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Life History of Two Goatfishes in Hawai'i and Alternative Methodologies in Life History Research

A thesis submitted in partial fulfillment of the requirement for the degree of Bachelor of Arts / Science in Biology from William & Mary

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Data-driven management of fisheries requires information on the life history of the species being managed. I provide new information on the life histories of two goatfish species in Hawai‘i, *Parupeneus insularis* and *Parupeneus cyclostomus*. Fish were collected using spearfishing from the reefs of O‘ahu between 2020 and 2023. Macroscopic and microscopic methods of assigning maturity and reproductive stage were used to estimate size at maturity and seasonality. *Parapeneus insularis* females are estimated to reach 50% maturity at 188 mm fork length (95% CI: 177mm, 197mm), and 95% maturity at 245 mm FL (95% CI: 226mm, 287mm). *P. cyclostomus* females reach 50% maturity at 250 mm fork length (95% CI: 237mm, 262mm), and 95% maturity at 294 mm FL (95% CI: 273mm, 316mm). Female *P. insularis* specimens with GSI above 2 and vitellogenic 3 or hydrated oocytes occurred between March and September with a peak around April, indicating a spawning season in Spring and Summer. Specimens with GSIs greater than 1 and vitellogenic 1 & 2 oocytes were found in Winter, indicating that oocyte maturation had begun. Female fish with vitellogenic 3 or hydrated oocytes were collected in all seasons for *P. cyclostomus*, and GSIs above 2 for all but winter. This is indicative of spawning in all seasons, but uneven sampling and a low sample size means that more data is needed. Wet mount maturity assignments were as accurate as histology when assigning maturity in female fish. For studies of fish life history where histology may be too expensive and GSI may require too many samples, wet mount is a more informative, low cost alternative.

**INTRODUCTION:**

More than ninety million tons of fish and invertebrates are harvested from the world’s fisheries every year by more than forty million people, creating one of the world’s most crucial commercial sectors (FAO 2022). Managing this is a serious concern, as the proportion of unsustainable fisheries operations has increased over the last 50 years (Figure 1). If the harvestable population, or stock, is being harvested faster than it can replenish itself, the fishery is experiencing overfishing, while a stock below the size required to replenish itself is overfished (Sass et al. 2014). In either case, fishermen must reduce their harvest to allow the stock to recover. If they do not, the stock could collapse, a drastic and potentially permanent loss of the target population with devastating consequences for the local ecosystem and economy. Sustainable fisheries management aims to prevent collapses by preventing overfishing and avoiding reaching an overfished level. This creates a fishery that is productive in the present and the future (Hilborn et al. 2020).

The number of fish in a management program varies by nation and region. Temperate fisheries, like those found in the North Atlantic, generally feature fewer fish species harvested in greater
numbers, whereas the tropics tend to feature more biodiverse fisheries with smaller per-species yields (Sass et al. 2014). Temperate fisheries are generally fished by developed nations using large, industrialized operations with large ships and industrial equipment (Ovando et al. 2021). In contrast, many tropical fisheries are much smaller operations using small boats and hand nets or lines, fished by people from developing nations with fewer resources to devote to fisheries management (Prince and Hordyk 2019, Ovando et al. 2021). Since higher latitude fisheries tend to be more industrialized, rely more heavily on a specific species and population, and have more resources available for harvesting, they generally feature more careful management of a specific species (Costello et al. 2016). In contrast, a small fishery in a developing nation generally has fewer resources to manage specific species, and more species to manage (Costello et al. 2012).

To ensure that a fishery remains sustainable and productive, fisheries managers can regulate the number, size, and time of year of harvest for fish populations. These decisions require knowing the fish’s life cycle and developmental trends, as well as trends in the fishery and harvest. In a data-rich, carefully managed fishery, regulations are established by modeling the growth and changes in the population (Prince and Hordyk 2019). These models use predictions of annual recruitment, the number of fish that will be added to the population in a year, as well as the size and structure of the population to adjust the regulations as needed. Smaller fisheries are managed with limits based on facts about the fishery and the species in question (Costello et al. 2012). However, all management requires understanding the life history of the population being harvested, and all fisheries will benefit from effective management (Pauly et al. 1998).

Figure 1: Trends in sustainability for global fisheries (Figure 19, FAO, 2020)
This graph shows the ongoing trend in fisheries sustainability over the last 50 years. The number of underfished fisheries has decreased steadily, and the number of unsustainable fisheries has increased, but to a lesser degree. This corresponds to a trend in data driven fisheries management.

Life history is the set of traits that govern growth and reproduction for a species based on their strategy for surviving in their habitat. Every species employs a different life history strategy to adapt to their environment and maximize their fitness. The variation in traits that influence life history is often described in terms of time functions - common traits include growth rate, time until maturity, and lifespan (Gaillard et al. 2016). These traits are highly variable in fish species; species with slow growth rates and long times until maturity are impacted more by commercial harvest, whereas those with rapid growth rates and short times until maturity can be fished more sustainably (Law 2000). Understanding life history is therefore essential for management. For a fishery, crucial traits include the growth rate, reproductive output, and the time between hatching and reproductive maturity. This information is sufficient to set basic regulations and improve the health of a fish population. However, smaller fisheries may not have the resources needed to obtain this information for all species, especially as these life history traits can vary widely in tropical regions (Winemiller 2005). This has led to a paucity of information for species in smaller tropical fisheries with high biodiversity and more complex reproductive schemes; the term data-poor fishery management is used to describe the basic elements of their fishery models.

Fish in temperate or arctic environments generally have well-described seasonal reproductive patterns, with annual changes in physiology and behavior at certain times of year. Due to the more consistent climate, tropical and subtropical fishes often use alternative reproductive schemes. Many species in tropical environments have partially or completely asynchronous spawning occurring with various degrees of seasonal specificity (Brown-Peterson et al. 2011). In a temperate species, a mature fish out of season will have undeveloped gonads and immature germ cells that resemble an immature fish. However, in a tropical species without synchronous spawning, reproduction can occur throughout the year, so a mature fish can be reproductively active or immature at any time.

Life history analysis must determine the seasonal patterns for a species and account for seasonal differences during sampling, or else the data collected may be inaccurate. In the spawning season, the gonads of mature fish should be significantly larger and demonstrate clear differences in gonad morphology (Winemiller 2005). Life history analysis must sample year round to determine if a and when a species’ season occurs. In a temperate environment, life history analysis will usually find a consistent spawning season in a specific date range. That season can be used to set catch seasons and determine sampling dates. However, tropical species may or may not be seasonal.
To determine the size at maturity and the seasonality of an organism, researchers examine the reproductive characteristics of the population throughout the year. Size at maturity is usually described with the length at which 50 and 95 percent of fish are expected to be mature (L50 and L95). This is shown on a growth curve ogive, which describes the estimated proportion of mature fish at each length. Fish with greater length are more likely to be engaged in reproduction during their spawning period.

Fish currently engaged in reproduction will have enlarged gonads with larger, more developed reproductive tissues, leading to a higher ratio of gonad mass to somatic tissue mass. The gonadosomatic index, or GSI, is the preferred measure of reproductive investment. GSI is the percentage of gonad mass in relation to total mass in an individual. A high average GSI indicates a spawning period for a population, often linked to a date range or season, or other environmental factors, such as water temperature and the phase of the moon. While GSI is easily measured, and sometimes used as a metric of maturity, it is not always an accurate measure of maturity (Winemiller 2005, Flores et al. 2019). High GSI is indicative of a mature fish, but low GSI is not indicative of an immature fish, especially when considering seasonality. A mature fish with a large enough body mass will have a smaller relative change in gonad mass, vs a smaller fish that will have greater fluctuations in GSI. The magnitude of changes in GSI are also dependent on sex and individual health. Since both body mass and gonad mass are highly variable, GSI-based methodologies have lower signal - noise-ratio, especially at the beginning and end of a spawning season. Managers may use GSI as a faster and cheaper method in fisheries, but this requires larger sample sizes due to the lower signal-to-noise ratio from GSI. Direct measurement of reproductive stages continues to be the gold standard for resource managers (Brown-Peterson et al. 2011).

Reproductive stages can be identified using a variety of techniques, either with microscopy or macroscopic analysis. Maturity can be assigned using macroscopic and microscopic morphological traits consistent with ontogenetic development. Depending on their sex and reproductive status, fish can have visibly different appearances, proportions, and reproductive or somatic organs, which researchers can use to assign maturity macroscopically. A juvenile fish can be visually distinct from a mature and reproducing fish based on the appearance of its gonads alone. In many species, researchers are able to assign maturity with visual cues during initial processing in the field or lab (Vitale et al. 2006, Flores et al. 2019). However, macroscopic maturity assessment is less effective than microscopic methods at assigning maturity, as it cannot identify specific spawning stages. Without specific information on oocyte stages, a developing fish may appear identical to a regenerating fish in an asynchronous spawning population. In addition, visual signs of maturity are species-specific and may require more training. As such, macroscopic assignment is usually less accurate than microscopic methods (Vitale et al. 2006).
**Figure 2: Examples of staging keys from macroscopic and microscopic techniques.**

These images show different methods of staging a fish using macroscopic (top) and microscopic (bottom) methods. The top row of images shows staging traits for macroscopic assignment of an ovary in an Irish Plaice (Figure A2.9, ICES 2007). Panels are arranged left to right, top to bottom. The bottom row (Figure 3 from Souza, 2021) show the stages of ovarian development in histology for an atlantic bluefish. The clusters of small cells in the leftmost ovary are Primary Growth (PG) oocytes. The center stages are developing stages, and the rightmost stages are the fully developed stages and post-spawning structures. An individual presenting both Post-Ovulatory follicles (POF) structures and primary growth oocytes will be similar to an immature fish, but is in the regenerating phase.

Due to the difference in concentration, size, and opacity of egg stages, the undeveloped oocytes in an ovary are visually distinct from mature oocytes in a standard compound microscope. This allows for rapid, simple assessment of maturity without fixatives or stains, and is accurate enough to identify maturity stages in ovaries. Gonad samples, prepared with wet mount or histology methods, can provide information, although wet mounts have significant limitations. While wet mounts are effective at assigning maturity and reproductive stage for females, they cannot assess prior history of spawning via post ovulatory follicles, and they struggle to identify reproductive stages for testes. As a result, the most common method of assignment is histology (Brown-Peterson et al. 2011).

Histology is the most labor and resource-intensive method, but offers more depth of information than wet mounts or macroscopic assignment. Histologists can identify not only maturity and
stage but also evidence of prior spawning and specialized tissue characteristics (Figure 2). This provides the most information per sample and maximizes both precision and accuracy (Brown-Peterson et al. 2011). Histology is generally accessible for many fisheries scientists, and so most studies will choose either histology or GSI based maturity assessment. Wet mounts are less represented in the literature, but do provide a less resource intensive alternative for assigning maturity in some situations. In my methodology, I outline a procedure for assigning maturity based on wet mounts that should be accessible to researchers wishing to accurately assign maturity without histology. This maturity data is critical for characterizing life history, and cheap, effective methods like wet mount for assessing gonad status have potential for fisheries managers.

Fisheries managers use knowledge of life history, particularly seasonality and size at maturity data, to set two major types of regulations - fishing seasons and size limits. Size limits set a minimum or maximum allowable size for a catch (Hilborn et al. 2020). Minimum size limits ensure that fish are not harvested before they have reached maturity and contribute to the population, while maximum size limits are used to protect the most fecund females (Hixon et al. 2014). These limits may vary by sex and are generally driven by the fish species’ reproductive strategy. Seasonal limits are generally used to protect a population during peak spawning periods, and managers can adjust the opening and closing date of a fishery to compensate for trends in the fishery (Sass et al. 2014).

Fisheries management in the reefs around the Pacific Islands is especially challenging. These reefs are highly biodiverse, with far more species to manage, lower populations of individual species, and lower management budgets per species (Costello et al. 2012). Data-driven management is difficult due to the scale of the research needed. A recent study in Hawai‘i found that life history data for reef fish species is frequently insufficient and that managers would particularly benefit from microscopic analysis of female fish L50s (Nadon et al. 2015). The scale of the management program has led to near-shore reef fisheries being understudied. Existing regulations are not always data-driven, and do not always cover the entire island chain (Longenecker and Langston 2008). The state of Hawai‘i is looking to expand their understanding of the life history of nearshore reef fishes.

Among the unregulated fishes in the nearshore fishery are two species of goatfish. Goatfish are a diverse clade found around the world in tropical and subtropical reefs (Figure 3), and a significant subsistence and recreational fishery in the Hawaiian islands (Echreshavi et al. 2022). They are benthopelagic carnivores that forage on sand flats and corals using a specialized set of barbels to sense prey (Gosline 1984). The Parupeneus genus is endemic to the Indo-Pacific and contains 27 species, including several unique to the reefs of Hawai‘i. Several species of goatfish in Hawai‘i are considered understudied, making regulation of the fishery challenging, since data is needed for each species.
Spearfishing is a popular method for collecting fish in the nearshore reef fisheries. Using a speargun or a Hawaiian sling, a hand spear propelled by an elastic cord, spearfishers can target individuals of a specific size and species with high specificity (Gillett and Moy 2006). This is more physically demanding and less efficient than using a net, but can be more efficient and lower bycatch than hook and line. Although some spearfishing happens from boats or on shore, the vast majority of spearfishing is done via diving. Spearfishers practice apnea diving (free diving), where they hold their breath and swim down to the seafloor to hunt. Spearfishing requires more physical ability than other fishing methods: most freedivers cannot dive deeper than 20 to 25 meters, and the type of fish that can be caught is dependent on the diver’s aim and skill. Spearfished populations adapt to the pressure and are more cautious of fishermen (Stamoulis et al. 2019). In regions like Hawai‘i where SCUBA spearing is permitted, spearfishers can stay underwater for more than an hour at 30+ meters. The use of SCUBA equipment allows skilled spearfishers to increase their yield and reduces the depth refuge for fishes compared to regions where only apnea diving is allowed (Gillett and Moy 2006, Lindfield et al. 2014).

Prior studies of the Parupeneus genus show the species are normally smaller and faster growing. Most species reach a maximum size in the 20 - 30 centimeter range, live for five or more years, reach maturity within a year of hatching, and do not show strong seasonal trends in reproduction (Reed et al. 2020). These studies can be helpful for predicting traits in other species in the genus, but reef fish ontogenetics are extremely variable. The Parupeneus genus uses gonochoristic reproduction (two sexes determined during embryonic stages) with only one species known to exhibit hermaphroditism. Life history studies on most goatfish species in Hawai‘i are scarce, and several fisheries are unregulated. Two species, *P. multifasciatus* and *P. porphyreus* are regulated statewide at 7 and 10 inches total length respectively, and a size limit has been set for all goatfish on the island of Maui at 8 inches total length (20 cm). However, the other two Parupeneus species, *P. insularis* and *P. cyclostomus* are unregulated for the rest of the state, including Oahu, which is the most densely populated and most heavily fished.
Parupeneus cyclostomus, the gold saddle goatfish or the moano kali, is distributed throughout the Indo-Pacific but is similarly understudied and unregulated. It occupies reefs between 2 and 125 meters deep, reaches a maximum size of around 50 centimeters, has an average size of around 35 centimeters, and the record weight is around 2.3 kilograms. The fish has yellowish and bluish colors and is generally solitary as an adult. Parupeneus insularis, called the Two Saddle Goatfish or the Munu, is a small species of goatfish endemic to reefs in Hawai‘i. No life history analysis has been conducted for the species (Randall 2004, Echreshavi et al. 2022), and no seasonal or size limits have been set at this time. It is generally dark red, with a white band on the tail, and found as deep as 80 meters. It reaches a maximum of around 30 cm and a maximum weight of around 1.4 kilograms (Randall and Myers 2002).

I conducted a life history analysis of *P. insularis* and *P. cyclostomus*, investigating trends in seasonal reproduction and size at maturity. This represents the first study of these life history traits for these species and one of only a few studies of goatfish life history in Hawai‘i. This research should provide actionable data for fishery managers, could lead to further studies of the species, and adds to the limited body of knowledge about Indo-pacific goatfish. It also represents one of the first investigations of wet mount methodology for the Mullidae family and for the nearshore reef fishery in Hawai‘i. My comparison of the effectiveness of maturity assessment methodologies could help provide an inexpensive technique to inform future life history studies in smaller fisheries. I determined two statistics of size at maturity, the L50 and L95, as well as the seasonality using the gonad condition and GSI at different times of the year. Finally, I aimed to quantify the accuracy of different methods of determining maturity in the target species.

**Methods:**

**Fishing:**
Fish were collected between 2020 and 2023 via spearfishing from a number of reef sites around O‘ahu, Hawai‘i with hand spears and stored on ice until processing. Although the majority of fishing was done via freediving, some samples were collected using standard or closed-circuit rebreather SCUBA to increase the efficiency and depth of sampling. This was supplemented by local fishermen at fishing tournaments or in the community who allowed us to process fish they caught. I participated in spearfishing and sample processing between June and August 2022. Fish prior to or during the summer of 2022 were processed by other researchers working on the project. Fish were sampled year-round to determine seasonality, but sampling was not uniform throughout the year.

**Sample preparation:**
Standard procedures were used to sample specimens after spearing. To process fish, the total length (TL) and fork length (FL) were measured to the nearest millimeter, the total mass was
recorded to the nearest gram, macroscopic observations were recorded, and samples were photographed. As goatfish have forked tails, the fork length, the length from the snout to the middle of the tail, was chosen for research, which is typical for fisheries research. The total length, measured from the snout to the tip of the top fin in the tail, was also recorded. Length will refer to fork length unless otherwise noted. For future analysis, various samples were collected but not analyzed: The sagittal otoliths for estimating age, a pectoral fin clip for genetic / genomic analysis, and the digestive tract for diet. The gonads were imaged and examined to sex the specimen, removed, and weighed to the nearest milligram. Maturity was estimated using macroscopic characteristics of the fish and gonads, and a cross section was taken for histology. Cross sections were placed in histology cassettes and fixed in 10% neutral buffered formalin. The remaining ovaries and indeterminate gonads were then stored on ice until wet mount slides could be prepared.

Wet mount slides were prepared by removing a small section of eggs from the center of an ovary with a scalpel after macroscopic maturity assignment and cassette preparation. These were smeared onto a microscope slide, diluted with saline, and covered with a cover slip. Photographs of wet mounts were taken using a Canon camera mounted onto a Meiji compound microscope at 4X and 10x magnifications.

**Analysis:**
Macroscopic staging was performed while determining the specimen’s sex, using the appearance and characteristics of whole gonads relative to the fish. General assignment of maturity was performed according to the ICES Report on the Workshop on Sexual Maturity Sampling (Figure 2, ICES 2007). Goatfish testes have a triangular cross-section, while female ovaries have a circular or oval cross-section. Mature ovaries generally have an opaque yellowish color with a distinct texture and are always larger than immature gonads. However, a mature fish not currently spawning could have gonads similar to an immature fish. A fish was classified as mature macroscopically if the gonads were enlarged, visibly similar to other mature gonads, or if eggs or semen were visible during processing. Oocytes were usually visible inside a mature gonad beyond the early developing stage. If gonads were small, difficult to locate, dense and rubbery, or dark red, they were considered reproductively immature. When ovaries in this state were located and wet mounted or examined histologically, these fish only had primary growth oocytes. These gonads could be described as “threadlike” or “stringy”, and could have a very small cross-section diameter. Smaller fish with particularly small gonads were considered immature, while larger fish with larger inactive gonads were considered mature but inactive.

Wet mount slides were prepared and reviewed without reviewing assigned macroscopic maturity. Slides or images of slides were assigned maturity using a modified version of the Brown - Peterson series (Brown-Peterson et al. 2011). The presence or absence of large hydrated eggs, the size of the eggs, and the opacity of the eggs were all used as visual cues for the stage of
oogenesis. Primary growth eggs would be dense, clear, and extremely small in a wet mount slide, whereas vitellogenic stage eggs appear larger and grainy. Since wet mount views the entire sphere of the egg, the granulation of vitellogenic eggs blocks most of the transmitted light, causing them to appear dark. Fully hydrated eggs were clear, round, and had distinct oil-drop yolks. Without stains or fixative, wet mounts could not reliably identify atresia, post-ovulatory follicles, cortical alveolar eggs, or tissue structures. In addition, hydrated or late stage vitellogenic eggs could lyse during the mounting process due to mechanical damage by the scalpel or the application of the cover slip; However, the wet mount method could reliably differentiate between primary growth, different vitellogenic stage eggs, and hydrated eggs, which was sufficient to assign maturity. Although post-ovulatory follicles and hydrated eggs can be confused during wet mount analysis, both provide evidence of maturity. Slides were not preserved or fixed, but images were taken of all wet mount slides, and verified between different researchers. Although the macroscopic and microscopic analysis was conducted separately, comparisons of immature and mature fish using both microscopic and macroscopic images were used to train researchers.

Preserved female gonads were mounted and stained using H&E stain by the Histology Core at the John A. Burns School of Medicine, University of Hawai‘i (NIH U54MD007601). Histology slides were analyzed and staged at the Virginia Institute of Marine Science using the protocols described by Brown-Peterson et al (2011). Histology slides were staged in August and September of 2022, while most wet mounts were reviewed in May - June. Slides were selected randomly and assessed without knowing the size of the fish or prior estimations of maturity; this helped blind the observer to potential bias but was not a fully blinded process. Mature fish were fish for which there were multiple vitellogenic or higher-stage eggs or histological evidence of prior reproduction (i.e., Stage 2 of the Brown-Peterson scale). After assessment, histology slides were reviewed by other researchers at the Virginia Institute of Marine Science. All maturity assessment of each type was performed or reviewed by the author as part of this honors project.
Figure 4: Staging legend and examples for *P. insularis*

Examples of macroscopic ovary images, wet mounts, and histology images for assigning maturity. All samples are *P. insularis*, scale bars represent 100 microns. 1A-1C are from an immature fish 224 mm fork length and 229 grams, with a GSI of 0.55. Row 2 is a mature fish 241 mm fork length, 344 grams, with a GSI of 1.44. 3A-3C is a mature fish 196 mm fork length, 179 grams, with a GSI of 1.96. Columns A and B show macroscopic staging information. 1A and 1B shows small red gonads are visibly underdeveloped, with no eggs visible inside. 2A and 2B shows gonads with more visible texture - the pebbly or cloudy texture inside indicates eggs. 3A and 3B, the yellow color, engorged size, pillowy shape, and cloudy opacity indicate hydrated eggs. Column C shows wet mount slide images: 1C shows tiny eggs that are densely packed, while 2C shows large eggs with visible yolks, and 3C shows several large, dense eggs with oil droplets forming. These are are tertiary stage vitellogenic eggs. Column D shows histology for the same gonads. 1D is densely packed with small eggs, these are cortical alveolar or primary growth eggs, indicative of an immature fish. 2D shows two large vitellogenic eggs, which are late-stage eggs preparing to hydrate and be released. 3D shows a mixture of late-stage eggs at various stages of development. Organisms with patterns similar to 2 or 3 in columns B, C, and D are clearly mature; however, while 3A is visibly mature, 2A is not.
Figure 5: Staging legend and examples for an Immature and Regenerating *P. cyclostomus*

The fish shown in the top panels (1) is a mature regenerating female *P. cyclostomus* collected on March 3rd, 2021, measuring 334 mm and 822 grams. The second row, from left to right, shows gonads (1B), the wet mounts of the gonads (1C), and a histology slide of the eggs found in the gonads (1D). The majority of the eggs in this fish are vitellogenic I or corticol alveolar indicating this fish is developing eggs. The bottom (2) is an immature female *P. cyclostomus* collected on March 24th, 2021, measuring 180mm long and 100 grams. The red circle in 2B
shows the gonads, which are clear and undeveloped. 2C shows a mass of primary growth oocytes while 2D shows the same PG oocytes in purple.

The size at maturity was calculated using the L50, the proportion of mature fish for a given size class. L50 and L95 analysis was performed using the Aquatic Life History package (version 1.0.4) by Jonathan Smart (Smart et al, 2016). This uses a GLM to predict the likelihood of a fish being mature at a given size using histology and wet mount data and graphed against fork length in a logistic regression. Similar calculations were performed to establish a mass at 50% maturity. The maturity of the fish was established using macroscopic and microscopic staging. When available, histology was used to assign maturity, and otherwise, wet mounts were used. If no microscopic assignment was available, maturity was assessed using macroscopic and GSI methods. Stages were used to assign mature or immature based on stage 2 in the Brown-Peterson Scale (Brown-Peterson et al. 2011). Analysis of seasonality, the annual timing of reproduction in the population, was performed using gonad mass and GSI, the ratio of gonad mass to total mass for an individual. GSI was calculated as \( \frac{\text{Gonad Mass}}{\text{Total Mass}} \times 100 \). Due to suggestions of lunar trends in prior studies, analysis of the relationship between GSI and lunar phase was conducted using the package Lunar (version 0.2-1). Data were graphed using the Tidyverse package (version 2.0.0).

Wet mount and GSI / macroscopic maturity assignment methods were compared to histology to assess the effectiveness of different methods. All statistical analysis was conducted in R (version 1.3.959).

**Results:**

**P. insularis:**

The *P. insularis* sample pool used 235 samples collected by collaborators between November 2020 and March 2023. Of these fish, 118 were identified macroscopically as males (50.8%) and 117 as females (49.2%), nine fish were indeterminate as gonads could not be recovered. These fish were too small to be reproductively mature, and are included in the female sample pool for this analysis. Of the samples used in the analysis, 61 were mature and 56 were immature. For female samples, fork length ranged from 134 mm to 279 mm, (mean ± s.d., 212 ± 27.4), and total mass ranged from 53 grams to 550 grams, (mean ± s.d., 235 ± 88.3). Ovarian mass ranged from 0.05g to 25g, (mean ± s.d., 2.7 ± 3.1). For male samples, fork length ranged from 163 mm to 350 mm (mean ± s.d., 248 ± 40.6), and total mass ranged from 89 grams to 1018 grams (mean ± s.d., 392.4 ± 1874). Testicular mass ranged from 0g to 6.67g (mean ± s.d., 0.43 ± 0.7).

Females were generally 36 mm and 157 grams smaller than males. Length-frequency analysis for different size classes of male and female samples showed overlap in the size range of fish (Figure 5A); distributions were found to be significantly different via a two-sided Kolmogorov–Smirnov two-sample test (D =0.47919, P = 1.597e-09). Fork length and total mass showed a clear log-linear relationship for both sexes (female R² = 0.95, male = 0.95; Figure 6A).
I found no clear pattern in seasonality in my samples (Figure 7A). Mature fish with high and low GSIs were found in all months of the year, which is characteristic of tropical species but not of species with clear spawning patterns. Clusters of primary growth oocytes were visible in most specimens, and often a mixture of primary growth, vitellogenic eggs, and hydrated eggs. This trend was characteristic of asynchronous oocyte development (Figure 4). Although statistically insignificant, GSI trended higher in the summer months for fish large enough to reproduce. Although some trends in the GSI based on the lunar phase and time of year appear to be present, statistical analysis was not feasible due to uneven sampling distribution and low per-category sample size (Figure 10).

Size at Maturity analysis was conducted for female specimens only. For *P. insularis*, female length at 50% maturity (L50) was estimated to be 187 mm FL (95% CI, 177-196 mm)) and the length at 95% maturity (L95) was 244 mm FL (95% CI 225 - 267 mm; Figure 8A). The estimated mass at 50% maturity was 161 grams (133 - 184 grams). The analysis was conducted in the package AquaticLifeHistory in R, using 2500 iterations.

*P. cyclostomus*:
I evaluated 115 *P. cyclostomus* samples. Of these, 65 fish were identified macroscopically as males (56.5%) and 50 as females (43.5%), seven fish were indeterminate as gonads could not be recovered. These fish are included in the female sample pool. For female samples, fork length ranged from 105 mm to 399 mm, (mean ± s.d., 254 ± 63), and total mass ranged from 24 grams to 822 grams, (mean ± s.d., 359 ± 214). Ovarian mass ranged from 0.02g to 20g, (mean ± s.d., 4.75 ± 6.22). For male samples, fork length ranged from 223 mm to 438 mm, (mean ± s.d., 334 ± 60), and total mass ranged from 221 grams to 1826 grams, (mean ± s.d., 839 ±440). Testicular mass ranged from 0.00g to 5.06g, (mean ± s.d., 0.83 ± 1.12). Females were generally 80 mm shorter and 480 grams smaller than males. Length - frequency analysis for different size classes showed overlap in the size range of fish (Figure 6B) and their distributions were found to be significantly different via a two-sided Kolmogorov–Smirnov two-sample test (D = 0.515, P = 5.676e-05). A log-linear relationship between fork length and total mass was clear for both sexes (female R² = 0.95, male = 0.96; Figure 7B). Analysis again showed 100% accuracy between wet mount and histology methods.

Due to a low sample size and uneven sampling, there was insufficient evidence to establish seasonality with the *P. cyclostomus* samples available (Figure 8b). GSI appears to peak for large fish with developed and developing / regressed gonads in the summer months, but due to the low distribution evenness, the sample size was too small to meet the assumptions for a statistical test. A wide distribution of egg stages was found in the ovaries of *P. cyclostomus* females at different sizes and stages. Although statistically insignificant, there was an increase in GSI in the summer
months for fish larger than the L50. Although some trends in the GSI based on the lunar phase and time of year appear to be present, statistical analysis was not feasible due to uneven sampling distribution and low per-category sample size (Figure 7b).

For *P. cyclostomus*, the female length at 50% maturity (L50) was estimated to be 250 mm FL (95% CI, 236-261 mm)) and the length at 95% maturity (L95) was 294 mm FL (95% CI 225 - 267 mm; Fig. 7b). The estimated mass at 50% maturity was 320 grams (278 - 356 grams). Predictive models in AquaticLifeHistory used 1000 iterations.

For *Parupenius insularis*, the L50 is estimated to be a fork length of 187 mm (95% CI: 177 - 197 mm), and L95 is estimated to be 245mm (225mm, 267 mm). In total length, this is a L50 of 214 mm, (95% confidence interval: 204 - 225 mm), and a L95 of 277mm (95% CI: 256, 303mm). For *Parupenius cyclostomus*, the L50 is estimated to be a fork length of 250 mm (95% CI: 237 - 262 mm), and L95 is estimated to be 295mm (273mm, 316 mm). In total length, this is a L50 of 285 mm, (95% confidence interval: 270- 295 mm), and a L95 of 330mm (95% CI: 308, 350mm).

Figure 6A:
Figure 6B.

**Figure 6:** (A) Density distribution of *P. insularis* sizes. The female sampling distribution trended smaller, with a greater density of samples between 200 and 250 millimeters, while male samples skewed larger, peaking between 250 and 300 millimeters. Significant differences were found in the distributions based on a two-sided Kolmogorov–Smirnov two-sample test (*D* = 0.47919, *P* = 1.597e-09) (B) Density distribution of *P. cyclostomus* sizes. The female sampling distribution trended smaller, with a greater density of samples between 200 and 300 millimeters, while male samples skewed larger, peaking between 300 and 400 millimeters. A two-sided Kolmogorov–Smirnov two-sample test (*D* = 0.515, *P* = 5.676e-05) showed significant differences in the sampling distribution.
Figure 7A:

Fork Length vs Total Mass

F

$y = -11 + 3x, R^2 = 0.95$

M

$y = -11 + 3x, R^2 = 0.95$

Figure 7B:

Fork Length vs Total Mass

F

$y = -9.7 + 2.8x, R^2 = 0.95$

M

$y = -11 + 3x, R^2 = 0.96$
Figure 7. Fork Length Vs Somatic Mass of samples. Size and mass increase linearly on a log-log transformed scale. Relationships were significant ($R^2 > 0.95$, $P < 0.05$) for females and males *P. insularis* (A) and *P. cyclostomus* (B).

![Reproductive Seasonality (Females > 180 mm)](image1.png)

A:

![Reproductive Seasonality (Females > 200 mm)](image2.png)

B: Figure 8. Distribution of mature sized samples throughout the year
This graph shows the month of sample collection and GSI for all female samples above the L50 for the species (Panel A shows *P. insularis*; Panel B shows *P. Cylsostomus*). Color indicates whether a species was classified as mature or immature microscopically. An immature fish with a high GSI is likely to have just completed spawning. *P Cylsostomus* (PACY) appears to have some trend by season, peaking in the spring and summer. The distribution of mature and immature specimens of different GSI at different stages is consistent with an asynchronous reproductive scheme. While there is some decrease in the Autumn months, the sampling distribution is uneven and insufficient to make statistical tests.
Figure 9: Length at Maturity ogives for *P. insularis* (A) and *P. cyclostomus* females (B). The red line estimates the length at which 50% of fish were mature in this study, while the blue line estimates the length at which 95% of fish were mature. Parentheticals show the 95% confidence interval for the value. The shaded region around the line is the 95% confidence interval, while black points represent the maturity of individual fishes according to microscopic analysis. *P. insularis* has an L50 of 187mm and an L95 of 255mm. *P. cyclostomus* has an L50 of 250 mm and an L95 of 294 mm.
Figure 10.
GSI vs Lunar Phase and Season for *P. insularis* specimen above 180 mm. Points represent the GSI for individual samples at different phases of the moon. As males are on average larger than females for both species, it is assumed that the male L50 is greater than the female L50, so all specimens included in this analysis would be mature. Sampling is too uneven to meet assumptions for statistical inferences for *P. cyclostomus*. *P. insularis* appears to have lunar trends within seasons.

**Maturity assignment:**
Histology slides were assessed for 51 female *P. insularis* samples and a wet mount maturity assessment was conducted for 117 female or indeterminate samples. Of the female samples analyzed using the wet mount method, 56 were immature, and 70 were immature. Of the 51 ovary cross-sections were analyzed histologically, 30 specimens were mature and 21 were immature. All fish classified as immature using histology were classified as immature using wet mounts and all fish classified as mature from histology were classified as mature from wet mounts. For *P. cyclostomus*, 126 female samples were analyzed using the wet mount method. Of these, 56 were immature and 70 were mature. Ovary cross-sections were analyzed histologically for 43 samples, with 25 mature and 18 immature. All fish classified as immature using histology were classified as immature using wet mounts and all fish classified as mature from histology were classified as mature from wet mounts. Results are shown in Table 1.
<table>
<thead>
<tr>
<th>Histology Stage</th>
<th>Immature (Wet Mount)</th>
<th>Mature (Wet Mount)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 or 2 (Immature)</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>3, 4, or 5 (Mature)</td>
<td>0</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 1: Table of false and true positives for wet mounts.

**Discussion:**
This study has established fundamental life-history information for two species of goatfish from the recreational coral reef-fish fishery primarily in Oahu, Hawai‘i.

Life-history information for these species in Hawai‘i is largely undocumented, and there is no prior description of their demography (Longenecker & Langson, 2008; Randall 2002). Future research should include an analysis of age-length relationships and population structures using otoliths (Longenecker & Langson, 2008). In addition, analysis of diet from preserved digestive tracts and genomes from fin clips may be helpful for understanding the ecology and evolution of the target species. *P. insularis* closely related to several other species of goatfishes (Randall and Myers 2002). Genetic and genomic analysis may provide insights into the evolutionary history and spread of the *Parupeneus* genus. The life history analysis demonstrated in this study may provide data to support establishing regulations for the fishery.

**Regulatory impact:**
The existing regulations set a size limit for all goatfish on the island of Maui at 8 inches (20.3 centimeters). My data indicates that 50% of *P. insularis* females reach maturity below this limit, but *P. cyclostomus* reaches maturity at 250mm, or 10 inches. The blanket approach to goatfish size limits in Maui is not appropriate for *P. cyclostomus*. Existing regulations for *P. cyclostomus* will likely have to be adjusted.

Seasonality estimates may require more sampling in the future, but my data currently suggest that *P. insularis* and *P. cyclostomus* do not have a clear and distinct spawning season. Since tropical fish can have complex reproductive patterns, reliably determining seasonality can be difficult. Our sample pool was weighted towards mature individuals. Many similar studies on Parapeneus goatfish have noted a skew towards larger specimen, but have generally found their samples to be representative of the population being described. Samples in this study frequently showed a mixture of primary growth and early vitellogenic eggs at various sizes. This is indicative of asynchronous spawning, consistent with other members of the genus.

In our samples, several individuals were reproductively inactive but developmentally mature based on the presence of limited vitellogenic oocytes and densely packed primary growth oocytes, consistent with regenerative phase development as described by Brown - Peterson et al, (2011). Research on these goatfish must take into account the gonad condition, GSI, and size of
the fish when assigning maturity. While a distinct spring/summer spawning season was not evident, it is plausible that other environmental cues trigger reproduction for these fish, or that more detailed studies are required. Longenecker (2008) identified the lunar cycle as a trigger for reproduction for multiple species in the reefs of Hawai‘i during the spring. The lunar cycle has been documented as a significant factor in predicting GSI for other species of Goatfish but is sometimes insufficient for Parapeneus goatfish (Reed et al. 2020). Since the lunar phase influences the relative height of the tide, these species may be spawning based on a tidal signal (Ikegami et al. 2014). This corresponds with the native Hawai‘ian lunar calendar, which relates different phases of the moon to fish catch and spawning (Poepoe et al. 2001, Jokiel et al. 2010). Investigating trends based on traditional knowledge should be an area of interest for future research.

**Maturity Assignment Methodologies:**
For smaller scale or lower budget studies, and for studies occurring in environments with limited resources, wet mount slides are a cost-effective way to study the reproductive characteristics of female fish and to assign maturity. Compared to histology, preparing and examining wet mount slides requires less time, training, and financial resources. A researcher simply needs a microscope and a box of standard glass slides for wet mount research, while histology requires specialized equipment, hazardous materials, and trained histologists. Macroscopic assignment of maturity was less reliable at this sample size - researchers expressed low confidence in some assessments and there was disagreement and confusion at times during maturity assignments. Due to the sample size and low seasonality, drawing clear conclusions using GSI or macroscopic assignment may be more difficult or result in larger confidence intervals. Additionally, macroscopic methodology is less statistically sound for a size-based analysis because researchers make judgments considering the size of the fish and the size of the fish’s gonads, introducing a bias in the relationship between maturity and size. As such, the primary concern is the difference in quality compared to the microscopic methods. For studies with similar target species and sample sizes, wet mounts should be a higher accuracy method without a significantly higher cost.

Histology has several advantages over wet mounts and remains the gold standard for life history analysis. Histology samples are fixed and preserved - samples do not need to be prepared and reviewed immediately as in wet mounts. Histology slides can be reviewed at a later date by other researchers, or reviewed for other studies, or new slides can be prepared from paraffin-embedded tissue blocks using different stains. The preservation of histology slides can be useful for future studies, but accurate imaging of wet mount slides can serve a similar purpose. Histology slides distort structures less, taking a fixed cross section of a sample, while wet mounts distort intact cells with a cover slip and displaying layers of intact oocytes. As a result, histology offers a more extensive set of data including cell measurements and quantification, identification of parasites and diseases, or analysis of unusual tissue structures. Wet mounts cannot identify signs of maturity like post ovulatory follicles, cortical alveolar eggs, or atresia. These are indicative of
past or recent spawning, i.e. maturation at an earlier size. As such, wet mounts do not offer the same level of depth and accuracy as histology, and so are not useful for all types of research questions addressing maturity.

Although histology can provide more information about the samples, data provided by wet mounts was sufficient for the analysis required in this study. In major commercial fisheries with complex, data-driven regulations, histology presents few downsides. So long as infrastructure and funding are available, histology is the optimal method for performing life history analysis (Winemiller 2005, Prince and Hordyk 2019, Ovando et al. 2021). For researchers without the equipment needed to fix and stain their own histology slides, most hospitals have a histopathology lab facility capable of processing samples. These facilities perform very high-quality work but generally have longer turnaround times and higher costs per slide, and may not have the capacity to process samples for research. The histology slides used in this study were prepared by the Research Corporation of the University of Hawai‘i, and were of excellent quality; however, due to the cost and time required for processing, we chose to only process our female samples. Histology presents other logistical challenges, such as samples storage and preservation, usage of hazardous materials, and training in histological techniques. These aspects may consume valuable time during a limited sampling season.

In future life history studies, researchers could use wet mounts for assigning maturity alongside or instead of histology. Wet mounts are faster, cheaper, safer, and accurate enough to assess maturity for female specimens. The wet mount technique can assign maturity to samples in field environments more easily, as it only requires access to a microscope. For a study looking to do initial life history analysis of a species, likely with GSI, but without the resources for a large sample pool, wet mount analysis of females could provide the initial data needed to assess seasonality and L50. The sample sizes needed for GSI studies are much larger than those needed for microscopic analysis. For species that are difficult to sample due to factors like overfishing and species rarity, wet mount offers an effective middle ground between histology and macroscopic methods. This could establish size limits or seasons more quickly and easily, and inform future studies if needed. Researchers can incorporate wet mount analysis into their initial workup, and still prepare histology cassettes if desired. The opportunities provided are of particular interest to fisheries researchers that find histology to be a significant logistical or financial challenge, fisheries managers who need to spread their sampling efforts across multiple species, and researchers who cannot acquire enough samples for GSI analysis. To process a few hundred samples, a researcher might spend multiple days and a significant portion of a grant fixing and staining samples instead of sampling in the field or exploring another dimension, like estimating chronological age via otoliths (Heher et al. 2016). The opportunity cost in smaller studies is significant, as a single day of sampling can add a significant amount to a sample pool. However, some training and proficiency is required to effectively evaluate maturity status using wet mounts.
For tropical fisheries with many species to analyze and manage, despite the same or fewer resources with which to do so, basic life history analysis will benefit from wet mount methods. With a smaller sample size and smaller budget, wet mounts are a better way to determine size at maturity than GSI or histology. The increase in efficiency and expanded access to quality data has the potential to be a useful tool for fisheries around the world.

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other than fishing. Ecological Indicators 96:579–590.


Size limits can be found at:
Appendix A:
Additional graphs:

Parapeneus insularis (top) and cyclostomus (bottom) Fork Length vs GSI.
Parapeneus insularis (top) and cyclostomus (bottom) Fork Length vs Total Length.
L50 curves with fall samples omitted. *Top is P. insularis*, bottom is *P. cyclostomus*. 