Maturation and Reproduction in Two Hawaiian Eteline Snappers, Uku, Aprion virescens, and Onaga, Etelis coruscans

Alan R. Everson, Happy A. Williams, and Bernard M. Ito

ABSTRACT: Size at sexual maturity, spawning season, and pattern of egg release were determined for two of Hawaii's commercially important snapper species: uku, Aprion virescens, and onaga, Etelis coruscans. Sexual maturity of females was assessed by macroscopic and microscopic (oocyte measurement and histology) techniques and gnosomatic indexes. Interspecific differences were noted in many aspects of the reproductive biology. Both species had protracted spawning seasons: uku spawned in May-October while onaga spawned in June-November. Female size at sexual maturity was 425-475 mm fork length (FL) for uku and 675-725 mm FL for onaga. Both species were determined to be multiple spawners, although the number of batches spawned per season could not be established.

Uku, Aprion virescens, and onaga, Etelis coruscans (Lutjanidae), are species of considerable importance in terms of total landings and value to bottom fish fisheries in southern Japan (Masuda et al. 1975), Guam, the Northern Marianas (Amesbury and Myers 1982), Vanuatu (Brouard and Grandperrin 1985). American Samoa (Western Pacific Regional Fishery Management Council (Council) 1986), and Hawaii (Ralston and Kawamoto 1985). In addition, many other Pacific island nations have subsistence and commercial fisheries for these species. In Hawaii, uku and onaga ranked second and third, after P. filamentosus, in total catch and value among bottom fish species in 1984 (Pooley 1987).

Both species are widely distributed throughout the tropical Indo-Pacific. Uku range from East Africa to Hawaii and from southern Japan to Australia (Allen 1985), and onaga extend to the Atlantic coasts of South America and Africa (Druzhinin 1970). Uku are caught at the surface by trolling gear and at ≤300 m depths by deepsea handline gear (Druzhinin 1970), whereas onaga are restricted to deeper waters between 220 and 320 m. In Hawaii, the greatest portion of the uku and onaga catches comes from the Penguin Bank region, which is southwest of Molokai in the main Hawaiian Islands (Ralston 1979).

Relatively few reproductive studies have been completed for the commercially important bottom fishes of the western Pacific, even though such information represents a critical component of the biological basis of management for the bottom fish and seamount groundfish fisheries in this region (Council 1986). Some information is available on Hawaiian stocks of P. filamentosus (Ralston 1981; Kikkawa 1984), Etelis carbyculus (Everson 1984), and S. dumerili (Kikkawa and Everson 1984), but none is available for uku and onaga. Thus, a study was undertaken to determine the size at sexual maturity, spawning season, and pattern of egg release of uku and onaga. Size at sexual maturity is a particularly important parameter used to assess and evaluate the impact of fishing mortality on spawning stock biomass and to determine levels of optimum fishery yield (Polovina 1987). During this study, we also attempted, within the constraints imposed by our sampling program, to develop an efficient method for determining gonad maturity. A third goal was to discern interspecific differences between the reproductive biology of the two species and to interpret those differences.


MATERIALS AND METHODS

Uku and onaga caught in 1984-86 and 1985-87, respectively, by commercial fishermen using deep-sea hook-and-line gear were weighed and measured for fork length (FL), and their capture locations were noted. Most were caught in the main Hawaiian Islands and were sold through the Honolulu wholesale fish auction. Following sale of the fish, viscera were extracted by the purchasing agent and refrigerated with an identifying tag. Gonad samples were collected later and preserved at the laboratory either in modified Gilson's fluid (Bagenal and Braum 1968) or Bouin's fluid.

Sexual maturity of females was evaluated by several methods. First, ovaries were staged macroscopically and given a preliminary maturity stage designation (Hilge 1977) (Table 1). To refine and confirm these macroscopic designations, at least one of the following three additional microscopic techniques was used: volumetric or cork borer subsampling, which is based upon the size and appearance of individual oocytes, or standard histological examination. Hilge's (1977) table was also used to assign a final stage designation to these ovaries. To avoid confusion, prespawning adults prior to vitellogenesis were classified as stage I immature, and prereproductive individuals as stage I juvenile.

To determine oocyte size-frequency distributions by volumetric subsampling, uku ovaries preserved in modified Gilson's fluid were examined. After adequate time for dissolution, connective tissues were removed, and the remaining "free" ova were placed in a flask, which was then filled with 200 mL of water. A homogeneous distribution of ova was obtained by using a magnetic stirrer (Van Dalsen 1977). A 3 mL sample was then pipetted onto a gridded petri dish and examined under a binocular dissecting scope at 50×. With an ocular micrometer, 100-200 oocytes were measured along their longest dimension. This method precluded the need to measure oocytes from various sites within the ovary to determine spatial homogeneity of development. Maturity stages were assigned based upon the largest oocyte mode and the degree of oocyte transparency (Table 1).

Subsamples of ovaries of both species preserved in Bouin's fluid were taken from the anterior portion with a cork borer and examined under a binocular dissecting microscope at 50×. The average diameter of the largest oocyte mode was determined, and the percentage of each maturity stage present was noted. Oocyte diameter frequency plots were constructed for uku ovaries in various stages of development and compared with similar plots constructed by using oocyte diameter data obtained by the volumetric method.

For histological examination, some ovaries of both species representing various visually identifiable maturity stages were transferred from Bouin's fluid to 70% ethanol. Portions of ovaries from 28 uku and 22 onaga caught at various times of the year were embedded in paraffin, sectioned at 5 μm, stained with hematoxylin, and counterstained with eosin. Each was as-

<table>
<thead>
<tr>
<th>Class</th>
<th>Stage</th>
<th>Oocyte developmental status</th>
<th>Gonad external appearance</th>
<th>Stage classification criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Immature</td>
<td>Oogenesis from oogonium to primary oocyte with cytoplasmic vacuoles beginning to appear</td>
<td>Genital ridge to definite gonad; individual eggs not discernible</td>
<td>Oogonia translucent</td>
</tr>
<tr>
<td>II</td>
<td>Developing</td>
<td>Vitellogenesis</td>
<td>Elongation of the ovary</td>
<td>Opaque yolked oocytes</td>
</tr>
<tr>
<td>III</td>
<td>Ripe</td>
<td>Hydration</td>
<td>Swollen; ovary wall thin</td>
<td>Transparent, ripe ova</td>
</tr>
<tr>
<td>IV</td>
<td>Spent</td>
<td>Atresia, general cell breakdown</td>
<td>Slack; shrinking ovary; ovary wall thick</td>
<td>Residual ova</td>
</tr>
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TABLE 1.—Ovary developmental stage designations used for study of the reproductive cycle of uku and onaga. Designations are adapted from Hilge (1977).
signed a maturity stage based on the criteria in Table 1. Oocytes were histologically identified using information provided in Crossland (1977). Sectioned ovaries also were examined for the presence of postovulatory follicles and oocyte atresia, features used to establish criteria for the estimation of spawning frequency and to separate juveniles from prespawning adults for determination of size at sexual maturity (Hunter and Macewicz 1985).

The results of these four visual methods were compared with gonosomatic index (GSI) values \((\text{gonad weight}/\text{body weight}) \times 100\). Gonosomatic indexes were calculated for both species to provide a rapid but preliminary indication of developmental stage, although an insufficient number of uku testes were obtained. Excluded from this analysis were individuals that had not yet reached size at sexual maturity. Spearman's coefficient of rank correlation (Snedecor and Cochran 1978) was used to ascertain whether a positive relationship existed between GSI and maturity stage during the spawning periods for females of each species.

Size at sexual maturity \((L_{50})\) was defined as the smallest length category in which at least 50% of the individuals were mature (i.e., stage II or beyond, \(\text{GSI} > 1.5\)) during the spawning season. The logistic equation was fitted to the percentage of mature individuals in each size class \((P_S)\) and FL (Gunderson et al. 1980; Ni and Sandeman 1984); that is

\[
P_S = \frac{100}{1 + e^{(aFL + b)}}
\]

where \(a\) and \(b\) are fitted parameters and \(L_{50} = -b/a\). We also calculated the percentage of maximum length (MAXLEN) at which sexual maturity occurred. This ratio has been used to compare proportionate size at maturity for species by habitat type, zoographic province, or depth range (Grimes 1987).

Sex ratios were compiled and examined for significant deviation from unity and to determine whether sex ratio and size (in 50 mm FL intervals) were independent using chi-square statistics. Two-way contingency table analysis was performed to determine whether the sex ratio differed during the year when pooled into bimonthly periods.

RESULTS

Spawning Season

Spawning season was determined from a wide size range of uku and onaga (Fig. 1). The Spearman rank correlation coefficients \((r_s)\) calculated by comparing GSI with stages I–III were \(r_s = 0.6205\) for uku \((P < 0.0001)\), and \(r_s = 0.8685\) for onaga \((P < 0.0001)\), indicating a positive relationship between GSI and stage of development for both species, although the correlation was considerably lower for uku. In addition, the range in GSI values representing stages II and III ovaries was greater for uku than for onaga (Fig. 2). Thus, rather than using GSI as the single method for estimating spawning seasonality by month or fish length, visual staging methods also were used.

Both species reached maturity in the spring and summer and spawned continuously until fall or early winter. Neither species reached stage II of development (vitellogenesis) at any other time of the year. Uku spawning began in May and peaked 1 month later in June, as evidenced by the sharp rise in GSI values and the presence of mature and ripe fish during this time (Figs. 3, 4).
From July to October, the mean GSI values gradually decreased as spawning activity tapered off. By November, all fish examined were either partially or completely spawned (stage IV). Stage IV fish were not found during the spawning season.

In contrast, female onaga began maturing in June. Fully ripe onaga were not found until July, and spawning activity did not peak until October (Figs. 3, 4). The GSI values dropped sharply in November as the incidence of completely spawned and partially spawned individuals abruptly increased. As with uku, completely spawned individuals were not found until the close of the spawning season. Mean monthly GSI values for male onaga reflected a similar pattern.

Size at Sexual Maturity

Uku matured at a substantially smaller size than onaga. Fifty percent of the female uku attained sexual maturity at 425-475 mm FL, as evidenced by elevated GSI's (Fig. 5) and by the percentage of fish judged mature by visual staging (Table 2). By the time the fish reached the
500 mm size class, 100% were mature. The smallest uku with vitellogenic (stage II) ovaries during the spawning season was 429 mm FL (1.27 kg), which is 41.7% of the maximum length (MAXLEN) recorded for the study animals. The smallest individual with ripe (stage III) ovaries was 477 mm FL (1.82 kg) or 46.4% of the MAXLEN. The predicted value of $L_{50}$ obtained from the logistic fit of percentage mature on FL (Fig. 6) was 449 mm FL ($a = -0.3444$, $b = $ ...
Interesting to note was the propensity for decreasing mean GSI’s in uku larger than 600 mm FL (Fig. 5). In contrast, the smallest mature onaga was 522 mm FL (2.22 kg) or 53.9% MAXLEN, and ripe individuals were not encountered until 605 mm FL (3.22 kg) or 62.4% MAXLEN. The predicted value of \(L_m\) obtained from the logistic fit of percentage mature on FL was 663 mm FL \((a = -0.0223, b = 15.462; \text{Fig. 6})\), and 77% of the onaga in the 675-725 mm FL class were found to be at stage II of development or beyond (Table 2). Onaga spawned over a narrower size range (600-900 mm FL; Fig. 5) compared with uku (450-1,050 mm FL; Fig. 5).

Histological examination of ovaries indicated that both species follow a similar pattern of development (Fig. 7). The progression from oogonia to hydration is typical of snappers and has been covered in detail by Crossland (1977) and Wallace and Selman (1981). Postovulatory follicles were not identifiable in tissue sections from ripe (stage III) ovaries of either species. None of the immature (stage I) ovaries examined histologically showed signs of atresia, indicating that these fish had not previously spawned. Identifiably atretic individuals in later stages of development were not observed until the end of the spawning season.

### Spawning Frequency and Pattern of Egg Release

The size-frequency distributions of oocyte diameter were constructed for ovaries of uku caught during the spawning season, using the volumetric method, and were found to be polymodal, suggesting that uku may release multiple egg batches (Fig. 8a). All ovaries possessed a...
Figure 8.—Size-frequency distributions of uku ovaries in various stages of development (cf. Table 1) preserved in (A) Gilson's fluid and (B) Bouin's fluid. Each distribution shown represents a single ovary.

mode of immature oocytes not represented in the figure. Stage II ovaries contained a mode of oocytes at 0.30-0.35 mm, representing various stages of vitellogenesis, while stage III ovaries included this developing mode and another mode (0.50 mm) nearing hydration. A large amount of variation was noted in the relative frequency of oocytes in the most advanced mode of the stage III ovaries. Similar results were exhibited for size-frequency distributions of uku ovaries constructed by the cork borer method. This method allowed delineation of the immature mode as well as the other advanced stages (Fig. 8b). Additional evidence that multiple batches of
The sex ratio of male to female onaga differed significantly ($\chi^2 = 8.99, P < 0.05$) from 1:1 in favor of males (61.4%, $N = 347$ individuals combined). The overall ratio of males to females was 1.59:1. There was a significant preponderance of males within the 50 mm FL intervals from 600 to 750 mm. However, females predominated above 850 mm FL, reaching 100% of the individuals in the 950 mm FL category. The two-way contingency table analysis suggested that sex and month of capture were not independent (Table 3).

This pattern would ensure optimum temperature conditions for developing larvae. A similar post-summer spawning decline associated with changing local environmental conditions was noted by Grimes and Huntsman (1980) for Rhomboplites aurorubens from North and South Carolina and by Everson (1984) for E. carbunculus. The extension of onaga's spawning season into November, with a peak in October, may reflect that the genus Etelis is restricted to much greater depths than are the other reef-associated lutjanid species. Seasonal changes in...
Temperature and photoperiod are much less pronounced at these depths. In Vanuatu, Brouard and Grandperrin (1985) found that seasonal changes in gonad maturation based on GSI values differed among species inhabiting discrete depths.

Grimes (1987) has suggested two distinct spawning patterns for snappers. One is a restricted pattern with spawning centered around the summer months, typical of species associated with continental habitats where peaks in production cycles occur because of nutrient run-off resulting from high rainfall. The opposing pattern is characterized by year-round spawning with peaks occurring in spring and fall, a pattern thought to be typical of less productive insular populations. Grimes (1987) has noted that Cuba and New Caledonia are large islands that follow the continental pattern, with spawning peaks arising during periods of high rainfall. *Etelis carbnculus* (Everson 1984) and *P. filamentosus* (Kikkawa 1984) have also been reported to follow a restricted spawning pattern in the Northwestern Hawaiian Islands. Both uku and onaga in our study also followed the restricted spawning pattern associated with continental habitats. Spawning took place over a protracted period centered around the summer months. Neither species was found in spawning condition at any other time of the year. Since temporal primary production cycles exhibit little seasonal variation throughout the Hawaiian Archipelago (Bienfang and Szyper 1981; Bienfang et al. 1984), the basis for this restricted pattern observed for uku and onaga is unclear. Apparently, the seasonal changes in day length and water temperature in Hawaii provide adequate spawning stimuli.

Interspecies differences in size at sexual maturity also were noted. The slope of the logistic curve fitted to the size at sexual maturity data was considerably steeper for uku compared with onaga (Fig. 6). Uku matured at 450-500 mm FL, with nearly 100% mature above 550 mm FL, and onaga matured at 550-800 mm FL, with 100% mature at 850 mm FL. Size at sexual maturity differed between species in terms of the percentage of MAXLEN at which maturity occurred. Uku began maturing at about 429 mm FL or 42% of their MAXLEN, whereas onaga began maturing at about 522 mm FL or 54% of their MAXLEN. Talbot (1960) reported that male and female *A. virescens* of East Africa reached maturity at 410 mm SL (51%) and 465 mm SL (58%), respectively. *A. virescens* off Vanuatu matured at 440 mm FL, a figure that Brouard and Grandperrin (1985) calculated from a MAXLEN coefficient of 57.6%, which was based upon the average values obtained from 34 tropical fish species from the west coast of Africa. The same coefficient, applied to Vanuatu populations of *E. coruscans*, indicated that sexual maturity was reached at 470 mm FL, although developmental staging data obtained for this species revealed that mature fish were first sampled at 330-380 mm FL. The actual size at which maturity commenced in all of these locations agreed closely with our data for uku in Hawaii, while the percentage of MAXLEN values differed considerably. However, Hawaii and Vanuatu populations of onaga matured at substantially different sizes.

Disparities in size at sexual maturity between areas may reflect differences in resource utilization and growth allocation. Grimes (1987) calculated the average percentage of MAXLEN at which sexual maturity occurred for lutjanid populations occupying similar zoogeographic locations and habitats. Insular and continental populations had average MAXLEN values of 51 and 43%, respectively, while the deep (> 91 m) and shallow (< 91 m) species were calculated at 49 and 43%. The MAXLEN value of 42% calculated for the study population of uku indicates that this species fits the shallow, continental pattern. As previously mentioned, onaga are found at much greater depths and therefore seem to be less influenced by continental effects than uku. These observations are substantiated by the fact that the MAXLEN value of 54% calculated for onaga conforms closest to the deep, insular pattern reported in Grimes (1987). He reasoned that these anomalies may result from regional differences in food production. Fish from a relatively resource-rich environment may mature at a proportionally smaller size than fish in less productive habitats. He further speculated that selection may favor maturation at a larger maturing size in insular regions because the cost of year-round spawning may be higher in these areas.

Estimates of von Bertalanffy growth parameters, derived from weight-frequency distributions for uku and onaga landed in Hawaii in 1984-86, indicate that uku mature at about ages 4-5 (429 mm FL), while onaga begin maturing at ages 5-6 (522 mm FL) (Ralston and Kawamoto, fn. 1). In the same study, Ralston and Kawamoto (fn. 1) calculated the size at entry to the fishery
as 650 mm FL for uku and 450 mm FL for onaga in the main Hawaiian Islands, indicating that, for onaga, the present fishery is capturing individuals that have not yet reached sexual maturity. Continuing this practice could lead to a serious decline in spawning stock biomass (Polovina 1987).

Sex ratio also differed between the two species. The ratio of male to female uku was judged not significantly different from the expected ratio of 1:1. In contrast, the onaga sex ratio was significantly different from unity in favor of males. Females dominated in the larger size classes for both species. The preponderance of large females has also been reported for other snapper species, including Lutjanus synagris (Reshetnikov and Claro 1976), R. aurorubens (Grimes and Huntsman 1980), and E. carbrunculatus (Everson 1984). This phenomenon is thought to be due to differential mortality of the sexes rather than to growth (Wenner 1972; Grimes and Huntsman 1980). The preponderance of male onaga in the smaller size ranges is more difficult to explain and may reflect intersexual behavioral differences. If smaller males feed more aggressively, they would be overly abundant in the catch. Differences in feeding behavior may also explain monthly variations in sex ratio reported for both species. The ratio of male to female uku increased markedly at the close of the spawning season, suggesting a heightened vulnerability to the fishing gear owing to what may be greater nutritional demands of postspawning females. Seasonally, the largest catch of uku occurs in summer (May-October), when fish are thought to form spawning aggregations (Ralston, fn. 2).

Numerous investigators have suggested that snappers are multiple spawners, based upon the presence of multiple size modes of developing oocytes (Min et al. 1977; Grimes and Huntsman 1980; Everson 1984; Kikkawa 1984; Grimes 1987). Other evidence reported as substantiating this phenomenon has been the wide variations exhibited in GSI's of L. griseus (Starck and Schroeder 1970) and R. aurorubens (Grimes and Huntsman 1980) during the spawning season. Ralston (1981) suggested that P. filamentosus is a multiple spawner because the ovaries of ripe females make up only about 4% of the total body weight, a relatively small percentage compared with that of a single spawning temperate species. These observations, the presence of multiple size modes of developing oocytes and the wide variations in GSI's, were noted for uku and onaga and suggested that these species also spawn repeatedly during the spawning season. Although it has been documented that the oocyte size-frequency distribution of many snapper species contains two or three distinct modes, the exact number of batches spawned per season is rarely reported. This is because the process of recruitment from the undifferentiated oocyte pool is dynamic and difficult to characterize (Grimes 1987).

Much of the above evidence is largely contingent on the assumption that multiple oocyte modes continue to develop and are successively spawned. Fouche and Beamish (1980) observed that, for Pacific hake, Merluccius productus, from the Strait of Georgia, oocyte development was multimodal during the spawning season, suggesting multiple spawning for this species. Histological examination of the ovaries revealed, however, that only the largest batch became hydrated and was spawned and all remaining residual yolked oocytes were resorbed. More direct evidence for this mode of spawning, as well as the delineation of the number of batches spawned, has been obtained through the process of identifying and ageing postovulatory follicles in species that exhibit these multiple modes of oocyte development. This method has been used to estimate spawning frequency in several engraulid species (Hunter and Goldberg 1980; Hunter and Macewicz 1980, 1985; Alheit et al. 1984; Parrish et al. 1986; Clarke 1987) and also for the skipjack tuna, Katsuwonus pelamis (Hunter et al. 1986). Ageing postovulatory follicles seems to work well for species normally found in large aggregations or schools but has yet to be applied to snappers. The ageing method using postovulatory follicles may be more difficult to apply to such species as snappers, which are known to occur in fewer numbers. Although our study attempted to identify postovulatory follicles in natural populations of uku and onaga, they could not be positively identified or aged. Future studies will have to address this problem, since the delineation of spawning frequency is important for accurate fecundity estimates.

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