Establishing size limits for coastal reef fish species of Funafuti, Tuvalu

Technical training manual

David J. Welch
C_2O Fisheries, February, 2022

C_2O Fisheries
Coasts | Climate | Oceans

Citing this document:

Welch, D.J. (2022) Establishing size limits for coastal reef fish species of Funafuti, Tuvalu: Technical training manual. Report to the Tuvalu Fisheries Department, C_2O Fisheries, Cairns, Australia. 41p.

Enquiries:

David Welch

C_2O Fisheries

Mob: +61 414 897 490

Email: d.welch@c2o.net.au

Web: www.c2ofisheries.net.au

Table of Contents

Report series

This manual represents Part 3 in a short report series produced through a consultancy to inform the processes for estimating size at maturity and establishing size limits for key coastal reef fish species of Funafuti, Tuvalu. The other reports in the series include: 1. Review of maturation and spawning seasonality for key species, and 2. Sampling program. These reports are intended as ongoing resources to guide Tuvalu Fisheries Department (TFD) staff in supporting implementation of the Funafuti Reef Fisheries Stewardship plan, and as outputs of the Pacific Islands Regional Oceanscape Program (PROP). A full glossary of terms used in this report series is found in this Part 3 training manual.

Glossary

Gonad - refers to the fish's reproductive organs.

Gonochorism – separate males and females during the lifetime.

Hermaphroditism - species that are capable of developing both male and female reproductive organs; can therefore change sex.

L_{50} – the estimated length by which 50% of the fish's population, on average, has reached maturity.

Life history – the full life cycle of a fish that includes all life stages.

L_m – the length at which the smallest individual of a fish population becomes mature, on average. N.B. this can be confused with other measures of maturity (e.g., L_{50}); this emphasises the need to accurately report the estimate derived.

Longevity – the length of a fish's lifetime, or their lifespan.

Maturity – the stage in life when a species is capable of breeding.

Metamorphosis – a biological process that involves the animal physically changing their body structure.

Milt – the fluid deriving from the testes in a mature male fish.

Natural mortality - refers to deaths of fish from all causes except fishing (e.g. ageing, predation, cannibalism, disease, etc.).

Ovary - refers to the female reproductive organs.

Ovipary – refers to egg-laying fish. These can be laid as demersal eggs (onto the ocean floor) or as pelagic eggs into the water column. The latter are termed **broadcast spawners**.

Protandrous - fish that mature first as a male and change sex later in life to a female. This is not common in coral reef fishes.

Protogynous - fish that mature first as a female and change sex later in life to a male. For coral reef fish this mode is found in parrotfish, groupers, wrasses and several species of emperors.

Recruitment – fish added to a fishable stock each year due to growth from the larval or juvenile stage, or fish migrating from elsewhere.

Settlement – the time that a fish change from using the pelagic habitat during their larval stage to using the demersal habitat they use as juveniles and/or adults.

Size class – a grouping of different sizes within upper and lower bounds. For example, for size classes of 2 cm, all fish of the length within the specified 2-cm groupings are included. That is for the size class 10-12 cm, all fish between 10 cm and 11.9 cm are included.

Testes - refers to the male reproductive organs.

Vivipary – refers to fish that give birth to live young. This mode is rare among finfish species and is used by most sharks and rays.

1. Introduction

The management of fisheries species relies on knowledge of key aspects of a fish's biology; including growth, maturity, natural mortality, reproduction and longevity (e.g., Jennings et al., 1998; Reynolds et al., 2005). These parameters can be highly variable within and among different species, but knowledge of these can significantly help fishery managers to understand the relative resilience of each species (and each stock) to fishing pressure, and to manage each species accordingly. For example, some species are able to withstand high fishing pressure and still be able to replenish and maintain their populations, while other less resilient species subjected to the same fishing pressure would collapse. Their level of resilience is determined by their life history characteristics. Therefore, being able to estimate key biological parameters for a species helps to then select management strategies that enable fishers to maximise how much they catch, while still allowing the species to naturally replenish itself. This is the key for fisheries managers in ensuring fisheries are sustainable.

1.1 Size at maturity and spawning seasonality

Two key biological parameters, that are both critical moments in the life cycle of a fish, are when they reach maturity and begin to breed (size and age), and the timing of when they come together to breed – their spawning season.

A key principle of fisheries sustainability is that, allowing a species to live long enough to reproduce at least once in its lifetime will help to ensure the maintenance of a healthy spawning stock biomass, and significantly decreases the likelihood of that stock being overfished (Myers and Mertz, 1998). For this to occur it is logical that a species should not be harvested before it has the opportunity to successfully breed. To achieve this a fisheries manager can use knowledge of the size at which a species reaches maturity to implement a minimum size of capture regulation (size limit); therefore, this size limit should be set above the estimated size at maturity.

Many factors contribute to the success of spawning, including environmental conditions, but for fisheries managers it is about ensuring spawning is not disrupted. Knowledge of the timing of when species spawn opens up a range of management options for fisheries managers (e.g., spatial and temporal closures) that can help to ensure successful breeding.

Therefore, knowledge of the size at maturity, and the spawning seasonality for a species, provides enough knowledge for a fisheries manager to implement management strategies that limits the impacts of fishing on a population for it to successfully breed each year, and thereby replenish its population (Myers and Mertz, 1998). These are two of the simplest life history characteristics that can inform relatively simple management strategies to ensure sustainable fishing.

1.2 Aim of this manual

Therefore, the content of this training manual focuses on education and training in: i) the life history and reproductive biology of fish, ii) the importance of reproductive data and how they can inform management decisions, iii) technical skills and knowledge for recording fish reproductive data, and iv) the analysis of these data to estimate size at maturity and spawning seasonality.

This manual therefore provides a resource to guide the collection of relevant data that informs management of coastal species in Tuvalu using size limits. A training video has also been developed that accompanies and complements this training manual, providing key visual elements of data collection. Although the video also serves as a stand-alone resource, it is **strongly recommended** that it is used in conjunction with this training manual. The manual also includes a data analysis section that provides the basis to analysis methods used to estimate size at maturity and spawning seasonality. The analysis section also provides step-by-step guides for conducting these analyses using simple and automated spreadsheets, that are also provided as accompanying tools to be used in conjunction with this training manual.

Overall, the aim of this manual is to provide the knowledge and skills for participants/students to accurately collect and use relevant data to enable estimation of size at maturity and spawning seasonality for key reef fish species.

2. Biology of key tropical reef finfish

2.1 Fish life cycles

Like all organisms, fish go through a number of stages during their lives as they complete their life cycle. Although the modes of reproduction among reef fish species varies (see section 2.3), the most common type of life cycle observed in marine coastal reef fish species can be illustrated by the diagram in Figure 1. Therefore, a typical fishes life cycle is comprised of the early life history stage (larvae), the juvenile stage and the adult stage.

Larvae hatch from fertilised eggs in the plankton and go through different developmental stages as they grow. Once developed enough, and when they locate suitable reef habitat, they go through a developmental transformation, or metamorphosis stage, and swim to the bottom and 'settle' on the reef as a juvenile. This process is called settlement. Juveniles spend a period of time living in their preferred juvenile habitat, which is usually reef flats, seagrass beds or mangrove forests. At a certain size, depending on the species and the conditions they experience as a juvenile, an individual fish will then mature as an adult that becomes capable of breeding. When the time is right, adults will come together to spawn, as a pair, a small group or sometimes as large aggregations of many fish, releasing sperm and eggs into the water column for the life cycle to continue (Figure 1).

Generally, there are several stages in a fish's life cycle, or life history, that are critical to the maintenance of populations and their overall productivity:

1. Larval stage.

Fish larvae hatch from eggs that are released into the water column and fertilised during spawning, and is the most vulnerable period in a fish's life. Following spawning, microscopic larvae drift away from reefs in the plankton where they develop over a period of between 8 and 120 days (most reef fish larvae develop over 20-30 days; Leis and McCormick, 2002), depending on the species. The mortality of larvae is extremely high and, depending on the prevailing environmental conditions during this larval development stage, can be 10-50% mortality per day. This is mainly due to predation but other factors such as temperature and salinity can also greatly affect growth and survival (Leis and McCormick, 2002; Hixon and Randall, 2019). Because conditions can be so highly variable from one season or year to another, survival of the larval

stage each season/year can also be highly variable. These good and bad 'recruitment' years are thought to be key drivers of the natural variability in fish population sizes and recruitment is often a good predictor of good and bad fishing years.

2. Size at maturity.

The size at which a fish becomes mature is a key life history stage as it is the time in their lives when they can begin to breed and can contribute to the replenishment of their populations. The size at maturity is highly variable among different species, and is also variable between individuals of the same species. During maturation, the gonads of the fish changes and the male or female tissue develop to become reproductively capable. That is, the fish becomes capable of breeding.

3. Spawning times.

The timing of spawning is a critical stage in a fish's life because it is when fish come together to breed, and usually occurs at least once per year. Each species has their own preferred timing of spawning which can range from one month to throughout the entire year. Not only does this vary among species, but also among latitudes for the same species. Generally, it is thought that spawning tends to be more extended where environmental conditions are least variable (Claydon et al., 2014), which tends to be at lower latitudes such as Tuvalu. Regardless, during the preferred spawning period all species tend to have a peak in spawning activity on or leading up to new and full moon phases (Hixon and Randall, 2019).

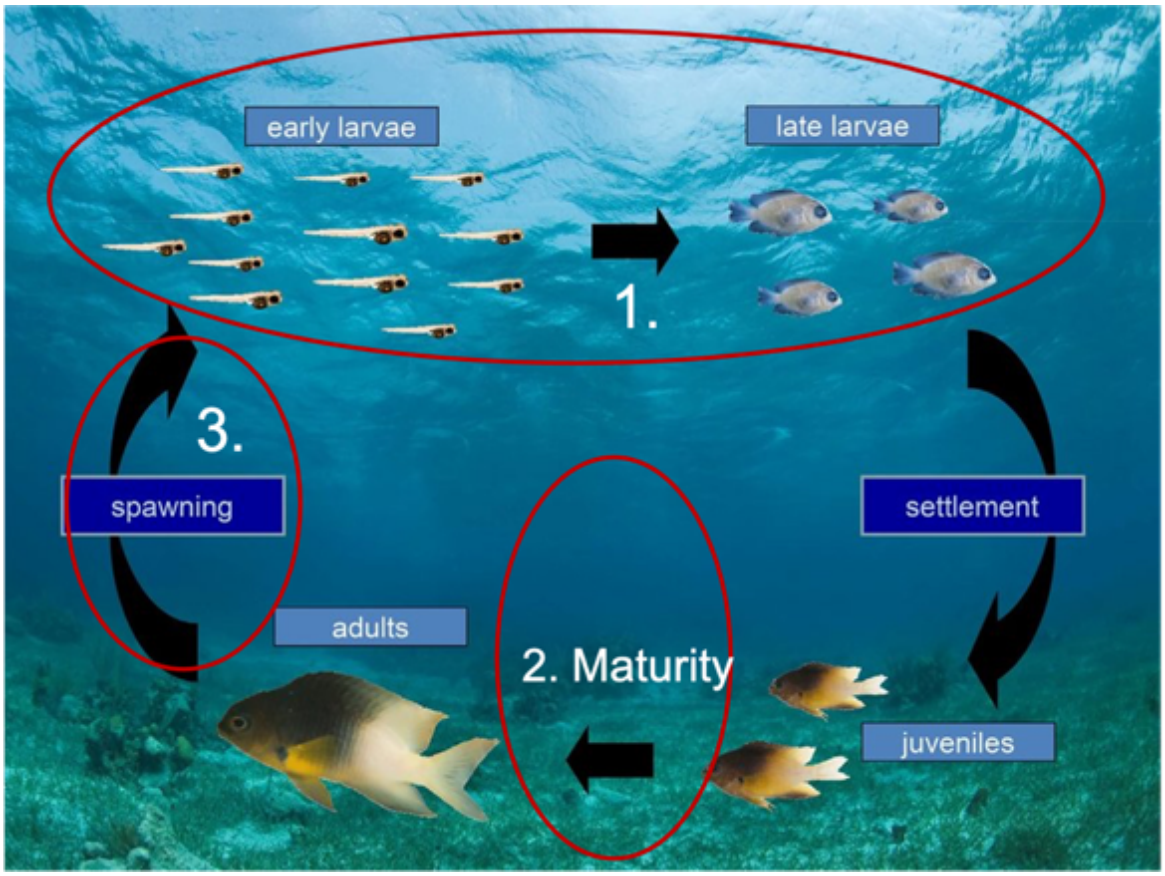


Figure 1. Representation of a typical coral reef fish life cycle showing three of the critical stages of development: 1. The larval stage, 2. Maturation, and 3. Spawning. Source: Hixon and Randall, 2019.

2.2 Productivity: variation in growth, maturity and spawning

Productivity is a term that describes how fast an animal can complete its life cycle; in other words, its population growth rate. The faster it can complete its life cycle the more rapidly it is able to recover from impacts such as fishing. Although there are some exceptions, generally:

High productivity species are those that produce many offspring, mature early in life, grow fast, and have a relatively short lifetime (longevity). Examples include Atule fakalaulau (Mackerel scad) and squid species.

Low productivity species are those that produce few offspring, mature late in life, grow relatively slowly, and may have a relatively long lifetime. Examples include Gatala pulepule (Camouflage grouper) and many shark species.

Therefore, high productivity species tend to be able to be fished harder than low productivity species. This is a key concept in understanding how fishing impacts different species, and therefore the different management requirements for different species (Hilborn and Walters, 1992). This concept also explains why in multi-species fisheries such as Tuvalu's coastal fisheries, that over time certain species tend to disappear from catches while other species continue to be common.

2.3 Finfish reproductive strategies

Reproductive modes

There are two main modes of reproduction in marine fishes: live bearers (vivipary) and egg-layers (ovipary). Live bearers rely on copulation (mating) and internal fertilisation of eggs, whereby young hatch and develop inside the parent for at least some period of their early development. Young are born live as free-swimming larvae or juveniles (Miller and Kendall, 2009). This mode tends to have relatively few young and is very rare among finfishes; this mode is used by most shark and ray species.

Egg layers either release eggs on to the reef floor (demersal egg layers) or into the pelagic water column (broadcast spawners). The vast majority of finfish are broadcast spawners with larvae largely at the mercy of ocean currents as they develop and disperse. Broadcast spawners tend to be highly fecund, with each female capable of producing from 10,000 to over a million eggs annually, depending on the species and their size (Hixon and Randall, 2019; Miller and Kendall, 2009). **All the key target reef finfish species of Tuvalu are broadcast spawners (see Part 1 report).**

Maturation and sexuality

Sexuality in fishes refers to gender differentiation and gender determination, and can be complex. Finfish can exhibit three modes of sexuality: bisexuality, unisexuality or hermaphroditism (Hixon and Randall, 2019; Miller and Kendall, 2009). Unisexuality, or parthenogenesis, refers to the development of young *without* fertilisation in some individual female live bearers, and is very rare in fishes and so won't be discussed further.

Bisexuality, or gonochorism, refers to the occurrence of separate males and females throughout a fish's life, and is by far the most common mode of sexuality in fish species. **Most of the key target reef finfish species of Tuvalu are gonochores** and includes species like trevallies, snapper and surgeonfishes.

Hermaphroditism refers to species that are capable of developing both male and female reproductive organs, and are therefore capable of producing both male gametes (sperm) and female gametes (eggs) during their lifetime. The vast majority of hermaphrodites in coral reef fish species are *sequential hermaphrodites*, meaning that they mature as one type early in life and change to the other type later in life (Hixon and Randall, 2019; Miller and Kendall, 2009).

Protandrous hermaphrodite: fish mature first as a male and change later in life to a female. This is not common in coral reef species, and is not evident in any of the priority target reef finfish species for Funafuti.

Protogynous hermaphrodite: fish mature first as a female and change later in life to a male. Although this mode is more common among coral reef fish species, for the key species targeted in Tuvalu, it is found almost exclusively in only three fish families:

- Parrotfish (Family Scaridae),
- Groupers (Family Serranidae), and
- Wrasses (Family Labridae).

Protogyny is also confirmed in six species of emperors (Family Lethrinidae), however none of these are in the priority (top 28) list of species for Funafuti. However, two of these species are in the top 45: *Lethrinus miniatus* and *L. variegatus* (Sadovy de Mitcheson and Liu, 2008). Therefore, of the priority finfish species for estimation of the size at maturity, 23 are gonochores (separate sexes) and five (4 groupers and 1 parrotfish) are protogynous hermaphrodites.

Video illustrating the complexity in fish reproductive strategies, using parrotfish as an example:

https://www.youtube.com/watch?v=FyTO-UHi-0c

Stages of maturation

For this training you will not be required to record the gonad maturation stage, therefore the required technical material is not included here. Despite this, it is useful that you understand the different stages of gonad development to facilitate more accurate data recording.

Maturation stages simply refers to the different levels, or extent, of gonad development in a fish. Once a fish becomes mature, it continually cycles through different stages of gonad development, often over the period of a year. These stages revolve around the timing of spawning for that species – their spawning season (Figure 2). During a fish's lifetime, they start life as immature individuals and are not capable of breeding. At a particular age and size, an individual fish's gonads will develop to become reproductively capable, either as a male or female, depending on their reproductive strategy. As a mature fish, their gonads will develop in size as they produce gametes (eggs and sperm) in preparation for spawning. Generally, there are four main stages of gonad development:

Developing – gonads are beginning to become enlarged and developed in preparation for spawning.

Spawning – gonads are in a 'ripe' condition with eggs and sperm fully developed and ready to be released as part of spawning. During this stage orange/pink eggs are visible in ovaries and white liquid milt (sperm) is readily squeezed from testes.

Post-spawning – gonads are obviously empty after releasing eggs and sperm following the spawning season, often appearing flaccid.

Inactive – gonads appear quite small and often indistinct. This is also referred to as the 'resting' phase in between periods of spawning.

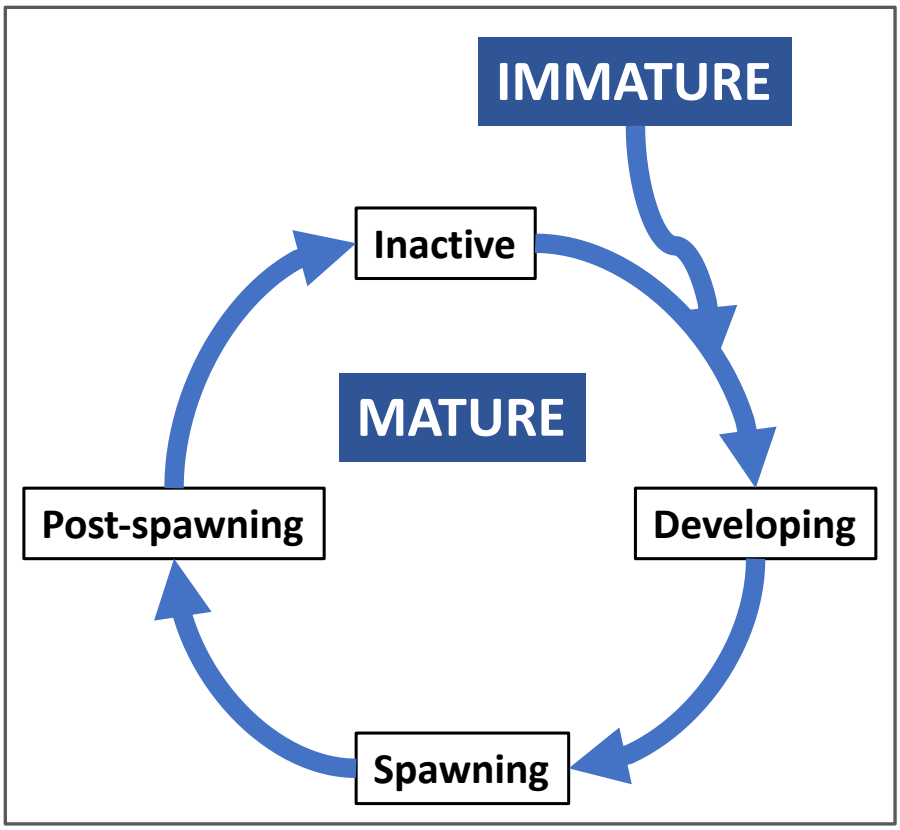


Figure 2. Diagrammatic representation of the different maturation stages in fish, showing that once a fish reaches maturity, gonads develop through different stages that are synchronised to their spawning season. Adapted from: Brown-Peterson et al. (2011).

During the non-spawning season when gonads are inactive, it can often be difficult to distinguish between an inactive or immature fish (Table 1). This is why the most reliable period to sample fish for maturity data is leading up to, or during, the spawning period when maturity is most obvious (i.e., developing or spawning stages) (Vitale et al., 2006).

Table 1. Generic description of male (testes) and female (ovaries) gonads for each of the different stages of maturation. See Table 2 for a description of male and female gonads.

Maturity/Stage		Description
IMMATURE		Small, often clear & thread-like. Blood vessels indistinct
MATURE	Developing	- Ovaries enlarged with blood vessels distinct. - Testes identifiable (see Table 2)
	Spawning	- Ovaries large with eggs visible, and blood vessels distinct. - Testes large, milt oozes out under pressure
	Post-spawning	- Ovaries flaccid, may have a dark red appearance. Blood vessels still distinct. - Testes reduced in size and no milt under pressure
	Inactive	- Ovaries small and blood vessels less obvious. Ovary wall may appear thickened. - Testes small and may be clear and threadlike

3. Data for estimating size at maturity and spawning seasonality

The collection of data that help to estimate the size at maturity for a species, and their spawning seasonality, although relatively simple, requires technical skills and knowledge. Firstly, it requires the skills and equipment needed for dissecting a fish. This section will go through different aspects of data collection including: i) the data to be recorded; ii) the required equipment; iii) technical skills for fish dissection, and iv) technical skills to help in the accurate recording of relevant reproductive data.

3.1 Overview of the data to be collected

Most of the data required are already collected as part of the Tuvalu Fisheries Department creel survey program, and so the additional data are not onerous and mainly relate to reproductive data collection. The creel survey data sheet template modified to include the required reproductive data is provided in the Part 2 Sampling program report. Below is a general description for each of these data fields as they relate to this training. These descriptions should be reviewed and understood to ensure accurate data recording.

Fish ID: each individual fish should be given a code or identifying number so that all data collected in the field and laboratory for each fish are correctly linked and recorded in the database.

Date of sample collection: this is required for data analysis to estimate the timing of spawning using the Gonadosomatic Index (GSI) (see data analysis section).

Location sample collected: this helps to understand any spatial patterns in size at maturity, spawning seasonality, and for any other use of the data.

Species: it is obviously critical that reproductive data are accurately assigned to the correct species. Several resources are useful for this (e.g., Moore and Colas, 2016).

Fork length: is measured from the tip of the nose, through the middle of the body, to the posterior mid-point of the tail. Generally, this is the most reliable length measurement because it is the easiest to measure accurately.

Total length: is measured to the longest length from the tip of the nose, through the middle of the body, typically to the back edge of the tail. It is useful to collect TL to generate a FL:TL conversion for each species so that length data can be readily converted if necessary, and can be compared with estimates from other studies if needed.

Fish total weight: this is required to estimate the timing of spawning using the Gonadosomatic Index (GSI) method. It can also be used to generate a Length:Weight relationship for each species. Weight should be recorded in grams.

Maturity: is data on whether the fish is mature OR immature, and is necessary for the data analysis to estimate the size at maturity. The fish is only mature if you can determine the fish's sex.

Sex: is data on whether the ***mature*** fish is female or male, and is necessary for the data analysis to estimate the size at maturity and spawning seasonality.

Gonad weight (GW): is the weight of the whole ***intact*** gonad for ***mature females only***, but can be either one lobe or two, depending on what is able to be extracted. Weight should be recorded in grams.

Lobes: is a record of how many gonad lobes were weighed.

3.2 Equipment

At least two people are recommended during fish dissections and data recording. Before beginning the process of fish dissections and data recording, certain equipment should be acquired and be ready for use (Figure 3). These are:

- Dissecting scissors
- Tweezers
- Scalpel if possible (or similar small sharp blade)
- Measuring board or ruler
- Scales for weight measurement (approximately 0.01 gram – 10 kg)
- Data sheet, and
- Pencil

Figure 3. Key pieces of equipment required for fish dissections.

3.3 Data collection before dissection

It is important to remember to record the basic data, including those requiring the whole fish, before beginning dissections and reproductive data collection. These data include fish ID, species, date, location, length (TL and FL recommended) and weight.

The first physical data collected on the fish should be the **total weight**, prior to any cutting of the fish. Ensure that the fish is intact and whole for weighing.

Figure 4. Photo of a Pinecone soldierfish (*Myripristis murdjan*) showing the correct way to measure both fork length (FL) and total length (TL).

3.4 Dissecting fish

Locating gonads

Before beginning to dissect a fish, it is important to have an understanding of the general layout of organs and tissues in the body cavity of different fish species. This is very important because identifying the gonad in a fish can sometimes be challenging, even for experienced practitioners. Therefore, knowing the general internal layout helps you to accurately locate the gonads and minimise the chances of damaging them during the dissection process. Importantly, there will always be a pair of gonads that sit symmetrically in the body cavity.

For most coral reef fish species, the gut sits in the base of the fish running forward from the vent (anus). The gonad also runs from the vent, usually attached by thin connective tissue, and sits above the gut and runs along the underside of the swim bladder (Figure 5).

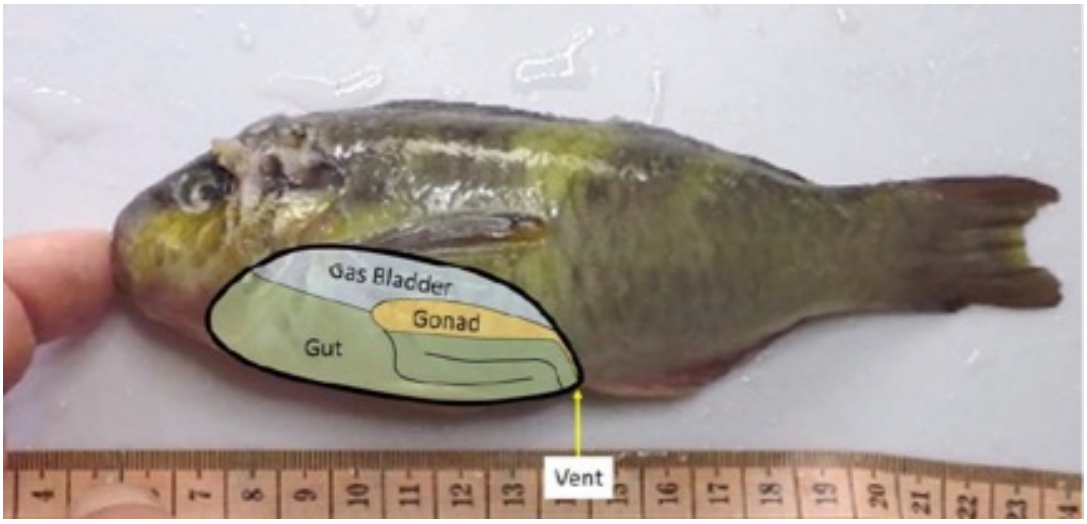


Figure 5. Generalised layout of internal organs in the body cavity of most coral reef fish. Source: Longenecker and Langston (2018).

Surgeonfish and unicornfish (Family Acanthuridae) have a modified body plan with the gonad located towards the back end of the body cavity, under the gut and generally running either side of the vent. The gonads usually sit in small cavities either side of the fish at the posterior end of the body cavity, and can be challenging to extract for weighing, so extra care is needed during dissections (Figure 6).

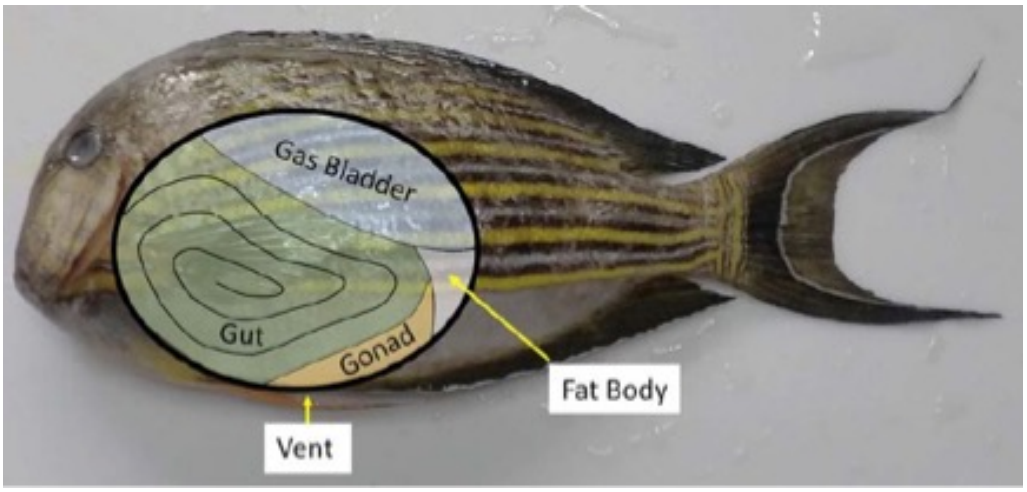


Figure 6. Modified layout of internal organs in the body cavity found in surgeonfishes and unicornfishes (Family Acanthuridae). Source: Longenecker and Langston (2018).

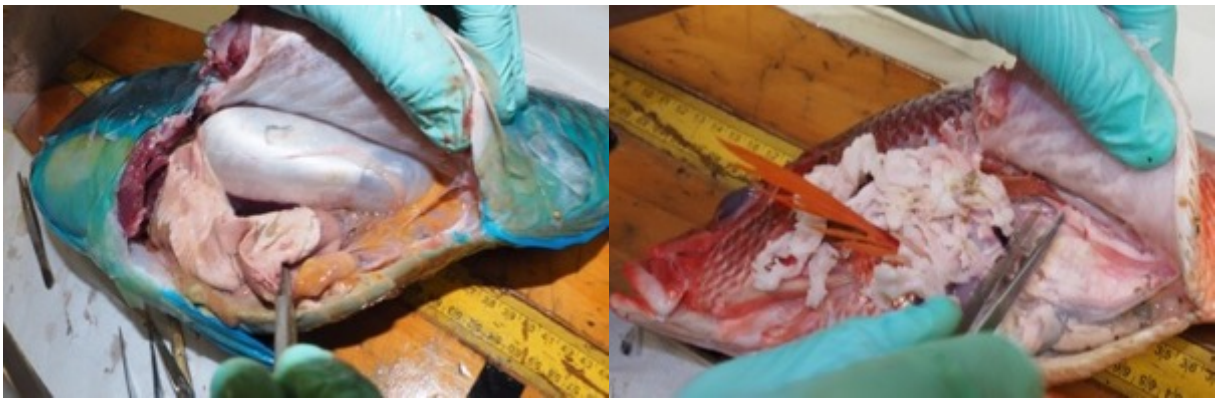
Opening the body cavity

There are several simple steps for opening up the fish's body cavity to locate and inspect the gonads:

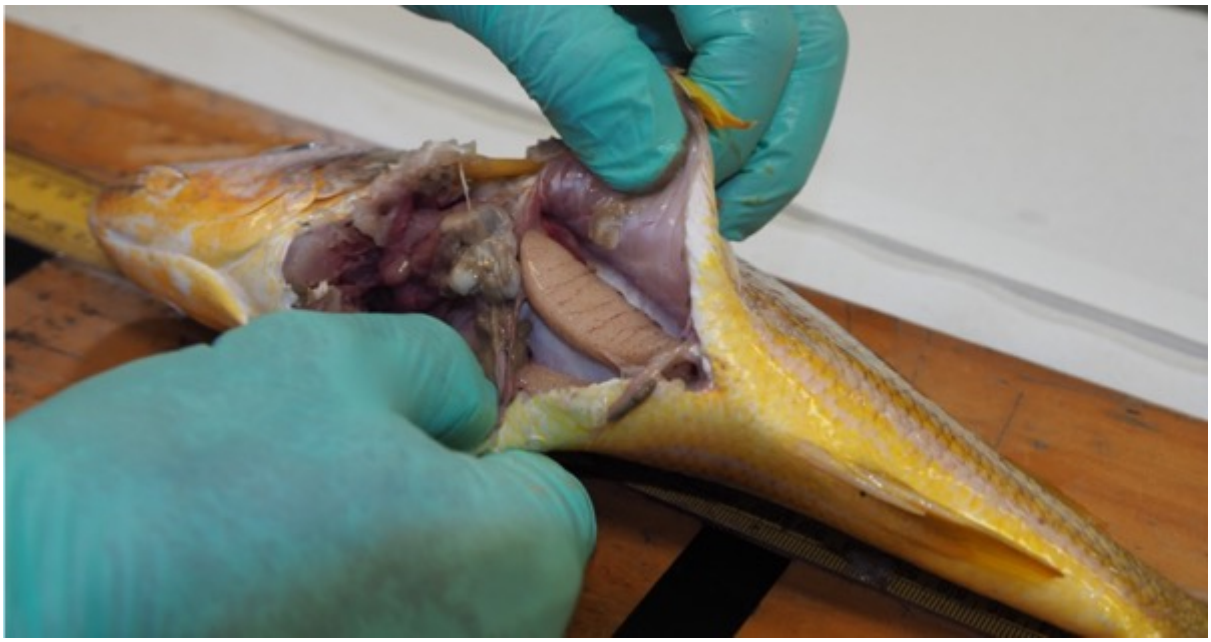
1. Using the dissecting scissors, a cut should be carefully made from the vent along the ventral surface to just behind the gills section and forward of the pelvic fins.

2. Next, cut upwards through the side of the belly flap and just behind the pectoral fin.

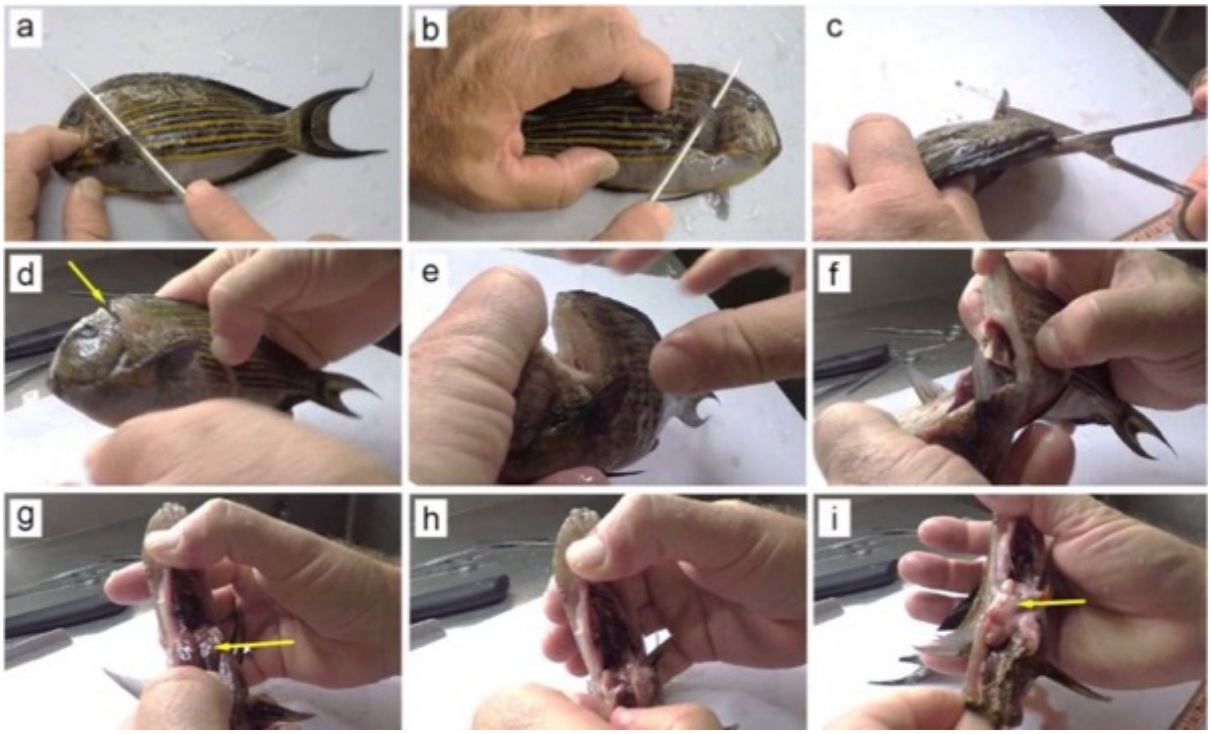
3. Make small cuts as needed until you can pull the belly flap away to expose the internal organs.
4. Use the tweezers to pull the gut forward towards the head, being careful not to damage the guts or the gonads sitting underneath.



5. Depending on the species, locate the gonads. These tend to be longer than one third of the length of the body cavity (see descriptions and images on following pages).



Because of the location of gonads in surgeonfish and unicornfish (Family Acanthuridae), an alternative dissection method is suggested by Longenecker and Langston (2018). You may need the scalpel or a sharp blade to make first cuts through the tough skin.



Sex determination in mature fish

If a fish is mature, there should be obvious indications that the fish is either a male or female, unless the gonad of a fish is in the inactive (resting) stage of maturation (Figure 2). **As a general rule, if you can determine the sex of the fish, it is mature**. If not, it is either immature or in an active stage (Figure 7).

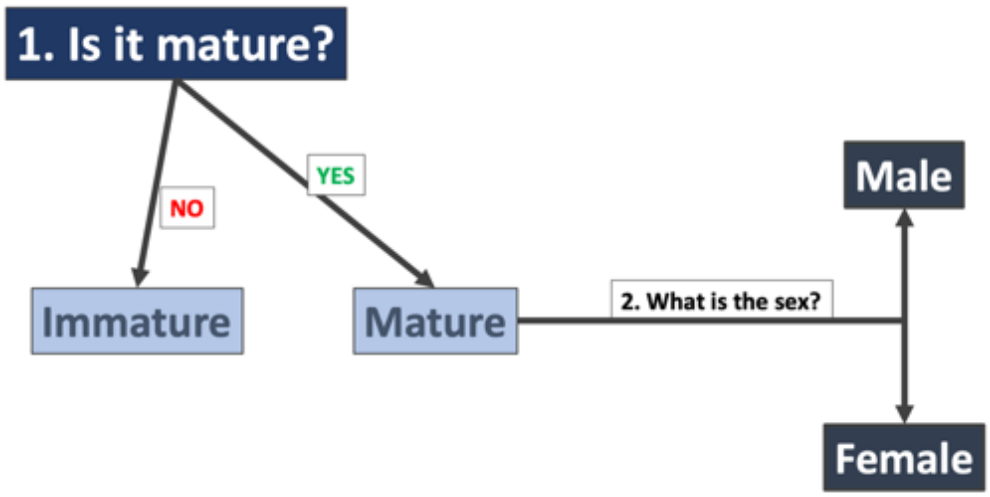
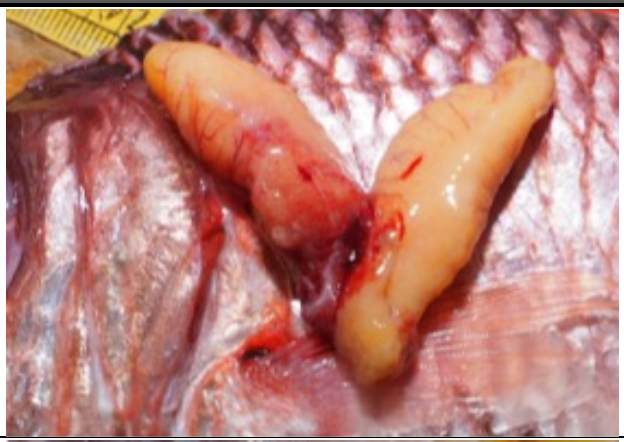
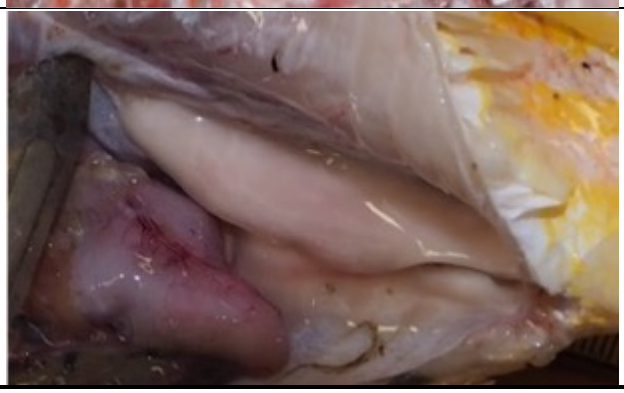


Figure 7. Simplified decision tree for interpreting gonads for reproductive data recording. If there is uncertainty at any step, no data should be recorded (i.e., don't guess!).

The general appearance of male and female gonads tends to be fairly recognisable (Table 2). Female gonads (ovaries) tend to be shaped like a sausage, are usually a shade of pink or orange, and have blood vessels that are usually obvious. Male gonads (testes) tend to be triangular shaped, are white in colour or a shade of white, and often appear very smooth and shiny (Table 2). However, if during examination of a fish you are not certain of the gender, **do not guess** – just leave the data field on the sheet blank. Several examples of mature male testes and mature female ovaries are given in the Appendices.

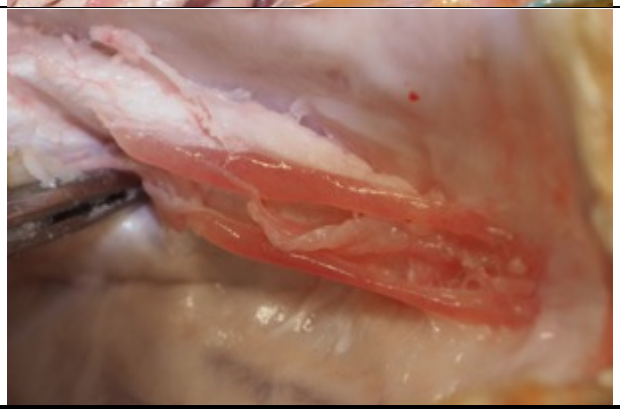
Table 2. Differences between mature male and female gonads. See Appendices for other examples of male and female gonads.

Gender	Description	Image
FEMALE	• Tend to be sausage, tubular or sack-like in shape • Are usually shades of orange or pink in colour • They often have obvious blood vessels	
MALE	• Roughly triangular in cross-section • Pale or whitish in colour • Often smooth and shiny in appearance	

Determination of maturity

Data on whether a fish is mature or immature is the key data for estimating size at maturity. As previously mentioned, making this assessment accurately can be challenging, especially if a mature fish's gonad is in the resting or inactive stage, because they may be difficult to distinguish to the inexperienced eye. The key difference is that an immature fish's gonad tends to be threadlike and clear in appearance, while inactive gonads tend to have a more thickened appearance (Table 3). Once again it is very important that, if you are not certain, you **do not guess** – just leave the data field on the sheet blank. Further, if you can confidently identify the gender of the fish, then it is NOT immature (Figure 7).

Table 3. General differences between immature and inactive (resting) gonads.

Maturation stage	Description	Image
IMMATURE	• Tend to be narrow and clear • Are often threadlike • Blood vessels are indistinct	
INACTIVE	• Small in size • Blood vessels may be evident • Often have signs of a thickened wall in ovaries	

4. Data analysis

The previous sections of this training manual, along with the sampling program, guide the collection of relevant data to be able to estimate the size at maturity and the spawning seasonality for key coastal fish species. The following sections describe the methods for analysis of data to obtain estimates of the size at maturity and for estimating a species spawning season. This section also discusses the need for periodic assessment of data collections, specifically to assess details about data types and the number of samples collected, to ensure there is sufficient data for robust outputs from data analyses. The sampling program should therefore be read in conjunction with this manual to guide the use of data for analyses. For these periodic data assessments to be as simple and efficient as possible, along with the preparation of data for analysis, is it is very important that all data are stored in a **common database** and can be readily accessed and queried as needed.

Key resources for the respective data analyses are the accompanying spreadsheet analysis tools that are mostly automated to provide the key results:

1. **Size at maturity analysis spreadsheet, and**
2. **Spawning seasonality analysis spreadsheet.**

4.1 Estimating size at maturity

This section briefly describes the statistical analysis method for estimating size at maturity, and details the steps for conducting the analysis using your own data and the '*Size at maturity analysis'* spreadsheet. This spreadsheet is developed to do all of the statistical work for you in estimating the size at maturity, making the analysis steps as simple as possible.

Statistical method

While there are several methods available for estimating the size at maturity in fishes, one of the simplest and most commonly used methods is to fit a model to data on the number of fish that are mature as a proportion of the total number, in relation to fish body size using different size classes. This readily enables the estimation of the proportion of the population that are mature based on the model that best fits the sampled data. For example, an estimate of the size at which 50% of the population are mature (L_{50}) can be predicted by the model (Figure 8).

Since the shape of maturity data is non-linear (it tends to be S-shaped), a logistic regression model is used (Chen and Paloheimo, 1994). In the example shown in Figure 8 below, the sampled (raw) data are presented as the proportion of fish that are mature in each size class, and are shown as the grey circles. During the data analysis, a logistic regression is mathematically fitted to the data as the best possible fit; shown as the blue line. This model is 'averaged' across the sampled data to provide a predicted relationship between the two variables (proportion mature and size). The model can then be used to estimate the size that a particular percentage of the population is mature (on average).

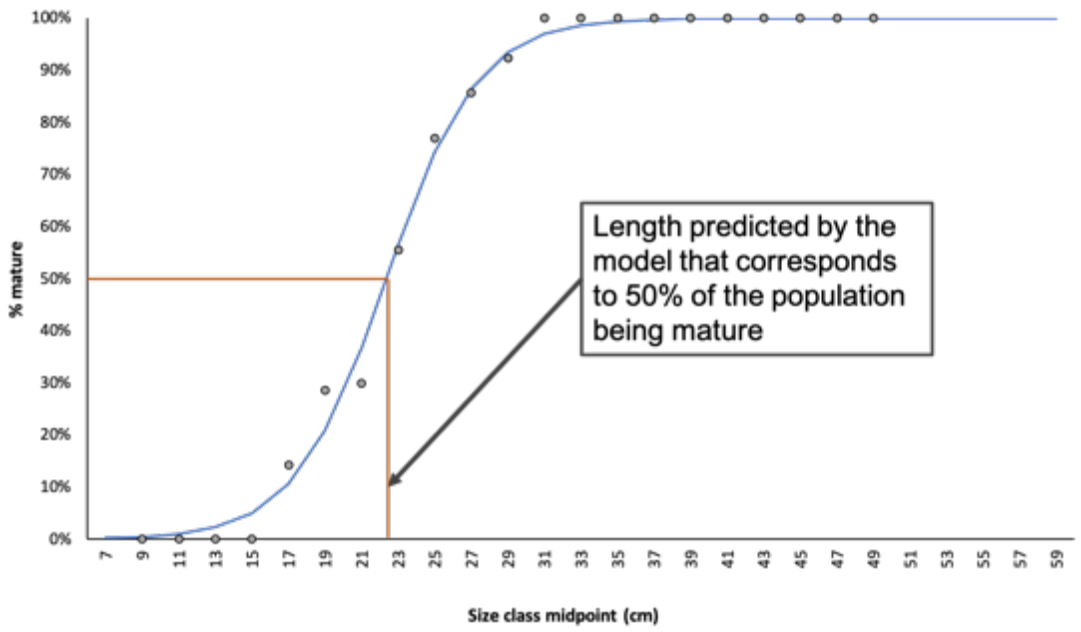


Figure 8. An example plot from fitting a logistic regression model (S-shaped blue line) to raw data of the proportion mature for different size classes. The model is able to then predict the size at which a certain % of the population is mature. For example, L_{50} ~ 22cm as indicated by the orange lines.

The logistic expression of the linear regression equation takes on the general form:

$$y = bLogx + a$$

where y = the proportion that are mature (dependent variable), x = size (independent variable), b ~ the slope, a = intercept (expected value of y when x =1).

To directly predict estimates of the average length when 50% and 95% of the population is sexually mature (L_{50} and L_{95}), the logistic equation is ultimately transformed to:

$$P = \frac{Pmax}{1 + e^{-\ln(19)\frac{SC - L_{50}}{\delta}}}$$

where *P* = the proportion of fish that are mature (adults), P_{max} = the asymptote (presumed to be 1 in a maturity model), *SC* = size class midpoint, L_{50} = the average length when 50% of the population is sexually mature, and δ = is the average increment after L_{50} to reach 95% proportion that are mature (as constrained by the natural logarithm of the odds ratio 19/1) (Hashiguti et al., 2018). In the analysis spreadsheet, the *a* and *b* model parameters equate to L_{50} and L_{95} respectively.

Spreadsheet analysis tool

The analysis for estimating the size at maturity is done in an Excel spreadsheet file which is set up to be fully automated; all you should need to do is enter the relevant data and press a button. The spreadsheet starts with an 'Instructions' page which provides a quick guide to the analysis steps provided in this manual in more detail.

Note that this spreadsheet contains a macro, and your computer settings may prevent the file to be downloaded with the macro included. See the Troubleshooting section below for instructions for how to resolve issues with downloading the file with macros intact.

Further, the macro uses the Excel Solver add-in. If you haven't used this before it will need to be "added" to your version of Excel. This is very simple to do and instructions are found in the Appendix (also see Troubleshooting section).

Example analysis sheets

There are also two contrasting examples of data analyses given in separate sheets. These examples are provided to help you to gain a visual understanding of completed analyses before you embark on using your own data. They can also serve as a useful reminder each time you use the analysis tool. These example sheets are colour-coded yellow:

i) **Example 1** uses 1-cm size classes and shows data where a species matures over a relatively large size range and with a relatively loose fit of the model (indicated by the smooth blue line) to the raw data (indicated by the grey data points). A loose fit simply means that some of the data points are at a distance to the fitted line. A good fit of the model to the data, and the goal of the analysis, is when the sampled data points all sit close to the fitted line predicted by the model. Although it is possible that this first example may still may provide useful estimates of the size at maturity, the data points that are furthest from the fitted line tend to have fewer samples (refer to the sample numbers in the green cells). This indicates that further sampling,

particularly for the size classes farthest from the fitted line, would greatly improve the accuracy of the size at maturity estimates.

ii) **Example 2** uses 2-cm size classes and shows data where there are more samples, particularly for the size classes across the range of sizes that this species matures. This results in a tighter fit of the model to the raw data and more accurate estimates of sizes at maturity.

Data analysis templates

Data analysis templates are colour-coded orange and are the sheets for you to use for analyses. The analysis sheets provide a choice depending on the size class grouping of your data: 1-cm, 2-cm, 3-cm, 4-cm or 5-cm size classes. **To preserve the integrity of the templates for future use, see analysis step 2 below for guidance on using these templates for analyses.**

Analysis steps

The goal of this analysis is to derive an estimate of the length at which 50% of the population is mature, denoted as L_{50}. The spreadsheet analysis tool generates estimates of both L_{50} and L_{95}.

Step 1. Assessing data for analysis

i) As outlined in the sampling program document, the primary data needed for estimating size at maturity for each species are:
   - fish size,
   - maturity (immature/mature), and
   - gender (male/female).

ii) To efficiently locate and organise the relevant data for analysis, all data should be stored in a common database that is readily accessed and queried. This will allow the database to be easily interrogated to assess the progress in accumulating maturity data across a range of sizes and sexes for each of the priority species. This will then give you an indication of which species may have sufficient data to conduct size at maturity analysis. Therefore, the first step involves extracting species data from the database so you can assess the amount of maturity data in a range of different size classes. The goal is for **7-10 fish sampled for each size class**, but see Table 2 in the sampling program.

iii) Selecting sexes for analysis – for each species you have the choice of analysing data based on females and/or males separately, or sexes combined (for gonochores only). Basically, it depends on how much data you have; the priority should be to estimate size at maturity for each sex, where possible.

iv) For protogynous hermaphroditic species, size at maturity for females is essentially first maturation and the analysis follows the same steps as for gonochores. To estimate the size at sex change (from female to male), the data used in the immature fish column in the spreadsheet is comprised of juveniles (immature) + mature females, while the mature fish column is comprised of mature males only.

v) Selecting size classes for analysis – the choice of size class groupings for your data comes down to the amount of data available, and is influenced by the species maximum size and the spread of your data across different sizes. Ultimately, there are no hard rules and the choice of size class groupings relies on the need to balance small enough size classes to gain precision, but having enough samples within each size class to generate an accurate estimate of the proportion mature

per size class. For example, grouping data into 5-cm size classes for a fish that reaches a maximum of 25 cm, would require the collection of less data but is highly unlikely to generate enough detail to robustly estimate the size at which they reach maturity. Similarly, grouping data into 1-cm size classes for a fish that reaches a maximum of 80 cm, although may provide robust estimates, would require a huge amount of data. See the sampling program for further details.

vi) Generally, the smaller the species, the smaller the size class groupings used. Remember, having sufficient data in the **size classes over which maturity occurs** is key to providing the most robust analysis results.

vii) Ultimately, if you're not sure there is no harm in conducting an analysis and assessing the results.

Step 2. Creating an analysis file

i) Before using the 'Size at maturity analysis' spreadsheet for the first time, save the original file as your **BACKUP MASTER FILE** and store it securely. This is very important to retain the integrity of the analysis tool in the event that errors occur with the file during working analyses. Save a second copy as your **WORKING ANALYSIS SPREADSHEET** to use **for all analyses**.

ii) Note that when you open the '*Size at maturity analysis'* spreadsheet, a dialog box will give you the option to enable or disable macros. You need to click '**Enable Macros**'.

Step 3. Preparing data for analysis

i) Once you've identified the species for analysis, the gender (or combined sexes), and the size class grouping for your data, the data need to be collated for input as per the analysis spreadsheet tool (Figure 9). To do this, you must:

   a) Group your maturity data into the size classes as per the spreadsheet. To make this simple to follow, the minimum size for all analysis sheets is 5cm. That is, the smallest size class for the 1-cm size class analysis sheet is 5-6 cm; for the 2-cm size class analysis sheet it is 5-7 cm; for the 3-cm size class analysis sheet it is 5-8 cm; and so on (see Figure 9).

   b) For each of the size classes, add up the total number of fish that are immature, and the total number of fish that are mature.

Step 4. Data input

i) Using your **WORKING ANALYSIS SPREADSHEET** file, and the relevant size-class analysis sheet, first enter the species name into cell B1.

ii) For each size class, insert the total number of fish that are immature into the first column (column C), and insert the total number of fish that are mature into the second column (column D). The data input cells are clearly indicated by the green shaded cells (see Figure 9).

iii) In each of the spreadsheets the cells are 'protected' except for the green shaded data input cells. Protected means that the information contained in certain cells can't be edited. This prevents any of the critical formulas in the analysis cells from being accidently altered. The chart is also not protected so you can save it – **be sure not to alter or edit the chart**.

iv) Entering a "0" or leaving a cell blank does not affect the data analysis outcomes – however, entering a "0" helps to ensure there are no errors in the data entry process.

v) Before moving to the data analysis step, check that your data entry is accurate.

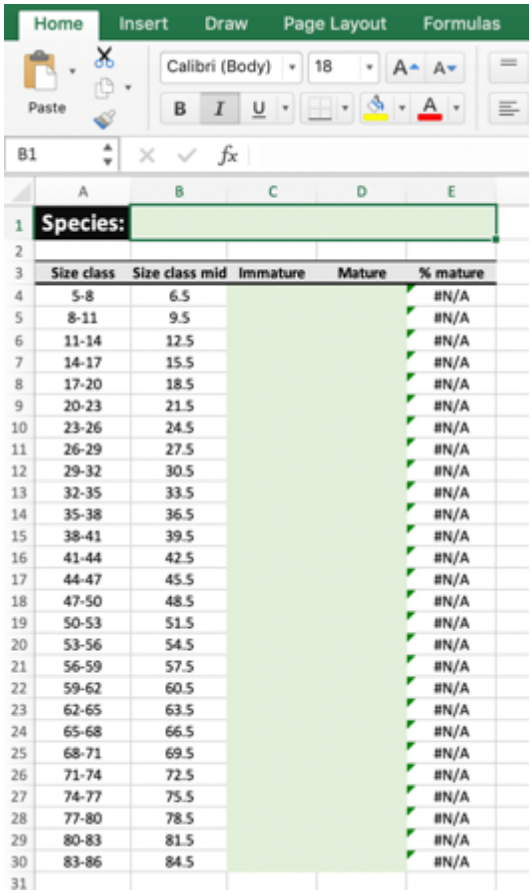


Figure 9. A screenshot of the 3-cm size class analysis spreadsheet showing the data input section, indicated by green shaded cells, with the size class groupings starting at a minimum of 5cm.

Step 5. Run analysis

i) All you need to do to run the analysis is to put the cursor over the grey '**Run Analysis**' button, located over cell G1, and 'left click' the mouse. Allow a few seconds for the analysis to run and then save the file.

ii) Note that the analysis may not run until you enter the 'save' button.

Step 6. Check analysis results

i) You should now see the blue line (relationship between % mature and fish size predicted by the model) on the plot fitted to the raw data (grey dots), and estimates of L_{50} and L_{95} in the cells Q4 and Q5 respectively ('Maturity estimates' table).

ii) Checking how well the model fits the raw data is done visually. That is, the blue line should travel approximately through the middle of all the data points in roughly an "S-shaped" pattern. Also, check that the estimates of L_{50} and L_{95} align with the plot AND that they make sense given the raw data and the species and their known sizes. Once satisfied, you now have your estimates of L_{50} and L_{95} for that species.

Step 7. Saving results

iii) Copy and paste the results (the chart and the maturity estimates table) to a separate spreadsheet and save it as a secure record of the analysis for that species. Be aware that if you use normal 'copy and paste', the results in the new file will refer to the working analysis file. Therefore, you need to 'Copy' and 'Paste Special' the chart 'As picture' into the new spreadsheet. Also, 'Copy' and 'Paste special' the Maturity table results (L_{50} and L_{95} estimates) as 'Values and Source formatting' (or just write them manually). This will preserve the results chart and values.

iv) Use the species to name the sheet, and/or be sure to record the species name on the sheet. Save the results file in a secure location.

v) Once finished, go back to the sheet you just used in the analysis working file and select the data in the green shaded cells, right click your mouse/cursor, and select 'clear contents'. Make sure that you retain the green shaded colouring for the data input cells.

vi) **The working file and the sheet are now ready for the next analysis.**

Troubleshooting

During the analysis you may encounter several issues with the file download, or the analysis and model fitting process, that can significantly affect the accuracy of your estimates of size at maturity. Below are some possible issues and what you can do to try and resolve them.

Problem: Your computer prevents you from loading the spreadsheet with the macros attached.

Solution: This may be due to the security settings on your computer disallowing macros. Because users may be using different versions of Microsoft Excel (e.g., Windows or Mac), you should refer to the Microsoft support pages online for 'Macros in Office files'. This is best sourced via the link https://support.microsoft.com/en-us/office/macros-in-office-files-12b036fd-d140-4e74-b45e-16fed1a7e5c6, or a Google search on your browser.

Problem: After running the analysis nothing on the sheet changes.

Solution: First try clicking on 'save' after each time you run an analysis for the model to fit the data and provide maturity estimates. If this doesn't work, it may be because the Solver add-in is not loaded in your version of Excel. See Appendix 4 for instructions on how to check this and load it if needed.

Problem: The model appears to fit the data poorly.

Solution: This may be due to low sample numbers. To test this try grouping the data into a larger size class and re-running the analysis using the relevant analysis sheet. If this doesn't improve the fit, you may need to collect more data to increase sample sizes. Experience in the analysis of different data sets will help you to make better judgements of model fits that are acceptable (see step 1 notes above, and the sampling program).

Problem: The model does not appear to fit the raw data at all.

Solution: This may be due to low sample sizes (see above), or may be that the starting values for the model parameters *a* and *b* (cells N4 and N5 respectively) are not close enough to the actual model values for the model to converge to a sensible solution. This is unlikely since the model is generally robust to different starting values. If you suspect it is the latter problem, you can try using different values of the *a* and *b* parameters (cells N4 and N5 respectively; these cells are not protected), and re-running the analysis. If this does not work you will need to contact the spreadsheet developer (see spreadsheet 'Instructions' sheet).

Problem: The analysis returns an error message.

Solution: There may be a problem with the macro. You can delete your working file, and create a NEW working file from the MASTER file, and try again. If problems persist, contact the spreadsheet developer.

4.2 Estimating spawning seasonality

This section briefly describes the statistical analysis method for estimating spawning seasonality, and details the steps for conducting the analysis using your own data and the '*Spawning seasonality analysis*' spreadsheet. This spreadsheet is developed to do all of the statistical work for you in estimating the size at maturity, making the analysis steps as simple as possible.

Statistical method

In fish, the relative stage of development of their gonad is considered a good indicator of reproductive activity and is used to determine a fish's spawning season. The relative developmental stage of gonad development is commonly measured using the Gonadosomatic Index (GSI). The Gonadosomatic Index is calculated by the equation:

$$GSI = \left(\frac{GW}{TW}\right) x100$$

where *GW* = the total gonad weight, and *TW* = the total fish weight. To determine a fish's spawning season, the average GSI is calculated and plotted over different months. Since the GSI follows gonadal maturation, reaching higher values at the ripe stage, and then decreasing after spawning, especially in females, the months with the highest GSI values indicate their spawning season. This is determined by examining plots of the data visually (Figure 10).

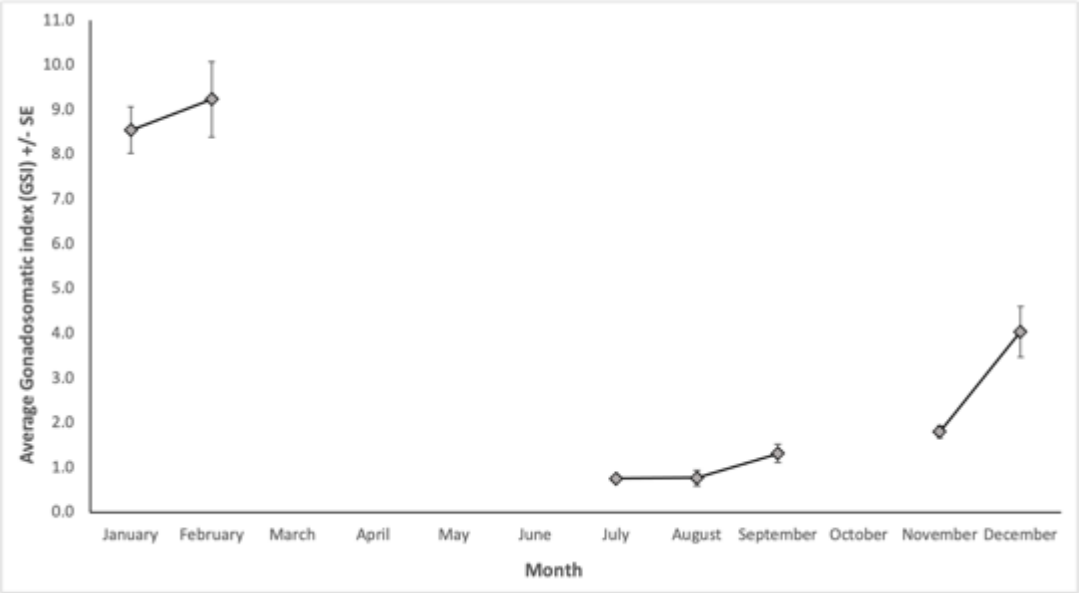


Figure 10. An example plot of average monthly GSI values. The highest values indicate the months where spawning is likely to be occurring. In this example, the plot suggests a spawning season from at least January-February, however also highlights the need for data to be collected after February to determine if spawning continues longer. The error bars are standard error.

Spreadsheet analysis tool

The analysis for determining spawning seasonality is done in an Excel spreadsheet file which is set up to be fully automated; all you should need to do is enter the relevant data. The spreadsheet starts with an 'Instructions' page which provides a quick guide to the analysis steps provided in this manual.

Example analysis

An example analysis of simulated data is given to help you to gain a visual understanding of completed analysis before you embark on using your own data. This can also serve as a useful reminder each time you use the analysis tool. The example sheet is colour-coded yellow.

The '*Data analysis – example*' sheet provides example data showing how it is entered into the green shaded cells. This can serve to remind you how data is entered if you are not sure. Note that the weight units are in grams. It is possible to use kilograms, however **it is critical that the same weight units are used** for fish and gonad weight data. Also note that data are given only for females. Only data for ovaries are collected because female reproductive biology is a more reliable indicator of the timing of spawning due to the greater relative change in gonad weight during different maturation stages (West, 1990).

All calculations and data plotting are done automatically after data have been entered into the green cells, with the results presented on the same page (scroll to the right). The results are given as a chart which refers to a table of average GSI values. The chart is all that is needed to interpret and present the results. The example results are shown in Figure 10 with peaks in GSI values indicating the months during which spawning occurs. The error bars are *standard error* and are an indication of the

variability in the data used in calculating the average GSI value. The orange text box on the example sheet provides an interpretation of the GSI plot in the chart.

Data analysis template

The data analysis template sheet is colour-coded orange. To preserve the integrity of the template for future use, a backup copy should be created along with a working file (see analysis step 2 below).

Analysis steps

Step 1. Assessing data for analysis

i) As outlined in the sampling program document, the primary data needed for estimating spawning seasonality for each species are:
    i) collection date,
    ii) fish weight,
    iii) gonad weight, and
    iv) gender (female only).

ii) To efficiently locate and organise the relevant data for analysis, all data should be stored in a common database that is readily accessed and queried. This will allow the database to be easily interrogated to assess the progress in accumulating the necessary data each month for each of the priority species. This will then give you an indication of which species may have sufficient data to conduct spawning seasonality analysis. Therefore, the first step involves extracting species data from the database so you can assess the number of fish and gonad weight data in any given month, across as many months as possible. The goal is for 7-10 fish sampled for each month, but see Table 2 in the sampling program.

iii) Only data for mature females are used for this analysis.

Step 2. Creating an analysis file

i) Before using the 'Spawning seasonality analysis' spreadsheet, save the original file as your **BACKUP MASTER FILE**. This is very important to retain the integrity of the analysis tool in the event that errors occur with the file during analysis. Save a second copy as your **WORKING ANALYSIS SPREADSHEET** to use for all analyses.

Step 3. Preparing data for analysis

i) Once you've identified the species for analysis, the data need to be collated for input as per the analysis spreadsheet tool (Figure 11). To do this, you must extract the data by the species of interest for **females only** and include date of collection, fish weight, gonad weight and the # of lobes data fields. These need to be arranged in columns in the order as shown on the example data entry sheet (see Figure 11).

Step 4. Data input

i) The first data input step should be to enter the species name into the cell starting with B2 (green shaded cell).

ii) Enter the data into the green shaded cells (columns A to E), being careful to ensure data are entered into the correct columns. There are 399 shaded green cells which means you can enter up to this number of rows of data (each row represents an individual fish). This should be more than adequate to carry out analyses for spawning seasonality on any species.

iii) In each of the spreadsheets the cells are 'protected' except for the green shaded data input cells. This prevents any of the critical formulas in the analysis cells from being accidently altered.
iv) Before moving to the next step be sure to check your data entry is accurate.

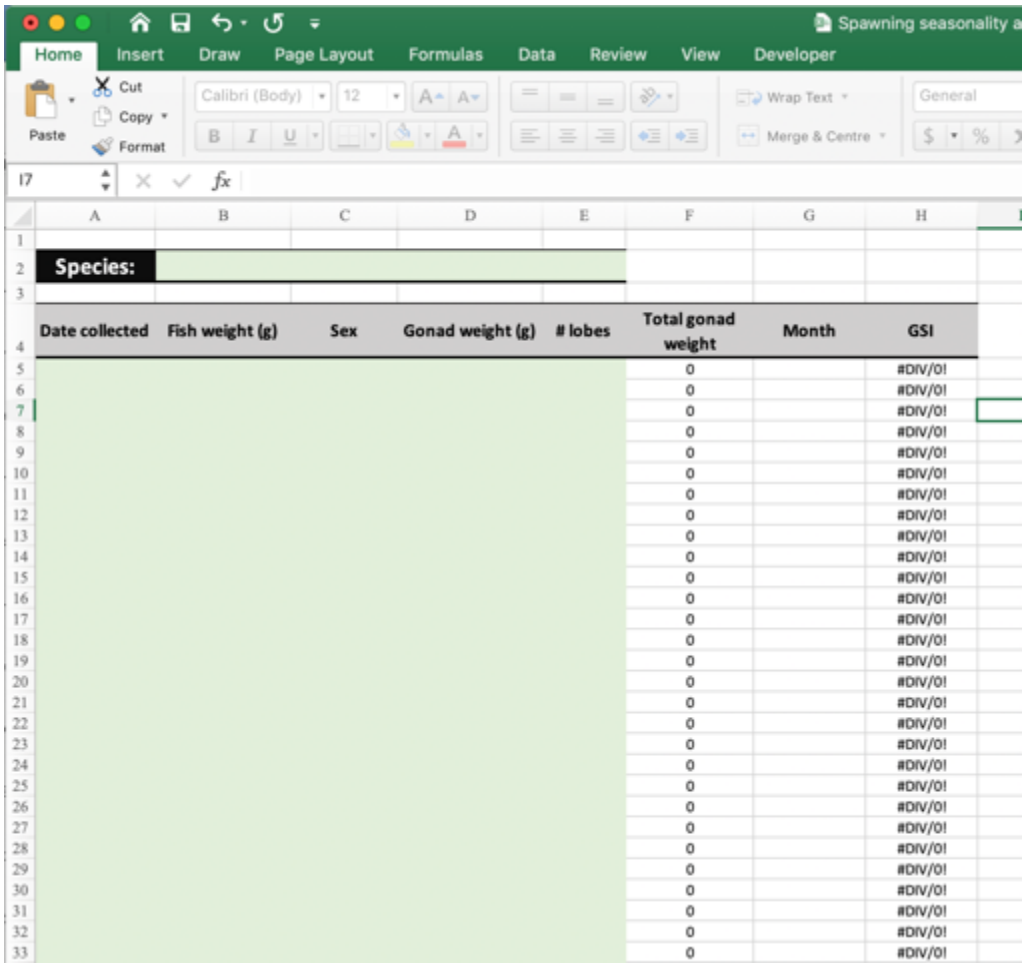


Figure 11. A screenshot of the spawning seasonality analysis spreadsheet showing the data input section indicated by green shaded cells.

Step 5. Check analysis results

i) Once you have entered your data, the analysis is done automatically and results are generated on the same sheet (scroll to the right).
ii) The months where the average GSI values peak indicate the months when spawning is occurring. Note that where GSI values are found to be relatively consistent throughout the year may indicate year-round spawning. Pay attention to the actual GSI values on the y-axis to help guide your interpretation of seasonality. For example, the difference between GSI values of 3 and 1 may not look much on the chart, however, remember that the higher value is 3x the lower value!
iii) The standard error bars are shown for each GSI value, and give an indication of the variability in the data. Large error bars may indicate a large variation in the data and is often seen when sample sizes are low. Therefore, they may indicate months where more data would be useful.

Step 6. Saving results

i) Copy and paste the results (chart) to a separate spreadsheet and save it as a secure record of the analysis. Be aware that if you use normal ‘copy and paste’, the results in the new file will refer to the working analysis file. Therefore, you need to 'Copy' and 'Paste Special' the chart **'As picture'** into the new spreadsheet. This will preserve the results chart.
ii) Use the species to name the sheet, and/or be sure to record the species name on the sheet. Save the results file in a secure location. This will provide a separate record of the analysis for each species that should be securely stored for updated versions with the addition of new data, if necessary, for publishing the data analysis results and for future reference.
iii) Once finished, go back to the sheet you just used in the analysis working file and select the data in the green shaded cells, right click your mouse/cursor, and select ‘clear contents’.
iv) **The working file is now ready for the next analysis**.

Troubleshooting

During the analysis you may encounter several issues that can affect the accuracy of your GSI values and therefore your interpretation of spawning seasons. Below are some possible issues and what you can do to try and resolve them.

Problem: After entering the data nothing changes on the rest of the sheet.

Solution: Click on ‘save’ after you’ve entered all your data. If this doesn’t work, carefully check that all your data are entered correctly into the green shaded data input cells.

Problem: Values don’t appear on the output plot.

Solution: Carefully check that all your data are entered correctly into the green shaded data input cells. Otherwise, this may be due to some cells and their formulas being accidentally altered. If so, contact the spreadsheet developer.

Problem: The chart output results should appear with lines connecting months ONLY where data exist (e.g., as seen in Figure 10). If lines appear to be continuous on your chart even when there are missing data (i.e., cells with #N/A), it is better to change the chart settings.

Solution: For instructions to change the chart to show cells with #N/A as empty, see Appendix 5.

References

Brown-Peterson, N.J., Wyanski, D.M., Saborido-Rey, F., Macewicz, B.J. and lowerre-Barbieri, S.K. (2011) A Standardized Terminology for Describing Reproductive Development in Fishes. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 3:52–70, 2011. DOI: 10.1080/19425120.2011.555724

Chen, Y., & Paloheimo, J. E. (1994). Estimating fish length and age at 50% maturity using a logistic type model. Aquatic Science, 56, 206–219.

Claydon, J.A.B., McCormick, M.I. and Jones, G.P. (2014) Multispecies spawning sites for fishes on a low-latitude coral reef: spatial and temporal patterns. Journal of Fish Biology (2014) 84, 1136–1163. doi:10.1111/jfb.12355

Hashiguti, D.T., Soares, B.E., Wilson, K.L., Oliveira-Raiol, R.D. and Montag, L.F.A. (2018) Comparing three methods to estimate the average size at first maturity: A case study on a Curimatid exhibiting polyphasic growth. Ecology of Freshwater Fish, 2018: 1-8. DOI: 10.1111/eff.12451

Hilborn, R. and Walters, C.J. (1992) Quantitative Fisheries Stock Assessment: Choice, Dynamics, and Uncertainty (Kluwer Academic Publishers, Boston).

Hixon, Mark A. and Randall, John E. (2019) Coral Reef Fishes. In Cochran, J. Kirk; Bokuniewicz, J. Henry; Yager, L. Patricia (Eds.) Encyclopedia of Ocean Sciences, 3rd Edition. vol. 2, pp. 142-150, Elsevier. ISBN: 978-0-12-813081-0

Jennings, S., Reynolds, J. D., & Mills, S. C. (1998). Life history correlates of responses to fisheries exploitation. Proceedings of the Royal Society B: Biological Sciences, 265(1393), 333–339. https://doi.org/10.1098/rspb.1998.0300

Leis, J.M. and McCormick, M.I. (2002) The Biology, Behavior, and Ecology of the Pelagic, Larval Stage of Coral Reef Fishes. Chapter 8 in: Coral Reef Fishes (pp.171-199), Academic Press. DOI:10.1016/B978-012615185-5/50011-6

Longenecker, K. and Langston, R. (2018) The Jungle Histology Atlas of Gonad Stages in Coral-Reef Fishes: 2nd edition. Pacific Biological Survey, Bishop Museum, Honolulu, Hawaii 96817, USA. 55p.

Miller, Bruce and Kendall, Arthur. W. Jnr. (2009) "Chapter 1. Fish Reproduction". In, Early Life History of Marine Fishes, Berkeley: University of California Press, 2009, pp. 9-38. https://doi.org/10.1525/9780520943766-005

Moore, B. and Colas, B. (2016) Identification guide to the common coastal food fishes of the Pacific Islands region. Noumea, New Caledonia: Pacific Community. 148 p.

Myers, R.A. and Mertz, G. (1998) The limits of exploitation: a precautionary approach. Ecological Applications, 8(1) Supplement, ppS165-S169.

Reynolds, JD, Dulvy, NK, Goodwin, NB and Hutchings, JA. (2005) Biology of extinction risk in marine fishes. Proc Biol Sci. 2005 Nov 22;272(1579):2337-44. doi: 10.1098/rspb.2005.3281. PMID: 16243696; PMCID: PMC1559959.

Sadovy de Mitcheson, Y. and Liu, M. (2008) Functional hermaphroditism in teleosts. Fish and Fisheries, 9: 1-43. DOI: 10.1111/j.1467-2979.2007.00266.x

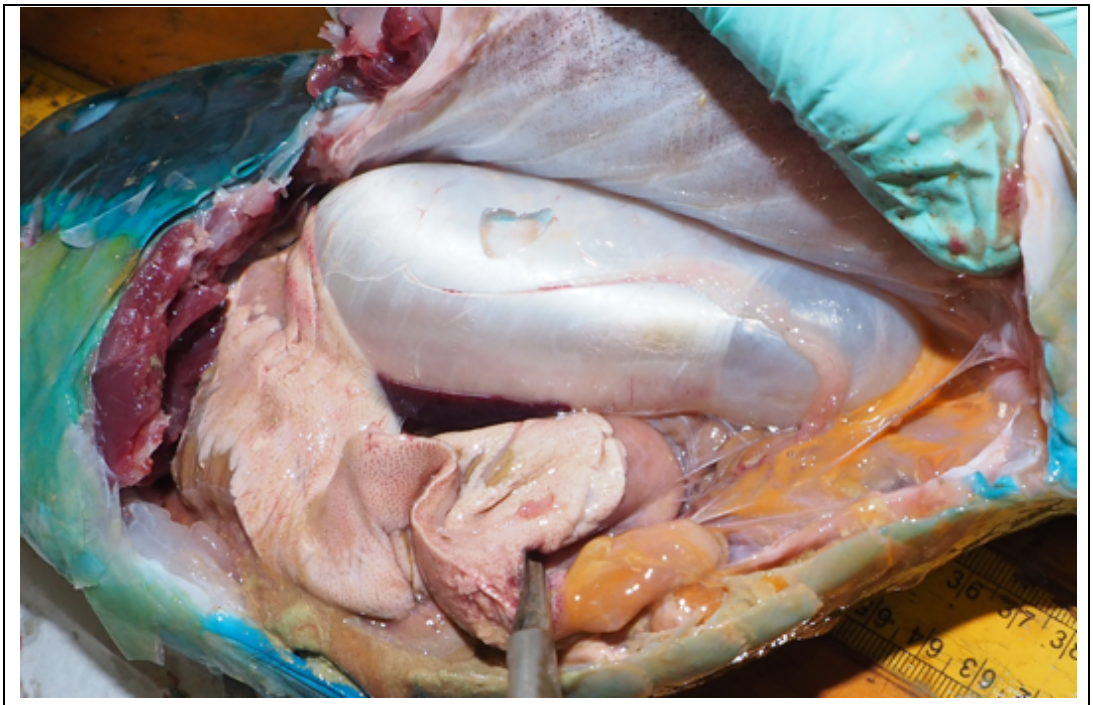
Vitale, F., Svedäng, H., and Cardinale, M. (2006) Histological analysis invalidates macroscopically determined maturity ogives of the Kattegat cod (*Gadus morhua*) and

suggests new proxies for estimating maturity status of individual fish. ICES Journal of Marine Science, 63: 485-492. doi:10.1016/j.icesjms.2005.09.001

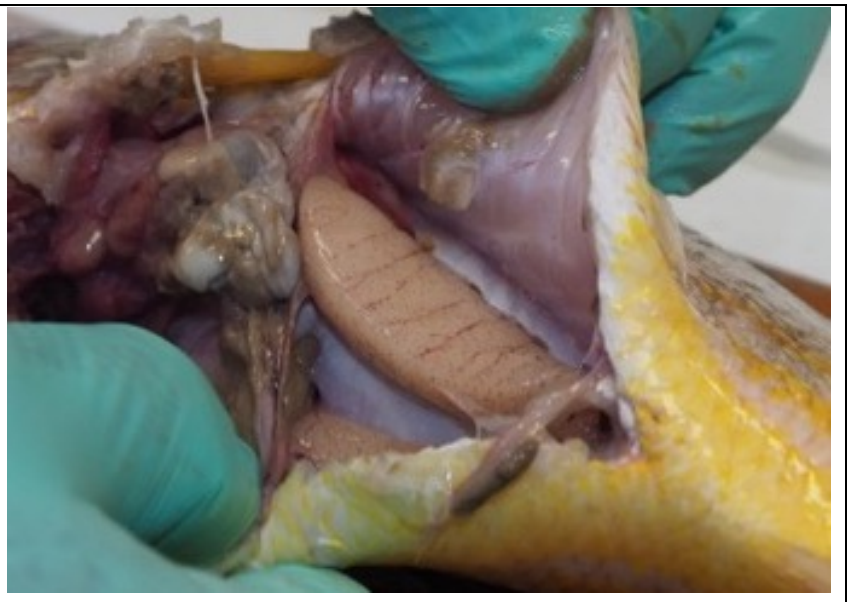
West, G. (1990) Methods of assessing ovarian development in fishes: a review. Marine and Freshwater Research 41(2): 199-222. http://dx.doi.org/10.1071/mf9900199

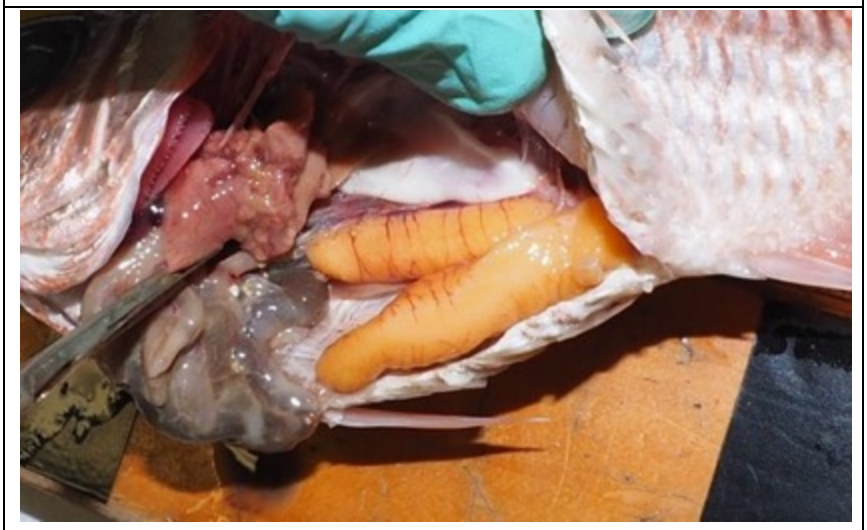
Appendices

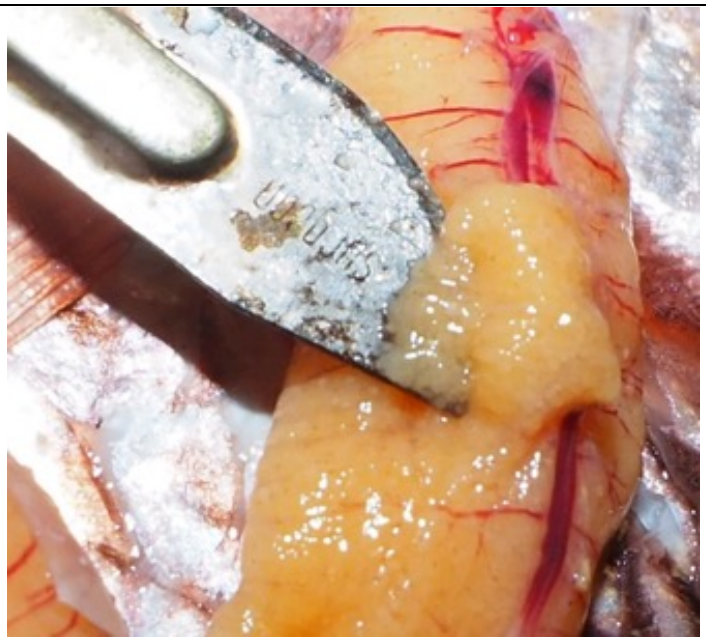
Appendix 1: Examples of immature fish

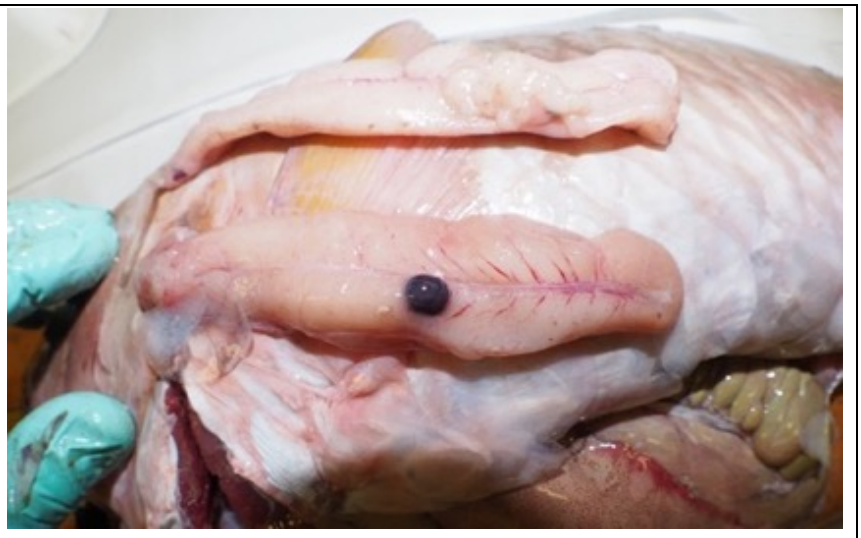


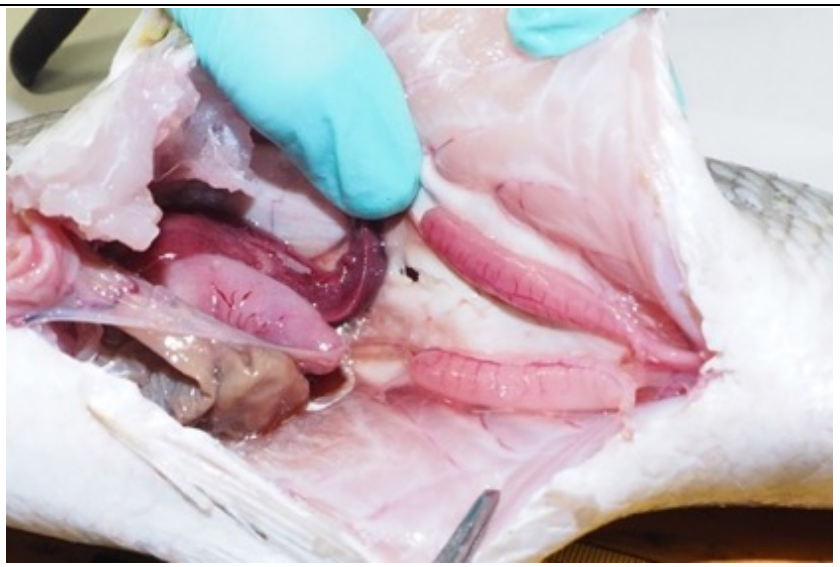
Appendix 2: Examples of mature females



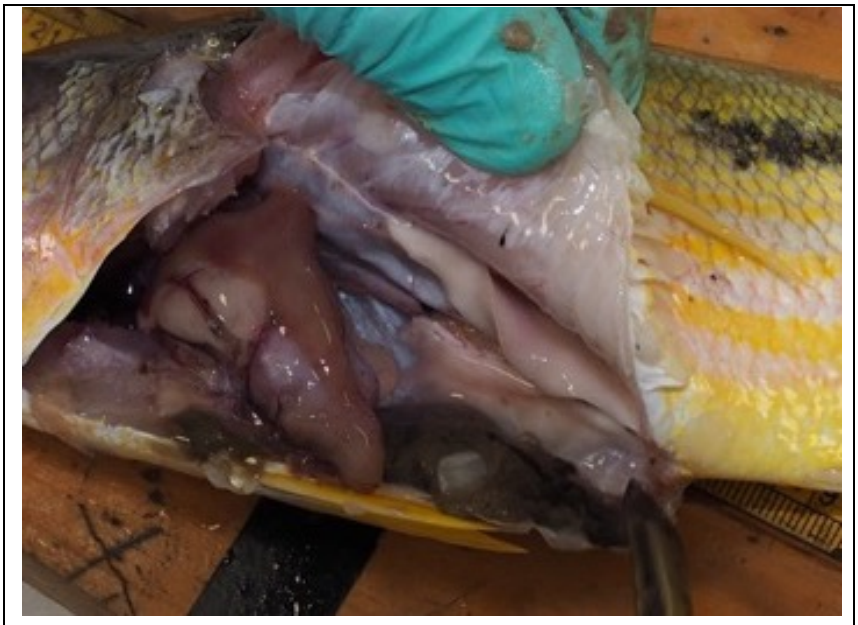


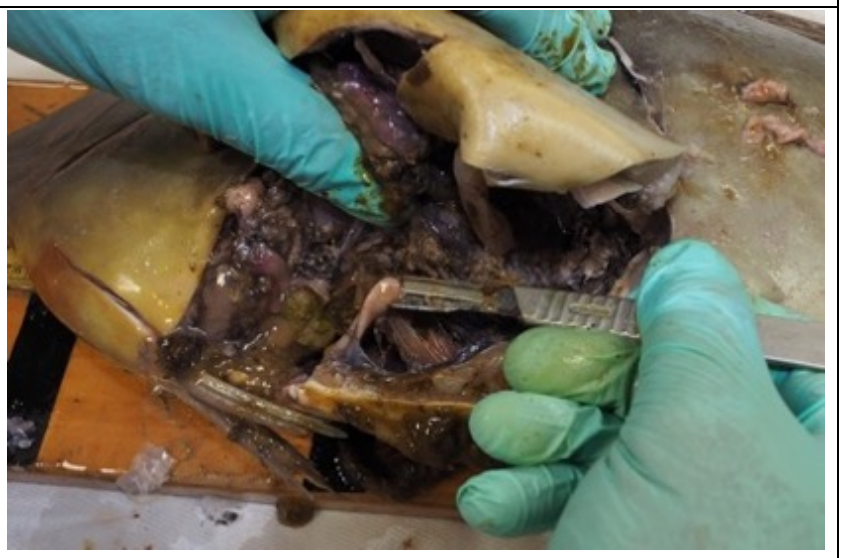


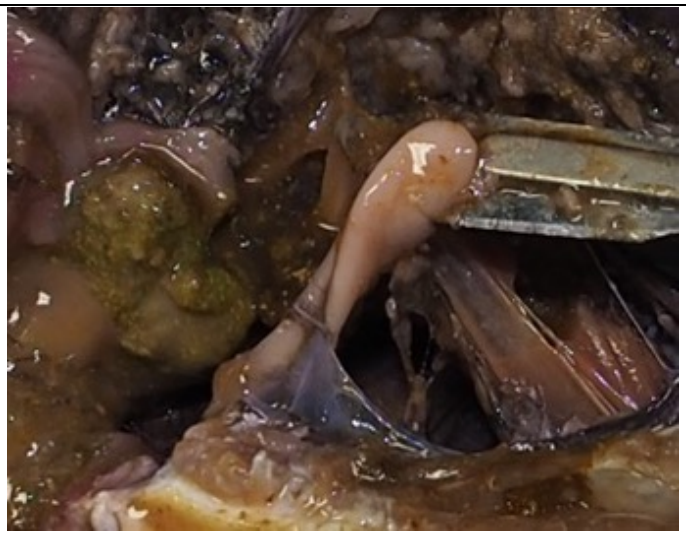


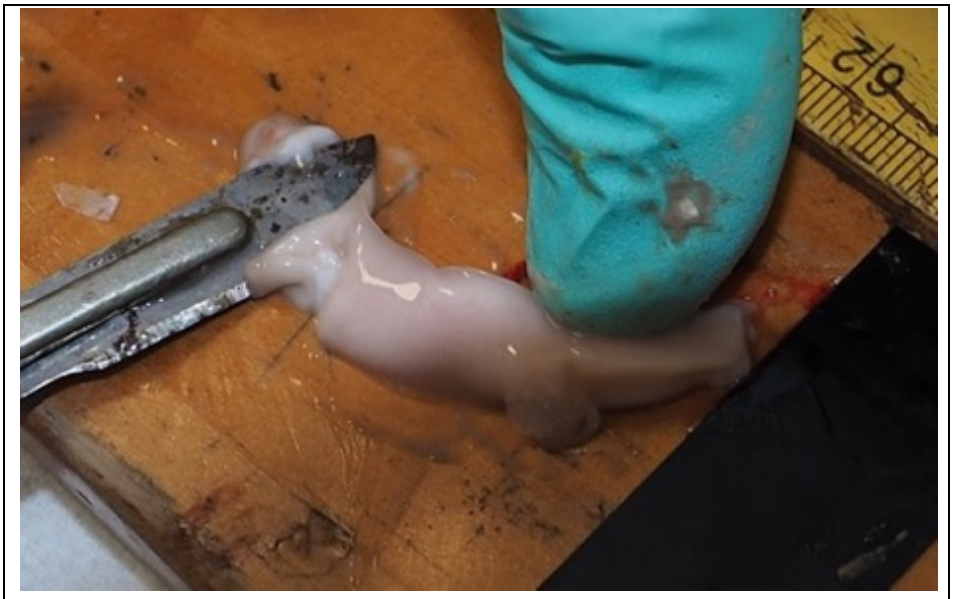


Appendix 3: Examples of mature males









Appendix 4: Instructions for the Excel Solver add-in

Below are instructions for ensuring that the Solver add-in provided with Microsoft Excel is loaded onto the program. This is necessary for the analysis to run successfully using the macro in the 'Size at maturity analysis' spreadsheet.

The instructions below are derived from the Microsoft support webpage for Windows, and the link is provided here also in the event that you are running a different version or operating system: https://support.microsoft.com/en-us/office/load-the-solver-add-in-in-excel-612926fc-d53b-46b4-872c-e24772f078ca#OfficeVersion=Windows.

Instructions

Step 1: Go to 'File' > 'Options'

Step 2: Click 'Add-ins', and then in the 'Manage' box, select 'Excel Add-ins'.

Step 3: Click Go.

Step 4: In the 'Add-Ins available' box, select the 'Solver Add-in' check box, and then click OK.

Step 5: After you load the Solver Add-in, the Solver command is available in the Analysis group on the Data tab.

Note:

- If the Solver Add-in is not listed in the Add-Ins available box, click Browse to locate the add-in.
- If you get prompted that the Solver Add-in is not currently installed on your computer, click Yes to install it.

Appendix 5: Instructions to show cells with #N/A as empty on charts

See steps below or: https://support.microsoft.com/en-us/office/display-empty-cells-null-n-a-values-and-hidden-worksheet-data-in-a-chart-a1ee6f0c-192f-4248-abeb-9ca49cb92274

Step 1: Click the chart you want to change.

Step 2: Go to 'Chart tools', then on the 'Design' tab, in the 'Data' group, click 'Select Data'.

Step 3: Click on 'Hidden and Empty Cell settings', and for 'Show empty cells as:', select 'Gaps'.

Coasts | Climate | Oceans