Introduction to Marine Fish Aquaculture:
Spawning and Larval Development of ʻŌpakapaka

Hawaiʻi Institute of Marine Biology
Education Program
Clyde Tamaru, Ph.D.
Malia Rivera, Ph.D
Kelvin Gorospe
Roxanne Haverkort

Part I: Pre-activities for the classroom

Science background

The majority of Hawaiʻi's fisheries are on a declining trend with estimates for some species showing as high as a 75% decrease in the commercial catch over the past century. The reasons for these declines are numerous and complex, but recent investigations point to over-fishing as being among the major causes, a trend that is intimately tied to a high market demand for fresh fish by our island residents (Fair Catch Hawaiʻi, 2008).

Management tools such as seasonal closures during the fish spawning seasons, restrictions on both the number of fish one can take (bag limits) and the minimum sizes of fish that are taken are largely based on biological information, namely the reproductive traits of the target fish species. Restricted fishing areas or creating no catch zones provide safe havens for groups of fishes to reproduce, and minimum sizes help mitigate the taking of fish that have not yet had a chance to reproduce and therefore contribute new individuals to the population. In addition to these management tools, for some species, scientists are now working to control reproduction in captive broodstock to develop methods for artificially propagating a species independent of wild stocks. This type of effort is called 'aquaculture', and is often used to enhance wild stocks (called stock enhancement) or to commercially produce fish for market.

Commercial production of a species can alleviate fishing pressure by providing an alternative source to meet market demand. A good example of this situation is with the captive production of the highly prized Pacific threadfin (or 'moi'), *Polydactylus sexfilis*, using open ocean cage technologies (Figure 1). Current outputs from just one farm exceed 300,000 lbs per year and outpaces the highest amount of moi ever recorded being harvested from the wild. This outcome is the result of the establishment of the first captive spawning moi broodstock at the Hawaiʻi Institute of Marine Biology (HIMB) beginning in the early 1970's, with research still ongoing for the refinement of current hatchery techniques and technologies.

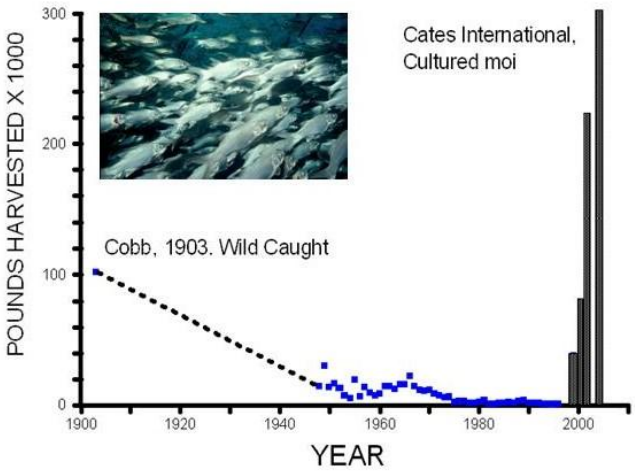

Figure 1: Comparison of commercial catch and cultured outputs of moi. Note the data point from Cobb (1903) followed by a precipitous decline that is typical of the majority of Hawaiʻi's commercial fish catch. Source: Division of Aquatic Resources and Ostrowski et al., (2001)

While the environmental requirements certain fish need to spawn in captivity are often a mystery, many species of fish will nonetheless spawn naturally when held under captive conditions. This was the case for the moi during the 1970's, and fortunately for researchers at HIMB, the deepwater pink snapper, *Pristipomoides filamentosus* (or ʻōpakapaka) juveniles caught in 1999 began to spawn naturally in 2001 while being raised in floating net cages, and have continued spawning ever since. Keeping track of the amount of eggs spawned and when spawning events occur allows for determining the spawning season of the captive broodstock (Figure 2). The source of eggs has provided a foundation from which researchers at HIMB hope to develop hatchery technologies that will result in production of large numbers of juveniles for this important bottomfish species.

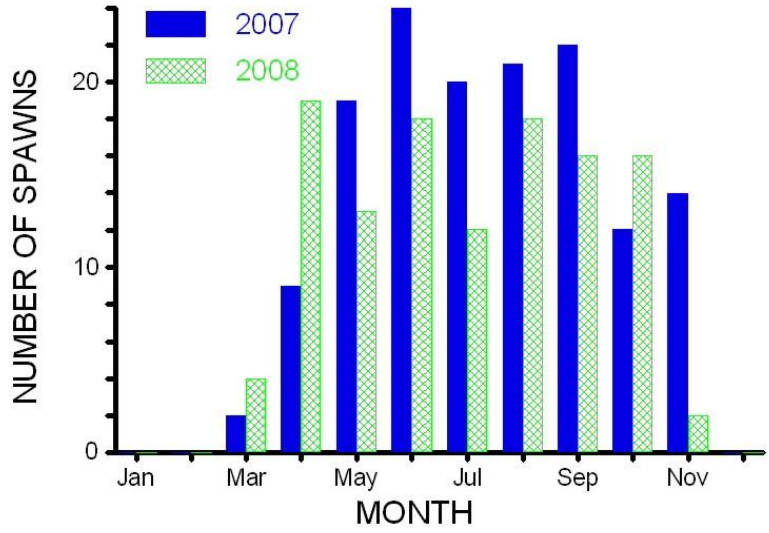

Figure 2: Temporal changes in the number of spawns per month from ʻōpakapaka broodstock held in net cages at HIMB.

When spawning is observed, aquaculturists start working to obtain data that helps to characterize the reproductive traits of the species. Basic biological characteristics such as when and how often spawning occurs and the number of eggs produced per spawning event provides fundamental information regarding the reproductive potential of the species. Correlating spawning events with environmental parameters (e.g., length of day, temperature, salinity) provides an indication of which of these variables could be manipulated to control maturation and spawning in captivity. The quality of the spawned eggs themselves comes under very close scrutiny because the best eggs are needed to mimic the normal process of embryonic development for the species in a captive setting. Furthermore, the spawned eggs will undergo rigorous testing in an effort to understand how much they can tolerate being handled.

Fish reproduction research at the Hawaiʻi Institute of Marine Biology

Mr. David Itano and Dr. Kim Holland have been involved with the project entitled, "Reproductive Biology of Yellowfin Tuna, *Thunnus albacares*, in Hawaiian waters and the western tropical Pacific Ocean". The focus of the project is to determine the seasonal and spatial variations in spawning of the yellowfin tuna, or ʻahi. Samples of yellowfin gonads (the male and female reproductive organs) were obtained from surface and longline fisheries throughout the region. Gonads were examined by cutting out small sections that are first preserved and then

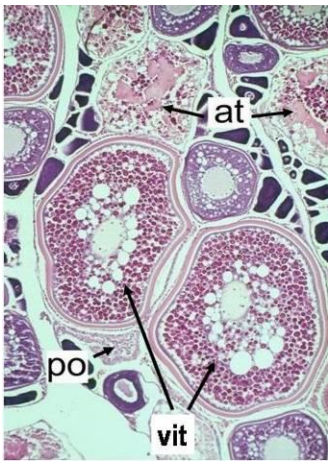

Figure 3: A histological section through a yellowfin ‘ahi ovary that possesses different stages of oocytes (eggs). This individual is a reproductively active female indicated by the presence of fully yolked or ‘vitellogenic’ oocytes (vit,) which will be released soon to potentially be fertilized by a male’s sperm. Oocytes that have released their eggs (‘ovulated’) are in the process of ‘atresia’ (at) and are being re-absorbed by the ovary. Follicles that have fully completed ovulation are ‘post-ovulatary’ (po) and have been almost entirely re-absorbed by the ovary. The latter stages are only found in females who have already spawned (atritic and post-ovulated). Using this method, researchers have been able to determine the peak periods in spawning activity of the yellowfin ‘ahi and discovered that they spawn continuously for several days. Photographed @100x. Source: Itano (2000).

processed in such a way that they can be sliced very thin, stained and then viewed under a microscope to determine reproductive state and time since last spawning (Figure 3). In addition, daily or near-daily spawning rates were also recorded. The major discovery of the project was the spawning season for yellowfin in Hawaiian waters occurs from April through October, peaking in June, July and August. Causing concern is the fact that the Hawai‘i based yellowfin fishing season also peaks during this same time period (which is likely an inshore spawning run), resulting in a high potential for interaction with fisherman. It remains to be determined whether the catching of fish during this spawning run should be allowed, as catching fish while they are reproducing directly conflicts with a basic principal of resource management.

Dr. Clyde Tamaru is principal investigator of the National Oceanic and Atmospheric Administration (NOAA) supported project entitled “Improving the hatchery output of the Hawaiian pink snapper, *Pristipomoides filamentosus*, to meet stock enhancement and open ocean aquaculture expectations”. More commonly known as the ‘ōpakapaka (Figure 4), research on this bottomfish at HIMB hinges on this species’ ability to naturally spawn from captive broodstock which are held in pens at Moku o Lo‘e (Coconut Island). Due to its over-fished status, this bottomfish fishery is now governed by the federal Magnuson-Stevenson Act[1] and the State of Hawai‘i has been mandated to devise ways in which the fishery can be restored by at

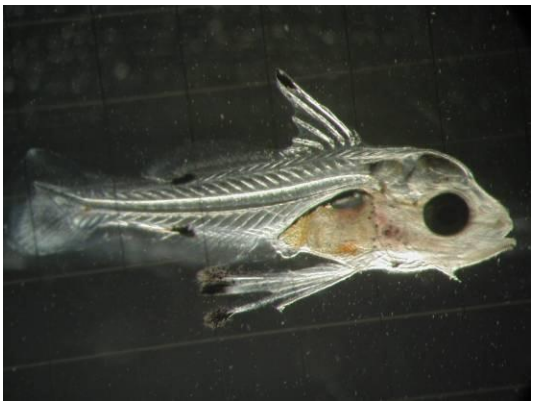

Figure 4: Photomicrograph of a two week post-hatched ‘ōpakapaka larvae. During the initial stages of the rearing process the larvae is feeding on copepod nauplii. To date, feeding the larvae live food organisms has resulted in the best survival. Challenges remain with regard to transitioning the larvae to the next live food organism, and this is the focus of current research activities. Photograph by Clyde Tamaru.

[1] The Magnuson-Stevens Fishery Conservation and Management Act mandates the use of annual catch limits and accountability measures to end overfishing, provides for widespread market-based fishery management through limited access privilege programs, and calls for increased international cooperation. See www.nmfs.nooa.gov/msa2007/index.html

least 20%. The bottomfish research at HIMB is a collaborative effort between scientists from the University of Hawai‘i and the Division of Aquatic Resources to develop hatchery produced juveniles for several purposes including: 1) to use tagged juveniles to assess the effectiveness of the restricted fishing areas, 2) to ascertain whether sufficient numbers of juveniles can be produced in which they can be used for stock enhancement, and 3) to determine whether sufficient production of hatchery raised juveniles would provide an opportunity for commercial-scale production such as an open ocean cage culture system.

Classroom Laboratory: The buoyancy of an egg

One characteristic of marine fishes, particularly for those that release their eggs into the water column, is that their eggs float in seawater and must do so until they hatch. If the eggs are unable to float, they will perish when they sink to the seafloor. Interestingly, the immature egg called an ‘oocyte’ (that is still inside the female), is not capable of floating unless it goes through a process known as ‘final maturation’ (Figure 5). During this process the female literally drinks seawater in order for her to hydrate her oocytes, and in doing so the eggs become equal to or less dense than seawater. The hydration of the oocyte takes place with the dissolving of the yolk granules and the coalescence of the oil vacuoles into a single oil droplet. The oocyte will transform from a completely opaque cell into one that resembles a glass bead when it is ready to be spawned. Because spawned eggs are either equal or less dense than seawater, they will float in or on the surface of the water column. For the ‘ōpakapaka broodstock that are held in net cages at HIMB, their eggs will float to the surface where they can be easily collected by skimming the surface of the water. We will demonstrate this concept in the following classroom exercise.

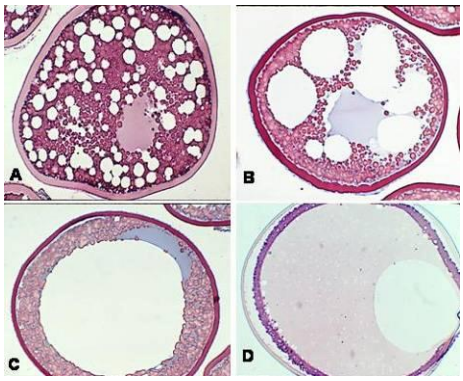

Figure 5: Histological changes that a striped mullet oocyte goes through in preparation to be spawned. A) An oocyte at the start of the final maturation process. Note the yolk vesicles (red balls) and oil droplets (clear balls). Stages B and C show the dissolving of the yolk and coalescence of the oil droplets as the oocyte begins to take in water (hydration). In stage C) the breakdown and dissolving of the nucleus (blue body) can also be seen. D) Completely hydrated oocyte with a single oil droplet ready to ovulate and be spawned. Source: Tamaru et al., (1991).

Density is a measure of how much matter there is in a given amount of space or volume. The more matter you can pack into a space, the denser it is. So if we take a fixed amount of ordinary water, add salt and dissolve it, we would make that water more dense.

Carry out the following activity:

1. Fill two 1-L glass beakers with tap water.
2. Add 1 tablespoon of salt to one of the glasses of water and mix well. This will be the treatment vessel. The other that did not get any salt is the control.
3. Place an uncooked chicken egg in each of the glass beakers and note if there are any differences between the two.
4. Place another tablespoon of salt into the treatment beaker and dissolve the salt.

5. Continue this process until the egg begins to float in the water column. Keep track of the number of tablespoons of salt that was needed and by weighing one tablespoon of salt you will be able to roughly calculate the salinity (g/Liter) necessary to float the egg.
6. How does the salinity needed to float the chicken egg compare to that of seawater?

What to expect during the field trip day

During your field trip to HIMB you are going to become hatchery managers for the production of ʻōpakapaka juveniles. Part of your initial tasks will be preparing spawned eggs for a larval rearing trial. This will require collection of eggs from the broodstock net pens (Figure 6) followed by:

- separating eggs from other plankton and debris
- quantifying the number of spawned eggs available
- determining the percent fertilization
- estimating when spawning took place
- estimating when hatching will occur

Please bring a copy of this lab with you to HIMB

Figure 6. Chris Demarke transferring an ʻōpakapaka broodstock during the annual changing of the net cages. Photo by Clyde Tamaru.

Part II: Field trip day at HIMB

Introduction

When the class arrives at HIMB, students will be divided into small groups. Each group will visit the ʻōpakapaka broodstock to collect all eggs from the assigned area of the net cage. The activities of this lab are the same steps that are taken by scientists to quantify and prepare eggs for stocking into the larval rearing tanks.

Guiding Questions

During the lab you should be able to answer the following questions:

- Why is it important to separate the spawning eggs from the debris?
- What can you say about the reproductive potential (e.g. when they spawn, how many eggs, percent fertilization etc.)?
- Are all of the eggs at the same stage of development?
- When did the broodstock spawn? When will the hatching occur?
- What triggered the spawning event?
- Are these eggs good for larval rearing? How do you determine this?

Tools available

At HIMB there are ʻōpakapaka broodstock that are being housed in floating net cages and spawning naturally. These will be the source of the spawned eggs (Figure 7) to be used for this lesson.

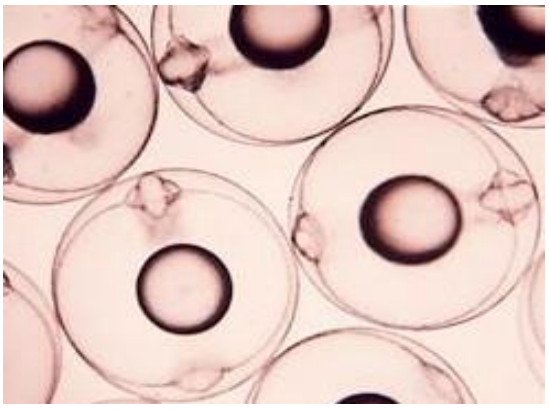

Figure 7. Spawned fish eggs viewed through the 4x objective of a compound microscope. Note the single oil droplet in the center of the egg that will provide the much needed energy for the embryo to develop. The fish embryo can be seen developing around a large yolk sac. Clearly visible are the developing eyes and tail. Photograph by Clyde Tamaru.

Materials available:

- Depression slides
- Petri dishes 60 x 15 mm
- One liter glass beakers
- 20 L buckets
- Compound microscope
- 10 mL pipettes
- Bulb pipette
- Hawaiian salt
- Portable aerators
- Magnifying glass
- Flashlight

Task 1. Collecting the eggs. Each group will be equipped with one 20 L bucket. When on the net pens, fill the buckets to the 10 L mark with seawater from *outside the pens*. The buckets will be labeled 'northeast', 'southeast', 'northwest', 'southwest' or 'center' to indicate the area of the net pen where each group will collect. The covers from the net pens will be opened for your group and using the fine mesh dip net provided, you will skim the surface of the water in your designated area of the net pen (e.g., northeast corner) and place the collected contents into the bucket. The eggs look like little glass beads that are slightly larger than the size of the mesh of the net. Repeat until you are confident you have skimmed all of the surface area in the section you are in. Groups should return to the class laboratory immediately. The buckets will be heavy so students should take turns carrying them. When back in the lab, turn on the portable aerator making sure the contents of the bucket are being aerated.

Task 2. Quantifying the number of eggs. Each group of students will have access to a 10 mL pipette and a petri dish. Making sure that the contents in the bucket are being well mixed by the aerator, aliquot 10 mL into the petri dish. Using a flashlight and magnifying glass, each student will then count the number of eggs present in their 10 mL samples and record the data on the data sheet provided in the Appendix. To help with counting, students can draw on a piece of paper a gridded outline of their petri dish. Students should then estimate the total number of eggs in their buckets by multiplying the number of eggs they found in their sample by 1,000 and record in the appropriate column. The reason is that 10 mL is $1/1000^{th}$ of 10 L. List the results and find the range (lowest and highest value) and average in the estimates of the number of eggs present in each bucket. Finally, we will average each group and then sum the averages of eggs from each of the buckets to estimate the total number of eggs that were spawned. Usually, the number of spawned eggs is in the tens of thousands and occasionally is in the hundreds of thousands.

Task 3. Separating Eggs From Other Organisms and Debris. Continue to aerate the contents of the buckets with the portable aerators. In this activity you will take advantage of the differences in density of seawater and freshwater to separate the spawned eggs from the debris that was also collected. You are about to add tap water to the container that holds the eggs and you should be able to answer the questions below before starting:

Figure 8. Besides spawned eggs a number of different kinds of organisms will inadvertently be collected such as phytoplankton (left column) and zooplankton (right column). These will all need to be separated from the spawn eggs and forms the basis for this particular task. Photomicrographs by Clyde Tamaru.

- By adding tap water what will happen to the salinity of the solution?
- What will happen to the density of the solution?
- What will happen to the density of the spawned eggs?
- What is going to happen to the spawned eggs?

Using a 1L beaker, each group will scoop out 700 mL from their bucket and place the beaker on the lab bench. Allow the contents to stand for a few minutes and students should observe the amount of debris (e.g., algae, zooplankton) that is in the sample (Figure 8). Fill the beaker to the 1 L mark with tap water and gently mix the contents with your pipette and allow the mixture to stand for a few minutes. Can you see what happened to the eggs? Did the eggs behave as you expected?

Using the 10 mL pipette, skim the debris from the surface of the 1 L beaker and discard the solution. Continue until all the debris on the surface is removed. When finished, add approximately 2 to 3 teaspoons of Hawaiian salt to the beaker and mix until the salt is dissolved. Based on your previous experience, what should happen to the eggs? Allow the contents in the beaker to stand for about 5 minutes. Use a pipette to collect the eggs from the surface of the water.

Task 4. Calculating percent fertilization. Use a 10 mL pipette to remove as many eggs as possible from the surface of the 1L beaker from Task 3 and transfer to a small petri dish. Then, use a bulb pipette to transfer approximately 20 eggs onto a depression slide. It is a good idea to also add a drop of tap water in order for the eggs to sink to the bottom of the depression slide so that they will not move around while being viewed under the microscope. Place the

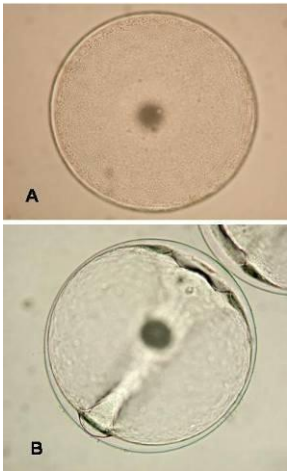

Figure 9. Photomicrograph of unfertilized (A) and fertilized (B) ʻōpakapaka eggs. The unfertilized egg is usually opaque and have no signs of any embryonic development taking place within it. In contrast, depending on the stage of development, the fish embryo will be clearly visible within the fertilized egg. Photographs by Aaron Moriwake.

depression slide under the compound microscope and determine the number of eggs that do not have an embryo developing within it (Figure 9A) and those that do (e.g. Figure 9B shows an early stage embryo). Calculate the percent fertilization by dividing the number of fertilized eggs by the total number of eggs counted and multiplying by a hundred, and record your results in the Appendix. Normally, the percent fertilization is very high (e.g., > 90%) and is one of the indicators that the egg quality of the spawn is very good. Spawns that have percent fertilization values that are below 80% are usually discarded.

Task 5. Determining time of spawning and time to hatching. From the hatchery manager's perspective, the time to hatching is an important piece of information to have because of the preparation (e.g., tank, water, aeration, live feeds, etc.) that must take place before stocking the spawned eggs into the larval rearing tank. Fortunately, researchers at HIMB have constructed a developmental series (Figure 10) for the ʻōpakapaka showing the embryonic stages from early stages of cleavage within the fertilized egg and ending with hatching of the larvae. This developmental series will allow you to estimate both the time that spawning took place and the time to hatching based on the stage(s) of development that the eggs are in when you observe them.

The first task is to scan through at least 20 eggs as a group to see if there are different embryonic stages that are present in the spawned eggs. If two different embryonic stages are observed, then at least two females which have spawned at different times have contributed to the spawned eggs that were collected. In most cases there is only one stage of development indicating either just one single female produced the eggs or that the spawning was synchronized among individuals (i.e. that the group spawned all together). The latter situation is not uncommon among other marine fishes and is the normal situation for the ʻōpakapaka rather than the exception.

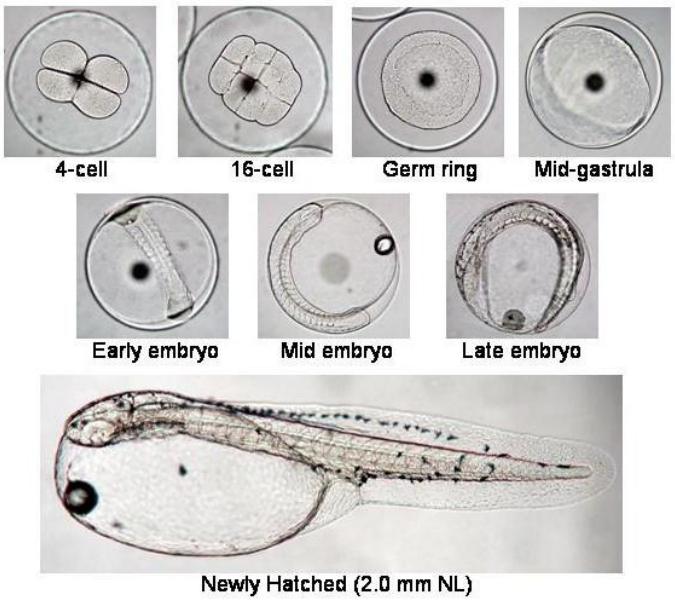

Figure 10. Photomicrographs of the embryonic stages of developing ʻōpakapaka. Images of the embryos were photographed with a compound microscope and are all from a single spawning event. Incubation temperature was 26^{O} C. Photos taken by Aaron Moriwake.

Identify what stage of embryonic development the eggs are in and record the time that you made the observation in the appropriate box of the data sheet supplied in the Appendix. To estimate the time of spawning, you will have to subtract the age of the embryo in hours from the current time they are being observed. For example, it is 12 noon and the eggs are at the mid embryo

stage of development. This would mean that they are 19 hours old and that spawning took place at approximately 7 pm the previous evening. From the developmental series we also know that hatching occurs when the embryos are 31 hours old, which means an additional 12 hours must pass before they hatch. That would mean the estimated time to hatching would be midnight that evening, and all preparations in the larval rearing tanks must be completed before then. You can take home your samples and continue to observe developmental changes until hatching. If your school classroom does not have microscopes, you might even be able to see the larvae with the naked eye.

What you will do with the data back in the classroom

The class will have gone through a set of tasks that are needed to prepare the eggs for stocking into the larval rearing tank. While back in your classroom, take some time to reflect on those tasks as they provide insight into the biology of the ʻōpakapaka that can be easily missed. Access to naturally spawned eggs provides an opportunity to investigate biological processes that would normally be difficult if not impossible in the wild.

Part III: Post-activities back in the classroom

A) Begin with discussing the abundance and fertility of the eggs in the broodstock cages. Are these eggs good for larval rearing? Are the number of eggs each group calculated from Task 2 the same for each area of the pen (e.g. 'northeast', 'southeast', 'northwest', 'southwest' vs. 'center')? If they are different can you think of an explanation for the observed data?

B) In Figure 2, you can see that the number of eggs being collected each month is not the same throughout the year. Based on the historical spawning data would you be able to estimate what the chances of getting another spawn would be within the next month? Because fishes are poikilotherms (e.g., their body temperature is dependent on the temperature of their surrounding environment), water temperature can have profound influences on their reproduction. What would be some other environmental parameters (e.g., length of day, salinity, dissolved oxygen, temperature, etc.) that might be important in mediating spawning in ʻōpakapaka? How would you go about exploring whether these factors influence reproduction in ʻōpakapaka? Normally ʻōpakapaka adults live at depths of 100 fathoms (600 feet) and some research is still needed to find out the differences in environmental factors that occur at that depth versus the surface. Based on your observations in this exercise, can you hypothesize what environmental parameters are most important? Would you have expected the broodstock to be spawning in captivity at such shallow depths?

C) The number of eggs produced for each spawn is an important value considering we as hatchery managers have to be able to provide sufficient food for the resulting larvae. For example, if we had 100% fertilization and assuming 100% hatch, how many ʻōpakapaka larvae are going to be produced from the spawn that you worked with at HIMB? Discuss how overfishing could impact the ʻōpakapaka population. How would you devise a management plan that would ensure an abundance of ʻōpakapaka and yet meet the market demand for this popular food fish?

D) The number of eggs produced should also provide a glimpse of the tremendous number of organisms present in the ocean necessary to support all of vast array of life in the ocean. It is estimated that one larva at first feeding will consume approximately 10 copepod nauplii, or first stage larvae, on the very first day it begins to feed. This amount will easily double each day over the course of the next five days. So based on the number of eggs you determined and again assuming 100% fertilization and hatch, how many live food organisms are needed to support just this group of larvae on just the first day of feeding? Hatchery managers have to grow the appropriate number of food organisms in a captive setting, so how does mother nature ensure that there are sufficient amounts of food in the wild?

Lab report

For your laboratory exercise at HIMB, you will be expected to eventually produce an in-depth laboratory report including the following independent sections:

- **Title**: summarize the entire laboratory exercise in several words.

- **Introduction**: in one half to three-quarters of a page, describe the status of our ʻōpakapaka fishery and the possible ways being studied to manage them.

- **Materials and Methods**: develop and describe, in detail, the experiments you conducted to prepare the fertilized eggs for rearing. ; include all of the materials you used to complete it as well.

- **Results**: compile your data and express them visually and where appropriate in graphs, tables, or figures.

- **Discussion**: analyze your data in essay form; discuss the results and emphasize what the results obtained mean to you and your group (e.g. What do you think about aquaculture as a method of relieving pressure from overfished wild populations of ōpakapaka? Is there any way it can be improved?). You should also propose future experiments that can further add to our knowledge of this species' reproductive biology.

- **Conclusion**: in a paragraph or so, summarize your results and make concise conclusions about them; also include a sentence or two conveying your general conclusions about your results in the context of the status of our fisheries and how we should go about managing it.

References

Science background information condensed and/or compiled from the following sources:

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6) Tamaru, C. S., C. D. Kelley, C.-S. Lee, K. Aida, I. Hanyu and F. Goetz, 1991. Steroid profiles during maturation and induced spawning of the striped mullet, Mugil cephalus L. Aquaculture, 95: 149-168

Relevant Hawai‘i Content and Performance Standards III

SC.BS.1.2: Design and safely implement an experiment, including the appropriate use of tools and techniques to organize, analyze, and validate data

SC.BS.1.3: Defend and support conclusions, explanations, and arguments based on logic, scientific knowledge, and evidence from data

SC.BS.1.5: Communicate the components of a scientific investigation, using appropriate techniques

Acknowledgements

The HIMB Education Program would like to thank Karen Brittain, Dr. Bradley ‘Kai’ Fox, the HIMB Community Education Program volunteers, and graduate student assistants Kelvin Gorospe and Roxanne Haverkort for providing additional comments and suggestions.

APPENDIX

Task 2: Data Sheet for quantifying number of eggs collected.

Group Results
Bucket Label (collection locale):_____

Name	No. of eggs/10 mL	No. of eggs in bucket
Range/Average		

Class Results

Bucket Label (collection locale)	No. of eggs in bucket
Northeast	
Northwest	
Southeast	
Southwest	
Center	
Total	

Task 4: Data sheet for number of eggs fertilized and percent fertilization.

Group number	No. of eggs fertilized	No. of eggs unfertilized	Total	Percent fertilization

Task 5: Data Sheet for estimating time to spawning and time to hatching.

Stage of development	Hours post spawning	Time observed	Date & time of spawn	Estimated time/day until hatching
4 cell	2			
16 cell	4			
Germ Ring	8			
Mid Gastrula	11			
Early Embryo	15			
Mid Embryo	19			
Late Embryo	27			
Hatched Larva	31			