INDUCED SPAWNING OF A TUNA, *EUTHYNNUS AFFINIS*

Investigations into the biology of young tuna have been hampered by the difficulty of capturing and maintaining live larvae or early juveniles from the wild. The production of young in captivity would provide an obvious solution; however, the inherent difficulties of maintaining and working with such powerful, fast-swimming, pelagic fishes under conditions of close confinement have discouraged attempts to artificially stimulate their spawning. The following describes what we believe to be the first induced spawning of any tuna, accomplished with specimens of *Euthynnus affinis*, one of the smaller sized of the true tunas (taxonomic relations of tunas were recently reviewed by Collette (1978)). These were held captive in tanks at the Kewalo Research Facility of the Southwest Fisheries Center, Honolulu Laboratory, National Marine Fisheries Service, NOAA.

The specimens used in these spawning trials were three females in captivity for 2-4 wk and three males in captivity for about 4 mo. All were captured by hook and line and transported to the laboratory in the baitwells of either a commercial fishing vessel or the NOAA Ship *Townsend Cromwell*. They were maintained outdoors in circular, 7.3 m in diameter by 1.1 m deep tanks with water flow of about 3.0-3.5 l/s and temperatures ranging from 23.3° to 26.2° C. They were fed thawed surf smelt, *Hypomesus pretiosus*, and squid *Loligo opalescens* at a rate of about 15-20% of their body weight per day.

Sex and gonadal maturation of each specimen were determined by biopsy, with samples obtained by catheterization through the urogenital aperture (Shehadeh et al. 1973). While the fish were physically restrained for the biopsies, each was marked with an identifying numeral or letter with silver nitrate (Thomas 1975). Males in advanced stages of maturation could be recognized by the presence of milt in the catheter, while females in any stage of maturation could be identified from the biopsied ovarian tissue. The diameters of 25 ova of the largest size class present in the catheterized sample were measured with an ocular micrometer and the average diameter calculated as an index of ovarian maturation.

Gonadal maturation of captive specimens was monitored by monthly biopsies, starting in March 1979 with 37 specimens maintained since the preceding January and February. After March, newly captured specimens were also biopsied on the day of delivery to the laboratory. The most advanced ovaries in our captive fish between March and August 1979 were in three females delivered to the laboratory on 6 June. Their largest ova averaged 0.54, 0.48, and 0.44 mm on arrival and 0.55, 0.53, and 0.50 mm, respectively, when biopsied again 2 wk later on 19 June. The first two (females A and B, Table 1) were used in the first spawning trial, which started that same day, on 19 June. The third female (female C, Table 1) was biopsied again after an additional 2 wk, and its largest ova had increased to an average diameter of 0.56 mm. It was used in the second spawning trial, which started that same day, on 2 July. Males from groups captured earlier in January and February were also biopsied on the starting dates of the spawning

TABLE 1.—Spawning responses of *Euthynnus affinis* to treatment with salmon pituitaries (SP), human chorionic gonadotropin (HCG), and pregnant mare serum (PMS). Ova sizes represent mean diameters of 25 spawned ova after preservation in 3-5% Formalin,[1] or the largest size class in fresh biopsied samples.

	Day 1			Day 2			Day 3		
	Ova (mm)			Ova (mm)			Ova (mm)		
Specimen	Mean	SD	Treatment	Mean	SD	Treatment	Mean	SD	Response
Trial 1:									
Female A	0.53	0.03	SP 5 mg HCG 100 IU	0.54	0.04	SP 5 mg HCG 100 IU PMS 1,000 IU	0.55	0.04	Did not spawn.
Female B	0.55	0.05	SP 10 mg HCG 500 IU	0.57	0.04	SP 10 mg HCG 500 IU PMS 2,000 IU	0.77 0.98	0.02 0.02	Spawned (>35,000 released spontaneously; <10,000 stripped and fertilized).
Male A			HCG 100 IU			HCG 100 IU			No hydration of milt.
Male B			HCG 500 IU			HCG 500 IU			Hydration of milt.
Trial 2:									
Female C	0.56	0.03	SP 10 mg HCG 500 IU	0.70	0.04	SP 10 mg HCG 1,000 IU PMS 2,000 IU	0.78 1.02	0.03 0.03	Spawned (>185,000 released spontaneously; >40,000 stripped and fertilized).
Male C			HCG 1,000 IU			HCG 1,000 IU PMS 1,000 IU			Hydration of milt.

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

trials, and specimens yielding milt in the catheter were selected. The selected males and females were transferred to a spawning tank identical in dimensions to the holding tanks but with water depth reduced to 0.61 m. Two females and two males were used in the first trial, and one female and one male in the second. Their sizes ranged from 47.0 to 52.9 cm fork length and 2.25 to 3.04 kg.

Hormone treatments administered were modified after those used by Leong (1977) to spawn Pacific mackerel, *Scomber japonicus*. Combinations of triturated, acetone-dried salmon pituitary glands (SP), human chorionic gonadotropin (HCG), and pregnant mare serum gonadotropin (PMS) were injected into the dorsal musculature with tuberculin syringes and 24-gage needles. The hormone preparations were suspended or dissolved in physiological saline and administered in injection volumes of 0.10-0.50 ml (Table 1). Each treatment consisted of two series of injections 24 h apart, starting at 2 p.m. in the first trial and 6 p.m. in the second. Responses of females were monitored by ovarian biopsies taken immediately preceding each series of injections, and spawning was detected by the appearance of the buoyant, pelagic ova in plankton nets (egg strainers) placed in the outflow from the treatment tank. Responses of males were based on subjective evaluation of hydration of milt, on the basis of whether milt could be expressed by moderate stripping pressure, and the fluid consistency of the milt obtained.

In the first trial, neither female showed any significant increases in sizes of largest ova within 24 h after the first injections (Table 1). However, ova were found in the strainers the next morning at about 8:15, about 19 h after the second injections. Biopsy of both females indicated that ovulation had been induced only in the one treated with higher dosages; similarly, examination of both males indicated that milt could be expressed only from the individual treated with the higher dosages. Eggs and milt were stripped from the responsive pair to effect fertilization. Most of the ova from this spawning were not viable and sank when placed in seawater, and of those that remained buoyant none progressed beyond early cleavage stages. This poor viability was probably caused by our failure to detect or respond to this induced ovulation early enough, with a consequent deterioration of the ovulated eggs within the ovarian lumen (Stevens 1966).

In the second trial, biopsy of the female indicated that its largest ova had increased in mean diameter from the original 0.56 to 0.70 mm within 24 h after the first injections. Ova were found in the strainer at 5:30 the next morning, 12.5 h following the second injections. Milt could be easily expressed from the male at this time, and the pair were stripped to effect fertilization. At incubation temperatures which ranged between 21° and 26° C, the first cleavage divisions were observed about 1 h after fertilization and the first hatching at about 31 h. With limited facilities for maintaining the developing embryos, we were able to sustain only a small fraction of the fertilized eggs obtained. About 300 larvae were hatched and they developed and absorbed their yolk sacs over 2 d. It was not our intent or purpose at this stage of these investigations to attempt to feed or rear these larvae, and they all died by the end of the third day.

Previous attempts to produce young tuna in captivity have been concentrated in Japan, and along two lines of effort. One is the fertilization at sea of ova obtained by stripping freshly captured, running-ripe fish (Yasutake et al. 1973; Harada 1978; Ueyanagi 1978). Since running-ripe females are only infrequently taken in current commercial fishing operations, these efforts have produced only occasional successes. The other approach is to maintain adult-sized specimens in large, netted enclosures in coastal waters with the hope that they will eventually start spawning naturally. (By coincidence, the first known spawning of a species of tuna in such a situation was reported to have occurred with bluefin tuna, *Thunnus thynnus*, on 20 June 1979 (Sōgō 1979) at the same time that our first induced spawning of *E. affinis* was taking place.) Whether either of these two approaches can be developed into reliable spawning operations for tunas remains to be determined. The two consecutive hormone-induced spawnings that we achieved with *E. affinis* suggest that this technique can be developed into a routine procedure, at least for this species. Work is expected to continue at the Honolulu Laboratory on refinement of effective treatments and to be initiated on rearing of the resultant larvae.

Acknowledgments

We thank Roderick Leong of the Southwest Fisheries Center La Jolla Laboratory, National Marine Fisheries Service, NOAA, for providing salmon pituitaries and for his words of encouragement.

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CALVIN M. KAYA

Southwest Fisheries Center Honolulu Laboratory
National Marine Fisheries Service, NOAA
Honolulu, Hawaii
Present address: *Department of Biology*
Montana State University
Bozeman, MT 59717

ANDREW E. DIZON
SHARON D. HENDRIX

Southwest Fisheries Center Honolulu Laboratory
National Marine Fisheries Service, NOAA
Honolulu, HI 96812