## RAPID AND SPONTANEOUS MATURATION, OVULATION, AND SPAWNING OF OVA BY NEWLY CAPTURED SKIPJACK TUNA, KATSUWONUS PELAMIS

This study was designed to test a hypothesis, formulated on the basis of preliminary observations, that skipjack tuna, Katsuwonus pelamis, captured in Hawaiian waters during their breeding season and maintained alive would ovulate spontaneously within a few hours after capture. If such did occur, and on a consistent and predictable basis, this would be of practical value in attempts to spawn these fish in captivity.

## Methods

These investigations took place at the Kewalo Research Facility of the National Marine Fisheries Service Honolulu Laboratory. Six deliveries of live skipjack tuna were received from two commercial fishing vessels during June and July 1980, within the normal spawning season of the species in Hawaiian waters (Brock 1954; Matsumoto 1966). The fish had been caught by standard pole-and-line methods and transported to the receiving dock of the laboratory in baitwells. Upon delivery they were transferred to circular tanks, 7.3 m diameter by 1.1 m deep, provided with continuous flow of seawater. Time of capture for all groups was between 1500 and 1700 ; time elapsed between capture and delivery to the laboratory ranged from 3.5 to 8 h , with a mean of 5.5 h . Sea temperatures at the capture sites were not measured, but were probably between $25^{\circ}$ and $30^{\circ} \mathrm{C}$. Water temperatures in the holding tanks were about $25^{\circ}$ to $26^{\circ} \mathrm{C}$. With all except the first group, a siphon and straining net were used to sample water continuously from the holding tanks to detect the release of their slightly buoyant, pelagic ova. For the last four of the six groups, we arranged also to receive specimens fished from the same school but refrigerated on ice immediately after capture. All the specimens were between 40 and 50 cm in fork length (FL) and 1.4 to 2.2 kg ; tunas larger than this are difficult to keep alive in the baitwells of these vessels (about 145 by 165 by 130 cm deep on the vessel which delivered five of the six groups). Skipjack tuna of this size are between 1 and 2 yr old and are probably in their first spawning season (Brock 1954; Yoshida 1971).

We determined gonadal maturation states of specimens at various specified times following
their capture, either through biopsies on live specimens or through postmortem dissections. Unless a specimen is already running ripe, neither its sex nor gonadal maturity can be determined through external appearances. Biopsies involved extraction of gonadal tissue by catheterization through the urogenital pore of restrained, unanesthetized fish. Ova were teased free from unpreserved, fresh or refrigerated ovarian tissue, immersed in a $0.9 \%$ saline solution, and the diameters of 25 from each of the largest and second largest developing groups were measured with an ocular micrometer. Also, since we were interested primarily in the occurrence and progress of ovulation, we classified females into the following four categories: Un-ovulated--ripe ova not present in ovarian lumen, developing ova enclosed within follicles; ovulat-ing-some ripe ova present in ovarian lumen but not easily stripped from females, follicles contain large, preovulatory ova 0.80 to 1.0 mm in diameter; ripe-ovarian lumen filled with large quantities of ova which can be easily stripped from females; spent-few residual ova present in ovarian lumen, follicles with relatively small ova of $<0.5 \mathrm{~mm}$ diameter.

## Results

Responses of each sex remained constant among the six groups. Testes of males sacrificed after 7 to 8.5 h appeared identical to those sacrificed and refrigerated on capture. All males had testes that were mature, white, and firm and had thick, viscous milt in the sperm ducts. None yielded milt when moderate stripping pressure was applied. To fertilize ova stripped from females, we had to squeeze milt directly from testes dissected from sacrificed males.

Observations on all six groups of female skipjack tuna received from 8 June to 31 July are summarized in Table 1. None of the 16 specimens killed and refrigerated on capture was in an ovulatory state. The maturing ova in the largest modal group averaged 0.59 to 0.64 mm in 14 of these females and 0.74 mm in another, while the remaining individual had relatively immature ovaries (Table 2). Nine females which died in transit to the laboratory were placed in refrigeration. Times of death had not been recorded by the fishing crews, but were $<5 \mathrm{~h}$ after capture in all cases. None of these females had yet ovulated, and the ova in their largest developing modal groups averaged from 0.60 to 0.93 mm in diame-

TABLE 1.-Ovulatory status of skipjack tuna at different times following capture during June and July 1980.

| Time | No. | Unovulated | Ovulating | Ripe | Spent |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Refrigerated <br> immediately after <br> capture | 16 | 16 | 0 | 0 | 0 |
| Captive females, <br> $0-5 \mathrm{~h}$ after <br> capture' | 9 | 9 | 0 | 0 | 0 |
| Captive females, <br> $5-6 \mathrm{~h}$ after <br> capture | 13 | 1 | 12 | 0 | 0 |
| Captive females, <br> $7-8.5$ h 'after | 12 | 3 | 1 | 8 | 0 |
| capture | 20 | 1 | 0 | 0 | 19 |
| Captive females, <br> $15-65 ~ h ~ a t t e r ~$ <br> capture |  |  |  |  |  |

'Refrigerated after dying in transit to the laboratory; individual times of death not known, but $<5 \mathrm{~h}$ after capture in all cases.
could be completed within 8 h after capture and occurred even in females that were so seriously traumatized that they died within a few hours after this time. Unless manually stripped, the ripe females released ova into the holding tank, and by the next day, 15 to 24 h after capture, were in a spent condition. Spawning behavior was not observed to occur. Instead, their behavior was invariably abnormal, as is typical for skipjack tuna during their first days in captivity, with individuals swimming aimlessly about the holding tanks.

The ovulated ova, both those released spontaneously into the tanks and those stripped from

Table 2.-Mean sizes (mm) ${ }^{1}$ of ova in largest and second largest modal group of developing ova in skipjack tuna killed and refrigerated immediately after capture, or refrigerated after dying in transit to the laboratory.

| Date | Refrigerated on capture |  |  | Died in transit |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | No. | Largest group | Second group | No. | $\begin{aligned} & \text { Time } \\ & (\mathrm{h})^{2} \\ & \hline \end{aligned}$ | Largest group | Second group |
| 15 July | 4 | $\begin{aligned} & 0.62 \\ & 0.61 \\ & 0.59 \\ & 0.60 \end{aligned}$ | Not measured Not measured Not measured Not measured |  |  |  |  |
| 21 July | 7 | $\begin{aligned} & 0.60 \\ & 0.60 \\ & 0.60 \\ & 0.59 \\ & 0.59 \\ & 0.60 \\ & 0.59 \end{aligned}$ | $\begin{aligned} & 0.42 \\ & 0.39 \\ & 0.40 \\ & 0.41 \\ & 0.40 \\ & 0.39 \\ & 0.40 \end{aligned}$ |  |  |  |  |
| 22 July | 3 | $\begin{aligned} & 0.22 \\ & 0.64 \\ & 0.74 \end{aligned}$ | $\begin{aligned} & 0.10 \\ & 0.41 \\ & 0.44 \end{aligned}$ | 7 | $<4.5$ | $\begin{aligned} & 0.84 \\ & 0.93 \\ & 0.76 \\ & 0.69 \\ & 0.72 \\ & 0.70 \\ & 0.72 \end{aligned}$ | $\begin{aligned} & 0.44 \\ & 0.43 \\ & 0.44 \\ & 0.44 \\ & 0.42 \\ & 0.42 \\ & 0.45 \end{aligned}$ |
| 31 July | 2 | $\begin{aligned} & 0.62 \\ & 0.62 \end{aligned}$ | $\begin{aligned} & 0.41 \\ & 0.43 \end{aligned}$ | 2 | $<5$ | $\begin{aligned} & 0.67 \\ & 0.60 \end{aligned}$ | $\begin{aligned} & 0.41 \\ & 0.41 \end{aligned}$ |

${ }^{1}$ Standard deviations 0.02-0.04.
${ }^{2}$ Time between capture and death.
ter (Table 2). Of those kept alive, all but 1 of the 13 specimens examined 5 to 6 h after capture were ovulating but not yet ripe, while 8 of 12 examined after 7 to 8.5 h were ripe. Such ripe individuals yielded about 100,000 to over 150,000 ova when stripped. All but 1 of the 20 specimens examined 15 to 65 h after capture were spent. On all five occasions when the holding tanks were monitored for the presence of spawned ova, large numbers of ova were evident by the morning following delivery.

These observations clearly demonstrated that female skipjack tuna caught and kept alive during this time of year rapidly underwent the final stages of ovarian maturation and then ovulated ripe ova into the ovarian lumen. This response
ripe females, were normal in size and appearance. They were spherical, transparent, averaged about 1.0 mm in diameter, and had a single oil globule about 0.24 mm in diameter. The fertilization rate of ova stripped from females about 8 h following their capture was only about $40 \%$ to $50 \%$; this may reflect the quality of the ova or the small amounts of viscous milt squeezed from the dissected testes. The embryos hatched in about 30 to 31 h at $25^{\circ}$ to $26^{\circ} \mathrm{C}$ and started feeding on the third day after hatching. Although they fed actively on rotifers, Brachionus sp., and copepod nauplii, we were not able to rear any beyond the 12th day.

Numerous investigators have described the multimodal size distribution of developing ova in
the ovaries of maturing tunas. All of the ova in the most advanced modal group (about 0.60 mm or larger in these specimens) appeared to undergo final maturation and ovulation during this response but the second largest modal group seemed not to be affected. Ovaries from "control" specimens killed and refrigerated on capture and from those that died within 5 h contained an advanced modal group of maturing ova, as previously described, and a second, smaller modal group in which the ova averaged between 0.39 and 0.44 mm in diameter (Table 2). Ovaries from fully ovulated, ripe females and from recently spent females contained a residual modal group of similar, unovulated ova that averaged 0.39 to 0.49 mm in diameter (Table 3). These latter observations support the common assumption that in species with multimodal size distributions of developing ova, only the most advanced modal group will mature and be ovulated for a given spawning.

Table 3.-Mean sizes (mm) ${ }^{1}$ of ova in largest modal group of unovulated ova in ripe or recently spent skipjack tuna.

| Date | Hours after capture | Status | Ova diameter |
| :--- | :---: | :--- | :---: |
| 28 June | 8 | Ripe | 0.46 |
|  | 8 | Ripe | 0.46 |
| 17 July | 46 | Spent | 0.43 |
|  | 46 | Spent | 0.40 |
| 21 July | 7 | Ripe | 0.43 |
| 22 July | 20.5 | Spent | 0.42 |
|  | 25 | Spent | 0.39 |
| 23 July | $232-39$ | Spent | 0.40 |
|  | $32-39$ | Spent | 0.43 |
|  | $32-39$ | Spent | 0.49 |
| 31 July | $26.5-15$ | Spent | 0.45 |

'Standard deviations 0.02-0.04.
${ }^{2}$ Found dead in holding tanks, time interval since last seen alive.

## Discussion

This rapid ovarian maturation, ovulation, and spawning appears to be a unique response to capture not previously reported. The trigger to this response is not known but appears related to stresses associated with capture and confinement. Witschi and Chang (1959) earlier concluded that ovulation of vertebrates could be facilitated by stress, but there has been a lack of direct evidence to support this conclusion. Indirect evidence for such a relationship within teleosts is suggested by ovulatory responses of certain species to treatment with corticosteroids (Hirose 1976; Sundararaj and Goswami 1977) and with epinephrine (Jalabert 1976), both of which have been reported to increase rapidly in serum concentrations following such stresses as handling and increased temperature (Mazeaud
et al. 1977; Strange et al. 1977; Cook et al. 1980). The handling associated with being hooked, transported in crowded baitwells, transferred to shore tanks, and confined is obviously stressful and often fatal to newly captured skipjack tuna. Thermal stress may occur when they are confined in warm surface waters and prevented from returning to cooler depths after feeding.

Many additional aspects of this postcapture ovulatory response are not yet understood. Several aspects would be of particular interest: 1) the state of ovarian maturation that would be prerequisite for rapid egg development in females; 2) the seasonal availability of responsive females; 3) whether the time to complete ovulation, about 7 to 8 h in this study, will vary depending on such factors as water temperature, ovarian maturation, or time of day the fish are caught; and 4) whether this apparent response to acute stress is entirely an artificially produced anomaly, or whether it does have some relation to their natural spawning biology.

Past efforts to rear tunas in captivity (briefly reviewed by Kaya et al. 1981) had not heretofore resulted in dependable spawning procedures for any species. However, the occurrence and predictability of the ovulatory response to capture have now been applied to establish a routine procedure for spawning skipjack tuna at the Kewalo Research Facility. Additional spawnings have thus been accomplished during the summer of 1981, the second season of trials, and the response has been observed also in a second species of tuna-kawakawa, Euthynnus affinis. It would be of interest to determine whether other species will undergo a similar response to stresses of capture and confinement.

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## ESTIMATING AND MONITORING INCIDENTAL DOLPHIN MORTALITY IN THE EASTERN TROPICAL PACIFIC TUNA PURSE SEINE FISHERY

Each year the purse seine fishery for yellowfin tuna, Thunnus albacares, in the eastern tropical Pacific is responsible for the incidental kill of thousands of small cetaceans, ${ }^{1}$ primarily dolphins or "porpoise." Yellowfin tuna are often associated with small cetaceans in this region and fishermen have used this association since 1959 to catch tuna (McNeely 1961; Perrin 1969; Fox 1978). During the purse seining operation, cetaceans encircled with yellowfin tuna by the net may become entangled and accidentally drown. In such cases, the fishermen retain the tuna and discard the cetaceans at sea.

The Marine Mammal Protection Act of 1972 requires the tuna fishery to be managed so that the dolphin populations are maintained at specific population levels and that incidental mortality be reduced to insignificant levels. The National Marine Fisheries Service (NMFS) has the responsibility of monitoring the dolphin mortality and of assessing the impact of dolphin mortality on dolphin populations. NMFS carries out research on the abundance and distribution of dolphins, their biology, the level of incidental mortality, and methods for reducing incidental mortality.

Beginning in 1971, the NMFS regularly placed observers on purse seiners to collect data on the incidental mortality of dolphins. Prior to 1974, however, only a few observers were hired to collect data; the amount of data collected, therefore, is too small to produce a precise estimate of total incidental mortality. Most estimates from this period place the total at about 300,000 to 500,000 animals/yr. Estimates for 1974 and 1975, which are more precise, are 140,000 and 157,000 animals killed, respectively, for the U.S. fleet ( $\mathrm{Smith}^{2}$ ).

After a U.S. District Court ruling in 1976, the NMFS set an annual quota of 78,000 animals for

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[^1]:    ${ }^{1}$ Dolphin, in this paper, is used as a general term referring to all small cetaceans impacted in the fishery. Mortality or kill refers to dolphin mortality incidental to the catch of yellowfin tuna. The unit of fishing effort "set" is defined as a single deployment of a purse seine net around an aggregation of dolphin or tuna. Tuna catches are expressed in the unit short tons, as it is the most common form in which these statistics are reported.
    ${ }^{2}$ Smith, T. D. Report of the status of porpoise stock workshop. Southwest Fish. Cent. Adm. Rep. LJ-79-41, 120 p.

