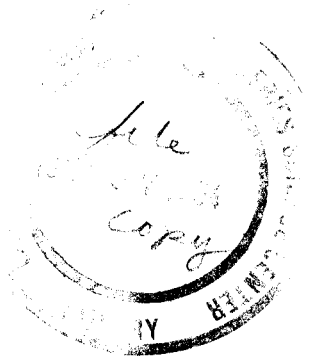

P R O J E C T R E P O R T

Submitted to

National Marine Fisheries Service, Honolulu Laboratory

Research Topic: Reproduction of Tuna in Captivity, Induced Spawning and Larval Rearing

Project Period: June 1 to July 31, 1980

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These investigations took place in Honolulu, Hawaii at the Kewalo Research Facility of the National Marine Fisheries Service. Six deliveries of live skipjack tuna were received during June and July 1980 from two local commercial fishing vessels, the Bluefin and the Neptune. The fish were caught by standard pole-and-line methods and transported to the receiving dock of the laboratory in live baitwells. All the specimens were between 40 and 50 cm in fork length and 1.4 to 2.2 kg in weight; fish of this size are about the largest that can be kept alive in reasonable condition in the baitwells of these vessels (about 145 X 165 X 130 cm deep in the Bluefin). Skipjack of this size are between one and two years old and probably in their first spawning season (Brock 1954; Yoshida 1966, 1971).

Gonadal maturation states of females were determined at various times following their capture, as will be specified, either through biopsies of live specimens or through postmortem dissections. Biopsies involved extraction of gonadal tissue by catheterization through the urogenital pore of restrained, unanesthetized fish (Shehadeh et al. 1973). Ova were teased free from unpreserved, fresh or refrigerated ovarian tissue placed in 0.9% saline solution and the diameters of 25 each from the first and second largest developing groups were measured with an ocular micrometer. Ovarian maturation stages of skipjack tuna have been described by several investigators (Buñag 1956; Shaefer and Orange 1956; Raju 1964; Yoshida 1966; Simmons 1969; Batts 1972). Since the occurrence and progress of ovulation was a primary interest of this study, females were divided into the following categories:

1. Unovulated. Ripe ova are not found loose in the ovarian lumen. Maturing ova vary in size according to maturation state of the female, the largest group of ova ranging from .10 mm or less in immature

specimens to about .90 mm in specimens nearing ovulatory condition. Biopsies of live females yield ovarian tissue, consisting mostly of developing ova within follicles, in the catheter. Dissections reveal ovaries that are yellow, firm, and of varying sizes depending on maturation state.

2. Ovulating, not yet running ripe. Some ripe ova are found loose in the ovarian lumen; females release few to no ripe ova on moderate to firm stripping pressure. Biopsies produce a mixture of ripe ova and enlarged, preovulatory ova in the catheter from females that are just starting to ovulate, and only ripe ova in those that already have considerable numbers of ova released into the lumen. Dissections reveal ovaries that are bright yellow, swollen, and soft. The ovarian walls are greatly thickened with the presence of the enlarged, pre-ovulatory ova.

3. Ovulated, running ripe. Ovaries are swollen with ripe, ovulated ova which can be readily expressed from the females with slight to moderate stripping pressure. Females of the size used in this study could be stripped of about 100,000 to over 150,000 ova when in this state. Biopsies yield only ripe ova in the catheter. Dissections reveal ovaries that are extremely swollen, soft, and with a yellow, slightly transluscent appearance. The ovarian lumen is greatly enlarged with the mass of ripe ova.

4. Recently spent. Ovaries contain a few to a few thousand ova loose in the lumen. Moderate to firm stripping pressure produces from no ova to a few thousand from the females. Ova appear normal in those which have spawned within a few hours previously, but have indented or

collapsed membranes in those examined later. Biopsies yield residual ova, or a mixture of residual ova and ovarian tissue containing the next group of developing ova, in the catheter. Dissections reveal ovaries that are soft, flaccid, and bloody in appearance.

The first group of skipjack were delivered to the laboratory on the night of June 18 and were biopsied at 9:30 the next morning. The purpose of this biopsy was to determine whether any females had ovaries sufficiently mature to make them suitable for hormonally induced spawning trials. However, all 8 females biopsied turned out to be recently spent. (There were actually 10 females in the group, as later determined by dissections, but for various reasons not all specimens in a group are successfully biopsied during any given session. Some struggle too violently to be effectively handled, and others are released after an unsuccessful biopsy attempt. Male skipjack of the size used in this study are difficult to biopsy because of the small size of their urogenital aperture, and specimens in which the catheter could not readily be inserted were released as probable males. On postmortem examination some of these would later turn out to be females, the number depending on the skill and experience of the investigator and the circumstances attending the given biopsy session. These other two females were examined five days later and also turned out to be spent.)

The spent condition of all the females was a disappointment at the time since it meant that none could be used for hormonally induced spawning attempts. I recalled that we had previously biopsied one other group of skipjack on the morning following their capture and delivery, during the preceding June of 1979, and all three females in that group also had been recently spent. The spent condition of all these females contrasted sharply with the maturing ovaries of similar sized specimens being concurrently sampled from the local cannery

(M. Queenth, National Marine Fisheries Service, Honolulu, Hawaii, unpublished data). These observations suggested that the skipjack might be spawning during their first night in captivity, a possibility strengthened by a report that anchovies (Engraulus mordax) will do so (Hunter and Goldberg, 1980).

When the next group of 13 live skipjack were delivered to the laboratory the evening of June 28, I set up a siphon and strainer to sample water from their holding tank. The strainer was checked at 6:05 the following morning and was found to contain more than 100,000 ova. The fish were biopsied that same morning and three females were found, all recently spent. Two other specimens, females, had died during the transfer from the fishing vessel to the holding tank at a time about 8 hr following their capture. When their refrigerated ovaries were examined the next morning, both pairs were found to be in a running ripe condition, swollen with masses of ovulated ova. These observations demonstrated that the skipjack were indeed spawning during the first night in captivity, and also suggested that females could be in a running ripe condition within 8 hr following their capture.

Before the next delivery of live skipjack, the boat captains involved were asked to immediately refrigerate specimens taken from the same school so that the gonadal condition of freshly captured fish could be determined. The next delivery, of 8 skipjack, was received on July 15. They had been captured at about 4:30 p.m. and were delivered at the laboratory dock at about 8:00 p.m. The 8 fish were watched for any spawning behavior in their holding tank until 12:30 a,m., but none was observed. At this time, 8 hr after capture, the fish were biopsied. Three were determined to be females, of which two were running ripe while the other had not ovulated. Three males were identified by the

presence of milt in the catheter, but none could be stripped of milt. Two males were sacrificed and their testes dissected out. About 100,000 ova were stripped from each of the two ripe females and fertilized with milt squeezed from the dissected testes. Both females were also sacrificed after being stripped. Again, large numbers of ova were found the next morning in the strainer sampling water from the holding tank. Two other females had remained in the tank and died the second day following capture. Both were found to have spent ovaries and were therefore responsible for the ova spawned out in the holding tank.

Thirteen refrigerated specimens were received at the same time, four females and nine males. The four females provided a distinct contrast to those that had been kept alive for 8 hr. All four had not ovulated and instead had ovaries containing maturing ova with the largest group averaging from .59 to .62 mm in diameter. All males had mature testes grossly indistinguishable from those of the males sacrificed 8 hr following capture.

Three more groups of both live and refrigerated specimens were received on July 21, 22, and 31, and in each instance produced results similar to those of July 15. Observations on all six groups of female skipjack received from June 18 to July 31 are summarized in Table 1. None of the 16 specimens killed and refrigerated on capture were in an ovulatory state. The largest group of maturing ova averaged .59 to .64 mm in 14 of these females and .74 mm in another, while the remaining individual had relatively immature ovaries (Table 2). Nine females were placed in refrigeration after dying in transit to the laboratory, with times of death not recorded by the fishing crews but less than five hr in all cases. None of these females had yet ovulated, and their largest group of developing ova averaged from .60 to .93 mm in diameter (Table 2).

Table 1. Ovulatory Status of Skipjack Tuna at Different Times Following Capture during June and July 1980.

	No.	Not Ovulated	Ovulating, Not Yet Running Ripe	Ovulated, Running Ripe	Recently Spent
Refrigerated Immediately After Capture	15	15	0	0	0
Captive Females, 0 to 5 Hr After Capture[1]	9	9	0	0	0
Captive Females, 5 to 6 Hr After Capture	13	1	12	0	0
Captive Females, 7 to 8.5 Hr After Capture	12	3	1	8	0
Captive Females, 15 to 65 Hr After Capture	20	1	0	0	19

[1]Refrigerated after dying in transit to the laboratory, individual times of death not known but less than 5 hr in all cases.

Table 2. Mean Sizes (mm)[1] of 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.

	Refrigerated on Capture			Died in Transit			
Date	No.	Largest Group	Second Group	No.	Time (hr)[2]	Largest Group	Second Group
July 15	4	.62	not measured				
		.61	" "				
		.59	" "				
		.60	" "				
July 21	7	.60	.42				
		.60	.39				
		.60	.40				
		.59	.41				
		.59	.40				
		.60	.39				
		.59	.40				
July 22	3	.22	.10	7	< 4.5	.84	.44
		.64	.41			.93	.43
		.74	.44			.76	.44
						.69	.44
						.72	.42
						.70	.42
						.72	.45
July 31	2	.62	.41	2	< 5	.67	.41
		.62	.43			.60	.41

[1] standard deviations .02 to .04

[2] time between capture and death

With those kept alive all but one of the 13 specimens examined 5 to 6 hr after capture were ovulating but not yet running ripe, and 8 of 12 examined after 7 to 8.5 hr were running ripe. All but one of 20 specimens examined 15 to 65 hr 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 during this time of the year responded to some factor associated with being captured or confined by undergoing the final stages of ovarian maturation and ovulating ripe ova into the ovarian lumen. This response could be completed within 8 hr. Unless manually stripped the females proceeded to spawn out these ova into the holding tank and by the next day, 15 to 24 hr after capture, were in a spent condition with from few to a few thousand residual ova loose in the ovarian lumen.

Numerous investigators have described the multimodal size distribution of developing ova in ovaries of maturing tuna. The entire complement of the most advanced group of ova (about .60 mm or greater in these specimens) appeared to undergo final maturation and ovulation in this "stress response", but the second largest group seemed not affected. Ovaries from "control" specimens killed and refrigerated on capture and from those that died within 5 hr contained an advanced group of maturing ova, as previously described, and a second, smaller group that averaged between .39 and .44 mm in diameter (Table 2). Ovaries from fully ovulated, running ripe females and from those recently spent contained a residual group of similar, unovulated ova that averaged .39 to .44 mm in diameter (Table 3).

Table 3. Mean Sizes (mm)[1] of Largest Modal Group of Unovulated Ova in Running-Ripe or Recently Spent Skipjack.

Date	Hr After Capture	Status	Ova Diameter
June 28	8	running ripe	.46
	8	running ripe	.46
July 17	46	spent	.43
	46	spent	.40
July 21	7	running ripe	.43
July 22	20.5	spent	.42
	25	spent	.39
July 23	32-39[2]	spent	.40
	"	spent	.43
	"	spent	.49
July 31	6.5-15[2]	spent	.45

[1]Standard deviations .02 to .04

[2]Found dead in holding tanks, time interval since last seen alive

Testes of males sacrificed after 7 to 8.5 hr appeared identical to those sacrificed and refrigerated on capture. All males had testes that were mature, white, and firm and had a thick, viscous milt in the sperm ducts. None of the males yielded milt on moderate stripping pressure. This indicates that either the males do not have a corresponding seminal hydration response to capture and confinement, or that their response occurs very quickly after capture and their testes recover to an original appearance within a few hours. Despite this seeming lack of response by the males and although we did not observe any apparent spawning behavior in the holding tanks, a very small fraction of eggs released spontaneously into the holding tanks and recovered by the strainer were found to be fertilized and undergoing embryonic development (e.g. less than 50 found among about 100,000 collected in the strainer the morning of June 29).

The ovulated ova, both those released spontaneously into the tanks and those stripped from ripe females, were normal in size and appearance. They were spherical, transparent, averaged around 1.0 mm in diameter, and had a single oil globule about .24 mm in diameter. The fertilization rates of ova stripped out of females about 8 hr following their capture were only about 40 to 50%, but this may reflect the quality or quantity of the small amounts of viscous milt squeezed from the dissected testes. The embryos hatched out in about 30-31 hr at 25-26^{o}C, and started feeding on the third day after hatching. Although they fed actively on rotifers (Brachionus sp.) and copepod nauplii, we did not manage to rear any beyond the 12th day after hatching. Rearing trials are expected to continue.

Conclusions

1. The "stress-induced" maturation and ovulation of skipjack tuna, discovered and documented by this study, means that fertilized eggs, embryos, and larvae can be produced reliably and predictably from captive specimens of this species,
2. Questions remain to be resolved (see recommendations) in order to better understand this phenomenon and to determine or improve the predictability of this response relative to time of day, season, and year,
3. Because of the inability of the laboratory to obtain live kawakawa from the commercial fishing boats during the study period and because of the ovulatory response and consequent spent condition of the skipjack females, appropriately mature females were not available for hormone-induced spawning trials on either species. However, inducement of spawning through hormone treatments, although demonstrated to be feasible with kawakawa (Kaya et al, 1981), now appears an academic problem no longer necessary for practical spawning operations with skipjack tuna.
4. Attempts to rear skipjack larvae have not yet produced survival beyond the 12th day following hatching, even though the larvae appeared normal and healthy and fed actively. Solution of this aspect will depend on further rearing trials, experimentation with different larval foods, monitoring of water quality in the rearing tanks, and determination of possible effects of egg quality on larval viability (see recommendations).

Recommendations

Since fertilized eggs of skipjack tuna can now be produced "on demand" at least during the summer months, a broad spectrum of experimental observations on embryos, larvae, and (with the development of effective rearing techniques) juveniles will be made possible. To better define the potentials for and the limitations of applying this ovulatory response to such practical purposes, I recommend that priority be given to continuing or initiating the following studies at the Kewalo Research Facility:

1. The larval rearing efforts should be continued and given highest priority. With access to fertilized eggs now assured, these efforts should eventually develop effective procedures and provide the means to produce advanced young routinely.
2. The relationship should be determined between the annual reproductive cycle of skipjack in Hawaiian waters and the availability of responsive females. The following should be done toward this objective:
   A. Weekly sampling of ovaries from skipjack delivered to the cannery should be continued at least until August 1981.
   B. Live skipjack should be obtained on at least a monthly basis until August 1981 and each group received should be subjected to the routine egg monitoring (siphon and strainer) and biopsy and stripping techniques to test for ovulation.

   These observations should provide the following:
   (1) Determine the portion of the year when skipjack can be expected to undergo the ovulatory response.

(2) Determine the size to which ova must develop before the females will respond, and the occurence of females advanced to this state at different times of the year.

(3) Provide larvae for further rearing trials.

3. An attempt should be made to determine whether there is a relationship between maturity of ova (as indicated by size of largest maturing group) and fertilization rate, hatching success, and time of survival of larvae. This could be examined by comparing the ova diameters from refrigerated specimens and the fate of fertilized eggs and larvae produced from members of the same school.

4. Responsiveness of other species of tuna should be tested:

   A. Kawakawa should be obtained during the spring and summer breeding season and subjected to the same procedural routine to determine whether they will also undergo an ovulatory response to capture.

   B. Consideration should be given to designing a similar experiment to be conducted at sea, either on a ship with a large baitwell or through the use of a floating enclosure, on larger species like yellowfin tuna.

5. The relationship between time after capture and stages of ovarian response should be better defined, This would involve periodic sampling of captive fish until specimens bcome running ripe. Because of the short time to completion of the response, such observations would have to be conducted, or at least initiated, on board the fishing vessel.

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