

Sex Determination and Mating Strategies of the Bluehead Wrasse, *Thalassoma bifasciatum*: A Role-play Simulation

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This lab explores key concepts in animal behavior, including the proximate and ultimate causes of behavior, phenotypic plasticity, and female choice. Students conduct role-play simulations of competitive and mating interactions of the bluehead wrasse, *Thalassoma bifasciatum*, a coral reef fish with socially-mediated determination of initial sex and of female-to-male sex reversal. Four distinct scenarios simulate wrasse mating on differently sized reefs as well as with and without female choice. Students calculate the reproductive success among fish with differing strategies for the four scenarios and develop proximate and ultimate hypotheses related to initial sex ratios and female-to-male sex reversal.

Keywords: animal behavior, mating behavior, sex reversal, phenotypic plasticity, role-play, coral reef, wrasse

Introduction

This lab provides an opportunity for students to explore several essential concepts in animal behavior, including the proximate and ultimate causes of behavior, phenotypic plasticity, and female choice. Students participate in simulations of mating behavior of the bluehead wrasse (*Thalassoma bifasciatum*), a sex-reversing coral reef fish, playing the roles of females, small “initial-phase” males and large “terminal-phase” males. These simulations highlight the competitive interactions among males as well as mating interactions between males and females. Four distinct scenarios explore these interactions on differently sized reefs, as well as with and without female choice. After the simulations are run, students calculate the average reproductive success of the females and of the two types of males for each scenario and examine how the reproductive success of each of these phenotypes depends upon the particular conditions (i.e. small versus large reef; female choice versus no female choice). Along with the key concepts, students develop a tangible understanding of the complex interactions among these fish and the effects of the social environment on reproductive success.

The set-up of this lab is not time-consuming (approximately 1 hour for one lab) and primarily involves pre-loading the gonads with gametes (i.e. filling the plastic bags with beans) and setting up territory boundaries for each scenario. Some trial-and-error is involved in delineating territory boundaries in a way that achieves the expected rela-

tive values for reproductive success, but the boundaries are also somewhat forgiving due to the rules provided for each role. The sweeping up of beans between scenarios and the resorting of beans after the lab is somewhat time-intensive, but this effort can possibly be mitigated if the lab is run outdoors with alternative “gametes” such as sunflower seeds or other foods that can safely be eaten by local fauna.

This lab requires a 3-hour lab period. It is well suited for upper-division animal behavior and ecology courses, but is also appropriate for introductory biology courses that cover animal behavior or behavioral ecology. Ideas for altering the lab to suit different instructional levels can be found in the “Notes for the Instructor” section.

Student Outline

Concepts Covered in this Lab

General

1. Ultimate causes of behavior are the selective pressures that drove, and/or continue to drive, the existence of particular behavioral traits during the evolutionary history of the animal. Ultimate causes are sometimes referred to as the “why” of behavior.
 - When thinking in “ultimate” terms, we posit that particular behaviors help to maximize an animal’s lifetime reproductive success, or “fitness”. For example, in many animal species including the bluehead wrasse (the focal species for this lab), some males expend a considerable amount of time and energy, and even risk their lives, to establish and defend territories. An ultimate hypothesis to explain this behavior would be, “Territory-holding males father more offspring than those without territories.”
 - The potential link between a behavior and reproductive success is not always so obvious. Some behaviors may indirectly promote the fitness of an animal by aiding in its survival and growth, rather than being directly linked to reproductive success. For example, juvenile fish often dart into crevices when larger fish come close to them. An ultimate hypothesis to explain this behavior would be, “Juvenile fish that dart into crevices in response to larger fish are more likely to survive to reproductive age than juvenile fish that remain in the open when larger fish are present.”
 - In developing ultimate hypotheses, both historic and current adaptive values of the observed behaviors are considered.
2. Proximate causes of behavior are the underlying genetic, morphological, and physiological factors that enable a behavior to occur, as well as the environmental cues that may trigger a particular behavior. Proximate causes are sometimes referred to as the “how” of behavior.
 - When thinking in “proximate” terms, we explore the underlying mechanisms of the behavior rather than the behavior’s role in maximizing reproductive success. For example, a proximate hypothesis related to territorial behavior would be, “The hormone testosterone triggers territorial behavior in male bluehead wrasse.” As another example, a proximate hypothesis related to the behavior of juvenile fish darting into crevices would be, “The sight of larger fish less than two meters away causes juvenile fish to dart into crevices.”
 - The development of a particular behavior is the result of interactions between genes and the environment. “Environment” includes both the body’s internal environment (e.g. hormones) as well as the external environment (both abiotic and biotic factors, including social interactions).
 - Like all hypotheses, both ultimate and proximate hypotheses must be testable and falsifiable.
3. Phenotypically plastic characteristics and behaviors are those that are strongly influenced by environmental factors.
 - A classic example of phenotypic plasticity was observed in the spade foot toad, *Spea multiplicata*. Tadpoles of this species that fed on fairy shrimp and cannibalized other tadpoles developed relatively large mouths and powerful jaw muscles suited to a carnivorous lifestyle, while those that fed on detritus and algae developed comparatively smaller mouths and jaw muscles (Pfennig, 1992).
 - Traits that we might consider to be only minimally affected by the environment, such as an individual’s sex, actually exhibit considerable plasticity in many animal species.

Mating Systems

1. A “mating system” refers to who mates with whom within a population of animals. This is not always easy to determine! In monogamous mating systems, males and females each have a single partner. In polygynous mating systems, at least some males mate with at least two females. Finally, in polyandrous mating systems, at least some females mate with at least two males. Polygyny and polyandry may occur simultaneously in some species, including the bluehead wrasse.
2. Determination of mates in polygynous mating systems often involves male-male competition and female choice.
3. In resource-defense polygyny, as occurs for the bluehead wrasse, males hold and defend territories that contain one or more resources sought by females.
 - The reproductive success of territory-holding males depends upon the extent to which they can defend against other males and control access to the resource.
 - The level of female choice may affect the reproductive success of territorial males.

Background

The bluehead wrasse, *Thalassoma bifasciatum*, is a coral reef fish found throughout the Atlantic and Caribbean Oceans. This species is fairly common, and the large, terminal-phase males, with their prominent blue heads set off by vertical black and white stripes, are easily recognizable. The small males and the females exhibit a less conspicuous initial-phase coloration, horizontally striped in yellow, black, and white, and sometimes with dark vertical bars. At approximately noon each day during the mating season, bluehead wrasses of both sexes and all manner of stripes temporarily forego foraging for a bout of mating close to the downcurrent end of the reef. This location is ideal because the eggs can be swept away from the reef and associated predators, and also allows for effective dispersal of the offspring. The terminal-phase males establish and defend distinct mating territories that are visited by several females each day. Within each territory, the terminal-phase male and one female at a time dart quickly upward into the water column, their bellies coming into close contact as they release their sperm and eggs nearly simultaneously. Small initial-phase males singly and collectively attempt to sneak in and release their sperm in the vicinity of the mating pair, so that some of the small males' sperm may fertilize the female's eggs. The females may also participate in group spawns with large groups of initial-phase males, a behavior much more common on larger reefs.

To complicate matters further, fish of this species are not fated to become male or female based on sex chromosomes during early development; rather, larval bluehead wrasse that settle onto a reef may initially develop either into small males (referred to as primary or initial-phase males) or into females (Munday, 2006; Fig. 1). The large, blue-headed males (i.e. terminal-phase males) are generally the result of sex reversal by a large, dominant female, although large, primary males may occasionally shift from the striped initial-phase coloration to the blue-headed, terminal-phase coloration (Munday, 2006; Fig. 1). Such flexibility in gender and behavior are examples of phenotypic plasticity, wherein the phenotype that develops is strongly influenced by the internal and/or external environments. In the bluehead wrasse, social interactions appear to be the strongest drivers of sexual development both early and later in the wrasse's life (Munday, 2006; Warner and Swearer, 1991).

Intriguingly, relatively few primary males (0-20% of initial-phase individuals) are found on small reefs; instead, virtually all of the newly settled fish become females, and males are almost all of the large, terminal-phase type. In contrast, on intermediately sized reefs, approximately 15-30% of newly settled fish become initial-phase males, and on the largest reefs, up to 50% of the newly settled fish become initial-phase males (Warner, 1984).

In this lab, you will explore:

1. The ultimate causes of sex determination and sex reversal.
 - What is the adaptive value, for an individual fish, of almost always initially becoming a female if it settles on a small reef, but having a greater possibility of becoming a male if it settles on a larger reef? In other words, can the difference in proportion of primary males on small reefs compared to larger reefs be explained by principles of natural selection?
 - What is the adaptive value of sex reversal for females that transform into males when they are relatively large?
2. The proximate causes of sex determination and sex reversal.
 - Which environmental conditions would make it more likely that a newly settled fish initially becomes a male?
 - Which environmental conditions could trigger female-to-male sex reversal later in life?
3. The role of female choice.
 - Does female choice affect the fitness of different male types (initial-phase versus terminal-phase)?

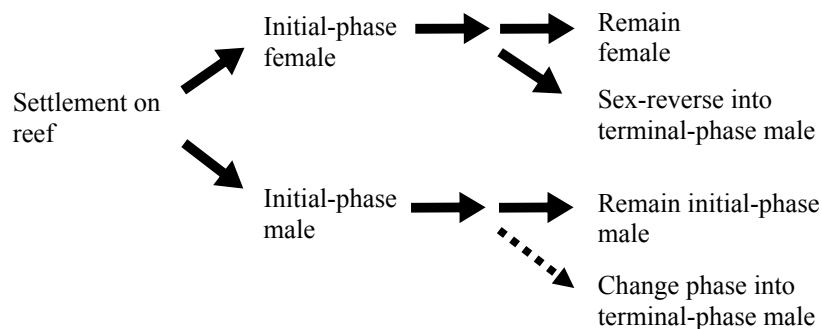


Figure 1. Phenotypic plasticity in the bluehead wrasse. The divergent arrows indicate the potential of individual fish to become one of two different phenotypes after initial settlement, and again, later in life. The dashed arrow indicates that phase change by initial-phase males is possible but uncommon.

Methods

Overview

You will participate in role-play simulations of the mating system of the bluehead wrasse. As a class, we will simulate mating on differently sized reefs, as well as with and without female choice. After our simulations, you will calculate and compare the reproductive success of the three phenotypes (initial-phase [IP] males, terminal-phase [TP] males, and females) for each scenario and answer related discussion questions. Finally, you will develop hypotheses related to 1) the differences in the proportions of initial sex ratios observed on differently sized reefs, and 2) the occurrence and timing of female-to-male sex reversal. **Be sure to wear footwear appropriate for walking and running (e.g. gym shoes or running shoes).**

General Rules

1. All individuals will be assigned a role. Your gender and size will be assigned to you for each scenario, and may change between scenarios.
2. You will be provided with “gametes” appropriate to your role. Females will receive large, plastic eggs. Your instructor will tell you how many actual eggs each plastic egg represents. Males will receive beans or peas of a particular type that will be considered “sperm”. For simplicity, all IP males will have one type of sperm and all TP males will have a different type of sperm unless otherwise instructed. Your role is to fertilize or be fertilized according to the particular rules of each scenario (see next section). TP males will be identified by blue hats.
3. You will be assigned a set of behaviors and restrictions in accordance with your role. These are meant to accurately reflect the advantages that one type of individual has over another in a particular mating system on a particular type of reef. For example, in the more territorial system on the small reef, all females will remain inside the territory during the designated mating period.
4. Your instructor will point out the territory boundaries and access points. Each scenario will have a territory set-up that simulates the particular reef size and population density.
5. At the end of each scenario, the contents of all the females’ plastic eggs will be consolidated and labeled with the scenario number. We will count gametes and calculate reproductive success once all scenarios are complete.
6. You will assist in sweeping up the “wasted” gametes as needed between scenarios and after all simulations have been run.

Scenarios

1. A system of TP male territories on a small reef (Fig. 2a); female choice occurs.

- STARTING SET-UP: TP males and females within designated territories, IP males outside of territories.
- Territories are relatively small, with only one entryway to access each territory.
- Each TP male guards his own territory, chasing away IP males. He also mates (i.e. provides “sperm”) to females in his territory as quickly as possible! A TP male should not enter another TP male’s territory.
- IP males attempt to gain access to females through the designated entryway. Their goal is to mate with any and all females within a territory while avoiding being chased away by the TP male. Any IP male may attempt to access the territory of any TP male.
- A chase is simulated by a shoulder touch by the TP male to the IP male, or if the IP males have been equipped with flag football belts, by the TP male detaching at least one flag of the IP male.
 - If the TP male successfully chases the IP male (i.e. with a shoulder touch or flag pull) the IP male must move to one of the designated penalty locations outside the territory (and replace his flag if applicable) before returning to any mating territory.
- Female choice: females must close their egg containers when not mating with the blue-headed male.
- Mating is finished within a territory when either the females’ eggs have all been fertilized (as simulated by a full egg container) or once all of the dominant male’s sperm is depleted.

2. A system of TP male territories on a small reef, no female choice.

- Same rules as Scenario 1 except:
 - Females must keep their eggs uncovered at all times.
 - A female must not seek out a particular male type, but should freely mate with any male who approaches her.
 - However, a female must stop mating with an IP male once the TP male executes a successful chase against the IP male. The IP male must move to one of the designated penalty locations outside the territory (and replace his flag if applicable) before returning to any mating territory, just as in Scenario 1.

3. An intermediately sized reef where females travel to the ideal spawning site occupied by TP males (Fig. 2b); female choice occurs.

- **STARTING SET-UP:** The TP males set up their territories at the designated downcurrent end of the reef. The IP males initially locate themselves just upcurrent of the TP male territories but can move downcurrent if that becomes more effective. Females start at the upcurrent end of the reef and travel to the downcurrent end of the reef once the simulation begins.
- There are no strict boundaries for the TP male territories, so IP males can approach a female from any angle.
- TP males may move to intercept and defend against mating by IP males. The rules for the chase, and penalties for a successful chase, are the same as in the first two scenarios, except that the penalty locations may have changed.
- **Female choice:** Females must close their containers except when mating with the TP male at/near the downcurrent end of the reef.
- Mating is finished when either the females' eggs have all been fertilized (as simulated by a full egg container) or once all the dominant males' sperm is depleted.

4. An intermediately sized reef where females travel to the ideal spawning site occupied by TP males; no female choice.

- Same rules as Scenario 3 except:
 - Females must keep their eggs uncovered at all times.
 - A female should not seek out particular males. Her goal is to mate with any male at/near the downcurrent end of the reef.

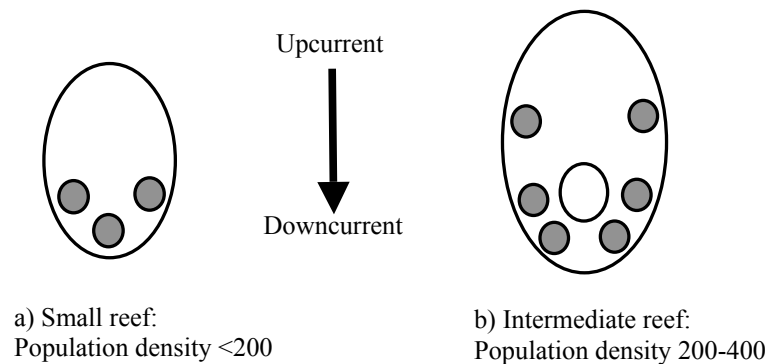


Figure 2. Arrangement of spawning territories on small reefs compared to intermediate reefs. Filled circles indicate terminal-phase male territories, while the larger, open circle indicates the group-spawning site for initial-phase males (after Warner, 1984).

Results/Data analysis

Instructions and Calculations

- After running all scenarios, we will count the sperm from IP males and sperm from TP males separately for each scenario. Sort first and then count each type.
 - If each male had his own characteristic sperm, separate and count accordingly so that individual reproductive success may be calculated.
- Class data will be entered onto the master data table (Table 1).
- You will calculate the average reproductive success (RS) for each phenotype (female, IP male, TP male). You need to develop the formulas for these calculations!
 - NOTE: If each male had his own characteristic sperm, you should calculate individual reproductive success as well as averages and standard deviations.
- Once all calculations are complete, you will answer the discussion questions (separate page) and then we will regroup for a closing discussion.

Table 1. Data and Calculations.

Data									Calculations		
Scenario 1, 2, 3, 4	Reef size	Female choice?	# of females	Eggs, total #	# of IP males	# of TP males	IP sperm, total #	TP sperm, total #	Ave RS, female	Ave RS, IP male	Ave RS TP male

Discussion Questions (answer on separate paper):

- Based on our data, for which reef size were the initial-phase males most reproductively successful and why (from a proximate perspective)? Consider only the variable of reef size without confounding the results with female choice. (HINT: Compare particular pairs of trials rather than all four together.) Also, if the results were not what you expected, speculate as to why not.
- Define “phenotypic plasticity” and provide an example from this lab.
- For the simulation in which the initial-phase males performed the poorest, would they have been better off if they had initially become females? Explain your answer based on our data.
- Based on our data, did female choice improve or decrease the reproductive success of initial-phase males? Explain these results from a proximate perspective. Also, if the results were not what you expected, speculate as to why not.
- Based on our data, which appeared to affect the reproductive success of initial-phase males more, reef size or female choice?
- Based on our data, should a female ever reverse sex? If so, which environmental condition(s) would be likely to trigger this reversal and how could reversal improve reproductive success?
- Given the potential to improve reproductive success via sex reversal, why don’t more females do so?

Based on our results, develop several hypotheses related to sex determination on reefs. Make sure that the independent and dependent variables for your hypotheses are clearly presented.

8. Develop an ultimate hypothesis for why IP males are not found on small reefs.
9. Develop a proximate hypothesis related to environmental triggers for initial sex determination.
10. Develop an ultimate hypothesis for why some of the larger females sex-reverse into males.
11. Develop a proximate hypothesis related to environmental triggers for female-to-male sex reversal later in life.

Literature Cited

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- Warner, R. R. 1984. Mating behavior and hermaphroditism in coral reef fishes. *American Scientist*, 72: 128-136.
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Materials

These numbers assume a class of 25 students, with the assigned roles of 10 females, 12 initial-phase males, and 3 terminal-phase males. The “per individual” numbers are also provided if you wish to change the ratios of fish types or have a different number of students.

Required

1. Plastic fillable Easter eggs, approximately 75 mm height and 50 mm diameter.
 - Quantity = 10 eggs; one per female.
 - These are widely available at grocery stores approximately a month before Easter. Alternately, they are available online through several suppliers (e.g. Amazon.com).
2. Dark-colored beans (e.g. black beans) for terminal-phase males. These must be the same size as the light-colored beans for initial-phase males (see next item).
 - Quantity = 4 kg. This is enough to fill 12, 0.5 liter (1 pint) plastic bags with 0.33 liters (1.5 cups) of beans, one per terminal-phase male for each of four trials.
 - If you would like to determine the reproductive success of individual males, you will need three different (and easily distinguishable) varieties of beans (one type per male) of approximately the same size.
 - If you are able to run the simulation outdoors, beans could be substituted for sunflower seeds or another or another food item that is non-toxic to local wildlife, using different varieties to distinguish between the different male types.
3. Light-colored beans for initial-phase males. These must be the same size as the dark-colored beans terminal phase males.
 - Quantity = 16 kg. This is enough to fill 48, 0.5 liter (1 pint) plastic bags with 0.33 liters (1.5 cups) of beans, one per initial-phase male for each of four trials.
 - If you would like to determine the reproductive success of individual males, you will need 12 different (and easily distinguishable) varieties of beans (one type per male) of approximately the same size.
4. Half-liter (1 pint) plastic bags (60) for gametes
5. 4 liter (1 gallon) plastic bags (60) labeled “Scenario 1”, “Scenario 2”, “Scenario 3”, and “Scenario 4”, for collecting the gametes after each trial.
6. Blue party hats for TP males (3).
7. Several brooms and dustpans for sweeping up wayward gametes.

8. Computers or calculators.
 - I generally have students use Microsoft Excel™ to calculate the fitness values. The calculations can be done more quickly than by hand and gives students the opportunity to work with spreadsheets.

9. Safety glasses (25) to prevent eye injury from bean projectiles.
10. Footwear appropriate for walking and running (e.g. gym shoes or running shoes) should be worn by all students.

Optional

1. Chairs with flagging tape or traffic cones (for use as barriers).
2. Flag football belts. Available online through several suppliers (e.g. Amazon.com). Cost is approximately \$15.00 for 10 sets of belts with flags.
3. Balances (four to eight) if you wish to weigh rather than count the beans.

NOTE: One liter of dry beans weighs approximately 1 kg. (English measurements: One cup of dry beans weighs approximately 0.5 pounds.)

Notes for the Instructor

Overview

This lab is not for the faint-of-heart instructor. During the time the scenarios are in motion, the students are fully engaged in chaotic mating frenzies, with “sperm” and “eggs” strewn everywhere, not unlike what occurs in wrasse mating on a real reef. Even the most introverted students seem to abandon their inhibitions. Yet, after all of the gametes are counted and the calculations are completed, you will hopefully find that this lab is effective at helping your students define and understand concepts related to fitness, proximate and ultimate hypotheses, phenotypic plasticity, and the role of female choice in mating systems that appear to be dominated by male-male competition. As a bonus, students truly enjoy this lab. I have successfully implemented this lab at all levels, from lower-division Principles of Biology courses to an upper-division Animal Behavior course, and it would also be appropriate for courses in Ecology or Evolution. Students readily relate to this lab, and I find that the lower-division students are often able to grasp the more advanced concepts.

Additional Background

This lab is based on the work of Warner (1984), Warner and Swearer (1991) and Munday et al. (2006). Warner (1984) used a comparative approach to explore ultimate hypotheses for hermaphroditism, focusing on coral reef fish. He exam-

ined the size advantage model of sex-reversal, wherein initial gender determination and timing of sex reversal later in life serve to maximize lifetime reproductive success. This model suggests that mating systems in which larger males are able to monopolize females, such as when large males hold territories with resources the females seek, favor female-to-male sex reversal later in life. In contrast, random pairing may favor male-to-female sex reversal since a larger female can produce more eggs, whereas even small males are presumed (in this model) to produce plenty of sperm. (This assumption of unlimited sperm may not be valid for males with relatively high mating frequencies or those subject to high levels of sperm competition [Judson, 2002]). For monogamous pairs, the model predicts that each member of the pair will maximize its reproductive success if the larger member of the pair is female, again assuming that egg production is more limited by animal size than is sperm production.

For bluehead wrasse on small reefs, males are able to dominate the ideal spawning sites at the downcurrent end of the reef, and low population densities allow the large males to effectively defend distinct mating territories and prevent intruding initial-phase males from successfully interrupting their mating events (Warner, 1984). In contrast, while the terminal-phase males still dominate the downcurrent end of the intermediately sized reefs, their territories are somewhat less defined, and the initial-phase males aggregate in group spawning sites just a bit upcurrent from the large male territories (Warner, 1984). The combination of females actively participating in group spawns, along with the greater difficulty of territory defense due to the larger reef size and higher population density, apparently gives the initial-phase males a greater chance of success. Our scenarios roughly mimic the small and intermediately sized reefs described by Warner (1984), except that Scenarios 1 and 2 have a stricter harem system than actually displayed by this species.

Proximate causes of initial gender determination and sex reversal in the bluehead wrasse appear to be socially mediated. Munday et al. (2006) found that newly settled bluehead wrasse collected from reefs and reared in isolation almost always became females, whereas when reared in groups of three, one of the new settlers would usually develop into a male. Similarly, Warner and Swearer (1991) demonstrated that female-to-male sex reversal in this species may be triggered by removal of males from local populations. The simulations in this lab should lead students to consider the role of population density and the social environment in influencing sex determination and sex reversal. Note that larger reefs also have larger population sizes. During mating, all the bluehead wrasse crowd into the downcurrent region of the reef, creating a temporarily elevated population density that is more pronounced on larger reefs. The simulation for the intermediately sized reef is set up in a way that mimics this higher population density.

When running this lab for an upper-division course, I have students read the related papers (or at least the Warner [1984] paper) only after they have completed the lab. This

allows them to develop their own ideas about the proximate and ultimate hypotheses for sex determination and sex reversal in the bluehead wrasse before examining the ideas and results of experts in this field. For further reading on the evolutionary basis of mating systems, including a myriad of excellent examples, I highly recommend *Dr. Tatiana's Sex Advice to All Creation* (Judson, 2002). Other concepts covered in this lab can be found in an animal behavior textbook (e.g. Alcock, 2013).

Logistics

Preparation and Clean-up of "Gametes"

It generally takes less than 30 minutes (per class of 25) to prepare the bags of beans. Also, the beans that end up in the fillable plastic eggs are sorted by the students and can be collected for the next time you run the lab. The swept-up beans may take 1 hour or more to sort for re-use, depending upon the vigor of your spawning events. As described in the "Materials" section, one possible alternative is to run the simulations outdoors and replace the beans with sunflower seeds or another food type that is suitable for the lab and non-toxic to local wildlife, using different varieties to distinguish between the different male types. The different varieties must be the same size; otherwise, the reproductive success will be overestimated for the male type with smaller gametes.

I generally run this lab with just one gamete type for each phenotype (see Materials section), but for more advanced courses, keeping track of gametes for individual males would help students understand the high level of variability in reproductive success of individual males in this type of mating system. Similarly, females could be of different sizes, with larger females having greater numbers of eggs as occurs for most fish. Even if you do not actually run the scenarios in this more complex way, individual variability is an important topic for discussion.

Territory Set-up, Sex Ratios and Chases

The most critical part of the set-up is selecting the appropriate boundaries and rules for each scenario. For Scenarios 1 and 2, where students simulate the small reef on which terminal-phase males control the prime downcurrent sites, I generally create territories of approximately 1.5 m by 2 m, with access blocked on three of the four sides of the territory so that initial-phase males can only enter the territory on one of the 1.5 m sides. It is worth experimenting with larger territories to allow more movement; the key is to make it relatively difficult for the initial-phase males to enter and mate with the females. You could use the lab benches to simulate natural barriers (a.k.a. "coral heads"), or if you are conducting the simulation outside, you could place chairs with flagging tape or use cones to limit entry. Also, you need to designate a penalty location approximately 3 m from each territory entrance for the tagged initial-phase males. In Scenarios 1 and 2, be sure to begin with the females and terminal

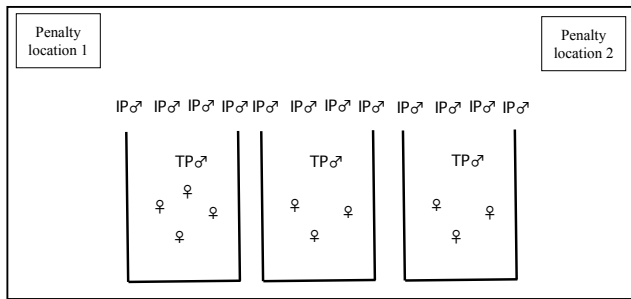


Figure 3. Scenarios 1 and 2: Territory organization and starting positions for each phenotype.

phase males already in designated territories and the initial-phase males outside all territories (Fig. 3).

For Scenarios 3 and 4, a long hallway or an elongated section of floor in the lab (approximately 2.5 m by 6 m) serves as the reef. One end is designated the downcurrent end and includes the somewhat fluid territories of the terminal phase males (organized similarly to Fig. 2b but without the two most upcurrent territories). The terminal phase males should begin these two scenarios in their territories while the initial-phase males should begin in a group just upcurrent of the terminal-phase males. The females begin at the upcurrent end of the reef and move to the downcurrent end. The penalty location should be approximately 3 m from the downcurrent end of the reef. Alternately, since all of the action is at the downcurrent end of the reef (Fig. 4), the penalty area could be located at the upcurrent end of the reef.

The numbers of females, initial-phase males and terminal-phase males will affect the outcomes of the scenarios. For consistency, I run all trials with approximately the same fish ratios. For a class of 25 students, there would be 3 terminal-phase males (identified with blue hats), 10 females (divided into three groups of three to four fish for the small reef trials but merely identified as “female” in the large-reef trials), and 12 initial-phase males. All roles are randomly assigned and not based on one’s human sex. I usually have students keep the same role for Scenarios 1 and 2, and then switch to a different role for Scenarios 3 and 4. For smaller classes, there must be at least two females per terminal phase male, otherwise the reproductive success of each female will be as high or higher than any male, which is not a realistic result for this mating system. (See Appendix A, which includes both ratios and actual results for classes of varying sizes.)

The rules for what a “chase” actually is must be made clear (see Scenarios section), and I have been pleasantly surprised to find that students really do stick to the rules. Students are allowed to move quickly, but the only physical contact they are allowed is the shoulder touch or flag pull, depending upon the selected chase method. The tagged initial-phase male must then go to the penalty location before returning to a territory.

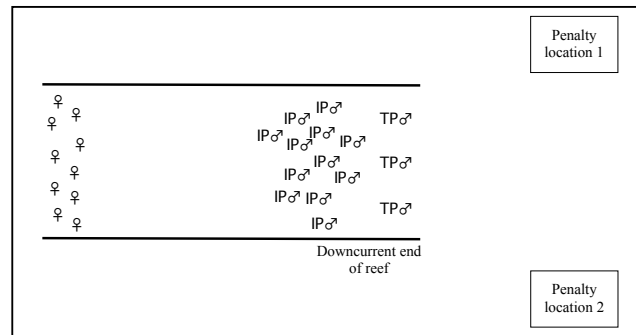


Figure 4. Scenarios 3 and 4: Territory organization and starting positions for each phenotype.

In the flag football belt version, initial-phase males must get a new flag at the penalty location. While the shoulder touch method is a bit easier to run, depending upon the make-up of your student population and whether you are conducting this lab during flu season, you may elect to use the flag football belt method. While I have mostly run this lab with the shoulder touch method, I tested the flag football belts with several lab sections and they worked well.

Other Considerations in Running the Lab

It is essential that students read this lab prior to coming to class. However, I generally review the concepts and background material in the student outline before assigning roles and describing the territory boundaries. You might consider having the students develop hypotheses and/or predictions for each of the scenarios prior to running the simulations; however, I do not do this myself because I am concerned this will cause students to skew their behavior during the scenario, consciously or subconsciously, towards (or perhaps contrary to) the expected results.

As you run the simulations, be sure to deposit the contents of the eggs for each scenario into the appropriately labeled bag. All students should participate in sweeping up the gametes prior to moving to the next scenario. When all scenarios are completed, students divide into groups, with each group counting the different gamete types for a particular scenario. Take care to make sure that gametes from different scenarios do not get mixed together prior to or during the counting process!

After counting the gametes, results for all scenarios are posted to a master spreadsheet, and the students then calculate the reproductive success (see *Calculations* section below). I require advanced students to develop the formulas for the data table (Table 1) themselves, whereas I provide more guidance to students in introductory labs. Once students have completed their calculations, we regroup so they can check their values and proceed to answering the discussion questions. All students should be able to answer Discussion Questions 1-7, whereas more advanced students should be

challenged to develop proximate and ultimate hypotheses guided by Discussion Questions 8-11.

I evaluate answers to the discussion questions based on whether the objective portion of each answer is consistent with the class data, and whether the explanations are logical and do not contradict the concepts or life history information presented. Additionally, I evaluate the hypotheses (Questions 8-11) based on whether the student has demonstrated the ability to distinguish between proximate and ultimate hypotheses and also whether each hypothesis has both an independent and a dependent variable. Sample answers to discussion questions are available upon written request to the author.

I usually have students initially work through the discussion questions and hypothesis development on their own or in student groups. If they become confused about questions related to proximate causes (e.g. questions asking about environmental stimuli or “triggers” for initial sex determination or sex reversal), have them think carefully about the social environment during the simulations and on a real reef, considering both the abundance/density of particular phenotypes during mating in the different scenarios as well as interactions among different fish phenotypes. I leave time (at least 20 minutes and ideally 30 minutes) to regroup and discuss the results, not only to make sure that students understand the results within the context of proximate and ultimate hypotheses, but also because this lab promotes discussion of a variety of topics related to sex determination and mating behavior.

Calculations

The calculations are based on the number (#) of successful sperm that made it into the eggs.

$$1. \text{ Average reproductive success per female} = \frac{\# \text{ of eggs}}{\# \text{ of females}}$$

$$2. \text{ Average reproductive success for IP males} = \frac{\# \text{ of IP sperm}}{\# \text{ total sperm} \times \# \text{ IP males}} \times \text{total \# of eggs}$$

$$3. \text{ Average reproductive success for TP males} = \frac{\# \text{ of TP sperm}}{\# \text{ total sperm} \times \# \text{ TP males}} \times \text{total \# of eggs}$$

4. If you keep track of individual males, then each male's success is:

$$\frac{\# \text{ of particular male's sperm}}{\# \text{ total sperm}} \times \text{total \# of eggs}$$

In general, the initial-phase males never do better than the females (Appendix A). However, the instructor should guide the students into recognizing that the relative reproductive success of initial phase males compared to females does change, with relatively greater success (closer to that of females) on the intermediate-sized reef than on the small reef.

Hopefully, students will be able to extrapolate from their own data that as reef size increases beyond what was possible in the simulation, the relative success of initial-phase males will increase to a point where they achieve reproductive success that is equal to or greater than the females. Thus, developing into an initial-phase male, rather than a female, after arrival on the reef may be adaptive on larger reefs. This is also a good time to discuss individual variability and how the range, rather than the average, could be important from an ultimate perspective.

If you don't obtain results that are similar to the expected results, possibly due to your boundaries, rules, and/or particular students, you should definitely discuss the differences between your simulations and the real reefs. You might then decide to have students work with the sample data (Appendix A) in order to answer the questions and develop their hypotheses.

Acknowledgments

I would like to thank the students in my Animal Behavior and Principles of Biology courses as well as the participants in my major workshop at ABLE 2013 who provided constructive feedback on the lab. I also owe a debt of gratitude to Ross and Kristi Turner, founders of Guided Discoveries and Catalina Island Marine Institute (CIMI), who encouraged a creative and active approach to teaching and learning science long before the concept of active learning became widespread. I created the first version of this lab for high school students at Guided Discovery's Catalina Sea Camp in the summer of 1987.

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Citing This Article

Haberman, K. L. 2014. Sex Determination and Mating Strategies of the Bluehead Wrasse, *Thalassoma bifasciatum*: A Role-play Simulation. Pages 90-102 in *Tested Studies for Laboratory Teaching*, Volume 35 (K. McMahon, Editor). Proceedings of the 35th Conference of the Association for Biology Laboratory Education (ABLE), 477 pages. <http://www.ableweb.org/volumes/vol-35/?art=6>

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Appendix A

Sample Results

Data									Calculations		
Scenario 1, 2, 3, 4	Reef size	Female choice?	# of females	Eggs, total #	# of IP males	# of TP males	IP sperm, total #	TP sperm, total #	Ave RS, female	Ave RS, IP male	Ave RS TP male
Lab Group A (12 students): Two TP males, 6 females (split into two groups of 3, each group with one TP male), and 4 IP males											
1	S	Y	6	300	4	2	90	1801	50	3.6	142.9
2	S	N	6	300	4	2	235	2090	50	7.6	134.8
3	I	Y	6	300	4	2	454	1467	50	17.7	114.5
4	I	N	6	300	4	2	856	2026	50	22.3	105.4
Lab Group B (16 students): Two TP males, 8 females (split into two groups of 4, each group with one TP male), and 6 IP males											
1	S	Y	8	400	6	2	17	2480	50	0.5	198.6
2	S	N	8	400	6	2	908	1465	50	25.5	123.5
3	I	Y	8	400	6	2	467	3223	50	8.4	174.7
4	I	N	8	400	6	2	1496	2080	50	27.9	116.3
Lab Group C (10 students): Two TP males, 4 females (split into two groups of 2, each group with one TP male), and 4 IP males											
1	S	Y	4	200	4	2	87	1267	50	3.2	93.6
2	S	N	4	200	4	2	449	1197	50	13.6	72.7
3	I	Y	4	200	4	2	585	1385	50	14.8	70.3
4	I	N	4	200	4	2	989	1639	50	18.8	62.4
Lab Group D (13 students): Two TP males, 6 females (split into groups of 3, each with one TP male), and 5 IP males											
1	S	Y	6	300	5	2	10	2138	50	0.3	149.3
2	S	N	6	300	5	2	316	1652	50	9.6	125.9
3	I	Y	6	300	5	2	551	1405	50	16.9	107.7
4	I	N	6	300	5	2	784	2017	50	16.8	108.0
Lab Group E (11 students): Two TP males, 5 females (split into groups of 3 and 2, each with one TP male), and 4 IP males											
1	S	Y	5	250	4	2	80	1509	50	3.1	118.7
2	S	N	5	250	4	2	252	2518	50	5.7	113.6
3	I	Y	5	250	4	2	167	2030	50	4.8	115.5
4	I	N	5	250	4	2	436	2246	50	10.2	104.7