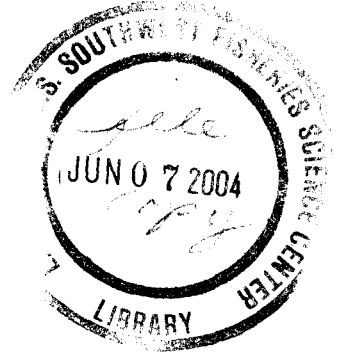




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FACTORS AFFECTING THE GROWTH AND SURVIVAL OF SKIPJACK TUNA,  
KATSUWONUS PELAMIS, LARVAE REARED IN THE LABORATORY

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Final contract report to the National Marine Fisheries Service,  
Southwest Fisheries Center, Honolulu Laboratory  
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## PREFACE

This report was prepared under contract (Purchase Order No. 81-ABA-492) by Sharon D. Hendrix, graduate student, University of Hawaii. The contract objectives were to collect and analyze data on the effects of various diets and rearing conditions on growth and survival of skipjack tuna, Katsuwonus pelamis, larvae reared under laboratory conditions. These data will provide information and insight on the early life history of this commercially valuable species. The data will also provide a guide for future studies such as those designed to measure the effects of normal environmental fluctuations and pollutants on survival and growth of this fish species' larvae in the wild. Since the report has been prepared under contract, the statements, findings, conclusions, and recommendations herein are those of the contractor and do not necessarily reflect the view of the National Marine Fisheries Service.

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December 17, 1982

## INTRODUCTION

Skipjack tuna, Katsuwonus pelamis, represent an important commercial fishery resource, yet little is known about their early life history. An understanding of the mechanisms affecting mortality of early life stages is important for the management of the skipjack tuna fishery. Mortality rates during early life stages are influenced by environmental factors which in turn effect future recruitment to commercial fisheries.

Rearing skipjack tuna larvae in controlled laboratory conditions provides information concerning the early life history of skipjack tuna that is otherwise difficult if not impossible to acquire. The objective of this study is to examine the factors that affect the growth and survival of skipjack tuna larvae reared in the laboratory.

## MATERIALS AND METHODS

Fertilized skipjack tuna eggs were obtained by stress-induced spawning of captive adult skipjack tuna at the National Marine Fisheries Service's Kewalo Research Facility (Kaya et al., in press). The fertilized eggs were stocked in rearing tanks at a density of 20 eggs/l. The rearing tanks were cylindrical, black fiberglass tanks (4 ft in diameter, 16 in. deep) immersed in temperature controlled water baths. Fluorescent lamps suspended over the tanks provided illumination.

The tanks were initially filled with 200 l of filtered seawater, however, additions of algae and food organisms increased the volume to as much as 300 l. The tank volume was maintained between 200 and 300 l by siphoning water from the bottom of the tanks periodically, which also

served to keep the tanks clean. Algae was added to the tanks prior to egg hatching; food organisms were added the second day after hatching. Mild aeration was started in the rearing tanks 3-4 days after hatching.

Larvae were initially fed laboratory cultured rotifers, Brachionus plicatilis, with daily additions of 4-8 l of algae (Tetraselmis sp. and Isochrysis sp.) to provide food for the rotifers. When available, cultured copepods (Tigriopus sp. and Euterpina acutifrons), Artemia nauplii or collected zooplankton were obtained and fed to 4-5 day old larvae.

Growth rates were determined by sampling 10 or more larvae periodically, however, as the numbers of larvae in a tank population declined, fewer larvae were sampled. Length (SL), eye diameter and body depth were measured while larvae were alive, using a calibrated micrometer mounted in a dissecting microscope.

Behavioral observations were made as frequently as possible, noting feeding behavior and swimming speed. Swimming speeds were determined by tracing a larva's swimming path on a piece of clear plastic using a pen mounted in a cross-hair device. The path distance was determined using a map reader. Ten observations were made daily, with observation times between 10 and 67 sec.

Attempts to rear skipjack tuna larvae from hatching through metamorphosis during June through August 1980 resulted in limited success, with larvae surviving up to 12 days after hatching. All 1980 rearing experiments were conducted at 24° C, using a 12 h day/12 h night light regime, and prey concentrations of 10-20/ml rotifers with additions of collected zooplankton at 1-2/ml when available.

In 1981, detailed studies were undertaken to determine the causes of the high larval mortalities observed in 1980. These studies examined several factors that affected growth and survival of skipjack tuna larvae, including temperature, light regime, dissolved oxygen, nutrient concentration, and water quality. Experiments were conducted for each of the spawnings as follows:

		Tank A	Tank B	Tank C
1st spawn	Temperature:	26° C	24° C	24° C
	Light:	12 h D/12 N	14 h D/10 N	24 h D/0 N
	Prey:	~15/ml <u>Brachionus</u> , ~1/ml <u>Artemia</u> nauplii and <u>Tigriopus</u> sp.		
2d spawn	Temperature:	24° C	24° C	22° C
	Light:	14 h D/10 N	14 h D/10 N	12 h D/12 N
	Prey:	~15/ml <u>Brachionus</u> , ~1/ml <u>Euterpina acutifrons</u>		
	Extra			
	procedures:	Monitor dissolved oxygen, examine larvae for bacterial fouling.		
3d spawn	Temperature:	24° C	24° C	24° C
	Light:	All tanks 14 h D/ 10 h N		
	Prey:	~15/ml <u>Brachionus</u> for all tanks.		
	Extra			
	procedures:	Monitor dissolved oxygen, mild aeration used if D.O. <80% saturation. Nutrients monitored in tanks A and B only. ~5,000 eggs shipped to NMFS La Jolla for rearing.		
4th spawn	Temperature:	All tanks at 24° C.		
	Light:	All tanks 14 hr D/10 hr N		
	Prey:	~15/ml <u>Brachionus</u> , 0.5-2/ml <u>E. acutifrons</u>		
	Extra			
	procedures:	Monitor dissolved oxygen, mild aeration used if D.O. <80% saturation. Nutrients monitored in tanks A and B. High algal concentration maintained in tank A. ~10,000 eggs shipped to NMFS La Jolla for rearing.		

Dissolved oxygen was monitored with an oxygen probe and YSI oxygen meter. Water samples were analyzed for phosphate, nitrate and nitrite, and ammonia by University of Hawaii HIMB Analytical Services. Eggs were shipped to NMFS La Jolla Laboratory in plastic bags and containers filled with autoclaved seawater. Larvae were reared at La Jolla using similar materials and methods. The purpose of the co-rearing was to determine if the difference in water quality at the two laboratories affected larval growth and survival.

## RESULTS

### General observations

Skipjack tuna eggs obtained from stress-induced spawnings were transparent and spherical, ranging from 0.9 to 0.96 mm in diameter with a single oil globule averaging 0.24 mm in diameter. Eggs hatched in 31-32 h at 26° C, and in 34-36 h at 24° C.

Larvae had an average length of 2.94 mm at hatching with a yolk sac averaging 0.73 mm long by 0.43 mm wide. The second day after hatching, larvae had pigmented eyes, functional jaws and remnants of the oil globule. At this time larvae had not started to feed but were exhibiting feeding and searching behavior. Larvae were actively feeding by the third day after hatching, larvae that did not initiate feeding died between 3 and 7 days. At day 3, many larvae were observed to have jaw abnormalities which apparently prevented them from capturing and ingesting sufficient quantities of food. The percentage of deformed larvae varied with different spawnings, in most groups 20-30% of day 3 larvae had deformed jaws.

Spination of the head region and jaw dentition was observed in 8-10 day old larvae. Larvae were essentially transparent, with pigmentation concentrated in the head and stomach regions and scattered along the trunk.

### Survival

Most of the skipjack tuna larvae died by day 7, with 2 groups of larvae surviving to day 12 (Table 1). Heaviest mortalities were observed on days 4 and 5, with losses of 50% or more of the tank population. Dying larvae usually swam slowly, 1-3 cm above the tank bottom, intermittently sinking in a head-down position. These larvae usually had shrunken stomachs characteristic of starvation, but were not necessarily smaller than actively feeding larvae (Table 2).

### Growth

Skipjack tuna larvae grew slowly in length, with no apparent effect of temperature on growth (Table 1). The 24-h day light regime resulted in total mortality by day 3, therefore the 14 h D/10 h N light regime was used in most rearing trials since it best approximated normal light conditions for that time of year.

Two groups of larvae (July 16, 1980 and May 31, 1981 spawns) exhibited significant growth and survival (Table 1). Statistical comparison of the two growth equations resulted in no significant difference between slopes and intercepts ( $P < 0.05$ , ANOVA, Sokal and Rohlf, 1969). Length data obtained from these two rearing groups were pooled and the results shown in Figure 1. All other rearing experiments resulted in insignificant growth (Table 1).



### Swimming and feeding behavior

Skipjack tuna larvae swam at an average speed of 0.45 cm/sec (~1.2 body lengths/sec) from day 2 to 10, averaging 1.64 (S.D. = 0.836) feeding attacks per minute.

Larvae continually searched the water column for prey. After selecting a prey, a larva would then stop and monitor it's prey while flexing the tail back into a tightly curved position. The strike involved a quick forward movement achieved by releasing the tail from the curved position and opening the mouth so that the prey was engulfed. This type of feeding behavior is similar to that seen in Pacific mackerel (Hunter and Kimbrell, 1930). Usually larvae would attack the same prey several times until the prey was ingested if the initial attack was unsuccessful.

Larvae fed mostly on rotifers, although with a jaw width of about 0.30 mm at first feeding, larvae were capable of ingesting much larger prey. Large prey, such as copepods or Artemia nauplii, were usually avoided by 5-7 day old larvae. Eight-ten day old larvae occasionally chased and attacked larger prey, however, only a few successful strikes were observed. Observations were made of 8-10 day old larvae seizing adult copepods and Artemia nauplii and then releasing their prey without subsequent pursuit. Many 8-10 day old larvae were seen along the side walls of the rearing tanks, apparently feeding on copepodites and copepod nauplii.

### Water quality

Dissolved oxygen remained above 80% saturation before food organisms were added to the rearing tanks. After food organisms were added, the daytime dissolved oxygen levels remained above 80% saturation but pre-dawn

levels dropped to 70% saturation or lower. The nighttime dissolved oxygen decrease was prevented by starting mild aeration on day 3, preventing pre-dawn dissolved oxygen levels from dropping below 85% saturation.

Buildup of nutrients (phosphate, nitrate and nitrite, and ammonia) occurred in rearing tanks as waste products from the larvae and food organisms accumulated (Table 3). Average ammonia levels increased in the tanks fivefold from the time of hatching (156.8 ppb) to day 5 (800 ppb) (Table 3). Average nitrate and nitrite levels increased sixfold in the same time period from 39.6 to 249.2 ppb, while phosphate levels increased fourfold from 32.2 to 126.2 ppb (Table 3).

Of the two groups of eggs shipped to La Jolla Laboratory, the Aug. 4 1981 spawn group survived the longest with larvae reared to day 4. Larvae from the same spawn reared at Kewalo laboratory survived to day 5. Since no significant increase in survival was obtained when eggs were reared in La Jolla, water quality at Kewalo laboratory may not be the sole cause for high larval mortality.

#### DISCUSSION AND RECOMMENDATION

The egg and early larval stages of skipjack tuna are similar to early stages of other scombrids, such as the yellowfin tuna, Thunnus albacares (Harada et al., 1971; Inoue et al., 1974a; Harada et al., 1980), the bigeye tuna, T. obesus (Houde and Richards, 1969), the frigate mackerel, Auxis rochei (Inoue et al., 1974a), A. tapeinosoma (Harada et al., 1973a), A. thazard (Harada et al., 1973b), the Pacific mackerel, Scomber japonicus (Hunter and Kimbrell, 1980), and several other scombrid species (Mayo, 1973). All of these species have slow, linear growth from hatching to 3 or 10 days and are about the same size at hatching.

Although the larvae of several scombrid species have been reared beyond metamorphosis, larvae of skipjack tuna have never been reared beyond 12 days after hatching. In an attempt to culture larval skipjack tuna, Japanese scientists obtained eggs from a ripe skipjack tuna female caught in a purse seine and dry fertilized the eggs (Inoue et al., 1974b; Ueyanagi et al., 1974). The subsequent larvae suffered substantial mortalities, with all larvae dying by the fifth day after hatching (Inoue et al., 1974b; Ueyanagi et al., 1974). Mayo (1973) attempted to rear skipjack tuna larvae from eggs caught in a plankton net. These larvae survived to 6.5 days after hatching, reaching a maximum length of 4.17 mm.

Skipjack tuna larvae are proving to be more difficult to culture than other scombrid larvae. The reasons are not obvious for the marked survival and growth of the July 16, 1980 and May 31, 1981 spawns, or for the high mortalities observed in the other groups. The stress-induced spawning method may cause adult skipjack tuna to hydrate and ovulate less than full-term eggs which would result in eggs with less than normal quantities of yolk (Hunter, pers. commun.). Low yolk reserves may result in energy deficiencies that could affect development as well as growth and survival of larvae.

The adults used for stress-induced spawnings are usually of small size, perhaps in the first season of spawning activity, which may also result in the spawning of eggs having low quantities of yolk or spawns with a high percentage of nonviable eggs. However, the stress-induced spawnings yielded normal appearing eggs, with high hatching rates, therefore not all of the problems associated with culturing larvae can be attributed to the spawning method.

If stress-induced spawning produces eggs with low quantities of yolk, it should be possible to compensate for energy deficiencies by feeding larvae prey of high caloric content. Rotifers have a caloric content of  $3.54 \times 10^{-4}$  calories/individual (Theilacker and McMaster, 1971), and copepod nauplii have a caloric content of  $6.8 \times 10^{-3}$  calories/individual (Laurence, 1977). Therefore if a larva ingested a copepod nauplius instead of a rotifer, it could obtain nearly an order of magnitude more calories. Rotifers may not supply adequate energy for skipjack tuna larval growth and survival, yet the rotifer appears to be adequate for first feeding larvae of other scombrids (Inoue et al., 1974a; Hunter and Kimbrell, 1980).

At sea, skipjack tuna larvae have a noticeably different diet than other tunas, skipjack tuna larvae prey predominantly on Appendicularians (Uotani et al., 1981). Perhaps appendicularians have a high caloric content and are necessary in the diet of skipjack tuna larvae for optimal growth and survival.

Water quality, especially the accumulation of ammonia, may affect the successful culture of skipjack tuna larvae. Unionized ammonia is toxic to many species of marine fish larvae at concentrations as low as 300 ppb (Brownell, 1980). Skipjack tuna larvae probably do not encounter ammonia concentrations of 300 ppb or more at sea, which suggests that they may be particularly sensitive to low levels of ammonia.

It is doubtful that water quality alone is causing the high mortalities observed in rearing skipjack tuna larvae, since attempts have been made by several scientists at different laboratories to rear these larvae. It seems that a combination of several factors--diet, water

quality, and egg quality are affecting successful rearing of skipjack tuna larvae in the laboratory. I recommend continuing the rearing effort with the following studies:

1. Eliminate water quality as a causative agent for larval mortality. This can be done by regular monitoring of nutrients and water exchange in the rearing tanks.
2. Establish a criterion for egg quality, egg diameter would probably be a good criterion. It would be simple to compare the results of rearing experiments of spawnings from different adult females with different mean egg diameters. Perhaps larvae reared from spawns of large eggs (0.95-1.0 mm diameter) would grow and survive better than larvae reared from spawns of small eggs (0.90-0.94 mm diameter).
3. Spawn larger fish, using larger sized females and males that are more mature than adults used in past spawnings.
4. Determine the effect of rearing larvae on various prey types and perform stomach analysis of locally caught larvae to determine what types of prey are ingested.
5. Study the energetics of growth of skipjack tuna larvae, comparing various stages of growth and conditions of larvae. An energetic study of a series of larvae reared from spawns of different egg sizes would indicate the relationship between energetic content and egg quality and the effect on larval growth and survival.

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Table 1. Growth of skipjack tuna larvae summarized for rearing experiments from 1980 and 1981. Mean length (mm SL) and standard deviation, spawn date, rearing tank and mean temperature are given.

29 June 1980	29 June 1980	16 July 1980	16 July 1980	21 July 1980	1 August 1980	1 August 1980
Tank A	Tank C	Tank B	Tank C	Tank C	Tank B	Tank C
24.07°C	23.99°C	24.54°C	24.74°C	24.60°C	24.48°C	24.67°C
n x SD	n x SD	n x SD	n x SD	n x SD	n x SD	n x SD
12 2.53 0.17	12 2.71 0.17	15 3.46 0.09	15 3.48 0.08	11 3.64 0.10	15 3.44 0.15	15 3.31 0.15
1 12 3.43 0.22	8 3.33 0.09	20 3.45 0.10	7 3.49 0.10			
2 12 3.56 0.10						
3 10 3.67 0.09	2 3.68	15 3.80 0.24	15 3.70 0.27	11 3.72 0.19	15 3.30 0.16	15 3.35 0.12
5 15 4.10 0.22				15 3.87 0.20		
6 2 4.12				3 3.86 0.09		
7 1 4.46						
8 1 4.37						
9						
10						
11						
31 May 1981	31 May 1981	25 June 1981	25 June 1981	25 June 1981	28 July 1981	4 August 1981
Tank A	Tank B	Tank A	Tank B	Tank C	Tank B	Tank A
26.52°C	24.32°C	24.13°C	23.63°C	22.87°C	24.07°C	24.67°C
n x SD	n x SD	n x SD	n x SD	n x SD	n x SD	n x SD
12 3.57 0.33	15 3.44 0.22	15 3.53 0.10	15 3.55 0.07	15 3.59 0.78	15 2.83 0.14	15 3.54 0.09
1 15 3.58 0.12	15 3.65 0.14			15 3.62 0.07	15 3.12 0.12	15 3.40 0.10
2 15 3.67 0.07	15 3.78 0.17			15 3.60 0.06		
3 15 3.50 0.18	15 3.74 0.17			10 3.60 0.09		
4 15 3.76 0.14	10 3.66 0.24			10 3.51 0.10		
5 5 3.58 0.21	5 3.58 0.21			5 3.63 0.08		
6 2 3.75	2 3.75			4 3.69 0.09		
7 1 4.30						
8 1 4.35						
9						
10						
11						
4 August 1981	4 August 1981					
Tank B	Tank C					
23.76°C	26.04°C					
n x SD	n x SD					
15 3.08 0.15	15 3.53 0.11					
1 15 3.58 0.07	15 3.50 0.09					
2 15 3.59 0.11	15 3.57 0.08					
3 15 3.71 0.11						
4 10 3.72 0.07						
5 15 3.63 0.12	1 3.80					



Table 2. Comparison of lengths of skipjack tuna larvae that are dying (near the bottom) with larvae that are normal (feeding near the surface). Significance levels of statistical analysis (ANOVA, Sokal and Rohlf, 1969) for differences in length are given with the mean lengths (mm) and standard deviations for each group tested. Also noted are the percentage of larvae with jaw abnormalities found in each sample.

Age (days)	Spawn date	Tank	Length (mm)		ANOVA results	
			Normal larvae	Dying larvae		
4	31 May 81	B	$\bar{x}$	3.746	3.606	Significant difference in length ( $P < 0.01$ )
			S.D.	0.168	0.134	
			% abnormal	20%	27%	
4	25 June 81	C	$\bar{x}$	3.595	3.563	No significant difference in length
			S.D.	0.086	0.069	
			% abnormal	not noted		
2	4 Aug. 81	B	$\bar{x}$	3.577	3.537	No significant difference in length
			S.D.	0.105	0.103	
			% abnormal	40%	67%	
4	4 Aug. 81	B	$\bar{x}$	3.533	3.715	Significant difference in length ( $P < 0.001$ )
			S.D.	0.119	0.071	
			% abnormal	60%	100%	
5	4 Aug. 81	B	$\bar{x}$	3.63	3.61	No significant difference in length
			S.D.	0.127	0.159	
			% abnormal	100%	100%	

Table 3. Average nutrient concentrations (and standard deviations) of phosphate, nitrate + nitrite, and ammonia in ppb of water in rearing tanks A and B, 4 Aug. 1981 spawn.

Age (days)		Phosphate	Nitrate + nitrite	Ammonia
0	$\bar{x}$	32.24	39.62	156.8
	S.D.	10.52	2.376	6.534
1	$\bar{x}$	54.56	42.63	192.22
	S.D.	10.52	4.653	36.826
2	$\bar{x}$	71.61	46.62	303.31
	S.D.	0.877	2.772	58.11
3	$\bar{x}$	75.79	65.94	404.39
	S.D.	15.56	3.762	101.87
4	$\bar{x}$	92.76	124.46	510.61
	S.D.	13.72	10.64	113.24
5	$\bar{x}$	126.17	249.20	800.80
	S.D.	0	51.87	175.62

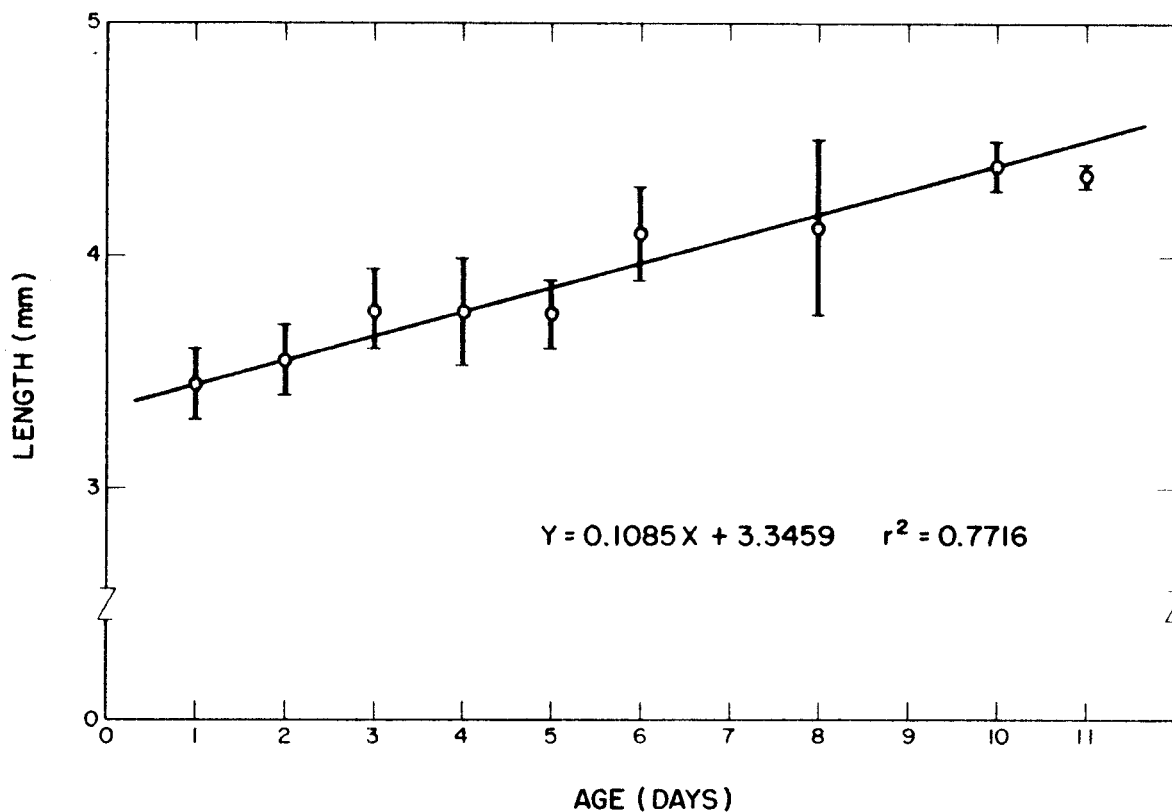


Figure 1. Growth of skipjack tuna larvae (16 July, 1980 and 31 May, 1981 spawns) to 11 days after hatching. Mean lengths  $\pm$  1 standard deviation are given for larvae from combined spawns. (Statistical analysis of separate regression lines obtained from each spawn group indicated no significant difference between slopes or intercepts ( $P < 0.01$ ) therefore data from both spawns were combined).