Report to the Division of Aquatic Resources
Dingell-Johnson Sport Fish Restoration

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Size-dependent and age-based female fecundity and reproductive output for three Hawaiian goatfish (Family Mullidae) species, *Mulloidichthys flavolineatus* (yellowstripe goatfish), *M. vanicolensis* (yellowfin goatfish), and *Parupeneus porphyreus* (whitesaddle goatfish)

The goals of this project were to address current deficits of knowledge regarding aspects of reproductive biology and life history for three Hawaiian goatfish species *Mulloidichthys flavolineatus* (yellowstripe goatfish), *M. vanicolensis* (yellowfin goatfish), and *Parupeneus porphyreus* (whitesaddle goatfish). Specifically, the activities of this project as proposed were as follows:

(i) collect females of each species throughout Fall 2008 and identify when spawning ends in order to establish the duration of the 2008 reproductive season for each species;

(ii) continue collections throughout Spring 2009 to determine the timing of the start of the 2009 reproductive season and compare this with the timing of the 2008 onset, in order **to identify possible annual variation in the timing of onset** (and by inference, possibly the duration) **of successive reproductive seasons**;

(iii) target small, medium-sized and large adults at the beginning of the 2009 reproductive season to increase existing sample sizes for size/fecundity analyses in order **to improve the predictability of instantaneous female fecundity estimates** based on size alone; and

(iv) carry out an otolith-based size-at-age analysis to **develop growth curves and growth rate estimates, estimates of age at first maturity, typical life span, and age-based variation in female fecundity**.

Three species of goatfish were investigated for this project: the yellowstripe goatfish, *Mulloidichthys flavolineatus*; the yellowfin goatfish, *Mulloidichthys vanicolensis*; and the whitesaddle goatfish, *Parupeneus porphyreus*. Because of difficulties obtaining sufficient sample sizes from natural populations in the field, the majority of specimens were obtained from local fish markets. Each of three species was sampled monthly for six months (October 2008- March 2009) to establish temporal boundaries of the spawning season. All collected fish were measured (TL, standard length or SL, fork length or FL), then the gonad removed; both gonad and remaining body weight were independently weighed and recorded in order to calculate the gonadosomatic index (GSI).

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Figure 5: SEM photograph of a ground and polished transverse section of a female *P. porphyreus* measuring 19 cm FL. White arrowheads indicate daily circuli.

Preliminary otolith examination indicated that otolith circuli may be used to obtain good estimates of fish age (Fig. 5).

Otoliths were removed, cleaned of soft tissue, dried and stored. Gonadal tissues were fixed in formalin-based Dietrich's fixative. Both gonadal tissues and otoliths were identified by a matching catalogue number unique to the fish from which they were removed. Aging was carried out by first grinding and hand-polishing otoliths (protocol modified from Santana et al., 2006) then using both scanning electron microscopy (SEM) and light microscopy to image the otoliths and to provide circuli counts to generate age estimates.

Age-size and age-growth curves were constructed by co-plotting otolith-based age estimates with fork and standard lengths. A subset of fish from each sampling period were also be used for gonad histostructure analysis.

Histological and GSI data were compiled to meet the following goals:

- characterize gonadal state (immature, inactive, active) of collected females using oocyte characteristics established in the 2007-08 DAR Dingle-Johnson funded study to develop estimates of the proportion of reproductively active females present in the female population;
- document the occurrence of spawning activity, based on the presence of mature gametes and/or post-ovulatory follicles resulting from recent ovulation, to determine the onset of the 2009 spawning season; and
- calculate instantaneous fecundity among reproductively active females based on counts of mature ova per unit gonadal weight to add to the existing data set to improve current estimates.

Age estimates from otolith analyses were used to:

- develop species-specific female age/length relationships and growth curves;
- based on age/length relationships, calculate species-specific age/fecundity relationships; and
- determine earliest age of maturation for all three species

FECUNDITY-BASED DATA ANALYSES AND RESULTS

Length of the annual spawning season

The three goatfish species examined in this study all exhibited spawning seasonality, but showed slightly different seasonal patterns. The spawning period of the yellowstripe goatfish (*M. flavolineatus*) in 2008 was from April through June (Fig. 2). Over this same time period, the yellowfin (*M. vanicolensis*) had an extended spawning season, being reproductively active almost year-round, with lowest female fecundity occurring in the months of November, December and January (Fig. 3). During 2008, the whitesaddle (*P. porphyreus*) was only reproductively active from January through March (Fig. 4).

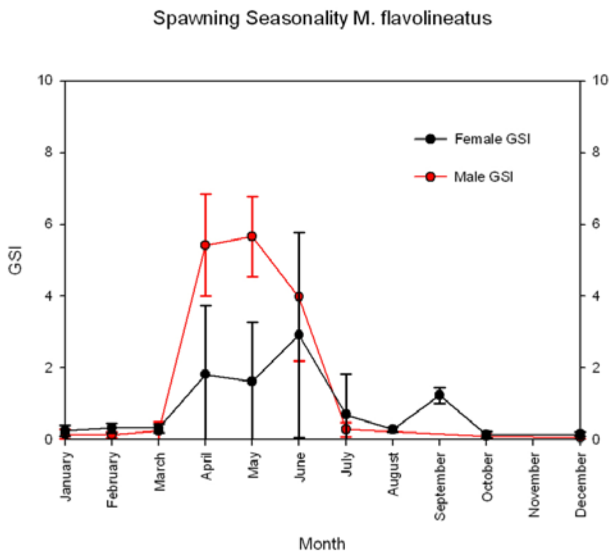

Figure 2. Female and male *Mulloidichthys flavolineatus* monthly GSI during 2008.

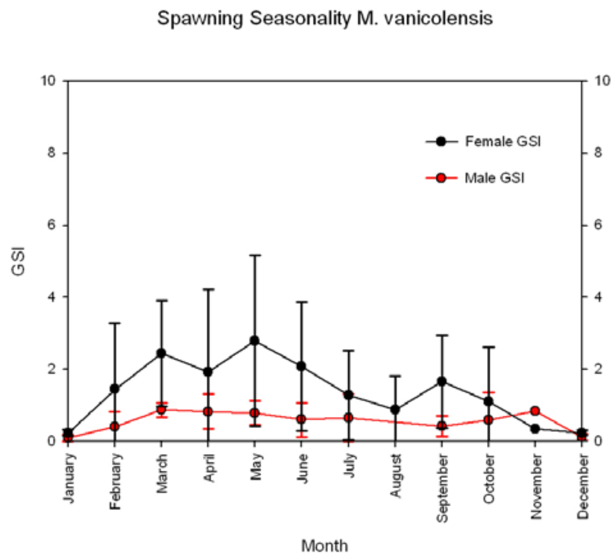

Figure 3. Female and male *Mulloidichthys vanicolensis* monthly GSI during 2008.

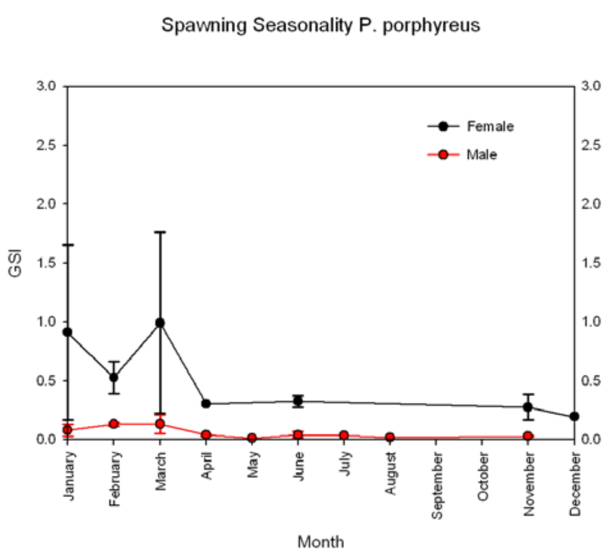

Figure 4. Female and male *Parupeneus porphyreus* monthly GSI during 2008.

Annual variability in the onset of the spawning season

An absence of sufficient data for *M. flavolineatus* in both 2007 and 2009 precluded making estimates of annual variation in spawning season timing (Fig.5). *Mulloidichthys vanicolensis*, for which there was a larger data set for all three years, showed similar patterns of spawning season onset and some variability over two years for winter declines in spawning activity (Fig.6). Due to extremely low sample sizes, no inter-annual analysis was possible for *P. porphyreus*.

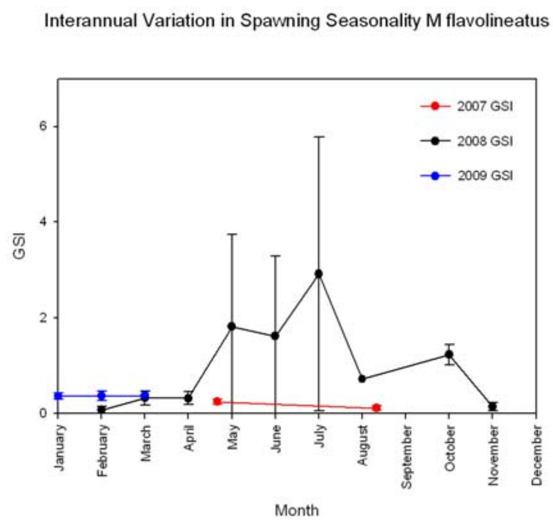

Figure 5. Female *M. flavolineatus* monthly GSI's during summer 2007, all of 2008 and early spring 2009.

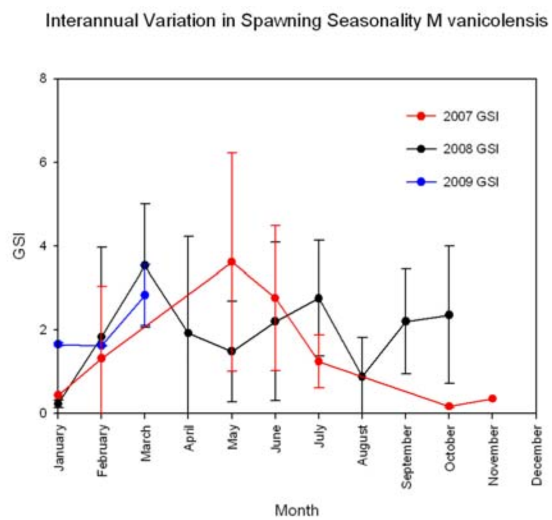

Figure 6 Female *M. vanicolensis* monthly GSI's during 2007, 2008 and early spring 2009.

Estimates of instantaneous fecundity

The relationships between batch fecundity and fork length for both *M. vanicolensis* and *M. flavolineatus* females were best explained by a power function indicating that batch fecundity increases exponentially with an increase in fish length (Figs. 7 and 8) . Due to a very low sample size of mature females, batch fecundity was determined for only two *P. porphyreus* females, with 15,141 and 4,002 ripe oocytes (24 cm FL and 22.5 cm FL respectively). There was considerable variation in batch fecundity among females, ranging from 20,339 to 279,217 ripe oocytes for *M. vanicolensis* and ranging from 44,898 to 379,772 oocytes for *M. flavolineatus* (Figs. 7 and 8) indicating that females within the same size likely do not have equal reproductive output.

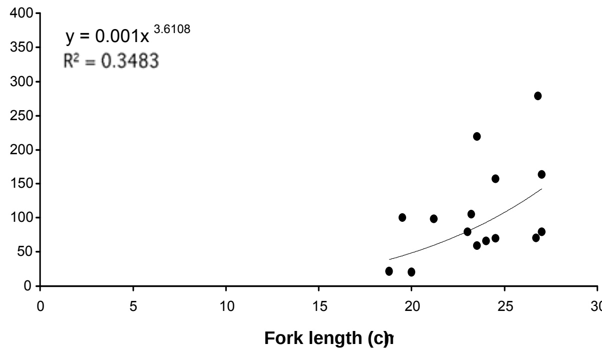

Figure 7. Batch fecundity vs. fork length for *M. vanicolensis*, n=16.

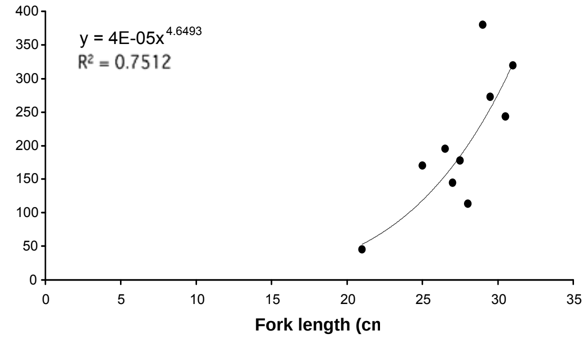

Figure 8. Batch fecundity vs. fork length for *M. flavolineatus*, n=10.

Otolith Aging-based Data Analyses

A single sagittal otolith from 50 *Mulloidicthys vanicolensis*, 41 *Mulloidicthys flavolineatus* and 47 *Parupeneus prophyreus* was weighed, sectioned and read following the methods of Choat and Axe (1996) and Claisse et. al. (In Press).

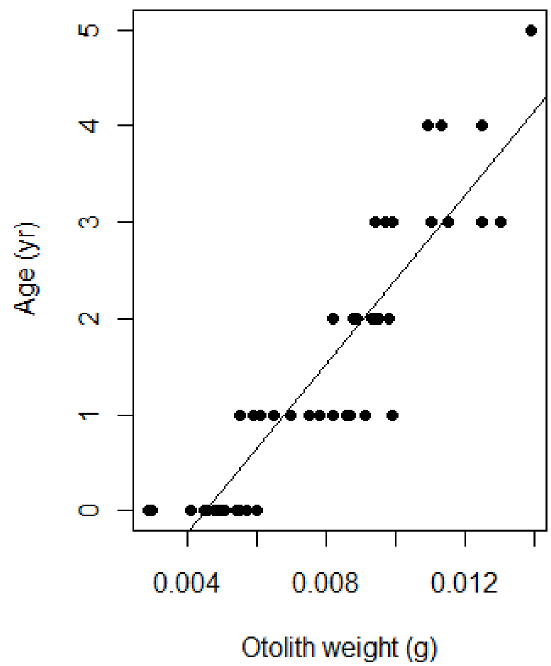

Figure 9. *Mulloidicthys vanicolensis*. Age vs. otolith weight linear regression (R^2 = 0.83, $p < 0.05$).

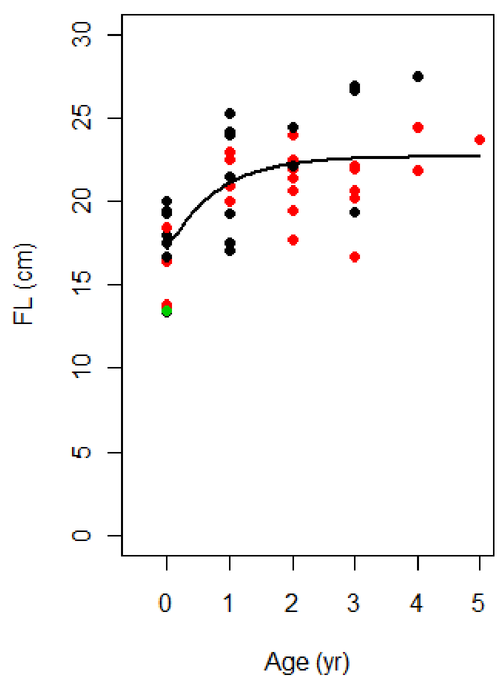

Figure 10. *Mulloidicthys vanicolensis*. Size vs. age relationship: females (black circles), males (red circles), unidentified sex (green circle) and VBGF (black line). Note: mean size at age 0 may be over-estimated due to limited sampling of smaller individuals.

Mulloidichthys vanicolensis otoliths displayed a clear pattern of increments that were assumed to be annual. While annual periodicity of increment formation has not been validated for *M. vanicolensis,* it has been for surgeonfish (Claisse et al. In Press) and parrotfish (Howard 2008) in Hawaii, and *M. vanicolensis* increments had a very similar structure.

There was a strong relationship between estimated *M. vanicolensis* age and otolith weight (Figure 9, linear regression: $R^2 = 0.83$, $p < 0.05$) which supports this assumption. The pattern of increments exhibited by *M. flavolineatus* and *P. prophyreus* otoliths were very irregular, providing inconsistent readings and therefore did not permit provide consistent readings and therefore did not permit age estimation.

The size vs. age relationship for *M. vanicolensis* (Figure 10) was modeled with the von Bertalanffy growth function (VBGF). VBGF parameter estimates were: $l_\infty = 22.7$, $k = 1.3$, and $t_0 = -1.1$. The mean size at age 0 (i.e. mean size of individuals less than 1 yr) may be over-estimated due to limited sampling of smaller individuals. While there was some evidence of sexual size dimorphism (i.e., females larger than males), there was not adequate data to fit a separate VBGF to each sex.

The relationships between gonad weight and age (Figure 11), and gonado-somatic index (GSI) and age (Figure 12) were also plotted. There appears to be an increasing trend both for males and for females starting at age 1.

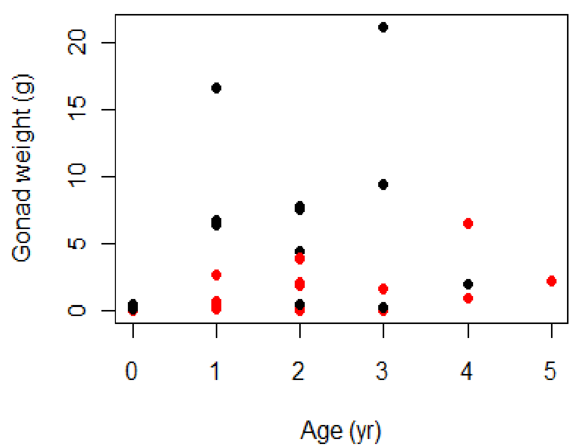

Figure 11. *Mulloidicthys vanicolensis*. Gonad weight vs. age: females (black circles) and males (red circles).

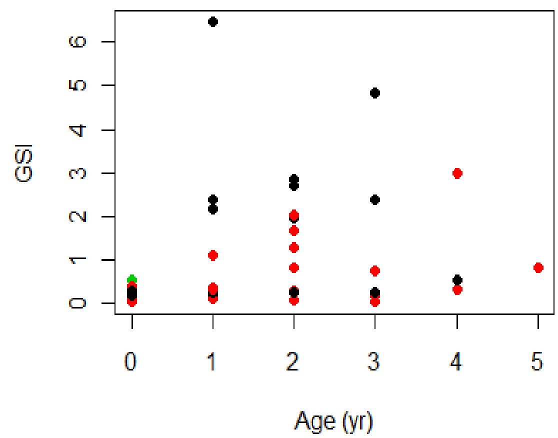

Figure 12. *Mulloidicthys vanicolensis*. GSI vs. age: females (black circles), males (red circles) and unidentified sex (green circle).

Otolith Aging Summary

Unvalidated aging based on otolith circuli counts indicate that individuals *of M. vanicolensis* can live to at least 5 years of age. In the absence of making circuli counts, otolith weight may be used as a reasonable predictor of age in this species. Growth, as measured by fork length, starts to level off between 1 and 2 years of age. Both gonad weight and GSI show an increasing trend with age.

Species-specific female age/length relationships and growth curves

We were unable to construct species-specific female age/length growth curves due to a lack of robust female specific data for all three species studied. In addition, growth curves were constructed for *M. vanicolensis* only, with compiled data from both males and females. The otoliths sampled from *M. flavolineatus* and *P. porphyreus* females provided inconsistent readings and were unsuitable for ageing.

Species-specific age/fecundity relationships

Species-specific age/fecundity relationships were not constructed for *M. flavolineatus* or *P. porphyreus* because ageing was not completed for those species. From comparing age/length and fecundity/length relationships for *M. vanicolensis* we can conclude that there is a general trend towards increased fecundity with age, however, with a large degree of variation among individual females. For example, a 26 cm (4 year old) female can have nearly 300,000 eggs, whereas an 18 cm (1 year old) fish may only produce 20,000 eggs per batch. Additionally, variation in reproductive output for similar aged females may be due to seasonal affects, nutrition, and overall fish health. More work is necessary to determine the ages of females of known fecundity and those of *M. flavolineatus* and *P. porphyreus* in order to compare among the species.

Earliest age of maturation

Counts of otolith daily microincrements indicated that most females sampled were reproductively mature prior to reaching one year of age (Table 1). These micro-increment counts correspond well with the size versus age relationship constructed from *M. vanicolensis* otolith annuli (Fig. 10), indicating fish less than one year old ranging from 15 to 20 cm in length. The smallest reproductively active females observed were 16.5 cm FL and 18 cm FL for *M. vanicolensis* and *M. flavolineatus* respectively. Three-parameter logistic equations were used to estimate female size at 50% maturity (L_{50}) for *M. vanicolensis* and *M. flavolineatus*, but not for *P. porphyreus* because females smaller than 19.5 cm FL were not obtained. Fifty percent of *M. vanicolensis* females were mature by 17.5 cm FL and 100% mature at 20 cm FL and larger (Fig. 13). For *M. flavolineatus*, 50% of females were mature by 18.5 cm FL and 100% mature at 20 cm FL and larger (Fig. 14). Only one *P. porphyreus* female, measuring 24 cm FL, had a mean microincrement count in excess of 365 (Table 1).

Table 1: Daily micro-increment counts from transverse otolith sections from the smallest reproductively mature females of three Hawaiian goatfish.

Species	**FL (cm)**	**# of Micro-increments**		
		Count 1	Count 2	Average
Mulloidichthys vanicolensis	16.5	233	259	246
	18	210	236	223
	19.5	193	226	209.5
Mulloidichthys flavolineatus	19	303	336	319.5
	19.5	262	333	297.5
	21	285	300	292.5
Parupeneus porphyreus	24	396	445	420.5
	19	276	294	285
	21.8	296	317	306.5

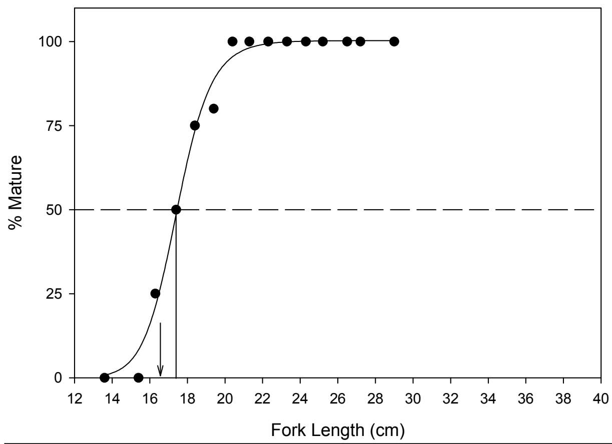

Figure 13. Logistic regression model estimating female size at reproductive maturity (L_{50}) as a function of fork length for *Mulloidichthys vanicolensis* (n = 118); smallest mature female (arrow) was 16.5 cm FL; L_{50} = 17.5 cm FL.

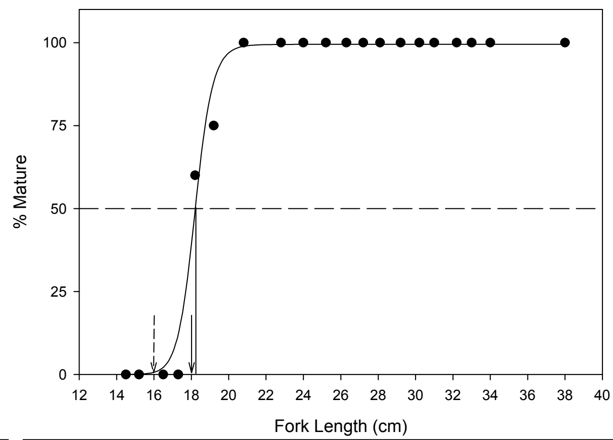

Figure 14. Logistic regression model estimating female size at reproductive maturity (L_{50}) as a function of fork length for *Mulloidichthys flavolineatus* (n = 95); smallest mature female was 18 cm FL; L_{50} = 18.25 cm FL.

These new findings, in conjunction with current data sets derived from the DAR Dingle-Johnson 2007-2008 funded study can be used to develop management and conservation policies designed to protect existing goatfish populations that they may continue to play a role in economic and culture-based fisheries in Hawaii.

Overall Summary:
The development of appropriate protocols and expertise to provide valid estimates of reproductive status and reproductive output is critical to the successful long-term management of commercial and recreational fishery species. Species-specific characteristics of reproductive biology among fishery species are typically determined by collecting gonadal and total weights of individuals throughout the year (expressed as a ratio called the gonadosomatic index, or GSI) in order to determine length of spawning season and size-related variations in female fecundity. This allows for rapid estimates of seasonal reproductive output, but suffers from a lack of information on sources of significant variation in individual fecundity including age, size, condition, operational sex ratio and population density, to identify but a few. In short, all fish are not equal in terms of their ability to produce gametes.

Numerous Hawaiian goatfish species provide both a commercial and recreational fishery in Hawaiian waters, and there is good evidence that mullids respond well to protective measures (Williams et al. 2006). There have been some biological studies that have focused on, or included, one or more Hawaiian goatfish species (e.g. Maha 1969, Hobson 1972, 1974, Moffit 1979, Sorden 1982) but there is little detailed and consistent information on reproductive output. At present, a study in reproductive output, age and growth is underway for the manybar goatfish, *Parupeneus multifasciatus,* (R. Langston & K. Longenecker in prep., R. Langston, pers. comm.) but similar detailed information for other Hawaiian goatfishes is lacking. Documentation of gonad morphology and stage-specific characteristics of developing oocytes and ova among females in each of the yellowstripe (weke'a), yellowfin (weke'ula) and whitesaddle (kūmū) goatfish were carried out with previous DAR Dingell-Johnson funding. Those findings provided a temporal series of gonadal tissues that indicated that the reproductive season for these three species starts in early spring.kj

However, there is still much that is unknown regarding the reproductive biology and reproductive output of these three Hawaiian goatfish species. The results of this DAR 2008-09 grant have significantly increased the predictive power of estimates of size-dependent and age-based female fecundity, species-specific durations for the spawning season and in some cases, annual variation in the onset of the spawning season.

Management significance:

In addition to the detailed reproductive biology findings that have been generated for the three targeted species of goatfish, the results of this project have had two additional important outcomes. The increase in available data generated from the 2008-09 DAR Dingle-Johnson funded study has greatly enhanced our ability to: (i) identify significant sources of variation in reproductive output (female fecundity) associated with size and age; and (ii) identify by size and/or age those females that make the greatest contribution to the reproductive output of the population and therefore should receive the greatest protection.

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