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BIOLOGY AND FISHERY OF DOLPHINFISH AND RELATED SPECIES. *E. MASSUTÍ and B. MORALES-NIN (eds.)*

Genetic homogeneity of dolphinfish (*Coryphaena hippurus*) in the western Mediterranean and the eastern Atlantic*

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SUMMARY: Dolphinfish (*Coryphaena hippurus*) is an epipelagic, highly migratory species distributed worldwide in tropical and temperate waters including the Mediterranean Sea. Protein electrophoresis analyses can provide knowledge of the genetic population structure of the species and therefore be used as a tool for fishery management. Areas sampled include the islands of Majorca and Sicily in the western Mediterranean and the Canary Islands in the eastern Atlantic. The results of the protein electrophoresis reveal a level of genetic variability similar to other highly migratory species. No differences were found among locations, and it was not possible to reject the null hypothesis of one panmictic population in the area studied.

Key words: dolphinfish, *Coryphaena hippurus*, protein electrophoresis, population genetics, Mediterranean Sea, Eastern Atlantic.

INTRODUCTION

Dolphinfish (*Coryphaena hippurus*) is an epipelagic species distributed worldwide in all tropical and temperate oceans limited to the 20ºC isocline (Gibbs and Collette, 1959). In the eastern Atlantic it is limited north to the Bay of Biscay and it is found all over the Mediterranean Sea except in the Black and Adriatic seas (Shcherbachev, 1973; Collette, 1986). Important fisheries exist in the Western Mediterranean in the islands of Majorca, Sicily and Malta, where dolphinfish is exploited by traditional and artisanal fisheries (Massutí and Morales-Nin, 1991).

Very little is known about the biology, migratory patterns and population structure of the species in the Mediterranean area. Genetic analysis is useful for describing the evolutionary patterns of the population structure in fishes and may lead to a redefinition of existing populations and stock concepts based on morphological data (Utter, 1991; Kinsey *et al.,* 1994).

In recent years great progress has been made towards a proper understanding of the genetic structure of fish species thanks to the application of molecular techniques such as protein electrophoresis. The principles of protein electrophoresis were first applied to population genetics studies in the 1960s in organisms such as drosophila (Lewontin and Hubby, 1966) and humans (Harris, 1966). In the 70s the technique was applied to fish population studies (Avise, 1974; Utter *et al.*, 1974), reaching its maximum applicability in the 80s (Ryman and Stahl, 1981; Allendorf *et al.*, 1987; Utter *et al.*, 1987; Utter, 1991).

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TABLE 1. – Summary of sampling location.

Location	1995 sample size	1996 sample size	Total sample size	Harbours
1 Gran Canaria	18	0	18	Arguineguín, Mogán
2 Northwest Majorca	223	76	299	Andratx, Pollença, Sóller
3 Southeast Majorca	61	41	102	Cala Figuera, Cala Rajada, Portocolom
4 Tyrrhenian Sea	104	50	154	Balastrate, Santa Agata, Trapani
5 Ionian Sea	40	23	63	Siracusa
6 Strait of Sicily	51	48	99	Lampedusa, Linosa
Total	497	238	735	

MATERIAL AND METHODS

A total of 735 juvenile dolphinfish samples were obtained during the 1995 and 1996 fishing seasons using fish aggregation devices (FADs). Areas sampled for this study included the Canary Islands in the eastern Atlantic and the islands of Majorca and Sicily in the western Mediterranean. Six different locations were considered for the genetic analysis: Gran Canaria (1), Northwest Majorca (2), Southeast Majorca (3), South Tyrrhenian Sea (4), Ionian Sea (5) and Strait of Sicilia (6). Table 1 summarises the sample size obtained in each location studied and the harbours where samples were collected.

Tissues analysed were heart, liver and skeletal muscle. Tissue extraction, electrophoresis and procedures for visualizing proteins generally followed the methods of Aebersold *et al*. (1987) with minor modifications (García-Marín *et al*., 1991). The electrophoresis was carried out in 11% starch horizontal gels. Genetic interpretation of banding patterns produced by the enzyme systems followed the principles outlined in Utter *et al*. (1987). Genetic nomenclature followed the suggestions of Shaklee *et al*. (1990). A summary of the enzyme systems analysed in this study and the nomenclature is:

AAT (aspartate aminotransferase), bBGA (N-acetyl-b-galactosidase), EST (esterase), FH (fumarate hydratase), G3PDH (glycerol-3-phosphate dehydrogenase), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), aGLU (a-glucosidase), GPI (glucose-6-phosphate isomerase), IDDH (L-iditol dehydrogenase), IDHP (isocitrate dehydrogenase), LDH (L-lactate dehydrogenase), aMAN (a-mannosidase), MDH (malate dehydrogenase), MPI (mannose-6-phosphate isomerase), PEP-GL (peptidase glycyl-leucine), PEP-LGG (peptidase leucyl-glycyl-glycine), PEP-PAP (peptidase phenilalanyl-proline), PGDH (phosphogluconate dehydrogenase), PGM (phosphoglucomutase), PK (pyruvate kinase), SOD (superoxide dismutase) and XDH (xanthine dehydrogenase). The buffer and tissue giving the best results for each enzyme analysed are summarised in Table 2.

Electrophoretic data were analysed with the BIOSYS Program (Swofford and Selander, 1981). Initially, allelic and genotypic frequencies were calculated. The genetic variability of the species was studied by determining the levels of polymorphism and heterozygosity. Polymorphism was examined on

TABLE 2. – Summary of enzymes studied for *Coryphaena hippurus* with locus abbreviation, Enzyme Commission number (E. C. number), best buffer and tissue used and result.

Locus	E. C. number	Buffer	Tissue	Result
AAT*	2.6.1.1.	AC	M	M
bBGA*	3.2.1.30.	AC	L	M
EST*	3.1.1.1.	TCLB	L	P
FH*	4.2.1.2.	AC	M, L, H	M
G3PDH*	1.1.1.8.	AC	L	P
GAPDH-1*	1.2.1.12.	AC	M	M
GAPDH-2*		AC	M	M
aGLU*	3.2.1.20.	AC	L	M
GPI*	5.3.1.9.	TCLB	M	P
IDDH*	1.1.1.14.	AC	M	M
IDHP-1*	1.1.1.42.	AC	M	P
IDHP-2*		AC	L	P
LDH-1*	1.1.1.27.	AC	M, L, H	M
LDH-2*		AC	M, L, H	M
aMAN*	3.2.1.24.	AC	L	M
MDH-1*	1.1.1.37.	AC	M, L, H	M
MDH-2*		AC	M, L, H	M
MDH-3*		AC	M, L, H	P
MPI*	5.3.1.8.	AC	M, L	P
PEP-GL*	3.4.13.-.	TCLB, AC	M, L, H	M
PEP-LGG*	3.4.13.-.	TCLB, AC	M, L, H	M
PEP-PAP*	3.4.13.-.	TCLB, AC	M, L, H	M
PGDH*	1.1.1.43.	AC	M	M
PGM*	5.4.2.2.	TCLB	M	P
PK-1*	2.7.1.40.	AC	M, L, H	M
PK-2*		AC	M, L, H	M
PK-3*		AC	M, L, H	M
SOD-1*	1.15.1.1.	AC, TCLB	M, L, H	M
SOD-2*		AC, TCLB	M, L, H	M
XDH*	1.2.1.37.	TCLB	L	M

Buffers, AC: electrode- (pH 7.1) 0.04M Citric acid, add N-(3-aminopropil)-morpholine until pH is reached/gel- (pH 7.1) Electrode buffer diluted 1:20. TCLB: electrode- (pH 8.1) 0.06M Lithium hydroxide, 0.03M Boric acid/ gel- (pH 8.5) 0.076M Tris, 0.05M Citric acid.
Tissue: H heart, L liver, M muscle.
Result: M monomorphic, P polymorphic.

TABLE 3. – Allele frequencies for all polymorphic loci found in all locations.

Locus	Allele	Gran Canaria (1)	Northwest Majorca (2)	Southeast Majorca(3)	Tyrrhenian Sea (4)	Ionian Sea (5)	Strait of Sicily (6)
EST*	*100	1.000	0.987	0.985	0.977	0.992	0.995
	*90	0.000	0.013	0.015	0.023	0.008	0.005
G3PDH*	*100	1.000	0.987	0.969	0.972	0.968	1.000
	*60	0.000	0.013	0.031	0.028	0.032	0.000
GPI*	*100	0.944	0.935	0.941	0.935	0.976	0.955
	*80	0.028	0.043	0.015	0.036	0.008	0.025
	*120	0.022	0.022	0.044	0.029	0.016	0.020
IDHP-1*	*100	0.778	0.820	0.867	0.832	0.724	0.847
	*120	0.228	0.180	0.133	0.168	0.276	0.153
IDHP-2*	*100	0.800	0.695	0.654	0.690	0.721	0.750
	*90	0.200	0.302	0.346	0.310	0.279	0.250
	*120	0.000	0.003	0.000	0.000	0.000	0.000
MDH-3*	*100	1.000	0.990	0.995	0.994	0.992	0.990
	*110	0.000	0.010	0.005	0.006	0.008	0.010
MPI*	*100	0.861	0.902	0.908	0.885	0.852	0.866
	*120	0.139	0.098	0.092	0.145	0.148	0.134
PGM*	*100	1.000	0.990	0.990	0.990	1.000	0.995
	*70	0.000	0.010	0.010	0.010	0.000	0.005

the basis of no criterion (a locus is considered polymorphic when two or more alleles are present) and on the basis of the 95% criterion (the frequency of the most common allele is equal to or less than 95%).

A preliminary analysis compared all samples within locations and tested annual differences by checking deviations from the Hardy-Weinberg equilibrium using the independence chi-square test and the exact probabilities test. The pooled sample for all locations was also tested for Hardy-Weinberg equilibrium. Differences in allele frequencies among samples were tested by contingency chi-square analysis (Sokal and Rohfl, 1981). Genetic diversity was estimated using the F statistics: Fis, Fit and Fst (Wright, 1951). The Fst value was calculated to check differentiation among sample locations. Relationship between samples was calculated using standard genetic distances according to Nei (1972).

RESULTS

Thirty genetic loci were examined for mendelian variation. After analysing all locations, 22 loci were found to be monomorphic: AAT*, bBGA*, FH*, GAPDH-1*, GAPDH-2*, aGLU*, IDDH*, LDH-1*, LDH-2*, aMAN*, MDH-1*, MDH-2*, PEP-GL*, PEP-LGG*, PEP-PAP*, PGDH*, PK-1*, PK-2*, PK-3*, SOD-1*, SOD-2* and XDH*. For the rest of the loci, 4 were highly polymorphic: GPI*, IDHP-1*, IDHP-2* and MPI*; the other 4 loci were only polymorphic when no criterion was applied: EST*, G3PDH*, MDH-3* and PGM*. Table 3 shows allele frequencies for all polymorphic loci found in the different sample locations.

The polymorphism value ranged between 13.33 and 26.67% using no criterion. Using the 95% criterion, the polymorphism ranged between 10.00 and 13.33%. The lowest amount of polymorphism was found in the Gran Canaria location. The level of expected heterozygosity ranged between 0.035 and 0.041. Table 4 summarises the genetic variability found in each location as judged by the levels of polymorphism and heterozygosity.

No deviations were found when each location was tested for Hardy-Weinberg equilibrium. The comparison of annual samples in each location also showed no differences. The pooled sample for all locations also did not deviate from Hardy-Weinberg equilibrium.

The comparison of allelic frequencies among locations using the contingency chi-square analysis (Table 5) showed only small differences at one locus, IDHP-1* ($p < 0.035$). For the rest of the loci no differences were found (total $p < 0.302$).

Genetic differentiation analysis among locations is shown in Table 6. All Fst values were very low, including the mean value for all loci (0.010). The

TABLE 4. – Genetic variability found in each location including level of polymorphism (using no criterion and using the 95% criterion) and heterozygosity (Ho-observed heterozygosity; He-expected heterozygosity).

Location	Sample size	Polymorphism(no criterion)	Polymorphism(95% criterion)	Ho	He
1 Gran Canaria	18	13.33	13.33	0.037	0.035
2 Northwest Majorca	299	26.67	13.33	0.041	0.037
3 Southeast Majorca	102	26.67	13.33	0.039	0.037
4 Tyrrhenian Sea	154	26.67	13.33	0.047	0.040
5 Ionian Sea	63	23.33	10.00	0.048	0.041
6 Strait of Sicily	99	23.33	10.00	0.043	0.036

TABLE 5. – Contingency chi-square at all loci for all locations.

Locus	Nº alleles	χ^2	Degrees of freedom	p
EST*	2	3.762	5	0.58420
G3PDH*	2	8.896	5	0.11329
GPI*	3	10.973	10	0.35960
IDHP-1*	2	11.961	5	0.03533
IDHP-2*	3	7.450	10	0.68242
MDH-3*	2	1.021	5	0.96083
MPI*	2	5.430	5	0.36569
PGM*	3	10.421	10	0.40441
Totals		59.914	55	0.30210

TABLE 6. – Summary of F statistics at all loci.

Locus	Fis	Fit	Fst
EST*	-0.024	-0.013	0.011
G3PDH*	-0.037	-0.023	0.014
GPI*	0.062	0.067	0.006
IDHP-1*	-0.260	-0.242	0.014
IDHP-2*	-0.105	-0.089	0.014
MDH-3*	-0.012	-0.006	0.006
MPI*	0.159	0.151	0.007
PGM*	-0.009	-0.003	0.007
Totals	-0.138	-0.134	0.010

average genetic distance was < 0.001 between any location compared, precluding the construction of any informative dendrogram.

DISCUSSION

The genetic variability found for dolphinfish in this study is moderately high as judged by the levels of polymorphism and heterozygosity. The highest polymorphism found was 26.67% using no criterion. When the 95% criterion was used this value decreased to 13.33% due to the fact that only four loci are highly polymorphic. The results obtained were similar among locations. Nevertheless, the lowest levels of polymorphism were found in the locations where less individuals were sampled. Thus, Gran Canaria with 18 individuals showed the lowest value. The level of heterozygosity found in this study is similar to the level reported by Pla *et al*. (1995) for other highly migratory pelagic species such as bluefin tuna (0.029 to 0.039) or bonito (0.036 to 0.051), and slightly lower than the mean heterozygosity value reported by Ward *et al*. (1994) for marine fishes (0.064).

No significant deviations were found within locations. Thus we can consider each location to fit separately under Hardy-Weinberg equilibrium as no differences were observed between the different catches of a same location. No differences were found in the comparison of the samples collected during the 1995 and 1996 fishing seasons in each location. Consequently, the several annual samples for each location were pooled and regarded as only one sample.

The comparison of the allele frequencies among locations using a contingency chi-square test showed no significant deviations and we can consider the six locations studied to have the same allelic frequencies. The Fst value, which measures the genetic divergence, was very low and not significant, showing no differences among locations. This was confirmed by the genetic distances, which were extremely low for any two locations compared. Both Fst and Nei's distance data suggested a lack of substructuring for dolphinfish in the area sampled.

Accordingly, treating all samples as a single location (pooling together the samples for all six locations) yielded no significant deviations from Hardy-Weinberg equilibrium, which means that based on this study we fail to reject the null hypothesis of one panmictic population of dolphinfish in the area studied. Although no differences were found, the sample size of Gran Canaria should be enlarged to confirm this fact.

This lack of structuring and the similarity between locations must be related to the highly migratory behaviour of dolphinfish. The seasonal

behaviour of the species is supposed to be similar to bluefin tuna, which inhabits the eastern Atlantic in the winter months, enters the Mediterranean Sea through the Strait of Gibraltar to spawn during the summer and returns again to the Atlantic at the end of the fall (Pujolar *et al.*, 1998). This hypothesis is supported by the dolphinfish cath data in the Canary Islands, where the species is only collected until the end of May. The amount of gene flow caused by migrations can lead to the homogenisation of geographically separated populations, not allowing differentiation or structuring. This conclusion should be taken with reservation until additional studies involving other genetic markers such as mtDNA sequences or microsatellites provide further evidence.

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