

## Microsatellite markers in Aquaculture: Application in Fish population genetics

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**Abstract:** Microsatellites or Simple Sequence Repeats (SSRs) represent an abundant source of genetic markers which are highly abundant and dispersed evenly throughout eukaryotic genomes. They have become the markers of choice for a wide range of applications in population genetic, conservation and evolutionary biology. Microsatellites represent ideal molecular markers because they have multiple alleles which are highly polymorphic among individuals. Polymorphism is achieved by having variable numbers of tandem repeat motifs resulting in size variation which can then be visualized by PCR with pairs of locus-specific flanking primers, followed by electrophoresis of the amplification product. Microsatellite motifs occur once every 10kb in fishes. They are inherited in a co-dominant fashion, and are fast and easy to assay. They are co-dominant in nature with high levels of polymorphism and can reproduce very well. Hence, giving better information than the dominant marker. This makes them a choice maker for estimating population structure and genetic diversity.

**Keywords:** Microsatellites, markers, fish, population, genetics

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### I. Introduction

Molecular genetic markers are powerful tools to detect genetic uniqueness of individuals, populations or species (Doveri *et al.*, 2008). Modern sequence based marker systems for genetic analysis such as Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSRs) are now predominantly used (Duran *et al.* 2009).

However, Microsatellites have become the marker of choice for application in fish population genetic studies (Beckmann and Soller, 1990). They have multiple alleles which are highly polymorphic among individuals. The polymorphism obtained with microsatellite markers has provided powerful information to be considered in the management of fish stocks (Alam and Islam, 2005), population analysis and biodiversity conservation (Romana-Eguia *et al.*, 2004). Simple Sequence Repeat (SSR) markers are preferable because they are potentially co-dominant and highly polymorphic. In addition; microsatellites have a wide distribution in the genome and can be efficiently identified, which is essential in studies about genetic variability of populations (Boris *et al.*, 2011).

High cost of developing species-specific markers has been the main challenge of microsatellite markers in the past is the (Castoe *et al.*, 2010). Now, this has been alleviated with the advent of Next-generation sequencing, which allows the detection and characterization of SSR loci easily achievable with simple bioinformatics (Abdelkrin *et al.*, 2009).

Recently, molecular markers have been commonly used for population studies (Al-Atiyat *et al.*, 2012). Microsatellites have been used to study the genetic diversity of farmed and wild population's fishes (Norris *et al.*, 1999; Boris *et al.*, 2011).

In Nigeria, molecular marker based on PCR techniques has been used to determine population structure, and genetic diversity of fish (Ahmad *et al.*, 2012; Mojekwu *et al.*, 2012). Though there is little information in this area.

Therefore, this write up x-rays the applications and relevance of SSR markers to fish population genetics in aquaculture.

### Population Genetics

Population genetics is the study of genetic variation among species, individuals and populations; fundamentally, it shows that distribution of genetic variability is affected by evolutionary forces of mutation, migration, selection, and random genetic drift (Hansen, 2003).

Assessing genetic diversity in aquaculture stocks or wild fish populations is crucial for effective management, interpretation, and understanding of fish populations or stocks.

Many characteristics and methods have been used to analyze stock structure in fish populations; they include ecological, tagging, parasite distribution, physiological and behavioural traits, morphometrics and meristics, calcified structures, cytogenetics, immunogenetics and blood pigments (Samaradivakara *et al.*, 2012).

Unfortunately, environmental variables often affect the relationship between genes and their phenotypic expression significantly.

Thus, the population geneticists mainly focused on Mendelian traits in species widely used in laboratory studies or on available pure breeds of few species (Hallerman, 2003).

The development of DNA amplification using the PCR (Polymerase Chain Reaction) technique opened up the possibility of examining genetic changes in fish populations over the past years (Ferguson *et al.*, 1995). Today many molecular methods are available for studying various aspects of wild populations, captive brood stocks and interactions between wild and cultured stocks of fish and other aquatic species (Yudha *et al.*, 2012). The choice of markers for particular method is not straightforward and mostly depends on the experience of the investigators, laboratory facilities and available fund. Thus, there is a need for occasional reviews of the developments in techniques, applications and interpretations of the data gathered.

## **II. Microsatellite**

Microsatellites are highly abundant in various eukaryotic genomes including all aquaculture species studied to date. In most of the vertebrate genomes, microsatellites make up a few percent of the genomes in terms of the involved base pairs, depending on the compactness of the genomes (Zhan *et al.*, 2009).

In fish, one microsatellite was found every 1.87 kb of DNA. For comparison, in the human genome, one microsatellite was found every 6 kb of DNA (Beckmann and Weber 1992). It is reasonable to predict that in most aquaculture fish species, one microsatellite should exist every 10 kb or less of the genomic sequences, on average (Wright, 1993; Duran *et al.*, 2009).

Their high polymorphism, and PCR based analysis has made them one of the most popular genetic markers ((Duran *et al.* 2009; Boris *et al.*, 2011).

Some microsatellite loci have very high numbers of alleles per locus (>20), making them very useful for applications such as parent-offspring identification in mixed populations, while others have lower numbers of alleles and may be more suited for population genetics and phylogeny (Al-Atiyat *et al.*, 2012). Primers developed for one species will often cross-amplify microsatellite loci in closely related species (Boris *et al.*, 2011). Genotyping of microsatellite markers is usually straightforward (Castoe *et al.*, 2010).

However, its disadvantage include the requirement for existing molecular genetic information, the large amount of up front work for microsatellite development, and the tedious and labor intensive nature of microsatellite primer design, testing, optimization of PCR conditions and cost (Telles *et al.*, 2010).

### **Applications of Microsatellite Markers in fish population**

Microsatellite markers are ideal for many types of applications in aquaculture. They give crucial information in aquaculture fish population, such as: (i) identification of Genetic variability between and within stocks; (ii) monitoring Genetic changes in stocks; (iii) parentage and pedigree analysis in selective breeding ;(iv) Genomic mapping and detection of quantitative trait loci (QTL) (Fjalestad *et al.*, 2003;Subasinghe *et al.*, 2003; Chistiakov *et al.*,2005).

Several papers has reviewed the use of Microsatellite markers in aquaculture, in particular their integration into breeding programmes (e.g.Fjalestad *et al.*, 2003; Taniguchi, 2003; Cross *et al.*, 2004; Boris *et al.* 2011; Al-Atiyat *et al.*,2012).

### **Identification of Genetic Variability between and within Fish Stocks**

Molecular markers can be useful tools in stock identification and monitoring potential changes in broodstock, called DNA fingerprinting (Fjalestad *et al.*, 2003; Ahmad *et al.*, 2012; Mojekwu *et al.*, 2012). Almost all major molecular markers from allozymes to microsatellite have been used in determination of between and within genetic variations in hatchery stocks (Sekino *et al.*, 2002; Ramos- Paredes and Grijalva-Chon, 2003; Samy-Yehya *et al.*, 2012).

Studies have shown loss of genetic variation in hatchery stocks (e.g. in salmonids) as a result of different factors including a low effective number of parents, domestication selection or the mating design ( Samaradivakara *et al.*, 2012). Sekino *et al.* (2002) assessed genetic divergence within and between hatchery, and wild populations of Japanese flounder (*Paralichthys olivaceus*) by means of microsatellite and mtDNA sequencing analysis. Desvignes *et al.* (2001) studied the genetic variability of French and Czech strains of hatchery stocks of common carp (*Cyprinus carpio*) using allozymes and microsatellites. They detected a more pronounced discrimination between the strains of the two countries by the microsatellite markers. Bartfai *et al.*

(2003) analyzed the whole broodstock of two Hungarian common carp farms (80 and 196 individuals) by using RAPD assay and microsatellite analysis. Microsatellite analysis was more informative than RAPD assay. Microsatellites are the assay of choice for the discrimination of culture stocks (Duran *et al.*, 2009; Boris *et al.*, 2011).

### **Monitoring Genetic Changes in stocks**

Microsatellite assays have been very successful in detecting the genetic impact of culture (Zhan *et al.*, 2009). The higher sensitivity Microsatellite to phenomena such as genetic drift and founder effect make this marker ideal for monitoring the consequences of founding and propagation in aquaculture than of mtDNA and allozyme (Duran *et al.*, 2009).

Microsatellite markers have been used for minimizing inbreeding in rainbow trout (Fishback *et al.*, 1999). Analysis of the F1 generation of a Greek gilthead sea bream broodstock revealed a 15% reduction in the number of alleles and a homozygosity increase of 1.5% (Magoulas, 1998).

Thus, SSR markers have important applications in monitoring inbreeding depression. For example, microsatellite markers can be used to locate the specific chromosomal regions responsible for inbreeding depression. This would be most feasible with cultured species where parents and their progeny can be managed and traced within a closed system. It will be possible to use mapped genetic markers to trace the inheritance of specific chromosomal arms in progeny (Morelli, 2007). The polymorphism obtained with microsatellite markers have provided strong information utilized in the management of fish stocks (Alam and Islam, 2005).

### **Parentage and Pedigree Analysis in Selective Breeding**

Selection programmes uses both information on the candidates for selection and their relatives in order to increase the precision of selection and hence selection responses.

One of the applications of Microsatellite markers in brood stock management is in the assessing of the contribution of possible parents in a mass spawning. Typically limited numbers of broods are used in spawning and some putative parents apparently fail to spawn. In this case SSR and other genetic markers, becomes useful in quantifying the relative success of the potential parents. Parentage can be determined using minisatellite or microsatellite markers after spawning (Moran *et al.*, 1996; Thompson *et al.*, 1998).

One of the most important hindrances to applying effective selective breeding programmes for fish is that newborn individuals are too small to be tagged physically.

Thus, selective programmes making use of family information have needed to keep families separated until the fish are large enough to be individually tagged. This is costly, limits the number of families available for selection and can induce environmental effects common to the members of the same family (Doyle and Herbinger, 1994).

This problem can be resolved by applying Microsatellite and other DNA-based genetic markers. Consequently, more families can be kept in the breeding stock without the need for using separate tanks at early ages. These markers have been used to assess family/parentage identification in many species and can be used to discriminate fish in mixed family groups (e.g. Herbinger *et al.*, 1995; Fjalestad *et al.*, 2003; Cross *et al.*, 2004; Duran *et al.*, 2009; Boris *et al.*, 2011; Al-Atiyat *et al.*, 2012).

A breeding programme can be initiated with a previously unselected farm raised strain by using a method, termed walk-back selection (Doyle and Herbinger, 1994). In general, this will involve the physical tagging and biopsy of individuals when they are large enough to be marked, with microsatellite analysis based on the biopsy used to assign individuals to family.

Several studies have empirically used microsatellite loci to successfully reconstruct pedigrees in fish populations with families mixed from hatching (Norris *et al.* 2000; Morelli 2007; Zhan *et al.*, 2009; Olivatti *et al.*, 2011).

Villanueva, developed deterministic predictions for the power of microsatellites for parental assignment and compared with stochastic simulation results. Their results showed that the four loci that are more informative are enough to assign the offspring to the correct pair up to 99% with 100 crosses that involves 100 males and females respectively. Doyle *et al.* (1994) used them to discriminate family groups of cod (*Gadus morhua*). In these cases, offspring assignment was to known parental types.

However, with sufficient levels of variability, family or parental discrimination may also be achievable in the absence of parental information (Norris *et al.*, 2000). However, microsatellites appears more popular because of their potential for high variability even among individuals of the same strain (Liu and Cordes 2004).

### **QTLs of aquatic organisms**

A high number of QTL research has been conducted for sex determination and sex linkage in fish using microsatellites because of the great interest in producing monosex populations of certain species.

Sex linked inheritance in fish was first reported by Aida (1921) in medaka. Waldbieser *et al.* (2001) examined 293 polymorphic microsatellite loci in channel catfish, and seven of these loci were closely linked to the sex-determining chromosome region. *Oreochromis* QTLs for sex and colour have been mapped (Kocher *et al.*, 2002).

Ozaki *et al.* (2001) used 51 microsatellite markers to identify several chromosome regions containing putative QTL genes that affect resistance to infectious pancreatic necrosis (IPN) in rainbow trout. Tanck *et al.*

(2001) utilized 11 microsatellites and found that they were correlated with stress-related plasma cortisol levels and basal plasma glucose levels in common carp.

QTLs for fitness traits and survival have been identified in fish and shellfish. A microsatellite accounted for 7.5% of the variance in thermal tolerance in unselected populations of rainbow trout (Perry *et al.*, 2001).

**Table of some examples of linkage maps constructed with microsatellite markers in aquaculture species.**

Species	Common name	References
<i>Salmo trutta</i>	Brown trout	Gharbi <i>et al.</i> , 2006
<i>Dicentrarchus labrax</i>	European sea bass	Chistiakov <i>et al.</i> , 2005
<i>Oreochromis</i> spp.	Tilapia	Lee <i>et al.</i> , 2005
<i>Plecoglossus altivelis</i> <i>Xiphophorus</i>	Ayu	Watanabe <i>et al.</i> 2004, Walter <i>et al.</i> , 2004
<i>Salvelinus alpinus</i>	Arctic char	Woram <i>et al.</i> , 2004
<i>Salmo salar</i>	Atlantic salmon	Gilbey <i>et al.</i> , 2004, Moen <i>et al.</i> , 2004
<i>Oncorhynchus mykiss</i>	Rainbow trout	Sakamoto <i>et al.</i> , 2000, Nichols <i>et al.</i> , 2003
<i>Ictalurus punctatus</i>	Channel catfish	Waldbieser <i>et al.</i> , 2001
<i>Danio rerio</i>	Zebrafish	Shimoda <i>et al.</i> , 1999
<i>Crassostrea gigas</i>	Pacific oyster	Hubert and Hedgecock 2004
<i>Crassostrea virginica</i>	Eastern oyster	Yu and Guo 2003
<i>Seriola quinqueradiata</i> and <i>Seriola lalandi</i>	Yellowtails	Ohara <i>et al.</i> , 2005
<i>Cyprinus carpio</i>	Common carp	Sun and Liang 2004
<i>Paralichthys olivaceus</i>	Japanese flounder	Coimbra <i>et al.</i> , 2005

Source: Zhanjiang, J. L. (2007).

### Microsatellite application to fish Population genetics in Nigeria.

The applications of molecular-based knowledge in fish genetics and stock management have been confined to developed and some Asian developing countries.

Several papers have reviewed the use of microsatellite markers in aquaculture and fisheries, (e.g. Fegurson *et al.*, 1995; Davis and Hetzel, 2000; Fjalestad *et al.*, 2003; Taniguchi, 2003; Cross *et al.*, 2004; Alam and Islam 2005; Boris *et al.*, 2011; Al-atiyat *et al.*, 2012; Yudha *et al.*, 2012).

In Nigeria, quite a number of published work exist on molecular markers in fish characterization using RAPD markers (Ahmad *et al.*, 2012; Mojekwu *et al.*, 2012; Megbowon and Bombata, 2013; Mojekwu *et al.*, 2013).

However, there are little or no publications and information to the best of my search on the use of microsatellite markers to study fish population in Nigeria.

Though Microsatellite markers have been applied in Nigeria to study population and genetic diversities in livestock; chicken (Olowofeso *et al.*, 2005), Insects; malaria mosquitoes and plants (Fatokun, *et al.*, 2008; Ogunkanmi *et al.*, 2010).

### III. Conclusion

Microsatellites have enabled the assessment of genetic variations at much smaller scales than has been possible with other markers (Sunnucks, 2000; Boris *et al.*, 2011).

The polymorphism obtained with microsatellite markers has given useful and detailed information in fish stocks management (Alam and Islam, 2005), biodiversity conservation and population analysis (Romana-Eguia *et al.*, 2004). They are optimal for mapping “causal” genes, whether these are responsible for single or multifactorial traits (QTLs). They are also the best markers for determining parenthood in mass spawning and/or rearing even for evolutionarily related genera ( Zhan *et al.*, 2009) , tracing escapes from cultured to wild populations and estimating coefficients of kinship among individuals drawn from a population (Hansen *et al.*, 2001).

Many studies have successfully demonstrated heterologous amplification of a target specie with SSR primer (Barbosa *et al.*, 2006; Hatanaka *et al.*, 2006, Morelli *et al.*, 2007), thereby lowering the costs of future projects.

Nevertheless, their major drawback remains the high cost and labour intensity involved in the development of primers (Telles *et al.*, 2010). Another disadvantage is the existence of null alleles that is alleles that do not amplify in PCR reactions (O'Really and Wright, 1995).

## References

- [1]. Abdelkrim, J., Robertson, B.C., Stanton, J.A and Gemmell, N.J (2009). Fast, cost-effective development of species – specific microsatellite markers by genomic sequencing. *Biotechniques*, 46:185–192.
- [2]. Ahmad J.N., Azizah, M.N., Adikwu, I.A., Istifanus, W. A., Abalis, G.E and Muchlisin, Z.A (2012). Diversity and distribution of fishes of Gaji River, Bauchi state Nigeria. *Advances in Environmental sciences-International journal of the bioflux society* vol 4, issue 2. Pp.50–54.
- [3]. Aida, T. (1921) On the inheritance of color in a freshwater fish, *Aplocheilichthys latipes*, Temmick and Schlegel. *Genetics* 6, 554–573.
- [4]. Alam S and Islam S (2005). Population genetic structure of *Catla catla* (Hamilton) revealed by microsatellite DNA markers. *Aquaculture* 246: 151–160.
- [5]. Al-Atiyat, R.M., Tabbaa, M.J., Salameh, N.M., Tarawneh, K.A., Al-shamyla, J. and Al-Tamimie, H.J (2012). Analysis of genetic fat tailed sheep in southern region of Jordan. *Asian J. Anim. Vet. Adv.*, 7:376–389.
- [6]. Bartfai, R., Egedi, S., Yue, G.H., Kovacs, B., Urbanyi, B., Tamas, G., Horvath, L. and Orban, L (2003). Genetic analysis of two common carp broodstocks by RAPD and microsatellite markers. *Aquaculture*, 219: 157–167.
- [7]. Barbosa ACDR, Corrêa TC, Galzerani F, Galetti-Junior PM. (2006). Thirteen polymorphic microsatellite loci in the Neotropical fish *Prochilodus argenteus* (Characiformes, Prochilodontidae). *Mol. Ecol. Notes* 6: 936–938.
- [8]. Beckmann, J.S and Solter, M (1990). Toward a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. *Bio/Technology* 8, 930–932
- [9]. Beckman, J.S and Weber, J.L (1992). Survey of human and rat microsatellites. *Genomics*, 12, pp. 627–631.
- [10]. Boris Brinez, Xenia Caraballo O, Marcel Salazar V (2011). Genetic diversity of six populations of red hybrid tilapia, using Microsatellite genetic Markers. *Rev.MVZ Cordoba* 16 (2): 2491–2498.
- [11]. Castoe, T.A., Poole, A.W., Gu, W., De Koning, A.P and Daza, J.M (2010). Rapid identification of thousands of copperhead snake (*Agkistrodon contortrix*) microsatellite loci from modest amounts of 454 shotgun genome sequence. *Mol Ecol Resour* 10:341–347.
- [12]. Chistiakov DA, B Hellemans, CS Haley, AS Law, CS Tsigenopoulos, G Kotoulas, D Bertotto, A Libertini, and FA Volckaert (2005). A microsatellite linkage map of the European sea bass *Dicentrarchus labrax* L. *Genetics*, 170, pp. 1821–1826.
- [13]. Cross, T., Dillane, E. and Galvin, P (2004). Which molecular markers should be chosen for different specific applications in fisheries and aquaculture <http://www.ucc.ie/ucc/research/adc/molmark/index.html>. Last accessed on 23 March 2004.
- [14]. Davis, G.P. and Hetzel, D.J.S. (2000) Integrating molecular genetic technology with traditional approaches for genetic improvement in aquaculture species. *Aquaculture Research* 31, 3–10.
- [15]. Desvignes, J.F., Laroche, J., Durand, J.D. and Bouvet, Y (2001). Genetic variability in reared stocks of common carp (*Cyprinus carpio* L.) based on allozymes and microsatellites. *Aquaculture*, 194: 291–301.
- [16]. Doveri S, Lee D, Maheswaran M, Powell W (2008). Molecular markers: History, features and applications. In *Principles and Practices of Plant Genomics*, Volume 1, C.K.a.A.G. Abbott, ed. (Enfield, USA: Science Publishers), pp. 23–68.
- [17]. Doyle, R.W. and Herbinger, C.M (1994). The use of DNA fingerprinting for high intensity, within-family selection in fish breeding. In: *Proceedings of the 5<sup>th</sup> World Congress on Genetics Applied to Livestock Production*, Guelph, Canada. 19: 23–27.
- [18]. Doyle, R.W., Perez-Enriquez, R., Takagi, M. and Taniguchi, N. (1994) Selective recovery of founder genetic diversity in aquacultural broodstocks and captive endangered fish populations. *Genetica* 111, 291–304.
- [19]. Duran, C., Nikki, A., David, E and Jacqueline B (2009). *Molecular Genetic Markers: Discovery, Applications, Data storage and visualization*. *Current Bioinformatics* 11, 37–41.
- [20]. Fatokun, C.A., Ogunkanmi, A., Ogundipe, O.T and Ng, N.Q (2008). Genetic diversity in wild relatives of cowpea (*Vigna unguiculata*) as revealed by simple sequence repeats (SSR) markers. *Journal of Food, Agriculture & Environment* Vol. 6 (3&4): 263–268.
- [21]. Ferguson, A., Taggart, J.B., Prodohl, P.A., McMeel, O., Thompson, C., Stone, C., McGinnity, P. and Hynes, R.A (1995). The application of molecular markers to the study and conservation of fish populations, with special reference to *Salmo*. *Journal of Fish Biology*, 47:103–126.
- [22]. Ferguson, M.M. and Danzmann, R.G (1998). Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? *Can. J. Fish. Aquat. Sci.*, 55:1553–1563.
- [23]. Fishback, A.G., Danzmann, R.G., Sakamoto T. and Ferguson, M.M (1999). Optimization of semiautomated microsatellite multiplex PCR systems for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 172:247–254.
- [24]. Fjalestad, K.T., Moen, T. and Gomez-Raya, L (2003). Prospects for genetic technology in salmon breeding programs. *Aquaculture Research*, 34: 397–406.
- [25]. Hallerman, E.M (2003). *Population genetics: principles and applications for fisheries scientists*. American Fisheries Society, Bethesda, Maryland: 458 pp.
- [26]. Hansen, M.M (2003). *Application of molecular markers in population and conservation genetics, with special emphasis on fishes*. DSc Thesis, Faculty of Natural Sciences, University of Aarhus, 68 pp.
- [27]. Hansen, M.M., Ruzzante, D.E., Nielsen, E.E. and Mensberg, K.L.D. (2001) Brown trout (*Salmo trutta*) stocking impact assessment using microsatellite DNA markers. *Ecological Applications* 11, 148–160.
- [28]. Hatanaka T, Henrique-Silva F and Galetti PM Jr (2006). Population substructuring in a migratory freshwater fish *Prochilodus argenteus* (Characiformes, Prochilodontidae) from the Sao Francisco River. *Genetica* 126: 153–159.
- [29]. Herbinger, C.M., Doyle, R.W., Pitman, E.R., Paquet, D., Mesa, K.A., Morris, D.B., Wright, J.M. and Cook, D. (1995) DNA fingerprint-based analysis of paternal and maternal effects on offspring growth and survival in communally reared rainbow trout. *Aquaculture* 137, 245–256.
- [30]. Kocher, T.D., Albertson, R.C., Carleton, K.L. and Streebman, J.T. (2002) The genetic basis of biodiversity: genomic studies of cichlid fishes. In: Shimizu, N., Aoki, T., Hirono, I. and Takashima, F. (eds) *Aquatic Genomics: Steps Toward a Great Future*. Springer-Verlag, New York, pp. 35–44.
- [31]. Liu ZJ and JF Cordes (2004). DNA marker technologies and their applications in aquaculture genetics. *Aquaculture*, 238, pp. 1–37.
- [32]. Magoulas, A (1998). Application of molecular markers to aquaculture and broodstock management with special emphasis on microsatellite DNA. *Cahiers Options Mediterranes*, 34: 153–168.
- [33]. Megbowon I and Fashina-Bombata, H.A (2013). Molecular Characterization of an Unidentified Cichlid, Commonly Called, ‘Wesafu’, Using RAPD Markers. *IOSR Journal Of Environmental Science, Toxicology And Food Technology*, 5 (2): 22–26
- [34]. Mojekwu, T.O, Oguntade, O.R, Oketoki, T.O and Usman, A.B (2012). Genetic variability of Tilapia in different water bodies using RAPD markers. *Proceedings of the 25<sup>th</sup> Annual Conference of the Biotechnology Society of Nigeria*. Held at National Open University, Abuja. 26<sup>th</sup> – 31<sup>st</sup> August, 2012.
- [35]. Mojekwu, T.O, Oguntade, O.R, Oketoki, T.O Usman, A.B and Omidiji, O (2013). Molecular characterization of Tilapia in different water bodies using RAPD Markers. *African Journal of Applied Biotechnology Research*, 1(1):1–12.

- [36]. Moran, P., Pendas, A.M., Beall, E. and Garc a-Vazquez, E (1996). Genetic assessment of the reproductive success of Atlantic salmon precocious parr by means of VNTR loci. *Heredity*, 77: 655–660.
- [37]. Morelli KA, Revaldaves E, Oliveira C and Foresti F (2007). Isolation and characterization of eight microsatellite loci in *Leporinus macrocephalus* (Characiformes: Anostomidae) and cross-species amplification. *Mol. Ecol. Notes* 7: 32-34.
- [38]. Norris, A.T., Bradley, D.G. and Cunningham, E.P. (1999) Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations. *Aquaculture* 180, 247–264.
- [39]. Norris, A.T., Bradley, D.G. and Cunningham, E.P. (2000). Parentage and relatedness determination in farmed Atlantic salmon (*Salmo salar*) using microsatellite markers. *Mol Ecol*, 11, pp. 155–165.
- [40]. O’Connell, M. and Wright, J.M (1997). Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries*, 7: 331-363.
- [41]. Ogunkanmi, L.A., Ogundipe, O.T and Fatokun, C.A (2010). Comparative analysis of genetic diversity and genetic relatedness between cultivated and wild cowpea [*Vigna unguiculata* (L.) Walp.] based on agronomic traits and microsatellite markers. *World Cowpea Conference Abstracts USA*
- [42]. Olivatti A.M, T.A. Boni, N.J. Silva-J nior, L.V. Resende, F.O. Gouveia and M.P.C. Telles (2011). Heterologous amplification and characterization of microsatellite markers in the Neotropical fish *Leporinus friderici* Genet. Mol. Res. 10 (3): 1403-1408 .
- [43]. Chowfeso, O., Wang, J.Y., Dai, D.J., Yang, Y., Mekki, D.M and Musa, H.H (2005). Measurement of genetic parameters within and between Haimen chicken populations using microsatellite markers. *International Journal of Poultry Science*, 4: 143-148.
- [44]. O’Reilly, P. and Wright, J.M (1995). The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. *J. Fish Biol.*, 47: 29-55
- [45]. Ozaki, A., Sakamoto, T., Khoo, S., Nakamura, K., Coimbra, M.R., Akutsu, T. and Okamoto, N. (2001) Quantitative trait loci (QTLs) associated with resistance/susceptibility to infectious pancreatic necrosis virus (IPNV) in rainbow trout (*Oncorhynchus mykiss*). *Molecular Genetics and Genomics* 265, 23–31.
- [46]. Perry, G.M.L., Danzmann, R.G., Ferguson, M.M. and Gibson, J.P. (2001) Quantitative trait loci for upper thermal tolerance in outbred strains of rainbow trout (*Oncorhynchus mykiss*). *Heredity* 86, 333–341.
- [47]. Ramos-Paredes, J. and Grijalva-Chon, J.M (2003). Allozyme genetic analysis in hatchery strains and wild blue shrimp, *Penaeus (Litopenaeus) stylirostris* (Stimpson), from the Gulf of California. *Aquaculture Research*, 34: 221-234.
- [48]. Romana-Eguia MRR, Ikeda M, Basiao ZU and Taniguchi N (2004). Genetic diversity in farmed Asian Nile and red hybrid tilapia stocks evaluated from microsatellite and mitochondrial DNA analysis. *Aquaculture* 236: 131-150.
- [49]. Samy-Yehya, E., Mohammed Morsi, M.A., Mohamed, E.S and Walid, N.E (2012). phylogenetic differentiation of wild and cultured Nile Tilapia (*Oreochromis niloticus*) populations based on phenotype and genotype analysis. *African Journal of Agricultural Research* vol. 7(19), pp.2946-2954.
- [50]. Samaradivakara, S.P., Hirimuthugoda, N.Y., Gunawardana, R.H., Illeperuma, R.J., Fernandopulle N.D., De Silva A.D and Alexander, P.A (2012). Morphological variation of four Tilapia population in selected Reservoirs in Sri Lanka. *Tropical Agriculture Research* vol. 23 (2) : 105-116.
- [51]. Sekino, M., Hara, M. and Taniguchi, N (2002). Genetic Diversity Within and Between Hatchery Strains of Japanese Flounder *Paralichthys olivaceus* Assessed by Means of Microsatellite and Mitochondrial DNA Sequencing Analysis. *Aquaculture*, 213: 101-122.
- [52]. Subasinghe, R.P., Curry, D., McGladdery, S.E. and Bartley, D (2003). Recent Technological Innovations in Aquaculture. In: *Review of the State of World Aquaculture*. FAO Fisheries Circular No.886, Rev. 2, Rome: 59-74.
- [53]. Sunnucks, P (2000). Efficient genetic markers of population biology. *Trends in Ecology and Evolution*, 15: 199-203.
- [54]. Tanck, M.W.T., Palstra, A.P., Van De Weerd, M., Leffering, C.P., Van Der Poel, J.J., Bovenhuis, H. and Komen, J. (2001) Segregation of microsatellite alleles and residual heterozygosity at single loci in homozygous androgenetic common carp (*Cyprinus carpio* L.). *Genome* 44, 743–751.
- [55]. Taniguchi, N (2003). Genetic factors in broodstock management for seed production. *Reviews in Fish Biology and Fisheries*, 13: 177–185.
- [56]. Telles MP, Resende LV, Brondani RP, Collevatti RG (2010). Isolation and characterization of microsatellite markers in the armored catfish *Hypostomus gymnorhynchus* (Loricariidae). *Genet. Mol. Res.* 9: 1770-1774.
- [57]. Thompson, C.E., Poole, W.R., Matthews, M.A. and Ferguson, A (1998). Comparison, using minisatellite DNA profiling, of secondary male contribution in the fertilisation of wild and ranched Atlantic salmon (*Salmo salar*) ova. *Can. J. Fish. Aquat. Sci.*, 55:2011-2018.
- [58]. Waldbieser, G.C., Bosworth, B.G., Nonneman, D.J. and Wolters, W.R. (2001) A microsatellite-based genetic linkage map for channel catfish, *Ictalurus punctatus*. *Genetics* 158, 727–734.
- [59]. Wright, J.M. (1993) .DNA fingerprinting in fishes. In: W.Hochachka, P. and Mommsen, T. Eds., *Biochemistry and Molecular Biology of Fishes*, Elsevier, Amsterdam, 58-91.
- [60]. Yudha, T.A., Jan-Lung, C and Jin- chywan, G (2012). Genetic diversity of Indonesian Milkfish (*Chanos Chanos*) using Amplified Fragment Length Polymorphism (AFLP) analysis. *African Journal of Biotechnology* vol. 11 (13) pp.3055 -3060.
- [61]. Zhan, A., Wang, Y., Brown, B and Wang, H.P (2009). Isolation and characterization of novel microsatellite markers for yellow perch (*Perca flavescens*). *Int.J.Mol. Sci.* 10:18 -27.
- [62]. Zhanjiang J. L (2007). Microsatellite Markers and Assessment of Marker Utility *Aquaculture Genome Technologies*, Blackwell publishing 1 (5) 43-57.