Is there an association between elongation factor 1-α overdominance in the seastar *Pisaster ochraceus* and "seastar wasting disease"?

John Wares and Lauren Schiebelhut

28 October, 2015

Abstract

In recent years, a massive plague has killed millions of seastars, of many different species, along the Pacific coast of North America. This disease, known as 'seastar wasting disease' (SSWD), is thought to be caused by viral infection. In the affected seastar *Pisaster ochraceus*, previous work had identified that the elongation factor 1-α locus harbored an intronic insertion allele that is lethal when homozygous yet appears to be maintained at moderate frequency in populations through increased fitness for heterozygotes. The environmental conditions supporting this increased fitness are unknown, but overdominance is often associated with disease. Here, we evaluate seastars from 3 regional populations of *P. ochraceus* to identify the relationship between SSWD and genotype. Although our data suggest that there may be decreased infection or mortality rates in individuals that are heterozygous at this locus, the effect is small and not statistically significant.

Introduction

One of the more stunning recent news stories pertaining to ocean health was the massive die-off of seastars on both coasts of North America via a necrotic syndrome now known as sea star wasting disease (SSWD) (Hewson et al. 2014). Similar die-offs have happened in earlier decades (Eckert, Engle, and Kushner 1999; Becker 2006), though none as extensive as in 2013-2014. Hewson et al. (2014) identified a candidate densovirus that is in greater abundance in diseased sea stars, and may be a causal agent; however, there is much yet to be learned. As seastars are key predators in marine benthic ecosystems, the impacts of disease on these organisms could dramatically restructure coastal communities (Paine 1966). Thus, we address here the potential for one species to respond to disease via natural genetic variation.

During disease outbreaks, biologists are keen to know whether populations will exhibit any resistance to a pathogen. Thus, management studies may include surveys of genetic diversity to identify the potential for evolving resistance, or genetic rescue from other regions (Whiteley et al. 2015); such studies may also provide insight into the extent of population structure and gene flow among regions. Following a routine analysis of genetic variation in the seastar *Pisaster ochraceus* (Harley et al. 2006), Pankey and Wares (2009) identified an insertion mutation in an intron of the elongation factor 1-α gene (hereafter EF1A) that appeared to exhibit overdominance. In this case, the insertion is lethal when homozygous (Pankey and Wares 2009), yet the average frequency of the insertion allele was ~0.24 along the Pacific coast of North America. These observations suggest that the heterozygote has a significant fitness advantage in an unknown environmental setting. Overdominance is often associated with resistance to disease or toxins, however, and Pankey and Wares (2009), referring to what is now called SSWD, speculated that

> "widespread die-offs on the west coast of North America... could exert a substantial selective force on *Pisaster*. Given the prevalence of pathogen resistance in earlier studies of overdominance, we believe this to be a probable explanation for the maintenance of the described ... polymorphism."

There is concern that elevated sea temperature is a component of the SSWD outbreak (Bates, Hilton, and Harley 2009; Hewson et al. 2014). The relationship between expression of EF1A and thermal tolerance

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1464v1 | CC-BY 4.0 Open Access | rec: 30 Oct 2015, publ: 30 Oct 2015

has been identified in other metazoans (Stearns, Kaiser, and Hillesheim 1993; Buckley, Gracey, and Somero 2006), and functions in part through rapid co-production of proteins associated with the heat shock response. Though Pankey & Wares (2009) were not able to detect EF1A expression differences among individuals of differing genotype, we were not able at the time to control for a number of environmental factors nor the possible action of splice variants. Here, we posit an indirect mechanistic relationship between temperature, the effect of expression of EF1A, and SSWD.

With as little as is known about this disease and marine disease in general (L. D. Mydlarz, Jones, and Harvell 2006), this is at best an educated guess. However, it is useful to know what potential *P. ochraceus* and other seastars have for surviving this outbreak and natural patterns of genetic variation, and whether subsequent generations will be more resistant or tolerant of similar pathogens. Here we evaluate this simple polymorphism from populations of *P. ochraceus* collected prior to and following the SSWD outbreak, as well as focus on particular individuals and their disease status. We ask whether there are frequency shifts of the two genotypes at this locus that may be associated with resistance to infection, and evaluate efforts to explore similar genomic variation in other species.

Methods

Pisaster and Disease Status

Collections were made in 2014 from locations in central California, sites near Friday Harbor (Washington), and Nanaimo (Vancouver), and categorized by health status using the Pacific Rocky Intertidal Monitoring Network classification (Table 1). Individuals from Friday Harbor were *a priori* separated into juvenile (arm tip to center $\leq$ 50mm) and adult cohorts. Complete information on collection location, individual sizes, and other metadata are in Supplemental Data S1.

Region	SSWD +/+	SSWD +/ins	healthy +/+	healthy +/ins
California	8	1	11 (11)	7 (11)
Friday Harbor (juvenile)	7	10	3	5
Friday Harbor (adult)	18	7	12	12
Nanaimo	8	4	7	5

Table 1. *Sample sizes from each regional collection of individuals (see Supplemental Table 1 for additional sampling information); samples are listed by health status as well as genotype (+/+ wild type, +/ins for the heterozygote genotype). Individuals from the California collection that were of uncertain health status but did not meet the criteria for SSWD (using the Pacific Rocky Intertidal Monitoring Network classification) are listed in parentheses; further information on classification is in Supplemental Data S1. Individuals from Friday Harbor were separated into juvenile (<50mm center to arm tip) and adult size classes prior to analysis.*

Temporal comparisons

In addition to individuals explicitly assessed for health status, we also considered the potential for genotype (and allele) frequency change following a related disease outbreak. Previous EF1A genotype/allele frequency information from specimens collected in 2003-2004 are available for many locations along the Pacific coast (Pankey and Wares 2009). In central California, tissues from 4 locations (Sonoma County) were obtained in both 2012 (pre-outbreak) as well as 2014 (the tissues noted in previous section from these sites). Thus genetic frequencies from 3 time points can be assessed. In the Friday Harbor (WA) region, the tissues noted in previous section (from 2014) can be compared to the genetic frequency information from 2003-2004 tissues.

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1464v1 | CC-BY 4.0 Open Access | rec: 30 Oct 2015, publ: 30 Oct 2015

Molecular Methods

As in Pankey and Wares (2009), primers PisEF1-F (5'-*aggctgccgataccttcaa*-3') and PisEF1-R (5'-*gctagtatctgtttctgtgtgactgc*-3') were used to determine individual EF1A genotypes by scoring length-polymorphic PCR products on 2% (or greater) agarose gels. About 10% of individuals were multiply genotyped so that genotype error rate (Pompanon et al. 2005) could be assessed. An example of this polymorphism is shown in Figure 1.

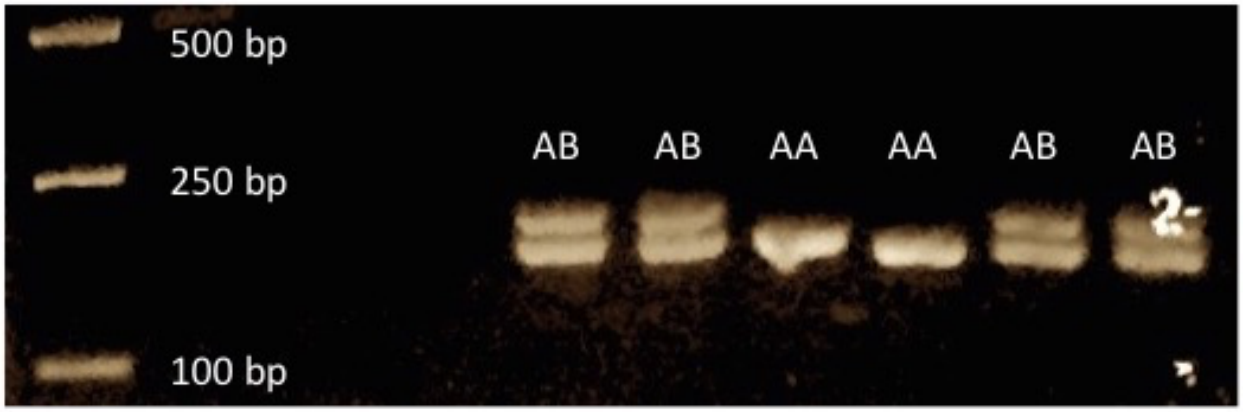

Figure 1. *Results from analysis of 6 individuals (right side of image) on 2% agarose gel following PCR amplification as noted in Methods. Fragments only vary by 6bp in length so gel must be run for ~60 minutes under typical conditions. Heterozygotes are denoted 'AB' and homozygotes denoted 'AA' on gel image (white "2" at far right is a marking on gel tray). Size ladder is shown at left side of gel.*

Statistical Analyses

Our first approach is to ask whether the frequency distribution of the two EF1A genotypes differs between diseased and healthy individuals of *P. ochraceus*. Samples from distinct sample sites are grouped by region (California, Friday Harbor, Vancouver) and each regional sample is evaluated separately as well as combined. Separate analyses recognize the potential for heterogeneity at related quantitative traits despite apparent phylogeographic homogeneity (Pankey and Wares 2009), as well as distinct environmental influences, while pooled analysis augments statistical power.

Regional and combined data are analyzed first with a Fisher's exact test. Additionally, following Gerrodette (2011), we estimate the effect size of genotype on SSWD mortality. Here we assume that the genotype frequencies are binomially distributed (with associated sampling error) and estimate the difference in proportion of disease incidence (also here assumed binomial) between homozygotes and heterozygotes. Again, these probability distributions are estimated for each individual regional/temporal sample. These same statistical methods were applied to evaluate genotype frequency changes between population samples from before and after the 2013-14 SSWD outbreak, as described above.

To evaluate the probability that heterozygous individuals have a higher probability of avoiding or surviving SSWD, logistic regression of the complete dataset is performed with both individual size (when available, measured from center of disk to tip of an arm) and EF1A genotype. All statistical analyses were performed using R (R Core Team 2015).

Results

The genotype error rate was 0 for 21 individuals that were repeatedly genotyped. Two individuals initially presented a very faint second band on gel, but subsequent repeat amplification of one of these confirmed it as

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1464v1 | CC-BY 4.0 Open Access | rec: 30 Oct 2015, publ: 30 Oct 2015

homozygous (note: this polymorphism has been assessed as an overdominant Mendelian locus (Pankey and Wares 2009), so we do not think this represents variation in paralog amplification success).

Data from each location (Table 1) were analyzed with a basic Fisher's exact test. Our sample from central California (n=27) suggested an increase of 0.296 probability of infection in individuals that are EF1 homozygotes (p-value 0.201). When individuals of uncertain disease history or status were included in the 'healthy' group (n=49), the genotype x disease differential was actually less (0.214) and still not significant (p-value 0.1265285). Note that some of these uncertain status individuals could be argued for being included in part or in whole with either factor group, but do not satisfy the criteria for SSWD.

Evaluating the samples from the Friday Harbor region, we separately analyzed data from 'juvenile' seastars (size < 50mm) and adult seastars of *P. ochraceus*. For the juveniles, there was no indication of an increased probability of infection in homozygotes (Fisher's p-value 1). However, among the adult cohort sampled in 2014, the pattern of genotype x health status was similar to that from California (p-value 0.1481603). There was little effect of genotype on disease status in the sample from Nanaimo (Fisher's p-value 1)

The individual probability distributions of effect attributable to genotype are shown in Figure 2. Certainly a component of individual regional/temporal samples is that with modest sample sizes, statistical power is low. Only in samples from California and the "adult" sample from Friday Harbor is the hypothesis supported for a consistent effect of genotype on disease prevalence. Combining these two samples alone (only mentioned because the original design of this exploration would have only used these two samples) leads to marginal support for our hypothesis both in terms of the effect distribution and the statistical support from an exact test (p-value 0.0558914). Combining all data sets leads to an equivocal result (Figure 2, p-value 0.2050854).

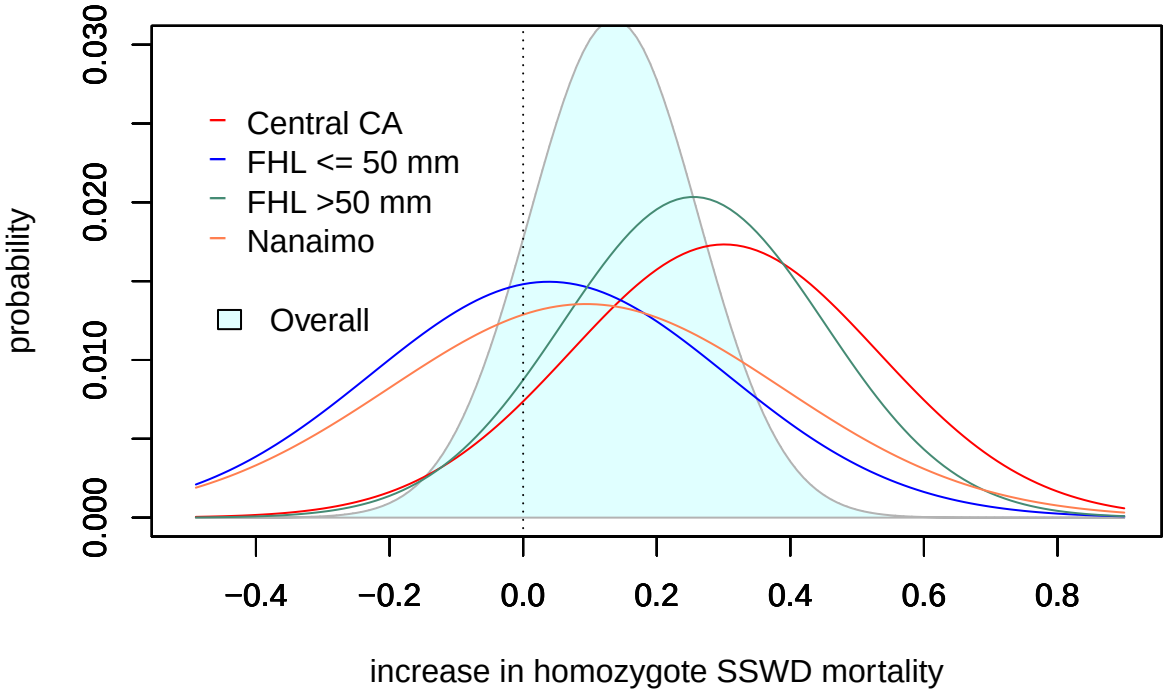

Figure 2. *Estimated probability distribution function assuming binomial error terms; the biological effect (x-axis) is the increase in SSWD mortality associated with an individual being homozygous for the EF1 polymorphism rather than heterozygous. Plots are shown for individual samples (spatial, temporal) as well as a combined inference for all samples.*

Logistic regression using radius of the individual and genotype as factors confirms the result of Hewson et al. (2014) indicating that size is only a marginally significant factor in disease presence ($p = 0.0772$). The same multifactor regression model indicated little support for genotype as a factor ($p = 0.173$; Table 2).

Despite the hypothesis of increased fitness for EF1 heterozygotes under these conditions, the frequency of the insertion (*ins*) allele in central California only appears to decrease through time, from approximately 0.27

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1464v1 | CC-BY 4.0 Open Access | rec: 30 Oct 2015, publ: 30 Oct 2015

(n=33) in 2003-4 (Pankey and Wares 2009) to 0.24 (n=40) in 2012 and 0.21 (n=40) in 2014. However, with sampling error these frequencies are effectively unchanged and a larger sample comparison may be necessary to explore this component of our evaluation. At Friday Harbor, the frequency of the *ins* allele increased slightly from 0.27 in 2003 (n=62) to 0.3 in 2014 (n=25) juveniles; the frequency in adults (0.23, n=46) is considerably lower, again not supporting a hypothesis of selection increasing or maintaining the *ins* frequency - however, this frequency includes both healthy and sick individuals selected *for that comparison*, so it is a non-random sample.

Discussion

Our data show that our hypothesis for a relationship between disease status (SSWD) and an apparent overdominant polymorphism in *P. ochraceus* is not well supported - results from each sample are in the predicted direction, but no single sample nor the combination of all samples confirms the proposed relationship. Certainly more replication is warranted, as the current samples include a modest number of individuals from central California (data from which suggest an increase of 0.296 probability of infection in individuals that are EF1 homozygotes), a sample of juveniles (radius <50mm) from the Friday Harbor region as well as a range of size classes from Nanaimo that do not support an effect at all, and a sample of 'adults' from the Friday Harbor region that again suggest an increased probability (0.25) of infection/mortality for homozygotes. It is likely that each of our regional samples has been exposed to distinct temperature profile histories (Bates, Hilton, and Harley 2009), and it is possible that despite apparent population genetic homogeneity (Harley et al. 2006) that undetected evolutionary changes have led to distinct reaction norms among regional samples. With little replication, it is hard to say whether separation of samples at Friday Harbor by size class - which was done *a priori* but arbitrarily - clarifies the relationship between polymorphism and SSWD-associated mortality, though the ecology of juveniles and adults is likely to differ in many ways.

Size as determinant?

Hewson et al. (2014) tested whether size was a predictive factor in disease status and found no clear statistical association (though it appeared there is a negative relationship between densovirus abundance and size in *Pycnopodia helianthoides*). Given our limited data on size, disease status, and genotype, there is some question whether the effectively accidental partition in the Friday Harbor animals between small (center to arm tip diameter $\leq$ 50mm) and large individuals is meaningful (note that all central CA seastars were larger than this, as were most individuals from Nanaimo). Binomial regression of the data, separated by size and into the two genotypic classes, does not suggest that the relationship between size and sickness differs between genotypes. However, examination of the size distribution across all of our data suggests that healthy individuals (99.7297297 $\pm$ 41.6699515mm diameter) tend to be only slightly larger than sick individuals (88.9491525 $\pm$ 34.1600814mm).

If smaller individuals are better able to tolerate exposure or otherwise avoid heat stress, perhaps the relevant fitness gain from EF1A regulation (and heat shock response) is minimal. Though it has been suggested that, for biomechanical reasons, the smaller-sized seastars could better tolerate such stressors, recent empirical work shows that for animals that have taken low-tide refuge (e.g. crevices or under macroalgae), there are no differences in lethal temperature or performance distributions (Monaco 2014). Although smaller *P. ochraceus* do tend to be found higher in the intertidal, these distributional differences are likely mediated by behavior and refuge size rather than thermal ecology (Monaco 2014) (*n.b.,* the work by Monaco et al. is based on body weight; our measurements are based on diameter; and (Hayne and Palmer 2013) shows that the relationship between size and mass is plastic and exposure-specific; also (Fly et al. 2012) notes that intertidal emersion appears to be a negligible temperature stress relative to interannual variation in water temperature).

One remaining notable component of this analysis that is difficult to overcome because of sampling limitations, almost all of the "juvenile" individuals assessed in this study come from a geographically restricted sample on Orcas Island, WA. The "adult" individuals from the Friday Harbor region include 13 out of 50 individuals from the same location, however. Whether there are additional components of geography, tidal height, sex,

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1464v1 | CC-BY 4.0 Open Access | rec: 30 Oct 2015, publ: 30 Oct 2015

or other factors remains to be considered with additional samples; however these frequencies for the *ins* allele varied little across the entire Pacific coast in previous surveys (Harley et al. 2006).

Evolutionary response

Despite the apparent and predicted effect in our samples (Figure 2), we do not see the hypothesized evolutionary response - the frequency of the *ins* mutation has not increased in recent years. If EF1A in *P. ochraceus* truly evolves via overdominance, where the heterozygote is significantly more fit under certain environmental conditions, then we would expect this allele to increase in frequency when exposed to a relevant mass mortality event. However, it is also not entirely clear what proportion of individuals have died in recent years as a result of SSWD (though estimates from intertidal surveys are high enough that some frequency response is warranted), and there is some indication that infection and death via SSWD results in mass gamete release - that is, the plagues of SSWD may reduce survival, but not fecundity, of affected individuals. Thus, detection of this change could be masked in part by simple stochastic changes (e.g. genetic drift) in local populations of *P. ochraceus*. Of course, there are other forms of mortality in seastars like *P. ochraceus* (Jurgens et al. 2015), and so it is still likely that we are seeing an indirect interaction between recent die-offs and individual-level responses that appear to be genotype dependent.

Disease in seastars

With limited understanding of immune response in most echinoderms (L. Mydlarz, Jones, and Harvell 2006), the problem of SSWD is difficult enough to explore in *P. ochraceus*, let alone the many other species affected in the recent outbreak. In another species of seastar (*P. helianthoides*), Fuess et al. (2015) have identified some of the genomic components that are upregulated in response to viral exposure; however, we know of no similar (apparent) overdominant system in these other asteroids, and as yet still know very little about how this polymorphism in *P. ochraceus* influences EF1A expression, alternate splicing events, or what genes may be linked to this region and thus affected. Our interest in exploring this particular case has little to do with solving the problem of disease, and more about the question of what demographics will be like for *P. ochraceus* in an increasingly warmer - and disease-affected - environment (Harvell et al. 2002). If disease like SSWD was interacting with the EF1A polymorphism noted here, and the frequency of the deleterious *ins* allele increased, this could indicate increased reproductive loss through homozygous lethality, which could decrease the potential for populations to rebound from crashes.

Parallels with Malaria

Aidoo et al. (2002) note that "sickle cell trait" (carrying a single *S* allele of hemoglobin) provides ~60% protection against overall mortality, mostly in the first 16 months of life; being a carrier is not a guarantee against infection. Other studies have focused on specific malarial parasites, and note that children heterozygous for the *S* hemoglobin allele have approximately one-tenth the mortality risk from *Plasmodium falciparum* as those homozygous for normal alleles (Cholera et al. 2008). In the absence of cohort data, it is difficult to estimate the level of disease or mortality protection that any single polymorphism can provide (Aidoo et al. 2002). Until such recent studies, the claim of overdominant selection on hemoglobin genotypes, based on the relationship between the frequency of the *S* allele and the prevalence of malaria (Allison 1954), was only correlative. This is still a clear case of overdominance, but is illustrative that increased heterozygote fitness does not require absolute protection against the associated risk factor - *e.g.*, that all individuals of *P. ochraceus* with SSWD would be homozygous for the wild-type EF1 allele identified in Pankey & Wares (2009), nor that healthy individuals would all be heterozygotes - as there are many components to disease avoidance, tolerance, or resistance.

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1464v1 | CC-BY 4.0 Open Access | rec: 30 Oct 2015, publ: 30 Oct 2015

Conclusions

At this time, with limited sampling, it is fair to say that there is little support for a relationship between SSWD outbreaks and the EF1A polymorphism in *P. ochraceus*. However, the results of this study are suggestive, in that the direction of effect is consistent, but the magnitude of this effect is likely small. Pankey and Wares (2009) indicated, based on simulation work, that the fitness differential between the genotypes would have to be ~0.2; our overall sample (see Figure 2) appears to have a biological effect of similar magnitude, and perhaps continued sampling would change the statistical outcome. Nevertheless, we do not see an increase in the frequency of the *ins* allele over time and so we remain curious about what drives overdominance at the EF1A locus.

It is possible, however, that regulation of EF1A is influenced by this polymorphism in a way that alters an individuals' tolerance or capacity for heat stress, and in a warming climate and ocean it is known that disease and mortality are higher in large part because of physiological stress modifying an organisms response to pathogens. Further work is needed not only to examine the association shown here, but also to identify (*i*) whether size or maturity is important in this relationship, (*ii*) whether individuals of different genotype do have distinct constitutive or regulated patterns of expression of EF1A or related/linked genes, and (*iii*) whether there are genotype-driven differences in mortality of individuals under thermal stress (which can affect feeding rates as well as physiological factors in *P. ochraceus*; (Sanford 2002)).

In the meantime, we emphasize that this system is so easy to explore as a low-budget research project or teaching tool that there are opportunities to work as a community to greatly expand our understanding of the maintenance of the overdominant EF1A diversity in *P. ochraceus*, perhaps for other pertinent variables of interest. We would encourage any interested colleagues to ensure that sufficient metadata are associated with any such comparative study so that data can continue to be updated, and we will facilitate this crowd-sourcing of analysis by maintaining a dynamic analytical database through JPW.

Acknowledgments

The authors would like to thank Dr. Collin Closek (Wares Lab alumnus) and Mo Turner for coordinating tissue samples from FHL, Mike Hart and John Drake for discussion of the idea and early drafts of the manuscript, California Sea Grant College Program grant #2012-R/ENV-223PD and National Science Foundation grant OCE-1243958 and OCE-1243970 to Michael Dawson, Rick Grosberg and Brian Gaylord, and NSF-Ecology of Infectious Diseases funding (award no. 1015342) to Wares and UGA colleagues, members of the Wares Lab at UGA and colleagues at the Odum School of Ecology. Mr. Tim Makinde balanced his pre-dental training with learning how to genotype seastars and enabled the quick turnaround on this project. We thank Sarah Abboud, Charlsie Berg, Anny Calderon, Lorely Chavez, Brendan Cornwell, Michael N Dawson, Madlen Friedrich, Brian Gaylord, Alehandra Guzman, Brittany Jellison, Laura Jurgens, Shawn Knapp, Kelly McClintock, Holly Mondo, Mira Parekh, Emily Ramirez, Mariana Rocha de Souza, Adam Rosso, Stephen Sanchez, Holly Swift, Sabah Ul Hussan, and Jesse Wilson for assisting with California collections in 2012-2014. Thanks to Mike Hart, Vanessa Guerra, and David Breault for specimen collection and handling of Nanaimo tissues in 2015, and Pete Raimondi for organizing collection of tissues along Oregon coast (analysis pending).

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1464v1 | CC-BY 4.0 Open Access | rec: 30 Oct 2015, publ: 30 Oct 2015

Aidoo, M., D. J. Terlouw, M. S. Kolczak, P. D. McElroy, F. O. ter Kuile, S. Kariuki, B. L. Nahlen, A. A. Lal, and V. Udhayakumar. 2002. "Protective Effects of the Sickle Cell Gene Against Malaria Morbidity and Mortality." Journal Article. *Lancet* 359 (9314): 1311–2. doi:10.1016/S0140-6736(02)08273-9.

Allison, A. C. 1954. "Protection Afforded by Sickle-Cell Trait Against Subtertian Malareal Infection." Journal Article. *Br Med J* 1 (4857): 290–4. http://www.ncbi.nlm.nih.gov/pubmed/13115700.

Bates, A. E., B. J. Hilton, and C. D. Harley. 2009. "Effects of Temperature, Season and Locality on Wasting Disease in the Keystone Predatory Sea Star Pisaster Ochraceus." Journal Article. *Dis Aquat Organ* 86 (3): 245–51. doi:10.3354/dao02125.

Becker, B. J. 2006. *Status and Trends of Ecological Health and Human Use of the Cabrillo National Monument Rocky Intertidal Zone (1990–2005)*. Report. National Park Service.

Buckley, B. A., A. Y. Gracey, and G. N. Somero. 2006. "The Cellular Response to Heat Stress in the Goby Gillichthys Mirabilis: A CDNA Microarray and Protein-Level Analysis." Journal Article. *J Exp Biol* 209 (Pt 14): 2660–77. doi:10.1242/jeb.02292.

Cholera, R., N. J. Brittain, M. R. Gillrie, T. M. Lopera-Mesa, S. A. Diakite, T. Arie, M. A. Krause, et al. 2008. "Impaired Cytoadherence of Plasmodium Falciparum-Infected Erythrocytes Containing Sickle Hemoglobin." Journal Article. *Proc Natl Acad Sci U S A* 105 (3): 991–6. doi:10.1073/pnas.0711401105.

Eckert, G. L., J. M. Engle, and D. J. Kushner. 1999. "Sea Star Disease and Population Declines at the Channel Islands." Journal Article. *Proceedings of the Fifth California Islands Symposium* 5: 390–93.

Fly, E. K., C. J. Monaco, S. Pincebourde, and A. Tullis. 2012. "The Influence of Intertidal Location and Temperature on the Metabolic Cost of Emersion in Pisaster Ochraceus." Journal Article. *Journal of Experimental Marine Biology and Ecology* 422: 20–28. doi:10.1016/j.jembe.2012.04.007.

Fuess, L. E., M. E. Eisenlord, C. J. Closek, A. M. Tracy, R. Mauntz, S. Gignoux-Wolfsohn, M. M. Moritsch, et al. 2015. "Up in Arms: Immune and Nervous System Response to Sea Star Wasting Disease." Journal Article. *PLoS One* 10 (7): e0133053. doi:10.1371/journal.pone.0133053.

Gerrodette, T. 2011. "Inference Without Significance: Measuring Support for Hypotheses Rather Than Rejecting Them." Journal Article. *Marine Ecology-an Evolutionary Perspective* 32 (3): 404–18. doi:Doi 10.1111/J.1439-0485.2011.00466.X.

Harley, C. D., M. S. Pankey, J. P. Wares, R. K. Grosberg, and M. J. Wonham. 2006. "Color Polymorphism and Genetic Structure in the Sea Star Pisaster Ochraceus." Journal Article. *Biol Bull* 211 (3): 248–62. http://www.ncbi.nlm.nih.gov/pubmed/17179384.

Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. "Ecology - Climate Warming and Disease Risks for Terrestrial and Marine Biota." Journal Article. *Science* 296 (5576): 2158–62. doi:Doi 10.1126/Science.1063699.

Hayne, K. J. R., and A. R. Palmer. 2013. "Intertidal Sea Stars (Pisaster Ochraceus) Alter Body Shape in Response to Wave Action." Journal Article. *Journal of Experimental Biology* 216 (9): 1717–25. doi:10.1242/jeb.078964.

Hewson, I., J. B. Button, B. M. Gudenkauf, B. Miner, A. L. Newton, J. K. Gaydos, J. Wynne, et al. 2014. "Densovirus Associated with Sea-Star Wasting Disease and Mass Mortality." Journal Article. *Proc Natl Acad Sci U S A* 111 (48): 17278–83. doi:10.1073/pnas.1416625111.

Jurgens, L. J., L. Rogers-Bennett, P. T. Raimondi, L. M. Schiebelhut, M. N. Dawson, R. K. Grosberg, and B. Gaylord. 2015. "Patterns of Mass Mortality Among Rocky Shore Invertebrates Across 100 Km of Northeastern Pacific Coastline." Journal Article. *Plos One* 10 (6). doi:Artn E0126280 Doi 10.1371/Journal.Pone.0126280.

Monaco, Cristian J. 2014. "Thermal Ecology and Physiology of an Intertidal Predator-Prey System: Pisaster Ochraceus and Mytilus Californianus."

Mydlarz, L. D., L. E. Jones, and C. D. Harvell. 2006. "Innate Immunity Environmental Drivers and Disease Ecology of Marine and Freshwater Invertebrates." Journal Article. *Annual Review of Ecology Evolution and Systematics* 37: 251–88. doi:Doi 10.1146/Annurev.Ecolsys.37.091305.110103.

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1464v1 | CC-BY 4.0 Open Access | rec: 30 Oct 2015, publ: 30 Oct 2015

Mydlarz, LD, LE Jones, and CD Harvell. 2006. "Innate Immunity, Environmental Drivers, and Disease Ecology of Marine and Freshwater Invertebrates." Journal Article. *Annu. Rev. Ecol. Evol. Syst.* 37: 251–88.

Paine, R. T. 1966. "Food Web Complexity and Species Diversity." Journal Article. *American Naturalist* 100 (910): 65–65. doi:Doi 10.1086/282400.

Pankey, M. S., and J. P. Wares. 2009. "Overdominant Maintenance of Diversity in the Sea Star Pisaster Ochraceus." Journal Article. *J Evol Biol* 22 (1): 80–87. doi:10.1111/j.1420-9101.2008.01623.x.

Pompanon, F., A. Bonin, E. Bellemain, and P. Taberlet. 2005. "Genotyping Errors: Causes, Consequences and Solutions." Journal Article. *Nat Rev Genet* 6 (11): 847–59. doi:10.1038/nrg1707.

R Core Team. 2015. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.

Sanford, Eric. 2002. "Water Temperature, Predation, and the Neglected Role of Physiological Rate Effects in Rocky Intertidal Communities." *Integrative and Comparative Biology* 42 (4): 881–91. doi:10.1093/icb/42.4.881.

Stearns, S. C., M. Kaiser, and E. Hillesheim. 1993. "Effects on Fitness Components of Enhanced Expression of Elongation Factor EF-1alpha in Drosophila Melanogaster. I. the Contrasting Approaches of Molecular and Population Biologists." Journal Article. *Am Nat* 142 (6): 961–93. doi:10.1086/285584.

Whiteley, A. R., S. W. Fitzpatrick, W. C. Funk, and D. A. Tallmon. 2015. "Genetic Rescue to the Rescue." Journal Article. *Trends in Ecology & Evolution* 30 (1): 42–49. doi:10.1016/j.tree.2014.10.009.

PeerJ PrePrints | https://dx.doi.org/10.7287/peerj.preprints.1464v1 | CC-BY 4.0 Open Access | rec: 30 Oct 2015, publ: 30 Oct 2015