive fishing waters off the Maine coast, it has been present along the entire coast of Maine since at least 2003 when 1 in every 10 000 lobsters was observed as symptomatic (Wilson 2005). Recent sampling has observed an increase with 1 in 500 lobsters being recorded in 2011 (C. Wilson, Maine Department of Marine Resources, personal communication). With the incidence of shell disease on the rise particularly in these primary fishing grounds, it is important to collate current data on ESD to create predictive models so that future efforts to understand and manage the disease can be as effective as possible.

ESD became a concern in American lobsters in the late 1990s (Cobb and Castro 2006). It remains enigmatic, as there is no clear etiological agent despite the recent collaborative research effort to understand the causes and consequences of this disease in wild American lobster stocks (Castro et al. 2012; Gomez-Chiarri and Cobb 2012). Hess (1937) was the first "modern" research to publish on shell disease in American lobster, and it was not until the work of Smolowitz (Smolowitz et al. 2005, 1992) that the multiple forms of the disease, including burnt spot, impoundment, enzootic, and epizootic, were noted.

As part of the creation of a body of work to better understand ESD in American lobsters, a limited number of models have been proposed. These efforts were rooted in the adoption of the Snieszko's (1974) environment–host–pathogen Venn diagram by Tlusty et al. (2007). This work was fundamental in reinforcing the need to examine this disease as the interplay of these three factors. How-

Received 10 July 2013. Accepted 22 February 2014.

Paper handled by Associate Editor Yong Chen.

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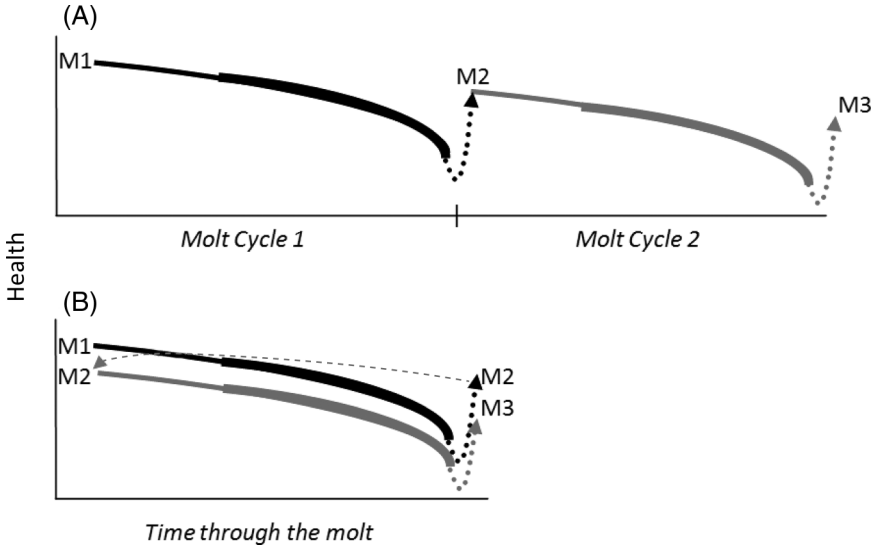
Fig. 1. (A) Health trajectories in American lobster over subsequent molt cycles. Health is on the *y* axis and can be the inverse probability of disease (e.g., (1 – shell disease)) or the actual measure of a health (e.g., hemolymph) parameter. Curves represent an individual through time and thicken when shell disease is apparent. A discontinuity (dotted line) occurs at the end of the cycle as the old shell is discarded and the new shell exhibits no shell disease. (B) To make an easier comparison of subsequent molt cycles within a single lobster, the molts are stacked, in which case transition to the second molt (M2) necessitates a wrapping back to the beginning of the next molt cycle, as indicated by the thin dashed line.



ever, this approach is limited by not creating specific testable hypotheses. Castro et al. (2006, 2012) developed preliminary epidemiological models for population-level ESD dynamics. At this time, the proposed models are considered working versions, although admittedly, they are incomplete (Castro et al. 2012).

While epidemiological models are applicable at a population level, they overlook some of the specific mechanisms by which individual lobsters transition to a disease state. Individual-based models are necessary to elucidate the causal mechanisms of the disease. These causal factors can then be adopted to the broader population models. A prior effort to create an individual-based model of ESD focused on the thickness of the shell as a function of internal deposition modulated by bacterial consumption (Tlusty et al. 2007). However, laboratory-based observations failed to support the hypothesis that shell thickness and bouligand layering was correlated to ESD prevalence or severity (Tlusty and Metzler 2012). Recent evidence proposed a protective, basic hydroxyl boundary layer on the external surface of the epicuticle that provides important antimicrobial function (Kunkel and Jercinovic 2013; Kunkel et al. 2012). Thickness may not be a factor in preventing shell disease within individual American lobsters, but it has the potential to explain evolutionary differences in resistance to microbial attack across the multiple lobster species (Davies et al. 2014; Tarsitano et al. 2006).

In creating an individual-based model, there are a few key factors that play a role in the etiology of all forms of shell disease. These relevant key points include (1) molting, (2) new shell hardening, (3) stress as an organizing component in new shell growth, and finally (4) the idea that shell disease is inevitable. These factors have been observed as early as the 2nd century AD, when Oppian wrote of shell pathologies in the decapod crab, *Cancer pagurus* (Mair 1928; superscripts identify the key points above):

> But when the sheath is rent and slips off[1], then at first they lie idly stretched upon the sands, mindful neither of food nor of aught else, thinking to be numbered with the dead and to breathe warm breath no more, and they tremble for their new-grown tender hide. Afterwards they recover their spirits again and take a little courage and eat of the sand; but they are weak and helpless of heart until a new shelter is compacted about their limbs[2]. Even as when a physician tends a man who is laden with disease, in the first days he keeps him from tasting food, blunting the fierceness of his malady, and then he gives him a little food for the sick, until he has cleared away all his distress and his limb-devouring aches and pains[3]; even so they retire, fearing for their new-grown shells, to escape the evil fates of disease[4].
>
> (Oppian, in *Halieutica*, I. pp. 281–306)

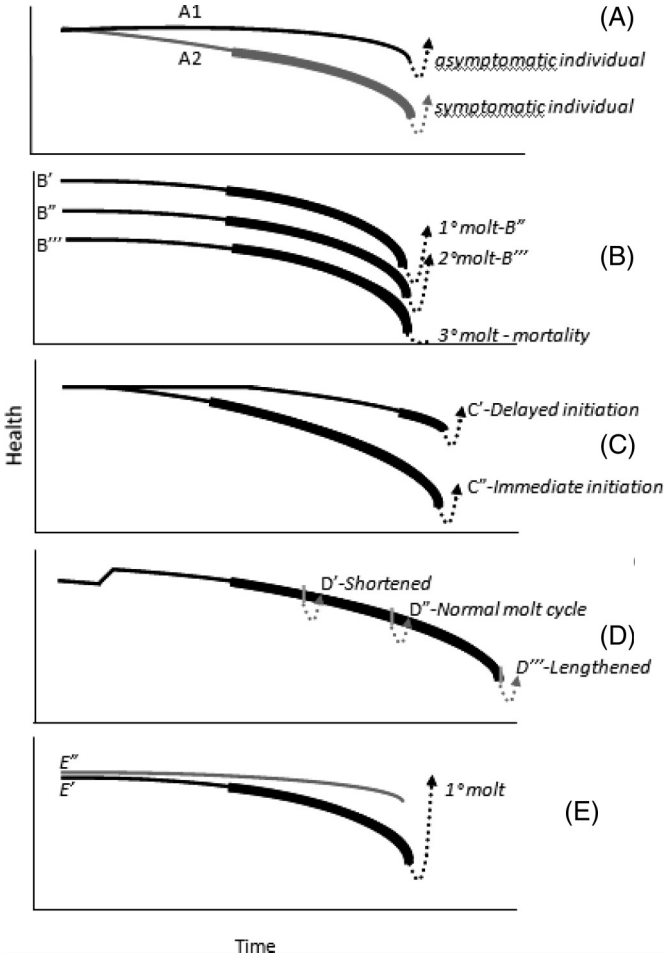
The remainder of this manuscript collates these concepts with recent advancements in our understanding of ESD (Castro et al. 2012) to form an individual-based model for ESD in American lobsters. This model can ultimately be integrated into population-level epidemiological models to better describe the factors affecting host susceptibility to ESD.

"Individual health trajectories" as a base model

The basic premise is that ESD in lobsters is bacterial in nature (Chistoserdov et al. 2012; Quinn et al. 2012*a*). For healthy and asymptomatic American lobsters, it appears that there is some normal bacterial community (Hsu and Smolowitz 2003; Meres et al. 2012) on the shell and that the "normal" community may include the bacteria responsible for the initial breach of the shell (Quinn et al. 2012*a*). ESD is also inextricably linked to the molt cycle (defined here as the time from the appearance of the new shell to the point at which the old shell is discarded and the next shell is physically present). Over the course of the molt cycle, bacteria may overcome the lobster's shell-based defenses (Kunkel and Jercinovic 2013; Kunkel et al. 2012) and open up a breach in the shell. In response, the lobster will elicit a phenol-based melanistic inflammatory response (Smolowitz et al. 2005), which can be observed as a darkening or spotting of the broached area (Tlusty and Metzler 2012). As the bacteria continue to breach the shell, the defense mechanisms do not keep pace, and eventually a lesion forms (Tlusty et al. 2008). The bacteria continue to act on the shell for the remainder of the cycle, and the lesion continues to worsen until the animal molts and a new shell is present. This new shell is characteristically assumed to be disease-free.

The lobster molt cycle can be presented graphically by showing health (*y* axis) as a function of stage of the molt cycle (*x* axis; Fig. 1A). Health starts at some level and slowly decreases through the molt cycle, particularly in cases where shell disease is present (Fig. 2A). The health axis is a continual variable indexing the prob-

Fig. 2. A model of shell disease. The molt is represented by the transition to the dotted portion of the curve, which returns the individual to the beginning of the next molt cycle. In panel A, two separate individuals are represented (one symptomatic and one asymptomatic), whereas in panels B–E, the same individual is represented through subsequent molt cycles. The trajectories in panel B represent a gradual decrease in health over subsequent molts until the lobster perishes. Panel C demonstrates the effect of delaying the initiation of the lesion, where a delay in onset results in a less severe disease state. Panel D exhibits the impact if an individual hastens or delays the molt. Panel D also has a hypothetical shift up in health at the beginning of the molt cycle corresponding to a period of shell hardening. Panel E shows that in the subsequent molt the lobster is less susceptible to shell disease, as would occur with increased immune function.



ability of mortality ($1 - P_M$), where the individual perishes ($P_M = 1$) when health is a minimum ($y = 0$). "Health" here is considered a general construct and can be measured in a number of ways, including the presence of shell disease (Tlusty and Metzler 2012; Tlusty et al. 2008) or any one of a myriad of hemolymph or physiological parameters (Basti et al. 2010; Chang 2005; Robohm et al. 2005). Currently, hemolymph protein (Theriault et al. 2008), immune function (Homerding et al. 2012), and gene expression (Tarrant et al. 2012) are all disrupted in lobsters with shell disease. If one of these physiological metrics is adopted, then the lower boundary would be the defined minimum for the parameter in question.

At the end of a molt, the lobster sloughs off the old shell, revealing the new shell. This represents a discontinuity between adjacent molt cycles. There is a sudden shift in bacterial load, as well as prevalence of shell disease. Graphically, it is represented as a break and substantial increase in health status at the transition between molt cycles (Fig. 1A). The cycle then repeats with the new shell. ESD, as well as all forms of shell diseases, appear to be iterative, as total animal health is integrated across molt cycles (Tlusty et al. 2008). From a graphical modeling perspective, it can be difficult to observe differences in subsequent molts when the impact is presented linearly (Fig. 1A). We propose to simplify such comparisons by overlaying the plot of subsequent health trajectories (Fig. 1B, Fig. 2). The stacked graphical representation of the individual health trajectory (IHT) makes it necessary to change the *x* axis to "time", as the length of subsequent molt cycles is not consistent. In the stacked IHT, the discontinuity from one molt cycle to the next remains, and the curve wraps around on itself, which is indicated by similar notation in Fig. 1B.

The stacked IHT is a special case of the "personalized health curve" (PHC) as developed by Schneider (2011). PHCs are biphasic plots showing the relationship between host and pathogens. The PHC model begins with a healthy individual, who is infected with a pathogen that increases to a state where the health of the host deviates from normal. Health may deflect negatively (pathogenesis), while the model also acknowledges that positive health benefits (mutualism) may occur. Under a pathogenic condition, the host may mount a defense response. In the recovery process, its health state, as well as the microbe numbers, will return back to their original values. In Schneider's PHC model, both axes vary continuously and can increase or decrease independently (and thus travel in any direction on the 2D plot). For shell disease in American lobsters, there is not a complete understanding of the dynamic between microbe number and shell disease severity. Thus, this model is already beneficial in identifying that when conceptualizing shell disease as a biphasic plot, it is apparent that a more complete understanding of the link between bacterial abundance and shell disease severity is required. Because lobster shell disease cannot plot health versus microbe number, the stacked IHT model necessarily differs in that one axis is time/molt cycle and is constrained given its unidirectionality. The stacked IHT is beneficial in that it provides a means to conceptualize changes in health across subsequent molt cycles and identify parameters (e.g., microbe number) that need to be better elucidated to complete the model.

Development of the ESD–IHT model

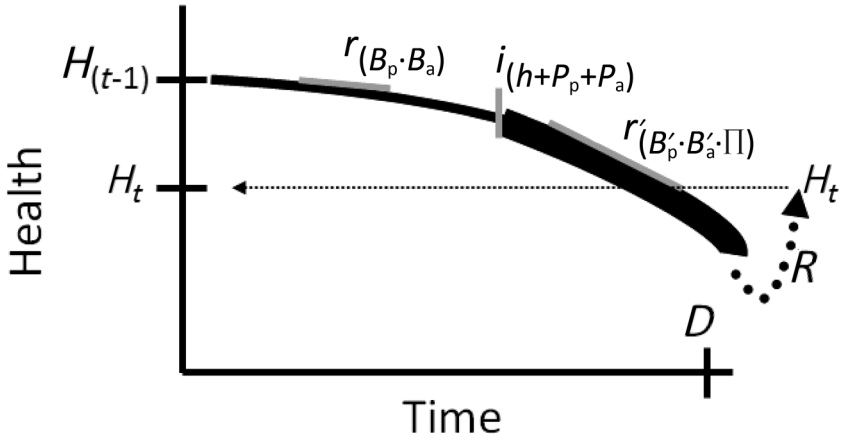
The ESD–IHT model is derived to graphically determine the status of an individual's health (H_t) throughout a molt cycle and to elucidate at what point shell disease occurs on an individual. The model in basic form construes that the status of an individual lobster's health after ecdysis (H_t) is a result of the heath at the start of the prior mole cycle (H_{t-1}) affected by the bacteria both prelesion (PL), as well as once the lesion is formed (ESD). Health is positively affected by recovery after the molt process (R, see eq. 1).

$$H_t = H_{t-1} + \mathrm{PL} + \mathrm{ESD} + R \quad (1)$$

Graphically, health is represented as a trajectory, and an asymptomatic lobster will retain a relatively high health level (Fig. 2A, asymptomatic individual). There is typically a downward trend in health just prior to molt based on the normal physiological process of ecdysis, which is often measured as a sudden change in hemolymph parameters (Mercaldo-Allen 1991). An individual that is susceptible to shell disease will become more susceptible (Tlusty et al. 2007) and have a general erosion of health (an increased downward slope (r) of the trajectory) until shell disease occurs (graphically represented as a thickened portion of the curve; Fig. 2A, symptomatic individual). When shell disease occurs, the lesion may allow for increased secondary infection, and slope of the health trajectory will likely steepen ($r'_{ESD} < r_{PL}$).

Fig. 3. The general equation for estimating the health of a lobster after it experienced epizootic shell disease (ESD) and the relevant components of the ESD – individual health trajectory (IHT). H indicates lobster health before ($t-1$) and after (t) molt in which the lobster had shell disease. D represents the length of the molt cycle, while R is the recovery after molt. The slope of the trajectory is divided by the point at which the lesion is formed ($i_{(h+P_p+P_a)}$). Lesion formation is determined by hardening (h), plus passive (P_p) and active (P_a) portals of entry. The slope (r) of the trajectory is dependent on the bacterial abundance (B_a) and pathogenicity (B_p), with these being different before and after lesion formation, as well as additional pathogens once the lesion is formed (Π).

$$H_t = \left(H_{(t-1)} + \left[r_{(B_p \cdot B_a)} \cdot i_{(h+P_p+P_a)} \right] + \left\{ r'_{(B'_p \cdot B'_a \cdot \Pi)} \left[D - i_{(h+P_p+P_a)} \right] \right\} \right) + R$$



There can be a variety of factors leading to the difference in susceptibility (Tlusty et al. 2007) between the asymptomatic and symptomatic individual (Castro et al. 2012; Gomez-Chiarri and Cobb 2012), including synergistic effects of multiple stressors (Robohm et al. 2005). After molting, this model then recovers (R) to the new shell (dotted line in Fig. 1A, Fig. 2A) that is not burdened with disease. From this point, the individual's trajectory can be remapped in the subsequent molt. In the case of succeeding bouts of ESD, there may be a general erosion of health (Tlusty et al. 2008) until a mortality event occurs (Fig. 2B, 3° molt).

The ESD–IHT model as presented in eq. 1 used general terms for both the changes in health prelesion (PL) as well as once the lesion formed (ESD). Prior to lesion initiation, the rate of declining shell health is associated with the bacteria to first breach the shell (Chistoserdov et al. 2012; Quinn et al. 2012*a*). Bacteria would influence shell health prior to lesion initiation both through their presence (B_p) and abundance (B_a). Breaching the shell will only occur when specific pathogenic strains or combinations of bacteria that make them more invasive are present. The carapace of American lobsters was more susceptible to microbial fouling than that of European lobsters (*Homarus gammarus*), and the American lobsters also experienced more severe lesions (Davies et al. 2014). More bacteria would also have a greater probability of breaching the shell; thus, the rate that health changes before lesion formation would be noted as $r_{(B_p \cdot B_a)}$ (Fig. 3). Once the lesion forms (ESD), the composition of bacteria affecting the shell would be different (Homerding et al. 2012). There may also be additional pathogens in the lesion (Π); thus, the comparable term for decrease in health after lesion formation would be $r'_{(B'_p \cdot B'_a \cdot \Pi)}$.

The division between PL and ESD is a function of how long it takes the lesion to form. The time of initiation (i; Fig. 3) of shell disease can impact the overall severity of shell disease. If the initial attack is delayed for any reason, then the total amount of time that the bacteria have to act on the lesion will be reduced (Fig. 2C). If there is a delay in the initiation of the lesion, then the individual will experience a lesser degree of shell disease and hence will recover back to a higher health status (Fig. 2C, C′ compared with C″). The association between a longer time to initiation and a lower shell disease severity has been observed in laboratory experiments (Tlusty et al. 2008).

Shell disease severity is a combination of how rapidly a lesion is initiated along with the amount of time the lesion is present on the shell. The lesion duration is calculated as the length of time between lesion initiation (i) and the total length of the molt cycle (D). The total time of the molt cycle (D; Fig. 3) was observed to modulate the level of shell disease based on the fact that females with eggs that have their shells for longer will exhibit elevated levels of shell disease (Castro et al. 2006, 2012). Similarly, while bacteria grow faster at higher temperatures, a laboratory study demonstrated that shell disease was significantly elevated in lobsters at intermediate temperatures, largely because of a longer molt cycle (Tlusty and Metzler 2012). In the graphical model, this can be demonstrated by adjusting the length of the molt cycle (see Fig. 2D). Lengthening the time of the molt cycle will result in a more severe incidence of shell disease, and likely poorer health following the molt (D'''; Fig. 2D), provided all other model parameters remain constant. It follows that an early molt may be an active defense against shell disease (D'; Fig. 2D) (Somers 2005). Laufer et al. (2005) found higher levels of molting hormones in lobsters with shell disease, suggesting an active means of defense to limit damage.

The initiation of the lesion (i) is the dividing point between the PL and ESD portions of the molt cycle (Fig. 3). The factors affecting how soon initiation begins include portals of entry for the bacteria, as well as how rapidly the shell hardens after ecdysis. There are two means for a portal of entry (P) to be opened into the lobster shell. A passive route of entry (P_p) would include errors in shell formation such as a gap between the different structural components of the shell (Kunkel et al. 2012). The portal of entry could also include active routes (P_a) such as mechanical damage to the shell through contact with abrasive surfaces (Davies et al. 2014; Quinn et al. 2012*b*). The time to initiation is also affected by the process of shell hardening (h; Laufer et al. 2012). Within the ESD–IHT model, hardening can be accounted for at the beginning of the cycle (Fig. 2D). One of the suggested modes by which pollutants such as alkylphenols may exacerbate shell disease is through a delay in the hardening response (Laufer et al. 2012). This can have the opposite effect of the time of initiation, in that the time to harden represents a time of initial decreased health early in the molt cycle (Fig. 2D). The longer it takes the shell to harden, the

more susceptible the lobster may be to shell disease (Fig. 3). Any interference with the hardening process may lead to steeper slope and thus a more rapid and severe onset of shell disease, as occurs in the difference between rapid and delayed onset of the disease (Fig. 2C). Thus, the full initiation term would account for hardening and the portals of entry: $i_{(h+P_p+P_a)}$.

Incorporating the above factors into the PL and ESD components of eq. 1, we respectively create time-weighted rates of health decline for each:

$$\text{PL} = \left[r_{(B_p \cdot B_a)} \cdot i_{(h+P_p+P_a)}\right] \quad (2)$$

$$\text{ESD} = r'_{(B'_p \cdot B'_a \cdot \Pi)} \cdot \left[D - i_{(h+P_p+P_a)}\right] \quad (3)$$

Substituting these values into the model as presented in eq. 1 provides the full model represented as

$$H_t = \left(H_{(t-1)} + \left[r_{(B_p \cdot B_a)} \cdot i_{(h+P_p+P_a)}\right] + \left\{r'_{(B'_p \cdot B'_a \cdot \Pi)}\left[D - i_{(h+P_p+P_a)}\right]\right\}\right) + R \quad (4)$$

Finally, in an ideal situation, organisms would gain some benefit after disease (Schneider 2011). This would be particularly true if they could mount an immune response. This would be modeled as upon molting, health may exceed that of the starting point in the first molt cycle (Fig. 2E, curve E′). The resultant trajectory (E″) would not exhibit the same level of decline as during the previous molt cycle. This series of trajectories would imply active incorporation of genetic, immune, chemical, or structural defenses. It could also occur if the causative factors were removed.

Summary

This model augments our understanding of how individual lobsters are affected by and potentially mitigate the disease. It is intended to elucidate the processes occurring at the individual level that can then be integrated into population-level epidemiology models (Castro et al. 2012). This model affords a mechanistic approach to examining the environment–host interface, which enhances the Snieszko (1974) model. Laboratory studies (Tlusty and Metzler 2012) indicated that temperature influenced molt duration (D), which was correlated to disease severity. In the wild, temperature may also affect bacterial presence and abundance (B_p, B_a, B'_p, B'_a), as well as pathogens that would opportunistically colonize the lesion (Π). Each environmental parameter that is correlated to shell disease (Castro and Somers 2012; Gomez-Chiarri and Cobb 2012; Shields et al. 2012) needs to be similarly evaluated to assess the mechanisms by which individual lobsters become more susceptible to the disease.

There is one main difference between the model for crustacean shell disease presented here and the original biphasic IHT as originally put forth by Schneider (2011). The original plotted health versus pathogen abundance, whereas the model for crustacean shell disease plots health against time. In crustacean shell diseases, the overall health appears not to be a simple function of bacterial abundance. Thus, to develop this model, time was selected for the x axis, since this represents progression through the molt cycle. If future work identifies a continual relationship between bacteria and crustacean health, then the model presented here can be easily modified to a biphasic form.

This model for shell disease in crustaceans, as it currently stands, is consistent with many of the laboratory and nature-based observations of shell disease in American lobsters. However, as with any model, each of the parameters discussed needs to be further explored and better elucidated. This model is intended to create a means to create a priori hypotheses that will lead to a more robust and comprehensive experimental approach to examining this disease.

Acknowledgements

S. Cobb was the first to point out the writings of Oppian, and we thank him graciously for his intellect and wit. D. Schneider and three anonymous reviewers provided comments on a draft of this paper. We thank C. Wilson for sharing observations of the spread of shell disease into Maine.

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