A population study of *Diplodus annularis* (Perciformes: Sparidae), low genetic variation through allozyme analyses for the Maltese populations

Noel VELLA, B.Sc. (Hons)
Assistant Lecturer, Deptartment of Chemistry, University of Malta
Junior College, Malta
e-mail: noel.vella@um.edu.mt

Adriana VELLA, Ph.D.
Senior Lecturer, Department of Biology, University of Malta, Malta
e-mail: adriana.vella@um.edu.mt

Abstract: *This study focuses on the genetic structure of the population of the annular seabream, Diplodus annularis. A number of specimens were collected from thirteen sites around the Maltese Islands (Central Mediterraenan), and were analyzed for seven allozyme loci. This investigation showed a low genetic variation for the sampled population. Also this investigation explored the possibility of utilizing non-invasive sampling for allozyme analyses.*

Keywords: *Allozymes, Sparidae, population structure.*

Introduction

In the Mediterranean, the Family Sparidae (Order: Perciformes), commonly known as seabreams, is represented by at least 11 genera and 23 species, of which at least 17 species known to reside around the Maltese Islands.[1] This family is composed of a variety of fish, some of which are excellent food fishes and consequently giving this Family a high economic value. In fact in the Mediterranean Sea, the Family Sparidae constitutes an important fishery resource, with the guilted seabream (*Sparus aurata*), being commercially exploited through aquaculture. However, even though this group of organisms have been studied for their commercial use, little

[1] Fisher, Schneider, and Bauchot 1987; Lanfranco 1993.

data has been collected on the wild stocks and their population genetic structure throughout their home range.

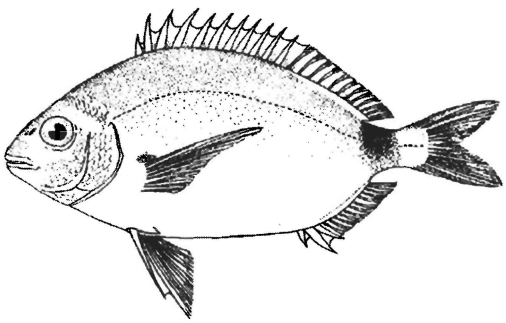

Figure 1. A diagram of *Diplodus annularis* (maximum total length 182 mm – in this study)

This study tackles one species from this Family, and goes through a population genetics study of the annular seabream, *Diplodus annularis* (Figure 1). *D. annularis* is a one of the smallest sparid species encountered around the Maltese Islands. Like other sparids, this species' life-history can be split in to two key phases: a dispersal larval phase and a demersal adult phase. Eggs are externally fertilized, and developing larvae stay in the pelagic phase for around one-and-a-half months,[2] meaning that the larvae of different areas can mix depending on the sea currents present at the time. In the Maltese scenario, the most predominant sea currents along the coast are north-west currents, though at the surface these are quite likely to change due to the direction of the winds.[3] *D. annularis* larvae are known to settle at total length of around 8 to 9 mm in order to start the second phase of their life cycle. Little is known about the choice of habitat for larval settlement, but the transitional period between these two phases is considered to be a crucial step due to changes in the morphology, general habitat, diet, and activity patters. After settling, individuals tend to move to deeper waters as they grow in size, thus the size class structure depends on the depth and also on the type of coast present.[4] Locally *D. annularis* is commonly encountered and plays an important ecological role in the ecosystems it resides in, but its stocks have been exploited by artisanal and hobby fishermen. Furthermore other anthropogenic factors such as habitat modification, pollution, and disturbances are also playing a role on this species' life history, especially in the most sensitive parts of its life-cycle mainly being the larval stages. Thus detailed population studies of this species' phylogeography and

[2] Harmelin–Vivien *et al.* 1995.
[3] Drago 1991; Drago *et al.* 2003.
[4] Harmelin-Vivien *et al.* 1995; Pajuelo *et al.* 2001.

distribution are imperative as to safeguard the current stocks, their genetic pools including all the characters that made this species adapted to survive in the local marine habitats.

To increase the knowledge on the population structure and population genetics of this species, a technique known as allozyme electrophoresis was utilized to identify genetic differences at the level of proteins. Allozyme analysis has proven to be powerful genetic technique in the understanding of populations, as it helps to discriminate between individuals at and within the species level.[5] The intraspecific variation makes it easier to differentiate between different stocks and populations of various organisms especially in populations where physical barriers are very difficult to identify, such as in the marine environment.

Methodology

A total of 110 *D. annularis* specimens were collected from 13 sites around the Maltese Islands (Figure 2 and Table 1) using artisanal fishing gear. All individuals showed adult morphology and, once captured, they were identified to the genus level using general morphological features such as the basic body shape; the silvery-yellow body and the number of spikes on dorsal and anal fins. Then identification to the species level was done according to the features described by Fisher,[6] where a number of characters were used to positively discriminate between different *Diplodus* species and identify *D. annularis*.

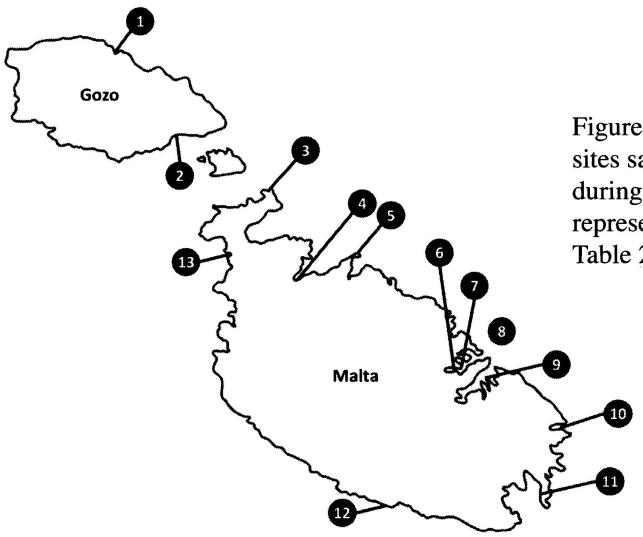

Figure 2. A map of Malta showing the sites sampled for *Diplodus annularis* during this study. (Numbers representing the sites are described in Table 2)

[5] Buth and Murphy 1999; Richardson *et al.* 1986; Shanklee *et al.* 1998.

[6] W. Fischer, M. Schneider, and M.L. Bauchot (1987), 'Sparidae' In Fiches FAO d' Identification des Especes pour les Besoins de la Peche – Mediterranee et Mer Noire, Zone de Peche 37, Vol. II, Rome, 1343–63.

Table 1 A table showing a list of the sites sampled (numbers coincide with Figure 1); Average sampling depth in (m); Sample size (number of specimens); maximum and minimum total body length (mm); and the mean total body length (mm) including its standard deviation (mm).

	Sites sampled	Average sampling depth (m)	Sample size	Total body length range (mm)	Mean total body length (mm)
1	Marsalforn	7.5	1	n/a	113.0 n/a
2	Mġarr	4.9	13	95–151	122.2 ±15.2
3	White Tower Bay	3.5	2	94–107	100.5 ±9.2
4	Xemxija	0.8	14	80–105	93.5 ±7.2
5	Qawra	3.0	6	103–157	134.8 ±24.9
6	Pieta	8.0	6	122–140	130.0 ±7.9
7	Ta' Xbiex	4.3	10	104–177	144–1 ±24.9
8	Dragut Shoal	10.0	12	113–148	132.8 ±9.3
9	Vittoriosa	8.0	15	102–182	131.1 ±18.3
10	Marsascala	3.2	9	106–160	137.9 ±18.9
11	Marsaxlokk	0.6	13	87–144	107.4 ±16.5
12	Għar Lapsi	6.0	7	92–122	112.0 ±10.8
13	Anchor Bay	6.9	2	96–127	111.5 ±21.9
	Total of 13 sites	5.1	110	80–182	122.3 ±21.9

On identification, the total length of each individual was measured to the nearest mm and recorded (Table 1). Specimens were immediately cooled to –20°C and within few hours, muscle tissues, fins, and fish scales were dissected from each specimen and stored at –80°C to avoid enzyme degradation. Each sampled tissue was homogenized using 1:1 w/v extraction buffer composed of 0.05M Tris-HCl pH7.5, 1% w/v NaCl and 0.1% v/v mercaphtoethanol.[7] The macerate was centrifuged at 12000rpm for 30mins at 2°C to separate the lysate from the cellular debris. The lysate collected after no more than three freezing thawing cycles was loaded on to a 12% w/v starch gel. The running conditions consisted of two buffer systems, one buffer system was discontinuous Lithium-Borate (pH 8.5/pH 8.1) and the second was a continuous buffer Tris-Citrate (pH 8.0).[8] Electrophoresis was conducted for 5 hours to separate the allozymes according to charge and size, with the discontinuous buffer system using 242V, while the continuous buffer using 160V. Gels were sliced to stain multiple enzymes simultaneously using the staining protocols from May 1992.[9] A number of enzymes were tested at different buffer systems, but the best scorable zymograms were obtained for G3PDH, GPI, LDH,

[7] Murphy *et al.* 1996; May 1992; Richardson *et al.* 1986.

[8] McAndrew and Majumdar 1982; Martinez *at al.* 1991.

[9] Morizot *at al.* 1990 and Murphy *et al.* 1996. Any bands observed were interpreted according to Buth 1990, Richardson *et al.* 1986; and Murphy *et al.* 1996.

and MDH, covering a total of 7 loci (Table 2). The data collected was statistically analysed to calculate the expected heterozygosity, Nei's Genetic Identify, and Nei's Genetic Distance for each sampled site using equations from May 1992.

Table 2 List of enzyme analysed (abbreviation) including the E.C. number, the tissue assayed, the buffer system used and the number of loci analysed per enzyme.

Enzyme (abbreviation)		E.C. number	Tissue	Buffer System	Number of loci
Glycerol-3-phosphate dehydorgenase	(G3PDH)	1.1.1.8.	muscle	Tris-Citrate	1
Glucose-6-phosphate dehydrogenase	(GPI)	5.3.1.9.	muscle	Lithium-Borate	2
Lactate dehydrogenase	(LDH)	1.1.1.27.	muscle	Tris-Citrate	1
Malate dehydrogenase	(MDH)	1.1.1.37.	muscle	Tris-Citrate	3

Results

The molecular data collected during this study showed that the genetic variability in terms of polymorphism was negligible. In fact from the seven loci analyzed, all of them exhibited one allele, with the only exception of locus *MDH-1** which exhibited a second allele but since it was recorded only in one individual, then it had to be rejected at a P-value of 0.99. Thus all loci were monomorphic and no heterozygotes were identified. In view of this, comparisons of sites against each other lead to a Nei's Genetic Identity value of one and a Nei's Genetic Distance of zero, when the sampled sites were compared against each other. This was due to the fact that no genetic differences were identified between the various areas and thus it was not possible to discriminate between each sampled site, so it was impossible to infer the population genetic structure of this species at the sites studied.

Moreover, one drawback of allozyme studies is that some loci exhibit tissue specificity (Figures 3–6), while significant amounts of tissues are required.[10] In general, muscle and other vital organs such as liver, heart, and eye tissues are used for fish allozyme studies. But this study also explored the possibility of utilizing small amounts of fin tissues or scales (easily obtainable without sacrificing the fish) to attain results for a few loci. In the present study, only *MDH-2** exhibited a degree of activity from such tissues. The importance of such analyses is that being applied as a non-invasive sampling method especially if this technique is to be utilized to study a number of allozyme loci of endangered species.

[10] Martinez *et al.* 1991; Shanklee *et al.* 1998.

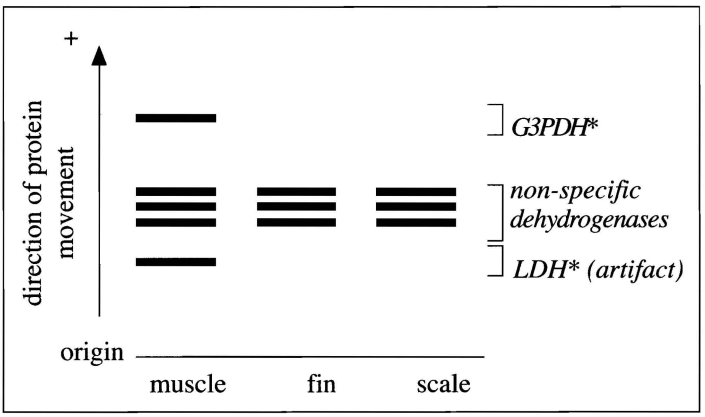

Figure 3.

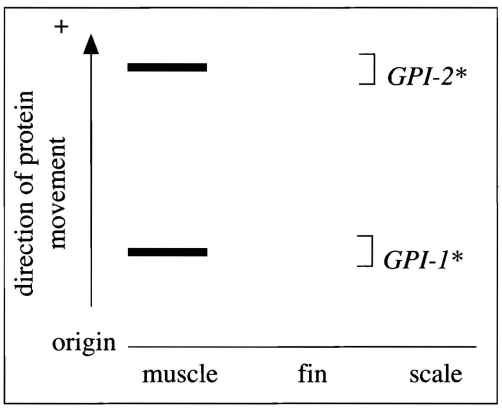

Figure 4.

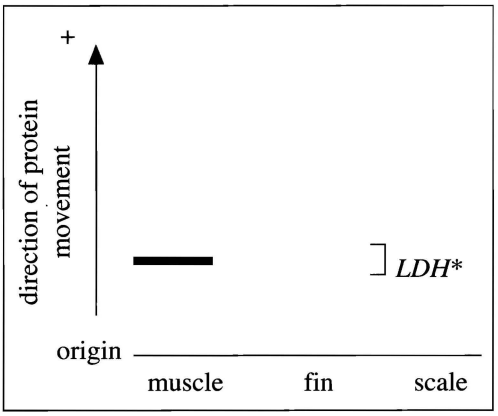

Figure 5.

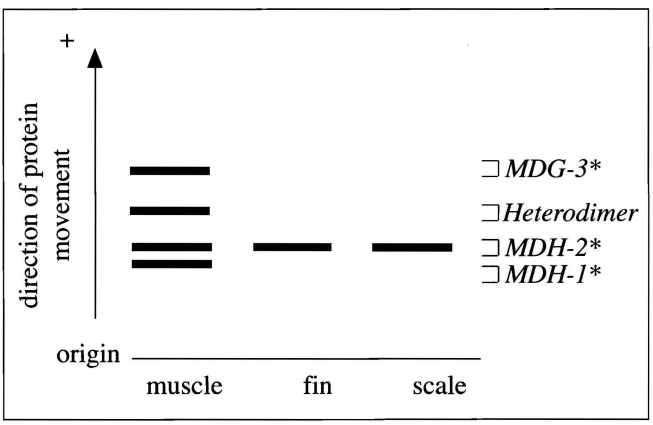

Figure 6.

Figures 3–6. *A diagrammatic representation of the banding patterns observed for the loci studied, including the labels of each band respectively.*

Discussion and Conclusions

Several factors may have attributed to the low genetic variability observed in this study. First of all allozymes tend to overlook certain genetic differences, especially if they are due to neutral mutations or if the genetic differences do not lead to a difference in the mobility of the proteins being analysed through the starch gel electrophoresis. The effect of the latter was minimized as starch gel was used since it is able to separate proteins according to both size and charge, while various electrophoretic conditions were used to maximize the possibility of separating different alleles from each other. Additionally, the choice of the loci analysed in this study was based on results obtained for other sparid species.[11] The latter two studies have shown that a number of loci (including the ones analysed in this study), happened to be polymorphic for most species of *Diplodus*, including *D. sargus* which is the most closely related species to *D. annularis*.[12] So even though the loci chosen for this study were polymorphic in several *Diplodus* species, they have shown no level of polymorphism in *D. annularis* during the present study.

In addition to the above, one has to consider the natural processes leading to a decrease in polymorphism. This can be attributed to a combination of factors, with the major promoters of such phenomenon being a population decline towards bottleneck or a founder effect. The former can be supported if low levels of heterozygosity are found within a specific geographical area, while sister groups exhibit higher levels of polymorphism. However, this cannot be proved since there are no other studies on this species on a regional scale to enable any comparisons. On the other hand, the founder effect may hold since if the population is set off by a small group of individuals, then any population rising from the founder individuals would only have the genetic character of the alleles present in the initiation founder population. So if no new alleles are brought in the population through migration and no new alleles are produced within the population through mutation, then the chances of genetic variation would be extremely low. Moreover, if the founder population is very small, then the above can be corroborated with inbreeding and genetic drift, thus the chances of loss of certain alleles would be enhanced leading to an increase in homogeneity.[13]

[11] Reina *et al.* 1992; Alarcon *et al.* 1999.

[12] Basaglia *et al.* 1991; De La Herran *et al.* 2001.

[13] Allendorf and Luikart 2006; Hunter and Gibbs 2007.

References

Alarcon, J.A. and M.C. Alvarez (1999), 'Genetic identification of sparid species by isoenzyme markers: application to interspecific hybrids', *Aquaculture*, 173: 95–103.

Allendorf, F.W. and G. Luikart (2006), *Conservation and the genetics of populations*, Singapore.

Basaglia, F. and M.G. Marchetti (1991), 'Study of soluble white muscle tissue proteins from fifteen Sparidae species', *Journal of Fish Biology*, 38: 763–72.

Buth, D.G. and R.W. Murphy (1999), 'The use of isoenzyme characters in systematic studies', *Biochemical Systematics and Ecology*, 27: 117–29.

Buth, D.G. (1990), 'Genetic principles and the interpretation of electrophoretic data', in D.H. Whitmore 1990, *Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management*, Florida, 1–21.

De La Herran R., C. Ruiz Rejon, M. Ruiz Rejon, and M.A. Garrido-Ramos (2001), 'The molecular phylogeny of the Sparidae (Pisces, Perciformes) based on two satellite DNA families', *Heredity*, 87: 691–97.

Drago A. (1991), *Physical oceanography in the coastal waters of the Maltese Islands*, Malta.

Drago A., R. Sorgente, and A. Ribotti (2003), 'A high resolution hydrodynamical 3D model of the Maltese Shelf area', *Annales Geophysicae*, 21: 323–44.

Fischer W., M. Schneider, and M.L. Bauchot (1987), 'Sparidae' In *Fiches FAO d' Identification des Especes pour les Besoins de la Peche – Mediterranee et Mer Noire, Zone de Peche 37*, Vol. II, Rome, 1343–63.

Harmelin-Vivien M.L., J.G. Harmelin, and V. Leboulleux (1995), 'Microhabitat requirements for the settlement of juvenile sparid fishes on the Mediterranean rocky shores', *Hydrobiologia*, 300/1: 309–20.

Hunter M.L. and J.P. Gibbs, (2007), *Fundamentals of Conservation Biology* (5th edition), Massachusetts.

Lanfranco, G. (1993). *The fish around Malta*, Malta, 4–38.

Martinez, G., I. McEwen, B.J. Andrew, and M.C. Alvarez (1991), 'Electrophoretic analysis of protein variation in two Spanish populations of the European seabass, Dicentrarchus labrax L. (Pisces: Moronidae)', *Aquaculture and Fisheries Management*, 22: 443–55.

May B. (1992), 'Starch gel electrophoresis of allozymes', in A.R. Hoelzel (ed.), *Molecular genetic analysis of populations – A practical approach*, Oxford, 1–25.

McAndrew, B.J. and K.C. Majumdar (1983), 'Tilapia stock identification using electrophoretic markers', *Aquaculture*, 30:249–61.

Murphy R.W., J.W. Sites Jr., D.G. Buth, and C.H. Haufler (1996), 'Proteins: Isoenzyme electrophoresis', in D.M. Hills, C. Moritz, and B.K. Mable (eds.), *Molecular systematics* (2nd edition), Massachusetts.

Pajuelo J.G. and Lorenzo J.M., (2001), 'Biology of the annular seabream, *Diplodus annularis* (Sparidae), in coastal waters of the Canary Islands', *Journal of Applied Ichthyology*, 17:121–5.

Reina J., G. Martinez, A. Amores, and M.C. Alvarez (1994), 'Interspecific genetic differentiation in Western Mediterranean sparid fish', *Aquaculture*, 125:47–57.

Richardson B.J., P.R. Baverestock, and Adams M., (1986), *Allozyme electrophoresis: A handbook for animal systematics and population studies*, Sydney.

Shanklee J.B. and P. Bentez (1998), 'Genetic identification of stocks of marine fish and shellfish', *Bulletin of Marine Science* 62(2): 589–621.