

**Impacts of Sea Star Wasting Syndrome on Larval *Pisaster ochraceus*: a pilot study  
on pathogen exposure**

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## **Abstract**

A recent outbreak of sea star wasting syndrome (SSWS) along the U.S. West coast has decimated populations of many intertidal and subtidal sea star species. If populations are unable to recover quickly, SSWS is likely to cause significant changes to rocky intertidal and subtidal community structure and functioning in the Northeast Pacific ocean. Recovery will likely depend in large part on the success of larval recruitment, but the impacts of SSWS on larvae are unknown. We raised embryos and early stage *P. ochraceus* larvae in treatments of sea water that varied in filtration exposure to potential pathogens of SSWS. We assessed treatment impacts on larval survival development and larval size. We found a significant effect of water treatment and filtration level on two measures of larval survival. Further studies are needed to better understand the direction and magnitude of these and other effects of SSWS on larval fitness.

## **Introduction**

Beginning in 2013-2014, many sea star species along the West coast of the United States have been impacted by a “sea star wasting” syndrome at epidemic proportions, virtually eliminating populations from intertidal and subtidal habitats ranging from California to southern Canada (Stokstad, 2014). Species affected include many large predators, such as *Pynopoda helianthoides*, *Pisaster brevispinis*, and *Pisaster ochraceus*, which play important roles in community food webs. If populations are unable to recover quickly, SSWS is likely to result in important changes to rocky intertidal and subtidal community structure and functioning (Schrope, 2014). These changes may follow similar trajectories seen in Washington by Paine in the 1960s, where experimental removal of *P.*

*ochraceus* resulted in the mussel *Mytilus californianus*, the dominant space occupier in the mid-intertidal, crowding out the diverse species of algae and invertebrates that occupy the low intertidal zone (Paine, 1974). A plausible prediction is that removal of sea stars from the intertidal due to wasting syndrome will have similar effects on the ecosystem.

The potential for population recovery is uncertain. The history of prior, smaller-scale disease outbreaks in sea stars indicates a range of potential population trajectories. Decimation of *Asterias vulgaris* populations in Maine in 1972 was followed by high recruitment the following season and indications of population recovery (Menge 1974). In contrast, mass mortality of *Heliaster kubiniji* in the Gulf of California led to virtual local extirpation of the species (Dungan et al. 1982).

The causative agent for SSWS syndrome has not yet been identified, but likely in part involves a viral pathogen that compromises sea star epidermis (Drew Harvell, personal communication), which may expose the sea star to secondary infection and often leads quickly to death. In adult stars, the first visible symptom of SSWS is typically lesions on the epidermis, followed by tissue loss, twisting of arms, arm loss and lesions across most of the body surface, and eventually total disintegration and death (Bates et al. 2009).

Healthy larval recruitment to affected areas is necessary for population recovery. Previous studies indicate that echinoderm larvae possess antimicrobial enzymes and are capable of immune responses (Metchnikoff, 1891, rev. Dyrinda et. al. 1995). However, although symptoms and consequences of SSWS are recognizable in adult sea stars, impacts of pathogen exposure on reproduction and larval fitness are unknown.

Our study provides preliminary information on the impacts of SSWS on early *P. ochraceus* life history stages. We assumed in this study that the SSWS-causing agent could be transmitted to larvae indirectly through contact with water collected from near symptomatic adults. We further assumed that this indirect contact with SSWS would cause a measurable and immediate response in infected larvae. We tested the hypothesis that contact with SSWS would have negative impacts on larval fitness, as measured by changes in larval survival, development and growth for those larvae exposed to water collected from tanks as compared with sibling larvae not exposed to SSWS. We reared larvae in five water treatments in an attempt to discern the effects of different causative agents on larval fitness, as follows: (1) filtered sea water from Friday Harbor, mimicking natural ocean conditions; (2) filtered sea water collected from tanks containing asymptomatic adults, to control for adult presence and mimic natural conditions near an adult population; (3-5) sea water collected from tanks containing symptomatic adults filtered at high, medium, and low levels to include virus particles, bacteria, and ciliates successively with each lower level of filtration.

### **Materials and methods**

Adult *P. ochraceus* were collected from three sites in the San Juan Islands on 26 and 27 July 2014: north of Point Caution (San Juan Island), north of Point George (Shaw Island), and on Brown Island (in Friday Harbor basin on San Juan Island). Six asymptomatic and nine symptomatic sea stars were collected, eight of which displayed one lesion and one of which was deflated, losing grip, and beginning to disintegrate. The Allen lab at Friday Harbor Labs donated one additional symptomatic sea star from Shady Cove, San Juan Island.

Six symptomatic and six asymptomatic animals were kept individually in isolation tanks at 12-16° C. Each tank had ambient seawater inflow and aeration systems. Three symptomatic stars were kept together in a separate flow-through tank to generate potentially pathogenic water for culture treatments. Water temperature and adult sea star condition were monitored daily.

### *Spawning*

Five symptomatic and five asymptomatic stars were each injected with 9 to 12 mL 100 µM 1-methyladenine (depending on body size) to induce spawning (Strathmann 1987). Because this experiment was conducted in late July, after normal *P. ochraceus* spawning season (April - June; Strathmann 1987), we were unable to induce spawning and instead obtained gonads through dissection of one arm per star. We fertilized eggs from one symptomatic female with sperm from one symptomatic male to stock culture treatments at an initial density of 100 fertilized eggs per 250 mL container.

### *Treatments*

Five water treatments were prepared for larval cultures: (A) bag filtered seawater; (B) bag filtered seawater from tanks containing asymptomatic sea stars; (C) seawater from tanks containing symptomatic sea stars that was filtered with 0.22 µm mesh (to remove ciliates and bacteria, but not viruses); (D) seawater from tanks containing symptomatic sea stars that was filtered with 0.45 µm mesh (to remove ciliates, but not bacteria and viruses); and (E) bag filtered seawater from tanks containing symptomatic sea stars (Fig. 1). Bag filtered water was passed through mesh filters with a nominal pore size to remove large particulates without affecting ciliates, bacteria, or viruses. Culture water was changed every other day using water from at least two tanks of isolated adults

and prepared as described. Each treatment had five replicate cultures, which were constantly stirred and kept at ambient seawater temperature (12-16° C) in a water table through six days of development.

### *Metrics of Larval Health*

We measured four responses, including: (1) average developmental stage at 4-7 hours post-fertilization. Embryos were staged (2, 4, 8, and >8 cell stages according to George et al. 2012) 4-7 hours post-fertilization. Although development progressed during the three hours spent determining stage, we sampled cultures in random order so we do not expect systematic deviations in development time across treatments; (2) survival to hatching Hatched larvae at the blastula stage were separated from non-viable eggs and counted 2-3 days post-fertilization for survivorship; (3) average length at day five. Five days post-fertilization, fifteen larvae per treatment (5 per culture) were imaged using an Infinity2 camera and InfinityCapture (Version 5.1.0.3) for biometric analysis. Larval diameter was measured in ImageJ (Version 1.48) (Fig. 2); and (4) survival from hatching to day six of the experiment in all cultures.

### *Statistical methods*

Because mortality rates were high in our cultures, assumptions made in statistical analyses were limited by sample size. We used Kruskal-Wallis ANOVA to test the null hypothesis that there were no differences in group medians of response variables as a function of treatments. Treatments included water source – control (filtered sea water); symptomatic (from tanks containing symptomatic *P. ochraceus*); and asymptomatic (from tanks containing asymptomatic *P. ochraceus*) – and water filtration level – bag filtered, 0.45 µm mesh, and 0.22 µm mesh.

We calculated average developmental stage in each treatment by multiplying counts of 2, 4, 8, and >8 cell embryos by a factor of 2, 3, 4, and 5, respectively, and summing these values to create a single value indicating the weighted cell stage for each replicate culture. We then divided the weighted cell stage by the number of cells counted to create a scaled value of average stage in each replicate culture. All statistics were done using RStudio version 0.97.551.

## **Results**

### *Adult Condition*

Of the 16 stars collected at the beginning of the experiment, 7 died of wasting syndrome by day 6 – all of which were initially symptomatic. Of the two spawned parent stars, the symptomatic female progressed from stage 2 (lesions) to stage 3 of wasting (lesions and arm loss) and the spawned symptomatic male remained in stage 2 (stages according to Bates et al. 2009). Symptom progression in the female may have been accelerated by the removal of arms to extract gonads. Four of six asymptomatic stars remained free of disease symptoms through the end of the study and were used throughout to generate culture water for treatments.

### *Larval results*

There was no evidence treatment, water filtration level, or water source affected developmental stage 5-7 hours post-fertilization, survival to hatching, or length at day 5 (see Table 1). However, survival of larvae to day six of the experiment depended on treatment (Kruskal-Wallis test  $p = 0.037$ ; see Table 1, Fig. 3), and on water filtration level (Kruskal-Wallis test  $p = 0.013$ ; Table 1). Box plots indicate that culture D,

containing water from symptomatic adults filtered through 0.45  $\mu\text{m}$  mesh, had higher survival compared to other treatments (Fig. 3).

## **Discussion**

To our knowledge, this study represents the first attempt to identify and quantify effects of SSWS on early sea star life history stages. Our results are inconclusive regarding the potential impacts of SSWS on larvae, but provide experimental protocols and useful preliminary data upon which to base future experiments. We observed differences in the survival of larvae raised in cultures with water of varying levels of exposure to SSWS, indicating the possibility of consequences of wasting syndrome on larval sea star fitness. However, the circumstances of SSWS and of our study design limit our scope of inference. We cannot be sure that sea stars that do not show symptoms of SSWS are actually healthy individuals and not just in the early, asymptomatic stages of the syndrome. Because we spawned *P. ochraceus* outside of their natural spawning season, gamete quality was likely compromised. The gonad from which we obtained eggs was probably from a female that had already spawned, so oocytes were those remaining in the gonad. 1-MA induced germinal vesicle breakdown, but fertilization envelopes did not lift off the egg surfaces very far (ca. 10-15  $\mu\text{m}$ ). We observed high rates of abnormal development before hatching and highly variable mortality over the first six days of development (30-100% mortality per culture) even in the control cultures with filtered seawater. Additionally, useable gametes were collected from only two parent sea stars, each with symptoms of SSWS. Thus, we are unable to account for parental effects, including potential vertical transmission of SSWS, on larval fitness and susceptibility to wasting. The responses of symptomatic individuals to wasting syndrome may be varied,

and effects on gametes may be equally variable. Further studies using more symptomatic and asymptomatic parent sea stars and assessing the potential impact of pathogen exposure on gametes (i.e. sperm motility and swimming), will help determine importance of parental effects and possible incidence of vertical transmission. Additionally, further metrics of larval health, such as behavioral changes or survival over a longer time interval (ideally throughout the course of development from embryo to juvenile), are needed to provide more comprehensive information about the impacts of SSWS on *P. ochraceus* larvae.

The 2013-14 outbreak of wasting syndrome provides a unique opportunity to quantify ecosystem response to widespread removal of multiple top predators. To accurately interpret impacts of this event, and to gain insights into the resilience of the rocky intertidal system, we must understand the pathology of wasting syndrome on all *P. ochraceus* life history stages and the species' capacity for recovery. This study makes a crucial first step towards that understanding.

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## Figures

Table 1. Results of non-parametric ANOVAs (Kruskal-Wallis tests) showing relationships between response and explanatory variables measured. Bold values indicate significance at the 0.05 level.

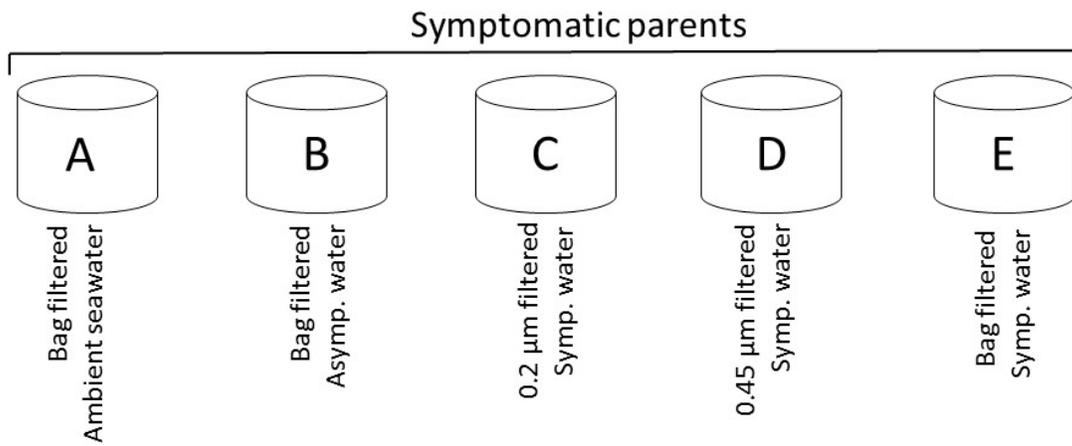
Figure 1. Schematic of culture and treatment experimental layout.

Figure 2. Five day old *P. ochraceus* larvae from 0.22  $\mu\text{m}$  filtered water from symptomatic star tanks with length measurement made in ImageJ.

Figure 3. Box plots showing percent of larvae that survived to day seven of the experiment in each of five culture treatments: (A) bag filtered seawater; (B) bag filtered seawater from tanks containing asymptomatic stars; (C) seawater from tanks containing symptomatic stars that was filtered with 0.22  $\mu\text{m}$  mesh; (D) seawater from tanks containing symptomatic stars that was filtered with 0.45  $\mu\text{m}$  mesh is bag filtered; and (E) bag filtered seawater from tanks containing symptomatic stars.

Table 1.

| Response   | Explanatory  | X <sup>2</sup> | d.f. | Significance |
|--|--------------|----------------|------|--------------|
| Developmental stage 5-7 hours post-fertilization | Treatment    | 7.42           | 4    | 0.115        |
|  | Filtration   | 4.56           | 2    | 0.102        |
|  | Water source | 2.41           | 2    | 0.300        |
| Survival to hatching                             | Treatment    | 5.22           | 4    | 0.265        |
|  | Filtration   | 2.02           | 2    | 0.364        |
|  | Water source | 1.86           | 2    | 0.394        |
| Survival from hatching to day 6                  | Treatment    | 9.43           | 4    | 0.051        |
|  | Filtration   | 8.54           | 2    | <b>0.014</b> |
|  | Water source | 2.28           | 2    | 0.319        |
| Length of larvae at day 5                        | Treatment    | 4.58           | 4    | 0.333        |
|  | Filtration   | 3.03           | 2    | 0.219        |
|  | Water Source | 1.57           | 2    | 0.456        |
| Survival to day 6                                | Treatment    | 10.19          | 4    | <b>0.037</b> |
|  | Filtration   | 8.70           | 2    | <b>0.013</b> |
|  | Water source | 3.05           | 2    | 0.2174       |



5x replications per treatment, for a total of 25 cultures

Figure 1.

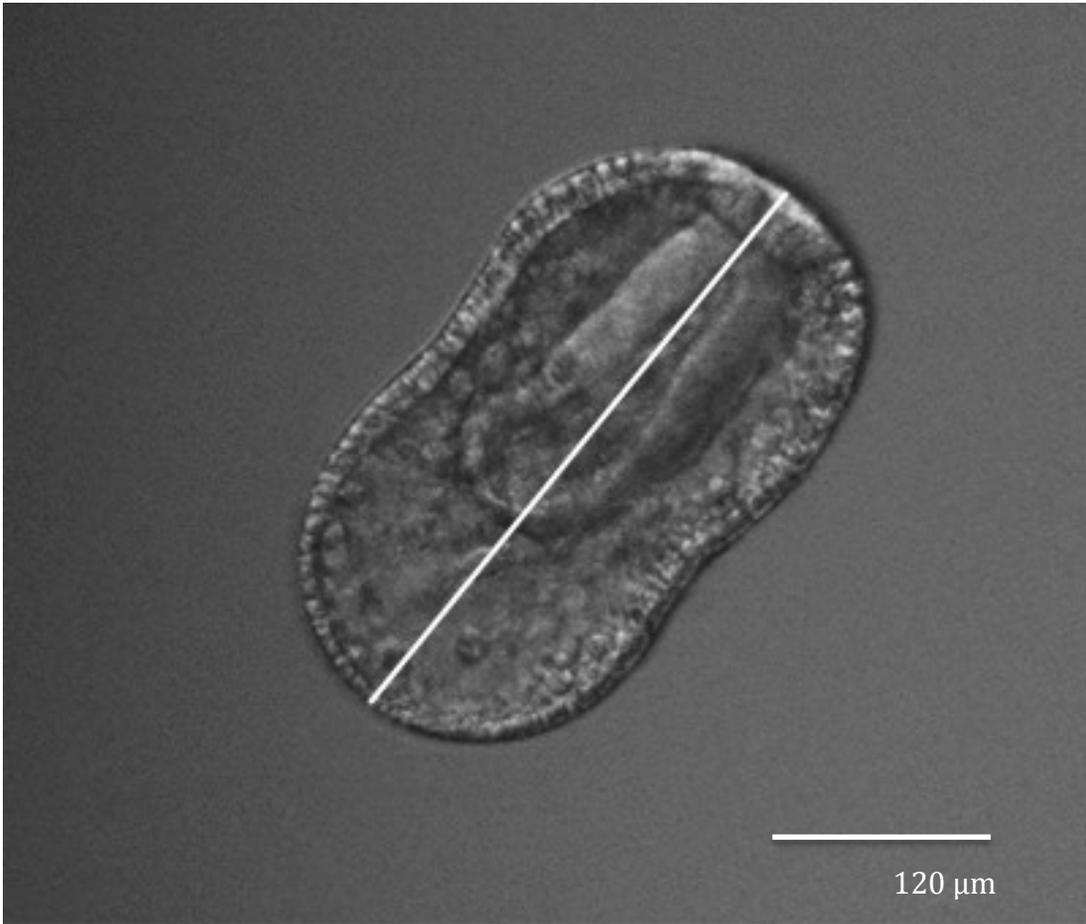


Figure 2.

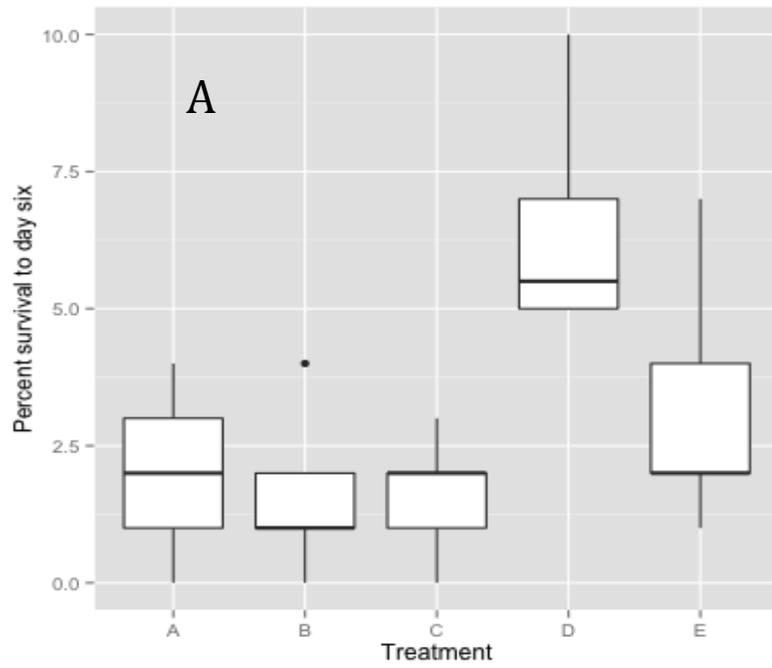


Figure 3.