Marine Biology Lab 362
Jellyfish Polyp Experiment Lab Report Assignment

Motivation for Studying Jellyfish Populations

Jellyfish, a term used here to collectively describe cnidarians, are a common occurrence in many coastal and estuarine systems. When they appear in high numbers, they can affect the ecosystem and human activities in many ways (Fig. 1). From an ecological perspective, these species are often dominant pelagic predators, and can exert intense top-down controls that impact trophic dynamics. Jellyfish can play an important role in carbon cycling in coastal systems, releasing high amounts of carbon-rich dissolved organic matter and shunting energy away from higher trophic levels (Condon et al. 2011). When blooms crash, dead jellyfish biomass may be rapidly decomposed by bacteria,

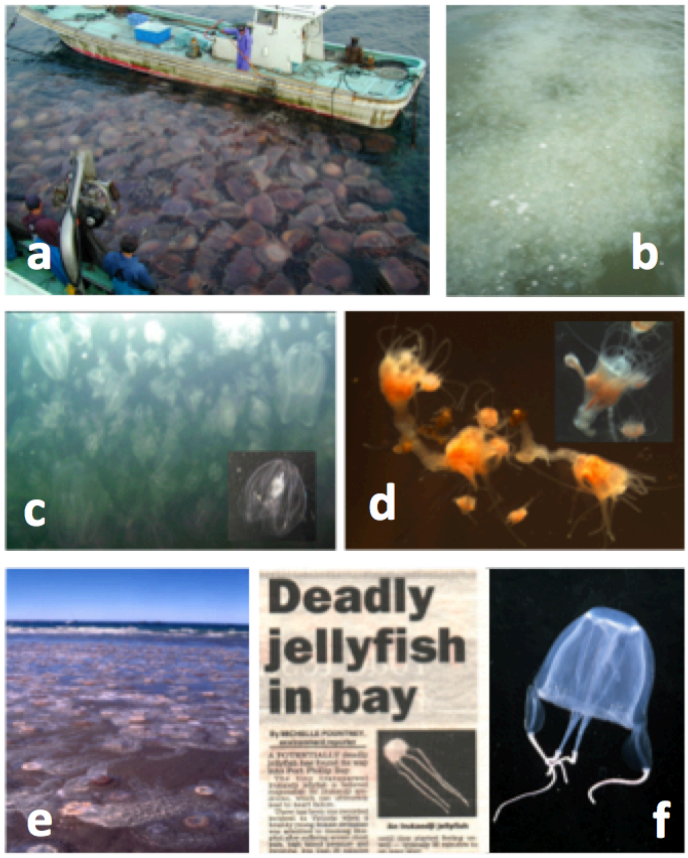

Fig. 1: Examples of jellyfish that regularly form blooms or impact human activities. (a) *Nemopilema nomurai* medusae off the coast of Japan (Photo credit: Shin-ichi Uye). (b) *Aurelia* sp. medusae bloom from Chesapeake Bay (Photo credit: Steve Kupiec). (c) *Mnemiopsis leidyi* ctenophores in the Baltic Sea. (d) *Aurelia* sp. Polyps showing asexual phases that lead to production of ephyrae. (e) *Chrysaora plocamia* medusa strandings on coast of Patagonia (Photo credit: HW Mianzan). (f) *Carybdea alata* from Port Phillip Bay, Australia. Media headline from *Herald Sun* newspaper.

creating an oxygen demand that potentially leads to localized hypoxia (Pitt et al. 2009), increase transfer efficiency of the biological pump (Billett et al. 2006, Lebrato et al. 2012), and provide food for deep-sea organisms (Sweetman et al. 2014). From a human perspective, jellyfish blooms are most often noticed due to their negative impacts. Blooms of giant jellyfish in Japan have been shown to clog fishing nets and even overwhelm boats when in large enough numbers. Stings are also a concern, and when biomass is high, entire beaches can be shut down to public access.

Within recent years, public interest and awareness in the ocean has increased. Children's books and television shows focus a spotlight on ocean life, and jellyfish are no exception. Most all humans who have interacted with the ocean have some type of "jellyfish story", whether it concerns a stinging event, or have heard about jellyfish populations on the news. Jellyfish are a major drawcard at public aquaria worldwide and generate ecotourism revenue in Palau (e.g., *Mastigias papua*) and Japan (e.g., *Nemopilema nomurai* giant jellyfish; Graham et al. 2014). The increase in general public curiosity and increased media coverage has lead to a certain perception of jellyfish and increasing trends in our world's oceans (Condon et al. 2012). We will cover these issues in greater detail in lecture late in the semester.

Currently, very little is known about the natural and anthropogenic environmental drivers initiating jellyfish populations (*Note:* correlation doesn't mean causation), which limits our ability to make future predictions about the extent of jellyfish populations and impacts on society. A recent paper by the Global Jellyfish Group (Condon et al. 2013) showed that rather than a sustained global increase, jellyfish populations and their blooms (1940 – 2011) exhibited synchronous, multidecadal cycles centered around a stationary baseline, including a rising (positive) phase in the late 1990s when jellyfish were initially

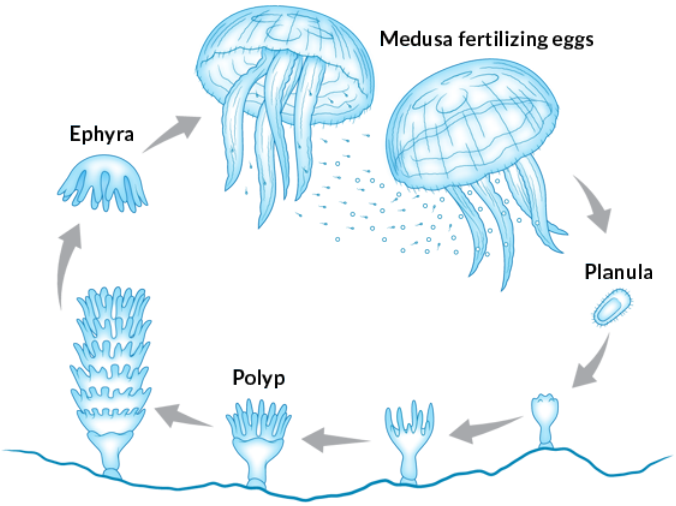

Fig. 2: Scyphozoan life cycle of *Aurelia aurita*. Male and female medusae sexually reproduce forming a planula, which settles on hard substrate where it metamorphoses into a polyp. After overwintering, the polyp asexually produces baby medusae ('ephyrae'), which detach and form adult medusae. Not shown is the ability of polyps to asexually clone themselves, physically detact and move to a new location, and form cysts if conditions are not correct. Cubozoan polyps directly form juvenile medusae

thought to have increased globally – (*Note:* for the record this is a myth and should be evaluated when critiquing the Brotz et al. paper). The research focus to date has been on the pelagic medusa phase of the life cycle, but the key to our understanding of jellyfish populations lies in the benthic polyp phase of the life cycle (Fig. 2). The primary purpose of the polyp is to undergo asexual propagation (cloning) and to produce several microscopic jellyfish called ephyrae, which when released grow rapidly into adult medusae. Virtually nothing is known about the behavior and ecology of polyps, not even their natural distribution is known to science.

The Primary Task

In this assignment, you will work in small groups of 4-5 students and conduct an experiment examining the behavior and ecology of jellyfish polyps. The target organism

is the moon jellyfish (*Aurelia aurita*) that is cosmopolitan in distribution and is one of the most studied and well-known cnidarian species. Other species (e.g., box jellyfish polyps) might be available but it would depend on the type of project. The experimental topic will be chosen by each group but will need to be approved by your TA. We will start the experiment in week 3 of the semester, after you have presented a summary and plan of attack to the class. It is more than likely that your initial proposal will need to be refined, don't be perturbed by this, as this is part of the process of science. Remember the simplest questions are often the easiest to perform and provide the most interesting insight for testing scientific theory and big picture questions. Once you have received the seal of approval from your TA, your group will have 5 weeks to finish the experiment, using each lab time to take measurements. You will also more than likely need to check on your experiment (e.g., feed polyps, take a timepoint) outside of lab hours as it is your group's responsibility to upkeep your experiment. Time management and organization is essential, but this is a good skill to learn in science. Your TA is there to help guide and facilitate the process and can provide feedback, <u>but they are **not** there to do the work for you</u>.

As you will experience and learn, polyps are relatively easy to observe and measure their morphology, and there are some very interesting and unique questions and hypotheses you can test relating to marine biology and ecology. I have listed some topics here (with brief comments on the applicability to jellyfish populations) but feel free to choose a topic outside of this list:

Polyp growth in the light vs. dark: In the field polyps tend to congregate in dark places, typically under rock ledges and over hangs where they are not exposed to sunlight. However, many researchers conduct lab experiments in the light. The question is do polyps behave and grow differently in the light vs. dark, and as a result could this lead to false information about jellyfish populations?

Do polyps grow better when facing up, down or on the side? As a follow on from the previous idea, many lab experiments are conducted with polyps facing up. However, in the field polyps tend to face downwards or on the side (e.g., on a pier pilling). Do polyps grow the same regardless of orientation?

Polyp growth under different salinity regimes: Many jellyfish species live in estuaries and are therefore potentially exposed to a wide range of salinity regimes, which can put tremendous osmotic stress on the organism and influence its growth and survival. In terms of translating polyp ecology to water column processes, it would be pertinent to know whether jellyfish polyps are osmoregulators or osmoconformers and whether this translates to more efficient growth at a particular range of salinities.

Do jellyfish polyps exhibit intraspecific and interspecific competition for space? Do polyps grow better when isolated or when aggregated? Jellyfish polyps require hard substrate to attach to, therefore competition for space (just like intertidal communities) is paramount, especially as parent polyps can quickly utilize settled space through asexual budding (cloning) of daughter polyps. Some preliminary results suggest that jellyfish

polyps can 'fight' for space by 'wrestling' other polyps using their tentacles, and this might be a good interspecific strategy when other species are present, but this comes as an energy loss term for the polyp which could influence survival and potential ephyrae production under the right conditions.

Are there predators of jellyfish polyps? Does the presence of a predator influence their behavior? Forming aggregations of polyps reduces the chances of being eaten (e.g., nudibranchs are natural predators of jelly polyps and use their prey's nematocysts), but is disadvantageous because of increased competition for food. The impact of predation might lead to increased substrate available for polyp settlement or could decimate a population entirely. The positive or negative effects of predation are poorly understood currently and beckon investigation.

Do artificial substrates provide increased habitat for polyps? Humans have greatly influenced the coastal morphology of the ocean through the addition of artificial structures, including metal, wood, rock, aquaculture (e.g., oyster shells (live vs. dead) are a preferred substrate for polyps in Chesapeake Bay) and plastics. The addition of artificial substrate is potential habitat for jellyfish polyps, however this idea is poorly understood. The implications of artificial substrate for jellyfish populations is that if polyps favor a human-mediated substrate then this might favor increased ephyrae production.

How does temperature influence asexual growth of polyps? How much cold and warm water does a jellyfish polyp need to produce ephyrae? Metabolism, including production/growth, reproduction, excretion and respiration, is a rate process that is centrally mediated by temperature, and several researchers have speculated that warmer oceans will favor organisms with the gelatinous body plan (see Acuna 2011). While this may suggest that with climate change increased jellyfish polyp growth would be favored, preliminary evidence suggests polyps require a certain exposure to cold temperature (<15°C) in order to trigger strobilation and the production of ephyrae. Thus, climate change may not favor an increase in medusae because the benthic polyps may not produce as many ephyrae when exposed to lesser periods of cold temperatures during winter and spring, but this is dependent on rates of asexual propagation by established benthic populations. A series of complementary experiments could be performed to tease apart these interactions.

How does food type or amount influence polyp growth? Shrimp vs. phytoplankton. Are jellyfish polyps able to utilize dissolved amino acids in the water for growth? What type of functional type curve? All heterotrophic organisms have intracellular elemental and stoichiometric requirements in order to satisfy physiological and growth processes. Similarly, prey are comprised of different total and relative amounts of elements depending on a variety of physiological (genotype) and environmental (phenotype) factors. Thus, the quality (i.e., how much C relative to N relative to other elements) and quantity of prey can influence overall growth rates of polyps. For example, if there is not enough C then growth will be limiting. Very little is known (if any) about the diet of jellyfish polyps. In experiments, brine shrimp are typically used as prey but there is some

evidence to suggest that polyps consume microzooplankton and smaller particles. Even preformed compounds such as dissolved amino acids (e.g., leucine, glycine etc..) are thought to be utilized by polyps for their metabolism. Thus, there is a need to quantify the diet of polyps as this might be linked to the ability to survive and produce ephyrae. As part of this understanding, knowing the functional response curve for different prey types would be desirable.

Do polyps ingest plastics? Are they directly ingesting plastics or are they consuming food items that have accumulated plastics prior to being eaten? Recently, much attention has been given to the effects of microplastics on food web processes, which can be toxic to organisms, and can bioaccumulate throughout the food web when consumed by predators. The size of these anthropogenic particles is well within the prey size range for polyps, but the effects of microplastics on jellyfish are unclear.

The End Goal: Written Report

The end goal of this assignment is to individually write up your group's experimental results in the format of a scientific journal (i.e., each member in the group will hand in their own report). The report should be in the style of *Marine Ecology Progress Series* and should be a maximum of 10 double-spaced pages in length (including tables and figures). Instructions to authors are usually listed in the first few pages of each volume of the journal, and also on the journal's website (http://www.int-res.com/journals/meps/guidelines-for-meps-authors/). Refer to these instructions to find out article format e.g. how references are cited. Sections to include: Abstract, Introduction, Methods, Results, Discussion, References, Tables and Figures. One of the main challenges in science is to write concisely and to the point but still being able to tell an interesting story. This assignment will provide practice in this area.

This assignment is worth 38% of the total Marine Biology lab grade. You will turn in three drafts of this report and receive feedback on each draft (from your peers on drafts 1 & 2 and from your TA on draft 3), so crafting the report will be a semester long project with multiple revisions and opportunity for improvement. Each draft of the lab report is required and constitutes an increasing proportion of your lab grade (first: 2%, second: 4%, third: 7%, final: 25%). Final written reports will be marked out of 100 points and the breakdown will be approximately as follows:

Abstract	5
Introduction	10
Methods	15
Results	20
Discussion	20
References	5
Tables & Figures	10
Journal formatting	5
Overall participation	10

Here are some tips to some of the key sections but also refer to the Lab Report Instructions pdf and the rubric spreadsheet for more information:

Abstract. This section provides a brief summary of the entire paper and should include the hypothesis, main result and main conclusion of the study. No references in this section. Because it is a summary I usually leave this to last. A good way to do this is to record you summarizing the work in your own words as if you were explaining the work to a friend (see Kwok et al. paper 'Two minutes to impress').

Introduction. Introduction is not just a statement of the problem, but it should indicate motivation to solve the problem and motivate the interest in the problem. A hypothesis must be included to receive any points for this section.

Materials & Methods. The Method section should include the approach and the caveats associated, including details on fieldwork, statistical analyses, reference to previous methods used but should not include any results. Enough detail should be provided so that the reader can repeat the study.

Results. The Results section is a brief summary of the main results. Be as brief and clear as possible in explaining the results (e.g., short sentences to the point are appreciated) including both statistically significant and non-significant findings. Be sure to reference figures or tables and definitively statistics (e.g., p-value, degrees of freedom and test score) when referring to the level of significance. *Tip:* If you keep a running spreadsheet of your results this will be a good way to streamline analyses and identify any problems with the experiment.

Discussion. This section includes your interpretation of the results. Was your hypothesis supported or rejected? Did the experiment work? If not, how can it be improved? How relevant are your findings to other studies and to the bigger picture? You should end this section with some concluding statements and potentially future directions.

Important Dates

Week 1: Intro to topic. TAs will give overview and walk you through some equipment you will require. Overview of scientific method.

Week 2: No lab but there is a group homework assignment that will be the pre-lab for the week 3 lab.

Here are some points and questions your group will need to consider:

What is the process of science?
What is the question? Hypothesis?
How will you replicate your experiment?
How will you control your experiment?

What equipment will you need? What do we need to borrow?
How will you record the data?
When should you feed the polyps?
What is your timeline? When will you time points be?
Is this experiment feasible? References? What could go wrong?
Do you have a plan B?
How is your experiment applicable to advancing science and to the big picture?

To help streamline your thoughts, your group will collectively provide all this information in the online form (http://condonlab.weebly.com/jellyfish-polyp-experiment.html). This will also help you prepare your summary presentation for the week 3 lab. The online form will be received by your TA in advance of the week 3 lab so that they can be thinking about shortfalls in the experimental design and help refine the approach.

Week 3: Present summary and plan of attack to the lab. You do not need to prepare PowerPoint presentation but you will be expected to explain your experiment by drawing a conceptual diagram on the whiteboard. Get all clear from TA (refine if necessary).

Weeks 3 – 8: Conduct experiment. *Note*: after hour measurements and husbandry of the experiment are likely. We will conduct the experiment in DO 129 which is usually open from 7.30am to 9pm. I will set up several microscopes in DO 129 so that you can take measurements. There will also be seawater, brine shrimp and other items you identify in the initial summary presentation so that you can conduct your time points efficiently. It is important that contributions are consistent from all team members and across different groups (after all there are 10 points up for grabs for this criteria). I will have a log sheet in DO 129 that you will sign in and out for when you conduct your time points or anything related to the experiment.

Week 4: Show you TA at least 10 **peer-reviewed** references that relate to your experiment. No Google or internet references.

Week 6: Draft 1 of lab report to include at least 'Introduction' and 'Methods' sections. If you have preliminary results please include these as well.

Week 8: Finish experiment

Week 10: Draft 2 of lab report

Week 12: Draft 3 of lab report

Week 14: Final lab report due.

Good luck and I hope that you enjoy and embrace the experience of doing actual scientific research. Who knows, your results may be so significant that you can publish them! Now that would be awesome and good science!