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A CENTRAL NORTH PACIFIC SPAWNING GROUND FOR STRIPED MARLIN, *TETRAPTURUS AUDAX*

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ABSTRACT

Istiophorid billfishes are notoriously difficult to identify to species, especially early life history stages. Traditional use of morphology and pigment based characters for species separation of larvae can fail when faced with moderate levels of intra-specific phenotypic plasticity. Unfortunately, as the early life history of marine fishes can play an important role in reproductive success and management strategies, we are potentially missing or confounding valuable data by misidentifying specimens. We recently presented a DNA based method of identification for near real-time processing of ichthyoplankton samples. Using this method we have unambiguously identified seven striped marlin larvae from Hawaiian waters. Additional analysis of DNA sequence data shows that these larvae all have unique mitochondrial haplotypes indicating they each had different mothers and were not the result of a single chance event. This represents the first detection of spawning activity in an area that historically has been regarded as only nursery habitat for this species. This finding has important ramifications for fishery management as striped marlin represent a significant portion of the billfish catch in the Hawaii-based longline fishery.

Despite their importance in commercial and recreational fisheries, relatively little is known concerning the early life history and reproductive strategies of billfishes (Istiophoridae and Xiphiidae). Until recently, fertilized eggs of the Pacific istiophorids remained unknown, but through the use of digital imaging and genetic identification techniques, the fertilized eggs of blue marlin, *Makaira nigricans* (Lacépède, 1802) and shortbill spearfish, *Tetrapturus angustirostris* (Tanaka, 1915) have been described. Small larvae (< 20 mm TL) are somewhat common but larger juveniles are exceedingly rare. Identification to species has been a particular problem for many larval istiophorids (Richards, 1974; Collette et al., 1984; Nishikawa and Rimmer, 1987; Nishikawa and Ueyanagi, 1992), usually relying upon slight differences in pigmentation (Nishikawa, 1991) and morphometric characters. This inability to reliably identify larvae to species further complicates the collection of early life history data. Despite the difficulty of larval identifications, the distributions and seasonality of billfish larvae have been well documented (Howard and Ueyanagi, 1965; Matsumoto and Kazama, 1974; Nishikawa et al., 1978; Nakamura, 1985). The advent of DNA based techniques (Chow, 1994; Innes et al., 1998; McDowell and Graves, 2002; Hyde et al., 2005) has served to validate previous identification methods and to increase the throughput and accuracy of current studies.

We previously reported on a technique used to identify eggs and larvae in near real-time (Hyde et al., 2005). Using this technique we have documented spawning activity for striped marlin (*T. audax*) for the first time off the Kona coast of Hawaii. Past studies have shown striped marlin larvae to occur primarily in waters of the northwestern Pacific, southwestern Pacific (Howard and Ueyanagi, 1965; Matsumoto and Kazama, 1974; Nakamura, 1985), and to a lesser extent in the northeastern Pacific (Armas et al., 1999). Though well sampled, there was no evidence for spawn-

ing of striped marlin around the Hawaiian Islands in the central north Pacific (Matsumoto and Kazama, 1974). The larval distribution atlas of Nishikawa et al. (1978) reported collections of striped marlin larvae from the central Pacific, suggesting that spawning may occur around the Hawaiian Islands.

Common in Hawaiian waters, striped marlin are caught in high numbers by the Hawaii-based longline fleet. For the period 1991–2002, striped marlin represented roughly half of the istiophorid billfish catch, with some uncertainty due to known misidentification problems (Curran et al., 1996; Walsh et al., 2005). These misidentifications are primarily striped marlin misidentified as black, *Makaira indica* (Cuvier, 1832) or blue marlin due to morphological similarity and inexperienced fishery observers (Walsh et al., 2005). Despite their abundance in this region, these animals are primarily sub-adults, using this area as a feeding ground (Matsumoto and Kazama, 1974). Striped marlin first appear in the fishery around 11 kg and remain for approximately 2 yrs (45 kg), at which point they are believed to migrate to spawning areas in the west Pacific (Matsumoto and Kazama, 1974). Size at first reproduction has been reported as 29 kg in the Coral Sea (Hanamoto, 1977). Animals of reproductive size are less common, but do occur off Hawaii (Matsumoto and Kazama, 1974; Squire and Suzuki, 1990; Brill et al., 1993). Tagging studies have shown movement of reproductive size fish from the northeastern Pacific into Hawaiian waters (Bromhead et al., 2004). Though striped marlin dominate the longline billfish catch, blue marlin and shortbill spearfish dominate in collections of larval istiophorids in the central Pacific (Matsumoto and Kazama, 1974; Hyde et al., 2005; Humphreys, Jr., unpubl. data).

Methods

Larvae and eggs were collected using a 1.8 m opening Isaacs-Kidd trawl fitted entirely with 0.505 mm nylon mesh and a rigid PVC cod-end. Sample tows were conducted 2–25 mi offshore of Hawaii, targeting surface slicks when possible. Salinity and temperature measurements as well as acoustic Doppler current profiler (ADCP) measurements were taken continuously during sample tows. All tows were conducted during daytime hours at the surface, sampling the neuston and upper 1 m of the water column. Surface tows were conducted exclusively as the majority of billfish eggs and larvae tend to be captured at or just below the sea surface (Matsumoto and Kazama, 1974; Humphreys, Jr., unpubl. data).

For a detailed description of the genetic identification techniques see Hyde et al. (2005). Briefly, DNA was extracted from eye tissue of seven istiophorid larvae using a boiling technique, queried through the use of a multiplex species-specific polymerase chain reaction (PCR), electrophoresed through an agarose gel, and species identification was inferred by comparing the size of the resulting DNA amplicon to those generated from reference specimens.

Though multiplex PCR has proven a reliable method for species identification we chose to confirm the unusual identifications of striped marlin larvae by sequencing a portion of the mitochondrial cytochrome *b* gene. PCR was performed using primers universalbillF (5′ AAT GAA TYT GAG GAG GCT TCT C) and universalbillR (5′ GTC GGA ADG TTA GGC CTC G). Briefly, 10 µL reaction volumes containing (67 mM Tris-HCl pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 10 mM β-mercapto-ethanol, 2 mM $MgCl_2$, 800 µM dNTPs, 0.4 µM each primer, 0.5 units *Taq* DNA polymerase (New England Biolabs), and 1 µL of DNA template) were amplified using the following temperature profile in a PTC200 DNA Engine (MJ Research); 94 °C (2:00), 35 cycles of [94 °C (0:30), 55 °C (1:00), 72 °C (1:00)], followed by three min at 72 °C. Products were electrophoresed through a 2% (w/v) agarose gel in 1 X Tris-Borate-EDTA buffer, stained with ethidium bromide and visualized via an UV-transilluminator. Reactions were digested using

ExoSAP-IT (USB Corp.) to remove unincorporated primers and deoxynucleotides prior to cycle sequencing. Products were cycle sequenced with BigDye v.1.1 (Applied Biosystems) and analyzed on an ABI 3100 automated capillary sequencer (Applied Biosystems).

DNA sequences were aligned and edited using Sequencher v4.5 (GeneCodes, Inc). Consensus sequences from the five species of Pacific istiophorid billfishes, Genbank accession #AY319369-AY319373, were combined with larval sequences and were evaluated for evolutionary model testing using Modeltest v3.7 (Posada and Crandall, 1998) as implemented using the PAUP* (v4.b10) (Swofford, 2001) framework. The data best fit the model of Tamura and Nei (1993) considering a gamma shape distribution. A maximum likelihood analysis was employed using this model; 1000 iterations of a 50% nonparametric jackknife were used to assess confidence of identifications. All larval sequences grouped with the striped marlin consensus sequence with 98% jackknife support. Cytochrome *b* sequences have been deposited in Genbank under the following accession numbers, DQ111996-DQ112002. In order to estimate the minimum number of mothers that produced the seven larvae, the 5′ hyper-variable portion of the mitochondrial control region was sequenced. PCR conditions were as previous, with the exception that primers ThrRF (5′ GAG GAY AAA GCA CTT GAA TGA GC) and DRF (5′CCT GAA AAT AAG AAC CAA ATG CCA G) were used. PCR amplicons were sequenced and edited as before. Sequences were compared to each other to determine the number of unique haplotypes present. Each larva possessed an unique haplotype. As mitochondrial DNA is inherited maternally, this indicates that these larvae came from different mothers. These sequences have been deposited in GenBank under the following accession numbers, DQ115565-DQ115571.

Morphology.—In addition to genetic means of identification, the seven larvae were compared to morphology and pigment character keys. All larvae lacked pigmentation on the lower half of the lower jaw and branchiostegal membranes, diagnostic of either blue or striped marlin (Ueyanagi, 1963). Following Matsumoto and Kazama (1974) snout/orbit length ratios (Table 1) were plotted against standard length and compared to their data. All larvae clustered around the line of best fit for striped marlin, though as noted by the authors, smaller specimens (SL < 6 mm) are not reliably separated from blue marlin using this metric. The orientation of both pterotic and preopercular spines were noted following Ueyanagi (1974). In this case, three of the seven larvae did not fit the described condition of pterotic spines being nearly parallel to the axis of the body (Fig. 1A,B,D). It should be noted that these outliers were smaller specimens; the largest specimens conformed well to this description.

Results and Discussion

Multiplex PCR and DNA sequence data clearly revealed that the seven istiophorid larvae were striped marlin. Additional DNA data showed that all larvae came from different mothers. This finding is of note as spawning for this species was not thought to occur around the Hawaiian Islands despite the abundance of fishery-caught animals (Matsumoto and Kazama, 1974). As all seven larvae came from different mothers, additional support is provided that this was not a single chance event, but that the Kona coast of Hawaii is a spawning area for this species.

The Kona coast has been shown to be a productive spawning ground for blue marlin, shortbill spearfish, and swordfish, *Xiphias gladius* (Linnaeus, 1758). Several of the Hawaiian Islands, especially Hawaii, consistently generate eddy fields due to their size and unique oceanography (Lobel and Robinson, 1986). Eddies generated by island wakes are believed to act as retention mechanisms for reef larvae and may act to concentrate prey items (Lobel and Robinson, 1986; Wolanski and Sarenski, 1997). This may be particularly important for fast growing predators, such as billfish, that need an abundant food supply to satisfy metabolic demands. Tracking and catch

Table 1. Summary of collection data for striped marlin larvae collected off Hawaii. Genbank accession numbers represent cytochrome b and control region sequences, respectively.

Station ID	Sample ID	Standard length (mm)	Snout length (mm)	Orbit length (mm)	Tow end points	Date	Mean salinity	Mean SST (°C)	Tow speed (kts)	Genbank accession #'s
8	OES0507008BF005	8.0	1.6	1.6	19°14.1 N 155°59.0 W	27 May 2005	34.46	26.15	3	DQ111996
					19°16.6 N 155°57.3 W					DQ115568
9	OES0507009BF003	4.6	0.7	0.8	19°16.5 N 155°58.0 W	27 May 2005	34.38	26.42	3	DQ111997
					19°19.1 N 155°59.0 W					DQ115569
10	OES0507010BF004	6.2	1.0	1.0	19°19.3 N 155°59.0 W	27 May 2005	34.44	26.31	3	DQ111998
					19°19.4 N 155°55.8 W					DQ115570
21	OES0507021BF007	6.4	1.5	1.6	19°14.5 N 155°58.0 W	28 May 2005	34.44	25.94	3	DQ111999
					19°17.0 N 155°56.7 W					DQ115571
22	OES0507022BF001	26.7	9.4	2.6	19°19.4 N 115°55.6 W	28 May 2005	34.44	26.16	3	DQ112000
					19°19.2 N 155°58.8 W					DQ115565
24	OES0507024BF004	6.1	0.9	0.9	19°19.2 N 155°55.3 W	28 May 2005	34.45	26.31	3	DQ112001
					19°19.4 N 155°53.9 W					DQ115566
46	OES0507046BF003	15.2	4.0	2.5	19°26.2 N 156°06.2 W	30 May 2005	34.52	26.55	3	DQ112002
					19°26.2 N 156°09.3 W					DQ115567

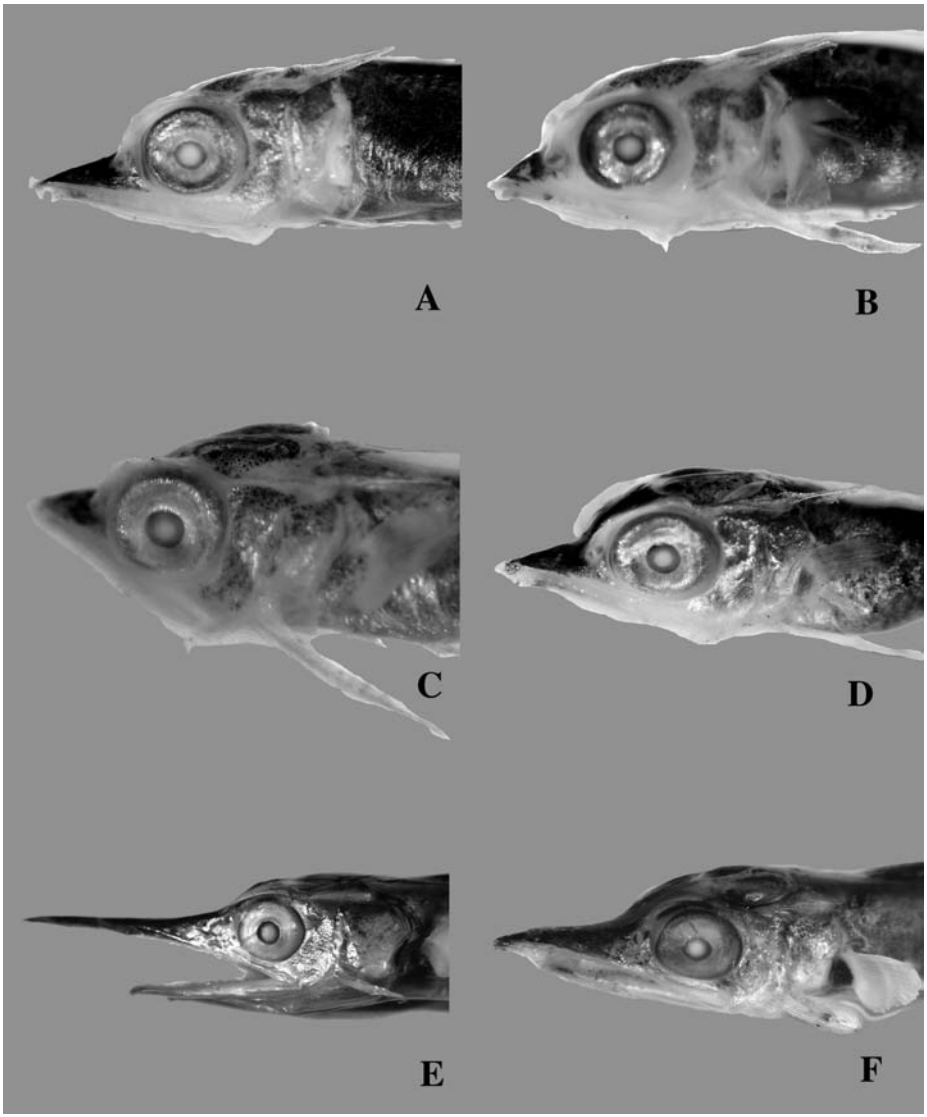

Figure 1. Photographs of six larval striped marlin showing head spination patterns: A (OES-0507008BF005), B (OES0507009BF003), C (OES0507010BF004), D (OES0507021BF007), E (OES0507022BF001), and F (OES0507046BF003).

record studies of adult billfish have shown they have an affinity for these eddies. Brill et al. (1993) used ultrasonic tags to track striped marlin off Hawaii. They found that several of their tagged striped marlin tracked and maintained themselves within these eddies over the course of the study. Similarly, Seki et al. (2002) noted a shift in the catch of blue marlin, likely due to the presence of a cyclonic eddy and its relation to local fishing grounds.

The striped marlin larvae were found within the shoreward front of an anti-cyclonic eddy offshore of Kealakekua Bay that maintained location and intensity over the course of the cruise. Though anti-cyclonic eddies are known to capture and concentrate passive particles we found no billfish eggs or larvae within the eddy core.

Collections within the eddy were depauperate in fish eggs and larvae when compared to tows along the periphery. Though downwelling dominates within anti-cyclonic eddies, shear along the periphery produces areas of small-scale upwelling (Mizobata et al., 2002). These patchy vortices of enhanced productivity may aid both in the retention and provisioning of prey items for larvae. On several occasions when large numbers of billfish eggs and larvae were encountered, we repeatedly sampled the same station to assess patchiness. In all instances our catch rate declined precipitously from the original tow suggesting that the water mass containing the eggs and larvae was small, as it was able to exit the study site within an hour or two. In addition to the striped marlin larvae, eggs and larvae of blue marlin, shortbill spearfish, and swordfish were found within the same net tows. The concurrent finding of four species of billfish larvae and three species of billfish eggs within the periphery of an anti-cyclonic eddy suggests that it may be an important element of nursery habitat in this region for the early life stages of these species.

It is possible that larvae of striped marlin have not previously been documented in Hawaiian waters as they were mistakenly identified as another, more abundant species. There may, however, be a more intriguing hypothesis. As noted by Hinton and Bayliff (2002), the mean size modes of longline caught striped marlin have declined dramatically in the northeastern Pacific over the past 30 yrs. Fishing pressure has been shown to cause shifts in life history parameters favoring fish that mature younger and at smaller sizes (Olsen et al., 2005). It may be that the observed spawning activity near Hawaii is a result of such fishery-induced early maturation and warrants further investigation.

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Literature Cited

Armas, R. G., O. Sosa-Nishizaki, R. F. Rodriguez, and V. A. L. Perez. 1999. Confirmation of the spawning area of the striped marlin, *Tetrapturus audax*, in the so-called core area of the eastern tropical Pacific off Mexico. Fish. Oceanogr. 8: 238–242.

Brill, R. W., D. B. Holts, R. K. C. Chang, S. Sullivan, H. Dewar, and F. G. Carey. 1993. Vertical and horizontal movements of striped marlin (*Tetrapturus audax*) near the Hawaiian Islands, determined by ultrasonic telemetry, with simultaneous measurement of oceanic currents. Mar. Biol. 117: 567–574.

Bromhead, D., J. Pepperell, B. Wise, and J. Findlay. 2004. Striped marlin: biology and fisheries. Bureau of Rural Sciences. Canberra. 260 p.

Chow, S. 1994. Identification of billfish species using mitochondrial cytochrome *b* gene fragment amplified by polymerase chain reaction. Int. Commission for the Conservation of Atlantic Tunas. Report of the 2nd ICCAT billfish workshop. ICCAT XLI: 549–556.

Collette, B. B., T. Potthoff, W. J. Richards, S. Ueyanagi, J. L. Russo, and Y. Nishikawa. 1984. Scombroidei: development and relationships. Pages 591–620 *in* H. G. Moser, W. J. Richards, D. M. Cohen, M. P. Fahay, A. W. Kendall, and S. L. Richardson, eds. Ontogeny and systematics of fishes. Spec. Publ. No. 1. Amer. Soc. Ichthy. Herpet. 760 p.

Curran, D. S., C. H. Boggs, and X. He. 1996. Catch and effort of Hawaii's longline fishery summarized by quarters and five degree squares. NOAA-TM-NMFS-SWFSC-225. 72 p.

Cuvier, G. and A. Valenciennes. 1832. Histoire naturelle des poissons. Tome huitième. Livre neuvième. Des Scombéroïdes. Hist. Nat. Poiss. i-xix. 509 p.

Hanamoto, E. 1977. Fishery oceanography of striped marlin I. Fishing season, fishing ground and movement pattern of the fish in the southern Coral Sea. Bull. Jap. Soc. Sci. Fish. 43: 649–657.

Hinton, M. and W. Bayliff. 2002. Status of striped marlin in the Eastern Tropical Pacific Ocean in 2001 and outlook for 2002. BBRG-1: 15th Meeting of the Standing Committee on Tuna and Billfish, Honolulu, Hawaii. In press.

Howard, J. K. and S. Ueyanagi. 1965. Distribution and relative abundance of billfishes (Istiophoridae) of the Pacific Ocean. Stud. Trop. Oceanogr. 2: 134 p.

Hyde, J. R., E. Lynn, R. Humphreys Jr., M. Musyl, A. P. West, and R. Vetter. 2005. Shipboard identification of fish eggs and larvae by multiplex PCR, and description of fertilized eggs of blue marlin, shortbill spearfish, and wahoo. Mar. Ecol. Prog. Ser. 286: 269–277.

Innes, B. H., P. M. Grewe, and R. D. Ward. 1998. PCR-based genetic identification of marlin and other billfish. Mar. Freshw. Res. 49: 383–388.

Lacépède, B. G. E. 1802. Histoire naturelle des poissons. Hist. Nat. Poiss. i-xliv. 728 p.

Linnaeus, C. 1758. Systema Naturae, Ed. X. (Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata.) Holmiae. Systema Nat. 10 i-ii: 824 p.

Lobel, P. S. and A. L. Robinson. 1986. Transport and entrapment of fish larvae by ocean mesoscale eddies and currents in Hawaiian waters. Deep. Sea. Res. 33: 483–500.

Matsumoto, W. M. and T. K. Kazama. 1974. Occurrence of young billfishes in the Central Pacific Ocean. Pages 238–247 *in* R. S. Shomura and F. Williams, eds. Proc. Int. Billfish Symp. Kailua-Kona, Hawaii, 9–12 August 1972. Part 2. Review and Contributed Papers.

McDowell, J. R. and J. E. Graves. 2002. Nuclear and mitochondrial DNA markers for specific identification of istiophorid and xiphiid billfishes. Fish. Bull. 100: 537–544.

Mitzobata, K., S. I. Saitoh, A. Shiomoto, T. miyamura, N. Shiga, K. imai, M. Toratani, Y. Kajiwara, and K. Sasaoka. 2002. Bering Sea cyclonic and anticyclonic eddies observed during summer 2000 and 2001. Prog. Oceanogr. 55: 65–75.

Nakamura, I. 1985. FAO species catalog. Vol. 5. Billfishes of the world. An annotated and illustrated catalogue of marlins, sailfishes, spearfishes, and swordfishes known to date. FAO Fish. Synop. 5: 65 p.

Nishikawa, Y. 1991. On the melanophore patterns on lower jaw of the larvae of striped marlin. Bull. Nat. Res. Inst. Far. Seas. Fish. 28: 15–19.

_____ and D. W. Rimmer. 1987. Identification of larval tunas, billfishes, and other scombroid fishes (suborder Scombroidei): an illustrated guide. CSIRO Marine Laboratories Report 186: 20 p.

_____ and S. Ueyanagi. 1992. On the larvae of the black marlin *Makaira indica*. Bull. Nat. Res. Inst. Far. Seas. Fish. 29: 1–7.

_____, S. Kikawa, M. Honma, and S. Ueyanagi. 1978. Distribution atlas of larval tunas, billfishes, and related species—results of larval surveys by R/V Shunyo Maru and Shoyo Maru, 1956–1975. Far Seas Fish. Res. Lab. Shimuzu: 100 p.

Olsen, E. M., G. R. Lilly, M. Heino, M. J. Morgan, J. Brattey, and U. Diekman. 2005. Assessing changes in age and size at maturation in collapsing populations of Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 62: 811–823.

Posada, D. and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Philippi, R. A. 1887. Historia natural—Sobre los tiburones i algunos otros peces de Chile. [Plus:] Apendice sobre el peje-espada, peje-aguja, peje-perro i vieja negra. An. Univ. Santiago Chile: 535–574.

Richards, W. J. 1974. Evaluation of identification methods for young billfishes. Pages 62–72 *in* R. S. Shomura and F. Williams, eds. Proc. Int. Billfish Symp. Kailua-Kona, Hawaii, 9–12 August 1972. Part 2. Review and Contributed Papers.

Seki, M. P., R. Lumpkin, and P. Flament. 2002. Hawaii cyclonic eddies and blue marlin catches: The case study of the 1995 Hawaiian Int. Billfish Tournament. J. Oceanogr. 58: 739–745.

Squire, J. L. Jr. and Z. Suzuki. 1990. Migration trends of striped marlin (*Tetrapturus audax*) in the Pacific Ocean. Pages 67–80 *in* R. H. Stroud, ed. Planning the future of billfishes. Research and management in the 90's and beyond. Proc. 2nd Int. Billfish Symp., Kailua-Kona, Hawaii, 1–5 August 1988. Part 2. Contributed papers.

Swofford, D. L. 2001. PAUP* Phylogenetic analysis using parsimony (*and other methods), 4.0b10. Sinauer Associates, Sunderland.

Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10: 512–526.

Tanaka, S. 1915. Figures and descriptions of the fishes of Japan including Riukiu Islands, Bonin Islands, Formosa, Kurile Islands, Korea and southern Sakhalin. Fig. Descr. Fish. Japan: 319–342.

Ueyanagi, S. 1963. Methods for identification and discrimination of the larvae of five istiophorid species distributing in the Indo-Pacific. Rep. Nankai Reg. Fish. Res. Lab. 17: 137–150.

_____. 1974. On an additional diagnostic character for the identification of billfish larvae with some notes on the variations in pigmentation. Pages 73–78 *in* R. S. Shomura and F. Williams, eds. Proc. Int. Billfish Symp., Kailua-Kona, Hawaii, 9–12 August 1972. Part 2. Review and Contributed Papers.

_____ and P. G. Wares. 1975. Synopsis of biological data on striped marlin, *Tetrapturus audax* (Philippi, 1887). Pages 132–159 *in* R. S. Shomura and F. Williams, eds. Proc. Int. Billfish Symp., Kailua-Kona, Hawaii, 9–12 August 1972, Part 3. Species synopses.

Walsh, W. A., R. Y. Ito, K. E. Kawamoto, and M. McCracken. 2005. Analysis of logbook accuracy for blue marlin (*Makaira nigricans*) in the Hawaii-based longline fishery with a generalized additive model and commercial sales data. Fish. Res. 75: 175–192.

Wolanski, E. and J. Sarenski. 1997. Larvae dispersion in coral reefs and mangroves. American Scientist. 85: 236–243.

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