

# MATERIALS FOR THE CHEMICAL IDENTIFICATION OF EARLY LIFE STAGES OF BLUEFIN TUNA, *THUNNUS THYNNUS*

SCRS/1994/077

Col.Vol.Sci.Pap. ICCAT, 44 (1) : 217-220 (1995)

*Richards, W.J., S. Kelley*

*National Marine Fisheries Service, Southeast Fisheries Center,  
75 Virginia Beach Drive, Miami, Florida 33149, USA*

## SUMMARY

Our research has shown that bluefin tuna eggs and a small percentage of larvae are impossible to identify or discriminate from congeners using conventional morphological means. However, chemical (biochemical or immunological) identification methodology has advanced in recent years which allows the identification of small organisms and also organisms which have been fixed in formalin or ethanol and preserved in ethanol. To investigate the efficacy of these new techniques we have assembled a large number of identified bluefin tuna larvae for analysis. These larvae were collected between 1977 and 1993 and were fixed and preserved in various combinations and strengths of formalin and ethanol. They will be examined with appropriate chemical techniques to determine appropriate methodologies.

We urge others holding early life history stages of bluefin tuna to submit their materials to this effort.

## RESUME

Notre recherche démontre qu'il est impossible d'identifier ou de distinguer les oeufs et un petit pourcentage des larves du thon rouge de ceux de leurs congénères avec des moyens morphologiques conventionnels. Toutefois, les méthodes d'identification chimique (biochimique ou immunologique) ont progressé ces dernières années et permettent d'identifier de petits organismes et des organismes stérilisés à la formaline ou à l'éthanol et conservés dans l'éthanol. Afin d'étudier l'efficacité de ces nouvelles techniques, nous avons rassemblé un grand nombre de larves identifiées de thon rouge pour les analyser. Ces larves ont été collectées entre 1977 et 1993, stérilisées et conservées dans différents mélanges et doses de formaline et d'éthanol. Elles seront examinées avec les techniques chimiques adéquates afin de déterminer les méthodologies appropriées.

Nous encourageons toutes les personnes qui disposeraient d'informations sur les premiers stades du cycle vital du thon rouge à nous remettre leurs éléments d'étude pour ce travail.

## RESUMEN

Nuestra investigación ha mostrado que los huevos de atún rojo y un pequeño porcentaje de larvas son imposibles de identificar o distinguir de congéneres que utilizan medios morfológicos convencionales. No obstante, la metodología de identificación (bioquímica o inmunológica) ha avanzado en los años recientes, lo que permite la identificación de pequeños organismos y también de organismos que se han fijado en formalina o etanol y se han preservado en etanol. Para investigar la eficacia de estas nuevas técnicas hemos juntado un gran número de larvas identificadas de atún rojo para análisis. Estas larvas fueron recolectadas entre 1977 y 1993 y se fijaron y preservaron en diversas combinaciones y proporciones de formalina y etanol. Serán examinadas con las técnicas químicas apropiadas para determinar las metodologías idóneas.

Instamos con urgencia a quienes posean datos sobre las etapas tempranas del ciclo vital del atún rojo a que presenten el material en su poder para ayudar en este esfuerzo.

## INTRODUCTION

The early life history stages of bluefin tuna (*Thunnus thynnus*) are comprised of egg, larval, and juvenile stages. Egg stages have been described, but the methodology has not been developed to separate this species from other similar species. The problem is that bluefin eggs may have discrete pigment characters, but these characters are lost upon preservation. This renders bluefin eggs indistinguishable from others. There is no practical way to identify eggs collected from plankton samples for several reasons: they develop rapidly (time from fertilization to hatching is less than 24 hours); possible diagnostic pigments are lost upon preservation; bluefin have a clear, smooth shell about 0.9-1.1 mm in diameter and most planktonic eggs fall within these boundaries. The embryo has 39 myomeres which are extremely difficult to count and are not unique in number, and the distribution of black chromatophores (melanophores) changes during development and is not dissimilar from many other species. The outlook for identifying eggs from plankton samples by morphological criteria is poor.

Larvae collected from plankton samples are relatively easy to identify. In two studies (Richards and Potthoff 1974; Richards et al. 1990) bluefin larvae can be identified morphologically from late post hatching until late larval stages by the distribution of melanophores on the tail. In some cases an examination of skeletal characters is required. For late larvae and early juveniles we routinely clear and

stain specimens to confirm identifications. Bluefin are unique in having both dorsal and ventral tail melanophores; internally they have 18+21 vertebrae and the first closed hemal arch is on the 10th vertebrae. Richards et al. (1990) provides additional information on larval characters.

Juveniles are difficult to identify morphologically, but they can be identified if great care is used. All juvenile tunas are very similar, but bluefin retain the short pectoral fin and have unique gill raker counts. In small fish the serrations on the liver are difficult to discern. Juveniles from 1.5-20 cm is the difficult size range. Capture of this size range is very rare - occasionally they are dipnetted or caught on hook and line, but most frequently they have been collected from birds. These specimens after spending several hours in the birds crop, are usually in poor condition and require a study of their osteology. Even with this approach many times key characters are lost (Potthoff and Richards 1970).

The purpose of this paper is to encourage the development of biochemical and immunological techniques to identify bluefin. We list the extant larval material in Tables 1 which can be made available for such studies. Additional bluefin larvae from the Gulf of Mexico are held by the SEAMAP Archivist. A ready source of eggs and small juveniles is not available.

#### DISCUSSION

Identification of eggs from plankton samples is needed in order to determine population sizes of spawning adults. Population sizes can be estimated from plankton caught larvae but mortalities are poorly known making such estimates with very wide confidence limits (Richards et al. 1981). With the reduction in larval catches in the last decade, these estimates are even more unreliable. However, it is generally assumed that eggs undergo minimal mortality, especially eggs which develop so rapidly and are exposed to mortality for such short time periods. A method which would reliably quantify the number of eggs in a quantitative plankton sample would easily yield spawning stock size estimates. A method which would just determine the presence or absence of bluefin eggs would be valuable, but would not offer any means to determine spawning stock size. Such a method would just show that bluefin recently spawned in the vicinity which can also be determined from larvae. Fish eggs are positively buoyant thus collection of surface samples and shipboard processing for presence or absence with a biochemical or immunological technique would be helpful, but quantification would require sampling throughout the depth range of eggs (assumed to be the range of adult bluefin). Bluefin are targeted by longliners in the 100-120m depth range. Our discrete depth plankton sampling has just been initiated and small bluefin larvae have been taken as deep as 80m in limited sampling. In our routine sampling we fish plankton nets to 200m (Kelley et al. 1993). If shipboard processing was to be done, a method for extracting eggs quickly would have to be developed.

Identification of larvae using biochemical techniques faces some of the same problems as for eggs. The larvae have to be manually separated from the other plankton components. This is a time consuming process and the material has to be preserved to avoid decomposition. In the past most plankton samples are fixed in formalin (usually 10%). Upon completion of sorting the larvae are transferred to 70% ethanol or

remain in 3% formalin. Our shipboard routine in recent years is to fix in formalin for 24 hours then transfer the sample to 70% ethanol. With the advent of interest in studying otoliths, shipboard collections omit the formalin and preserve the entire sample in 90% ethanol. Once sorting is completed the larvae are placed in 70% ethanol for permanent storage. Shipboard sorting is extremely difficult. The motion the sea has on the ship coupled with ship engine vibration makes microscope work virtually impossible. Rough sorting can be accomplished, but the high magnification needed to reliably identify bluefin larvae cannot be done under shipboard conditions. Biochemical/immunological methods would have to overcome these basic problems. And as with eggs, presence or absence is helpful, but quantitative data are desired.

Juveniles are difficult to catch but bird crops provide a very useful source. Currently this source requires crop content collections from a tern rookery at the Dry Tortugas, Florida. The number of birds is known thus sampling of contents can provide juvenile bluefin population estimates. The problem is that crop contents are very difficult to identify because of decomposition of the fish and often only heads and parts of the skeleton remain (Potthoff and Richards 1970; Miller et al in prep.). A biochemical/immunological technique would be extremely valuable for this work. Estimates of numbers from these bird rookeries would yield an index for determining age 0 stock sizes since mortalities are probably reduced through growth and transport away from bird feeding grounds.

In conclusion, new techniques should be found to utilize eggs, speed up larval and juvenile processing and identification. Chemical markers specific for bluefin early life history stages, or other species as well, would allow more efficient utilization of these stages in population assessment studies. The ideal situation would be collecting the plankton sample or stomach content sample, and, then by adding chemical solutions, the target species would be readily visible for enumeration. Carried a step further the enumeration could be done automatically. The drawback to this automation is the extreme clotting that occurs among plankton specimens, but perhaps this can also be solved.

#### LITERATURE CITED

- Kelley, S., J. V. Gartner, Jr., W. J. Richards, and L. Ejsymont. 1993. SEAMAP 1984 & 1985 - Ichthyoplankton. NOAA Tech. Mem. NMFS-SEFSC-317: 15 p. + 12 tables and 73 figs.
- Miller, R. J., J. A. Browder, J. Cramer, W. B. Robertson, Jr., W. J. Richards, and S. Kelley. In Prep. Biological assessment of tunas and other prey of sooty terns nesting in the Dry Tortugas, Florida. ICCAT Working Document. SCRS/94/.
- Potthoff, T. and W. J. Richards. 1970. Juvenile bluefin tuna, *Thunnus thynnus* Linnaeus, and other scombrids taken by terns in the Dry Tortugas, Florida. Bull. Mar. Sci. 20:389-413.
- Richards, W. J. and T. Potthoff. 1974. Analysis of taxonomic characters of young scombrid fishes, Genus *Thunnus*. Pages 623-648 in Blaxter, J. H. S. (Ed.). The early life history of fish. Springer-Verlag, Berlin, Heidelberg, New York. 765p.
- Richards, W. J., T. Potthoff, E. D. Houde. 1981. Abundance of bluefin tuna larvae and estimates of spawning stock sizes in the Gulf of Mexico in 1977 and 1978. International Commission for the Conservation of Atlantic Tunas. Coll. Vol. Sci. Pap. 15(2):273-277.
- Richards, W. J., T. Potthoff, and J.-m. Kim. 1990. Problems identifying tuna larvae species (Pisces:Scombridae:Thunnus) from the Gulf of Mexico. Fish. Bull. U. S. 88:607-609.

Table 1. List of bluefin tuna *T. thynnus* larvae available for chemical analysis

Oregon II Cruise 7705

Station	Latitude	Longitude	Date	No.fish	Net	Presv.
21973	2400.00N	08059.00W	77/IV/30	1	N947	FORM
21975	2401.00N	08200.00W	77/V/01	2	B505	FORM-ETOH
21981	2702.00N	08500.00W	77/V/02	1	B505	FORM
21987	2659.00N	09100.00W	77/V/06	2	B505	FORM
21989	2701.00N	09301.00W	77/V/06	1	B505	FORM
21990	2701.00N	09501.00W	77/V/07	1	B505	FORM-ETOH
21994	2759.00N	09159.00W	77/V/08	1	B505	FORM
21998	2800.00N	08900.00W	77/V/10	5	N947	FORM-ETOH
21999	2800.02N	08800.00W	77/V/10	7	N947	FORM
22001	2801.00N	08601.00W	77/V/10	7	B505	FORM
22001	2801.00N	08601.00W	77/V/10	34	N947	FORM-ETOH
22002	2800.00N	08501.00W	77/V/10	77	N947	FORM-ETOH
22004	2600.00N	08459.00W	77/V/12	8	N947	FORM
22006	2459.00N	08656.00W	77/V/12	1	N947	FORM-ETOH
22009	2503.00N	09259.00W	77/V/14	1	B505	FORM
22021	2134.00N	08646.00W	77/V/19	3	N947	FORM-ETOH
22026	2342.00N	08314.00W	77/V/21	1	B505	FORM-ETOH
22026	2342.00N	08314.00W	77/V/21	68	N947	FORM-ETOH
22028	2416.00N	08035.00W	77/V/22	4	N947	FORM

Oregon II Cruise 87

23876	2827.50N	08258.80W	78/V/02	1	B505	FORM
23876	2827.50N	08258.80W	78/V/02	1	B333	FORM
23902	2630.00N	09200.00W	78/V/08	12	B505	FORM
23955	2600.00N	08400.00W	78/V/23	13	N947	FORM
23958	2600.00N	08400.00W	78/V/23	16	N947	FORM-ETOH
23959	2800.00N	08500.00W	78/V/24	1	B505	FORM
23960	2830.00N	08531.00W	78/V/24	1	B505	FORM-ETOH
23961	2800.00N	08530.00W	78/V/24	1	B505	FORM
23961	2800.00N	08530.00W	78/V/24	2	N947	FORM-ETOH
23962	2730.00N	08500.00W	78/V/24	2	B505	FORM
23962	2730.00N	08500.00W	78/V/24	1	N947	FORM
23963	2701.00N	08501.00W	78/V/24	18	B505	FORM
23963	2701.00N	08501.00W	78/V/24	60	N947	FORM
23964	2630.00N	08500.00W	78/V/24	8	B505	FORM
23965	2600.00N	08500.00W	78/V/25	79	N947	FORM
23974	2331.50N	08700.50W	78/V/26	1	N947	FORM
23975	2330.00N	08730.00W	78/V/26	1	N947	FORM
23978	2400.00N	08730.00W	78/V/26	12	N947	FORM
23979	2400.00N	08700.00W	78/V/27	2	N947	FORM
23983	2500.00N	08600.00W	78/V/27	1	N947	FORM
23985	2500.00N	08659.00W	78/V/27	4	B505	FORM
23985	2500.00N	08659.00W	78/V/27	1	N947	FORM
23986	2530.00N	08700.00W	78/V/27	5	N947	FORM
23988	2559.50N	08630.50W	78/V/28	1	N947	FORM
23989	2600.00N	08600.00W	78/V/28	1	B505	FORM

Table 1 Cont.

Oregon II Cruise 87

23989	2600.00N	08600.00W	78/V/28	2	N947	FORM
23996	2729.50N	08600.00W	78/V/29	14	N947	FORM
23997	2800.00N	08600.00W	78/V/29	1	B505	FORM
23997	2800.00N	08600.00W	78/V/29	32	N947	FORM
23998	2800.00N	08630.00W	78/V/29	3	B505	FORM
23998	2800.00N	08630.00W	78/V/29	11	N947	FORM
23999	2800.00N	08701.00W	78/V/29	4	B505	FORM
23999	2800.00N	08701.00W	78/V/29	34	N947	FORM
24000	2800.00N	08730.00W	78/V/29	4	B505	FORM
24000	2800.00N	08730.00W	78/V/29	3	N947	FORM
24001	2830.00N	08730.00W	78/V/29	16	B505	FORM
24001	2830.00N	08730.00W	78/V/29	57	N947	FORM
24002	2830.00N	08659.00W	78/V/29	8	B505	FORM
24002	2830.00N	08659.00W	78/V/29	12	N947	FORM
24004	2830.00N	08601.00W	78/V/29	2	B505	FORM
24004	2830.00N	08601.00W	78/V/29	5	N947	FORM
24007	2900.00N	08700.00W	78/V/29	3	B505	FORM
24007	2900.00N	08700.00W	78/V/29	78	N947	FORM
24008	2922.00N	08730.00W	78/V/29	18	B505	FORM
24008	2922.00N	08730.00W	78/V/29	66	N947	FORM
24009	2922.00N	08730.00W	78/V/29	4	N947	FORM

Oregon II Cruise 117

34491	2359.70N	09500.20W	81/V/18	2	B333	FORM
34497	2752.00N	09349.84W	81/V/20	2	N947	FORM-ETOH
34498	2754.80N	09349.45W	81/V/20	2	N947	FORM
34501	2752.10N	09349.40W	81/V/20	1	B505	FORM
34506	2749.49N	09349.61W	81/V/20	50	N947	ETOH
34508	2752.20N	09349.50W	81/V/20	7	N947	ETOH
34510	2754.83N	09349.20W	81/V/20	2	N947	ETOH
34513	2752.80N	09349.70W	81/V/20	14	N947	ETOH
34514	2754.60N	09349.30W	81/V/20	9	N947	ETOH
34515	2749.70N	09349.10W	81/V/21	28	N947	ETOH
34516	2753.20N	09349.70W	81/V/21	131	N947	ETOH
34518	2800.00N	09300.00W	81/V/21	6	N947	FORM
34519	2659.90N	09259.80W	81/V/21	25	N947	FORM
34522	2500.10N	09200.10W	81/V/22	1	N947	FORM
34525	2759.50N	09200.90W	81/V/23	24	N947	FORM
34527	2700.50N	09100.30W	81/V/24	1	N947	FORM
34530	2500.30N	09000.50W	81/V/24	13	N947	FORM
34534	2700.30N	08959.80W	81/V/25	10	N947	FORM
34537	2859.40N	08759.20W	81/V/26	2	N947	FORM

Oregon II Cruise 146

41380	2700.00N	09330.00W	84/VIII/18	1	N947	FORM
41463	2747.00N	08508.00W	84/VIII/24	2	B333	FORM

Table 1 Cont.

Oregon II Cruise 159  
44091 2830.00N 08530.00W 86/V/21 1 N947 FORM

## Oregon II Cruise 166

45334 2600.00N 09330.00W 87/IV/30 5 N947 ETOH  
45351 2650.00N 08800.00 87/V/03 1 B333 ETOH  
45357 2830.00N 08800.00 87/V/03 2 N947 ETOH  
45361 2930.00N 08630.00N 87/V/07 2 N947 ETOH  
45362 2900.00N 08600.00W 87/V/07 1 N947 ETOH  
45366 2700.00N 08600.00W 87/V/08 1 N947 ETOH  
45369 2600.00N 08429.90W 87/V/08 1 N947 ETOH  
45375 2600.00N 08500.00W 87/V/09 2 B333 ETOH  
45376 2600.00N 08457.90W 87/V/09 4 N947 ETOH  
45377 2559.70N 08459.80W 87/V/09 13 N947 ETOH  
45394 2730.00N 08600.00W 87/V/11 1 N947 ETOH  
45396 2726.00N 08600.00W 87/V/12 2 N947 ETOH  
45397 2728.00N 08600.00W 87/V/12 1 N947 ETOH  
45398 2730.00N 08600.00W 87/V/12 1 N947 ETOH  
45399 2732.00N 08600.00W 87/V/12 1 N947 ETOH  
45400 2734.00N 08600.00W 87/V/12 19 N947 ETOH  
45401 2735.98N 08559.93W 87/V/12 1 N947 ETOH  
45414 2751.00N 08700.00W 87/V/13 4 N947 ETOH  
45416 2743.00N 08700.00W 87/V/13 48 N947 ETOH  
45417 2739.00N 08700.00W 87/V/13 8 N947 ETOH  
45422 2719.00N 08700.00W 87/V/14 1 N947 ETOH  
45429 2630.00N 08800.00W 87/V/15 2 N947 ETOH  
45435 2600.00N 08900.00W 87/V/16 2 N947 ETOH  
45436 2600.00N 08930.00W 87/V/16 2 N947 ETOH  
45437 2600.00N 09000.00W 87/V/16 38 N947 ETOH  
45438 2630.00N 09000.00W 87/V/16 6 N947 ETOH  
45439 2700.00N 09000.00W 87/V/17 19 N947 ETOH  
45444 2600.00N 09130.00W 87/V/17 3 N947 ETOH  
45449 2700.00N 09300.00W 87/V/18 1 N947 ETOH  
45450 2630.00N 09300.00W 87/V/18 1 N947 ETOH  
45455 2700.00N 09400.10W 87/V/19 1 N947 ETOH  
45456 2730.00N 09330.00W 87/V/19 1 N947 ETOH  
45460 2800.00N 09130.00W 87/V/20 2 N947 ETOH  
45465 2800.00N 08900.00W 87/V/20 1 N947 ETOH  
45467 2830.00N 08800.00W 87/V/20 1 N947 ETOH  
45471 2810.00N 08548.00W 87/V/22 1 N947 ETOH  
45472 2806.00N 08548.00W 87/V/22 5 N947 ETOH  
45477 2746.00N 08548.00W 87/V/22 1 N947 ETOH  
45479 2738.00N 08548.00W 87/V/22 1 N947 ETOH

## Oregon II Cruise 173

47543 2659.70N 08829.90W 88/IV/26 2 N947 ETOH  
47577 2814.80N 08559.50W 88/V/06 2 N947 ETOH  
47579 2804.80N 08550.70W 88/V/06 6 N947 ETOH  
47581 2755.20N 08559.80W 88/V/07 2 O/CB ETOH  
47582 2750.10N 08600.00W 88/V/07 2 N947 ETOH

Table 1 Cont.

## Oregon II Cruise 173

47582 2750.10N 08600.00W 88/V/07 5 O/CB ETOH  
47588 2507.50N 08512.40W 88/V/09 2 O/CB ETOH  
47637 2710.00N 08959.90W 88/V/21 1 N947 ETOH  
47647 2700.00N 09258.30W 88/V/22 1 N947 ETOH  
47648 2629.90N 09259.80W 88/V/22 1 N947 ETOH  
47649 2614.00N 09259.80W 88/V/22 15 N947 ETOH  
47658 2759.90N 09129.90W 88/V/24 16 N947 ETOH  
47660 2800.00N 09030.00W 88/V/24 1 N947 ETOH  
47662 2800.10N 08929.90W 88/V/25 2 N947 ETOH  
47664 2800.00N 08829.90W 88/V/25 4 N947 ETOH  
47666 2830.00N 08800.00W 88/V/25 6 N947 ETOH

## Albatross IV Cruise 8902

14 08 2648.33N 08601.12W 89/IV/27 2 MOC8 ETOH  
14 09 2648.33N 08601.12W 89/IV/27 2 MOC9 ETOH  
65 2900.00N 08615.10W 89/V/05 1 B333 ETOH  
73 2600.00N 08400.00W 89/V/07 1 B333 ETOH  
74 2518.00N 08400.00W 89/V/07 2 B333 ETOH  
77 07 2495.27N 08406.70W 89/V/07 2 MOC7 ETOH