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Exploring larval development and applications in marine fish aquaculture using pink snapper embryos

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Practical

Exploring larval development and applications in marine fish aquaculture using pink snapper embryos

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This biology investigation on *Pristipomoides filamentosus* larval development, survival, and aquaculture research was developed with three educational objectives: to provide high school students with (1) a scientific background on the biology and science of fisheries as well as overfishing, its consequences, and possible mitigations; (2) exposure to field and laboratory techniques in marine science; and (3) practical skills in scientific inquiry and investigation. We teach this investigation at the Hawai'i Institute of Marine Biology, where we have access to captive broodstock of the pink snapper, *P. filamentosus*. During this investigation students follow several steps scientists take to study aquaculture: collecting spawn from outdoor fish pens, quantifying the number of eggs, determining the percentage of fertilisation, and estimating the time of spawning and hatching. Additionally, students perform hypothesis-driven science activities with the embryos to test the effects of water quality on their development and survival. In this paper we discuss background information of aquaculture, specifically of *P. filamentosus*, and thoroughly describe the several components of delivering the investigation. Lastly, we provide possible outcomes of students' performances in the laboratory activities, and discuss how effectively the exercise met its educational objectives.

Keywords: inquiry-based learning; high school; place-based education; hands-on; *Pristipomoides filamentosus*

Introduction

As the world's marine fisheries resources are under enormous pressure, now is a crucial time to educate the next generation of scholars and stewards regarding the biology and science of fisheries as well as overfishing, its consequences, and possible mitigations. Knowledge of this subject is of importance to science students as it raises awareness of on-going efforts towards achieving a sustainable future.

One of the main causes of declining fisheries globally is the increase in the human population; as the demand for seafood increases, so does the impact on fish species and populations targeted for consumption (Jennings, Kaiser, and Reynolds 2001). This high demand has led to over-exploitation of the world's oceans and global crises in marine fisheries (Pauly et al. 1998; Jennings, Kaiser, and Reynolds 2001). In the United States, the demand for seafood is similarly increasing and will continue to do so (Barnaby 2006). Federal health guidelines call for Americans to double their consumption of seafood, while the harvest of wild-caught seafood will probably never keep pace with demand (Barnaby 2006). Therefore, the need for sustainable forms of aquaculture is now higher than ever before.

In Hawai'i, a decrease in commercial landings raised concerns about the status of the Hawaiian

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bottomfish fishery in the late 1980s (NOAA 2013). Recent fishery management plans have placed regulations and restrictions on the fishery to replenish bottomfish stocks (NOAA 2013). Such regulations include seasonal closures during the fish spawning seasons, restrictions on both the number of fish one can take (ie bag limits), and the minimum sizes of fish that are taken. These regulations are largely based on biological information, namely the reproductive traits of the target fish species. In addition to these regulations, 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 often used to enhance wild stocks and simultaneously commercially produce fish for market. Commercial production of a species can alleviate fishing pressure by providing an alternative source to meet market demand.

When spawning is observed, aquaculturists start working to obtain data that help to characterise 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 provide fundamental information regarding the reproductive potential of the species (Figure 1). Correlating spawning events with environmental variables (eg length of day, temperature, salinity) suggests which of these variables in the wild are important in governing reproduction of the target species and could be manipulated to control maturation and spawning in captivity.

This investigation was developed for *Pristipomoides filamentosus* in Hawai'i using embryos from Hawai'i Institute of Marine Biology (HIMB). The fish pen at HIMB is 3 m wide, 6.1 m long and 6.1 m deep. Spawned eggs were obtained from 20 *P. filamentosus* individuals, estimated to be approximately 13–14 years of age, and with an approximate male to female ratio of 1:1. However, in theory only one fertile fish of each sex is needed to ensure that spawning is

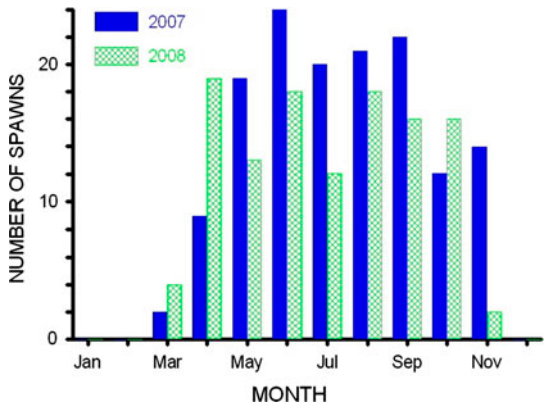

Figure 1. Temporal changes in the number of spawns per month from *Pristipomoides filamentosus* broodstock held in net cages at HIMB

frequent and likely to occur around the date scheduled for the activity. The activities described in this paper are meant to be used as a guide for modification in different geographic regions. In most cases collaboration will be needed with aquaculture practitioners or facilities in the area.

In order to reach our first educational objective (to provide high school students with a scientific background on the biology and science of fisheries as well as on overfishing, its consequences, and possible mitigations), we provide a lecture with the information discussed above. In order to reach our second educational objective (exposure to field and laboratory techniques in marine science), the lecture is followed with science activities where students follow several steps scientists take to study aquaculture: collecting spawn from outdoor fish pens, quantifying the number of eggs, determining the percentage of fertilisation, and estimating the time of spawning and hatching. Finally, to reach our third educational objective (practical skills in scientific inquiry and investigation), we then guide the students through hypothesis-driven science activities on the effects of water quality on larval development and survival.

Inquiry-based teaching (National Research Council 1996; National Research Council 2012) is a method known to be engaging and effective for connecting, recruiting, and retaining students in the STEM fields (Abell 1999; Schneider et al. 2002). Moreover, previous studies on inquiry and its relationship to student performance in science have highlighted the importance of lessons that have applications to the real world (Gee and Wong 2012). As a research institute, the Hawai'i Institute of Marine Biology, is up to date with the newest scientific findings in aquaculture research and is thus able to expose students to current techniques and research topics in aquaculture as well as on the status of fisheries in Hawai'i and globally.

In the following sections we shall thoroughly describe the several steps undertaken during this investigation, provide possible outcomes, and discuss possible alternatives. Lastly, in order to measure how effective these activities were in improving student understanding of fisheries, overfishing, and aquaculture techniques and utility, we test students with questions both before and after the lecture and science activities, using i>clicker® (i>clicker, USA), a wireless question and answer polling system (Figure 2). To examine whether students have developed skills in scientific inquiry and investigation and an increased interest in marine science we evaluate students' opinion after the lecture (Figure 3).

Materials and methods

The investigation consists of three components (Figure 4): (1) background reading, classroom lecture

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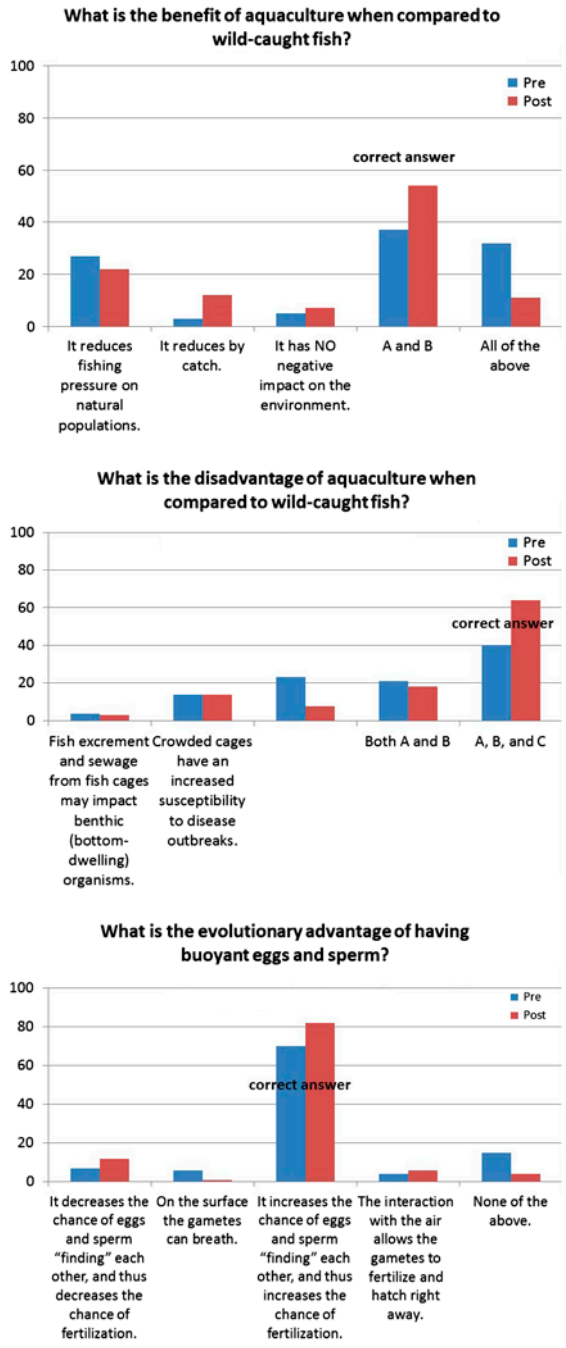

Figure 2. Student understanding of aquaculture related issues, before and after laboratory activities

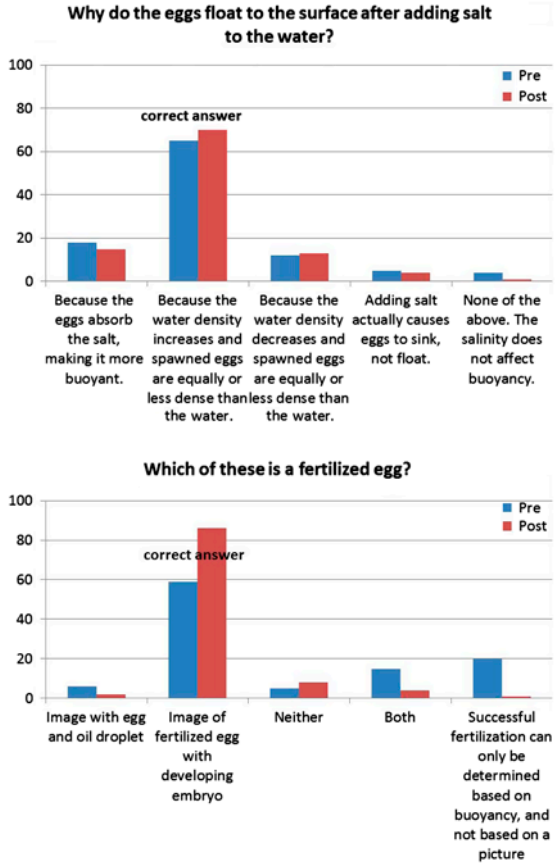

Figure 2. (*Continued*)

and a preparatory activity; (2) hands-on science activities conducted in field and laboratory environments; and (3) data analysis, discussion and scientific writing. In the following sections, we shall thoroughly describe these three components. More detailed descriptions of these components and guiding questions may be found in the original lesson plan (downloadable from: www2.hawaii.edu/~himbed/inquiry-fieldtrips.html). In addition to high-school students, the investigations can be applicable to a wider audience including middle school students, recent high school graduates, and even early undergraduates.

Component 1: Background reading, classroom lecture and a preparatory experiment

The first phase of this lesson consists of several classroom-based elements. Students should be introduced to the subject of fisheries, overfishing and aquaculture using the background information provided above. The lesson we teach at HIMB was developed for one particular fish species found in Hawai'i and across the Indo-Pacific, so modification of the curriculum will be necessary based on the type of fish broodstock available to teachers elsewhere. We encourage collaborations with aquaculture researchers in the geographic vicinity. As an assignment, students should perform a literature search on the advantages and disadvantages of aquaculture, and this should be followed by a teacher-led discussion on the results from the assignment. For this exercise, it may be interesting to divide the class into two groups; one with proponents and one with opponents of using aquaculture instead of wild catch. Sample guiding questions are available in Appendix A.

Component 2: Hands-on science activities

During this activity, students should be divided into groups of three to four students. The activities of this component are divided into six tasks (Figure 4); tasks

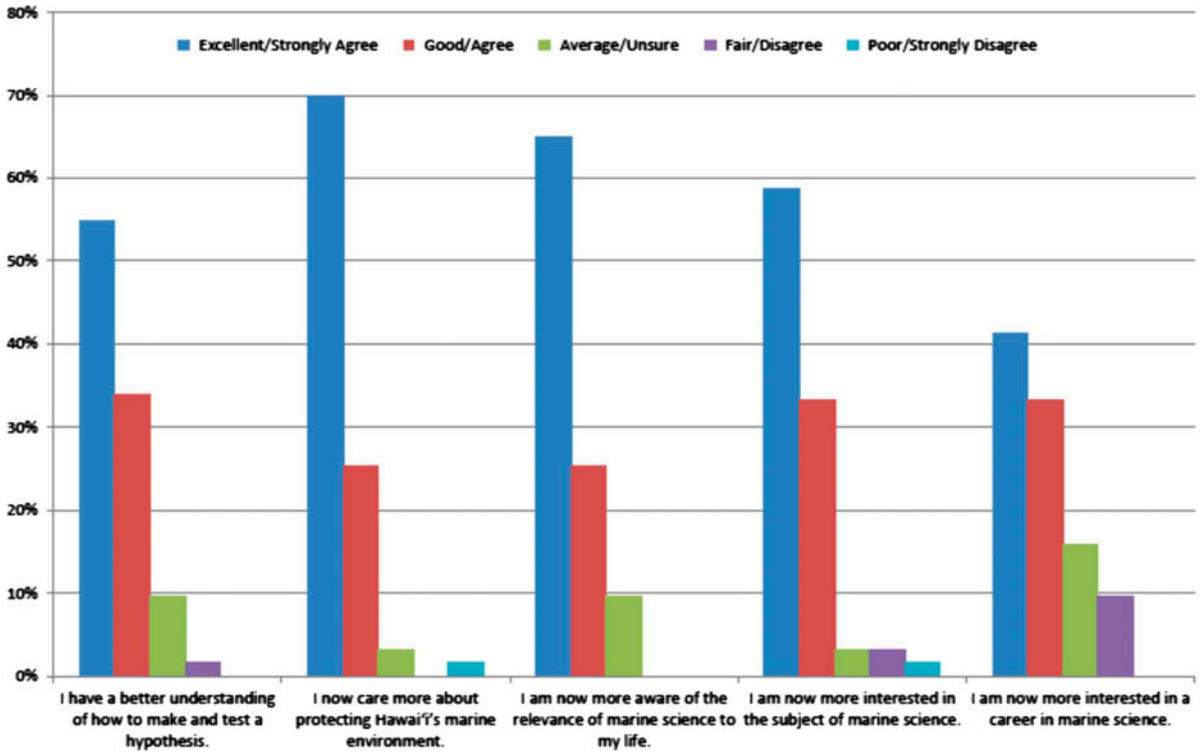

Figure 3. Student feelings about marine science after participating in the HIMB laboratory activities

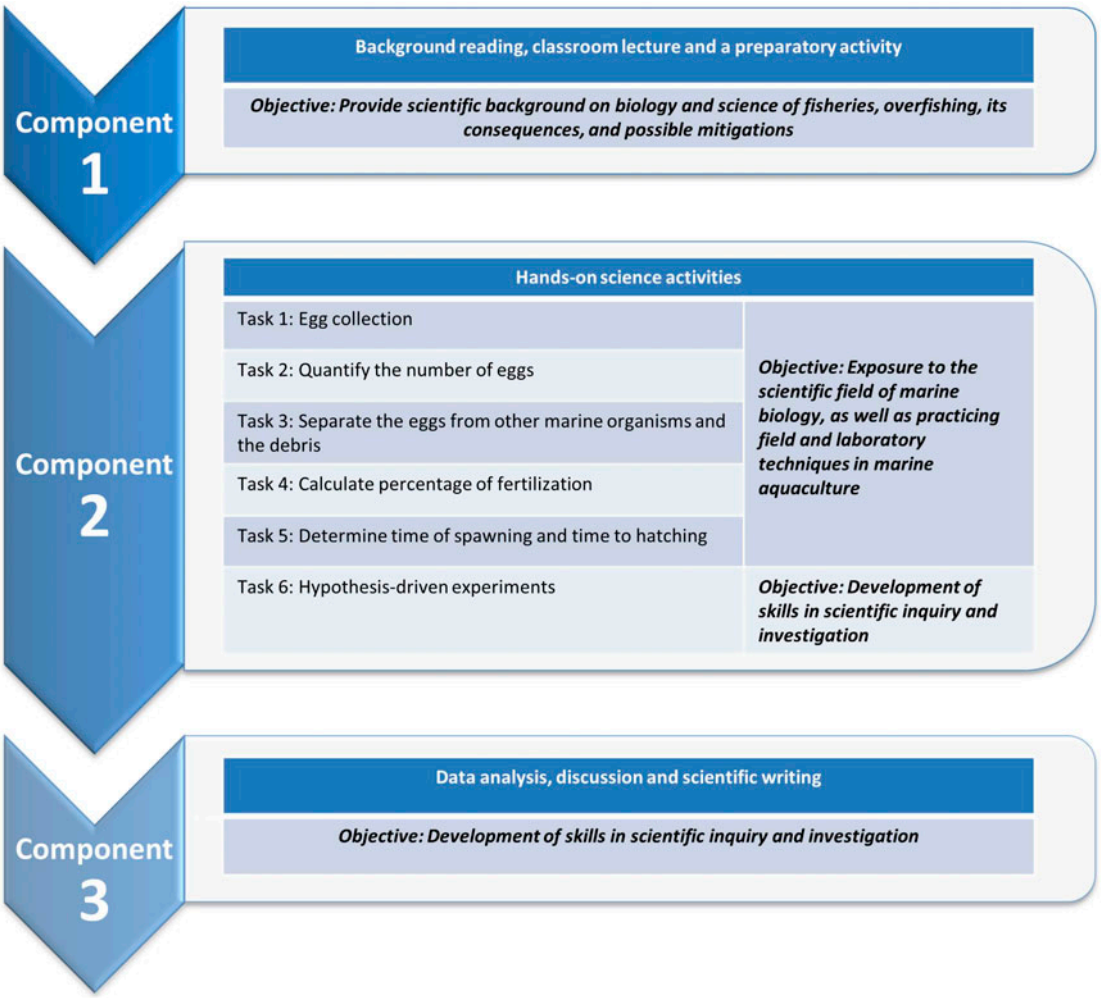

Figure 4. Hierarchical overview of the various components and tasks of the investigation, and their corresponding objectives

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1 through 5 follow the same steps that are taken by scientists to quantify and prepare eggs for stocking into the larval rearing tanks, whereas during task 6 students have the opportunity to develop and test their own hypothesis within the framework of water quality effects on *P. filamentosus* embryos. For task 2, 4 and 5, each group should write down their results in the tables provided in Appendix B.

Task 1 – egg collection. Each group should fill a 20 litre bucket with 10 litre of seawater. If possible, students should collect from different areas of the pen (eg divide the surface area into four quadrants). Using a fine mesh dip net, students should skim the surface of the water in their designated area of the net pen and place the collected contents into the bucket. When students have skimmed all of the

Figure 5. Besides fish embryos 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 fish embryos and form the basis for task 3

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surface area in their section, they should return to the laboratory where they install a portable aerator to *very lightly* oxygenate the contents of their bucket without producing excessive turbulence that may inadvertently damage the eggs.

Task 2 – quantify the number of eggs. Each student should mix the contents in the bucket with a 10 ml pipette so that it is homogenous, and aliquot 10 ml into a petri dish using the same pipette. Each student should count all eggs in 10 ml by eye, record the results, and calculate the group range and average (Appendix B). Then students should calculate the number of eggs in the bucket based on their individual results, and calculate the group range and average (Appendix B). Finally, students should collect data from all groups (eg average total number of eggs from each collection area) and estimate the total number of eggs collected by summing each group's average egg count.

Task 3 – separate the eggs from other marine organisms and debris. Before performing task 3, students need to be introduced to the concept of egg buoyancy, an important feature of fish reproduction for many species. Fish eggs are either equal or less dense than seawater and as a result float in seawater and must do so until they hatch; if the eggs are unable to float, they will sink to the seafloor and perish. Density is a measure of how much matter there is in a given amount of space or volume; the more matter contained in a given space, the denser that space is. The density difference between seawater, freshwater, the eggs, and the debris, can be used to separate spawned eggs from the debris and other marine organisms collected alongside the eggs. It is important to remove the debris and other marine organisms from the eggs because they may interfere with the rearing process. Using a 1 litre beaker, each group should scoop out 700 ml from the upper surface of the bucket contents to collect a large amount of eggs. Students should gently fill the beaker to the 1litre mark with tap water. The decrease in salinity causes the eggs to become negatively buoyant (ie they will sink), while most of the debris and other marine organisms continue to float on the surface. Using the 10 ml pipette, students should remove the debris and other marine organisms from the surface of the beaker. Students should use a dissecting microscope to identify these organisms and use Figure 5 as a guide. Finally, students should add 12–18 grams of sea salt to the beaker and mix until dissolved. The addition of salt should cause the eggs to become positively buoyant (ie rise to the surface).

Task 4 – calculate percentage of fertilisation. Each student should individually use a bulb pipette to transfer approximately 20 eggs from the surface of the beaker from task 3 onto a depression slide. Each student should then use a compound microscope to determine the percentage of fertilisation by counting eggs that do not have an embryo developing within it (Figure 6A) and those that do (Figure 6B). Students should calculate the average percentage fertilisation within their group (Appendix B).

Task 5 – determine time of spawning and time to hatching. For hatchery managers, the time to hatching is important information because of the preparation (eg tank, water, aeration, live feeds, etc.) required before stocking spawned eggs into larval rearing tanks. Aquaculture researchers and practitioners will have a developmental time series available for their species of interest as depicted in Figure 7 for *P. filamentosus*. Using the depression slide from task 4 and the developmental time series, each student should scan through approximately 20 eggs to identify the stage(s) of embryonic development of the eggs, and record time of observation. With these data and the information contained in the table of task 5 in Appendix B, students should estimate the time of spawning and hatching.

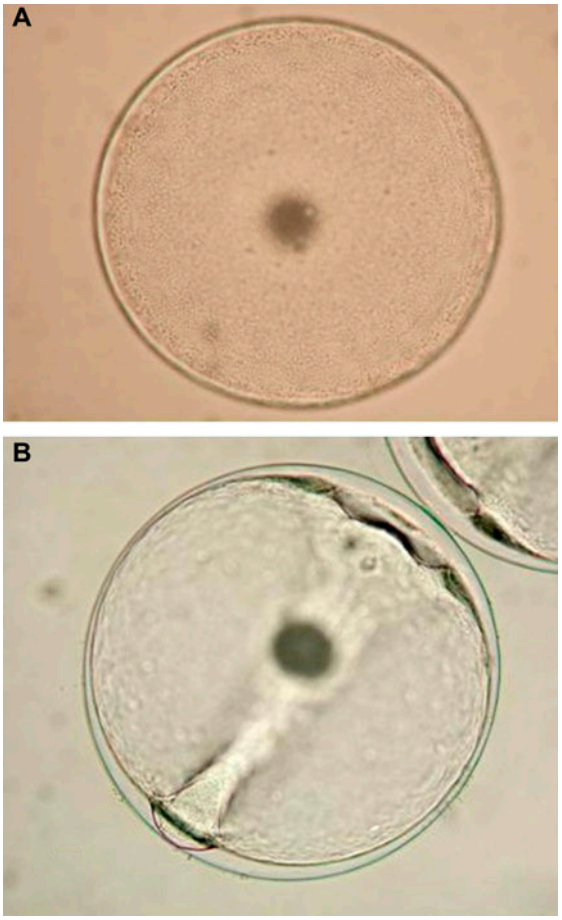

Figure 6. The unfertilised eggs of *Pristipomoides filamentosus* (a) are usually opaque and have no signs of any embryonic development taking place within them. In contrast, depending on the stage of development, the fish embryo (b) will be clearly visible within the fertilised egg. Photos: Aaron Moriwake

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Task 6 – Hypothesis-driven experiments. During task 6 students have the opportunity to develop and test their own hypothesis within the context of water quality effects on fish embryo and hatchling development and survival. Teachers should briefly lecture on water quality changes due to heavy rainfall, drought, or run-off as well as the concept of hypothesis-testing and the use of control experiments. For background information on water quality, teachers may download the lesson plan '*Sea Urchin Fertilization*' (from www2.hawaii.edu/~himbed/inquiry-fieldtrips.html). After the lecture, student groups should brainstorm about a hypothesis. At HIMB we provide sea salt and tap water to simulate the conditions of drought or heavy rainfall, respectively, and plant fertiliser to simulate pollution run-off. Students may make and test different concentrations, and bring additional solutions to class for manipulating the water quality of their experiments (eg household cleaning supplies). After the students form their hypotheses, teachers should proof-read and accept these before students start setting up their experiments. At this moment, teachers should ensure that each group has incorporated a control experiment. As a guideline, students should add 50 fish embryos to 1 litre of experimental ocean water, and monitor the fish embryos using a compound microscope during several time intervals (T=0, T=3 hrs, T=6 hrs). To monitor the embryos, each student should observe 20 embryos and note individual results (Appendix B, Task 6 table). Students should tally the proportion of embryonic stages in each experiment, and calculate the average among the group. When fish embryos or hatchlings discontinue development, it may be assumed they are dead. Once the embryo develops into a hatchling, a heartbeat may be observed.

Component 3: Data analysis, discussion and scientific writing

Once the various tasks are completed, students will have experienced the basic steps necessary to stock a rearing tank for growing larval fish. Once this is

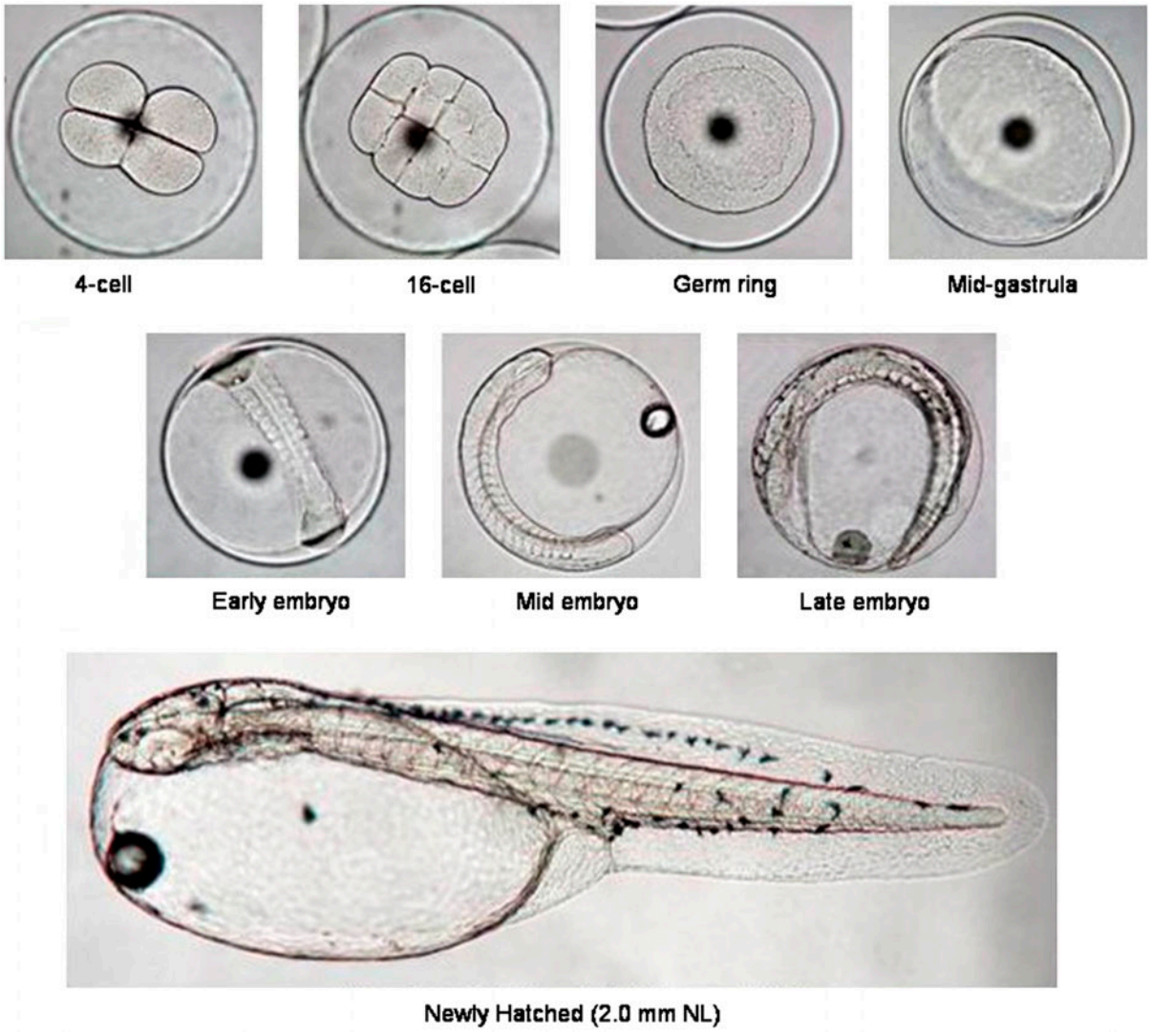

Figure 7. Several of the embryonic stages of developing *Pristipomoides filamentosus*. Photos: Aaron Moriwake

completed, the instructor should lead a class discussion, covering the various steps from components 1 and 2. Guiding questions are available in Appendix C. These questions and ensuing discussions help stimulate student thinking beyond the basic steps undertaken, helping them explore potential hypotheses to test and encourage interest in future scientific inquiry. Finally, each group of students should produce an in-depth laboratory report including a title, introduction, materials and methods, results, discussion, and conclusion, and/or an oral presentation of their work.

Results

The effectiveness of the various activities is measured through testing students both before and after the lecture and science activities using i>clickers® content questions. In addition to the content questions, we evaluate student opinions relating to developed skills in scientific inquiry and investigation and an increased interest in marine science after the lab. We always point out that the answers are anonymous to stimulate honest answering. Our surveys reveal an increased understanding of aquaculture related issues (Figure 2), and a significant increase in student interest in marine science as a result of our program (Figure 3). The results presented here are based on the responses of 138 high school students from six separate classes.

Discussion

The educational objectives of this investigation on *P. filamentosus* aquaculture research are to (1) provide high school students with a scientific background on biology and science of fisheries, overfishing, its consequences, and possible mitigations; (2) provide exposure to the science field of marine biology as well as practicing field and laboratory techniques in marine science; and (3) develop skills in scientific inquiry and investigation. Based on the i>clicker® questions which we uniquely designed for this particular laboratory activity, we successfully increase students' knowledge of the advantages and disadvantages of aquaculture versus wild-caught fish. Our activities are also effective in providing exposure to the science field of marine biology and our i>clicker® findings reveal that students understand the biology behind the various scientific tasks undertaken during the investigation. Moreover, our findings indicate that students develop skills in scientific inquiry and investigation, through our hypothesis-driven laboratory experiments (Figure 3). We have received overwhelming positive feedback from both visiting teachers and students, and therefore encourage use of these exercises to enhance student interest in marine science and aquaculture, and to develop awareness of overfishing and possible mitigations. Finally, alterations to the investigations to extend the curriculum to a broader audience are described in Appendix D.

Acknowledgements

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Appendix A:

Guiding questions for teacher-led discussion during component 1, regarding the advantages and disadvantages of aquaculture *vs*. wild catch.

(1) What are some of the environmental consequences of aquaculture (both positive and negative)?
(2) What are some of the economic implications of aquaculture?
(3) What kind of research needs to be done before one can start aquaculture farming?
(4) If you had to pick between aquaculture and wild catch, which would you choose and why?

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Appendix B:

Data sheets for student research findings during component 2.

Research findings

Date:_____
Group members:_____
Collection area:_____

Task 2: Data sheet for quantifying number of eggs collected
Individual student results

Student name	No. of eggs/10 ml	No. of eggs in bucket (10 litres)
Student 1:		
Student 2:		
Student 3:		
Student 4:		
Group range:		
Group average:		

Group results

Group name	Collection area	Group average no. of eggs/10 ml	Group average no. of eggs in bucket
Group 1:			
Group 2:			
Group 3:			
Group 4:			
Class range			
Class average:			
Class total:			

Task 4: Data sheet for number of eggs fertilised and percentage fertilisation

Student name	No. of eggs fertilised	No. of eggs unfertilised	Total	Percentage fertilisation
Student 1:				
Student 2:				
Student 3:				
Student 4:				
Range/Average				

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			

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Task 6: Data sheet for monitoring effects of water quality on embryo survival on several time intervals for both the test and control experiment; students should fill out the total number of embryos monitored and the total number in each stage (eg 18/20 Mid Embryo, 2/20 Late Embryo), and calculate the average among the group

	Test experiment			Control experiment		
Student name	**T=0**	**T=3 h**	**T=6 h**	**T=0**	**T=3 h**	**T=6 h**
Student 1:						
Student 2:						
Student 3:						
Student 4:						
Group average fertilised:						
Group average 2 cell:						
Group average 4 cell:						
Group average 8 cell:						
Group average 16 cell:						
Group average germ ring:						

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Appendix C:

Guiding questions and answers for s teacher-led discussion during component 3, on the various steps from components 1 and 2.

Questions	**Answers**
(1) During task 2, the quantification of the total eggs spawned, what was the total count of the class? Did you expect this? Why do you think fish produce so many eggs in comparison to humans and other mammals?	During the quantification of the total eggs spawned, a number in the tens of thousands and occasionally in the hundreds of thousands can be expected.
(2) Were the total number of eggs each group calculated during task 2 the same for each area of the pen? If they were different can you think of an explanation for the observed data?	Likely due to ocean current and wind-driven drifting of the eggs there were differences among different sections of the pen. In nature, many organisms (also those which are sessile during later life stages such as corals) are dispersed during early life stages by wind and currents. This is how for example new species of corals may arrive in remote places such as Hawai'i.
(3) As you can see in Figure 1, the number of eggs 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 are of getting another spawn on any given day within the next month?	Look at the figure to determine what the next month will be like. Teachers may acquire a similar figure from aquaculture practitioners/facilities who provided the fish zygotes. Interesting discussion points are also regarding which environmental parameters (eg length of day, temperature, salinity) instigate the spawning events. For example, for the pink snapper in Hawai'i, this is during the summer when the days are longer and the water is warmer.
(4) During task 4, what was the percentage of fertilisation? Did you expect this number? Do you think the percentage of fertilisation indicates anything about the egg quality?	The percentage fertilisation is normally very high (eg > 90%) and values in this range are one of the indicators that the egg quality of the spawn is very good. Spawns that have percentage fertilisation values that are below 80% are usually discarded.
(5) During task 5, did you see only one or multiple embryonic stages? What do you think may be the reason of multiple embryonic stages?	This means that at least two females have spawned at different times. In most cases, however, there is only one stage of development indicating either just one single female produced the eggs or that the spawning was synchronised among individuals. The latter situation is not uncommon among marine fish and is the normal situation for the *P. filamentosus* rather than the exception.

(*Continued*)

Appendix C. (*Continued*)

Questions	Answers
(6) Because fishes are poikilotherms (ie 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 that might be important in mediating spawning in pink snappers? How would you go about exploring whether these factors influence reproduction in pink snappers?	Examples of important parameters are length of day, salinity, dissolved oxygen, and temperature. If you want to test if salinity affects pink snapper spawning events, the fish should be exposed to various salinity concentrations, below and above the normal (test experiments), as well as to the normal salinity concentration (control experiment).
(7) Based on your findings of component 2, task 6, how may (a) global climate change, and (b) localised influences impact marine life?	a) Global climate chance results in more extreme weather and thus more extreme droughts and rainfall. As a result, fish zygotes, and also other marine life may be impacted by water quality changes (higher salt concentrations due to increased droughts, or lower concentrations due to increased rainfall). When fish zygotes are affected by certain altered concentrations, this may indicate that other marine life would be affected as well. (b) Localised influences such as contaminated water quality may be affected by run-off containing fertiliser (from farmland or golf courses), or other chemicals (such as cleaning solutions). Similarly as with salinity changes, when fish zygotes are affected by altered concentrations, this may indicate that other marine life would be affected as well.

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Appendix D:

Alterations to the lab.

At HIMB we have access to a *P. filamentosus* broodstock, which is housed in floating net cages where captive fish spawn naturally. The techniques we describe, however, should largely be transferrable to other species of marine fish. Teachers should try to connect with a nearby aquaculture research group through a university, private company or individual aquaculturalist in the vicinity. If there is no access to live broodstock, it is also possible to keep zebra fish in a classroom aquarium. A zebra fish breeding kit can be purchased (Carolina Biological Supply Company) which allows observing embryological development for three to four days before hatching. Although zebra fish are not an important fisheries species, they are an important model vertebrate organism used for developmental biology research. Zebra fish are freshwater fish, however, and therefore the salinity experiments during component 2, task 6, are irrelevant when using zebra fish. However, different concentrations of fertiliser or other household cleaning supplies may still be tested to explore the effects of water quality on development and survival. When using the Zebra fish kit, or any other type of fish, a developmental series such as in Figure 4 specific to that fish should be used. Alternatively, it is possible to visit a local pet shop and independently set up a zebra fish aquarium. For a more advanced lab, students can continue to monitor development and survival over time to obtain estimates of hatching success. In our experience, hatchlings can survive up to a week with gentle aeration.