**Physical environmental triggers of aggregating anemone (*Anthopleura elegantissima*) reproduction in a changing ocean**

Kahana Pietsch[1]

[1]School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat St, Box 355020 Seattle, WA 98195-5020

kahanp@uw.edu

## Abstract

The Phylum Cnidaria, containing the jellyfish, corals, anemones, box-jellyfish, staurozoans, and myxozoans, is extremely diverse and ecologically important throughout the world's marine environments. Despite this, much of cnidarian reproductive biology is poorly understood. Specifically, the triggering initiating different reproductive pathways have not yet been characterized in most cnidarians. The aggregating anemone, *Anthopleura elegantissima*, is one such taxa. It is a widespread and abundant anthozoan cnidarian native to rocky and intertidal areas of the west coast of North America. Here, we propose to expose *A. elegantissima* to an array of variation in physical environmental factors (pH, temperature, and salinity) in order to determine the effects each may have on an individual anemone's reproductive pathways.

## Introduction

With over 10,000 described species spread across 4 classes, the phylum Cnidaria is incredibly diverse (Zhang et al., 2011). This diversity is distributed throughout all of the world's oceans and is present in almost every available habitat, from the surface water plankton (Martell-Hernández et al., 2014) to the deep-sea benthos (Angeletti et al., 2014), and is composed of corals, jellyfish, anemones, box-jellyfish, and other less recognizable members. In order to survive in this wide range of environs, the cnidarians have developed an equally wide array of unique life strategies and physiological adaptations, developing processes as wide ranging as endosymbiotic photosynthesis (Fransolet et al., 2012) to an extreme, degenerate form of microparasitism (Hartigan et al., 2016).

The most biodiverse of the cnidarian classes, the Anthozoa, display notable variety in their reproductive strategies. Within the anthozoans are species which can reproduce sexually via broadcast spawning, in some instances brooding offspring internally or externally (Kahng et al., 2011). anthozoans can also reproduce asexually via budding, fragmentation, and binary fission (Figure 1; Sibly & Calow, 1982). Oftentimes, multiple or all of these reproductive methods can be seen in a single species or even single organism (Gilman et al., 2016).

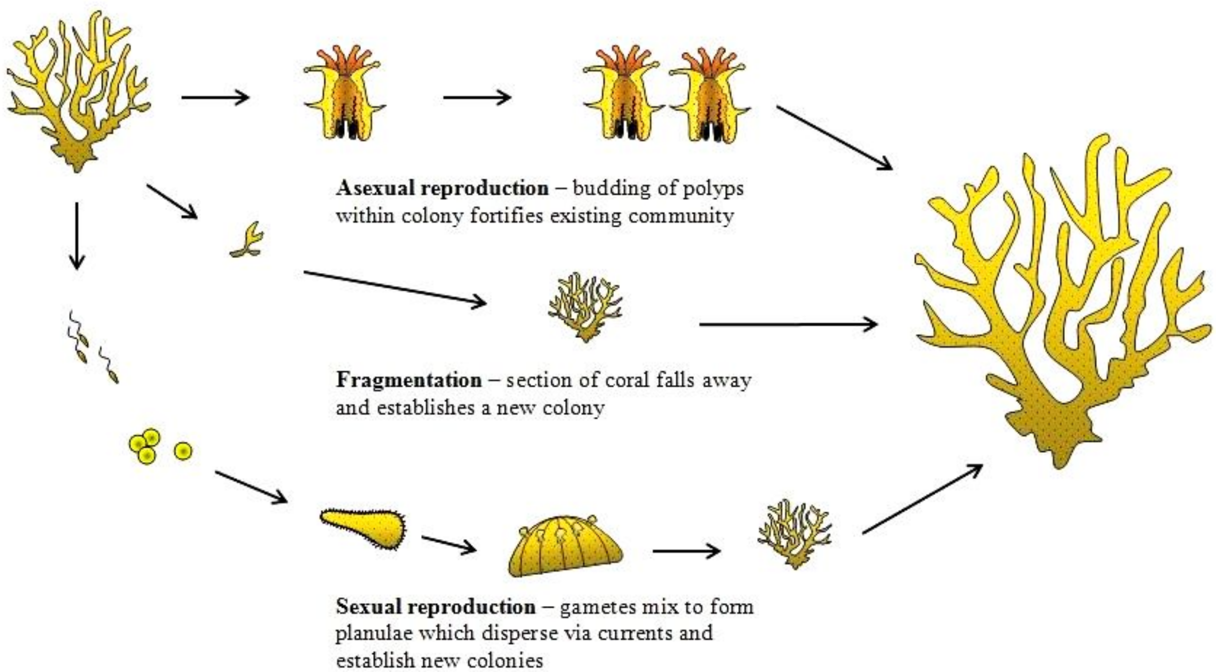


Figure 1: Typical anthozoan reproductive pathways. Figure originally appeared in Patel (2014).

One particular anthozoan, *Anthopleura elegantissima*, is a prime example of this reproductive diversity; it is known to reproduce through budding, fission, and sexual broadcast spawning and occurs both solitarily and in extensive, clonal colonies (Sebens, 1981). *A. elegantissima* is a sea anemone (subclass Hexacorallia) native to the Eastern Pacific along the western coast of North America. Its range extends from Southern Alaska, United States to Northern Baja, Mexico. It is an important member of intertidal and fouling communities throughout its range, operating both as a photosynth and generalist predator (Hiebert & Bingham, 2012). Past work by Kenneth Sebens (1982) exploring the diversity of its reproduction has found *A. elegantissima* typically spawn sexually between the early spring and late summer while fall and winter months are associated with fission. Another project of Sebens' (1980) investigated potential causal factors of reproduction in the species and found that asexual reproduction appeared to occur more frequently at higher temperatures, but looked at an unrealistically wide range of temperatures (5-25°C), making their results considerably less meaningful in the context of the natural environment of *A. elegantissima.*

In many other cnidarians, environmental reproductive triggers have been far more successfully characterized. For example, in many shallow water anthozoans, synchronized broadcast spawning is known to be directly linked to lunar cycles (Brady et al., 2016). In similar taxa, asexual reproduction can be triggered by storm events and current conditions (Kramarsky-Winter et al., 1997). In scyphozoans, temperature has been shown affect gonad maturation and spawn timing (Ohtsu et al., 2007). In contrast to other cnidarians however, the triggers of anemone reproduction are very poorly understood. While some have been shown to perform synchronous broadcast spawning like corals (Scott & Harrison, 2007), the cues involved in gamete release are not clear. Further, as discussed in *A. elegantissima*, triggers of asexual reproduction are even less clear.

While poorly understood, these environmental reproductive triggers may become increasingly important in coming years. Both long term datasets and recent observations indicate relatively rapid changes in the circulation patterns and currents of the world's oceans which might dramatically affect the conditions marine organisms experience (Winton et al., 2013). Both localized and global salinity, pH, and temperature conditions are shifting as positive radiative forcings in the earth's ocean atmosphere systems increase (IPCC, 2014), and these have already been shown to have considerable effects on some invertebrate taxa (Delorme & Sewell, 2014). Whether or not the physical environmental variables known to be changing have any effect on anemone reproduction may greatly inform how their population structures will or will not change in the future.

**Motivation for Research**

This lack of understanding of their reproduction coupled with the wide distribution and environmental importance of anemone taxa make their reproduction an important topic of study. Its broad distribution, high abundance, easy access and collectability, and history of use as a model organism in past assays make *A. elegantissima* an ideal candidate for exploring this topic.

While sea anemones currently have little economic importance, understanding how their ecological roles may shift as climates change could be important. For example, should asexual reproduction be highly favored under the warmer, lower pH conditions projected for the world's oceans, genetic diversity of anemone populations may suffer and, as a result, put them at risk. Conversely, the triggering of rapid reproduction could also lead to a dramatic increase in population abundance, potentially influencing the dynamics of the benthic, intertidal, and fouling communities they inhabit. The exact nature of how varying physical conditions may interact with their reproduction and ultimately population dynamics is not at all clear with the current understanding of anemone biology. A large, multivariable, comprehensive study is needed not only to fill this gaping hole in the academic literature on these taxa, but to better understand how their populations and ecological roles might shift in response to a changing climate.

**Research Question**

What environmental factor or factors activate each of the various reproductive pathways in *A.elegantissima*?

**Hypotheses**

$H_a$: A physical variable or variables (temperature, pH, salinity, photoperiod) are a triggering factor in one or more reproductive pathways in *A. elegantissima*.

$H_o$: None of the studied variables are correlated with any reproductive pathway in *A. elegantissima*.

**Methods**

*1 Overview*

*A. elegantissima* adults will be collected in from the wild, propagated artificially, and exposed to a suite of physical environmental variations. Rates of reproduction by different methods in each treatment will be measured and recorded.

*2 Collection of specimens*

*2.1 Field collection*

Past studies have shown genetics can play a role in this species' reproductive patterns (McFadden et al., 1997). As such, all specimens will be originated from one clonal colony. This colony will be the largest available in the rocky intertidal zone of the south side of San Juan Island, Washington State. This location was chosen as the notable series of studies on *A. elegantissima* reproduction by Sebens (1980, 1981, 1982) and a number of other past studies (Hiebart and Bingham, 2012; Ponce-McDermott et al., 2012), sourced their specimens from this location. Additionally, this location is in convenient proximity to the University of Washington's Friday Harbor Labs, a likely candidate facility for the propagation and experimentation phases of this study, described in the following sections.

*2.2 Propagation*

Anemones will be housed communally in a large, closed system during the propagation phase. As individual anemones reach 3 cm in diameter, they will be artificially propagated via bisection with a scalpel until the needed number of anemones (see below sections) is achieved. As the holding system reaches capacity, propagated anemones will be distributed to experimental systems. No assays will begin until the total number of required specimens has been produced.

*3 Assay*

*3.1 Variables tested*

Anemones will be exposed to a series of physical variables and combinations thereof (Figure 2). These will include salinity (15ppt, 20ppt, 25ppt, 30ppt, 35ppt, 40ppt), photoperiod (8-20hrs in 4hr intervals), temperature (ambient annual average, + and - 2°C), and pH (current ambient and high (7.75) and low (7.95) end estimates for ocean acidification from IPCC, 2014). These trials build considerably on past work of Sebens (1980), who explored temperature and food availability as determining factors in asexual reproduction, but did not examine salinity, pH, photoperiod or any cumulative effects thereof; further, as previously mentioned, Sebens' trials did not examine reproduction in the context of environmentally realistic temperatures.

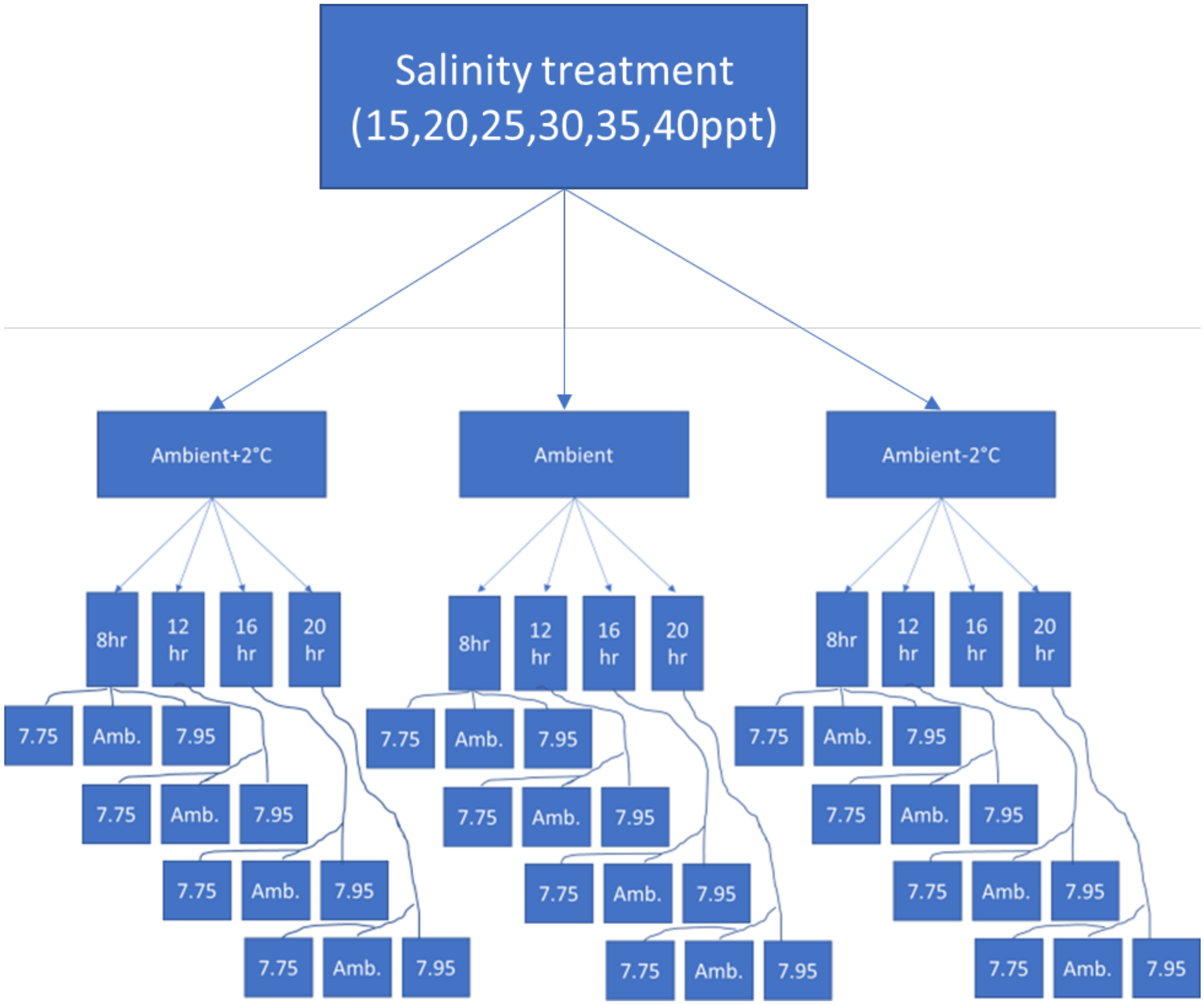


Figure 2: Branch diagram demonstrating all combinations of variables tested. The first tier are salinity variations tested, the second are temperatures, the third are photoperiods, and the fourth are pHs.

*3.2 Experimental Setup*

Once propagation is complete, anemones will be held in separate systems containing each of the tested treatments (Figure 2) in a controlled indoor environment. For each treatment, three groups of ten individual anemones of the standard size described in section 2.2 will be held in separated closed systems in order to avoid any potential tank effect. In total, there will be 216 treatments, 648 systems, and 19,440 individual anemones. Systems will be maintained for a period of 1yr, as in some other anthozoans reproduction has been shown to be an annual event (Brady et al., 2016). Food availability will be kept high in all systems throughout the study period in order to avoid any effect of stress that may result from starvation, especially given that Sebens (1980) determined relative food availability might act as a triggering factor of fission.

*3.3 Data collection and analysis*

All reproductive events will be recorded into one of the following categories: fission, budding, or sexual reproduction. Reproduction will be recorded both in terms of the total number of reproductive events and in the number of offspring produced. Every individual bud produced will be counted as a reproductive event. Rates of each reproductive strategy under differing conditions will be compared to establish a causal relationship between each given strategy and a certain set of environmental conditions. The rate and means of offspring production along the gradient of variation within each environmental condition will be compared to projected ocean conditions in order to characterize the effects of climate change on anemone population dynamics.

**Projected Results**

The lack of past study on this subject has given no background by which to predict which (if any) of the tested variables and combinations of variables will prove to be corelated to any of the three analyzed modes of reproduction. The known trend of fission dominating reproduction in winter months and sexual spawning taking place in summer months described by Sebens (1982) could suggest a relationship of each strategy with the thermohaline conditions associated with each of those seasons. However, Sebens' (1980) demonstration of higher fission rates at elevated temperature are in apparent contradiction to this, again complicating any sort of meaningful prediction. A set of results producing all desired information would show strong correlation between each reproductive strategy and at least one tested environmental condition. One potential result might, for example, demonstrate sexual reproduction occurs most frequently at low pH treatments while fission might occur more frequently at high temperature and budding at low salinity (Figure 3).

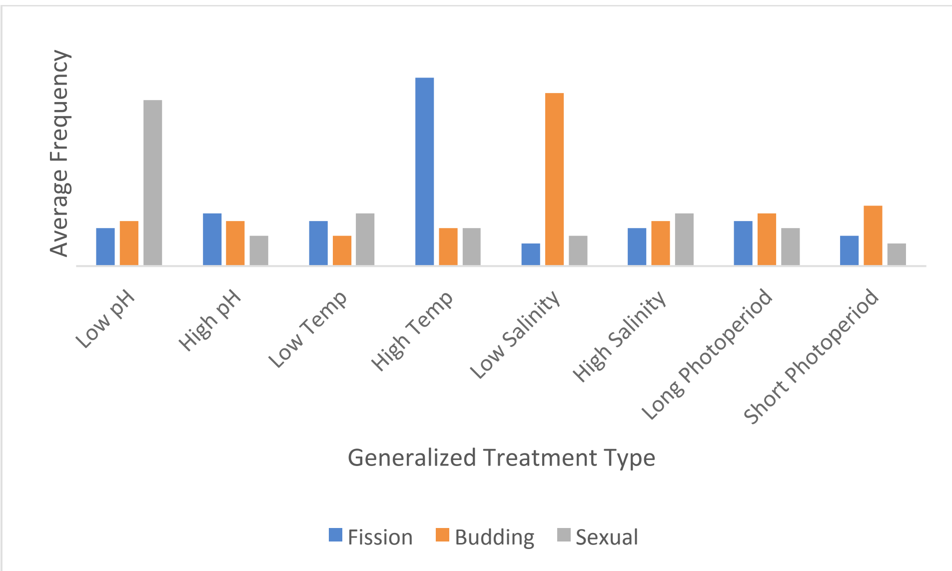


Figure 3: Bar graph showing one potential outcome demonstrating strong correlation of reproductive strategy to conditions. Treatment types are generalized into categories (High/Low for each variable) to avoid listing all 216 tested treatments.

**Discussion**

Upon its completion, this study will have provided the most comprehensive analysis to date of physical environmental reproductive triggers in *A. elegantissima* or any other anthozoan. The information it generates will greatly increase our understanding of the biology of this species and provide an in-depth framework to interpret reproduction in anemone species and anthozoans on the whole.

The primary limitation of this study is that, while comprehensive in regards to physical environmental variables, it does not examine any potential biological influences on reproduction, such as food availability, genetic variation, and proximity to mates. While there is no documented evidence of the influences of proximity to potential mates on reproductive strategy in other anthozoans, chemical signals from other individuals have been shown to induce spawning in some other invertebrates (Watson et al., 2003), making it an interesting area for further study. Additionally, food availability and symbiont state has been shown to influence lipid content of *A. elegantissima* considerably (Ponce-McDermott et al., 2012), potentially impacting development of gonads and was also identified as a potential trigger of asexual pathways by Sebens (1980). Biological factors were not included in this study to avoid potential difficulty of interpreting results that might arise from testing such a large number of variables. Indeed, there are already over 200 treatment groups examined without testing any additional variables.

Furthermore, this study only examines these effects in one genotype of one population of one species. Future study could potentially investigate the effects of the same factors explored here on other anemones, or a wider range of cnidarian taxa, in order to better understand how applicable our results are to species and populations separate from the one we looked at.

The above said, the expansion of the base of scientific knowledge of anemone biology this study will provide is not to be understated. There is a marked lack of understanding currently and the groundwork we intend to provide will greatly inform not only future studies, but critical knowledge of how wild populations will change in coming years. Should the projected ocean conditions we expose anemones to prove to seriously impact their choice of reproductive strategy, there is the potential for major changes in their population dynamics. Increased resource devotion to frequent sexual spawning could affect growth rate, body condition, and disease resistance of mature individuals. On the other end of the spectrum, increased asexual reproduction could greatly lower population genetic diversity, potentially impacting resilience to further ocean change or pathogen exposure. The net effects of potential changes in reproductive pathways on total population abundance and resilience are not known and cannot currently be predicted accurately. Given the ecological importance of anemones worldwide, this is an important lack of knowledge which this study intends to fill.

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