Development of Genetic Improvement in the African Catfish (*Clarias gariepinus*, Burchell, 1822)

A Thesis Submitted for the Degree of
Doctor of Philosophy

By

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Declaration

I declare that this thesis has been compiled entirely by me based on research I carried out. It has not been submitted for award of another degree or certificate elsewhere. All information and other sources of assistance have been duly acknowledged.

Suleiman Ihiabe Isa

January, 2019

Signature of Candidate ----------

Signature of First Supervisor ----------

Signature of Second Supervisor ----------

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Dedicated

To

The Departed Soul of My Beloved Father

(*Alh. Isah Ihiabe*)

Abstract

The African catfish, *Clarias gariepinus*, is the most important fish species for aquaculture in sub-Saharan Africa. Despite its long-standing history in aquaculture (since the 1950's) and current rapid expansion, little work has been done on its genetics and the genetic management/improvement of different populations globally. The industry, currently worth over USD 720 million in Nigeria, and with so much more growth potential, is faced with numerous challenges. To understand the extent of these challenges and possible areas/types of intervention, the Nigerian catfish aquaculture industry was reviewed. Inadequate supply of good quality fingerlings/broodstock and feeds were notably the most significant challenges. As a step towards addressing the former, a survey of the current practise in catfish hatcheries was conducted, to identify problems and prospects therein. Over 90% of the hatcheries surveyed use shooters (fast growers) as broodstock, use only farmed broodstock and have no broodstock management/replacement programmes. Findings from these studies informed research on the development of genetic improvement for *C. gariepinus*. Just as in salmon, tilapia, carp, etc., the use of molecular markers as tools for genetic management and improvement of *C. gariepinus* was explored. Problem-solving markers, separating *C. gariepinus* from its closest relative, *C. anguillaris,* were developed. A total of 24 diagnostic SNP markers were identified from double-digest restriction-site associated DNA sequencing (ddRADseq). Following validation using KASP assay, 8 of the 24 SNPs were tested on a total of 291 *Clarias* catfishes and 7 *Heterobranchus longifilis* (out groups). The *Clarias* samples were separated into 259 putative *C. gariepinus* and 32 putative *C. anguillaris.* These are the first diagnostic markers for separating these species, for which morphological features perform poorly (effectively cryptic species). A set of eight new microsatellite markers was developed from the ddRADseq data and microsatellite enrichment. These microsatellite markers, together with four others sourced from the literature were optimised, multiplexed and used to genotype populations of *C. gariepinus* being evaluated for suitability for aquaculture. Although incomplete (due to problems with parental DNA quality), preliminary assessment of the assignment power by simulation shows that over 90% of the offspring could be assigned to a pair of parents. The high parentage assignment power and polymorphic information content (>0.5), suggest the usability and reliability of these markers in genetic management and improvement in the *Clarias* catfish industry, enabling parental assignment and kinship studies, and for evaluation of practices such as the use of "shooters" as broodstock in the industry.

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List of Abbreviations

AIFP – Aquaculture and Inland Fisheries Programme

ARAC – African Regional Aquaculture Centre

AU – African Union

AWERB - Animal Welfare and Ethical Review Body

CAFAN – Catfish Farmers Association of Nigeria

CASI – Computer Assisted Self-administered Interview

DAPC – Discriminant Analysis of Principal Component

ddRADseq – Double Digest Restricted-Site Associated DNA Sequencing

DFID – Department for International Development

DFRRI – Directorate of Food, Road and Rural Infrastructure

DPH – Days Post Hatch

ECOWAS – Economic Community of West African States

EEZ – Exclusive Economic Zone

FAO – Food and Agricultural Organisation

FCR – Feed Conversion Ratio

FDF – Federal Department of Fisheries

FISON – Fisheries Society of Nigeria

FMARD – Federal Ministry of Agriculture and Rural Development

FMST – Federal Ministry of Science and Technology

GIFT – Genetically Improved Farmed Tilapia

GSI – Gonado Somatic Index

GST - Genomar Supreme Tilapia

HND – Higher National Diploma

ICLARM – International Centre for Living Aquatic Resources Management

IoA – Institute of Aquaculture

KASP - Kompetitive allele specific PCR

LASCAFAN – Lagos State Catfish Farmers Association of Nigeria

LGC - Laboratory of the Government Chemist

MPH – Months Post Hatch

NABDA – National Biotechnology Development Agency

NAS – National Aquaculture Strategy

NBS – National Bureau of Statistics

NGS – Next Generation Sequencing

NIFFR – National Institute for Freshwater Fisheries Research

NIOMR – National Institute for Oceanography and Marine Research

NNFP – Nigerian National Fisheries Policy

OND – Ordinary National Diploma

PAPI – Paper And Pencil Interview

PCA – Principal Component Analysis

PIT – Passive Integrated Transponders

RADseq – Restricted-Site Associated DNA Sequencing

RAS – Recirculating Aquaculture System

SNP – Single Nucleotide Polymorphism

UNDP – United Nations Development Programme

USAID – United States Aid for International Development

WAAPP – West African Agricultural Productivity Programme

WPH – Weeks Post Hatch

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction - The African catfish

African catfish (*Clarias gariepinus* (Burchell, 1822)) is an important species for aquaculture in sub-Saharan Africa, some parts of North Africa, South America, Asia and Europe (FAO, 2014b). By volume, it is the second most cultured species in Africa (FAO, 2017). Its fast growth rate, high fecundity, adaptation to varied culture environments and conditions, has made it the choice of many fish farmers in sub-Saharan Africa, and a good species for peri-urban aquaculture, livelihood and sustainable development projects. Domestication of *C. gariepinus* started in the 1950s, and in the mid -1970s, it was adopted as the most ideal catfish for African aquaculture due to its fast growth rate, adaptation to varied culture conditions and high fecundity (FAO, 2014a; Hecht *et al.*, 1996). Its ecology, naturally spanning many countries in Africa, makes it a very popular fish species already. *C. gariepinus* is increasingly gaining attention in many African countries beside Nigeria. Uganda, Kenya and Egypt are examples of countries with special focus on *C. gariepinus* (Ponzoni and Nguyen, 2008). In 2010, farmed *C. gariepinus* in sub-Saharan Africa accounted for 198,296 tonnes, while the total of farmed tilapia was 60,350 tonnes, making *C. gariepinus* the most important commercially farmed fish species in sub-Saharan Africa (FAO, 2012; Ondhoro *et al.*, 2015).

1.1.1. Classification (Ichthyology) of *Clarias gariepinus*

C. gariepinus belong to the family *Clariidae* and genus *Clarias* (Lagler *et al.*, 1977; Moyl and Cech, 1988). Currently, there are 16 recognised genera and 113 species belonging to the family *Clariidae* (Ferraris, 2007; (Ng and Tu, 2011). The nominate sub-genus *Clarias* (*Clarias*) is the most often used in aquaculture; popular amongst them are *C. gariepinus* and *C. anguillaris* in Africa. (Lagler *et al.*, 1977; Teugels, 1984; Teugels, 1986; Moyl and Cech, 1988; Na-Nakorn and Brummett, 2009).

1.1.2. Morphology of the Sub-Genus *Clarias*

Generally, *Clarias* catfish has a bony broad head (coarsely granulated in adults), elongated-cylindrical body shape tapering towards the caudal end of the fish, four pairs of barbels, a pair each of pectoral and pelvic fins, long dorsal and anal fin, round caudal fin, small eyes, villiform vomerine teeth on both jaws and no dorsal spine or adipose fin (Moyl and Cech, 1988, ADW, 2004; Na-Nakorn and Brummett, 2009). They are either dark grey-

greenish black or marble patterned (camouflaging) dorso-laterally and lightly cream coloured or whitish ventrally, with the adults having dot-like lateral lines (secondary sensory organ) running on either side of the body from the posterior end of the head to the middle of the caudal fin base (FAO, 2018a). The 4 pairs of barbels namely, the nasal, mental (inner mandibular pair), maxillary and mandibular (outer) pairs are used to search for food in dark and or murky waters as they house the taste buds (thousands) of the fish (FAO, 2009).

The total length is 5 - 9 times the body depth and 3.0 - 3.5 times the head length, with the head length averaging 1.50 - 1.66 times the width and twice the length of the caudal fin (de Kimpe and Micha, 1974; Teugels, 1982). The dorsal and anal fins comprise 61 – 80 and 45 – 65 soft rays respectively (Teugels, 1986). Each pectoral fin possesses a serrated spine and 9-12 soft rays while each pelvic fin comprises six soft rays. In the wild, *Clarias* has been reported to grow up to 1.7 m and 59 kg (ADW, 1986), while under husbandry conditions; they can reach up to 1 - 3 kg in a year.

1.1.3. Anatomy of the Sub-Genus *Clarias*

The family of *Clariidae* (Siluriformes) is commonly known as the air-breathing catfishes because they possess suprabranchial organs (Teugels and Adriaens, 2003). Common to all members of this family (including the sub-genus *Clarias*), the highly vascularised flowerlike arborescent organ located on the second and fourth branchial arches of *Clarias* enables them to utilise atmospheric oxygen, hence, making them capable of tolerating low dissolved oxygen levels (in water) and still meeting 80-90% of their oxygen requirements (de Kimpe and Micha, 1974; Moreau, 1988). This organ, which is a characteristic feature of the ancestral species of catfish, is shared exclusively (in somewhat modified form) by its evolutionary descendants, uniting different genera of catfish under the family *Clariidae*. They include *Bathyclarias, Channallabes, Clariallabes, Dinotoppterus, Dolichallabes, Encheloclarias, Gymnallabes, Heterobranchus, Horaglanis, Platyallabes, Platyclarias, Uegitglanis* and *Xenoclarias* (Teugels and Adriaens, 2003; Na-Nakorn and Brummett, 2009). This air-breathing ability, in combination with their fast growth rate, omnivorous feeding nature and high stress resistance makes them very attractive for aquaculture (Moreau, 1988; Na-Nakorn and Brummett, 2009). *Clarias* has a minimum of 16 and a

maximum of 110 gill rakers on its first branchial arch, with *C. gariepinus* having a higher maximum than other members of its genus (Teugels, 1982).

1.1.4. Physiology and adaptation (air breather, barbels, walking fins)

C. gariepinus are potamodromous in nature (Teugels, 1986). *Clarias* catfish was observed to have moved between a pool and a river covering a distance of up to 180m on a firm soil in about 1hr (de Kimpe and Micha, 1974). At the onset of rainy season, they normally migrate upstream to spawn, while in drying season, they burrow into muddy substrates at the base of seasonal pools, ponds or swamps (Lagler *et al.*, 1977; Teugels, 1986; ADW, 2004) to keep moist and sometimes to escape harvesting nets and predators. Their body is often covered by mucus slime, which protects them from infections, handling and helps to keep them moist under drying conditions (ADW, 2004). Despite having an optimum growing temperature of 28 – 30^{o}C (Teugels, 1986), *C. gariepinus* can tolerate temperatures of as low as 8^{o}C and as high as 35^{0}C (ADW, 2004).

1.1.5. Feeding Habits

C. gariepinus are naturally omnivorous benthic feeders, a characteristic that can be explained by their medium length intestine and sub-terminal/inferior mouthpart (Lagler *et al.*, 1977). They are nocturnal in nature and exhibit their predatory instincts mostly at night, an indication that they are not only limited to the benthos. They feed on diverse food materials, ranging from zooplankton, to aquatic insects and invertebrates, snails, crustaceans and smaller fish, to fish almost their size. Furthermore, they also feed on waste food items, fruits, dead animals etc. (ADW, 2004). However, they also predators, hence, spend some of their time around the surface and in the water column in search of smaller fish to prey on. Under intensive culture conditions, *C. gariepinus* are fed formulated diets in forms of extruded floating pellets or sinking pressed pellets.

1.1.6. Reproductive physiology

C. gariepinus do not exhibit obvious sexual dimorphism when young, but prior to maturity, a tube-like genital papilla becomes obvious in the males, while the females appear to have broader abdominal region (when gravid) with an opening on its ventral part (Bruton, 1979). They mature between the ages of 8 – 10 months and the females have a

gonadosomatic index (GSI) of about 15 ± 5% of their body weight, and depending on their age and sizes, produce an average of 600 ± 100 eggs g^{-1} of ovary (Hogendoorn and Vismans, 1980; de Graaf and Janssen, 1996; Brummett, 2008; Fleuren, 2008). The females are relatively more sensitive to temperature and photoperiod fluctuations as compared to the males (de Graaf and Janssen, 1996; Brummett, 2008). Fingerlings of *C. gariepinus* are found in huge numbers within the first few months of the rainy season (June - August), suggesting they could also be sensitive to rainfall and also that they are annual spawners.

As the water levels rise, mature male and gravid female *C. gariepinus* pair-up and mate, shedding their eggs on grasses and aquatic plants, mostly in areas with low current, where they hatch into larvae and begin to swim around in search of food about 2-3 days later. *C. gariepinus* do not exhibit parental care. The hatched fry fend for themselves immediately after yolk absorption, feeding on a wide range of food source (depending on availability), ranging from zooplankton, to aquatic insects and invertebrates, snails, crustaceans, and smaller fish, to fish almost their size etc. About 10 to 15 days after hatching, sex differentiation commences, (Brummett, 2008), with gonadal sex differentiation commencing 40 – 50 dph, evident through histology and expression of sex specific markers like dmrt1, sox9, foxl2, and aromatase (25–27) (Raghuveer *et al.*, 2011). The males ultimately becoming bigger in size (Skelton, 1993; ADW, 2004) and the cycle are heterogametic (Kovács *et al.*, 2000).

1.2. Global Perspective of the Clarias Catfish Aquaculture Sector

Over 800 million people across the world depend on fish for food, income and nutrition, amongst them are people from many developing food-insecure countries, where fish is often the cheapest and most accessible source of animal protein (WorldFish, 2017b). In 2016, global fish production was 171 million tonnes, out of which aquaculture accounted for 80 million tonnes and 88% of the total production was utilised for direct human consumption at an average of 20.3 kg per capita (FAO, 2018c). Many African countries fall below this average. Demands are often very high in the face of low production due to several challenges including dwindling of wild stock. While global annual capture fisheries declined from 92.7 to 89.5 tonnes between 2011 and 2016, global annual aquaculture production grew steadily from 61.0 to 80.0 million metric tonnes within the same period

(FAO, 2018c). This makes aquaculture a more reliable and promising means of meeting human fish protein needs, in the face of the growing human population. Although a relatively small volume is farmed when compared to species like carp, tilapia and salmon, the culture of torpedo shaped catfishes (*Clarias spp.*) has also grown from 353,000 to 979,000 tonnes per annum from 2011 - 2016, making it one of the fastest growing farmed species in the world (FAO, 2018c). Popular amongst them are *C. gariepinus* in Africa, and their Asian counterparts – *C. macrocephalus* and *C. batrachus*. The growth of aquaculture globally may be attributed to increased investment in research, policy, market demand and development of technology around aquaculture.

Clarias batrachus (Linnaeus, 1758), *Clarias macrocephalus* (Gunther, 1864) and *Clarias fuscus* (Lacepede, 1803) are the three most important *Clarias* species beside the African catfish *C. gariepinus*. They, together with their hybrids (with *C. gariepinus*) constitute the second largest group of catfish farmed in Asia, accounting for more then 500,000 MT/annum (FAO, 2014b; Na-Nakorn and Brummett, 2009). The total aquaculture production of *Clarias species* (Torpedo shaped catfishes nei) in 2016 was 979,000 MT, accounting for about 2% of total fin fish production (FAO, 2018b). Introduction of the hybrids with *C. gariepinus* have raised concerns and caused speculations of threats posed on the purity and viability of wild populations, which have evolved within farms and have been included in genetic management programmes (Na-Nakorn and Brummett, 2009). Just like the African catfish, little is reported about genetic variation in their Asian counterpart, and conservation efforts for these species has begun in many Asian countries (Argungu *et al.*, 2013).

1.3. Development of African Catfish Aquaculture

Equatorial African aquaculture development started with tilapia culture between 1946 and 1949 following the end of the Second World War, and this was followed with the building of several hectares of earthen ponds across different African countries and different states in Nigeria between the late 1950's and early 1960s (Hogendoorn and Vismans, 1980). For several reasons, including inadequate supply of fish feed and fingerlings, which were mostly sourced from the wild, aquaculture in this region (Nigeria in particular) declined until the last two decades. Fingerlings from species such as carps, tilapia and catfishes

were sourced from the wild and grown extensively in earthen ponds. Of all the warm water tropical fishes grown, the African catfish adapted best to varied and harsh culture conditions, grew faster, matured in captivity, tolerated high stocking densities, accepted and thrived on cheap feed, had higher survival rate and above all was readily acceptable to the consumers. It constitutes the highest percentage of fish species landed in the inland waters of Nigeria and its ecology naturally spans many countries in Africa (Hecht *et al.*, 1996; FAO, 2014a; National Bureau of Statistics, 2017). Domestication of *C. gariepinus* started in 1950, however, its adoption as the most ideal African catfish for aquaculture took place in the mid - 1970s. Ovulation was successfully induced in 1975 in Central Africa and successful artificial propagation was demonstrated in the late 1970s in Cameroun (Hogendoorn and Vismans, 1980; FAO, 2014a). Since then, its culture has extended to four continents of the world (**Figure 1.1**), namely Africa, South America, Asia and Europe (Ponzoni and Nguyen, 2008; FAO, 2014a).

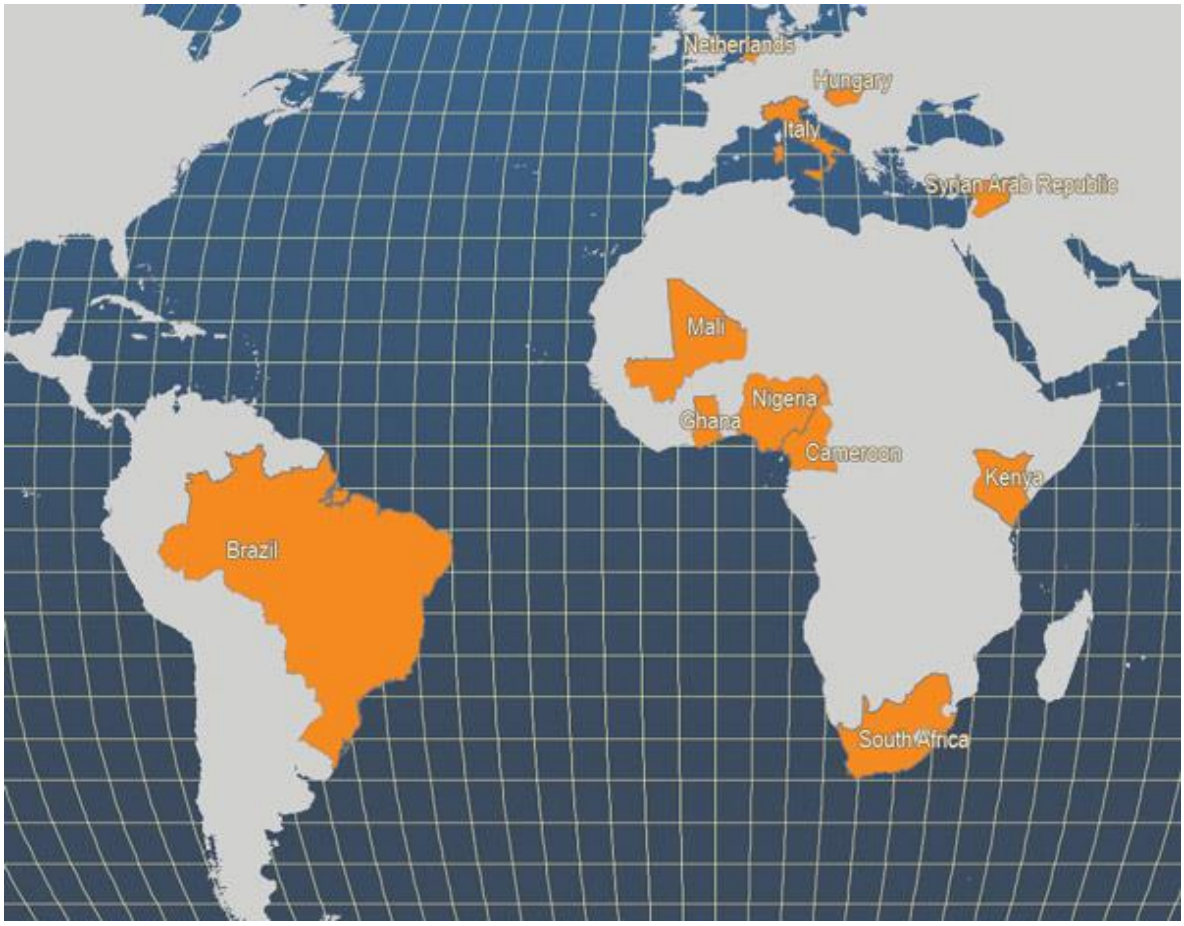

Figure 1.1. Map Showing countries producing C. gariepinus across four continents
Source: FAO, 2014a

A total of 33 farms in The Netherlands produce over 4,000 tonnes of the Dutch domesticated strain of *C. gariepinus* per annum, and this is largely done using heated recirculating aquaculture systems (RAS) (Fao, 2013). This strain was imported into The Netherlands for domestication, and it reported to have been improved to adapt to indoor farming. Other European countries producing this species in relatively very small quantities include Hungary, Poland and Belgium (FAO, 2014a).

C. gariepinus is increasingly gaining attention in many African countries beside Nigeria. Uganda, Kenya and Egypt are examples of the few countries with special focus on *C. gariepinus* (El-Hawarry *et al.*, 2016; Opiyo *et al.*, 2017; Ponzoni and Nguyen, 2008). By volume, *C. gariepinus* is now the most cultured fish species in the sub-Saharan Africa and the second most cultured fish species in Africa – second to tilapia, which largely comes from Egypt. In 2010 for instance, farmed *C. gariepinus* in sub-Saharan Africa accounted for 198,296 tonnes, while the total of farmed tilapia was 60,350 tonnes (FAO, 2012).

1.4. Nigeria - Current and Potential Resources for Aquaculture Development

It is projected that between 2016 and 2030, Nigerian aquaculture production (predominantly catfish production) will grow by 36% - i.e. from 310,000 tonnes to an estimated total of 418,000 tonnes in 2030 (FAO, 2018a). With over 13,000 sq km (1.3 million hectares) of inland water, 853 km of coastline and 200 nautical miles of Exclusive Economic Zone (EEZ) (Ita *et al.*, 1985; Ibeun, 2006), Nigeria is blessed with abundant water resources and water bodies with great potentials for aquaculture production. Located in West Africa and bordered by the Gulf of Guinea between Benin and Cameroun, Nigeria stretches northwards to the Sahel (shoring the Sahara Desert), covering a total of 923,768 sq km (Ibeun, 2006). The inland water comprises numerous rivers, lakes, streams, seasonal pools, dams, ponds, etc., and is said to be over 12 million hectares (Ibeun, 2006; Ita *et al.*, 1985). Most notable among these inland water bodies are Rivers Niger and Benue, and Lake Chad. The River Niger is the longest river in West Africa, and the third longest river after the Nile and Congo/Zaire Rivers in Africa (FAO, 1997). Originating from Sierra Leone, the R. Niger stretches over 4,184 km as it flows through Guinea, Mali, Niger, and into Nigeria through the Northwest, through Sokoto and Niger states, meeting the River Benue in Lokoja, Kogi state, where both flow a further 547 km into the Atlantic ocean

(Ibeun, 2006). The River Benue on the other hand, rises from Adamawa Plateau in Cameroun and some tributaries in Chad and Cameroun (FAO, 1997; Akaahan *et al.,* 2014). Originating from these central African countries, R. Benue stretches over 1,400 km (Ita *et al.*, 1985; Akaahan *et al.,* 2014), as it flows into Nigeria through the North - Eastern states of Adamawa, Taraba and Benue states, to meet the R. Niger in Lokoja, Kogi state and flow into the Atlantic Ocean. The inland water bodies in Nigeria are home to more than 230 commercially exploited fish species (FAO, 1993; Ita, 1993; Olaosebikan and Bankole, 2005; Fapohunda and Godstates, 2007), although this number could be higher as 775 species of fish is said to be in Nigeria (FishBase, 2019). Common among the species landed range from the very active predators such as *Lates niloticus, Gymnarchus niloticus, Channa obscura,* etc., to the intermediate omnivores such as *C. gariepinus, C. anguillaris, Heterobranchus bidorsalis, H. longifilis, Bagrus bayad* etc., to the common herbivores such as tilapia, grass carp, *Heterotis niloticus, Citharinus citharus*, etc. The most common catch is tilapia and *Clarias spp,* while the visually largest sized fish recorded are the *Lates niloticus*, followed by *Heterobranchus bidorsalis* and the most expensive fish in the country is *Gymnarchus niloticus* (costing between £ 8.00 - £ 10.00 per kg). Dwindling of catches from these water bodies in the recent years, partly due to increases in the number of fishermen competing for a finite resource (hence, overfishing and use of obnoxious fishing techniques), siltation and global warming, etc., in the face of a growing population have contributed to the present day focus on aquaculture. Attempts have been made by several government institutions and private farms to domesticate *Lates spp, Gymnarchus spp, Bagrus spp,* Carp*, Clarias spp, Heterobranchus spp, Heterotis spp, Citharinus spp,* etc. Limited successes were recorded in most species, while greater success was recorded in the *Clarias and Heterobranchus spp* due to their very high adaptability to different culture environment and conditions.

1.5. Development of the African Catfish Aquaculture in Nigeria

Between 1901-1960 the colonial government built more than 2,000 earthen ponds in Nigeria for subsistent aquaculture (Miller and Atanda, 2011). Two decades into independence saw a steady decline in production from these ponds, which were later abandoned due to lack of interest by the post-independence governments, and the fact that capture fisheries and the livestock industry were thriving, discovery of oil and lack of

inputs and skills to sustain production in these ponds (Hogendoorn and Vismans, 1980; Ramesh, 2013). The Directorate of Food, Road, and Rural Infrastructure (DFRI) in the 1980s initiated a nationwide pond construction project in response to the decline of fish production in the country, need to diversify the economy and for food security (Igoni-Egweke, 2018). Again, the lack of input i.e. good quality fingerlings and feeds, credit facilities and infrastructure such as storage cold rooms and processing plants, led to a very slow growth of the industry (Adeoye *et al*. 2012; Omobepade *et al.* 2015; Igoni-Eqweke, 2018).

In response to the aforementioned problems, government interventions through providing semi-skilled trainings, inputs and technical knowhow for different groups under different parallel and successive schemes took off, with concise evidence of success in meeting the actual goal of reducing unemployment and increasing food security. Extended government support to academic institutions, where, between the late 1990's to date, more than ten government-funded universities have introduced and are successfully running degree programmes in Fisheries and Aquaculture, and allied courses is a welcome development. These are in addition to the already existing universities already offering such courses or related ones. In 2006, it was reported that over 35 universities offered fisheries or allied courses (Ibeun, 2006), plus two Federal Colleges of Freshwater Fisheries (in Niger and Borno states respectively) and a Federal College of Marine Fisheries in Lagos state of Nigeria. Presently, this number is much higher and Fisheries has been introduced into the curricula of secondary education. Furthermore, there is increased funding for research at the National Institute for Freshwater Fisheries Research (NIFFR), established in 1968, with mandate focussing on freshwater fisheries and aquaculture research and training (Ibeun, 2006) and the National Institute of Oceanography and Marine Research (NIOMR), established in 1975, with a mandate for research on oceanography, marine fisheries research and training (Anyila, 2008). Affiliated to NIOMR, was the African Regional Aquaculture Centre (ARAC), established in 1980 by UNDP/FAO, with a mandate for research and training on local adaptive aquaculture techniques within the region (FAO, 1989; Ibeun, 2006). University and college students receive practical training from these institutions during industrial training, at the end of which students are equipped with the needed practical knowledge on fisheries and aquaculture management, especially as it relates to fish breeding, larval rearing, nutrition and water quality management in aquaculture, with a lot of focus on commercially important fish species, e.g. African

catfish. In addition, there is a Federal Department of Fisheries, and 36 State Department of Fisheries in Nigeria, charged with the responsibility of disseminating information to farmers, data collection, planning and regulating fisheries and recently aquaculture activities. The Aquaculture Unit of the National Biotechnology Development Agency, Abuja, Nigeria, is also involved in research into different areas of aquaculture, fish bioconservation and biotechnology. The fisheries society of Nigeria (FISON) and the catfish farmers association of Nigeria (CAFAN) are non-governmental professional organisations, charged with educating farmers and professionals on best management practices. Workshops, seminars and conferences have been organised within and outside the country, in areas of nutrition, fish health management, breeding technology, etc.

In 2008, the National Aquaculture Strategy (NAS) was produced, as reviewable policy document on aquaculture production and export promotion, supported both technically and financially by the FAO (FMARD, 2008). Reviewed in 2013, the Nigerian National Fisheries Policy (NNFP) guides interventions in both fisheries and aquaculture, which aims at increasing domestic production of fish, sufficient enough for domestic consumption and attract export market (FMARD, 2008).

These policies in addition to a ban on importation of fish, lower import tariffs on aquaculture equipment, tax holidays to fish farmers, access to inputs, profitability of catfish farming and already established markets with limited supply from the capture fisheries, saw a lot of private sector investments, with technical support provided by government institutions. It was estimated that aquaculture accounted for about 30% of new investments in Agriculture.

Other initiatives includes the creation of a Fish Farm Village in Ijebu-Ode, Ogun state, administered by the traditional ruler of Ijebu-ode **(Figure 1.2)**. Youths are trained and are given plots of land to dig ponds within the fish farm village. Within the cluster, there are sub-groups, where 4-6 farmers having ponds next each other have common workers and a small pellet mill to produce feed. Currently, there are over 600 youths in this fish farm village producing African catfish. On the other hand, the Fish Farm Estate in Lagos State **(Figure 1.3.)** is more in a residential area and as such has only concrete and plastic tanks. Pre-planned plots of land are sold to intended fish farmers to build their houses and concrete tanks for catfish farming.

Figure 1.2. Fish Farm Village Ijebu-Ode, Ogun State **Figure 1.3.** Fish Farm Estate Ikorodu, Lagos State

1.6. Current Demand and Status of Fish Production in Nigeria

There are an estimated 1.5 million fish-based livelihoods in Nigeria (FAO 2017; WorldFish, 2017), producing about 1.027 million MT **(Table 1.1)** of fish per year, with 710,331 and 316,727 MT coming from capture fisheries and aquaculture respectively (FAO 2017; NBS, 2017; WorldFish, 2017). Over 56% of fish species cultured in Nigeria are *C. gariepinus* (FAO, 2018a). Nigeria is the largest producer of African catfish in the world and has the second largest aquaculture industry in Africa (after Egypt) (Ponzoni and Nguyen, 2008). In 2010 the Nigerian catfish industry was valued at US$ 800 million (Adewumi and Olaleye, 2011). Consumers' preferences for catfish, due to its few intra-muscular spines, scale-less nature, relished taste under different processed forms and relatively good storage shelf life when smoked makes it a very popular fish in the country. Having over 6,000 small scale farmers and altogether employing over 13,000 people, the Nigerian aquaculture industry, which in 2010 was valued at USD 800 million (Adewumi and Olaleye, 2010; NBS, 2017; FAO, 2017; WorldFish, 2017) has shown an increase in production from 21,700 tonnes in 1999 to 316,700 tonnes in 2015, contributing 33% of the 0.5% GDP contributed by the fisheries sub-sector, equivalent to 0.166% of the nation's GDP, hence, prompting increased governmental attention and investment in aquaculture to about 30% of the total investment in agriculture (FAO, 2019).

Despite this growth, consumption is currently at 1.63 million tonnes, averaging a per capita consumption at 13.3 kg of fish per annum (WorldFish, 2017). This is due to the growing income level and very high and fast growing population of the country, estimated at 186

million people, growing at a rate of 17% between 2010 and 2016 (World Bank, 2016). Current demand for fish is estimated at 3.32 million tonnes per year, while annual importation is over 1 million tonnes/year, valued at over USD 1.2 billion/year (Igoni-Eqweke, 2018; FAO, 2019).

Table 1.1. Nigerian Fish Production (tonnes (metric ton)) in Year 2000, 2005, 2010 and 2015

Sector	**2000**	**2005**	**2010**	**2015**
Aquaculture	25,720	56,355	200,535	316,727
Artisanal (coastal, brackish and inland)	418,069	490,594	616,981	694,867
Commercial Trawlers	23,308	32,595	31,510	15,464
Total	467,098	579,544	849,026	1,027,058

- *Source: Akintola and Fakoya, 2017; NBS 2017*

Very accurate data on production levels of *C. gariepinus* from aquaculture alone is limited, and this is largely due to inefficient mechanisms for proper inventory of total number of catfish farmers at the state, zonal and country levels. Secondly, the fact that hybrids of *C. gariepinus* and *Heterobranchus spp.* are produced but not separately accounted for due to their relatively low volume/year makes accurate estimates difficult. Insecurity in the North-East, where a substantial amount of the wild fish comes from e.g. Baga area along Lake Chad in Borno State (which accounts for 30% of the artisanal fisheries production (Ibeun, 2006)) and parts of the R. Benue in Adamawa State makes fishing or accurate record of landings per year impossible due to inaccessibility resulting from the unrest. In 2010 for instance, the total fish production in Nigeria was estimated at 616,981 metric tonnes per annum, with aquaculture accounting for 200,535 metric tonnes of this total (FAO, 2018a). Studies show that artisanal fisheries have between years 2000 - 2010 contributed just about 400,000 tonnes (Miller, *et al.*, 2006; FAO, 2007; Grema, *et al.*, 20013; Oladimeji, *et al.*, 2013; NBS, 2017; FAO, 2018a). The sudden jump in artisanal fisheries production from 400,000 to over 700,000 (NBS, 2017; WorldFish, 2017; FAO, 2018a) tonnes in 2016 could therefore be attributed to increased access to the Lake Chad area following increased security and as a result of unintended closed seasons, the fisheries became richer. Furthermore, over exaggerated or underestimated figures reported by some farmers for some reasons, unreported account of quantities consumed or gifted by farmers, quantities smoked and added to those from the wild due to unavailability of the wild stock, and preference for wild smoked fish in some areas etc., makes it more difficult to make accurate estimations and predictions. For better understanding of the Nigerian catfish

aquaculture industry, the industry will be broken into: production of fingerlings, broodstock, grow-out and fish feed and their respective market segments.

The bulk of the cultured *Clarias* comes from the Southern part of Nigeria, mostly from the South-West and South-South, and then the North central. It is difficult to account for which of the other three geopolitical zones (North-West, North-East and the South-East zones) produce more than others, however, the following factors could account for their relatively lower production compared to South-West, South-South and North-Central.

The North-Eastern and North-Western part of Nigeria are the areas through which the Rivers Benue and Niger flow into the country, across different states in the respective zones, and down to the North central, where they meet and flow into the Atlantic ocean through some southern states. These two main rivers of Nigeria, together with other relatively smaller ones, dams and lakes in these zones account for a huge number of fish, which are captured and sold either live or processed (smoked or dried) within and outside their zones. Production is however seasonal and dwindling, therefore not very reliable, hence, the increasing attention on aquaculture.

Northern Nigeria (especially the Northeast and Northwest) account for over 90% of Nigeria's cattle and 70% its sheep and goat industry (Lawal-Adebowale, 2012), hence, local people have traditionally preferred meat to fish and have had a sustainable alternative to fish. Their distance from Nigeria's ports located in Lagos and Port Harcourt, has made aquaculture production (which relies largely on imported feed) relatively more expensive and hence not very favourable to fish farmers in these zones. This is especially true in the face of competition from whole sellers, who purchase fish produced in the southwest at cheaper rates, transport and sells them alive in these zones. This is in addition to the availability of alternative sources of animal protein, which are likely cheaper. On the other hand, the major plant proteins (soy and peanuts) and carbohydrates (maize and millet) used in fish feeds production are cultivated and thus cheaper in the Northern Nigeria.

The southeast on the other hand, has relatively fewer water bodies to depend on for fish. The undulating topography and soil type in many parts of the zone, did not favour the early extensive and semi-intensive forms of aquaculture in the earthen ponds. However, with the

advent of the use concrete, fibreglass and plastic tanks for aquaculture, coupled with increased demand, aquaculture production is now increasing in this zone.

The present level of experience of many farmers in the Nigerian aquaculture industry is partly from accumulated years of hands-on learning, and after series of mistakes, a lot of farmers, developed the art of catfish farming. This therefore, implies to some extent that those geopolitical zones with earlier exposure to aquaculture, for many reasons (e.g. due to lack of the wild sources or any cheaper alternative animal protein), have more experience in production than the others.

While most of the fish produced by farmers are mostly sold within their respective zones, some from the Southwest are transported to other parts of the country (e.g. Abuja, Enugu, Kaduna, Onitsha, Plateau and Rivers states) and sold alive. This is as a result of the fact that the price for catfish outside the Southwest can be as much as 25–50% higher per than the other zones of the country, due to the volume of production in the Southwest kg (Miller and Atanda, 2011). On the other hand, large quantities of the captured fish from North (especially along the Rivers Niger and Benue, and the Chad basin) are smoked and transported to the south (specially the southeast and south-west) and other parts of the country that pay higher amount of money for them. Often, during the rainy season (mostly April-October), the water levels rise and consequently cause fishing decline due to reduced access to the fish. This generally increases the value of fish (both captured and cultured), and sometimes, farmed fish are bought over, smoked and added to the wild ones whose value is relatively higher. The consistently huge marked demand for smoked fish has paved the way for multitudes of small-scale smoked fish businesses to spring up in the different parts of the country, especially in the regions with rapidly growing aquaculture production. Farmers enjoy more profit from smoked fish rather than selling them live, therefore, find smoking a safer means of making more profit through added value.

1.6.1. Characteristics of Fingerling Production Systems

Demand for African catfish seed (fingerlings) in Nigeria has steadily increased due to increased investment in aquaculture to meet the huge production and supply deficits of 2.30 and 1.69 million tonnes respectively, out of a total demand of 3.32 million tonnes of fish/annum. The inability to distinguish a poor quality from good quality fingerlings has

led to sceptical attitudes from buyers or intended farmers, and has in some cases also led to many farmers producing their own fingerlings without prior formal training, hence, a huge number of hatcheries of different sizes, types and using different systems in Nigeria. Consequently, there are very wide ranges in hatchability and survival to juveniles, from as low as 5 to as high as 200 eggs for every single juvenile produced (Fleuren, 2008). Numerous small-scale farmers (producing <200,000 fingerlings/year), using varied systems such as aerated, flow-through, non-flowing indoors and outdoors concrete, lined wood **(Figure 1.4)** and plastic tanks, earthen ponds and a combination of any of the aforementioned, dominate production. Broodstock are sourced from other farms or the wild (thought to comprise *C. gariepinus* and *C. anguillaris*, which occur sympatrically). The small sizes and production capacities of these hatcheries, coupled with the high fecundity of *Clarias spp.*, enables farmers to rely on few broodstock, potentially creating/increasing inbreeding depression. On the other hand, the few large-scale hatcheries (producing >200,000 up to 3-4 million fingerlings/year: Ponzoni and Nguyen, 2008)) use mostly intensive recirculating aquaculture systems (RAS) **(Figure 1.5)** and the Dutch domesticated *Clarias* broodstock, imported from the Netherlands. These systems can produce as high as 6000 juveniles/m^3, and the Dutch *Clarias* have undergone several generations of mass selection (Fleuren, 2008).

Figure 1.4. Small-scale catfish Hatchery in Lagos State

Figure 1.5. Large-scale catfish Hatchery in Adamawa State (360 Farms, Nigeria)

The relatively fast growth rates of *Clarias* from these intensive farms have turned them into sources of broodstock/broodstock replacement to the smaller hatcheries; a practice that potentially reduces variation and further poses problems of inbreeding depression, bioconservation and identification of the *Clarias spp*.

1.6.1.1. Shooters (Fast-growers)

Furthermore, the common practice of using shooters (fast growers) selected from batches of *Clarias* **(Figure 1.6)**, as broodstock, due to their unproven perceived superior genes, can potentially create "bottlenecks" (reduce effective breeding number) and lead to negative/unintentional selection for aggression and cannibalism. Although the actual cause of the fast growth is unknown, studies suggest establishment of social hierarchy or inherent genetic attributes such as prompt response to feeding, feed conversion efficiencies, protein and fat deposition pattern, etc., (Martins *et al.*, 2005). Shooters are highly cannibalistic especially in the early phase of their life-cycle (fry, fingerlings and juveniles) and constitute a major cause of economic loss in *Clarias* catfish hatcheries. At the moment routine and proper grading has been found to reduce the effect of shooters in hatcheries, while feeding and stocking density management has also been found useful. Studies suggest that although grading disrupts social hierarchy in a cohort of *C. gariepinus*, it does not necessarily encourage uniform growth in them, as graded stocks grew in the same pattern as ungraded stock of *Clarias* in an experiment involving a batch of *C. gariepinus* separated in to two groups, one of which was graded into three categories (shooters, intermediate and runts), while the others remained ungraded (Martins *et al.*, 2005). This study thus suggest passible inherent genetic attribute to the fast growth rate in shooters, however, it is important not to overlook the emergence of new set of shooters following grading of older ones in the same batch of hatchlings, as has been observed by many farmers in the field, further suggesting a possible environmental and/or social factor causing the fast growth nature or interacting with the genetics of the fish.

An interesting contrast is also the fact that shooters can be 6 – 10 times bigger than their sibling hatched on the same day and rose in the same tank. They easily eat smaller frys and fingerlings around them and continue to develop more appetite and predatory skills (for fish rather and feed) and thus continue to gain more size advantage. Not grading such fish out of the tank could result to loosing up three fish per shooter per day. This will result serious economic loss when 20 shooters are in a tank of 5000 fish, in less than a month, the number could fall below 500 fish in the tank. In Barramundi (*Lates Calcarrifa*), it was found that at 50% the size of a shooter, a sib could easily be eaten as a prey and a shooter could swallow a sib of up 78% of its total length (Catarina, 2015). It was also noted that once the shooters began cannibalism, they tend to continue to develop the cannibalistic

skills and thus growing better and causing more economic loss (Ribeiro and Qin, 2013; Catarina, 2015).

Figure 1.6. Shooters (the fast-growing bigger ones) in a tank, usually selected and kept as future broodstock

1.6.1.2. Cannibalism

Cannibalism in *Clarias* catfish is another major cause of mortality and economic loss. In the early days of *Clarias* aquaculture, cannibalism accounted for slightly ≤ 90% of mortalities in this fish species (De Kimpe and Micha, 1974; van der Waal, 1974; van der Waal, 1978 and Hecht and Appelbaum, 1988). *C. gariepinus* has been listed among fish species of economic importance displaying cannibalism (Hecht and Appelbaum, 1988; Hecht and Pienaar, 1993). Just as in some other fish species (Kohlmeier and Ebenhoh, 1995), cannibalism occurs naturally in the wild population of *Clarias* (Moyl and Cech, 1988). In the cultured population, cannibalism has been observed to start as early as immediately after yolk sac absorption by attacking and biting part of the prey fish from the tail end, upwards to the head. At this early stage, they usually have a small mouth gape and are similar in size to the prey, as such cannot swallow the prey as a whole. This type of cannibalism, often called "type-1cannibalism" has also been observed in the Eurasian perch, *Perca fluviatilis* (Baras, 1999), although in that species, it starts around 10 - 11 days post hatch. This later changes into complete swallowing of complete smaller fry, fingerlings or juveniles by the shooter amongst them, attacking from the head, somewhat fitting into the type-2 cannibalism in *Perca fluviatilis*, as described *by* Baras *et al.*, (2003).

While feeding, light intensity and culture conditions (stocking density, water quality, stress) are known to be environmental influences on cannibalism (Hecth and Pienaar, 1993; Kohlmeier and Ebenhoh, 1995; Baras and Jobling, 2002; Baras *et al.*, 2003; Krol *et al.*, 2014), genetic factors such as variation in growth rate (Hecht and Pienaar, 1993), feeding behaviour (carnivorous or omnivorous feeders) and individual/population aggression (Baras and Jobling, 2002) also influence cannibalism in fish. Hecht and Pienaar (1993) have also suggested that a conducive environment could reduce the propensity towards cannibalism. Almazán Rueda (2004) reported reduced aggression in *C. gariepinus* raised under 24:0 hours dark: light (DL) compared to 12:12 hours DL or more. Although the exact cause of cannibalism in larval culture of *Clarias* is yet to be completely understood, starvation, delay in first feeding and improper feeding regime (low and uneven feeding frequencies or quantities of feed), have been observed to trigger and or increase cannibalism in *Clarias* catfish. Furthermore, low stocking densities and sometimes, increased stress due to poor water quality, have also been observed to increase cannibalism in *Clarias* catfishes. The resultant uneven growth, coupled with the large mouth-gape, favours more cannibalism and the shooters tend to dominate and feed on other fish in the same tank. Social dominance (Hecht and Appelbaum, 1988; Pienaar, 1990) and cannibalism (Damme *et al.*, 1989; Kestemont *et al.*, 2003) have been reported to be one of the causes of size variation in fish, just as size variation is reported to cause cannibalism. Large mouth gape enables *C. gariepinus* larvae to prey on fish of almost its size, as a cannibal to prey ratio of 1.28:1.00 was reported in type-1 cannibalism in the works of Hetch and Appelbaum, (1988). Hseu *et al.*, 2003 reported that cannibalism could succeed in groupers if the predator to prey size difference is above 30 - 32% and 33% in snakehead, *Channa striatus* (Qin and Fast, 1996) and Baramundi, *Lates calcarifer*, (Jesu Arockiaraj and Appelbaum, 2011) respectively. Generally, cannibalism is more severe in the hatchery phase of catfish production. Inter-cohort cannibalism has been reported in the first week of culturing European catfish, *Silurus glanis,* (Krol, *et al.*, 2014) and in *Heterobranchus longifilis* (Baras, 1998). Although a genetic approach to minimise cannibalism is *Clarias* catfish seems to be lacking due to lack of proper understanding of the underlying factors, some management approaches are commonly used with some levels of success. At the hatchery phase, maintaining high stocking densities, good water quality and sorting/grading of fish are recommended. Grading at three weeks post hatch is recommended and this should continue fortnightly until they reach juvenile stage, when they are finally graded and sold or stocked for onward growth. Martins *et al.* (2006),

reported reduced aggression levels (improved welfare) in medium and larger *C. gariepinus* following grading. Controlled environmental conditions in European catfish larviculture (Krol *et al.*, 2014) and the use of rotifers and copepod as opposed to cladocerans and pellets, as feed in Koi carp, *Ciprinus carpio* larviculture (Altaff and Janakiraman, 2013), have been observed reduced cannibalism. Furthermore, maintaining constant darkness in barramundi culture (Jesu Arockiaraj and Appelbaum, 2011), supplementation of tryptophan (TRP) in juvenile Atlantic cod (Hoglund *et al.*, 2005) and grouper, *Epinephelus coioides* (Hseu *et al.*, 2003[a]) and increased stocking density in perch larviculture (Baras *et al.*, 2003) have also been reported to reduce cannibalism. Coulibaly *et al.* (2007) have reported a decrease in cannibalism with decreased stocking density in cage culture of *H. longifilis*.

While cannibalism is said to be less prevalent in the grow-out phase than the hatchery phase, it is important to mention that in the grow-out, different forms of cannibalism - both type-1 and type-2 cannibalism occur. The difference between the type-1 cannibalism in the grow-out and those of the hatchery is that, in the grow-out, it does not necessarily happen when the fish are hungry. It has been observed that whenever there is a bruise or any exposed injury in the catfish tanks, other fish in same tank tend to go after the injured fish, biting the same wounded area and gradually eating the into the flesh until the fish dies, after which they eat the carcass and leave the bony head. Secondly, it has also been observed that after changing the water in a fish tanks, there are times fish are lost as a result of cannibalism, and this sometimes starts from a small bruise from either biting each other, or from injuries caused by pectoral spines of other fish as they crowd at the base of the draining tank, struggle for space and water. It often ends up in a similar manner as described above. In the case of these forms of cannibalism, grading does not help reduce it, as sometimes the larger fish in the tanks are also victims. The losses due to such cannibalism can also be huge for it could be as often as the water is changed, and typically, in the all-in-all-out tank systems (typical of the Nigerian aquaculture industry), water is change from as low as fortnightly, to as high as biweekly. This is much worse if a farm is composed of multiple smaller tanks with a longer culture period. It is important to state here that most of the farmers that have reported this have said it increased with the age of the fish in grow-out tanks, admitting only noticing it 2-3 months after stocking onwards.
Although the exact cause of this form of aggression still needs to be thoroughly investigated, it is only fair to state that this phenomenon is on the increase, as this was not

the case in the last decade (personal experience and reports from some farmers). *Could this suggest an increased aggression in the present generations of the Clarias catfish in Nigeria?* However, it is also thought that not feeding the fish before changing the water (ideally) could partly be responsible for the aggression. Farmers have tried minimising these losses by only partially changing the water instead of completely draining the tanks, so as to minimise bruising. Furthermore, gradual feeding (adding little quantity at a time) immediately draining stops and filling of water starts is used, so as to distract fish from going after any injured ones. Although some farmers claim that adding salt immediately after changing the water reduces this incident, there is no scientific proof or basis for this yet, hence, this remained to be researched.

1.6.1.3. Other Issues Around Fingerlings Production

Government hatcheries, which were at some points the sources of fingerlings, have become incapable of meeting the demands for some reasons, amongst which is the fact that a lot of them are underutilised, maintained (Ponzoni and Nguyen, 2008), and sometimes underfunded. This therefore, provided very good opportunities to the private hatchery owners who now account for the majority of fingerlings produced in the country. Studies report that from 2000 to 2005, fingerling production increased 10 times, from 3 million to 30 million fingerlings per year (AIFP, 2005 in Ponzoni, 2008). On the other hand, indiscriminate setting up of hatcheries by people who have had short training/courses in fish breeding or those who have grown table fish and want to start breeding has further contributed to the uneven quality of fingerlings in the Nigerian aquaculture industry. The Ogun State Department of Fisheries introduced a certification scheme for fish breeders in the state. In another study, the total number of fingerlings supplied (including wild sources) as at 2007 was said to be, 55.8 million (FDF, 2007). It is important to state here that the West African Agricultural Productivity Programme (WAPP) in 2014 began a deliberate act to among others, increase the production of good quality fish seeds in Nigeria through rendering financial support and training to fisheries and aquaculture research institutions (NIFFR, NIOMR and NISPRI (the Nigerian stored products research institute)). This support is in the form of research grants, training of research staff, building laboratories and in part designating them as National Centres of Specialisation in Aquaculture (WAAPP, 2014; WAAP, 2016). This led to the distribution of over 25 million fingerlings by WAAPP in Nigeria 2016 and stimulating production and growth in the

sector to the then target of about 250 million fingerlings per year. Based on the current size of the catfish industry, it is safe to say that the fingerling demand of Nigeria is estimated at 364 million (i.e. assuming that the current demand of 3.32 million tonnes is composed of 1 kg sized fish and a mortality of only 10% occurs).

Mortality, high cost of inputs and management requirements (feeds, installation and operation), and unstable market conditions are some of the main challenges of hatchery production. Catfish broodstock are induced with synthetic hormone (ovprim, ovuline, ovatide, etc.) as opposed to some other countries like Uganda, where the pituitary of the male is extracted to induce females whose eggs will be fertilised by same male's milt (extracted and stored). Catfish fry require live feed immediately after yolk absorption (Hecht, 2013), and this is usually cultured zooplankton or live *Artemia*, although most farmers do not depend on cultured zooplankton due to inconsistent and inadequate quantities produced. Furthermore, the high cost and sometimes unavailability of Artemia, coupled with lack of constant power, limits its use. Farmers in Nigeria mostly use the shell-free Artemia, which is fed off the shelf and has been very dependable. Although it is very expensive, the fact that the shell-free Artemia is always available makes it the farmers' choice. Exogenous feed for frys and fingerlings are equally very expensive. This might be due to its high nutrient content, needed to meet the nutritional requirement of the young fry which is 50 – 55 % crude protein (CP) (Uys and Hecht, 1985; and Hecht, 2013).

Diseases are also common causes of mortality in *Clarias* catfish hatcheries, especially those without strict farm hygiene rules. Proper diagnosis of diseases is still lacking due to lack of or inadequate numbers of specialists in aquatic veterinary medicine and related courses in Nigeria. The source of water used on farms also greatly determines whether or not such farm or hatchery will be prone to certain pathogenic infection. Many hatcheries use underground water i.e. boreholes and wells and are mostly free from parasitic infections. On the other hand, those hatcheries that rely on streams or other open water bodies are prone to all kind of diseases. Common among diseases in the catfish hatchery include *Saprolegnia, ichth (white spot) and furunculosis*.

With intensification come diseases issues. In September 2013, reports emerged from across the country of mass mortality of fry at about 2-4 weeks with consequent failure to produce fingerlings and juveniles. The true reason behind this was however, not fully diagnosed.

While some believed it was nutritional, others blamed high temperature triggered by climate change (Anetekhai, 2013). In any case, both represent risk factors that could predispose the fish to infections and diseases. A study in two communities in the south of the country showed that 89.39% of fish farmers observed disease symptoms with haemorrhagic lesions being the highest (Adeyemo, 2013; Dauda and Ibrahim, 2015; Jibrin pers. comm., 2018).

There are very few aquaculture veterinarians or fish health experts in Nigeria. This could explain the very high losses recorded during outbreaks and lack of good understanding of fish diseases across different production systems. In an attempt to salvage the situation, some farmers resort to using crude practices such as indiscriminate use of salts (NaCl), antibiotics made for humans and poultry, and sometimes cocktails of antibiotics from different sources, which invariably primes bacteria for resistance and poses a serious threat to public health.

Nutritional deficiencies e.g. in vitamin C and calcium have been found to cause broken head disease in both fingerlings and table catfishes. Rancid feed has been reported to cause jaundice in adult catfishes. Routine monitoring to check the status of fish health and prevalence of disease causative agents have been advised (Nwabueze, 2012). Daily monitoring of water quality, observing fish behaviour and swimming activity especially during feeding is very important in minimising pollution triggered infections and for early detection and treatment of potential disease. Generally, prevention has proven to be the cheapest way out.

1.6.1.4. Market for Fish Fingerlings and Juveniles

Fish seeds (fingerlings and juveniles) are produced mostly in the Southwest, South-South and North-Central. However, the Southwest produces more fingerlings than any other part of the country to supply its region, which has the highest number of farms in the country and for trade to other regions of the country. In most parts of the country, fingerlings are traded at farm-gate and are manually counted. They are packed in 50 L water kegs, with both sides of the top, just beside the handle, opened up for ventilation **(Figure 1.7).** Depending on the size of the fingerlings or juveniles, stocking density could range from 1,000 – 2,000 fish/can and this number could either increase or decrease if the distance is

short or long. In such a container, about 15 – 20 L of water is added to each can before the fish is added, and 0.5 ml of palm oil is added to the water to minimise foaming when travelling a very long distance e.g. 800 kilometres. They are usually loaded on to a commercial vehicle and transported without aeration in most cases. Sometimes during seasons with hot weather, the fish are transported at night or provisions made to use ice blocks to cool the water temperatures down at intervals during a long journey. On the other hand, marketers of fish feed in different locations and markets become links, middlemen or nursery operators; by way of linking their customers (who buy catfish feeds) to reputable hatcheries for fingerlings/juveniles, buy directly from hatcheries/nurseries to sell to farmers alongside the feed or buying fingerlings from the Southwest or any reputable hatchery and grow them for 2 – 4 weeks old to become juveniles; a size which they can sell to farmers to on-grow. In some very few cases, feed marketers setup hatcheries to supply clients with fish seeds alongside fish feed.

Figure 1.7. From left to right shows the type of kegs in which fingerlings are transported and delivered

1.6.2. Characteristics of Broodstock Production Systems

Many hatcheries in Nigeria start and work with small populations that came from a single or two batches of fish grown on the farm or bought over from another farm. Furthermore, due to relatively high fecundity in *Clarias*, there are tendencies that few broodstock are used in breeding. Continuing on such practices reduce the effective breeding number (Ne) i.e. the number of individuals (broodstock) producing viable offspring in the next generation (Tave, 1993; FAO, 1995 and FAO, 1999), and as the Ne decreases, the chances of inbreeding depression increases. Applying selective breeding in such populations could lead to decreased Ne and genetic drift, especially in an un-pedigreed population (Tave, 1993). Most often due to limited facilities, selected recruits are pooled together and raised in few tanks or ponds with no effort to identify individuals or families at any point in time

(Personal observation). The type of crosses employed in the hatchery, the sex ratios, number of times a female is reused/year and the manner in which milt from males used to fertilise eggs (i.e. milt from different males is pooled together or used separately) could be responsible for inbreeding depression.

Traits of importance to most Nigerian farmers are growth rate, body length and survival. Due to the fact that catfish are mostly sold live or smoked in Nigeria, there is more concern about the weight and less focus on dressing percentage and meat quality (red or white) muscle for now, unlike the *Clarias* in Europe, where they are mostly sold as fillets (Fleuren, 2008). The length is an imrotant consideration as some market (e.g. restaurants) are particuar about number of portions per kg of catfish, hence, prefer the longer ones. Selection for survival is very important in aquaculture, and it is almost an immediate concern to farmers especially when faced with unexplainable mortalities. In the *Clarias* catfish industry, selecting for survival or disease resistance is still simply by avoiding any batch or source of fish with history or recent incidences of questionable mortalities. As important as prevention is, it is very difficult to fairly attribute all mortalities to the genetic make-up of the fish, especially when there are no proper diagnoses or post-mortem analysis to evaluation the root cause.

The lack of multiple tanks to accommodate different families of broodstock, improper record keeping and the inability of most farmers to identify and differentiate between fish of different families due to lack of tags, has made it almost impossible for farmers to monitor and/or control inbreeding and genetic variation. This is especially true in Nigeria, where the bulk of catfish fingerling producers are operating at a small scale. Central to any breeding programme is proper broodstock production and management. Identification of individuals and families (e.g. using PIT tags, separate tanks for each family, etc), good feeding regime (quality and quantity), water quality and other management issues are essential for successful broodstock management (Fleuren, 2008). Most catfish farmers do not have separate feed for broodstock in Nigeria. Often, they are fed the available commercial grow-out diets, which might not necessarily meet the nutritional requirement of broodstock (especially the females). Because there is no pronounced size variation that could encourage cannibalism between the male and female *Clarias* broodstock in Nigeria, most farmers raise/keep them in same ponds or tanks.

Due to the large number of eggs produced by a female and the fact that the females can be reused and the males are normally sacrificed during breeding, more males are kept in hatcheries that breed very often, to prevent scarcity. Females in well-managed farms can produce eggs quarterly **(Figure 1.8)**, while in the wild, they spawn only once a year. While it is not clear why this is happening, some authors ascribe multiple egg production per year in the farmed *Clarias* to residual effects of the hormonal induction (FAO, 2006). It is also thought that keeping broodstock indoors, under consistent temperature, water flow and water quality might be linked to multiple ovulation in farmed stock of *Clarias*. Matured *Clarias* broodstock show obvious sexual dimorphism. Although inbreeding depression is an undesired product of mating close relatives together, carefully controlled selective breeding can be used to enhance desired heritable traits in a population (Tave, 1993; Tave and FAO, 1995; Tave and FAO, 1999).

C. gariepinus broodstock are raised in varied culture environments e.g. in earthen ponds, fibreglass, plastic or concrete tanks, etc., depending on what is available to the farmer. Although they attain sexual maturity at the age of 8 months post hatch (MPH), they are mostly kept for longer before use. The females have a gonadosomatic index (GSI) of about 12-16 % of their body weight, and depending on their age and sizes, produce an average of 600 ± 100 eggs $g^{-1}$of ovary (de Graaf and Janssen, 1996; Yong-Sulem *et al.*, 2008; Fleuren, 2008). The females are relatively more sensitive to temperature and photoperiod fluctuations compared to the males (de Graaf and Janssen, 1996). They are ideally kept separately from the grow-out fish and depending on the level of intensity, fed a nutrient-rich diet with the aim of meeting their nutrient requirements, thus ≥ 35 % crude protein CP, 21.2 kj g^{-1} and a protein energy ratio of 20 protein kj g^{-1} gross energy (GE) (Ayinla, 1988**;** Ali and Jauncey, 2005; Ondhoro *et al.*, 2015)**.** Many farmers rely on commercial grow-out feed to raise their broodstock due to inadequate supply of broodstock feed.

1.6.2.1. Market for Broodstock

There is no farm specialised on only production and sales of catfish broodstock in Nigeria. Most broodstock are sourced from farms that raised them to along side grow-out, hatchery or a combination of all operations on the same farm. They are commonly sold per kg of body weight and many farmers select shooters from the hatchery or buy on-growing fish to be raised as broodstock for use and sales. As only few broodstock are used at a time, many

farmers source for broodstock with their zones, while those with large operations might source or sell to farmers in other zones of Nigeria.

Figure 1.8. Stripping of eggs from female *C. gariepinus* broodstock into an clean and dry bowl

1.6.3. Characteristics of Grow-out Production Systems

Production was mainly carried out in already existing earthen ponds (Miller and Atanda, 2011), constructed mostly by the government in the 1950s-1970s for aquaculture and burrow pits of abandoned mining sites (Adeoye *et al.*, 2012). Access to suitable land for construction of earthen ponds sometimes meant going outside the cities i.e. away from market, electricity and security. Although *C. gariepinus* thrive well in earthen ponds, they also thrive in varied culture environments, provided stocking density, water quality and feed are optimum. Most common sizes (surface area) of ponds are 100 – 300 m^2 and depth is averagely 1.5m. Liming and fertilisation are commonly done using $CaCO_3$ and poultry or livestock manure respectively. The rate of lime depends on the pH of the eventual pond water and lime is added to get it within the recommended optimum pH of 6.5 – 8.5 and 30 - 100 kg manure is commonly used per pond (100 - 300 m^2). Recently, high-density earthen ponds, with up to 100% weekly water exchange are replacing the large low-density ones with stagnant water. Such farms are sited close to rivers and they pump water daily into one of the several ponds to reduce waste and increase oxygen.

1.6.3.1. Transition from Earthen Ponds to Tanks (Peri-urban Aquaculture)

Over time, due to increased access to husbandry technology and limited access to suitable lands (secured, accessible and with good water retention capacity) to construct earthen ponds, the use of concrete tanks came into being, and this among other advantages, offered easy management of fish. Subsequently, the high cost of constructing durable concrete tanks, and the quest for aquaculture on/in rented land or homes (where tenants cannot build permanent structures) and in the peri-urban and urban areas (with readily available market), gave room for the introduction of fibreglass tanks and later different kinds, shapes and sizes of plastic tanks, the latest of which is the collapsible tanks; popularly known as the metal - frame family swimming pool in other continents of the world. Despite this transformation, it is important to state here that, the earthen ponds (built and owned or rented by individuals) and concrete tanks are still in use to date, at increasing levels of intensity (i.e. in terms of stocking densities, water exchange, aeration and intensity of feeding) **(Table 1.2)**. Small to medium scale farmers using a combination of the above systems (earthen ponds, concrete, fibreglass and plastic tanks), account for the largest number of fish farmers in the country (Ponzoni and Nguyen, 2008). Stocking densities are usually high, practicing mostly monoculture and harvests are mainly for domestic markets and the scale of productions are usually 0.5 – 20 tonnes per annum. Despite operating mostly stagnant water, they make efforts to aerate using air pumps or pump in freshwater to ensure water exchange (Emmanuel *et al.*, 2014)**.** Most of the farmers do not produce their own fingerlings or fish feed (except when by-products, bakery or kitchen wastes are available for use as feed or feed ingredients), thus, often enjoy lower profit margins compared to the large-scale intensive farms. However, many amongst them are part time farmers, engaged in other forms of agribusiness, trade or are gainfully employed in an organisation.

1.6.3.2. Recirculating Aquaculture Systems

Recirculating aquaculture systems (RAS) for *Clarias* catfish were introduced to Nigeria about two decades ago, in response to the need for very efficient production systems. This was partly an outcome of visits to The Netherlands, UK, Israel and other parts of Europe by potential investors and other stakeholders from Nigeria and a reciprocal visit by consultants/managers (Miller and Atanda, 2011). Presently, such systems have reached a record high stocking density of 375 kg/m^3. The high initial capital cost and lack of stable

electricity, has confined this system to only the affluent; who can afford to set up and operate such systems in urban areas, relying mainly on generators for electricity to power the system (pumps, aerators, UV lights, etc). Access to RAS also necessitated access to sources of good quality fingerlings (the Dutch *Clarias*) and fish feed (Coppens), which were originally lacking in Nigeria and thus few broodstock and feed were also imported during the RAS intervention era. This therefore, has put them among the large-scale catfish producers, whose annual production is in excess of 200 tonnes per year, and are important players in setting the industry standard in terms of harvest weight and farm-gate price/kg. This marked an important point in the history of catfish farming in Nigeria, as other farmers explored all available opportunities to improve their husbandry techniques and yield, thus driving the industry from predominantly semi-intensive systems, using earthen ponds, to very intensive outdoor and indoor systems in concrete and plastic tanks, earthen ponds and few recirculating systems.

1.6.3.3. Cage Culture

Cage culture, which is an experimentally old system in Nigeria, using bamboo structure (Ita, 1976) is only recently been tried out (in 2011) for commercial production of tilapia, at Epe Lagoon in Lagos State of Nigeria by Amolese Aquaculture Nigeria Limited who pioneered cage culture of tilapia in Nigeria (Tiamiyu, pers. comm. 2018). In 2013, this company expanded to Ikere Gorge Dam in Oyo State of Nigeria, producing both tilapia and the African catfish. Since then, other companies such as Choice Fisheries and Triton Aquaculture have set up cage sites for commercial tilapia production in Asejire Dam in Oyo State. Durante Fisheries, Ejide Farms and Premium Farms have been set up at Oyan Dam in Ogun State, of Nigeria. Frames, anchors, floating barrels and platforms are sourced/fabricated in Nigeria, while the nets are imported. Given the abundant water resources in Nigeria, it is for the government and other relevant stakeholders to pay attention to promote such systems by ways of enforcing policies promoting such efforts (domestic production) and ensuring profitability of such venture, access to funds and market regulation.

Table 1.2. Summary of catfish Production Practices in Nigeria, information here is based on answers provided by farmers interviewed and personal experience

Intensive Monoculture Systems for *C. gariepinus*			
Culture Systems	**Stocking Density (kg/m3)**	**Water Exchange (days)**	**Harvest Weight @ 6 months (kg/fish)**
Earthen Ponds	15 - 25	60	1 - 1.2
Outdoor Tanks (Concrete and Plastic)	15 - 25	3 to 5	0.9 - 1.1
Indoor Tanks (Concrete and Plastics)	25 - 30	2 to 3	0.9 - 1.1
Earthen Ponds	35 - 50	Continuous Flow Daily	1.2 - 1.5
Outdoor Tanks (Concrete and Plastic)	35 - 50	Continuous Flow Daily	1.1 - 1.3
Indoor Tanks (Concrete and Plastics)	35 - 50	Continuous Flow Daily	1.1 - 1.3
Recirculating Aquaculture System	200 - 250	Constant	1.2 - 1.5
Cage Culture		Constant	
FCR ranged from 1 - 1.7 using extruded commercial diets (mostly imported) in combination with Nigerian made feeds. The lower FCRs were recorded on farms that use imported feeds completely (RAS systems) or for longer period of time before using on-farm feeds or locally extruded pellets. This agrees with FCR records of: FCR = 0.94 ± 0.06 (Martins *et al.*, 2005); FCR = 1.02 – 1.09 (Baßmann *et al.*, 2017) FCR = 1.00 ± 0.086 (Marimuthu *et al.*, 2011) FCR = 1.59 ± 0.06 (Tunde *et al.*, 2016) FCR = 1.0 – 1.3 (Miller and Atanda, 2011)			

1.6.3.4. Market for Grow-out (Live)

In Nigeria, the bulk of the farmed catfish produced are sold live. They are often transported in mini-buses carrying 50 bowls (60 L each), with each bowl holding 25 kg of live catfish in 12 – 15 litres of water and 1 – 2 ml of palm oil to minimise foaming during transport. The bowls are covered with jute bags poly sacks fastened with elastic bands and layered in the minibus with planks between each layer **(Figure 1.9)**. Effectively, a minibus can transport 1,250 kg of fish from the South-West to the North-Central and other parts of the country where they are retailed. This system of transport has been mastered such that live harvested catfishes are transported over 800 km for up to 12 hours successfully. Over short distances e.g. between farms and local markets, 30 – 40 kg could be packed in on bowl, covered with sacks. Farm-gate prices for live catfish are relatively low compared to market prices, and are always based on live weight, with the bigger fishes fetching a higher price per kg than smaller ones. Many markets for live catfish have one thing in common – the fish are kept and sold in used metallic bathtubs, mostly white, enabling clear view of the fish and are rarely sold by weight, but rather by physical size. Most markets have unions who are mostly middlemen. Memberships are not easily accessible to farmers. They dictate the prices for the farmers and make more profit per kg of fish than the farmers who

have raised the catfish for 6 or more months, enduring the very high cost of feed. In some parts of the country e.g. in Southern Nigeria, these middlemen are mostly women (Babalola, *et al.* 2015), while in the Northern Nigeria, they are mostly men. A good initiative by the Ogun State's Department of Fisheries was to build a fish farmers' market, where farmers take turns (on appointed days of the week) to sell directly to consumers. As at early 2016, there was a 50% increase in price as consumers were happy to pay a price the middlemen couldn't.

Figure 1.9. Mode of transportation of live table fish from one location to another (in Nigeria)

1.6.3.5. Markets for Grow-out (Smoked)

Smoked farmed *C. gariepinus* is increasingly becoming popular. By volume, smoking is the most important means of value addition to fish in Nigeria and the second most important means of selling farmed *C. gariepinus*. Traditional smoking kilns and modified versions with low deposit of toxins (from burning wood) are currently in use **(Figure 1.10 and 1.11).** Properly smoked catfishes can last several months in storage and are exported from Nigeria to African communities in Europe (Miller and Atanda, 2011; Anatekhai 2013). Farmed catfishes ranging between 350 – 700 g (i.e. an average of 500 g) are the most preferred sizes by the fish smokers, marketers and even the farmers producing them. Respectively, these are due to the fact that the process of dehydration is more efficient and doesn't last as long as for a 1 kg fish, the size range offers a more attractive price (not too high for a poor man and not too low for a rich man) and the cost of production and FCR isn't as high as producing a 1 kg fish (which is the normal harvest size for fresh catfish). Numerous small-scale farmers, who now smoke their fish instead of selling live and fresh catfish, have attested to the profitability of this practice in the face of high cost of production (feed). The fact that farmed catfishes are not seasonal, and uniform or desired sizes can be maintained all year round **(Figure 1.10)** makes it a perfect option for fish smokers and marketers, who rely on farmed catfish in the absence of wild fish to supply their already established markets and networks. Farmed catfishes are smoked for 4 to 12 hours depending on the kiln and intended final shelf life. Most commonly, 1 kg of smoked fish is derived from 4 kg of live catfish (75% loss of fresh weight). It is important to study the effect of the different smoking techniques on moisture content, quality and shelf life of the finished product.

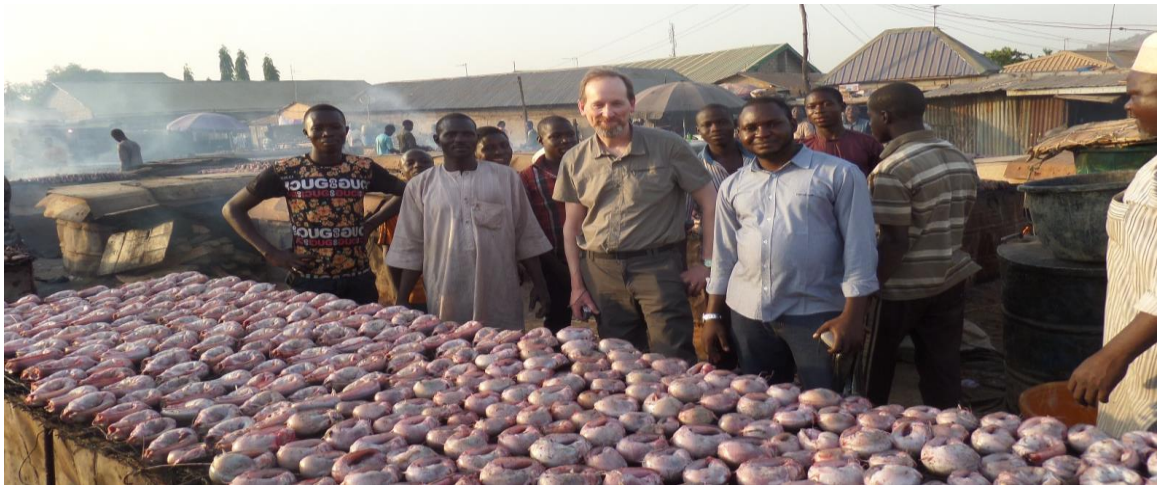

Figure 1.10. A fish smoking site at Kado Fish Market, Abuja, Nigeria

Figure 1.11. Process of curving, drying and smoking catfish on fish farms, and the bottom right shows how smoked fish are displayed for sale in markets in Nigeria (Kado Fish Market, Abuja)

1.6.4. Status of the Catfish Feed Industry

Presently, feed accounts for over 80% of the cost of *Clarias* catfish production. This is partly due to very high dietary protein requirement (45 – 55% at nursery stage and 35 – 40% at grow-out stage) of *Clarias* catfish, mainly sourced from the very expensive fishmeal and other high quality plant protein (soybean and groundnut cakes) making their feed very expensive (Anyila, 1988; Ali and Jauncey, 2005; Ondhoro *et al*., 2015).

Production of on-farm fish-feeds **(Figure 1.12)** by farmers has been successful to some extent (due to availability of ingredients locally), however, for the most part, challenging due to the high cost of ingredients outside the growing/harvest seasons, competition with human and other animals for some of the ingredients and poor physical quality of the pellets produced (low water stability, sinking and leeching of nutrients). Furthermore, due

to high humidity and inefficient methods of drying, the storage shelf life is of the feed is affected, thus forcing farmers to only produce little at a time.

Unlike the poultry and livestock feed, Nigerian aquaculture depends largely on the imported extruded fish feeds. Of the 3.8 million tons of animal feed produced by 620 animal feed manufacturers in Nigeria in the year 2000, fish feed accounted for only 1% by volume, which could only supply 30% of feed used in the aquaculture industry; the remaining >70% was imported from the Netherlands, Denmark, Israel, US, Thailand and South-Africa (Miller and Atanda, 2011; www.fishfeedmachinery.com, 2016; Udo and Dickson, 2017). This proportion had changed by 2015; 647,750 tons of catfish feed were produced out of a total of 5,300,000 tonnes of animal feed, making fish feed account for 12% of animal feed production and representing a growth rate of around 10% per annum between the years of 2000 - 2018 (Udo and Dickson, 2017). The imported catfish feeds are extruded pellets **(Figure 1.13)** of higher quality, offering better fish growth/yield compared to locally produced pressed pellets. Farmers prefer the extruded feeds because they float – hence, farmers can observe feeding and general activities of the fish, know when to stop feeding, thereby, minimising wastage and pollution from over feeding, aid in properly estimating quantities of feed to be fed, and lead to overall better management of the fish.

Furthermore, the imported feed offers better growth and FCR than most of the commercially available pressed pellets, hence, are more reliable. This could be as a result higher water stability (thus minimum leeching of nutrients) and very importantly better digestibility as a result of breakdown of long-chain carbohydrates into digestible simple ones during the extrusion process. The reduction in use of imported feed from 70% to in 2000 to less than 10% today despite its superior quality explains the current challenge facing the fish feed industry. Nigeria currently imports over 40,000 tonnes of fish feed amounting to over USD 60 – 75 million annually (Foramfera, 2018).

Figure 1.12. On-farm feed produced from a pellet press **Figure 1.13.** Commercial extruded Pellets

1.6.4.1. Market for Fish Feed

As described earlier, the Nigerian catfish aquaculture industry relied heavily on imported fish feed for more than a decade and a half. In 2014, the global drop in prices of crude oil led to the devaluation of the Naira (the local currency of Nigeria), consequently leading to a 220% rise in feed prices especially the imported ones (from USD 0.8 to 1.5 – 2.0 kg^{-1}). This made catfish feed to account for over 80% of the production cost, thus reducing profitability and sustainability of aquaculture, especially for the small-scale catfish farmers. The last two years however, has seen the rise in domestic production to ease the high cost of feed and farmers (especially the small scale ones are now able to have alternatives. This has led to a general drop in prices of feed, especially in 2018 – 2019, and some foreign feed companies are now withdrawing from the Nigerian market or concentrating on production of micro-diets (0.2 – 1.5 mm) and starter feeds (2 - 3 mm). **Table 1.3** shows the current prices of existing feed brands and companies behind them. It is very difficult to draw conclusions on the quality of the new feeds, and there is therefore a need for benchmarking of the various fish feeds in the market. However, the present rate of domestic fish feed production (647,750 tonnes/year) when compared to the annual cultured fish production (316,727 tonnes/year) signifies potentially high FCR values resulting from poor quality of feeds, management or genetics of the fish or an indication that not all catfish produced in Nigeria are captured in the estimates of total annual production. Admittedly, the growing tilapia industry could attract high tonnage of feeds with a higher FCR compared to the catfish feed.

Inputs such as fishmeal, premixes and other trace nutrients are normally imported into the country. As such, they are also affected by the global prices of crude and resultant devaluation. Other ingredients such as maize, soybean and peanuts are produced mostly in the northern parts of Nigeria. Hand feeding is still the most common form of feeding *C. gariepinus* in Nigeria. Demand feeders are not very popular in the industry. The feeding frequency starts from twelve times daily (at the fry stage) to as low as twice daily (adult stage).

Table 1.3. Current (2018) prices of existing feed brands and companies behind these

		Average Retail Price/kg (USD)		
Country of Manufacture	**Brand Name**	**Micro-Diets**	**2 - 3 mm**	**4 - 10 mm**
Netherlands	Coppens	2.78	2.22	1.94
Denmark	Aller-aqua	2.50	1.67	1.57
Israel	Multi Feed	-	-	-
Ghana	Raanan	-	-	-
Brazil/Multinationals	Aqualis	2.50	1.39	1.26
Multinationals in Nigeria	Skretting	2.50	1.81	1.30
Multinationals in Nigeria	Blue-Crown	-	1.15	1.02
	Aquaboom	-	-	0.83
Produced in Nigeria	Vital Feeds	-	1.11	1.02
Produced in Nigeria	Ideal Feeds	-	-	0.93
Produced in Nigeria	Top Feeds	-	1.11	1.04
Produced in Nigeria	Imperial Feeds	-	1.25	0.95

It is important to state here that there are also indigenous extruded fish-feed that are gaining acceptance by the day with increasing market presence **(Table 1.3)**. They are slightly cheaper than the imported ones and also offer reasonably better growth and FCR than the commercially available local press pellets. The very high costs of extruders and associated machines and high cost of running (due to lack of power) has slowed the growth of the aqua-feed industry in Nigeria. Government and non-governmental organisations such as WAAPP (ECOWAS), interventions through funding of purchase of such machines in some research institutes and Universities (e.g. NIOMR, NIFFR, NABDA, University of Calabar) is a good initiative, however, more needs to be done. Just as in the case of fingerlings production, the wide gap between demand and quantities produced has encouraged private investment in the aqua-feed industry, as more and more individuals purchase extruders (especially the cheaper ones available in China) for producing on-farm and commercial feed.

1.7. Genetic Improvement

Genetic improvement is the intentional change of trait(s) resulting from the transfer of genetic materials from generation to generation. There are many reasons for genetic improvement; one of the most common of which is to optimise performance in an organism, and this has been the case in different plants and animals. The oldest form of genetic improvement is selective breeding. It started happening in a "green-fingered" way since plants and animals were first domesticated. In the United Kingdom (UK), this started as far back as 2.5 centuries ago (in the 18th century) with the accurate records of performance of different animals by Sir Robert Bakewell (1725 – 1795) (Oldenbroek and van der Waaij, 2015). His aim was to enable objective selection and he was able to improve certain popular traits in animals e.g. developing the New Leicester breed of sheep, which had good fatty shoulders and good quality fleece, and the long-horned breed of cattle which used less feed and grew fast. He pioneered progeny testing as an evaluation tool for selection, "breeding the best to the best to get the best", without any knowledge of genetics. The establishment of the first herdbook in 1791 for horses that were winning races, was in response to the increasing need for accurate records of performance of important animals from generation to generation and this was followed by a herdbook for shorthorn cattle in 1822 (Oldenbroek and van der Waaij, 2015). Other countries followed suit and in 1876, the first international herdbook was established for the American Berkshire pigs, and animal breeding using yearbooks became a standard – thus paving the way for the development of different breeds of animals.

Today, herdbooks of different thoroughbred species and breeds of animal and plants registered in different associations, organisations and countries exist, and are largely based on generations of genetic improvement for different traits and from different forms of breeding programmes.

1.7.1. Aims of Genetic Improvement

Broadly, the aims of genetic improvement can be summarised as follows:

- To increase performance by way of improving growth rate, quality, yield, productive capacity of an individual, resistance to pests and diseases, and adaptation to adverse and/or varied culture or environmental conditions.

- To change or transform behaviour or appearance e.g. from wild to domesticated fish, from calm to aggressive dogs, from brown to white flowers.

There could be other aims of genetic improvements not mentioned here, some of which will be mentioned in the next section.

1.7.2. Tools for Genetic Improvement

1.7.2.1. Phenotyping

Phenotyping is the basic tool used in identification and selective breeding of plants and animals. Shape of seeds, colour of flowers, nature of fleece of sheep, colour of coat on dogs, weight of sheep, cattle, goats, pigs and broilers at slaughter, etc., have been used for genetic improvement in different plants and animals. These were all phenotypic based selection. In aquaculture, morphological markers are still very relevant in distinguishing some species from others. They include fins, teeth, body shape and size, presence or absence and location of certain features) and/or anatomy (such as gill rakers, arborescent organ, gas bladder) of the fish (Ezenwaji, 1982; Agnèse *et al.*, 1997; Laurene, 2015). Upon discovery of the influence of genotype and environment on phenotypes, selection shifted from only morphological markers to a combination of genotype and environment as well. Genotype by environment (GxE) selection became popular for adaptation and environmental based performance evaluation. Proper understanding of genotypes was enhanced by molecular biology and the discovery of molecular markers.

1.7.2.2. Genetic Markers

There has been a transition from the use of morphological markers which are visible to the eyes, to biochemical markers which are generally extractable proteins e.g. isozymes and storage proteins; to chromosomal markers e.g. G-banding; to more recent DNA/molecular markers e.g. microsatellite markers, single-nucleotide polymorphism (SNP) markers etc. (Raza *et al.*, 2016). Molecular markers are some of the most important tools for genetic improvement in the 20th and 21st century. In livestock, poultry and aquaculture, they have become indispensible. They are useful in identification of a wide range of genetic variations. The development of DNA-based genetic markers has revolutionised genetic studies, creating the possibility of studying evolutionary relationships among individuals,

observing and exploiting genetic variation in an organism's genome. Various applications of genetic markers are in areas of:

- Strain, population and species detection
- Detection of hybrids (inter and intra-specific hybrids)
- Detection of locations of origin of populations
- Parentage and kinship analysis in selective breeding early communal rearing
- Parentage contribution in mass spawning in selective breeding programme
- Sex determination, QTL mapping and estimation of effective breeding number, inbreeding and genetic variation.
- Genetic management of farmed stocks.

The advent of next-generation sequencing (NGS) altered the landscape of genomics and transcriptomics, discovery, validation and assessment of genetic markers in populations. Markers are ideally supposed to have recognisable phenotypes, be polymorphic and have low interactions between themselves. The limitation of DNA marker is that if markers are linked to a gene of interest, and are used in selection when the gene of interest is not known, inaccurate results due to recombination between the marker and gene of interest may occur (Lander *et al.*, 2004; Brem *et al.*, 2002). Although molecular markers are very important in genetic management of stocks, in some species, the correlation between molecular genetic variation in a population and genetic variation (heritability) for traits of interest are weak (Ponzoni and Nguyen, 2008).

1.7.2.2.1. Types of Genetic Markers

There are various types of molecular markers used for various kinds of genetic management/improvement. They are broadly categorised as type I and type II, based on their association with genes of known function or DNA sequence with anonymous function (O'Brien, 1991). Furthermore, when markers cause no change in phenotypic or metabolic traits, they are termed "neutral", and if they do, they are termed "functional" (Raza *et al.*, 2016). Selection and application of markers depends largely on appropriateness i.e. how informative they are (polymorphic), the cost per sample/run and user friendliness. The polymerase chain reaction (PCR) is the most common method used nowadays in detecting polymorphism in markers. The next section describes various types of genetic markers

used in aquaculture. **Figure 1.14** shows a chart of the different genetic markers and proportion of publications between 1983 and 2013.

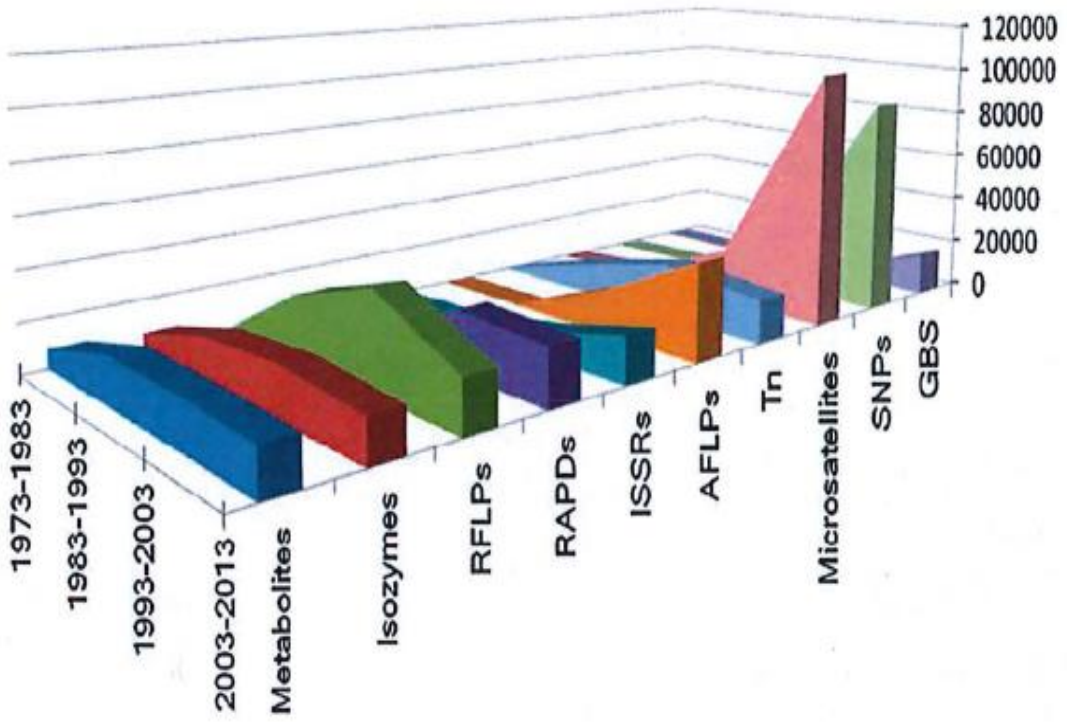

Figure 1.14. Number of publications on the use of different genetic markers (Grover and Sharma, 2016)

1.7.2.2.2. Isozymes

Also known as isoenzymes, these are multiple forms of enzymes, differing in amino acid sequence, but performing the same catabolic action. Isozymes aid in efficient metabolism aimed at meeting particular tissue or developmental need of an organism. They are either coded for by different alleles of same gene (allozymes) or by different genes making same enzyme or enzyme sub- units (isozymes) (El-alfy *et al.*, 2008). Isozymes are visualised using specific histochemical stains following separation in an electric field passing through a matrix such as polyacralymide, cellulose acetate or starch (Dunham, 2004).

Pros: they are inexpensive, fast, easy to use, can accommodate large sample sizes and their variation has been associated to growth performance, disease resistance, temperature tolerance, speed of development and salinity tolerance in fish (Park and Moran, 1994; Dunham, 2004). Isozymes have successfully been used in tracking inbreeding, stock identification, parentage assignment and QTL (linkage mapping) (Liu and Cordes, 2004).

Cons: Usually requires tissue from lethal samples, large tissue samples, and the limited number of such markers allows exploration of only small portion of the genome, hence, may not identify recently diverged species and may not capture variation at DNA sequence levels (Gheyas, 2006).

1.7.2.2.3. Mitochondrial DNA (mtDNA)

Mitochondrial DNA has been popular in phylogenetic studies in the 1980s due to its higher genetic variance relative to isozymes (Magoulas, 1998; Chauhan and Rajiv, 2010). Smaller effective population size and faster mutation rate as a result of lack of repair mechanism during replication is responsible for the observed sequence divergence in the mtDNA relative to nuclear DNA (Liu and Cordes, 2004).

Pros: Just like in the case of isozymes, mtDNA are fast, easy to use and inexpensive genetic tools. They are passed down unchanged from the mother to offspring, thus are useful in detecting ancestry in fish population (Nwafili and Gao, 2007 and Nwafili, 2013).

Cons: However, the relatively small coverage – in effect one locus compared to millions of potential loci in nuclear DNA studies, makes it less useful in the face of modern technology such as restriction site associated DNA sequencing (ddRADseq) that can generate thousands of SNP markers. Furthermore, in a situation of gender-based migration/introgression, only maternal based inheritance of the mtDNA might fail to capture and reflect true genetic structure and phylogeny (Birky *et al.*, 1989; Chow and Kishino, 1995).

1.7.2.2.4. Restriction Fragment Length Polymorphism (RFLP)

The use of restriction enzymes to create DNA fragments of different lengths (polymorphisms) due to a base substitution, resulting to deletion, insertion or sequence rearrangement at or between restriction sites is called RFLP (Vos *et al.*, 1995; Mueller and Wolfenbarger, 1999) . This is a popular technique for detecting markers e.g. SNPs and is useful in selecting multiple desired traits simultaneously.

Pros: RFLP shows co-dominant inheritance and data generated from this technique are easily of interpreted and scored (Agarwal *et al.*, 2008; Raza *et al.*, 2016).

Cons: Currently, this technique is not frequently used due to its low through-put and high time and labour requirement in the face of advances in next generation sequencing and other SNP discovery techniques with very high throughput, relatively shorter time and can accommodate large number of samples.

1.7.2.2.5. Restricted Amplified Polymorphic DNA (RAPD)

This is a PCR-based technique that uses short primers (8-10 bp) of random sequences to amplify unspecified regions of genomic DNA, usually at low annealing temperatures (36 – 40^{o}C). RAPDs are non-coding markers, with a relatively high polymorphism detection potential and have widely been used in population genetic studies, estimation of heteroses and phylogenetic studies (Liu and Cordes, 2004; Raza *et al.*, 2016).

Pros: This technique is easy to use, inexpensive and fast, as multiple products, each representing different loci can be amplified at once due to the short length of primers and low annealing temperature involved (Gheyas, 2006). RAPD requires no prior knowledge of the DNA of the target genome (Pandey *et al.*, 2018), and large number of markers can be generated from a small amount of DNA

Cons: It is no longer popular due to poor repeatability, reproducibility and shows dominant inheritance. It relies on a high quality DNA template, and as such does not work well on degraded DNA and has relatively low resolving power (Kumar and Gurusubramanian, 2011) compared to microsatellite markers.

1.7.2.2.6. Amplified Fragment Length Polymorphism (AFLP)

Yet another PCR-based technique, AFLP is similar to RFLP and RAPD in many respects except that known bases (adaptors) are added to the 3/ end of the PCR primer, thus, enabling selective PCR amplification (Vos *et al.*, 1995).

Pros: This technique offers highly polymorphic markers, is inexpensive, highly reproducible due to known parameter – annealing temperature and adapters and are useful in selecting multiple desired traits simultaneously (Raza *et al.*, 2016; Agarwal *et al.*, 2008).

Cons: It shows a dominant inheritance - sees one allele as a band, often cannot detect other alleles, making it hard to distinguish between homozygotes and heterozygotes, thus its no longer as popular (Raza *et al.*, 2016; Agarwal *et al.*, 2008).

1.7.2.2.7. Microsatellite

Microsatellite markers are often more variable than allozymes and PCR amplification can use very little and even degraded DNA. Both show differentiable loci with co-dominant alleles (Senanan *et al.*, 2004). They are also known as simple sequence repeats (SSR) and are tandem arrays of short nucleotide repeat (1 – 6 bp), interspaced throughout the genome (Tautz and Renz, 1984). Between 48 – 67% of microsatellites found in many species of animals are dinucleotides, while poly A/T mononucleotides are in the majority in primates (Wang *et al.*, 1994; Tóth, and Jurka, 2000). All except trinucleotides are more prevalent in the non-coding regions (Litt and Luty, 1989). Jarne and Lagoda, (1996) classified microsatellites into pure, compound and interrupted repeats, consisting of single sequence, contiguous arrays and intersperse arrays of motifs respectively.

Pros: Microsatellite markers have the highest polymorphic information content (PIC) value amongst all genetic markers, are dispersed and abundant in the genome (1/10,000 bp), obey Mendelian inheritance and can be rapidly assayed (Litt and Luty, 1989; Wright, 1993; Thodesen *et al.*, 2013; Wright and Bentsen, 1995; O'Connell and Wright, 1997; DeWoody and Avise, 2000; Lui and Cordes, 2004; Excoffier and Heckel, 2006). Despite the ease of setup using PCR amplification, and their high level of polymorphism (Excoffier and Heckel, 2006), there has been limited use of microsatellite markers in population genetic studies of African catfish, when compared to other fish species (Nguyen, 2008) and also in comparison with its use in identification of different species of *Clarias* (Ozouf-Costaz, 1990; Teugels, *et al.*, 1992; Agnese *et al.*, 1997, Nwafili *et al.*, 2013).

Cons: High cost of setup, time and labour requirements when compared to next generation sequencing, and the fact that there is presently limited advancement in microsatellite markers development technology, are the disadvantages of this genetic marker.

Though requiring very expensive set-up and run ($ 2-3/ microsatellite), the use of microsatellite markers in a breeding programme can be very helpful in the areas of identifying individuals, parentage assignment, reducing inbreeding, saving time, and increasing genetic gain (Ponzoni and Nguyen, 2008; Saad *et al.*, 2009; Ninh *et al.*, 2013).

The ability to combine and compare data between different laboratories/research groups and also with advanced techniques such as data from next generation sequencing techniques products i.e. SNPs makes it a relevant and reliable technology till date. This is possible through calibration of size standards between laboratories with a very minimal error rate (0.01 ± 0.002) between the laboratories and the number of alleles, their size ranges within a locus, repeats types, genotyping accuracy, etc., was not associated to the degree of error between laboratories (Ellis *et al.*, 2011). The high cost might be a limiting factor especially for medium to small-scale commercial farmers interested in a breeding programme.

1.7.2.2.8. Single Nucleotide Polymorphisms (SNPs)

The discovery of the extent of variation in single nucleotide polymorphisms (SNPs) from the sequencing of livestock genomes has revolutionised the world of molecular biology. It is relatively cheap, fast and very reliable when compared to microsatellite markers. Although the exact number of SNPs required for species identification, phylogenetic studies and parentage assignment in *Clarias gariepinus* is not yet know, next generation sequencing platforms are capable of finding and genotyping thousands of SNPs at once, and it is reckoned that 100 – 150 SNPs per individual should suffice for parentage assignment (Vandeputte and Haffray, 2014). RAD (Restriction site associated DNA) sequencing is a next generation sequencing technique that allows sequencing of massive numbers of short fragments of DNA which are next to specific restriction enzyme recognition sites **(Figure 1.15)**, from which SNPs are identified and genotyped (Braid *et al.*, 2008; Peterson *et al.* 2012). The first application of RADseq for genetic marker discovery was in the three-spine stickleback, which has its genome already sequenced

(Hohenlohe *et al.*, 2010). Polymorphisms resulting from point mutations at nucleotide position, where different alleles contain alternative bases, are called SNPs. SNPs have been characterized since the advent of DNA sequencing in 1977, but the ability to rapidly genotype large numbers was limiting until the deployment of gene chip technology and NGS platforms.

Pros: Their versatility and abundance makes them potentially very useful in different studies such as phylogenetics, parental assignment and family identification in breeding programmes, and QTL mapping. In theory, a SNP can produce up to four alleles in a locus, each containing either A, T, C or G at the SNP site, while practically, they are mostly bi-alleleic, having either of the two pyrimidines C/ T or the two purines A/G (Liu and Cordes, 2004). They have a lower PIC value than multi-allele microsatellites, but their abundance makes up for the shortcomings, e.g. usually for every one microsatellite, up to six to ten SNPs are required for parental assignment (Vandeputte and Haffray, 2014). SNP markers are inherited as co-dominant markers. Several methods of SNP genotyping exist and have evolved over time from heteroduplex analysis (Sorrentino *et al.*, 1992), SSCP analysis (Hecker *et al*., 1999), to direct DNA sequencing. Next generation sequencing is probably the most popular and accurate method for SNP discovery, as well as genotyping.

Cons: Unlike in microsatellite markers analysis, double digest RAD-Seq relies on good quality genomic DNA, which is digested using two restriction enzymes. However, other techniques for SNP analysis such as KASP, could work well on low quality DNA.

Due to advancements in science and technology, SNP genotyping is increasingly becoming affordable and accessible, with machines capable of genotyping 40 – 2,500 individuals in a run (depending on the type of machine and coverage required per sample) and advancement in bioinformatics, this high through-put technology is becoming more affordable while maintaining or improving on its very high accuracy. Although some applications of next generation sequencing (e.g. ddRADSeq) offer a low cost per sample, the high cost of the machines (454, Illumina Hiseq and Miseq, Iron Torrent Solid and Pacific Bioscience) with various capabilities limits the application of such technology to few laboratories.

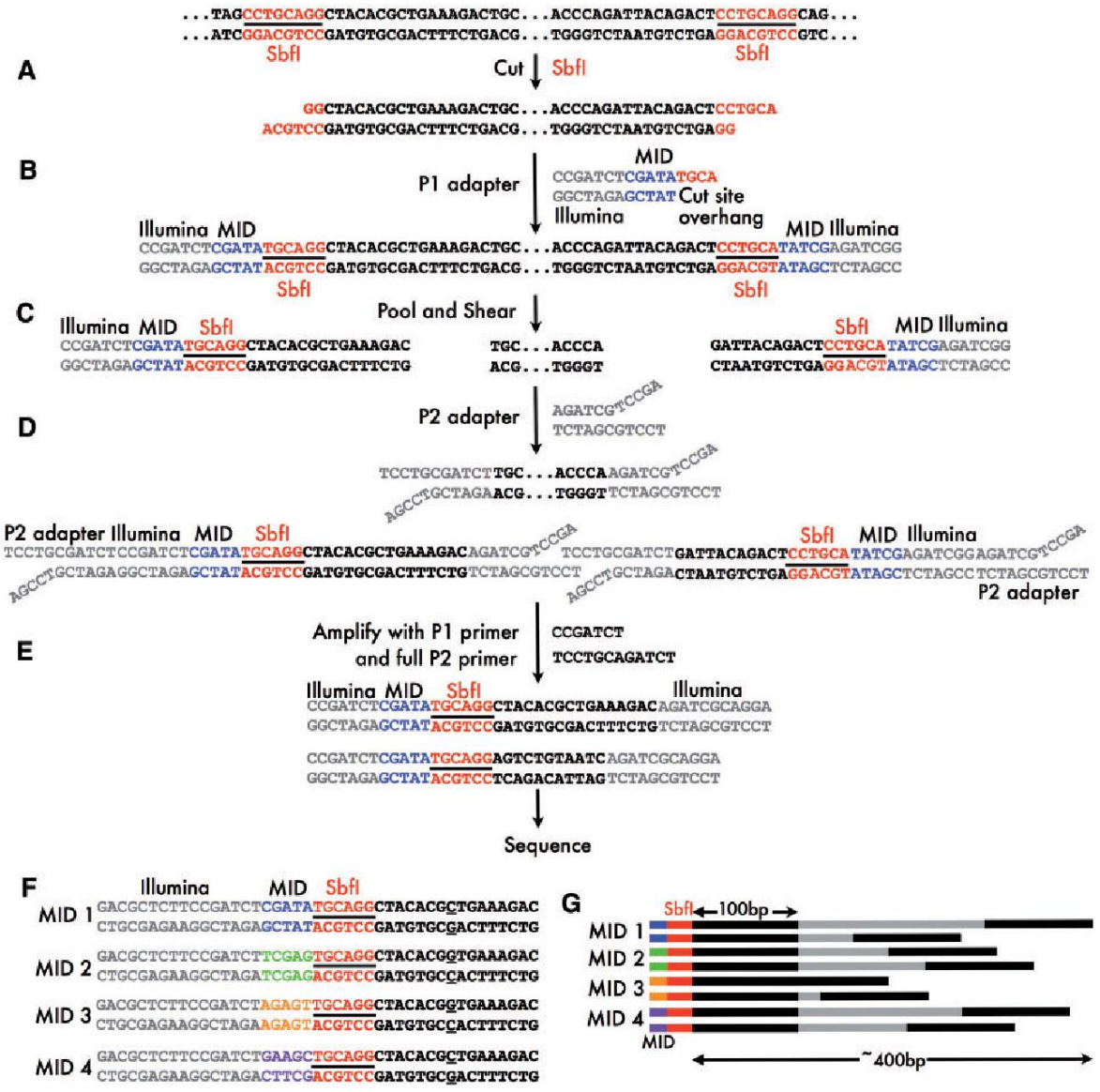

Figure 1.15. Illustration of the working principle of restriction site associated DNA (RAD) Sequencing (*Source:* (Davey and Blaxter, 2010) . SNP discovery and genotyping follows the production of stacks.

Table 1. 4 Types of DNA markers, their characteristics and potential application (**Source**: *Liu and Cordes, 2004*)

Marker Type	Acronym or alias	Requires Prior Molecular Information	Mode of Inheritance	Type	Locus Under Investigation	Likely Allele Number	Polymorphism or Power	Major Application
Allozymes		Yes	Mendelian, Codominant	Type I	Single	2 - 6	Low	Linkage mapping, population studies and maternal lineages
Mitochondrial DNA	mtDNA	No[a]	Maternal Inheritance	-	-	Multiple Haplotype	-	Maternal lineage
Restriction Fragment Length polymorphism	RFLP	Yes	Mendelian, codominant	Type I or type II	Single	2	Low	Linkage mapping
Random Amplified Polymorphic DNA	RAPD, AP-PCR	No	Mendelian, dominant	Type II	Multiple	2	Intermediate	Fingerprint for population studies, hybrid identification
Amplified Fragment Length Polymorphism	AFLP	No	Mendelian, codominant	Type II	Multiple	2	High	Linkage mapping, population studies
Microsatellites	SSR	Yes	Mendelian, codominant	Mostly Type II	Single	Multiple	High	Linkage mapping, population studies, paternity analysis
Expressed Sequence Tags	EST	Yes	Mendelian, codominant	Type I	Single	2	Low	Linkage mapping, physical mapping, comparative mapping
Single Nucleotide Polymorphism	SNP	Yes	Mendelian, codominant	Type I or type II	Single	2, but up to 4	High	Linkage mapping, population studies
Insertions/Deletions	Indels	Yes	Mendelian, codominant	Type I or type II	Single	2	Low	Linkage mapping

[a]Conserved PCR primers can be adopted from sequence information from a related species.

1.7.3. Genetic Improvement in Aquaculture

Over 800 million people across the world depend on fish for food, income and nutrition, amongst them are people from many developing food-insecure countries, where fish is often the cheapest and most accessible animal-source of protein (WorldFish, 2017b). In 2016, global fish production was 171 million tonnes, out of which aquaculture accounted for 80 million tonnes, and 88% of the total production was utilised for direct human consumption at an average of 20.3 kg per capita (FAO, 2018c). Many African countries fall below this average, as consumption levels are much lower, hence, demands are exceedingly high in the face low production due to several challenges including dwindling of wild stock. While global annual capture fisheries dwindled between 92.7 – 89.5 tonnes between 2011 and 2016, global annual aquaculture production grew steadily from 61.0 – 80.0 million metric tonnes within the same period (FAO, 2018c). This make aquaculture a more sustainable means of meeting the human fish protein needs, especially in the face of growing human population.

The sustainability of aquaculture however, is hinged on several factors, amongst which is the need for proper genetic management and improvement of aquaculture species to ensure better yield, survival and adaptation to: culture systems, high stocking densities and increased plant based diets, etc. As at 2012, it was estimated that only 10% of aquaculture production worldwide is based on genetically improved stocks (Gjedrem *et al.*, 2012; Thodesen *et al.*, 2013). This might be largely attributed to the difficulty and high cost of obtaining pedigree information (Vandeputte and Haffray, 2014). The fact that most fish species are only slightly domesticated portends a potential for very large genetic gain. Some of the current methods of genetic improvement for livestock and aquaculture are said to have been developed centuries ago (choice of breeds, cross-breeding and selective breeding), while the other methods (such as sex reversal, determination and sterilization, and triploidization) now deployed for improvement in aquatic organisms were developed relatively recently (Scott *et al.*, 1989; Kocher *et al.*, 1998; Kwon *et al.*, 2002; Lee and Penman, 2003; Penman and Piferrer, 2008; Cowan *et al.*, 2011) . Accurate estimation of breeding values of broodstock is key to a successful breeding programme (Bentsen and Gjerde, 1994). Absence of or improper genetic management over time is likely to reduce the performance of farmed stock. Reduced performance in farmed stocks could be attributed to inbreeding depression due to reduced genetic variation (reduced heterozygosity/increased homozygosity), thus reducing growth rate, survival, fecundity,

etc., especially in small closed populations, which are also prone to genetic drift. Genetic drift is the random change in allele frequency as a result of an error in sampling of gametes in a small population (BOD, 2018; Gheyas, 2006). Reduced variation in fish species could also be attributed to unintended selection resulting from poor hatchery practices and broodstock replacement. Due to improved aquaculture management practices (in some species), no external broodstock replacement may be involved in breeding programmes, at least for certain traits. An example is the Norwegian Atlantic salmon-breeding programme, which started in the early 1970's, using founder stocks solely from the Norwegian rivers, and up to 1997, introduced no extrinsic genetic material (Gjøen, 1997).

1.7.3.2. Selective Breeding

"Animal breeding involves the selective breeding of domestic animals with the intention to improve desirable (and heritable) qualities in the next generation (Oldenbroek and van der Waaij, 2015)*"*. Selective breeding is one of the oldest and most natural forms of genetic improvement employed by farmers, plant and animal scientists (Tave and FAO, 1995; Tave and FAO, 1999; Quinn *et al.*, 2012; Tillotson and Quinn, 2018). Selecting the best, and mating them to the best to get the best, has often been the norm. It seeks to improve the animals; changing the population average (genetic abilities) of important traits from one generation to another. The choice of traits for genetic improvement largely depends on the consumers (preference), the farmers (yield – growth and survival) and the feasibility of techniques/methods involved.

1.7.3.3. Steps Involved in a Selective Breeding Programme

A breeding program will be presented here as a circular activity. In each generation, the programme starts with formulating the breeding goal and ends with a critical review of the results obtained in the next generation. The evaluation might lead to a reconsideration of the breeding goal for the next round of selection.

1. Define the breeding goal
2. Record information on the genetic relatedness and performance of the animals
3. Identify animals with the best genetic potential
4. Select animals to be used in achieving genetic gains in the next generation
5. Mate the selected animals

6. Evaluate their offspring to see if the goals are met. And the cycle continues with some adjustments, modifications or in a similar manner as above, from one generation to another.

Genetic gains recorded from one generation to another cumulatively account for the overall improvement. As such it is important to maintain the same breeding goal for some generations before changing it (when satisfactory gain has been recorded) (Oldenbroek and van der Waaij, 2015). Nowadays, breeding of high valued fish species like Atlantic salmon is in the hands of multinational companies like Marine Harvest, which invest a lot of money in state of the art breeding programs. **Figure 1.16** is a diagrammatic representation of a breeding program as a circular activity, starting with formulating the breeding goal and ends with a critical review of the results obtained in the next generation.

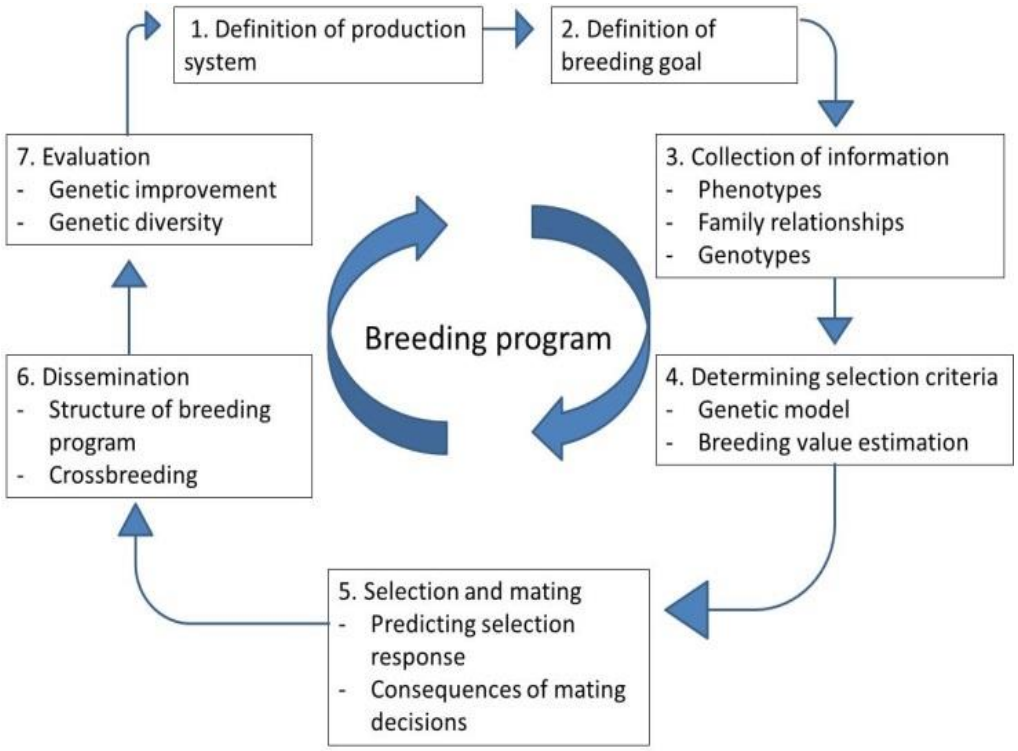

Figure 1. 16. Diagrammatic representation of a breeding program (*Source*: Oldenbroek and van der Waaij, 2015)

Largely influenced by research and developments in population, quantitative and molecular genetics, selective breeding could result to unexpected negative effects such as inbreeding depression. While inbreeding remains a very effective tool for genetic

improvement due to its role in developing particular breeds or strains, developing better broodstock, improving response to selection even under low heritability conditions and in cross-bred animals, etc., when not properly studied and managed, it could lead to inbreeding depression (Tave, 1993; Tave and FAO, 1995; Tave and FAO, 1999). Inbreeding depression is the loss of heterozygosity in the loci of chromosomes, resulting to poor survival, growth, viability, disease resistance, fecundity, etc., as a result of mating between relatives. Most often, this occurs in un-pedigreed populations and genetic improvement within such populations is difficult under such conditions.

There are 5 very important aspects that should be considered in animal breeding:

1. Traits under selection should be heritable.
2. Animals under selection should be genetically variable.
3. Select which animals to produce the next generation.
4. Assess the population average and phenotype from one generation to the next.
5. Assess overall success of the breeding programme by measuring the cumulative result of multiple generations of selection and then make breeding decisions with the future in mind.

Genetic improvement programmes in Atlantic salmon, Nile tilapia, common carp and channel catfish are examples of some successful breeding programmes in aquaculture. For the sake of illustration, genetic improvement in one temperate marine and one tropical freshwater species are briefly reviewed below: the Atlantic salmon (*Salmo salar*) and the Nile tilapia (*Oreochromis niloticus*).

1.7.4. Genetic Improvement in Atlantic Salmon (*Salmo salar*)

Norway is the largest producer of Atlantic salmon, with a current annual production of 1,236,354 tonnes (FAO, 2018b; Baklien and Steinset, 2018). The Norwegian Atlantic Salmon breeding programme, which started in the early 1970's, using founder stocks solely from the Norwegian rivers, has up till 1997 introduced no extrinsic genes (Gjoen, 1997). It maintained four sub-populations (separate lines), which showed over 90% of additive genetic variation for a trait within river (population) rather than between rivers, hence, crosses between rivers are established when there is reduced additive genetic variation resulting from random genetic drift (Gjoen, 1997). This practice is aimed at

reducing inbreeding depression and re-establishing genetic variation in the population, and as such, they have been able to maintain 10% genetic gain per generation for all the seven traits (body weight at slaughter, age of sexual maturation, survival in challenge tests with furunculosis and ISA, flesh colour, total fat content, and amount of fat tissues) in their selection programme. Early sexual maturation decreased harvest weight, as such selection for delayed maturation was employed – thus favouring the selection of female Atlantic salmon for culture. These traits were not all aimed at from the start of the selection programme, rather, were introduced in different years except for body composition (flesh colour, total fat content, and amount of fat tissues) as shown in **Table 1.5**. In a separate study in Canada, Quinton (2005) observed a favourable indirect selection response for higher colour score and pigmentation in Atlantic salmon directly selected for body weight. Thodeston *et al.* (1990) reported 1.3 – 4.6% selection response to FCR, while Gjedrem (2005) and Gjedrem *et al.* (2012) reported about 3 – 8% selection response to sexual maturation.

Table 1.5. Breeding goal in the Norwegian breeding programme. *(Source: Gjoen, 1997)*

Year	Trait
1975	Growth (G)
1981	G + Age of sexual maturation (SM)
1993	G + SM +Disease resistance (DR)
1994	G + SM + DR + Flesh Colour (FC)
1995	G + SM + DR + C Body Composition

Selective breeding for all the above listed traits **(Table 1.5)** and now egg quality has dominated the breeding programme for Atlantic salmon in recent years. Commercial companies have evolved and specialised in production of varied qualities of fertilised salmon eggs for hatcheries and nurseries, selecting stock based on issues not only limited to commercial value, but also industry, environmental and societal needs (Fletcher, 2018). Mowi and Aquagen in in Norway are examples of breeding companies that select and adapt salmon for different market needs, and breeding of disease resistant salmon to reduce the use of chemicals in disease and parasites (e.g. sea lice) treatments, and now selection for feed utilisation (adaptation to high plant protein and efficient digestion), reducing pressure on fishmeal and fish oils, and to reduce waste production in the aquatic environment. Infectious pancreatic necrosis (IPN), pancreatic disease (PD) and amoebic gill disease (AGD) are examples of diseases in which salmon is elected for. Aquagen

measures 22 traits in Atlantic salmon breeding programme (Aquagen, 2019). The economic benefit of salmon breeding programme (FCR and disease resistance) was assessed and found have led to increased profit of €275 and €291/ton/generation of family and genomic selections respectively (Fletcher, 2018).

1.7.5. Genetic Improvement in the Nile Tilapa (*Oreochromis niloticus*)

China is the largest producer of tilapia, with an annual production of 1.78 million tonnes per annum FAO 2018. By volume, it is the second largest farmed fish in the globe after carp and the Nile tilapia (*Oreochromis niloticus*) is the most popular species of tilapia due to is higher fecundity and faster growth rates. As the name implies it originated from the Nile and found naturally in most parts of Africa. Of the different forms of genetic improvement techniques (transgenesis, selection, cross breeding, hybridisation, sex control and chromosome manipulation), selective breeding enables a permanently increasing performance as traits are continuously passed from one generation to another (Ponzoni *et al.*, 2011). Several breeding programmes have been established for this species, mostly for intensive culture in Asia, with Africa (mainly Egypt and Ghana) producing tilapia on a very large scale only in the last 2 decades. Examples of the popular breeding programmes include GIFT- genetically improved farmed tilapia by the WorldFish (Eknath *et al.*, 1993; Eknath and Acosta, 1998), GET-EXCEL by the Government of Philippines and Norway (Tayamen, 2004), and GST by Genomar (GenoMar Supreme Tilapia, Zimmerman and Natividad, 2004).

The most popular genetic improvement for tilapia is selective breeding programme for Nile tilapia (from African and Asian origin) by the WorldFish, which gave rise to the GIFT strain and its multiple derivatives in the recent years. Spanning 10 years, the development of the GIFT strain was a collaborative project by The WorldFish Centre, the Institute for Aquaculture Research, Norway (AKVAFORSK), Bureau of Fisheries and Aquatic Resources and Freshwater Aquaculture Centre (BFAR) of Central Luzon State University, Philippines in from 1988 to 1997, and funded by the United Nations Development Programme (UNDP) and managed by WorldFish (then ICLARM – International Centre for Living Aquatic Resources Management) (Tayamen, 2004). The 8th generation GIFT provided to different collaborators was developed by crossing the best performing group from 8 genetically diverse strains of tilapia, the 13th generation from

within family selection, the Egyptian strain originating from eight location in Egypt, while the Kenyan strain is a progeny of the GIFT (Tayamen, 2004). The main traits of interest were growth rate and body traits (length, depth and weight). Following the dissemination of the GIFT strains after completion of phase 1 of the genetic improvement, and adoption by farmers in different countries, studies revealed that the GIFT strain performed better in terms of growth than other strains of farmed tilapia when compared in farm trials as shown in **Table 1.6.**

Table 1.6. Examples of On-farm Trials in which GIFT Had Significantly Higher Average Harvest Weights and Survival than Locally Farmed Tilapia Strains

Country	Farm Type	Increased Harvest Weight (%)	Increased Survival (%)
Bangladesh	Pond	+57.9a	
People's Republic of China	Pond/Cage	+17.5a	+3.3c
Philippines	Pond	+34.2b	+13.9c
Thailand	Pond	+32.3c	
Viet Nam	Pond	+32.3c	

Source: (AsianDevelopmentBank, 2006)

About 10 – 15% improvement per generation for more than six generations were recorded, without and companying undesirable correlated response in a study examining genetic improvement in Nile tilapia (Ponzoni *et al.*, 2008; Ponzoni *et al.*, 2011). In same study, evidence of genotype by environment interaction in markedly dissimilar environment were noted, however, the need for more than one selection programme to address that was viewed as less important since no universal guideline could be prescribed as at that time. Furthermore, there were no correlations between sex and body shape, thus, asserting limitations in selecting for sexual dimorphism and body shape. Another studies on 10 generation of two lines of the GIFT strain produced from 2002 – 2011 in Malaysia, revealed a high genetic variation, and a heritability value of 0.24 ± 0.031, despite recording an 11.9% improvement per generation (Hamzah *et al.*, 2014). Further studies on the indirect effect of genetic and non-genetic selection on harvest weight in the GIFT strains showed a suppression in the growth rates of social mates of tilapia by those with better genes in a competitive environment, thus suggesting the significance of underscoring social interaction and how it is affected by or it affects traits interest (Khaw *et al.*, 2016). Funded by the European Union (EU), continuation of genetic improvement in the GIFT strain by the WorldFish in 2016 produced the 15th generation, which has been

disseminated to Myanmar. A 5.7% improvement in growth rate above the 14th generation was estimated, and a 5 - 10% increase in growth rate per generation of the new GIFT strain has been observed (Khaw, 2016).

Selection for harvest weight in ProGIFT Nile tilapia (a derivative of the GIFT strain) in China showed a 60 -90% increase in weight over 6 generations (Thodesen *et al.*, 2013). The GET EXCEL tilapia project aimed at replacing the local strains of tilapia with genetically improved farmed tilapia in The Philippines (Tayamen, 2004). The Genomar Superior Tilapia (GST) is also a derivative of the GIFT strain (G10 – tenth generation), and as at 2013, it was already in the 22nd generation of selection, with harvest weight, stress tolerance and fillet yield as the traits of interest in the GST (Jamtøy, 2013). While the overall objective of developing the GIFT strain was to improve yield and thus better livelihood and income for the small scale and mostly rural farmers, the GST is an example of a very large-scale successful fingerling producer with stocks derived from the GIFT strain.

Unlike in the Atlantic salmon, most of the genetic improvement focussed more on yield and other body traits, and only recently are traits such a diseases resistance being considered. This could be as a result of the fact that tilapia is considered as hardy fishes, and the production systems have been predominantly semi-intensive – in fertilised earthen ponds. The advent of the tilapia lake virus (TiLV), described as a novel virus of the family *Orthomyxoviridae*, found in both fresh and brackish water (Eyngor *et al.*, 2014) and directly horizontally transmitted, causing severe health problems to the tilapia and economic losses to farmers has further necessitated the need for selection for disease resistance in tilapia.

Other forms of genetic management and improvement in tilapia such as the development of the YY- all male and YY sex reversed female tilapia to address the issue of stunted growth in mixed sex and avoid use of hormonal treatment for sex-reversal has been researched (Scott *et al.*, 1989; Kocher *et al.*, 1998; Kwon *et al.*, 2002; Lee and Penman, 2003; Penman and Piferrer, 2008; Alcántar-Vázquez *et al.*, 2014; Chen *et al.*, 2019). The DFID Fish Genetic Research Programme (FGRP), managed by University of Swansea, Whales, initiated the development of the YY super male for aquaculture purposes and this continued under the DIFID's Aquaculture and Fish Genetics Research Programme

(AFGRP) and managed by the Institute of Aquaculture, University of Stirling, Scotland, UK (Brink *et al.*, 2002). Fishgen, a UK company based in Swansea and Til-Aqua in the Netherlands is an example of a commercial companies producing the YY-male tilapia and sex reversed females (Hartley-Alcocer and Bink, 2001).

1.7.6. Status of Genetic Management and Breeding Programme for *C. gariepinus*

1.7.6.1. Identification

Proper identification and characterisation of species is important for conservation and genetic management issues (Hartvig *et al.*, 2015). The very close resemblance between different species of *Clarias*, the differences in identification keys and/or definition of keys used in identification of these species and the high intraspecific (individual) variation, have made proper identification often difficult (Ezenwaji, 1982; Hanssens, 2009), especially in the stocks used for aquaculture in Africa – *C. gariepinus* and its closest relative *C. anguillaris*.

Over the years, authors came up with different meristic indices for identifying *Clarias* species, notable among them were: length of and number of rays on its dorsal fins, type and nature of vomerine teeth, presence or absence of pigment bands on either side of the lower head, shape and size of the head, number of rakers on the first gill arch and in some cases colour of the eggs (Debouche *et al.*, 1979; Teugels and centrale, 1982; Teugels, 1986; Agnèse *et al.*, 1997; Rognon *et al.*, 1998; Yisa and Olufeagba, 2005; Hanssens, 2009; Wiecaszek and Antoszek, 2010; Compaoré *et al.*, 2015; Zakariah *et al.*, 2016)

The application of molecular/genetic markers in fish systematics has been seen to provide an opportunity to refine *Clarias* catfish classification and identification (Na-Nakorn, 2002; Na-Nakorn *et al.*, 2002). Molecular techniques for identification of *C. gariepinus* have been investigated using allozymes, microsatellites and mitochondrial DNA (Ozouf-Costaz *et al.*, 1990; Teugels *et al.*, 1992a; Agnese *et al.*, 1997). The same conclusions were reached based on two of the three aforementioned techniques i.e. allozymes and microsatellite markers. Both techniques although differentiated *C. gariepinus* from *C. anguillaris*, and agreed to the use of number of gill rakers on the first branchial arch as a valid meristic index for identification (Agnese *et al.*, 1997), they were all non-diagnostic.

Table 1. 7. Documented Keys and Indices for Identifying *C. gariepinus* and *C. anguillaris*, and genetic techniques used in distinguishing both techniques

	Clarias gariepinus	***Clarias anguillaris***	
Morphometric and Meristic Techniques	**Index**		**References**
Premaxillary (% of head length)	22.3		(Agnèse *et al.* (1997))
Vomerine tooth plate (% of head length)	21.5		(Agnèse *et al.* (1997))
Vomerine teeth	Slightly interrupted (with gap)	No gap	(Reed *et al.*, 1967; Yisa and Olufeagba, 2005)
No. of gill rakers on the 1st branchial arch	24 - 110	16 - 50	(Teugels and Centrale, 1982; Teugels, 1986; Agnèse *et al.*, 1997; Rognon *et al.*, 1998; Compaoré *et al.*, 2015)
Standard length/body depth	6 – 8 times		(Teugels, 1986; Agnèse *et al.*, 1997)
Pigmented band on sides of the head	Present	Absent	(Teugels and Centrale, 1982; Agnèse *et al.*, 1997; Rognon *et al.*, 1998)
Correlation of gill rakers to standard length	High ($0.122 \leq b \leq 0.267$)	Low ($0.013 \leq b \leq 0.060$)	(de Vos (1986) and Compaoré *et al.* (2015)
Head length (% of standard length)	26.6 – 25.0	21.0 – 26.10	(Agnèse *et al.* (1997))
Egg colour	Green	Brown	Used in Nigeria
Longitudinal line on the cleithrum	Present	Absent	(Benech *et al.* (1992))
Genetic Techniques	**Diagnosis**		
Chromosome Number	2n = 56	2n = 56	(Richter *et al.*, 1987; Ozouf-Costaz *et al.*, 1990; Teugels *et al.*, 1992b; Okonkwo and Obiakor, 2010) vs (Eyo (2005) and Teugels *et al.* (1992b))
Chromosome Number	2n = 54	2n = 54	(Maneechot *et al.*, 2016) vs (Aluko, (1998))
Allozymes	Private alleles non diagnostic loci	Private alleles non diagnostic loci	(Agnèse *et al.* (1997)). *Outcome suggested use of gill rakers as a correct morphometric index for identification*
Allozymes, Microsatellite markers ant mtDNA	Private alleles non diagnostic loci	Private alleles non diagnostic loci	(Ozouf-Costaz *et al.*, 1990; Teugels *et al.*, 1992a; Agnese *et al.*, 1997; Wachirachaikarn *et al.* 2009). *Outcome suggested use of gill rakers as a correct morphometric index for identification*
Mitochondrial DNA	Non-diagnostic mtDNA lineage	Non-diagnostic mtDNA lineage	(Ozouf-Costaz *et al.*, 1990; Teugels *et al.*, 1992a; Agnese *et al.*, 1997)
Cytochrome b	Three mtDNA lineages (non-diagnostic)	Suggested Lineage (non-diagnostic)	(Nwafili and Gao, 2007; Nwafili, 2013)

Another study on the genetic structure of the Dutch domesticated *C. gariepinus* farmed in Nigeria involving a comparison of the mtDNA (cytochrome b) of the exotic (Central Africa and Israeli strain), Dutch-domesticated and indigenous population, revealed high genetic distance/differences between the populations (Nwafili, 2013). In this study, the Dutch-domesticated populations were genetically differentiated from the indigenous ones and were themselves composed of two phylogenetically distinct populations, having at least three mtDNA lineages. The use of cytochrome b in his study enabled the establishment of genetic structure and differences between the cultured populations of *Clarias spp*. in Nigeria, suggesting possibly that the exotic spp is composed of three mtDNA lineages, presumably one of which was though to come from a *C. anguillaris*. The limitation of mtDNA in that it is only maternally inherited and is limited to only a locus as compared to SNPs makes it undependable and requires further research.

1.7.6.2. Genetic Improvement

Less than 10% of the world's aquaculture production comes from genetically improved stock (Gjedrem, 2012; Gjedrem *et al.*, 2012). There are shortages of specific information regarding the different populations of *C. gariepinus* within and between different countries in the continent (Nguyen, 2008). *C. gariepinus* exhibits different levels of phenotypic variations between strains, within strains, within populations and even within a batch or spawning set. The observed variations are either qualitative (such as skin colour, presence or absence of pigments, type and nature of vomerine teeth, colour of the eggs etc.), which may be linked to both genetic and/or ecological factors such as geographical location (Teugels *et al.*, 1998; Hanssens, 2009**),** or quantitative phenotypic variation (such as growth rate, disease resistance, FCR, etc.), which might be influenced by the level of management. The interest of most commercial food fish hatcheries is however, to exploit the quantitative phenotypic variation, with fast growth being the first to come in mind as mentioned earlier. Till date, no documentary evidence of the existence of any successful or on-going selective breeding programme for African catfish.

1.7.6.3. Dutch Domesticated *C. gariepinus*

The Dutch domesticated strain of *C. gariepinus* now present in Nigeria is said to have originated from several countries. Studies show that they were collected from Cameroun,

Central African Republic, Cote d'Ivoire, and Israel and domesticated in Wageningen University, The Netherlands in partnership with FAO (Richter *et al.*, 1987; Holcik, 1991; Miller and Atanda, 2011; Nwafili, 2013). The introduction of this domesticated strain of *C. gariepinus* by some Dutch consultants for intensive culture in concrete tanks and earthen ponds with accompanying influx of imported extruded catfish feeds marked a significant point in the history of the Nigerian aquaculture industry. Their impressive growth rate and harvest size encouraged farmers to abandon the native strains already undergoing domestication. It is important to mention that the Dutch-domesticated strain of *Clarias gariepinus* has been reported to have gone through several generations of selection for adaptation and it is possible that other desirable traits such as growth performance and feed utilisation would have unintentionally been selected for, as they have been known to have better growth performance and adaptation to tank conditions than the wild or domesticated wild population in Nigeria, weighing 1.2 – 1.9 times heavier in the works of Megbowon *et al.*, (2014). Today, they (the Dutch-domesticated strain of *C. gariepinus)* and their hybrids with *Heterobranchus spp*.**,** constitute the bulk of catfish production in Nigeria.

Studies on the genetics of catfishes commenced about 41 years ago as evident in studies on variation, hereditary, evaluation of strains' suitability for aquaculture, cytogenetics, protein variation and DNA nucleotide variation etc. (Volckaert and Agnèse, 1995). However, it is evident that a lot more needs to be done to support development of commercial production of African catfish. In Nigeria, attempts have been made to produce triploid catfish (specifically of *Heterobranchus longifilis* (Vandu) and *Clarias anguillaris* hybrids) using thermal induction (Aluko, 2000; Olufeagba *et al.*, 2000a; Olufeagba *et al.*, 2000b) and inter-specific hybrids of *Clarias spp* and *Heterobranchus spp*. While the former stopped only at laboratory levels, the latter was adopted commercially and different forms of inter-specific hybrids are now produced in Nigeria, and in some parts of Holland, where they are called *Claresse.*

1.7.6.4. Inter-Generic and Inter-Specific Hybrids

Inter-generic hybrids in *Clariidae* are products of crosses between members of the genus *Clarias* and *Heterobranchus*. The *Clarias spp* are mostly either *C. gariepinus* or *C. anguillaris*. The *Heterobranchus spp.* is either *H. longifilis* or *H. bidorsalis* **(Figure 1.17)**. The *Heterobranchus spp* are also members of the *Clariidae* family (Teugels, 2003) and are

found in different water bodies in Nigeria. Characterised by possessing adipose fins, the length of this adipose fin and skin pigmentation are the main distinguishing features. In *H. longifilis*, the length of adipose fin is equidistant to its dorsal fin, while in *H. bidorsalis*, the adipose fin is half the length of its dorsal fin **(Figure 1.17)**. *H. longifilis* are dark grey to black dorsally, with a very black spot at the posterior end of the adipose fin, while *H. bidorsalis* are reddish-brown dorsally and have no black spot on their adipose fins.

The third known member of this genus in Nigeria is *Heterobranchus isopterus,* which looks exactly like *H. longifilis*, except that it does not have a black spot. They are available in Nigeria, however, are not used in aquaculture due to their relatively poor growth rate (Olufeagba, 2006 pers. com.). It is worth mentioning that hybrids from *H. longifilis* are more common because both male and female *H. longifilis* attain sexual maturity within 1 year, making their broodstock more available than *H. bidorsalis*, in which the males mature after one year and the female after 2 years. Furthermore, *H. bidorsalis* are seasonal spawners (from May-August), with potential of growing out of usable proportion within a short time, especially if nutrition is not monitored and controlled.

Despite the small volume relative to pure *C. gariepinus*, success recorded in the adoption of inter-specific hybridisation in Nigeria is much so that is almost impossible to talk about the Nigerian catfish industry without mentioning these hybrids – *Heteroclarias* and *Clariobranchus*. The prefix in each name represents the species of the maternal parent. They grow relatively bigger at the grow-out stage (particularly in earthen ponds), have broader heads and are physically larger than same weight of *Clarias*. The sterility of hybrid is questionable as many field attempts have reported very poor to no viability. Legendre *et al.* (1992) observed considerably lower GSI and fecundity in female FI hybrids of *C. gariepinus* and *H. longifilis,* while the males had higher GSI, but 100 time lower spermatozoa concentrations and numerous abnormalities in their gonads. In the same study very few viable F2 offspring were produced, suggesting much lesser risk compared to completely non-sterile hybrids. The hybrids have higher dressing yield, making them relatively more expensive than *Clarias* both at fingerlings and grow-out stages. Legendre *et al.* (1992) reported better growth performance in the *H. longifilis* and its reciprocal hybrids with *C. gariepinus,* than the pure *C. gariepinus* stock. In the same study, the gonads of both reciprocal hybrids showed abnormalities under macroscopical and microscopical observation. A similar growth pattern was shown in another hybrid

involving *C. gariepinus* and *H. bidorsalis* (Owodeinde *et al.*, 2010). Due to the relatively higher hardiness and distinct feature (small adipose fin) in hybrids, on-growers find them more reliable than the pure *Clarias* in the face of decreasing and unpredictable growth and survival rates in *Clarias*. Despite all the above qualities, the production of these hybrids is still less than that of the pure *C. gariepinus* because *C. gariepinus* broodstock are relatively more abundant, they mature earlier and their females are more fecund. Their hatchery phase is relatively easier to manage because they are less aggressive than either the hybrids or the pure *Heterobranchus*, hence, relatively lesser cannibalism and more uniform growth is recorded. They respond to feeding better and grow faster than the pure *H. bidorsalis* and their hybrids at the hatchery phase and sometimes in indoor concrete and plastic tanks.

The hybrids are identified as having a shorter adipose fin (approximately $1/3^{rd}$ - $1/5^{th}$ of the length of the dorsal fin **(Figure 1.18)**). The colour of each hybrid tends to depend on the type of *Heterobranchus* parent used in the mating; hybrids from *H. longifilis* are dark grey to black dorsally, while those of *H. bidorsalis* are reddish-brown. In both cases, the head size is bigger than those of the *Clarias* parent, and a little smaller than the *Heterobranchus* parent.

Figure 1. 17. From top to bottom are *C. gariepinus (*with no adipose fin*), H. longifilis* (with an adipose as long as its dorsal fin) and *H. Bidorsalis* (with a slightly shorter adipose fin 1/2 of its dorsal fin)

Figure 1.18. Inter-generic hybrid between *C. gariepinus* and *H. longifilis* showing a very short adipose fin ($1/3^{rd}$ – $1/5^{th}$ the length of its dorsal fin). *Source:* (Roosendaal, 1995)

1.8. Current Gaps in *C. gariepinus* Industry

1.8.1. Research Gaps in Fingerlings and Broodstock Production and Management

C. gariepinus broodstock origin and quality has been an issue discussed at different fora in Nigeria and beyond (Ponzoni and Nguyen, 2008), however, detailed study on quality and improvement programmes is still lacking in the industry. Considering reports of poor, uneven and unpredictable performance of fingerlings (Adeoye *et al.* 2012) and high farm-level losses (Igoni-Eqweke, 2018), broodstock origin, selection, management and replacement strategies, mating design, culture systems, etc., needs to be studied with a view of setting up a breeding programmes. The fact that many hatchery owners in Nigeria select and grow shooters (fast growers) to be used as broodstock needs to be investigated to ascertain if the practice, which is based on the assumption that the shooters possess superior breeding value for growth, is appropriate or not. The chances of reduced variation and increased inbreeding in the industry could be high since, due to the fast growth nature of the Dutch strain, broodstock are only sourced from the farms with Dutch strains irrespective of their broodstock management strategies.

C. gariepinus is still not easily distinguishable from other members of its genus and sub-genus (Teugels and Centrale, 1982; Teugels, 1986; Agnèse *et al*., 1997; Nwafili and Gao, 2007; Compaoré *et al.*, 2015). This is especially true for its closest relative, *C. anguillaris*: several authors hinged the distinction (identification) on its ichthyology, morphology and anatomy. Vomerine teeth are the most popular means of identifying *Clarias* species especially when they are sourced from the wild. The accuracy of this technique and the

possibility of natural hybridisation between the two species (Dunham and Smitherman, 1985; Agnese *et al.*, 1997; Aluko, 1998) and indiscriminate crossing of both species in hatcheries further deepen the confusion.

1.8.2. Research Gaps in Grow-out Production Systems

There is need for more studies on carrying capacities of the various catfish production systems, under different operations and management conditions so as to standardise the operation. Maximum standing crop, quantity of waste discharged and environmental risks and impact of the various systems needs to be assessed, especially in the face of climate change and increased intensification. Lastly, genotype by environment studies needs to be conducted to ascertain the performance of various strains and crosses (e.g. hybrids) in the different environment, with an aim of providing the right environment for optimum expression of genetic potentials.

1.8.3. Research Gaps in *Clarias* Catfish Feed Industry

There is need for benchmarking of existing commercial catfish feed in Nigeria, examining their FCRs and economic value (as the most expensive feed per kg might not be the most expensive feed per kilogram of catfish produced). Furthermore, is the need for optimisation of the *C. gariepinus* feed, to develop a bioenergetics model especially the thermal growth coefficient. There is need to research into non-conventional and conventional raw materials and by-products for their potential as ingredients in *C. gariepinus* feeds, with an aim of developing affordable and reliable feed. This is an important area that requires urgent research. It is important to emphasize the need for research into the nutrient requirement of *C. gariepinus* broodstock, identify barriers to implementation of previous such research, and also underscore the nutrient requirements of the different live stages of *Heterobranchus spp*. and their hybrids with *C. gariepinus*, with an aim of developing diets that best suit each species, their crosses and live stages.

1.9. Scope of Research

While gaps have been identified in the different areas of catfish aquaculture in Nigeria, i.e. genetic characterisation and management of broodstock, fingerlings and feed quality, processing and marketing issues, disease and cannibalism related mortality issues, and

finally policy, economic and governance issues, it is only possible to address a specific set of objectives within the scope of a PhD. This PhD is focussed on investigating current hatchery practices, genetic characterisation and management of broodstock, developing tools to aid selection in a breeding programme and investigating the relationship between shooters and cannibalism, with an aim of improving fingerlings quality through development of selective breeding programme for *C. gariepinus*.

1.10. Research Questions

1. *What are the practices in Nigerian catfish hatcheries and the industry in general that relate to genetic management of the stocks being used, and what lessons can be learned from this that would be of benefit to the industry and to the present research?*
2. *In the face of suggestions of possible introgression between C. gariepinus and C. anguillaris (especially in the Dutch domesticated Clarias catfish farmed in Nigeria) is it possible to find genetic markers for clearly identifying each of these species?*
3. *Are SNPs and/or microsatellite markers derived from sequences generated from ddRADseq, suitable for parentage assignment and useful tools in selection in a breeding programme? What are the heritability and breeding values of individuals in the populations of farmed C. gariepinus in Nigeria?*
4. *How can the findings of the present research be applied to develop genetic improvement in C. gariepinus to the benefit of aquaculture in Nigeria and other countries growing this species?*

1.11. Aims and Objectives of this Research

The aims of this study is to develop markers for distinguishing *C. gariepinus* and *C. anguillaris*, develop markers for selection in a breeding programme for *C. gariepinus*, evaluate hatchery practices in the Nigerian Aquaculture Industry and evaluate the suitability of different populations of *C. gariepinus* for aquaculture and set up selective breeding programme to improve growth rate and curb inbreeding depression. Furthermore, this study will attempt to estimate variations (within and between the different

populations), heritability and breeding value for individuals within these populations. The study also aims to investigate the suitability of using shooters as broodstock and develop adaptable models of breeding programmes for farmers.

1.12. Structure of this Thesis

This PhD thesis is divided into five chapters, out of which chapters two, three, four and part of five are based on experiments conducted during the PhD research.

Chapter 1 provides a background to the study by way of reviewing genetic improvement in aquaculture, the African catfish and the status genetic improvement in catfish aquaculture in Nigeria. A review of the Nigerian catfish aquaculture industry was carried out, with an aim of identifying and understanding the status, problems and prospects of the *Clarias* catfish aquaculture in the country. Research gaps are identified from which the scope and aims of this thesis is derived.

Chapter 2 is a survey of hatchery practices in the Nigerian aquaculture industry, with special focus on *Clarias* catfish hatcheries. It also provides an up to date review of artificial spawning and the life cycle of *Clarias* catfish.

Chapter 3 describes the development of species-specific SNP markers for discriminating *C. gariepinus* from *C. anguillaris* using ddRADseq. It also describes the discrimination of populations from six different countries using KASP assay.

Chapter 4 describes the methods of discovering microsatellite markers from sequences generated from ddRADseq, primer design and optimisation for the microsatellite markers. It further, describes the process of enrichment for microsatellite markers discovery, experimental setup for species evaluation for selective breeding programme, kinship and cannibalism. It also provides details on simulation, testing the power of assignment of markers developed.

Chapter 5 Summarises the major findings, challenges and limitations of this research, relevance and application of the findings to *Clarias* aquaculture and the development of genetic improvement for this species.

CHAPTER 2. A STUDY OF THE CURRENT HATCHERY PRACTICES IN THE NIGERIAN AQUACULTURE INDUSTRY – FOCUS: *Clarias gariepinus*

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Suleiman Ihiabe Isa, designed questionnaire under the supervision of David J Penman and Brendan McAndrew, with inputs about structure and scope from David Little and William Leschen. Questionnaire was piloted and later administered by the author. All analysis of response from and compilation of this chapter were done by the author under the supervision of David J. Penman and Brendan McAndrew.

2.1. Abstract

Clarias gariepinus - the most popular aquaculture species in Nigeria and the second most popular in Africa - is increasingly gaining attention of both the government and private sectors in Nigeria. Fast growth rates, adaptation to adverse and varied environmental conditions, make this species a desirable candidate for aquaculture, particularly in sub-Saharan Africa.

Until recently, most studies on the aquaculture of this species have been based on nutrition, physiology and culture systems, with little known on the genetics and genetic management of different populations of wild and cultured *C. gariepinus* within and between different countries. As a prerequisite for setting up a selective breeding programme to optimise production of this species, a survey of different *Clarias* hatcheries and farms in Nigeria was carried out to evaluate current practices, problems and prospects.

Broodstock origin, selection, management and replacement strategies, mating design, culture systems, etc., were studied mostly from farms in different parts of Nigeria, plus a farm and a research institution in The Netherlands and Hungary respectively. A questionnaire containing 167 questions was prepared and administered in the form of a semi-structured interview, using the traditional paper and pencil interview (PAPI) in Nigeria and computer assisted self-administration interview (CASI) in The Netherlands and Hungary.

The results from the survey showed that 98% of broodstock used in the hatcheries were of farm origin, with more than 80% of farmers interviewed having a broodstock replacement plan. Average broodstock holding capacity was between 50 and 300 broodstock per annum, mostly below 25 families, of which less than 50% contribute to broodstock replacement. This practice significantly reduces the effective population size (N_e) and increases the chances of inbreeding depression. Furthermore, over 46% of farmers interviewed hatch and select their own broodstock, out of which above 90% of them select and use only shooters (fast-growing cannibalistic fish) for broodstock replacement; a practice based on the assumption that the shooters poses superior breeding value for growth. This was contrary to the reaction in the Hungarian and Netherland.

In conclusion, although the *C. gariepinus* catfish industry is growing rapidly, some of the current hatchery practices present potential threats to the sustainability of this growth, if their effects are not properly addressed.

2.2. Introduction

Fish are an important source of nutrient, livelihood, income and foreign exchange to many countries of the world. Global annual increase in fish consumption (3%) has doubled the annual increase in human population (2%), growing from 9.0 to 20.20 kg per caput between 1961 and 2015 (FAO, 2018c). Capture fisheries have levelled out for the past three decades (fluctuating between 85 and 95 million tons per annum), while aquaculture production has steadily increased within the same period (growing from below 18 to over 80 million ton per annum), now accounting for 47% of global food fish production (Waldron, 2009; FAO, 2018c). Central to the growth of global aquaculture has been the development of appropriate skills, knowledge and technology to domesticate, manage, feed and breed different fish species across the different aquaculture industries. While these have been very successful for some fish species in some countries (e.g. Atlantic Salmon in Canada, Norway and Scotland, Carps in China and India, Channel catfish in America), some of the required skills, knowledge and technology are limiting in some other species, countries and/or industries.

The introduction of this domesticated strain of *C. gariepinus* by some Dutch consultants for intensive culture in concrete tanks and earthen ponds with accompanying influx of imported extruded catfish feeds marked a significant point in the history of the Nigerian aquaculture industry. Their impressive growth rate and harvest size encouraged farmers to abandon the native strains already undergoing domestication. Today the Nigerian aquaculture industry is dominated by Dutch domesticated *C. gariepinus*, which in some cases have been bred with the local stock (due to scarcity of broodstock), and in many cases have themselves probably been inbred. Private sector interest and participation, hence, growth of the industry became more evident due to the resulting practicality and profitability of catfish farming in Nigeria. Access to high quality fish feed (imported extruded pellets) and improved culture systems (RAS systems, flow-through systems, indoor hatcheries, aerators, concrete tanks, etc.) marked the beginning of intensive aquaculture production in Nigeria, with many farmers abandoning the traditional pond rearing of fingerlings. Numerous small backyard farms and hatcheries proliferated to provide fingerlings for the growing aquaculture industry. The overreliance on imported extruded pellets has proven to be unsustainable due to exchange rate problems. It is

therefore safe to conclude that the main problems of the Nigerian Aquaculture Industry are inadequate supply of good quality fish feed and fish seeds (fingerlings and juveniles).

2.3. Life Cycle of *Clarias gariepinus*

A detailed study of the life cycle of *C. gariepinus* was carried out based on expert knowledge, information obtained from literature on current breeding and management practices from experienced catfish hatcheries to ensure accuracy of the life cycle analysis of this species. Details of the processes involved are illustrated in **Figure 2.1 a - h.** below.

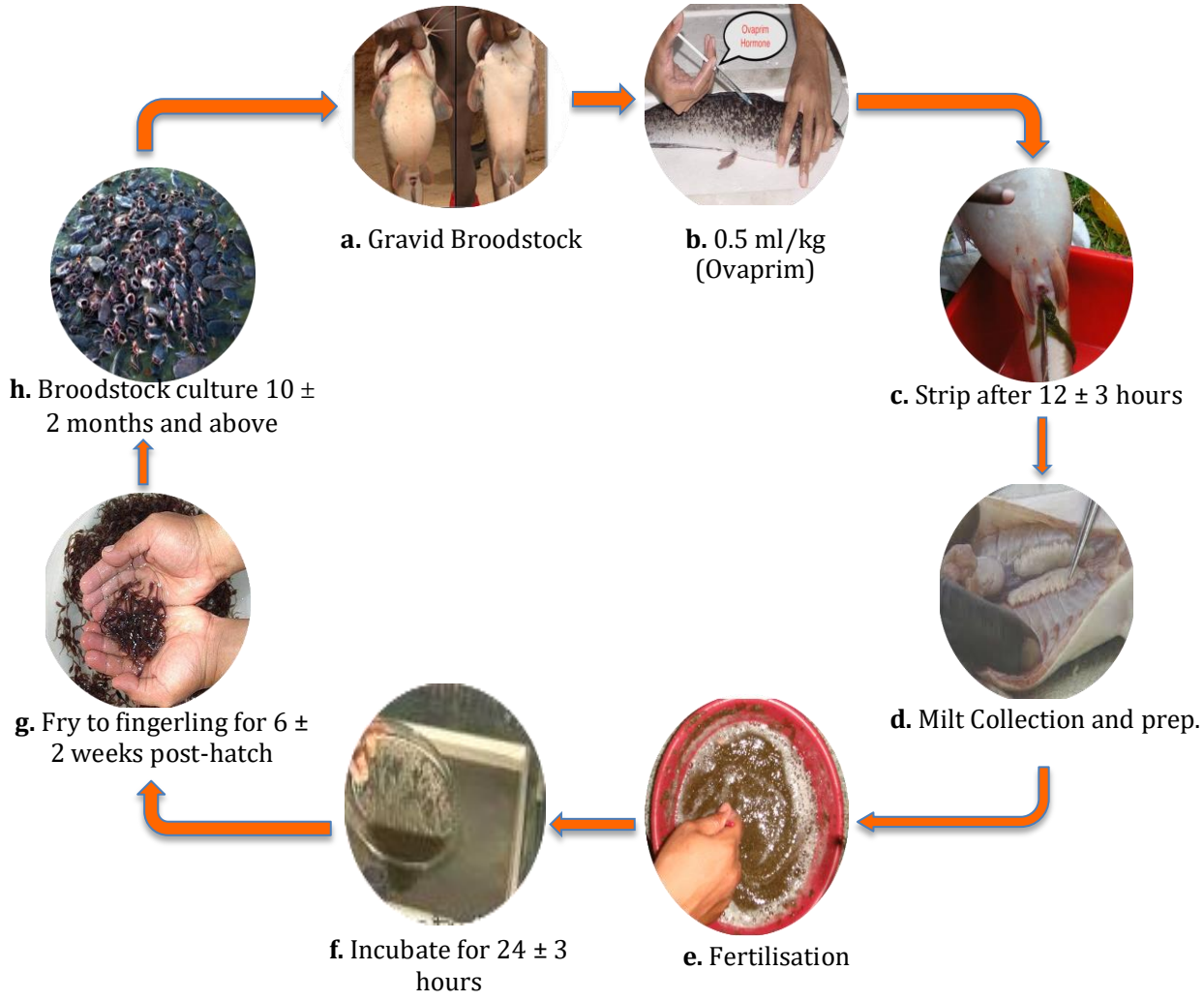

Figure 2.1 (a – h). Life cycle of African catfish *(C. gariepinus)* under hatchery conditions. Images were sourced from Google and assembled by Isa, I. I. (the author).

2.3.1. Broodstock Selection

C. gariepinus females exhibit obvious sexual dimorphism and maturity from the ages of 4 and 8 - 10 months respectively (Hogendoorn and Vismans, 1980). As illustrated in **Figure 2.1.a** mature females are identified by large, distended, soft and round abdomen, with a swollen and reddish ovipositor, through which few eggs flow upon application of a slight pressure (Hogendoorn and Vismans, 1980; de Graaf and Janssen, 1996). Females with eggs (visible upon application of slight pressure) of uniform diameter and dark point (nucleus – with careful observation) are selected. On the other hand, males are identified and selected based on vascularised genital papilla with occasional reddish tip (**Figure 2.1.a)**. Due to absence of a large distended abdominal region, they appear to be slimmer and slightly longer.

2.3.2. Hypophysation

Selected females are conditioned (depending on system and circumstances) for a period of 1 – 14 days before induction. The use of acetone-dried carp pituitary at 4 mg dried material kg^{-1} of female, homoplastic hypophysation (using fresh pituitary from a male whose milt will be temporarily stored to fertilise the induced female(s)) at 1:1 for fish of similar sizes and the use of different brands of GnRH analogues with dopamine antagonists at 0.5 ml kg^{-1} of body weight have been reported to successfully induce final oocyte maturation in female *C. gariepinus* (Hogendoorn and Vismans, 1980; de Graaf and Janssen, 1996; Mylonas *et al.*, 2010; Sharaf, 2012; El-Hawarry *et al*., 2016). The fish are injected inter-peritoneally or intramuscularly as illustrated in **Figure 2.1.d** above. Different products are available for induction in different countries. OVAPRIM, manufactured by Syndel in Canada (Karami, 2011; Akankali *et al.*, 2011) a salmon gonadotropin – releasing hormone, combined with domperidone (SnGnRHa) is the most popular hormone used to induce *C. gariepinus* in Nigeria (Karami, 2011). The average GSI for female *C. gariepinus* is 15 ± 5% of the body weight, and this varies between wild and farmed stocks, with the lower end recorded mostly for wild and the higher GSI rates for the farmed ones (Hogendoorn and Vismans, 1980; de Graaf and Janssen, 1996; Rocha, 2008; Eyo *et al*., 2014; Admassu *et al.*, 2015; Al-Deghayem *et al.*, 2017).

On the other hand, the aforementioned hormones are seldom used to induce male *C. gariepinus,* except during induced natural breeding. This is due to the fact that male *C.*

gariepinus cannot be hand-stripped like male salmonids or cyprinids as their ripe milt concentrates along the convex part of the testes and they are oligospermic (GSI ≤ 1%), with very low volume of sperm (Hogendoorn and Vismans, 1980; Urbányi *et al.*, 1999). Viveiros *et al.* (2002), observed very low spermatocrit, sperm concentration and hatching rates in an experiment conducted to observe the effects of various hormonal treatments in facilitating hand stripping of male *C. gariepinus*.

In the case of induced natural spawning, both sexes of broodstock (preferably conditioned separately prior to induction) are induced and placed together in a well-prepared earthen pond, plastic or concrete tank, etc., where courtship, release of eggs and fertilisation take place. The broodfish are removed (depending on the water temperature, they are left overnight in earthen ponds or until shed eggs become visible in tanks) before hatching commences to prevent cannibalism. This method ensures survival and multiple use of the same males; however, it is increasingly becoming unpopular amongst farmer for some reasons, amongst which is the difficulty in ascertaining male potency, hence, not very predictable fertilisation.

2.3.3. Ovulation, Stripping of Females and Dissection of Males

Induced females are kept in an enclosed and solitary container for a period of 12 ± 5 hours (latency period) depending on the water temperature (optimum 30 ± 2 ^{o}C) as shown in **Table 2.1**, after which the eggs are stripped with ease into a clean dry bowl **(Figure 2.1.c above).** Male *C. gariepinus* are dissected as illustrated in **Figure 2.1.d** above to access the kidney shaped lobes of testes for sperm collection by maceration. In most cases, the males are sacrificed, although Adebayo *et al.* (2012), recorded 100% survival and no significant difference between the sperm quality of regenerated testes (following a partial gonadectomy) and the sperm initially collected. The sperm are either applied directly onto the eggs for fertilisation or are extended using 0.9% saline solution, which was reported to improve hatching rate by 9% when compared to non-extended sperm (Hogendoorn and Vismans, 1980).

2.3.4. Fertilisation

Sperm (with or without extenders) are dropped evenly over the stripped eggs and gently stirred **(Figure 2.1.e)** for about 1 minute during which water may be added to activate the sperm. Activated sperm are only potent for 1 minute, a time within which fertilisation occurs under continuous gentle stirring using a bird's feather or plastic spoon.

Table 2.1. Time between hypophysation and stripping (latency period) of *C. gariepinus* female at different water temperatures (*Source:* Hogendoorn and Vismans, 1980; de Graaf and Janssen, 1996)

Water Temperature (°C)	**Latency Period (Hours)**
20	21.0
21	18.0
22	15.5
23	13.5
24	12.0
25	11.0
26	10.0
27	9.0
28	8.0
29	7.5
30	7.0

2.3.5. Incubation

Hatching troughs, vats, tanks, trays, etc., made of different materials such as fibreglass, plastics, glass, concrete, stainless steel, etc., are currently used to incubate *C. gariepinus* eggs. The sizes and number of incubating units may vary, however, it should contain clean, aerated flowing or non-flowing water with a substrate (nets **(Figure 2.1.f)**, caccabans, etc.,) onto which the eggs are dispensed. This is done immediately after fertilisation to prevent sticking of eggs together, which can severely reduce hatchability. Optimum water temperature range is 28 – 32 °C. **Table 2.2** below shows that the higher the temperature (within the optimum range), the shorter the incubation period.

Table 2.2. Time between fertilisation and hatching (incubation period) of *C. gariepinus* eggs at different water temperatures (*Source*: Hogendoorn and Vismans, 1980; de Graaf and Janssen, 1996)

Water Temperature (°C)	Incubation Period (Hours)
20	57.0
21	46.0
22	38.0
23	33.0
24	29.0
25	27.0
26	25.0
27	23.0
28	22.0
29	21.0
30	20.0

2.3.6. Fry Rearing

Under optimum temperature conditions, *C. gariepinus* fry absorb their yolk within 3 days post-hatch (DPH), after which they swim up in search for food. Naturally they feed on zooplankton (Daphnia, Moina, etc.,) at this stage, and this is readily available in well-prepared fertilised nursery ponds. Supplemented feed is added after 6 DPH. After three weeks, post-frys and fingerlings become more visible at the surface at the time of feeding. In intensive indoor production systems, different feeding practices are carried out depending on available resources and/or cost effectiveness. Live zooplankton or Artemia nauplii are fed at least up to 7 DPH (or longer), after which they are weaned onto preferably dried microdiets (usually with crude protein ≥ 50%). This practice ensures higher survival rates as their stomach develops only after 7 - 9 DPH and thus, they rely on exogenous enzymes for initial digestion of ingested feed, hence, source enzymes from live feed (Hogendoorn and Vismans, 1980; de Graaf and Janssen, 1996; Hecht, 2013). In some Sub-Saharan countries with unreliable power supply such as Nigeria, hatching of Artemia

nauplii becomes an uphill and expensive task. Hatchery owners rely on the ready-to-feed decapsulated and decysted Artemia as first feed, before weaning onto microdiets.

Feeding and water quality management routines depend on the design, scale and intensity of the hatchery. Due to the high level of cannibalism, hatcheries sort or grade the fish from 3 weeks post hatch (WPH) onward. Depending on temperature and management in place, *C. gariepinus* hatchlings could attain 1.0 ± 0.5 g **(Figure 2.1.g)** and 2.0 ± 0.5 g in weight at 6 ± 2 WPH and 8 ± 2 WPH respectively; the standard sizes for fingerlings and juveniles in Nigeria respectively.

2.3.7. Grow-out/Broodstock Production

C. gariepinus broodstock are raised in varied culture environments e.g. in earthen ponds **(Figure 2.1.h)**, fibreglass, plastic or concrete tanks, etc., depending on what is available to the farmer. Although they attain sexual maturity at the age of 8 months post hatch (MPH), they are mostly kept for longer before use. They are ideally kept separately from the grow-out fish and depending on the level of intensity, fed a nutrient-rich diet with the aim of meeting their nutrient requirements, thus; ≥ 35% crude protein (CP), 21.2 kj g^{-1} and a protein energy ratio of 20 protein kj g^{-1} gross energy (GE) (Ayinla, 1988**;** Ali and Jauncey, 2005; Ondhoro *et al*., 2015)**.** Many farmers rely on commercial grow-out feed to raise their broodstock due to inadequate supply of broodstock feed.

2.4. Aims and Objectives of this Research

Until recently, most studies on the aquaculture of this species has been based on nutrition, physiology and culture systems, with little known of the genetics and genetic management of the different populations of wild and cultured *C. gariepinus* within and between different countries (Hecht, 2013). The aim of this study is to survey of different *C. gariepinus* hatcheries and farms, to assess and evaluate current practices, problems and prospects. The objective is to provide baseline information requisite to setting up a selective breeding programme, to improve growth rate, survival, and reduce inbreeding depression, cannibalism, poor quality of fingerlings and uneven growth currently reported in the industry.

Approach: In order to achieve the above aim and objective, this study was approached in the following sequence. A detailed desk study of artificial propagation *C. gariepinus* was conducted all through its captive lifecycle. Information generated, together with industry experience and expected best practices informed the design and content of the questionnaire. The questionnaire was piloted and eventually distributed to source needed data for analysis and inferences. Information generated was assessed against expected best practices in the industry, and also drawing from experience of other similar aquaculture industries. Outcomes of the survey provided understanding of the current practices in the Nigerian *C. gariepinus* industry and will serve as a guide towards better genetic management of hatchery stocks.

2.5. Methodology

2.5.1. Preparation of Questionnaire

Pertinent questions around current hatchery practices, problem and prospects were outlined covering including, but not limited to, topics related to broodstock origin, selection, management and replacement strategies, mating design, culture systems. These questions were arranged into a questionnaire, which was distributed primarily to hatcheries in different parts of Nigeria, in addition to a farm and a research institution in The Netherlands and Hungary respectively. This questionnaire (**Appendix 1**), containing 167 questions was prepared, following the guidelines below.

In order to allow for comprehensive study and analysis of hatchery practices in Nigeria, both qualitative and quantitative approaches (mixed methods) were taken, using both open and closed questions (Bird, 2009). In appreciation of the limitations of semi-structured interviews, which include the ability of interviewers to dictate the direction and tone of interviews and the lack of specific information on the wordings of questions and phrases (Valentine, 2005; Hawkes and Rowe, 2008), the question format adopted was concise, phrased in simple and plain English (Bird, 2009; Sarantakos, 2016). The length of the questionnaire was so as to cover the key issues around hatchery practices. To ensure that respondents participate despite the length, most questions were short, multiple choice and closed, with check boxes and options of "others please specify", "don't know" or "not applicable" to give options beyond the given range. Furthermore, related questions were

grouped under same subheading or section, arranged in a logical sequence and transition, using open questions to create depth and quality (Bird, 2009), while representing (repeating) some questions in different formats and contexts, so as to cushion the possibility of respondents overestimating yield and underestimating losses. Such questions were checked against one another, within and between the different contexts to assess and ensure consistency in response. The questionnaire started with two introductory paragraphs containing a brief background and objective of the research, the researcher's preface and assurance of flexibility and confidentiality of data generated. Finally, with the understanding that some individuals might find personal questions intrusive or invasive (Parfitt, 2005), such questions were asked in the last section of the questionnaire as shown in **Appendix 1.** To further minimise the effect of low response rate, 300 questionnaires were distributed to different fish hatcheries in Nigeria, three times the number of expected participants. The questionnaire was piloted in 27 hatcheries and then administered to different 273 other hatcheries in the form of a semi-structured interview (face to face), using the traditional paper and pencil interview (PAPI) in Nigeria and computer assisted self-administration interview (CASI) in The Netherlands and Hungary.

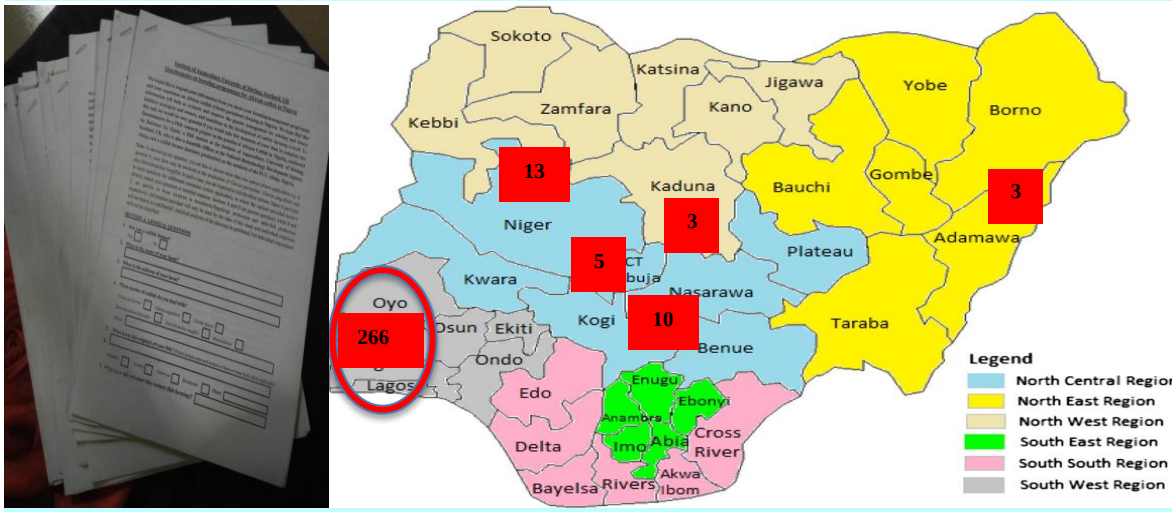

Figure 2.2. States of Nigeria where indicated number of farmers were interviewed

A combination of purposive and probable sampling method was used. South-Western (**Figure 2.2.** below) Nigeria is famous for aquaculture production, thus has more

hatcheries than other parts of the country that were surveyed, hence more hatcheries in Lagos, Ogun and Oyo states were covered in this study - purposive. On the other hand, within each state, hatcheries were randomly selected – probable. Of the 300, a total of 66 questionnaires were distributed to respondents in Ogun state, while Lagos and Oyo states respective had 100 each, making it a total of 266 questionnaires in the South-Western states. Numbers to other states are indicated in **Figure 2.2**.

The questionnaires were retrieved and brought back to the UK for analysis. All 300 questionnaires were examined and sorted based on level of completion of the broodstock, hatchery and nursery sections. Those with the most complete responses of above 70% of the questions were selected, and the minimum target of 120 respondents was met **(Figure 2.3)**. Response rate per question also varied, depending on the type of question and activity of the hatchery owner. Most of the questions had above 75% response rate. As indicated in **Figure 2.3,** the 99 selected questionnaires were 39 from Lagos, 28 from Ogun and 32 from Oyo states respectively. The number selected from other state is indicated in **Figure 2.3.**

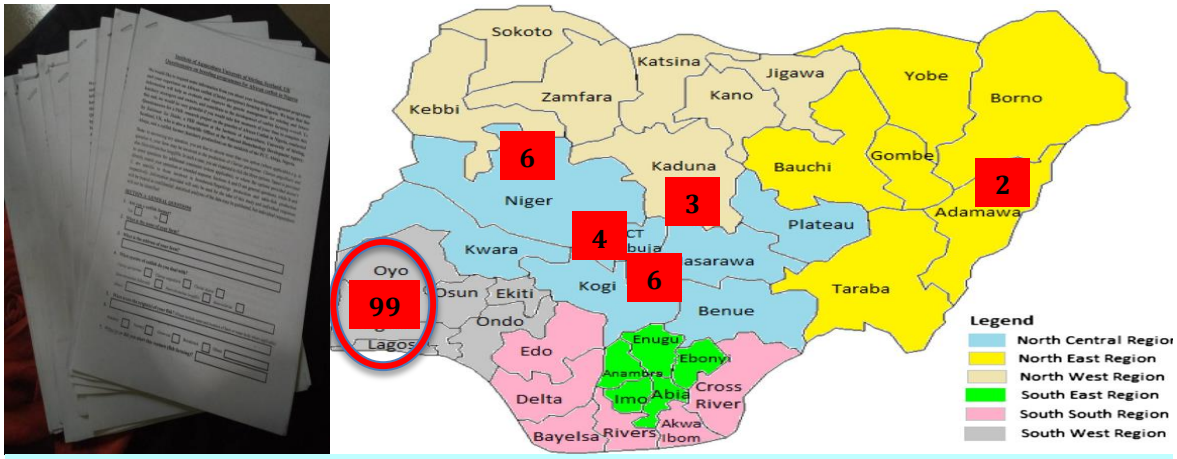

Figure 2.3. Number of questionnaires selected from states where responses were received

2.5.2. Ethical Approach

This study was initiated before the University of Stirling's new ethics approach. However, in line with the 25th May, 2018 European Union (EU) privacy law called the General Data Protection Regulation (EUGDPR, 2018), all personal information of all respondents are

treated as confidential and if for any reason it were to be used, a prior written consent must be sought. Information provided in this thesis are written as anonymous and cannot be traced to any individual, and where such traceability is possible, a written consent was sought where publication was absolutely necessary.

2.5.3. Statistical Analysis

All data was coded and entered into Microsoft Excel (2013). Analysis was based on descriptive and inferential statistics.

2.6. Results

Data generated from the survey included technical, financial and some personal details of the hatchery venture such as: year of establishment, source(s) of funds, address of the hatchery, number and gender of staff, names and levels education of respondents (primarily the hatchery manager, sometimes the hatchery owner), hence, the need to be anonymous under the current EU privacy policy.

2.6.1. Main Challenges of Hatchery Operation

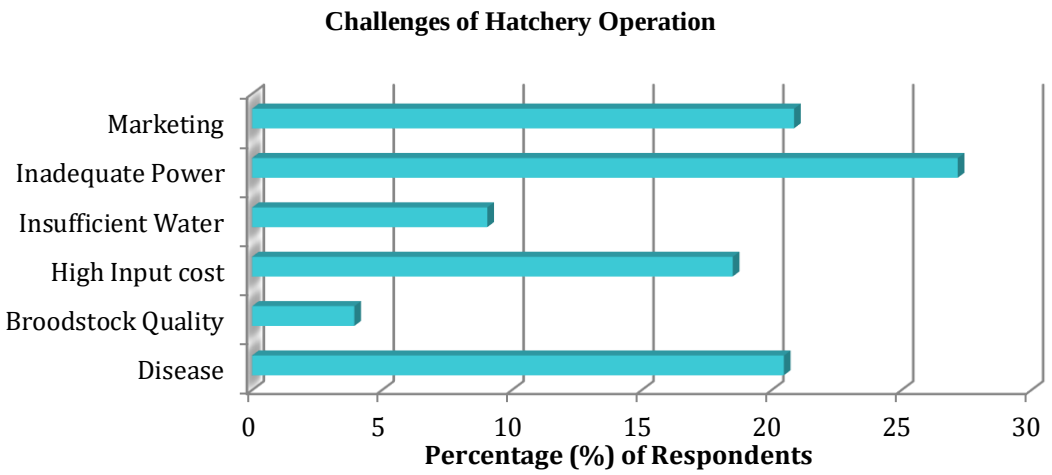

Figure 2.4. Challenges of hatchery operation in Nigeria (n = 120)
Where n = number of respondents

Hatchery owners were asked what they thought the main challenge to successful operation was. Inadequate power (electricity), market and disease issues appeared to top the

responses with 27%, 21% and 21% respectively. Surprisingly, only 4% of respondents mentioned broodstock quality as their main challenge (**Figure 2.4**). The remaining results of the survey will attempt to probe the situation from different perspectives.

2.6.2. Broodstock Types and Origin

The Nigerian catfish industry is dominated by *C. gariepinus*; however, *Heterobranchus longifilis* (possessing an adipose fin of equal length with the dorsal fin) and *Heterobranchus bidorsalis* (possessing an adipose fin that is about half the length of the dorsal fin) are also farmed and in many cases hybrids (Heteroclarias) between either of the *Heterobranchus spp* and *C. gariepinus* are produced. **Figure 2.5.** shows that 62% of hatcheries surveyed produced only *C. gariepinus*, 8% produce *C. anguillaris* and 30% produce hybrids of *Clarias spp* and *Heterobranchus spp* (*Heteroclarias*). Of those producing *C. gariepinus*, 84% of them sourced their original stock of *Clarias spp* from other hatcheries/farms, with 14% sourcing from Government institutions and only 2% of them sourced directly from the wild. Of those who claimed to produce *C. anguillaris*, they identified them from the vomerine teeth (the premaxillary tooth plate having no gaps as opposed to *C. gariepinus* which are thought to have gaps).

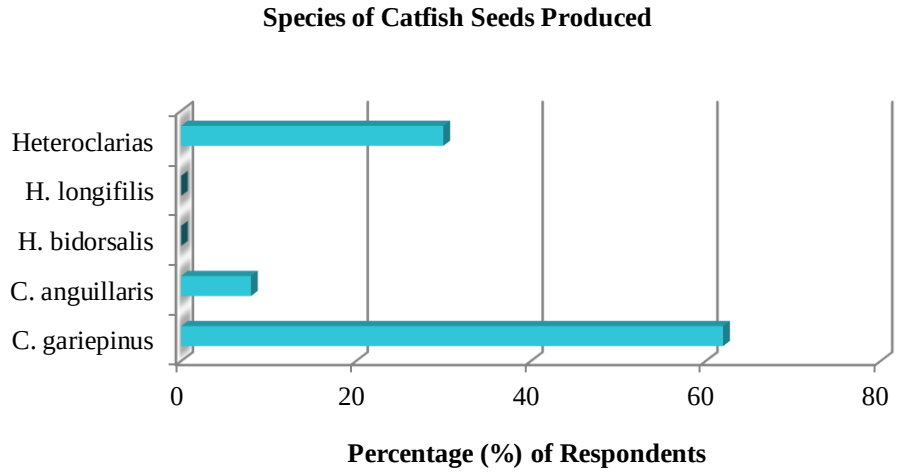

Figure 2.5. *Clarias* species and hybrids of catfish produced in the Nigerian catfish hatcheries (n = 120)
Where n = number of respondents

2.6.3. Broodstock Culture Systems

Of all the hatcheries surveyed, 44% and 40% grew their broodstock in outdoor tanks and earthen ponds respectively **(Figure 2.6).** The outdoor tanks varied in material, being either concrete, plastic, fibreglass and/or a combination of concrete and plastic. **Figure 2.7** shows

that 26% of respondents relied on wells, 41% relied only boreholes and 28% relied on a combination of both wells and boreholes as sources of water for their hatcheries. Thus 95% use underground water on their farm.

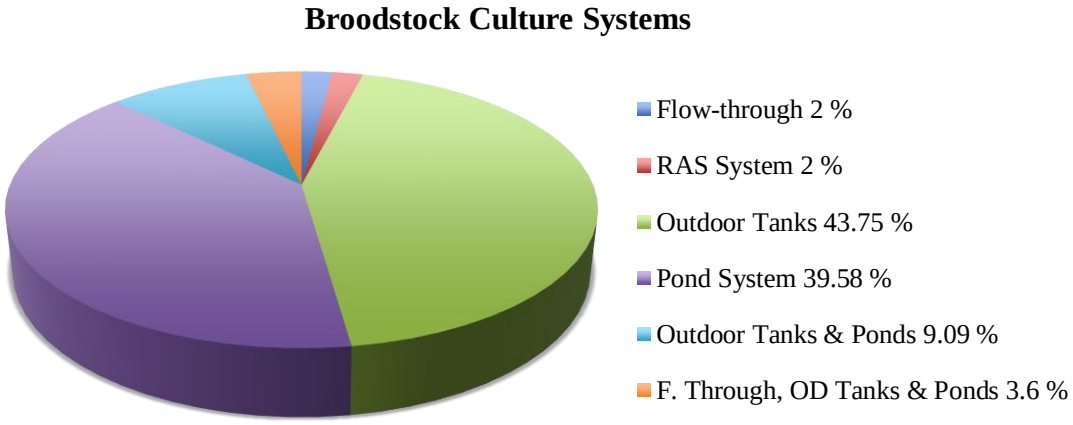

Figure 2.6. Broodstock culture/holding systems for *C. gariepinus* in Nigeria (n = 117)

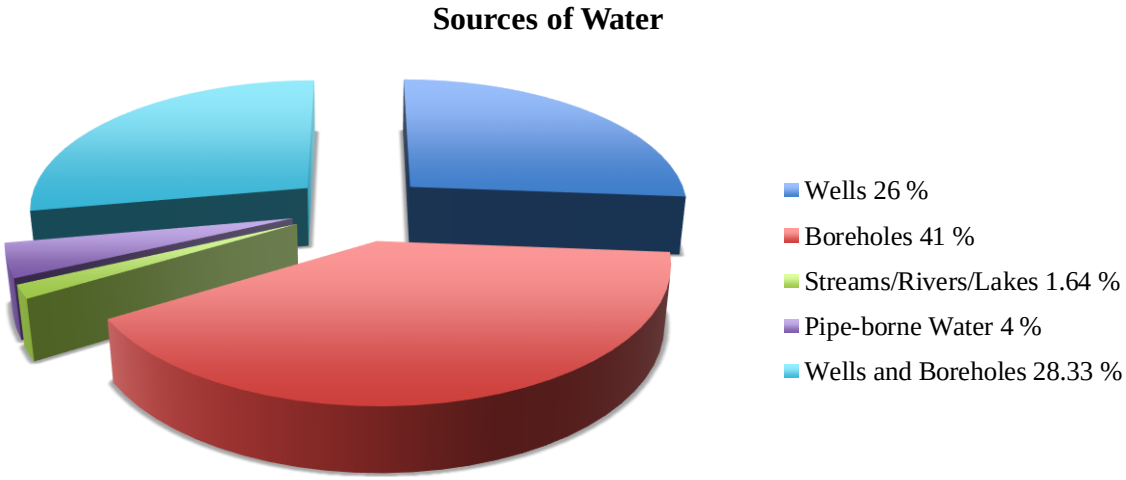

Figure 2.7. Sources of water used in hatcheries in the Nigerian Aquaculture Industry (n = 120)

2.6.4. Broodstock Evaluation

Although there seemed to be large numbers of broodstock kept by hatcheries annually (42% of respondents kept ≥ 300 and 45% of respondents kept between 100 – 299 broodstock year^{-1} respectively), 97% of all respondent's broodstock came from < 25 families **(Figure 2.8.).**

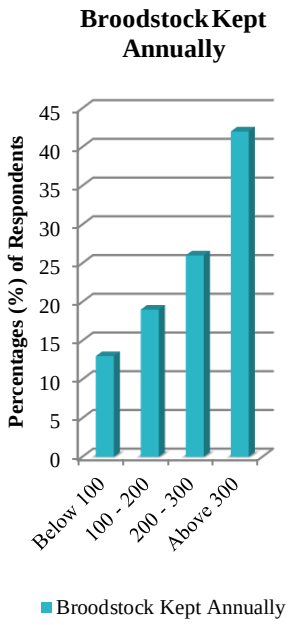

Figure 2.8. Annual broodstock population kept by respondents (n = 97)

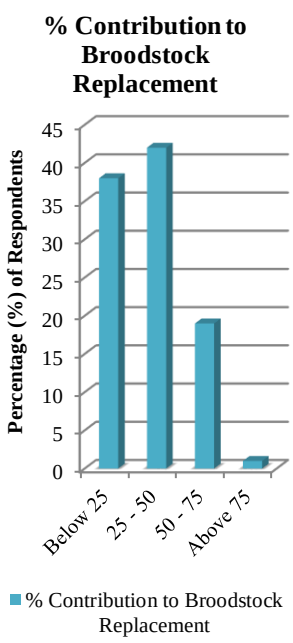

Figure 2.9a. Percentage contributing to the next generation of broodstock (n = 97)

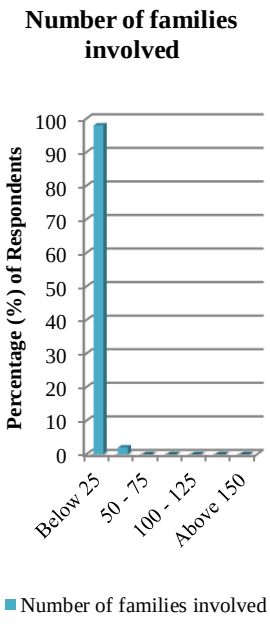

Figure 2.9b. Number of families they came from (n = 93)

81% of hatcheries surveyed had a broodstock replacement strategy, of which 41% of them replaced broodstock biannually and same percentage did so once a year **(Figure 2.9a.).** In 72% of the hatcheries surveyed, below 50% of the broodstock kept annually contributed to the next generation of broodstock, 76% of which were selected from ≤ 4 batches of fish. 98% of respondents' broodstock came from ≤ 25 families **(Figure 2.9b)**, and 93% of female broodstock were reused mostly 2 – 3 times year^{-1} for up to 1-3 years before discarding. 96% of respondents exclusively sacrificed the males while the remaining 4% sometime stitched some males back. The most common sex ratio was 2 males : 4 females. 80% of respondents used ≥ 12 months old broodstock, and 92% of farmers use ≥ 1.00 kg sized broodstock for breeding in their hatcheries. While 86% of respondents claimed female broodstock of same age are bigger than the males, only 15% of them think there are differences in the time of maturity, with the males maturing earlier than the females. 73.81% of respondents recorded GSI of 15 – 20%, hatchability of above 75% was recorded by 80% of respondents and more than 66% of respondents reported survival rates of ≥ 50% while most of the remaining respondents had no idea of the survival rate.

2.6.5. Broodstock Replacement Strategies

98% of respondents producing *C. gariepinus* did not source broodstock from the wild; 38% sourced them from other farms, 39% hatched them on their farms and 21% sourced part from other farms and hatched the remainder on their farms as shown in **Figure 2.10.** Of those who bought from other farms, 75.76% of them bought as table fish (grow-out) and raised them further to broodstock **(Figure 2.11.)**. The question about number of farmed sources was asked in three stages, if respondent bought recruits from a single or multiple farms at juveniles, table fish and broodstock stage.

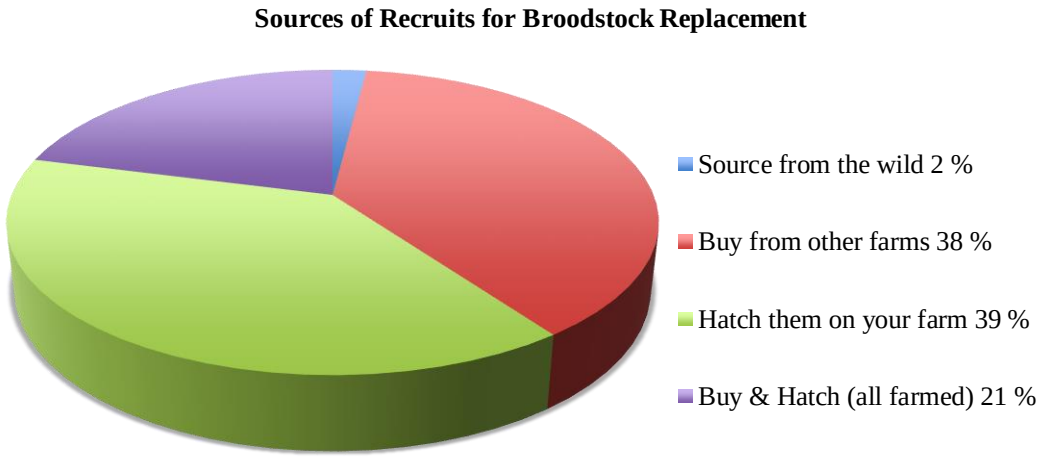

Figure 2.10. Sources of recruits for broodstock replacement (n = 119)

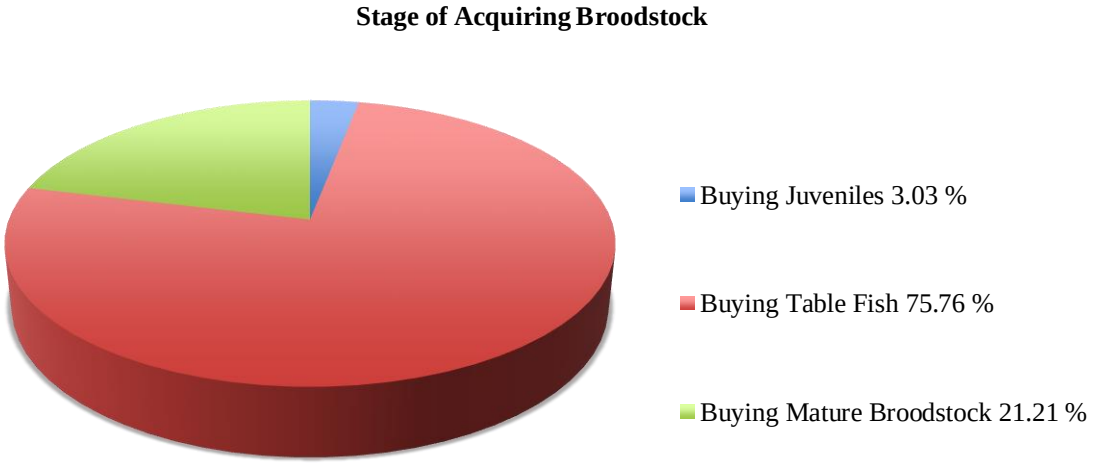

Figure 2.11. Stages at which recruits are acquired for broodstock replacement (n = 46)

The responses for the three stages were pooled together and average percentage of responses of either single, multiple or a combination of both computed as shown on **(Figure 2.12).** Interestingly, 57% of them bought from different farms, 38% bought from

both single and different farms, while only 6% of respondent bought recruits from a single farm. Of those who hatched them, 91.8% selected only the shooters (fast-growers (**Figure 2.13.**)) to raise them as broodstock as shown in **Figure 2.14.** Despite attempts by some respondents to select and/or buy broodstock from different batches of hatched fish and different farms respectively, 73% of them eventually raised all similar sized broodstock together in the same tank/pond, with no intention or ability to distinguish them. During mating, 77% of respondents use 1:2 male to female ratio for broodstock replacement (**Figure 2.15)**.

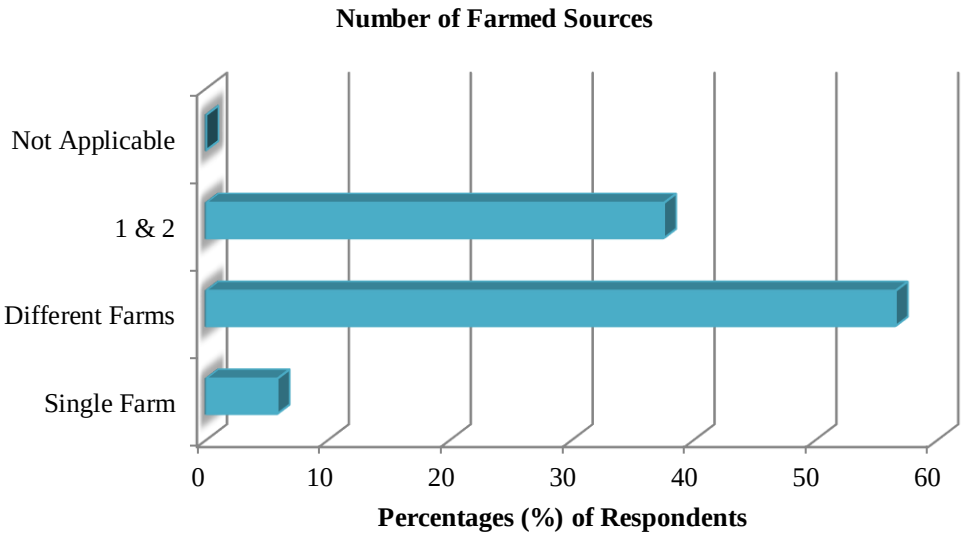

Figure 2.12. Number of sources of farmed recruits for broodstock replacement in Nigeria (n = 46)

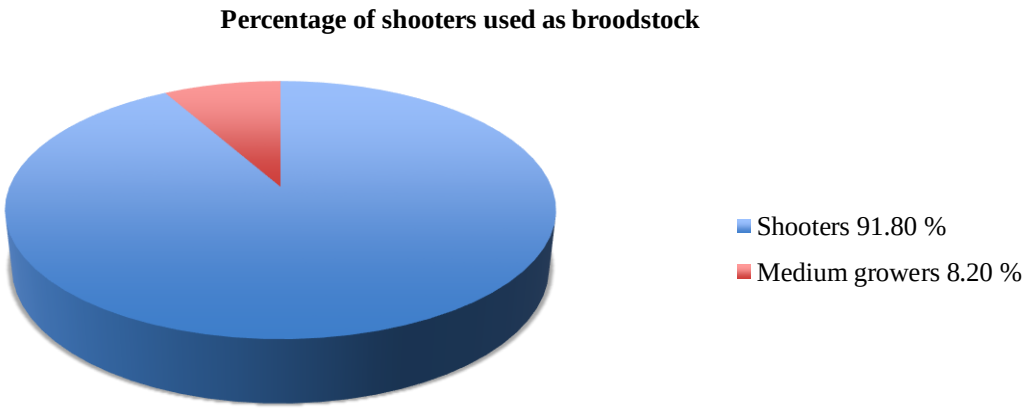

Figure 2.13. Percentage of shooters used as broodstock in Nigerian catfish hatcheries (n = 120)

Figure 2.14. A shooter (2.7 kg) and an average sized fish (0.63 kg), hatched on the same day, from the same parents and grown under similar conditions

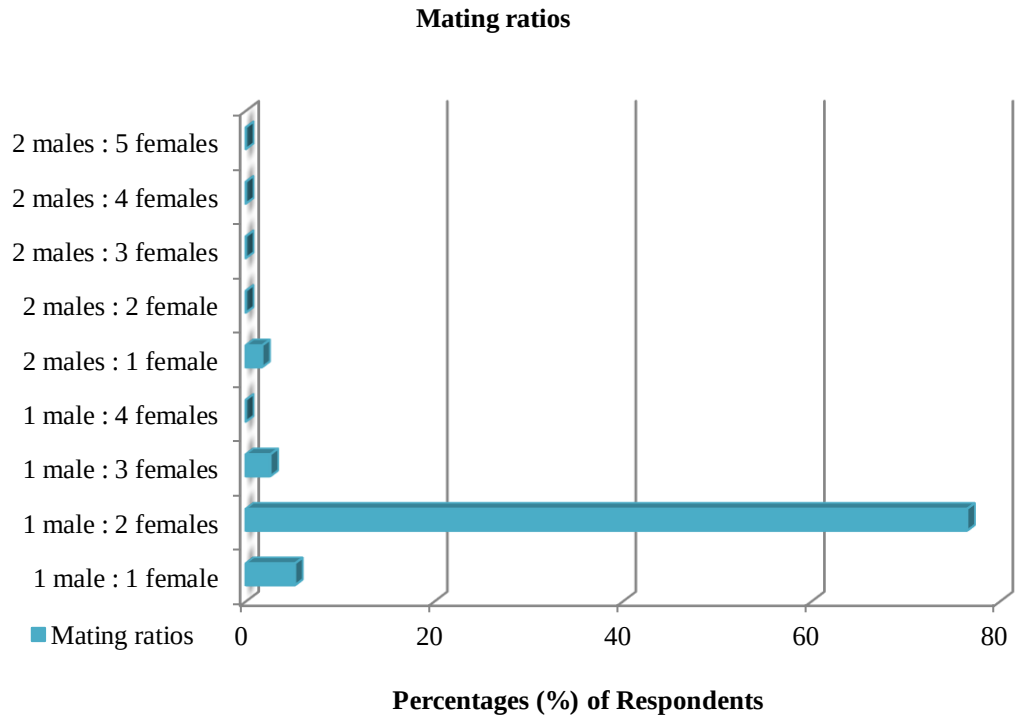

Figure 2.15. Mating ratios used by farmers (respondents) during breeding of *C. gariepinus* (n = 120)

2.6.5.1. Effective Breeding Number (N_e)

An estimate of the effective breeding number (N_e) was calculated based on the average number of broodstock kept, percentages contributing to the next generation, mating design and sex ratios. **Figure 2.8** shows a total of 58% of respondents keeping ≤ 300 broodstock per annum; **Figure 2.9b** shows that over 98% of them came from ≤ 25 families and that only about 50% (150 individuals) of them contribute to the next generation **(Figure 2.9a).** With a sex ratio of 1 male to 2 females (Figure 2.15), one would expect only 50 males and 100 females contribute to the next generation.

$N_e = (4(N_m \times N_f))/(N_m + N_f)$; where

N_e = Effective Breeding number

N_m = Number of breeding males = 50

N_f = Number of breeding females = 100

$N_e = (4(50 \times 100)/(50 + 100)$

$N_e = 133.33 = 1.33$

The inbreeding coefficient $\Delta F = 1/2N_e$, thus making it 0.602% inbreeding depression per generation.

2.6.6. Broodstock Management Techniques

37% of respondents used commercial broodstock feed, 37% of them used commercial grow-out feed, 13% used both of the aforementioned, 5% use on-farm broodstock feed and 6% used on-farm grow-out feed as shown in **Figure 2.16.** More than 93% of respondents fed to about 2 - 5% of bodyweight per day **(Figure 2.17.),** with the majority feeding 25 – 40% CP diets. 11% of hatcheries surveyed bred fish all year round, 84% breed at different times of the year between the months of April and December while 5% breed exclusively between January and June. The main reason for seasonality in breeding was attributed to availability or not of gravid females (15%), temperature (27%) and rainfall (38%). As shown in **Figure 2.18**, 40% of respondents who bred all year round manipulated temperature, 20% manipulated feeding, 30% of them manipulated both temperature and feeding, while 10% of them operated RAS systems, which meant optimum conditions for proper metabolism.

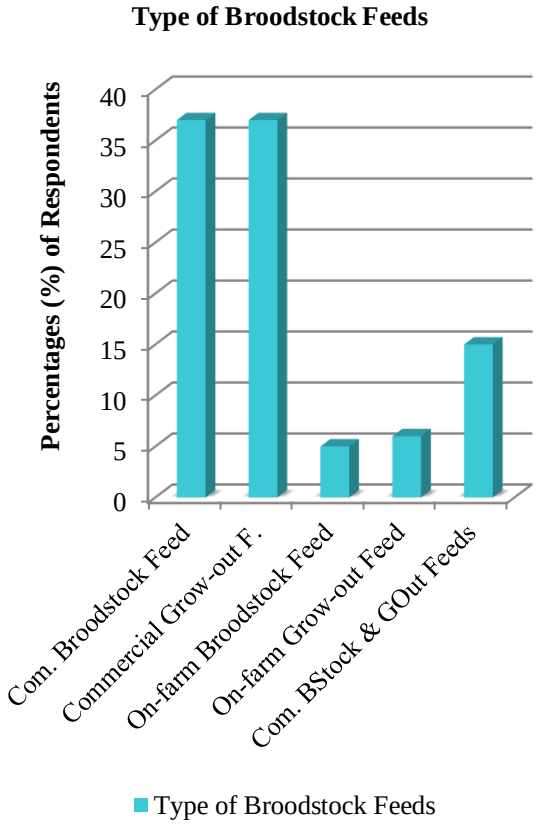

Figure 2.16. Types of broodstock feed used by respondents (n = 112)

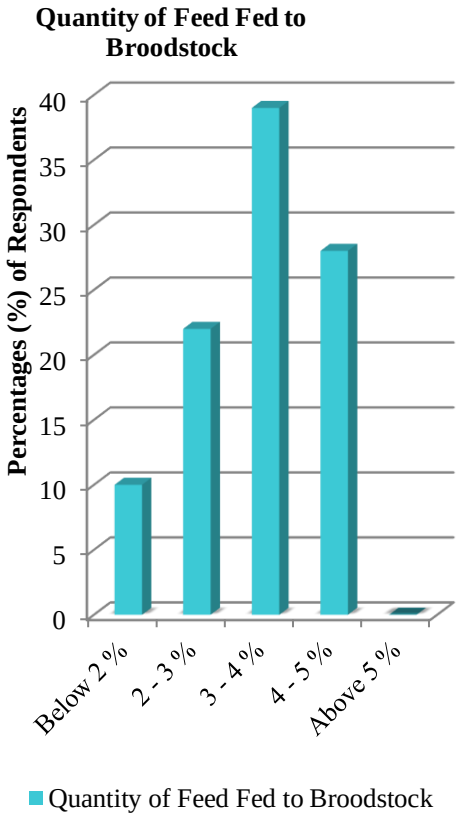

Figure 2.17. Quantity of broodstock feed fed as a percentage of body weight day^{-1} (n = 112)

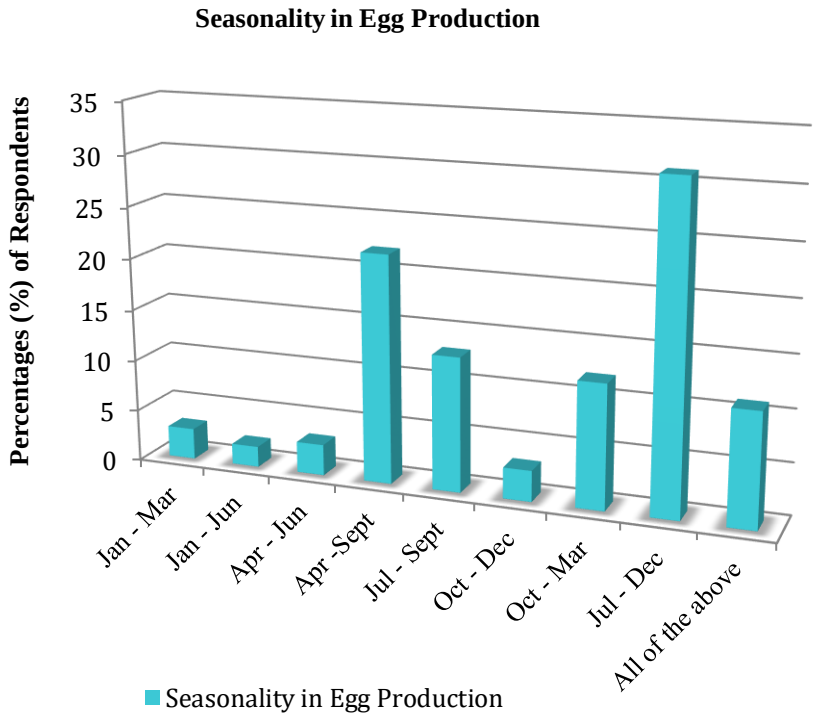

Figure 2.18. Seasonality of *Clarias gariepinus* broodstock production (n = 120)

The cost of broodstock production varied considerably as 55% of respondents spent ≤ $ 3.00 kg^{-1} of broodstock produced, 23% spent $ 3.00 – $ 4.20 kg^{-1}, while 21% spent above $ 4.20 kg^{-1} of broodstock produced. On a reassuring note, 94% of respondents profitably sold broodstock for $ 5.56 – $ 8.33 kg^{-1} and are largely patronised by hatchery owners (77%) from different parts of the state, zone and country.

2.6.7. Fingerlings/Juveniles Production and Management

This survey examined specialisation in different aspects of the hatchery value chain. The study revealed that 13% of respondents engaged exclusively in hatchery operations (breed and raised only to post-fry and fingerlings), 4% had exclusively nursery operation (bought post-fry and fingerlings and raised to juveniles), 24% are involved in both hatchery and nursery, 3% were exclusively involved in broodstock production and 30% were involved in hatchery, nursery, broodstock and grow-out operations, as shown in **Figure 2.19.** 85.22% of hatcheries surveyed used flow-through (in some cases with aerators), 10.43% used aerator systems and 4.35% used RAS. 74.29% of nursery operations used indoor flow-through tanks (plastic, fibreglass, metallic, wooden and concrete tanks), 2.86% used RAS systems, 5.71% used outdoor tanks (mostly made of concrete), 11% in earthen ponds, while 6.13% used a combination of indoor flow-through system and outdoor tanks / earthen ponds **(Figure 2.20.).**

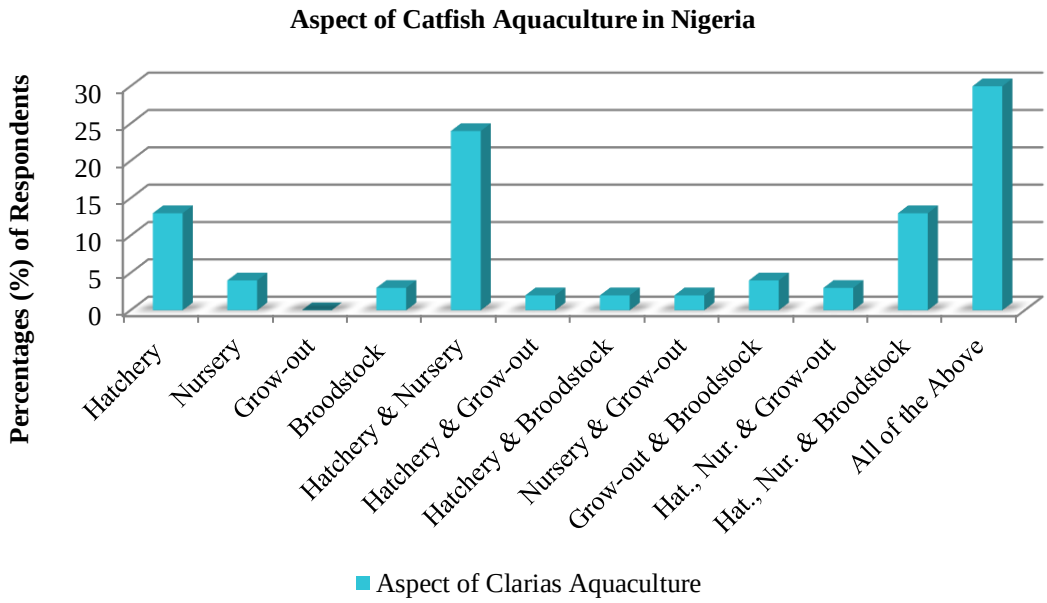

Figure 2.19. Different aspects of catfish aquaculture engaged in by respondents (n = 120)

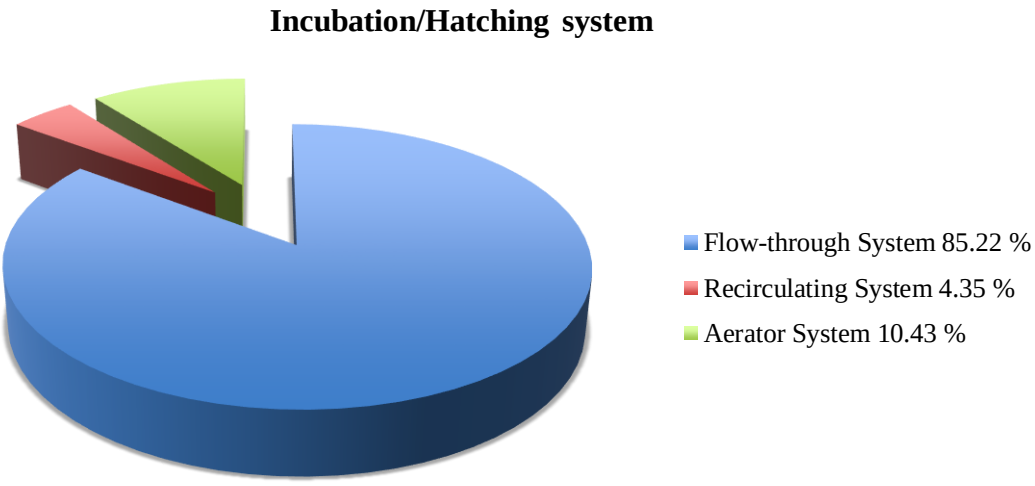

Figure 2.20. Incubation and hatching systems (n = 120)

The majority of respondents (86%) produced between 200,000 – 800,000 fingerlings/juveniles per annum as summarised in **Figure 2.21.** Stocking densities in the majority of hatcheries (97%) ranged between 2,000 – 5,000 fingerlings m^{-3}. Fingerlings in this context were 3 - 8 week old catfish with average weight of 1.0 – 1.5 g in weight (as attested to by 96% of respondents) and length < 4 - 6.0 cm (for 98% of respondents) **Figure 2.22.** On the other hand, stocking densities in majority of the hatcheries (95%) ranged between 1,000 – 3,000 juveniles m^{-3}. Juveniles according to respondents were 8 - 10 weeks old catfish, of 2.0 - 2.5 g in weight and ≥ 6.0 cm as shown in **Figure 2.23.**

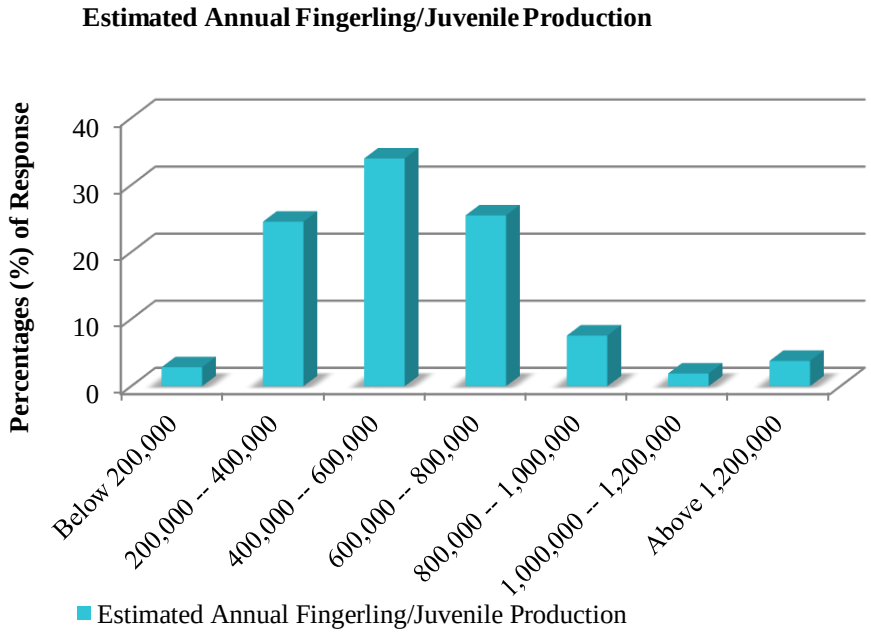

Figure 2.21. Seed (Fingerling and Juveniles) Production in Nigeria

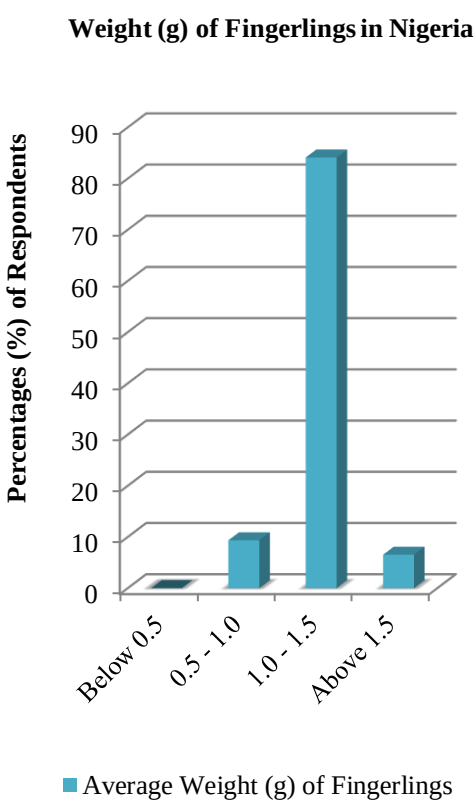

Figure 2.22. Average weight of fingerlings (n = 81)

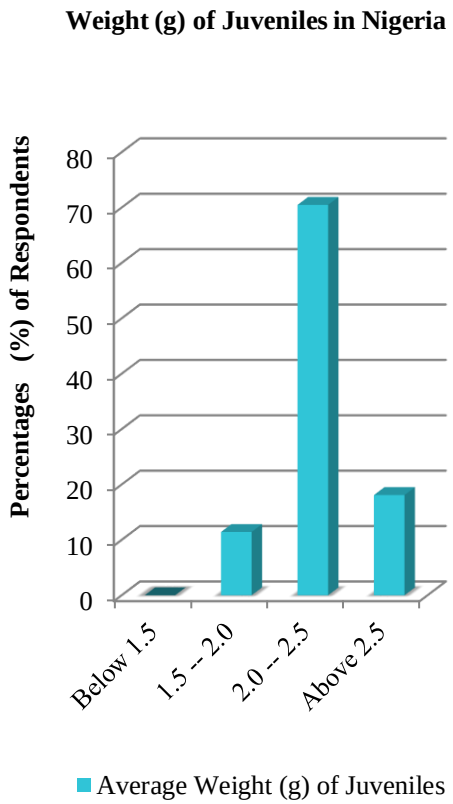

Figure 2.23. Average weight of juveniles (n = 81)

2.6.8. Other Issues

89% of respondents were sole owners of the hatchery for commercial production, while 5% were family businesses, 3% partnerships and 3% research hatcheries. **Figure 2.24.** shows that 77% of workers in the hatcheries surveyed were male while 23% were female. 54% of respondents had degrees and for higher national diplomas (HND) in different disciplines, including few with Fisheries and Aquaculture related courses, 26% had ordinary national diplomas (OND) and the remaining 20% had senior secondary certificates. Furthermore, when asked what the sources of skills and knowledge for catfish hatchery operation and broodstock management were, 47% of respondents learnt from family and friends, 32% attended short training sponsored by government empowerment programmes, 4% attended privately funded professional training, 3% had diplomas and 14% had degrees in fisheries and aquaculture related courses respectively **(Figure 2.25.** below**)**.

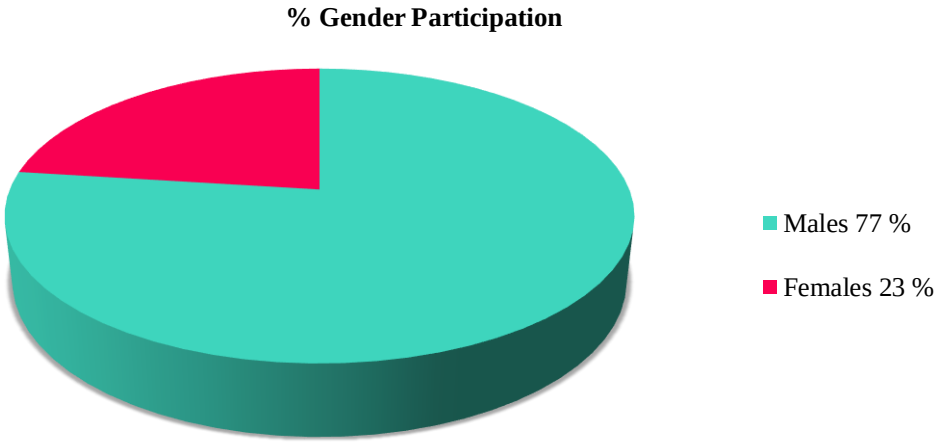

Figure 2.24. Percentage of male and female workers in *C. gariepinus* hatcheries surveyed

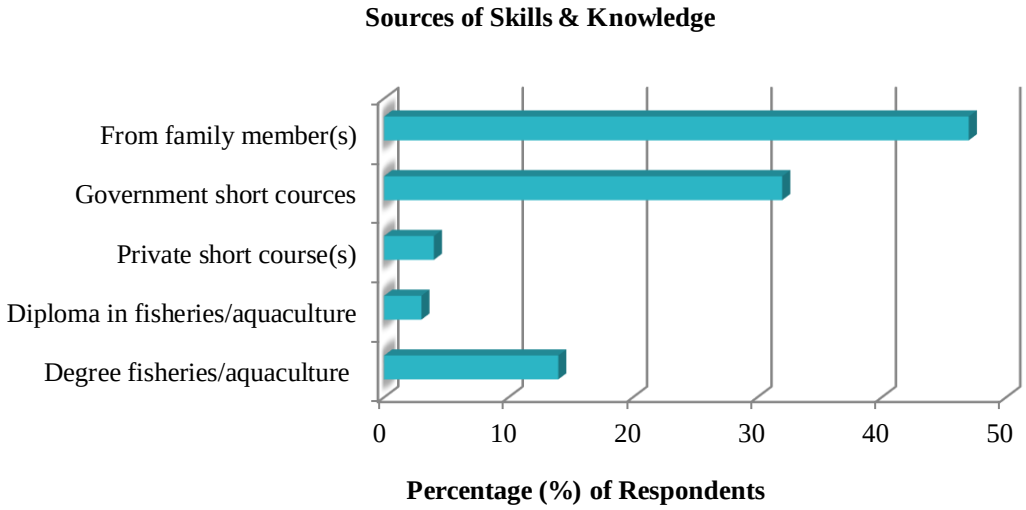

Figure 2.25. Sources of skills and knowledge for operating *C. gariepinus* hatcheries (n = 77)

2.7. Discussion

Just like many other developing aquaculture industries in the world, the Nigerian aquaculture industry is not without its challenges despite its successes and growth. Broodstock quality appeared to be the least concerning of the main challenges asserted to by respondents from this survey, however, many reports and publications have repeatedly mentioned inadequate supply of good quality fingerlings as one of the two major challenges of the Nigerian aquaculture industry (Adewumi and Olaleye, 2011; Akankali *et al.*, 2011; Emmanuel, *et al.*, 2014). The contradiction here might be due to the following:

a. The scale of other challenges, which when compared to broodstock quality could be perceived as more prominent. This is because in the past 2 decades, Nigeria has been gripped with unreliable power supply. In 2014 for instance, Nigerians experienced power outage 32.8 times in a month and each time, an outage lasted an average of 11.6 hours (World Bank, 2015). Inadequate power supply which 27% of respondents chose to be their major challenge in this survey meant increased production cost (cost of fuelling generators to supply electricity to pump water, aerate the fish tanks, etc.), reduced yield and limited ability to expand (decreased water quality and quantity, and increased disease and mortality rates), hence, limiting efficiency, productivity and profitability in hatcheries. This is especially a problem as many hatcheries continue to adopt peri-urban aquaculture, abandoning the semi-intensive culture systems in earthen ponds, and heading for the more intensive indoor production systems, in tanks, where optimum water quality is completely dependent on electricity. Insufficient market (21%) and high input costs (19%) (Artemia, feeds, broodstock, fuel, etc.,) recorded may be linked to the devaluation of the Nigerian currency (Naira) as a result of the drop in global prices of crude oil in 2014 (Emefiele, 2014; Abraham, 2016; Oxford Business Group, 2016). Being an oil-dependent economy, the drop in price indirectly led to increase in the prices of imported feed, upon which almost all hatcheries depend. The market of fingerlings was affected since many on-growers found aquaculture unprofitable (due high cost of feed and feed materials) and as such, a reduced demand of fingerlings. Motivated by the commercial viability of catfish hatchery business, many hatchery owners "as investors" will perceive inadequate power, market, water, disease issues and high cost of input as major challenges over broodstock quality, which only 4% of respondents acknowledged to be a major challenge.

b. The fact that many hatchery managers and or owners are non-scientists, the lack of knowledge and understanding of the genetics and genetic potentials of different strains and population of *C. gariepinus* could limit the ability to appreciate issues around broodstock quality in the Nigerian aquaculture industry, hence, the low result. Inbreeding depression for instance has been reported by some authors to be very prevalent in the Nigerian catfish industry

(Olatunji-Akioye *et al.*, 2010; Megbowon *et al.*, 2014; Ikpeme *et al.*, 2016; Iwalewa *et al.*, 2017; Nyunja, 2017; Awodiran and Afolabi, 2018). Without proper knowledge and assessment of stocks, it is difficult to know if the disease issues recorded as a major challenge in this study, is actually endemic or as a result of decreased disease resistance/increased susceptibility due to inbreeding depression. Also, the fact that many respondents buy males and females from separate farms is an indication of insight into an existing broodstock quality issues.

c. The fact that many hatcheries are also involved in grow-out, and/or sell fingerlings and juveniles within their locality makes performance evaluation beyond the hatchery phase more feasible. However, it is difficult to tell if the perception of poor vs good broodstock quality is based on (i) empirical data (e.g. from on-growers), (ii) experience or (iii) driven by the need to uphold the hatchery's business reputation of having good quality broodstock. It will be of immense contribution if a future study is conducted exclusively on the quality of catfish raised by on-growers. This will erode doubts and provide an unbiased insight into the quality of stocks raised in the industry.

2.7.1. Broodstock Types and Origin

The Nigerian aquaculture industry is dominated by *C. gariepinus* (62% of respondents) and their hybrids (30% respondents) – *Heteroclarias*, a cross between a male *Heterobranchus spp* and a female *C. gariepinus*, and vice versa for *Clariobranchus*. Since the inception of hatchery technology for catfish in Nigeria, *C. gariepinus* has been more popular than the other catfishes due to the relative ease of broodstock maturation, breeding and management. Although *C. anguillaris* was mentioned by only 8% of respondents, a study on the identification of *Clarias* catfishes **(Chapter 4)** has shown that the popular morphometric identification index used by farmers in Nigeria (i.e. the vomerine teeth) is unreliable, inconsistent and inaccurate, thus, cannot be relied upon to separate *C. gariepinus* from *C anguillaris*, which occur sympatrically. *Heterobranchus longifilis* (Valenciennes, 1840) matures at 12 – 14 MPH, has a lesser GSI, but grows faster in the hatchery than *C. gariepinus* (Legendre *et al.*, 1992; Adebayo and Fagbenro, 2004;

Azeroual *et al.*, 2010). *Heterobranchus bidorsalis* (Geoffrey, 1809) on the other hand, matures at the age of ≥ 12 and ≥ 24 months for males and females respectively. They have a lower GSI and a much slower growth rate in the hatchery phase when compared to both *Clarias gariepinus* and *H. longifilis*. Both species of *Heterobranchus* mature in captivity, however, their high rates of cannibalism, relatively lower fecundity and GSI both in males and females, delayed female maturation in *H. bidorsalis* and seasonality in egg production make them less popular/attractive to breeders when compared to *C. gariepinus*. At grow-out phase, the hybrids *(Heteroclarias and Clariobranchus)* however, have faster growth rate, tolerate higher stocking densities and have higher dress out percentage than *C. gariepinus* (Legendre *et al.*, 1992; Oellermann, 1996; Nguenga *et al.*, 2000; Toko *et al.*, 2007), hence, they are increasingly becoming popular. Some hatchery owners have reported higher survival rates in the hybrids when compared to pure *C. gariepinus* under same breeding and culture conditions. It is important to mention here that, unlike in *C. gariepinus*, broodstock and fingerlings of *Heterobranchus spp* are collected from the wild without resultant consequences of poor performance (e.g. growth rate, feed utilisation, fecundity), as recorded in some wild strains of *Clarias*, hence, only 2% of hatcheries surveyed source *C. gariepinus* broodstock from the wild in Nigeria. The remaining 98% source from other farms or government institutions, which currently is populated by the Dutch domesticated strain of *Clarias gariepinus* and some of South African origin. They grow faster, have better feed utilisation, higher fecundity and are well adapted to varied intensive culture systems, when compared to the indigenous or wild strains of *C. gariepinus* (Megbowon *et al.*, 2014).

2.7.2. Broodstock Culture Systems

The fact that more hatcheries (44%) now grow broodstock in outdoor tanks than in earthen ponds (40%) as reported from this survey is an indication that hatchery production is becoming more peri-urban. This is not surprising because, following the rise of peri-urban aquaculture in Nigeria, there are many more on-grower farms are springing up in urban centres (Miller and Atanda, 2011), where there are access to inputs, market, security, etc.. This is increasingly possible due to the increased availability of reliable underground water, plastic (collapsible, polypropylene (PP), PVC etc.,) tanks, fibreglass and concrete tanks, which can be fitted/constructed anywhere with little or no regard for the soil's water retention capacity, as required in the case of earthen ponds. The increasing presence of

these on-grower farms, overall increase in demand for good quality fingerlings and the fact that fingerlings are currently mostly produced in very intensive culture systems, using underground water, thus, requiring electricity and other inputs, may explain the increased presence of hatcheries to supply fish farms in the urban centres. Therefore, the increased use of outdoor tanks as broodstock culture/holding facility have been seen instead of earthen ponds in residential areas. The increased presence of hatcheries in cities has also encouraged female participation in fish hatcheries, as 23% of workers in hatcheries surveyed were females. Some hatcheries grew broodstock in earthen ponds and later transferred to holding tanks (concrete, plastic and or fibre) for conditioning. Furthermore, over 95% of respondents used underground water (wells and boreholes), thus further enabling broodstock production anywhere and perhaps explaining the low pathogenic disease issues recorded in the Nigerian aquaculture industry.

2.7.3. Broodstock Evaluation

Matured broodstock of ≥ 12 months old, weighing ≥ 1.0 kg were mostly used in Nigerian catfish hatcheries. They have been reported to have high GSI (mostly 15 – 20%), hatchability (mostly above 75%) and survival rates (mostly above 50%), and are mostly of farm origin as described above. The GSI recorded by most respondents agreed with the findings of Eyo *et al*. (2014) and Al-Deghayem *et al.* (2017), while the hatchability and survival rates agreed with the findings of Owodeinde and Ndimele (2011), Ondhoro *et al.* (2015) and El-Hawarry *et al.* (2016). Many hatcheries in Nigeria start and work with small populations that came from a single or two batches (mostly ≤ 4 families) of fish grown on the farm or bought over from another farm. Although results from this survey reveals relatively large number of broodstock are kept in hatcheries annually (42% of respondents keep ≥ 300 and 45% of respondents keep between 100 – 299 broodstock year^{-1} respectively), 97% of all respondent's broodstock came from < 25 families.

Due to the relatively high fecundity in *Clarias*, there are tendencies that few broodstock are used in breeding while others are sold. This survey revealed that < 50% of the broodstock (i.e. ≤ 12 families) contributed to the next generation (of broodstock replacement). The estimated N_e for majority of the respondents 133.33, while the census number is 150 (considering only 50% of the population contributing to the next generation). An N_e of 66.67 was estimated from a census number of 75 (when a maximum

of 25 families are considered). Coefficient of inbreeding of 0.75% and 0.60% per generation for the two respective scenarios seems like a healthy breeding population, going by Tave (1993) who recommended a maximum of 5 - 10% per generation involving a minimum of 50 broodstock. As commercial operations with such number of broodstock, it is expected that it will take several generations before inbreeding sets in. However, due to other factors such as mating siblings, skewed sex ratios, high fecundity and most importantly the possibility of founders effect as a result of the introduction in the Dutch domesticated strain of *Clarias*, one cannot rule out the possibility of inbreeding depression already existing in the *C. gariepinus* strains cultured in Nigeria. This is especially because only 6% of respondents who bought broodstock sourced exclusively from a single farm. As much as 56% make effort to source from multiple farms while the remaining 38% source from both single and multiple farms goes to tell that there are some levels of appreciation of variation and inbreeding depression. This awareness cutting across a total of 94% of respondents, who bought fish for broodstock replacement, could suggest the possibility of an underlying problem, hoping to be solved by genetic variation and cross breeding. The level of genetic variation in the farmed stock could have been significantly reduced (due to founders effect) so much so that the present mating system used in the industry can only sustain it only for a temporary period of time. There isn't documentary evidence of the exact time, number of times and number of broodstock brought in from the Netherlands, it is however, known to be in the early 2000s (Miller and Atanda, 2011) and there are farms producing catfish on a large scale in RAS and earthen ponds who have also brought some broodstock form The Netherlands and South-Africa. With a generation time of 8 - 12 months, the earlier introduced Dutch Domesticated strain of *C. gariepinus* would have spent almost 20 generations by now. Depending on their number and level of genetic variation within strain, 20 generations is long enough to allow for founder effect, inbreeding depression and bottlenecks to occur, possibly resulting to reduced survival and yield witnessed in the *C. gariepinus* aquaculture industry in recent times (WorldFish, 2017). There is also no empirical evidence against this claim as at yet, however, the development of molecular markers and evaluation of different strains and populations *C. gariepinus* for genetic management and suitability for aquaculture respectively in (**Chapter 4**) will seek to assess the levels of variation within and between the farmed strains and wild populations of *C. gariepinus*. This is with a view of setting up a selective breeding programme for *C. gariepinus* in Nigeria.

Continuing fish breeding against the possibility founder effect further reduces the N_e (i.e. the effective number of individuals (broodstock) producing viable offspring in the next generation) especially with evidence of skewed sex ratios and variance in contribution (e.g. survival rate) among families (Tave, 1993; Tave and FAO, 1995; Tave and FAO, 1999), and as N_e decreases, ΔF increases. Furthermore, the type of crosses employed in the hatchery, the sex ratios and the manner in which the milt is used to fertilise eggs (i.e. whether milt from different males is pooled together or used separately) could bear on the N_e. Although the most common sex ratios are 1 male : 2 females, in practical terms 2 males : 4 females are actual numbers bred by the respondents at most breeding times. Under such circumstances, it is very unlikely that milt from different males are not sometimes combined before fertilisation. In addition, the frequency with which the same females are used in their life time is a factor not to be overlooked, in that, farms in which potential recruits are selected from multiple batches in a year might select from siblings (mostly half-sibs) from the same female as most farms surveyed reuse the same female 2 – 3 times per year, for up to 3 years. This practice creates half-sibs from one female and different males, and these might be mated together at some point. The effect of the above practices is likely to reduce genetic variation and eventually increase inbreeding. If selective breeding is applied in such populations, there could be "bottle-necks", leading to decreased N_e and genetic drifts, especially in an un-pedigreed population (Tave, 1993; Tave and FAO, 1995; Tave and FAO, 1999).

2.7.4. Broodstock Replacement and Management Strategies

Only 2% of the respondents sourced *C. gariepinus* broodstock from the wild. This is not surprising due to the poor growth rate of the wild stock mentioned above. However, there is need to evaluate the wild stock (along side the farmed ones) to ascertain the level of genetic variation in them and potentials for genetic improvement. The fact that 76% of respondents who buy broodstock, buy them as table fish, from on-growing farms to further grow them to become broodstock (recruits) is an indication of the very high cost of actual broodstock in Nigeria. The number of batches, frequency of purchase, genetic relatedness of stocks on the farm(s) of purchase, manner in which broodstock are raised, mating design etc., determine the level of genetic variation or inbreeding within the eventual broodstock. Crosses made for sales of fingerlings to on-growers are often for the most parts 1 - 3 families, and a batch could be sold to multitude of farmers. Purchasing potential

broodstock by these farms meant potentially sourcing broodstock from very few families and possibly related individuals, hence, increasing the chances of inbreeding. Furthermore, due to limited facilities and knowledge, 73% of respondents raise similar sized recruits in the same tank and or pond, with no effort or means of identification, thereby, increases the chances of mating relatives, thus, increasing inbreeding. Similarly, the majority respondents who kept different batches separately mostly do not have means of identifying them to individual or family level.

Additionally, 92% of respondents who hatch and select broodstock on their farms select shooters from batches of *Clarias gariepinus* fingerlings or table fish produced for sale to use as broodstock. This is due to the perception that the fast growth rate in shooters is due to a superior genetic makeup – a perception derived from terrestrial animal husbandry (e.g. poultry, sheep and goats), whose feeding behaviour differ completely from shooters (the majority of which are cannibals). Although various management practices such as grading, increasing feeding frequencies, stocking densities etc., are practically employed to reduce mortality due to cannibalism by mostly shooters, it is imperative to investigate/study to ascertain if the fast growth rate in shooters is genetic, environment or an interaction of both (G x E). Such a study will also seek to understand the possible link(s) between cannibalism in shooters and aggression, and if such are heritable traits or not. Finally, it should also establish whether the shooters consume more feed or have better feed conversion efficiency.

It is unclear what sources of commercial broodstock feed are available in Nigeria. What is apparent from this study is that the crude protein (25 – 40% CP) and feeding rate (2 – 5%) of most respondents are in line with the recommendations of Ayinla (1988), de Graaf and Janssen (1996), Eyo *et al.* (2016), FAO (2010) and Ali and Jauncey (2005). The quality of commercial grow-out feeds in Nigeria is varied. Some studies suggest better fecundity with broodstock diet fed at 3% body weight day^{-1} when compared to grow-out diets, which the study noted to have produced better growth rather than fecundity (Eyo *et al.*, 2016). Accumulation of visceral fat is noticed in some dissected males fed commercial grow-out diets at high feeding rates, a practice that reduces spawning successes. Assessing the quality of broodstock diets only based on its crude protein content (as is the case by farmers in Nigeria), ignoring the protein energy ratios, digestible and metabolisable energies, and other essential nutrients (amino and fatty acids) therein, makes it impossible

to know if the commercial broodstock and grow-out feeds meet the nutrient requirements of *C. gariepinus* broodstock. Therefore, the industry will benefit from the availability of good quality, traceable, affordable commercial broodstock feed to reduce the current high production costs, thus, making broodstock more affordable, especially to smaller hatcheries.

Seasonality of egg production in *C. gariepinus* is very common amongst respondents (89%) as only 11% of them could produce gravid broodstock all year round. This is largely due to the fact that these broodstock are raised/held in outdoor tanks and earthen ponds, exposing them to the cold weather conditions of the months of December to middle of March, and its diurnal fluctuating temperatures from as low as 9 °C at night to as high as 30 °C in the afternoon. Sub-optimal and fluctuating temperatures affect feed intake, metabolism and egg production in *C. gariepinus* broodstock and growth in fingerlings (Hogendoorn and Vismans, 1980; Britz and Hecht, 1987; Richter *et al.*, 1987; Sapkale *et al.*, 2011). The absence of rain in these months in many parts of the country contributes to the lack of egg production in the outdoor broodstock facilities, as rainfall is one of the natural triggers of vitellogenesis in *C. gariepinus* in the wild.

2.7.5. Fingerlings/Juveniles Production and Management

Demand for African catfish seeds (fingerlings) in Nigeria has steadily increased due to increased investment in aquaculture to meet the huge production and supply deficits. Some literature has described the weight of standard fingerlings to range from 5 – 10 g (De Graaf and Janssen, 1996; Ponzoni and Nguyen, 2008). It is important to state that in the Nigerian context, according to the majority of respondents, average weight of fingerlings and juveniles range from 1.0 – 1.5 g and 2.0 – 2.5 g respectively. More than 95% of fish hatcheries are privately owned in Nigeria. With 85.71% of respondents producing between 200,000 – 800,000 fingerlings/juveniles per annum, in indoor flow-through systems (tanks), at stocking densities ranging from 2,000 – 5,000 fingerlings m^{-3}, using good quality micro-diets ≥ 50% C.P., it is safe to describe fingerling production in Nigeria as mainly an intensive indoor operation. Despite a very high stocking density (≥ 20,000 fingerlings m^{-3}), the high initial capital required and complete reliance on electricity, limits the adoption of RAS system in Nigeria. Breeding and management technology for *C. gariepinus* has been mastered by many hatchery managers/owners through training,

adaptation and years of experience, despite the challenges in the industry. Despite the cost of aeration and pumping, access to underground water enables the use of disease-free water, with fairly consistent and optimum temperature and other parameters in the hatchery. The introduction of shell-free Artemia into the Nigerian markets in the mid-2000s almost eliminated the long-standing problems of using inadequate zooplankton, the expensive/laborious and electricity-dependant live-Artemia, and inefficient compounded feeds. Furthermore, the availability of high quality, nutrient dense, mostly imported, expensive but efficient micro-diets and larval feeds enabled intensive culture of *C. gariepinus* seeds. The relatively low cost of investment and quick return on investment also made it an attractive venture. The inability to distinguish bad quality from good quality fingerlings has in some cases led to some farmers delve into producing fingerlings for themselves and for commercial supply without adequate training. The lack of regulation and control in the aquaculture sub-sector in most parts of the country, and all the aforementioned factors have contributed to indiscriminate establishment of *C. gariepinus* hatcheries across Nigeria.

While breeding and management of *C. gariepinus* seems straight forward, it is important to stress here that it is just one side of the coin. The other and most critical side of the coin is broodstock quality and management, hence, fingerling quality – a chicken and egg situation, which comes first? Despite the fact that 54% of respondents have tertiary educational qualifications, only 14% had degrees and 3% diplomas in Fisheries and aquaculture related courses, with the overwhelming majority of hatcheries owners/manager having only short trainings from family, friends or government short courses. It might be too demanding to expect proper understanding of issues around genetic management of broodstock, variation, inbreeding depression, heritability of traits, etc., which are the back bone of broodstock quality. It is therefore pertinent that selected farms, government institutions or groups of farms, etc., set up a breeding programmes centre, just as the National Biotechnology Development Agency, Abuja, Nigeria is pioneering for *C. gariepinus* in Nigeria. Regulation through certifications and trainings by the government, NGO or a collaborative effort should be put in place to ensure best practice and linkages with sources of inputs.

2.8. Conclusion

Since there is no data on the exact number of fish hatcheries in Nigeria, it is difficult to say what percentage the 120 hatcheries make of the total number of hatcheries in the country. However, the fact that most of the selected hatcheries were from south-western Nigeria (the region with the highest number of hatcheries and catfish farms in the country) and that members of catfish farmers association of Nigeria (CAFAN) were involved in the selection of farms and during the survey, could suggest an unbiased and representative choice of farms. The choice of states and manner in which the hatcheries were selected for the survey attempts to give a holistic picture of the practices, problems and prospect of hatcheries in the Nigerian aquaculture industry. What is apparent though is that although there are appropriate inputs and technology for hatchery operation, there is significant dearth of knowledge on proper broodstock management. This is due to the fact that many hatchery operators only had short training or courses, and as such, there is great need for knowledge, education, training and regulation of practices in the hatchery sub-sector. Due to poor yield, wild *C. gariepinus* broodstock are neglected as a source of broodstock. There is however, the need to evaluate the wild stock (alongside the farmed ones) to ascertain the level of genetic variation and potential for genetic improvement. The use of shooters as broodstock is a very common practice, as such there is need to investigate whether or not shooters actually have superior genetics and if such practices are beneficial or not. Commercial grow-out diets are also used to feed broodstock, as such; there is need for research into developing reliable and affordable broodstock feed. The Nigerian government needs to look into the power (electricity) needs of fish hatcheries, invest in research on the feasibility of solar-powered fish hatcheries, and provide subsidies on inputs and consultancy services to ensure growth in the hatchery subsector. Lastly and most importantly, there is need for improved genetic management and selective breeding to improve growth rate, feed utilisation and survival, to ensure sustainable growth and development of the Nigerian Aquaculture Industry.

CHAPTER 3. DEVELOPMENT OF DIAGNOSTIC SNPs TO SEPARATE *Clarias gariepinus* (Burchell, 1822), FROM IT CLOSEST RELATIVE *Clarias anguillaris* (Linnaeus, 1758)

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Experimental design, set-up and fieldwork were carried out by the author of this thesis under the supervision of David Penman John B. Taggart and Michael Beakert. The author carried out all fin clipping, measurement of phenotypes, DNA extraction and quantification. ddRADSeq was carried out by John Taggart and the author, while bioinformatics by Michael Beakert, John Taggart and Stefanie Wehner. Kerry Bertie and Bram Meersman did SNP assaying on for his MSc project, looking at some of the samples collected by the author. The author genotyped most of the samples and carried out all the statistical analysis, compiled the entire chapter. Every other content of this chapter not mentioned here was carried out and all the content compiled written by the author and under the supervision of David J. Penman.

3.1. Abstract

African catfish (*Clarias gariepinus* (Burchell, 1822)) is an important species for aquaculture in the Sub-Saharan Africa, some parts of South America, Asia and Europe. Despite this status, *C. gariepinus* is still not easily distinguishable from other members of its genus and sub-genus.

This study represents the first application of next generation sequencing to develop markers to distinguish between putative *C. gariepinus* and *C. anguillaris*. Double digest RAD-seq (using *Sbf*1 and *Sph*1 restriction enzymes), was used to generate over 2,500 SNP markers. These SNP markers were used to produce a phylogeny of the two putative species and an out-group (*H. longifilis*). Principal component analyses of this dataset clearly separate these two species and the out-group from one another. When 1-2 SNPs were allowed per ddRAD locus, 24 species-specific SNP markers were identified across the set of three species of *Clariidae*. Larger numbers of species-specific markers were found when more than two SNPs were allowed per locus. Screening of a set of such SNPs (8 selected SNPs) using a larger test panel of putative *C. gariepinus* and *C. anguillaris*, showed over 99% discrimination between species. All members of the Dutch domesticated strain of *C. gariepinus* from three farms in Nigeria, three farms in The Netherlands, a source of the Hungarian and Polish strains, all shared the same genotype as the wild *C. gariepinus* from Rivers Niger and Benue, and stocks from Egypt (the WorldFish centre) and Zambia. *C. anguillaris* had a distinct genotype and this was consistent across the batches sampled.

From this study, vomerine teeth and the number of gill rakers on the first branchial arch popularly used in the identification of both species in Nigeria and other parts of the world have been confirmed to be inaccurate.

Further studies on correlation between genotypes and morphological indices are recommended. Studies involving larger sample sizes, especially *C. anguillaris* from different locations/countries is also recommended. This approach looks auspicious and could be vital to studies investigating hybridisation, introgression, genetic and population structures in both morphologically identical species for aquaculture and conservation purposes.

3.2. Introduction

African catfish *Clarias gariepinus* (Burchell, 1822) is an important species for aquaculture in the Sub-Saharan Africa, some parts of North Africa, South America, Asia and Europe (FAO, 2014a). Its fast growth rate, high fecundity, adaptation to varied culture environments and conditions has made it the choice of many fish farmers in the sub-Saharan Africa and a good species for peri-urban aquaculture, livelihood and sustainable development projects. Its ecology, naturally spanning many countries in Africa, makes it a very popular fish species already. *C. gariepinus* is increasingly gaining attention in many African countries beside Nigeria. Uganda, Kenya and Egypt are examples of countries with special focus on *C. gariepinus* (Ponzoni and Nguyen, 2008).

Despite this, *C. gariepinus* is still not easily distinguishable from other members of its genus and sub-genus (Teugels and Centrale, 1982; Teugels, 1986; Agnèse *et al.*, 1997; Nwafili and Gao, 2007; Compaoré *et al.*, 2015). This is especially true for its closest relative, *Clarias anguillaris*: several authors hinged the distinction (identification) on their ichthyology, morphology and anatomy (Debouche *et al.*, 1979; (Teugels and centrale, 1982; Teugels, 1986; Agnèse *et al.*, 1997; Rognon *et al.*, 1998; Yisa and Olufeagba, 2005; Hanssens, 2009; Wiecaszek *et al.*, 2010; Compaoré *et al.*, 2015; Zakariah *et al.*, 2016)

3.2.1. Geographical Distribution

Different fishes belonging to the family *Clariidae* naturally occur in Africa, Asia-minor, the Indian subcontinent, and in South-East Asia (Teugels and Adriaens, 2003; Hanssens, 2009).

3.2.1.2. Geographical Distribution of *Clarias gariepinus*

C. gariepinus, with the common name "North African catfish" is found across almost all of Africa (pan-African distribution **Figure 3.1**), migrating laterally from large water-bodies where they feed and grow to smaller water bodies (e.g. lakes and seasonal pools) where they spawn (Hogendoorn and Vismans, 1980; Hecht, 1988; FAO, 2014a). It was also confirmed to be present in Asia Minor, including Jordan,

Israel, Lebanon, Syria and Southern Turkey (Turan *et al.*, 2005; FAO, 2018c). In 2014 the annual aquaculture production was 237,124 MT, with Nigeria being by far the largest producer of *C. gariepinus* in the world (Dauda, *et al*., 2018; FAO, 2018a). Other countries producing *C. gariepinus* include Thailand, Indonesia, Uganda, Malaysia, the Netherlands, Philippines, Hungary, Syria, Cambodia, Poland, Brazil, Kenya, Mali, Belgium, Togo, Romania, Italy, Cameroon and South Africa. In China, *C. gariepinus* has been adopted for culture within its rice fields, while Egypt pioneered research on selective breeding for this species (Ponzoni and Nguyen, 2008; FAO, 2018a).

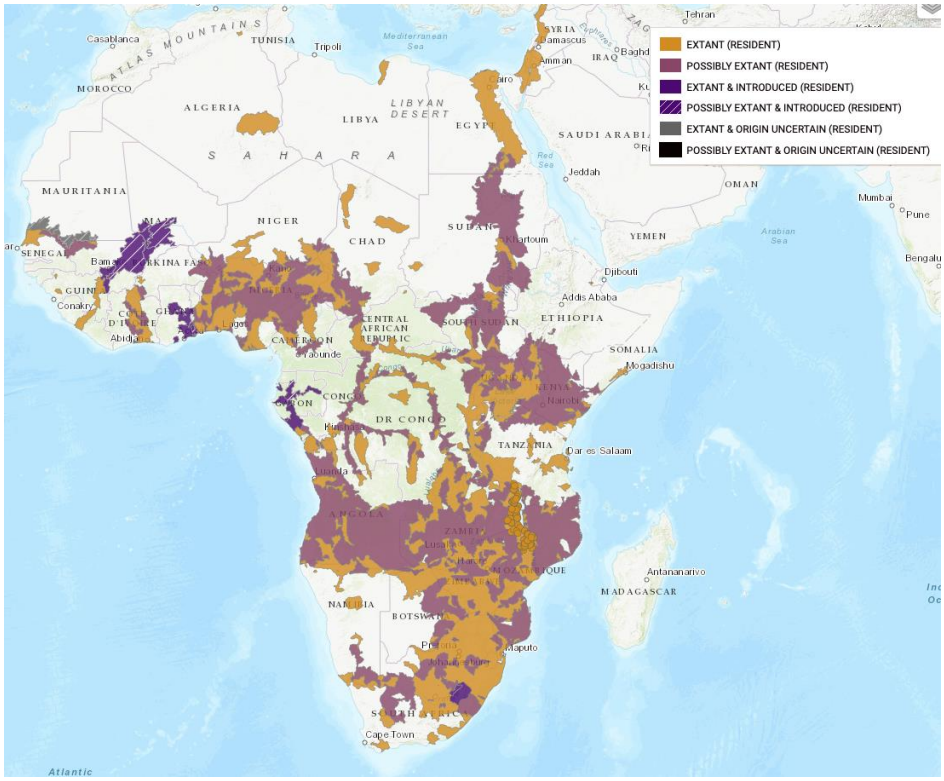

Figure 3.1. Geographical distribution of *C. gariepinus* (Source: Freyhof, *et al.*, 2016)

3.2.1.3. Geographical Distribution of *Clarias anguillaris*

C. anguillaris, first described in 1758 by Linnaeus, is commonly called the "Mudfish" (FishBase, 2000). Unlike its close relative, *C. anguillaris* only occurs naturally in Africa: mostly in West Africa and parts of the Nile as reported by Johnels (1957), Lévêque *et al.* (1991) and FAO (2018b). They extend from the lower Senegalese rivers, through the Gambia, Togo and Burkina-Faso, to the Rokkel basin and River Jong in Sierra Leone. Furthermore, they inhabit the Bandama basin in Cote d'Ivoire,

the Njala, Mano and Mattru water systems in Guinea Bissau, the Volta system in Ghana and the upper Niger River Basin spanning Guinea, Mali, Benin, Niger and Nigeria. *C. anguillaris* is also present in Cameroun, Chad (Chad basin) and through to South Sudan, Ethiopia, Sudan and Egypt **(Figure 3.2)**. Currently, Egypt is the largest producer of this species in the world, accounting for 99.2 % of the global production, with Senegal producing the remaining part. Total annual production across the globe (both capture and culture) in 2015 was estimated at 30,711 MT, fluctuating between 26,000 – 43,000 MT since the year 2000 (Tridge, 2018; FAO, 2018c). Unlike its closest relative *(C. gariepinus)*, documented annual aquaculture production (1989-1992) never exceeded 3 MT, suggesting a lesser aquaculture of this species.

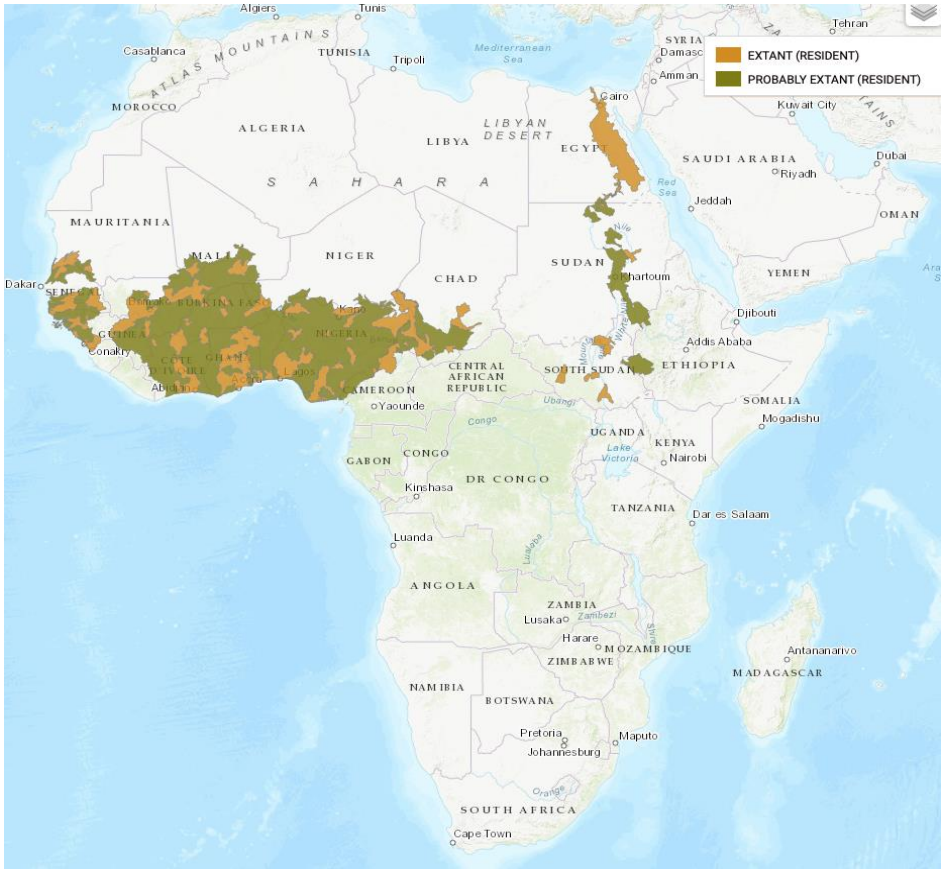

Figure 3.2. Geographical distribution of *C. anguillaris* (Source: Azeroual, *et al.*, 2016)

3.2.2. Classification (Ichthyology) of *Clarias*

C. gariepinus and *C. anguillaris* are freshwater fish species, inhabiting and migrating within and between different tropical water bodies (such rivers, lakes, seasonal pools,

dams, swamp etc.). They belong to the family *Clariidae* and genus *Clarias* (Lagler *et al.*, 1977; Moyl and Cech, 1988). Currently, there are 16 recognised genera and 113 species belonging to the family *Clariidae* (Ferraris, 2007; Ng *et al.*, 2011). Within the genus *Clarias*, there are 6 sub-genera (Teugels, 1984) and 56 – 58 different species, out of which 33 and 25 are of African and Asian origin respectively (Ferraris, 2007; Ng *et al.*, 2011; Na-Nakorn and Brummett, 2009). The nominate sub-genus *Clarias* (*Clarias*) is the most often used in aquaculture; popular amongst them are *C. gariepinus* and *C. anguillaris* in Africa, and *C. macrocephalus*, *C. batrachus* and their hybrids with *C. gariepinus* in Asia (Lagler *et al.*, 1977; Teugels, 1984; Teugels, 1986; Moyl and Cech, 1988; Na-Nakorn and Brummett, 2009).

3.2.3. Identification of Members of the Sub-Genus *Clarias*

Proper identification and characterisation of species is important for conservation and genetic management issues (Hartvig *et al.*, 2015). Due to the huge diversity in fish species and very close resemblance of some groups of species (such as tilapia and catfishes), identification sometimes becomes very complex and challenging, traditionally relying on the morphology (such as fins, teeth, body shape and size, presence or absence and location of certain features) and/or anatomy (such as gill rakers, arborescent organ, gas bladder) of the fish (Ezenwaji, 1982; Agnèse *et al.*, 1997; Laurene *et al.*, 2015). The choice of an index (method of identification) depends on how variable or informative it is. In some families, genera or sub-genera, where differentiating between certain species based on morphology and anatomy is difficult (e.g. *Clarias* spp), molecular techniques can be employed to overcome such problems.

The very close resemblance between different species of *Clarias*, the differences in identification keys and/or definition of keys used in identification of these species and the high intraspecific (individual) variation, have made proper identification often difficult (Ezenwaji, 1982; Hanssens, 2009), especially in the stocks used for aquaculture in Africa – *C. gariepinus* and its closest relative *C. anguillaris*. Furthermore, limitation of identification of *Clarias* to those found in a particular geographical or regional area, renaming of the same species by different authors and the use of few specimens in original descriptions, have often created overlaps

(Ezenwaji, 1982; Ozouf-Costaz*et al.*, 1990). *C. gariepinus* is found throughout Africa, while its closest relative *C. anguillaris* is restricted mainly to West Africa (Nwafili and Gao, 2007). They occur sympatrically in the wild and are genetically very close to one another, with estimates of genetic distance of 0.16 from a study investigating allozyme variation (Rognon *et al.*, 1998) and 0.04 from sequences of cytochrome b oxidase (Nwafili and Gao 2007).

3.2.4. Morphometric and Meristic Traits used for Species Identification

Over the years, authors came up with different meristic indices for identifying *Clarias* species, notable among them were: length of and number of rays on its dorsal fins, type and nature of vomerine teeth, presence or absence of pigment bands on either side of the lower head, shape and size of the head, number of rakers on the first gill arch and in some cases colour of the eggs (Debouche *et al.*, 1979; Teugels and centrale, 1982; Teugels, 1986; Agnèse *et al.*, 1997; Rognon *et al.*, 1998; Yisa and Olufeagba, 2005; Hanssens, 2009; Wiecaszek and Antoszek, 2010; Compaoré *et al.*, 2015; Zakariah *et al.*, 2016)

Reed, (1967) and Teugels (1982a) in their respective studies reported that vomerine teeth in *C. gariepinus* were mostly conical, sub-granular, forming a crescent band, which might be slightly interrupted in the middle. Agnèse *et al.* (1997) reported that width of the premaxillary and vomerine tooth-plates were 22.3% and 21.5% of their head length in *C. gariepinus*.

Although observations were limited only to Senegal, standard length, head length, length of the inter-orbital space, frontal fontanel and occipital process were used to differentiate between *C. anguillaris* and *C. gariepinus* (Debouche *et al.*, 1979). The accuracy of these techniques was confirmed to be limited to samples from Senegal in the works of de Vos (1986) and Agnèse *et al.* (1997).

In a study comparing *C. gariepinus* specimens from the Nile Basin (Lake Manzala, Chobra, Lake Victoria), Orange Basin (Orange River), Komati Basin (Sand River Dam), Senegal Basin (Senegal River), Niger Basin (Sankarani River) and Chad Basin (Chari River and Chari delta), with *C. anguillaris* collected from Senegal Basin

(Senegal River), Niger Basin (Sankarani River, Niger river), Chad Basin (Chari River and Chad delta) and the Ebrie Lagoon (Layo), Rognon *et al.* (1998) used the number of gill rakers on the first gill arch in combination 13 other meristic traits. They discovered that *C. gariepinus* showed a considerable morphometric variation in the number of gill rakers, compared to *C. anguillaris*. Benech *et al.* (1992) reported that the presence or absence of punctuations and longitudinal lines on the cleithra could be used to distinguish species in the Central Niger Delta when combined with the number of branchiospines/gill rakers. Presence was 100% associated with *C. gariepinus* while absence occurs mostly in *C. anguillaris.*

Based on these keys, *C. gariepinus* has a slightly shorter dorsal fin relative to other species of *Clarias*, slightly interrupted (an open gap) vomerine teeth (Reed *et al.* 1967; Yisa and Olufeagba (2005), pigment bands on either side of the lower head and a positively correlating standard length to the number of gill rakers (24-110) on the first gill arch (Teugels and Centrale, 1982; Teugels, 1986; Agnèse *et al.*, 1997; Rognon *et al.*, 1998; Compaoré *et al.*, 2015). Its standard length is 6 – 8 times its body depth and it has a relatively broader head (26.6-35% of the standard length against 21-26.1% in other species).

On the other hand, *C. anguillaris,* although very similar to *C. gariepinus* in many respects, has 16 – 50 rakers on its first branchial arch (Teugels 1986; Compaoré *et al.*, 2015) and is reported to have no gap on its vomerine teeth (Yisa and Olufeagba, 2005). The number of gill rakers also correlated positively to the standard length, however, this (correlation) is much lower than in *C. gariepinus* and this is further confirmed in the works of de Vos (1986) and Compaoré *et al.* (2015), who noted a significant difference between the two species using a regression equation to obtain a regression coefficient (b- value) of $0.013 \leq b \leq 0.060$ for *C. anguillaris* and $0.122 \leq b \leq 0.267$ for *C. gariepinus*.

3.2.5. Limitation of the Morphometric Identification Techniques

The existence of overlaps, amongst other reasons, makes it difficult to rely on morphometric and meristic indices for identification (Na-Nakorn *et al.*, 2002). The number of rakers on the first branchial arch has been widely used to identify *C.*

gariepinus from other members of the same genus. The overlap in these numbers between *C. gariepinus* and *C. anguillaris* (24 -110 and 16 - 50 respectively) and inconsistencies in the number of rakers (increasing with increase in standard length) in both species (from 24 – 110 rakers in specimens of *C. gariepinus* with standard lengths of 28 - 600 mm, and 16 – 50 rakers in specimens of *C. anguillaris* with standard lengths of 31.5 – 650 mm), makes this technique very confusing and difficult to completely rely on (Compaore *et al.*, 2015). The fact that there are higher number of these gill rakers in *C. anguillaris* found in the Nile-Chad area compared to the same species found in West Africa (Teugels, 1986; de Vos and Teugels 1986), possibly due to ecological factors and adaptation (available feed/food and feeding behaviour), further deepens the confusion in the use of this technique. On another note, for husbandry purposes, this technique might not be very suitable because the number of rakers on the gill arch can only properly be ascertained by removing the gills from the opercular cavity, a procedure that is laborious, time consuming and requires killing the fish. As both species occur sympatrically, sampling few individuals out of a population might not be representative of the actual composition of species within the population.

Similarly, the use of vomerine teeth (in addition to the egg colour) for identification of *Clarias* (though reported by very few authors) is a common practice in Nigeria. This technique is not very reliable. *C. gariepinus* are thought to have a gap in their vomerine teeth and green eggs (when ripe), while *C. anguillaris* has no gap on its vomerine teeth and the egg colours are deep brown. This technique(s) has traditionally been used across the country, without having to kill the fish. However, the accuracy of this technique and the possibility of natural hybridisation between the two species (Dunham and Smitherman, 1985; Agnese *et al.*, 1997; Aluko, 1998) and indiscriminate crossing of both species in hatcheries further deepen the confusion. The frequent lack of correlation between the egg colour and nature of vomerine teeth, the difference in egg colour depending on the stage of vitellogenesis and the inability to apply this key to small or male *Clarias* increases the unreliability of this identification technique.

The use of shape and size of head relative to the body length has not been widely documented. The validity of this technique remains to be proven, as the ecology of

water bodies has been found to determine the shape of the head and possibly other internal features e.g. gill rakers.

3.2.6. Molecular Techniques of Identification

Application of molecular/genetic markers in fish systematics has been seen to provide opportunity for refined *Clarias* catfish classification and identification (Na-Nakorn, 2002; Na-Nakorn *et al.*, 2002). Although not strictly a molecular technique, in a study based on karyotypes, *C. gariepinus, C. lazera* and *C. mossambicus* were found to be synonyms of the same species (*C. gariepinus*) following karyological studies conducted on samples which originated from Israel, Central African Republic and Ivory Coast (Ozouf-Costaz, Teugels and Legendre, 1990).

Attempts to distinguish between the two species using chromosome numbers have largely been difficult, partly because, no discriminatory karyotypes were found between the two species (Teugels *et al.*, 1992a) and different authors have reported different chromosome numbers for each of the respective species. Chromosome number 2n = 56 has been reported for *C. gariepinus* (Richter *et al.*, 1987; Ozouf-Costaz *et al.*, 1990; Teugels *et al.*, 1992b; Okonkwo and Obiakor, 2010). In separate studies, chromosome number 2n = 54 was reported for the same species (Omotayo, 2012; Maneechot *et al.*, 2016). Similarly, Aluko, (1998) reported chromosome number of 2n = 54 for *C. anguillaris,* while 2n = 56 was reported in the work of Eyo (2005) and Teugels *et al.* (1992b).

Molecular techniques for identification of *C. gariepinus* have been investigated using allozymes, microsatellites and mitochondrial DNA (Ozouf-Costaz *et al.*, 1990; Teugels *et al.*, 1992a; Agnese *et al.*, 1997). The same conclusions were reached based on two of the three aforementioned techniques i.e. allozymes and microsatellite markers. Both techniques differentiated *C. gariepinus* from *C. anguillaris,* and agreed to the use of number of gill rakers on the first branchial arch as a valid meristic index for identification (Agnese *et al.*, 1997). Examining 25 loci (13 of which were polymorphic) using principal component analysis on 35 alleles (in 38 samples analysed using allozymes), eight private alleles were detected for *C. gariepinus* and 84 private alleles for *C. anguillaris*. Using microsatellite markers, Agnèse *et al.*

(1997) detected 19 private alleles for *C. gariepinus* and 26 for *C. anguillaris*. However, no diagnostic loci were found between the two species in this study and the possibility of hybridisation between the two species was suggested due to an intermediate position in the result of a sample (one individual) in same study. Despite having as high as 84 private alleles in *C. anguillaris*, it was found not to be diagnostic probably due to very low frequencies of the alleles (Rosenberg, 2011).

In a study to elucidate the genetic diversity of African catfish in Thailand, using microsatellite loci, Wachirachaikarn *et al.* (2009) revealed that there were two distinct groups of strains of African catfish stock. Similarly, studies on the genetic structure of the Dutch domesticated *C. gariepinus* farmed in Nigeria involving a comparison of the mtDNA (cytochrome b) of the exotic (Central Africa and Israeli strain), Dutch-domesticated and indigenous population, revealed high genetic distance/differences between the populations (Nwafili, 2013). In this study, the Dutch-domesticated populations were genetically differentiated from the indigenous ones and were themselves composed of two phylogenetically distinct populations, having at least three mtDNA lineages. The use of cytochrome b in his study enabled the establishment of genetic structure and differences between the cultured populations of *Clarias* species in Nigeria, suggesting possibly that the exotic species is composed of three mtDNA lineages. However, due to limitation of mtDNA to maternal inheritance, and the fact that the technique is only based on mitochondrial DNA, it was difficult to conclude about the presence or not of a third (West African) lineage.

3.2.7. The Implications for Aquaculture

The Dutch domesticated stock now present in Nigeria is said to have originated from several countries. Studies show that they were collected from Cameroun, Central African Republic, Cote d'Ivoire, and Israel and domesticated in the Wageningen University, The Netherlands in partnership with FAO (Richter *et al.*, 1987; Holcik, 1991; Miller and Atanda, 2011; Nwafili, 2013, FAO, 2017). The introduction of this strain of *C. gariepinus* by some Dutch consultants for intensive culture in concrete tanks and earthen ponds with accompanying influx of imported extruded catfish feeds marked a significant point in the history of the Nigerian aquaculture industry. Their impressive growth rate and harvest size encouraged farmers to abandon the native

strains already undergoing domestication. Today the Nigerian aquaculture industry is dominated by Dutch domesticated *C. gariepinus*, which in some cases have been bred with the local stock (due to scarcity of broodstock), and in many cases have themselves probably been inbred.

On a separate note, the growth of the Nigerian catfish industry and the profitability of catfish aquaculture in Nigeria has attracted some multinational coporations to the industry. Large-scale intensive farms are springing up in different parts of the country and one issue common to these farms is the origin, quality and purity of broodstock. One option that has been taken has been broodstock importation. Countries such as The Netherlands, Hungary, Poland, South Africa, etc, have well developed intensive culture systems for *C. gariepinus* and are potential sources of broodstock for some of these new farms in Nigeria. Therefore, the possibility of moving broodstock into Nigeria cannot be overlooked, especially in the face of the poor growth performance of some of the indiginous wild strains. The genetic relatedness of imported stock to indigenous ones is largely unknown.

The purity of the Dutch-domesticated stain of *Clarias* was questioned in the works of Nwafili and Gao (2007), Nwafili (2013) and Nwafili (2017). As described earlier, they are thought to be products of crosses between *C. anguillaris* and *C. gariepinus*. If this is true, then escapes from hatcheries could threaten biodiversity and conservation.

3.2.8. Research Questions

1. *In the face of speculation of possible introgression between C. gariepinus and C. anguillaris (especially in the Dutch domesticated Clarias catfish farmed in Nigeria) is it possible to find genetic markers for clearly identifying each of these species?*
2. *In the face of paucity of information on the genetic structure of the African catfish (C. gariepinus) farmed in different countries in Africa and some parts of Europe, and the ease of transfer of Clarias broodstock from one country to another, are the stocks of Clarias gariepinus cultured across these different countries pure or introgressed with C. anguillaris?*

3.2.9. Aims and Objectives of this Study

The aim of this study was to develop genetic markers to distinguish *C. gariepinus* from its closest relative *C. anguillaris* and to assess the purity of different strains of *C. gariepinus* cultured in Nigeria and elsewhere.

Hypothesis: "This study is designed to assess the hypothesis that *C. gariepinus* are genetically distinct from *C. anguillaris*"

Approach: Initial samples of *C. gariepinus* and *C. anguillaris* were collected from three farms and two rivers in Nigeria. The wild ones from the two rivers were identified and selected based on the anatomy of the vomerine teeth. Together with *C. gariepinus* samples from Egypt (WorldFish) and *H. longifilis* (used as out-groups) from The Netherlands, their DNA were sequenced at the IoA, Stirling, and following analysis, they clearly separated into putative *C. gariepinus* and *C. anguillaris* based on 24 diagnostic SNPs. The SNPs were validated using KASP Assay on same samples sequenced and more samples from Netherlands, Hungary, Poland, Zambia (domesticated at IoA), and three farms in Nigeria, River Niger and River Benue. This showed that vomerine teeth weren't consistent and reliable with the SNPs, thus further samples were collected from the 4 distinct locations in the two rivers. During sampling meristic and morphometric traits were measured so as to ascertain which morphology matches the SNPs for ease of identification.

3.3. Materials and Methods

3.3.1. Ethics Statement

All working procedures complied with the UK Animals Scientific Procedures Act (Parliament of the United Kingdom 1986). This research was carried out with the approval of the University of Stirling Animal Welfare and Ethical Review Body (AWERB).

3.3.2. Biological Materials

3.3.2.1. Sampling Protocol

Samples were sourced from freshly killed fish from different origins. From each origin, live fish were collected and anesthetised using 0.25 ml clove oil/L of water; 4 times the upper dose recommended for anaesthesia + recovery for this species (Hamackova *et al.*, 2006). An initial verification of inactivity following the anaesthesia was carried out before stunning the cranium (to cause brain contusion) with a blunt pestle to ensure death and then enable measurements of meristic indices (vomerine teeth, gill rakers and number of rays on the dorsal fin) and fin clipping **(Figure 3.3 – 3.6.)**. Approximately 1 cm^2 of fin was collected, water removed using filter paper and fixed in 99% ethanol. This was stored at 4°C in a refrigerator and after 24 hours, the ethanol was renewed and kept at the same temperature until samples were brought to the UK for DNA extraction, sequencing, genotyping and analysis.

Figure 3.3. *Clarias spp.* (farmed *C. gariepinus*)

Figure 3.4. Gill Rakers on the first branchial arc

Figure 3.5. Vomerine Teeth of *Clarias* (without gap)

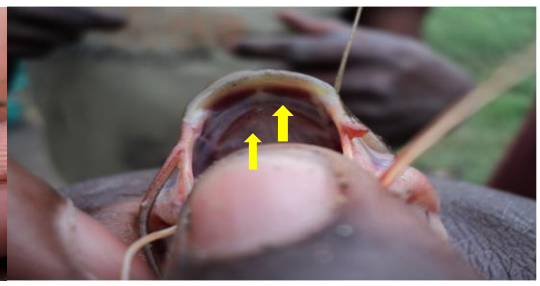

Figure 3.6. Vomerine Teeth of *Clarias* (with gap)

3.3.2.2. Sampling Phases and Sites

There were three phases of sampling altogether.

3.3.2.3. Phase 1 of Sampling

Fin samples were collected from 5 different sources (and six different locations) in Nigeria. These comprised 2 wild sources (Rivers Niger and River Benue (2 locations i.e. upper R. Benue and lower R. Benue)) and three catfish farms producing mainly the Dutch-domesticated stock of *C. gariepinus*. Treating the two locations on R. Benue as one, and using only vomerine teeth as an index for identification (as popularly used in Nigeria), a minimum of 7 provisionally identified *C. gariepinus* and 7 *C. anguillaris* were collected from each of the rivers **(Figure 3.7).** A total of 42 wild samples were collected from the two rivers. The owners described / presented all the three farmed-sources as *C. gariepinus*, because they are mainly the Dutch strain popularly called "*Hollandis*" or the "Dutch *Clarias*". From each farm, 24 samples were collected following the sample protocols described above.

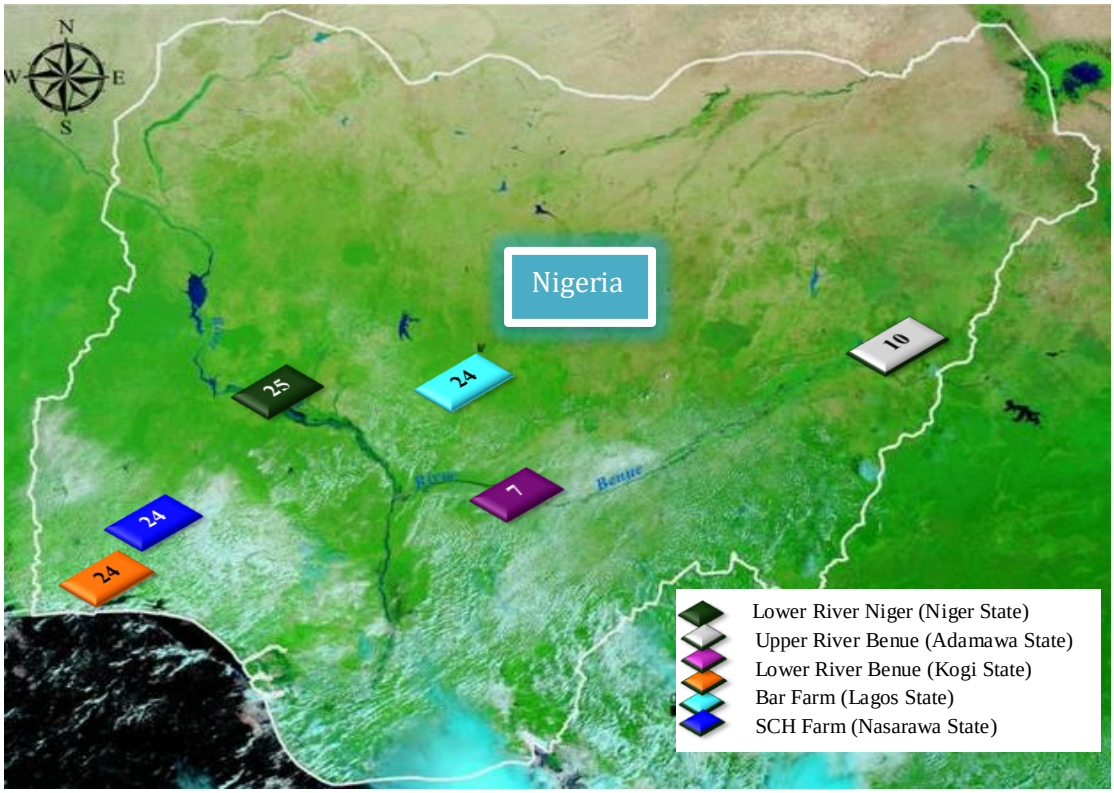

Figure 3.7. Sampling sites for wild populations and farmed *Clarias* catfish strains in Nigeria.
Phase one sampling, total number of wild samples = 42 and total farmed samples = 72

3.3.2.4. Phase 2 of Sampling

As shown in **Figure 3.8**, fin samples were collected from Hungary, Poland, The Netherlands, The WorldFish Centre in Egypt and mucus sample from a Zambian

strain kept in the tropical aquarium of the Institute of Aquaculture, University of Stirling, Scotland, UK. Number of samples received was different from the different sources, as such; samples used were proportional to samples received. A total of 33 and 20 samples received from the Netherlands and Egypt respectively, were used in this study, in addition to 3 fin samples from Poland, 4 from Hungary and 11 mucus samples collected from the Zambian strain **(Figure 3.8** and **Table 3.5)**. Again, the suppliers described / presented all the fin samples as *C. gariepinus*. Samples collected from The Netherlands were partly to compare their purity to those of the Dutch-domesticated strains currently used in the Nigerian Aquaculture Industry, thereby possibly identifying points of introgression, if any. A contact at Aquaculture, Consultancy and Engineering (ACE), a Dutch aquaculture company in the Netherlands facilitated and collected all the samples from the different farms in the Netherland, Poland and Hungary. The three farms in Nigeria were Spring Continental Harvest LtD, Banarly and ZARTECH.

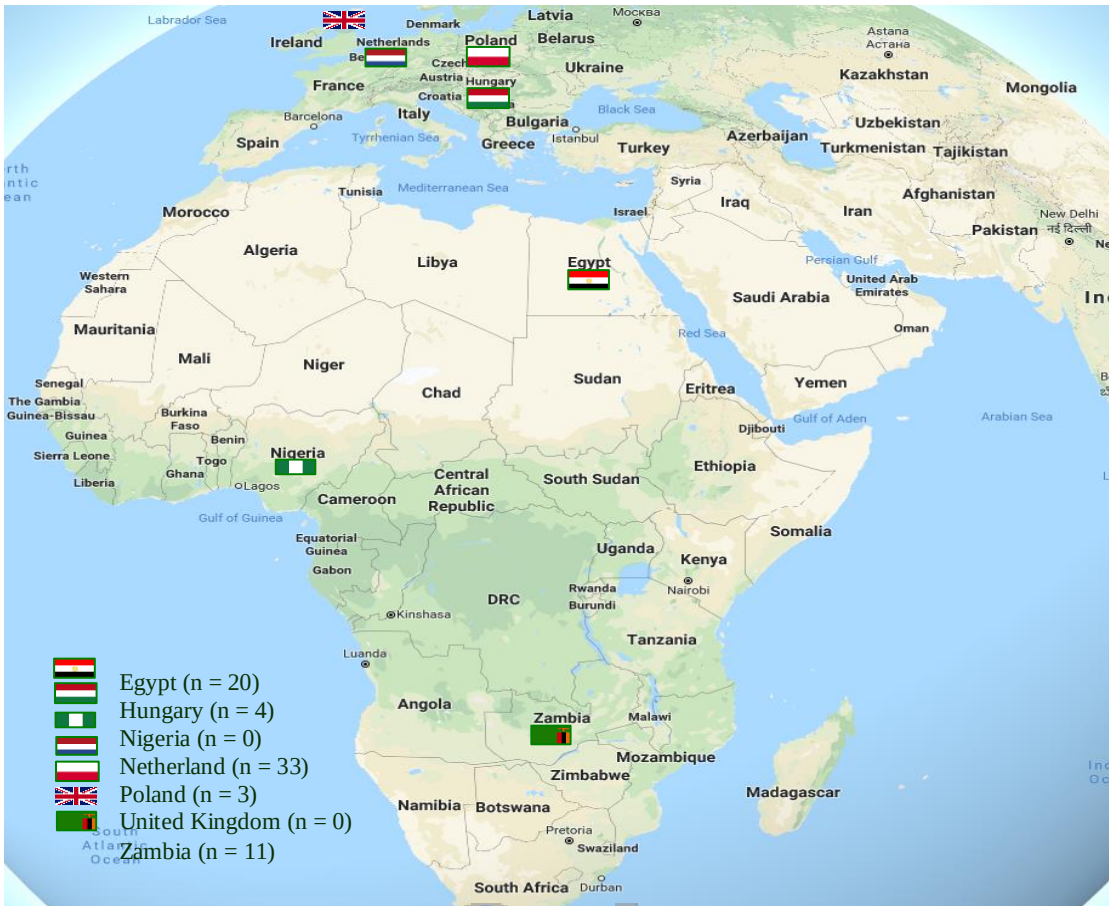

Figure 3.8. Countries where the second set of samples of *Clarias* species were collected from. Phase two sampling, total number of samples collected = 71. Farmed and wild samples have already been collected from Nigeria in stage 1.

3.3.2.5. Phase 3 of Sampling

Due to the mismatch between the phenotype (vomerine teeth) used in identifying the wild samples of *C. anguillaris* and *C. gariepinus* collected, there was need for another sampling, in which other phenotypic data of each individual was recorded. A total of 165 fin samples were collected from Rivers Niger and Benue. Meristic traits such as standard length, total length, number of rays on the dorsal fin, number of rakers on the first branchial arch, type of vomerine teeth (gapped or no gap) and cleithral bone were examined for each sample, recorded against its fin clip.

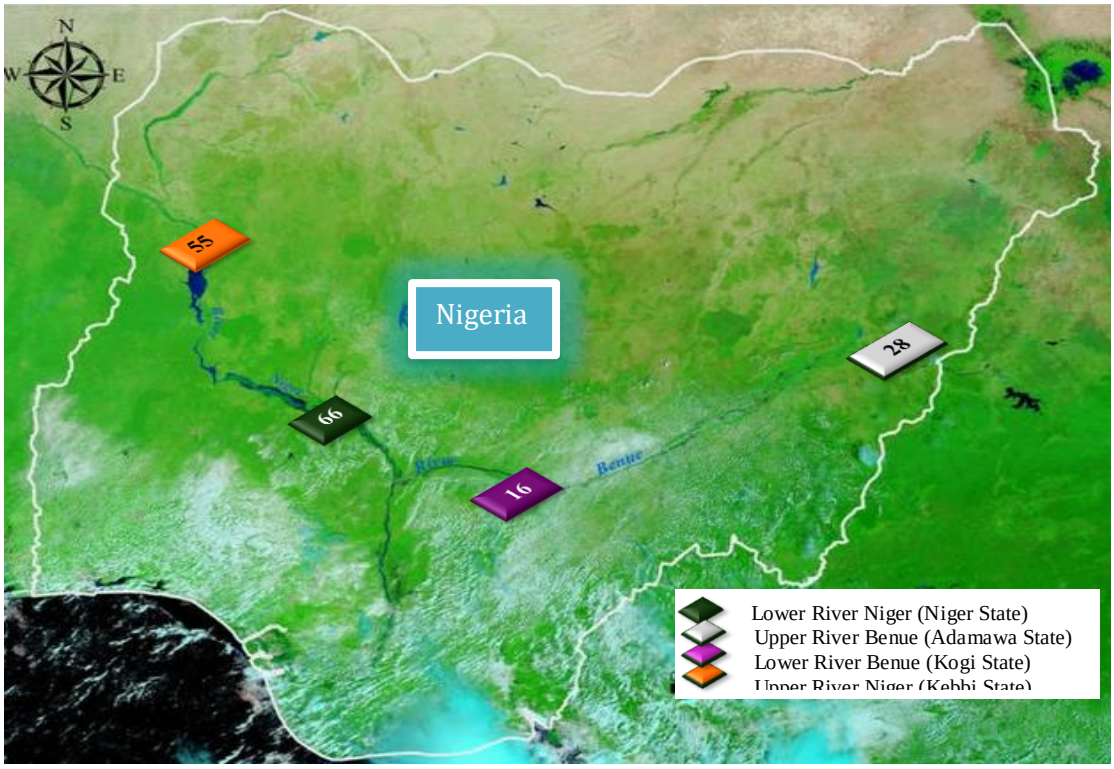

Figure 3.9. Sampling sites for wild *C. anguillaris* and *C. gariepinus* along Rivers Niger and Benue. A total of 165 samples were collected from the four spots on the two rivers in Nigeria.

Efforts were made to source the wild stock from distinct locations as shown in **Figure 3.9** (Upper R. Benue and Niger in Adamawa State and Kebbi States respectively, and Lower R. Benue and Niger in Kogi and Niger States respectively) and from locations very far away from any known fish hatchery or farm. This was to minimise the possibilities of same strain or origin and to ensure that they were purely wild stocks. On the other hand, farmed populations with relatively known histories, free from wild *Clarias* stocks of broodstock, were targeted to minimise the possibility of sampling hybrids between the two *Clarias spp* resulting from misidentified wild stock. The *H.*

longifilis strain used as out-group in this study was sourced by ACE in the Netherlands. More details of samples and origins and approximate locations of the sampling sites are listed in **Table 3.5.**

3.3.3. Genomic DNA Extraction

Total genomic DNA was extracted using a slightly modified protein salting-out and isopropanol precipitation method (SSTNE/SDS) described by Aljanabi and Martinez (1997). This method offers good quality and quantity of genomic DNA. Each precipitated DNA sample was finally re-suspended in 5 mM Tris at pH 8.5. DNA samples were left to dissolve at 4°C for a minimum of three days before quantification using spectrometry (NanoDrop ND 1000 Spectrophotometer, NanoDrop Technologies Inc.) and then by fluorimetry (Qubit® Fluorometer 2.0, dsDNA High Sensitivity assay kit; Invitrogen, ThermoFisher Scientific). The fluorimetry measures of DNA quality was carried out only on those samples that were used for ddRADseq, as high quality standardised DNA was required for the ddRADseq. The integrity of each sample was checked using agarose gel (0.8%) electrophoresis. For the purpose of double digest RAD sequencing, all the selected samples had high molecular weight genomic DNA, with both 260/280 and 260/230 OD ratios exceeding 1.8. Based on fluorimetry values, samples were diluted to 7 ng/µL with 5 mM Tris, pH 8.5.

3.3.4. Double Digest RAD Library Preparation and Sequencing

One library containing 25 samples was constructed. This entailed five *Clarias* samples from each of the four locations (Rivers Niger - putatively *C. anguillaris* and Benue, a farm producing the Dutch-domesticated strain and the Egyptian strain – all putatively *C. gariepinus* and five samples of *Heterobranchus longifilis* from The Netherlands as an out-group. Samples from R. Benue, Dutch-domesticated strain and the Egyptian Strain were putatively *C. gariepinus*. The ddRAD library preparation protocol was based on the methodology originally reported by Peterson *et al.* (2012) with modifications / refinements as described in Manousaki *et al.* (2016) and Brown *et al.* (2016). Briefly, for each library, individual DNA samples (21 ng – 3 µL) were simultaneously digested with two high fidelity restriction enzymes (RE): *Sbf*I (CCTGCA|GG recognition site), and *Sph*I (GCATG|C recognition site), both sourced

from New England Biolabs, (NEB) UK. Digestions were incubated for 40 min at 37°C, using 0.25 U of each enzyme in 1× CutSmart Buffer (NEB), in a 6 µL reaction volume. The reactions were then cooled to c. 22°C, 3 µL of a premade barcode / adapter mix was added to each digested DNA sample and incubated at 22°C for 10 min. The adapter mix included individual-specific barcoded combinations of P1 (*Sbf*I-compatible) and P2 (*Sph*I-compatible) adapters at 6 nM and 72 nM concentrations respectively, in 1× reaction buffer 2 (NEB). Adapters were compatible with Illumina sequencing chemistry (see Peterson *et al.* (2012) for details). The barcoded adapters were designed such that adapter– genomic DNA ligations did not reconstitute RE sites, while residual RE activity limited concatemerisation of genomic fragments. The adapters included an inline five- or seven-base barcode for sample identification. Ligation was performed over 3.5 h at 22°C by addition of a further 3 µL of a ligation mix including 4 mM rATP (Promega, UK), and 2000 cohesive-end units of T4 ligase (NEB) in 1× CutSmart buffer. The ligated samples were then heat denatured at 65°C for 20 min and cooled to room temperature. Samples for a library were combined into a single pool. The pooled library sample was column-purified (MinElute PCR Purification Kit, Qiagen, UK), and eluted in 80 µL EB buffer (Qiagen, UK). Size-selection of fragments, ranging from 320 bp to 590 bp, was performed by agarose gel separation. Following gel purification (MinElute Gel Extraction Kit, Qiagen, UK), the eluted size-selected template DNA (60 µL in EB buffer) was PCR amplified (13 PCR cycles; 24 separate 12.5 µL reactions, each with 1 µL template DNA) using a high fidelity Taq polymerase (Q5 Hot Start High-Fidelity DNA Polymerase, NEB). The PCR reactions were combined (300 µL total), and column-purified (MinElute PCR Purification Kit). The c. 50 µL eluate, in EB buffer, was then subjected to a further size-selection clean up using an equal volume of AMPure magnetic beads (Perkin-Elmer), to maximise removal of small fragments (less than c. 200 bp). The final library was eluted in 19µL EB buffer, QUBIT quantified and diluted to 10 nM stocks. The library was sequenced in house on an Illumina MiSeq run (v2 chemistry, 300 cycle kit, 161 base paired-end reads; Illumina).

3.3.5. Genotyping RAD-tags

The MiSeq generated reads were processed using a software pipeline designed specifically for RAD analysis, Stacks v1.46 (Catchen *et al.*, 2013). First, the *process_radtags* component was used to demultiplex the individual samples. During this step, sequence reads with quality scores below 20, missing either restriction site or with ambiguous barcodes were discarded. Barcodes were removed, and all sequences trimmed (3' end) to be no greater than 150 bases long. For the purposes of this analysis paired-end reads were treated as separate loci, read 2 sequences being appended to read 1 sequence files. These sequences were assigned to RAD loci using *de novo* genome-based approaches. The key parameter values employed to identify RAD loci for the *de novo* analysis were (m6M2n1): a minimum stack depth (m) of 6, a maximum of 2 mismatches (mutations) allowed in a locus (M) of an individual and up to a maximum number of 1 mismatch between loci when building the catalogue (n). Finally, the *populations'* component of Stacks was used to export filtered data (polymorphic loci) for further analysis.

3.3.6. Marker Identification

Polymorphic loci were defined as RAD-tags with one or more SNPs. Shared loci were defined as polymorphic loci present in at least 50 and 75% of the samples, while species-specific loci were defined as polymorphic loci exhibiting no intraspecific polymorphism but showing interspecific polymorphism and present in at least 75% of each taxon. A marker was defined as one particular SNP at a locus.

3.3.7. Phylogenetic Analysis Using SNP Data

SNP data from filtered shared loci was combined into composite genotypes for each individual (n = 25). Phylogenetic trees were constructed with RAxML v8.0.0 (Stamatakis, 2014). Maximum-likelihood phylogenetic trees were inferred using the GTR+CAT nucleotide substitution model (Lartillot and Phillippe, 2004) and bootstrap support values estimated from 10,000 replicate searches of randomly generated trees using R/*adegenet*. The best-scoring ML tree was visualised using FigTree v1.4.2.

3.3.8. Species Discrimination Analysis of SNP Data

Principal Component Analysis (PCA) and Discriminant Analysis of Principal Components (DAPC) were carried out on the SNP data using R v3.3.2 (R Core Team, 2016) and an associated R/*adegenet* package v1.4-1 (Jombart, 2008). PCA creates simplified models of the total variation within the dataset and DAPC identifies clusters of genetically related individuals (Jombart *et al.*, 2010).

3.3.9. PCR-based SNP Genotyping

A total of eight species-specific SNP markers were selected and SNP assays were designed and manufactured using KASP (Kompetitive Allele-Specific PCR) genotyping technology by LGC Genomics Ltd. These distinguishing markers were derived from *C. gariepinus* and *C. anguillaris* (see Results), and were designed to have an allele only found in that species e.g. *C. gariepinus* and not in the other two (i.e. *C. anguillaris* and *H. longifilis*), and the *C. anguillaris* - specific markers designed to have an allele found only in that species and not in the other two (i.e. *C. gariepinus* and *H. longifilis*). The out-group was predicted to behave as neither of the two species targeted by the marker when all 8 SNPs are evaluated, and was included as an additional controls. For primer design to be feasible, the SNP of interest at a given locus needed to be at least 20 bp from the end of a given sequence. However, 35 bp from either side of the SNP was considered to allow enough sequence for compatible primers to be designed. DNA samples were standardised and 8 ng of each sample was air-dried in a 96 well white PCR plate (Starlab) and genotyped in a 5 µL reaction volume. The PCR cycling conditions (TAdvanced thermocycler, Biometra) were as follows: an initial denaturation at 94°C for 15 min, 10 cycles at 94°C for 20 sec and touchdown 65°C to 57°C (dropping 0.8°C each cycle) for 1 min. This was followed by 35 cycles at 94°C for 20 s and 57°C annealing/amplification for 1 min. Fluorescence signals were measured at 22°C using a Techne Quantica® Real Time PCR Thermal Cycler (Techne) and genotypes assigned by allelic discrimination analysis using the Quansoft software v1.121.

3.4. Results

3.4.1. Double Digest RAD library sequencing

A total of 36,932,592 paired-end raw reads were produced from the single ddRAD library involving 25 individuals. Again, these 25 individuals comprised five individuals from each of the following five populations; wild *C. gariepinus* from R. Benue, wild *C. anguillaris* from R. Niger, Barner farmed population from Nigeria, Egyptian population from the WorldFish Centre and the out-group, *H. longifilis* from the Netherlands. After removing low quality sequences, ambiguous barcodes and orphaned paired-end reads, 69.90% of the raw reads (25,808,862) were retained. Altogether, the Stacks analysis identified 73,591 unique RAD-tags (*i.e.*, the total number of loci across all 25 individuals (5 populations and 5 individuals)), with overlapping subsets of loci among populations and species, and 20,808 polymorphic loci in the *de novo* analysis. The depth of reads per sample ranged from 724,176 – 1,394,274, with 9,508 – 11,306 unique stacks as shown in **Table 3.1.**

Table 3.1. Number of reads, unique stacks, polymorphic loci and SNP detected
This comprised sample of putative *C. anguillaris*, *C. gariepinus* and *H. longifilis*.

Sample ID	Read number	Unique Stacks	Polymorphic Loci	SNPs Found
Can_Niger_1	786,164	9,629	1,395	2,051
Can_Niger_2	736,058	9,546	1,282	1,824
Can_Niger_3	878,256	9,710	1,367	1,975
Can_Niger_4	956,228	9,700	1,369	1,920
Can_Niger_5	925,864	9,745	1,370	1,957
Cga_Baner_1	970,200	9,878	1,359	1,919
Cga_Baner_2	1,181,126	10,122	1,347	1,921
Cga_Baner_3	1,138,320	10,172	1,355	1,928
Cga_Baner_4	1,064,686	9,865	1,305	1,836
Cga_Baner_5	949,886	9,997	1,322	1,889
Cga_Benue_1	1,033,318	9,825	1,538	2,106
Cga_Benue_2	957,486	9,725	1,577	2,136
Cga_Benue_3	870,986	10,298	1,561	2,217
Cga_Benue_4	959,684	10,329	1,666	2,322
Cga_Benue_5	1,047,980	9,833	1,577	2,106
Cga_Egypt_1	1,254,486	10,602	1,702	2,369
Cga_Egypt_2	857,986	10,089	1,792	2,474
Cga_Egypt_3	1,139,714	9,948	1,355	1,869
Cga_Egypt_4	1,244,792	10,739	1,806	2,528
Cga_Egypt_5	1,281,172	10,551	1,730	2,381
Hlo_Carol_1	1,316,198	11,306	1,480	2,126
Hlo_Carol_2	1,394,274	11,162	1,481	1,992
Hlo_Carol_3	1,251,434	10,885	1,376	1,909
Hlo_Carol_4	888,388	10,321	1,431	1,994
Hlo_Carol_5	724,176	95,08	1,280	1,694

3.4.2. Sequence analysis and SNP-Based Tree Construction

Sequence analysis was done in three stages. To better capture the discriminant ability of the markers, a principal component analysis (PCA) was conducted at every stage and phylogenetic trees were constructed using *R/adegenet*. These stages were based on the assumptions that:

1. The different populations could represent different species of catfish, hence, potentially five species. Based on this assumption, a total of **2,587** shared loci (*i.e.*, biallelic RAD-tags with one to five SNPs and present in at least 50% of the samples for each population) were found and were used in subsequent analyses and for the phylogenetic reconstruction. **Figure 3.10** shows a principal component analysis and phylogenetic aligning into three groups. The groups from the River Benue, Egypt and Baner (all nominally C. gariepinus) all clustered closely together, while the group from the River Niger (nominally *C. anguillaris*) and *H. longifilis* formed two clearly separate clusters on the PCA. The three distinct clusters suggest that the five populations are actually three species.

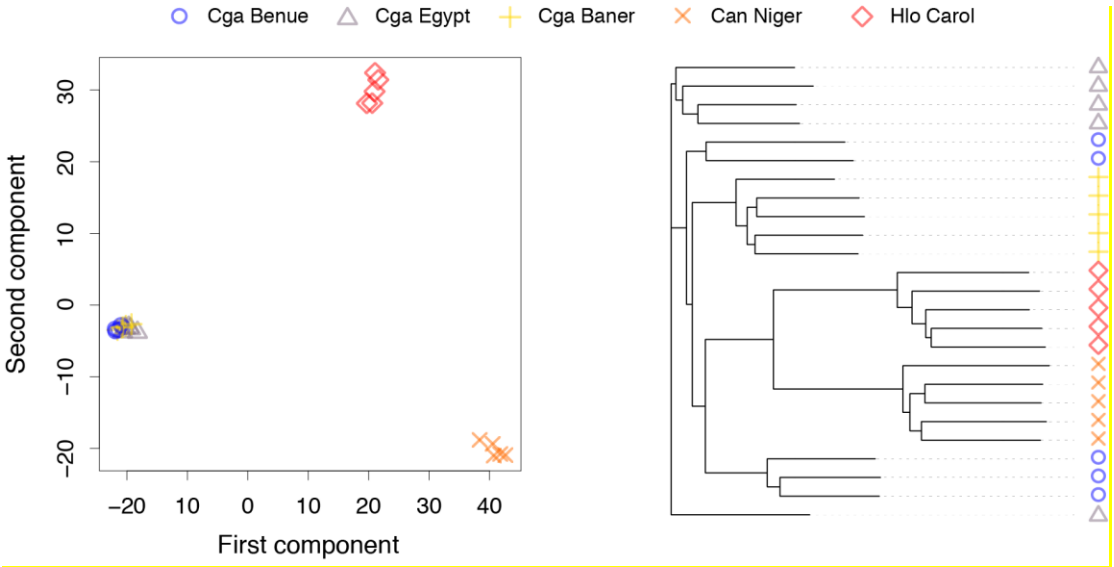

Figure 3.10. PCA and phylogenetic tree showing the relationship between the putative species – *C. anguillaris*, *C. gariepinus* and *H. longifilis*. PCA and tree based on 2587 markers for 50% coverage and up to 5 SNPs. Cga Benue and Can Niger are wild populations, while Cga Egypt and Cga Baner are farmed strains. Hlo Carol is *H. longifilis* – the out-group.

The phylogenetic tree also does clearly group the putative *C. anguillaris C. gariepinus* and *H. longifilis* into their respective separate clades. Although some members of *C. gariepinus* from R. Benue appeared at the bottom of the

tree, while others at the top, it is important to note that the vertical order of samples is not reflective of the phylogenetic relationship, as this positioning is an artefact of the visualisation.

2. Similar to the above assumption, the different populations could represent different species of catfish, hence, potentially five species, but in this case only biallelic RAD-tags with one or two SNPs and present in at least 75% of the samples were used. A total of 397 shared loci were found and were used in subsequent analyses and for the phylogenetic reconstruction. **Figure 3.11** shows a principal component analysis and phylogenetic aligning into three groups. Again, the three distinct clusters suggest that the five populations are actually three species. *C. gariepinus* from River Benue, Egypt and Baner (farmed Nigerian population) all clustered closely together, while *C. anguillaris* and *H. longifilis* formed their respective clusters. Again, the three distinct clusters suggest that the five populations are actually three species.

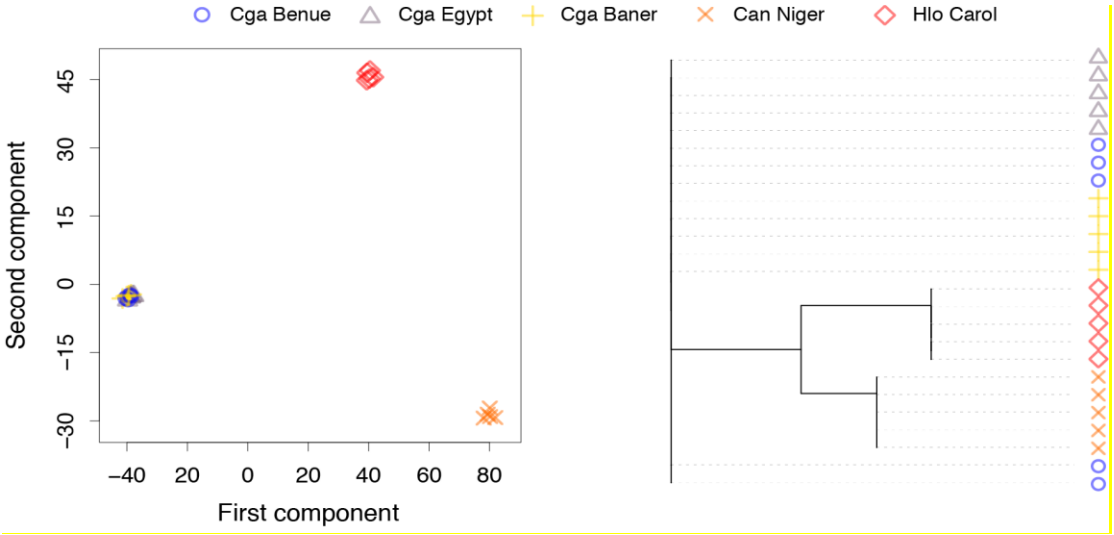

Figure 3. 11. PCA and phylogenetic tree showing the relationship between the putative species – *C. anguillaris, C. gariepinus* and *H. longifilis*. PCA and tree based on the 397 markers for 75% coverage and up to 2 SNPs. Cga Benue and Can Niger are wild populations, while Cga Egypt and Cga Baner are farmed strains. Hlo Carol is *H. longifilis* – the out-group.

The phylogenetic tree also does clearly group the putative *C. anguillaris C. gariepinus* and *H. longifilis* into their respective separate clades. Although some members of *C. gariepinus* from R. Benue appeared at the bottom of the tree, while others at the top, it is important to note that the vertical order of

samples is not reflective of the phylogenetic relationship, as this positioning is an artefact of the visualisation.

3. Based on the above, the different populations actually represent three distinct species of catfish based on the above groupings. Species-specific loci were identified as the RAD-tags exhibiting no intra-specific polymorphism but showing inter-specific polymorphism (*i.e.*, fixed differences between species so that one species had one allele that different from all other species) and present in at least 75% of each species with up to 2 SNPs: 24 loci (25 SNP markers) (Table 2). **Figure 3.12** shows a principal component analysis and phylogenetics aligning into three distinct clusters, again suggesting that the five populations are actually three species. The phylogenetic, does clearly group the putative *C. anguillaris, C. gariepinus* and *H. longifilis* into their respective separate clades. Again, the vertical order of samples is not reflective of the phylogenetic relationship, as this positioning is an artefact of the visualisation. As shown in **Figure 3.13,** *C. gariepinus* from River Benue, Egypt and Baner (farmed Nigerian population) all clustered closely together in clade 1, while *C. anguillaris* and *H. longifilis* formed their respective clusters in clades 2 and 3.

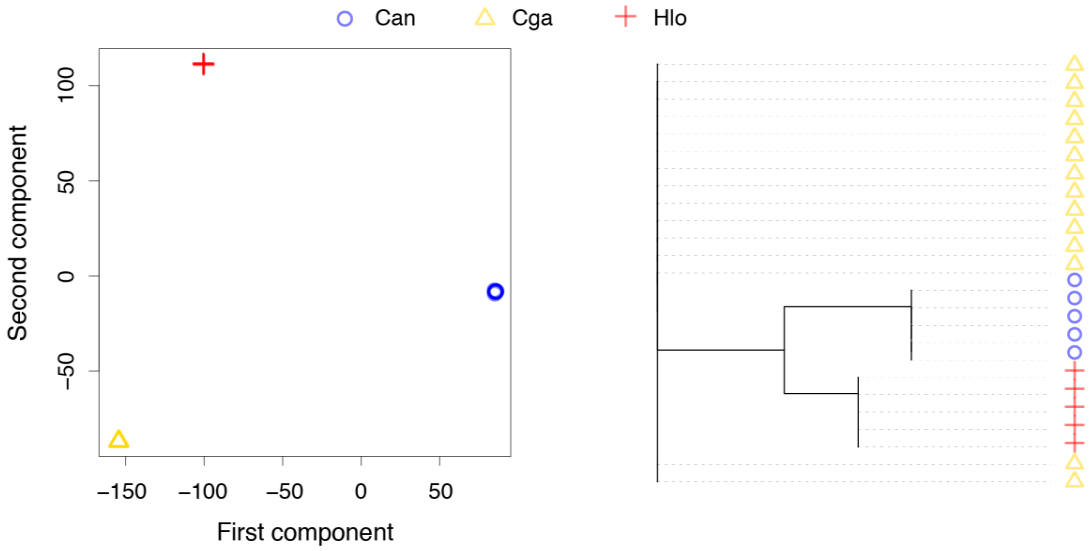

Figure 3.12. PCA and phylogenetic tree showing the relationship between the putative – *C. anguillaris, C. gariepinus* and *H. longifilis*. This is based on the 24/25 markers for 75% coverage and up to 2 SNPs. Cga Benue and Can Niger are wild populations, while Cga Egypt and Cga Baner are farmed strains. Hlo Carol is *H. longifilis* – the out-group.

3.4.3. Species-specific Markers for Aquaculture Species

The results of the phylogenetic analysis with bootstrap values, shown in **Figure 3.13,** reveals the genetic distances within and between the different species involved. The phylogenetic tree was based on the 126 markers (349 SNPs) as described earlier (only biallelic RAD-tags with one or two SNPs and present in at least 75% of the samples). There was over 80% of cumulative variance from the bootstrap values. 100% distance noted at the nodes of `Hlo, Can and Cga is a reflection of the genetic distances between the species and the lower values on the internal nodes shows the close relationships between the different individuals and populations (Cga).

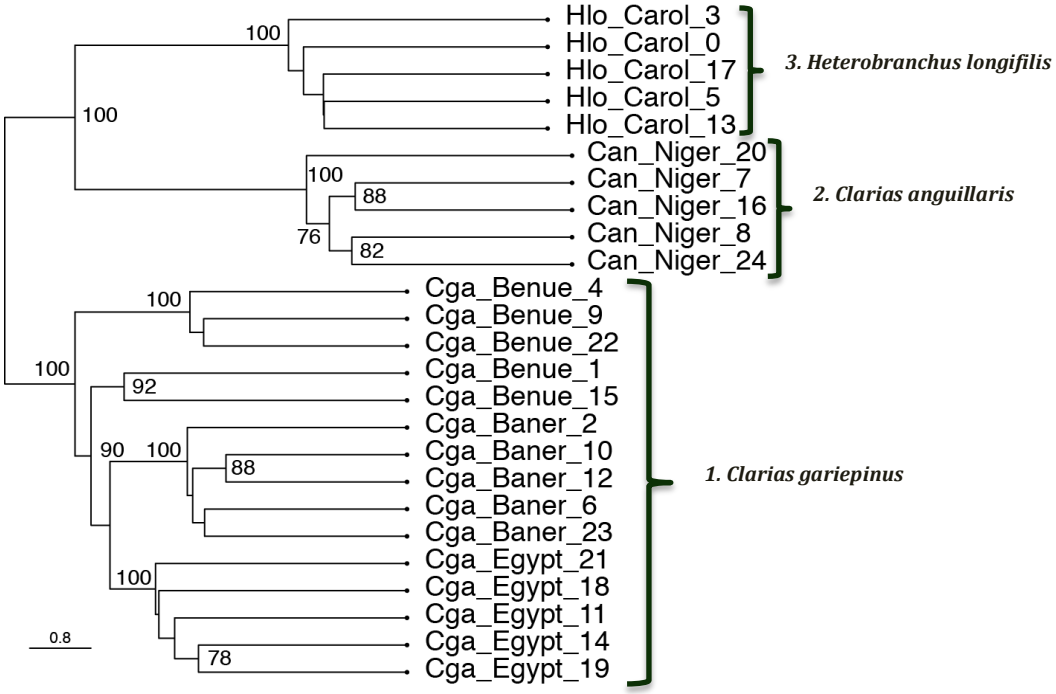

Figure 3.13. Phylogenetic tree based on the 126 markers (349 SNP) with bootstrap values
The tree is showing the relationship and or genetic distance within and between the putative C. *anguillaris*, *C. gariepinus* and *H. longifilis*. Cga Benue and Can Niger are wild populations, while Cga Egypt and Cga Baner are farmed strains. Hlo Carol is *H. longifilis* – the out-group.

Species-specific markers were discovered from the results of the ddRADseq were eleven for *C. anguillaris* and seven each, for *C. gariepinus* and *H. longifilis* respectively. These markers enabled each species to be distinguished from the other two. No subspecies-specific markers were found and there were no indication of hybrids or introgressed individuals from the results analysed**.**

When the set of species being compared were recognised as two instead of four *Clarias* species (plus 1 other species – *H. longifilis* (an out-group)), which, although occurring sympatrically in the wild, have rarely been hybridised in aquaculture (as speculated by some authors), 24/25 (24 markers/25 SNPs) species-specific markers were identified as above. These markers enabled each species to be distinguished from the other two and no subspecies-specific markers or hybrids were found.

The selection of species-specific loci with only one or two SNPs was to facilitate the development of PCR-based SNP assays from the resulting 24 diagnostic markers (25 SNP). A subset of eight markers (eight SNPs) was selected out these species-specific markers. A PCA and phylogenetic studies from the ddRADseq also group them in the same manner (three distinct species) as described earlier (**Figure 3.14)**.

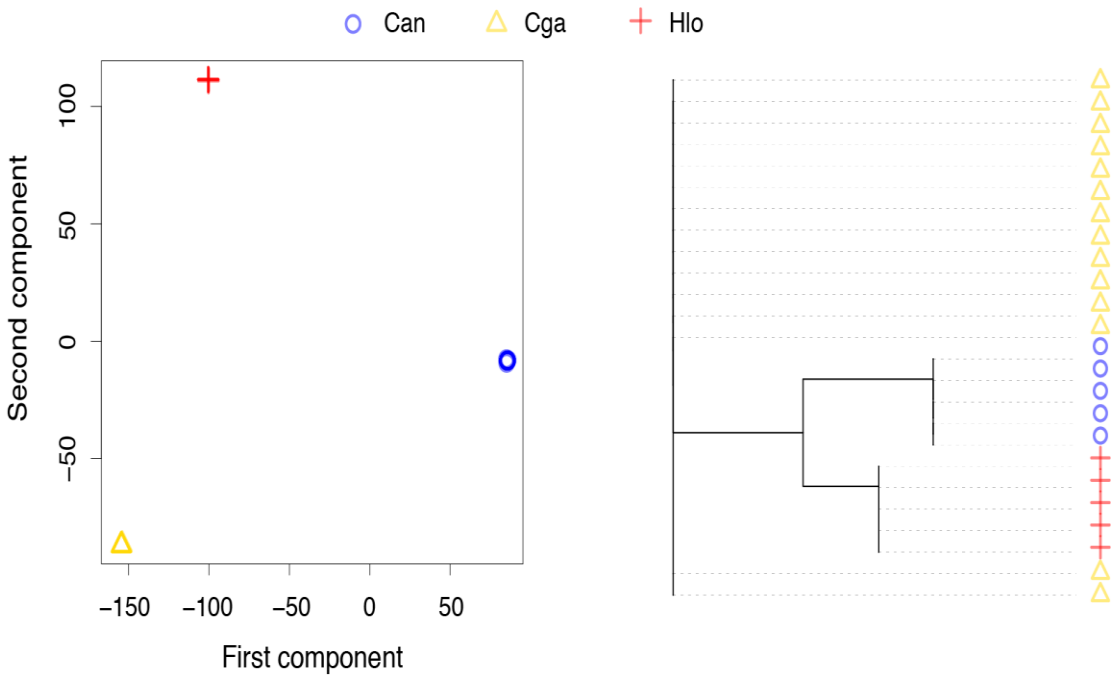

Figure 3. 14. PCA and tree based on the 8 SNP markers selected for KASP assay on *C. anguillaris, C. gariepinus* and *H. longifilis.* Can is *C. anguillaris*, Cga is *C. gariepinus* and Hlo is *H. longifilis*.

Further details on the approach taken to identify and select SNPs i.e. the scripts written and the selection criteria are provided in **Appendix II.**

3.4.4. SNP Assay Validation

KASP assays for eight of these putative species-specific markers (for *C. gariepinus* and *C. anguillaris*) were designed and tested. They were selected to be diagnostic for the two species based on the ddRADseq data. Individual genotypes for each of the eight markers tested by KASP are listed in **Table 3.2** and the allele frequencies are summarised in **Table 3.3.** Comparison of genotypes generated by KASP assays with ddRADseq data (based on 20 individuals (5 *C. anguillaris* and 15 *C. gariepinus*) and eight SNPs) showed 100% matches. The five *H. longifilis* behaved as predicted (outgroups; neither *C. gariepinus* nor *C. anguillaris*). There were no mismatches or heterozygotes in both the ddRAD and the KASP assay.

The subset of eight markers designed to discriminate the two species (*C. anguillaris* and *C. gariepinus*) were tested on a panel of 110 fish representing the two species from R. Niger, R. Benue, Egypt, Hungary, three farms each from Nigeria and the Netherlands, Poland and the Zambian stock (Bram's MSc project, 2016). There were mismatches between the genotype and the vomerine teeth used to identify and collect samples during the stage one sampling. It became apparent that although all of the samples analysed fell into two very distinct groups on the basis of the eight SNP markers, with composite genotypes matching one or other nominal species as seen in the ddRADseq analyses, the morphological trait (vomerine teeth) used to separate the samples initially was not accurate enough (mismatch between vomerine teeth and genotypes in 15 out of 32 of the remaining wild samples collected at stage one).

Table 3.2. Representation of the selected SNP's within their sequences.

	SNP ID	Repeats			Sequences and locations of SNPs Identified in Colours and Underlines in a Bracket
Ang.	1652_B	{GG}	{AA}	{AA}	TGCAGGTCTAAGGTCTTACTGAATATCGAAGGCCCCATTAGTCATATATGTG**[AG]**AGCACTGAATGTGGAAGGCCTCA TTAGTAATATAAGCTGCTGCCCAGGTCTTTGGTCAAATCAGACTCCTGCTATGAGGCCACACTC
Gar.	2766_B	{CC}	{AA}	{CC}	TGCAGGAATGTGTGTCTAAAAGCACGTTTGGCGGCGAGGCCGGGCCCAGATTACAG**[AC]**GCTCTCCTGTGATCATTTG TGCATCTCCCCTGCAAGATCACAAGGCACCATCACAGAGCCAGCAGCTGTGTCTTCAGAGCCTG
Gar.	2995_B	{CC}	{TT}	{CC}	CATGCAGCTGCGCTCTCGGGCCATGTATCCACTGTGCGGTTGTTGCTGGAAAAAGGAGCCATGGTGGACCCTCTGGAT GTGATGAAACACACTCCT**[CT]**TGTTCCGTGCCTGCGAGATGGGTCACCGTGATGTCATTCTCAC
Gar.	5288_A	{AA}	{CC}	{AA}	TGCAGGACAGCGCAGGCAGCTTGCCCTCCAGTCCGTACCGGCTGGCCCAGGACGA**[AC]**GACGAGTACGAGAGCACGC AGGAGTACCCGCCCTCACTGGAGCAACCAAAGAGAAGCAATGGACGCTGGCATAGGTCCAGACTG
Ang.	5437_A	{GG}	{CC}	{CC}	CATGCTGTGCCAACAGATAAAGCTGAACTCACTGCCTCCACTAGCTCTGTTTGCATTTGGCATAATGTCCCTGTCCCAC CCTTCCCCCATCCTCTACCACTCAAAAGACT**[CG]**CTGTCTACTTATGCTAAGTGATTGACAGC
Ang.	5661_A	{AA}	{GG}	{GG}	TGCAGGTTGCTGTAGCTGGAGGAGTGGGGCAGCCGGAATGCTGCAGGGCCAGGTGAAGGTCC**[AG]**GGTGAGGTTACC GGCTCGATGGCCTCCACCACACCCATGCTGAGTGAGACCGGCAGCACGGGCCGCTGGCACAGGCA
Ang.	6243_B	{GG}	{CC}	{CC}	CATGCTGGCGCGAAAGAGCATCATACCCGAGGAGTTCGCGCTGCCCGCGCTGGC**[CG]**TCGCGCGCGCCCCGGAAGCC GGTGTTCAGGGACCGCGTGAACAAGGCGCGCTTCATTGCCAAGAGCGGCGCGTGCAACCTGGCGC
Gar.	8167_A	{CC}	{TT}	{CC}	TGCAGGCAAAGCACACTCAGGAGGGCAGCACCTCATGGGGTGTTAACGG**[CT]**GAGACAGGCACTCTGGCAGACATGG CTGAGCTGGGAATCTGGGAGCCACTAGCTGTCAAAGCCCAAACATACAAGACAGCAGTAGAGGTA

Ang. = *C. anguillaris*; Gar = *C. gariepinus;* Het = *H. longifilis*

Table 3.3. SNP Assay Run on Quansoft for 8 if the selected SNPs

						Type 1	Type 3
Species	SNP ID	SNP	Ang.	Gar.	Het.	AlleleHEX	AlleleFAM
Ang.	1652	[A/G]	G	A	A	G	A
Gar.	2766	[A/C]	C	A	C	C	A
Gar.	2995	[C/T]	C	T	C	T	C
Gar.	5288	[A/C]	A	C	A	C	A
Ang.	5437	[C/G]	G	C	C	G	C
Ang.	5661	[A/G]	A	G	G	G	A
Ang.	6243	[C/G]	G	C	C	G	C
Gar.	8167	[C/T]	C	T	C	T	C

Ang. = *C. anguillaris* and Gar = *C. gariepinus*

Table 3.4. SNP Assay Validation (ddRAD vs KASP Assay)

	SNPs from ddRAD in Brackets and Coloured, while KASP Genotypes Separated with Slash							
SII ID	**1652[AG]**	**2766[AC]**	**2995[CT]**	**5288[AC]**	**5437[CG]**	**5661[AG]**	**6243[CG]**	**8167[CT]**
63	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
64	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
83	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
84	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
92	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
93	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
95	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
96	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
97	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
110	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
EM2	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
EM4	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
EM10	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
EF7	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
EF9	A/A	A/A	T/T	C/C	C/C	G/G	C/C	T/T
99	G/G	C/C	C/C	A/A	G/G	A/A	G/G	C/C
100	G/G	C/C	C/C	A/A	G/G	A/A	G/G	C/C
102	G/G	C/C	C/C	A/A	G/G	A/A	G/G	C/C
73	G/G	C/C	C/C	A/A	G/G	A/A	G/G	C/C
75	G/G	C/C	C/C	A/A	G/G	A/A	G/G	C/C
H6	A/A	C/C	C/C	A/A	C/C	G/G	C/C	T/T
H7	A/A	C/C	C/C	A/A	C/C	G/G	C/C	T/T
H8	A/A	C/C	C/C	A/A	C/C	G/G	C/C	T/T
H9	A/A	C/C	C/C	A/A	C/C	G/G	C/C	T/T
H10	A/A	C/C	C/C	A/A	C/C	G/G	C/C	T/T

The coloured letters are the Species-specific SNPs, with the green standing for *C. anguillaris* and red for *C. gariepinus*
SII ID 63, 64, 83, 84 and 110 are farmed Dutch-domesticated strain of *C. gariepinus* in Nigerian.
SII ID 92, 93, 95, 96 and 97 are putative samples of *C. gariepinus* from R. Benue in Nigeria.
SII ID EM2, EM4, EM10, EF7 and EF9 are putative samples of *C. gariepinus* from the WorldFish Centre in Egypt.
SII ID 73, 75, 99, 100 and 102 are putative samples of *C. anguillaris* from R. Niger in Nigeria.
SII ID H 6, H 7, H 8, H 9 and H10 are samples of *H. longifilis* from Netherlands, used as out-group in the experiment.

Due to this, more morphological traits used to separate these two species were further investigated, and it was postulated that the number of gill rakers on the first branchial arch was a better trait, perhaps in combination with standard length (due to possible correlation between the two). Nonetheless, vomerine teeth, total length, and number of rays on the dorsal fin were counted in addition to the gill rakers and nature of the cleithral bone. In the same manner, fin clips were collected and genotyped, with a resultant 99.49% diagnostic accuracy for the additional 149 samples from R. Benue and Niger. Four *H. longifilis* samples (out-groups) were added as controls, in addition to two non-template controls (NTC). Details of the exact number of individuals (included in the templates) per population are presented in **Table 3.5**. The eight selected SNPs comprised four *C. gariepinus* markers (*Cga*2766 (A/C), *Cga*2995 (C/T), *Cga*5288 (A/C) *and Cga*8167 (C/T)) and four *C. anguillaris* markers (*Can*1652 (A/G), *Can*5431 (C/G), *Can*5661 (A/G) *and Can*6243 (C/G)).

Table 3.5. Samples, Origin and KASP-based Genotypes

S/N	Source of Samples	Country of Origin	*C. anguillaris*	*C. gariepinus*	*H. longifilis*	No. of Individuals
1	River Benue	Nigeria	0	61	0	61
2	River Niger	Nigeria	32	114	0	146
3	Farm BANER	Nigeria	0	6	0	6
4	Farm SCH	Nigeria	0	7	0	7
5	Farm ZAC	Nigeria	0	7	0	7
6	The WorldFish	Egypt	0	20	0	20
7	Farm HU1	Hungary	0	4	0	4
8	Farm HU2	Poland	0	3	0	3
9	Farm Carol	The Netherlands	0	6	7	13
10	Farm ME	The Netherlands	0	10	0	10
11	Farm HP	The Netherlands	0	10	0	10
10	T. Aquarium	Zambia	0	11	0	11
	Total		**32**	**259**	**7**	**298**

3.4.5. Phenotypic – Genotype Correlation and Diagnosis

A total of 298 fish from different origins were genotyped **(Table 3.5)**. Out of this number, 87 originated from farms in different countries, while 207 were of wild origin (R. Niger and R. Benue). Although sample sizes were relatively few to conclude on the purity of farmed stocks from different countries, all the 71 samples from the 6 different countries plus additional 20 farmed Nigerian strains **(Table 3.5)** fell into one genetic group – logically the nominal *C. gariepinus*, and aligning with all the putative *C. gariepinus* from

both R. Niger and Benue in Nigeria. The wild stock (207) was composed of 32 putative *C. anguillaris* and putative 165 *C. gariepinus* altogether. Although some fish from R. Benue were initially identified (using vomerine teeth) as *C. anguillaris*, they all had SNP genotypes concluded to be *C. gariepinus*, and all the *C. anguillaris* genotypes were confined to R. Niger. While the eight SNPs clearly split these fish into two distinct genetic groups (nominally *C. gariepinus* and *C. anguillaris*), it is important to correlate the genotypes with data from the different phenotypes (morphometric and meristic trait) initially used to try to separate samples of both species. This is to assess which phenotype correlates better to the genotype, to enable farmers, scientists and/or conservationists to properly identify and distinguish between these closely related species of catfish. The farmed stocks genotyped had no records of morphological or meristic data, hence, are not included in the following analysis.

3.4.6. Correlation of Vomerine Teeth to Genotype

Popularly used in Nigeria, this morphological trait was used for provisional identification and selection of the wild samples in stage 1 and was recorded in stage 3 of sample collection due to preliminary result (from stage 1 and part of stage 2) showing some levels of disagreement. *C. gariepinus* were thought to have a gap (slightly interrupted) in the middle of their vomerine teeth, while *Clarias anguillaris* were not. As shown in **Figures 3.15** and **3.16**, using this morphology as an index of identification, 53% of the wild populations of identified as genotypic *C. anguillaris* had their phenotype agreeing to the genotype, while the remaining 47% had gaps on their vomerine teeth. Similarly, 65% of the wild populations of *C. gariepinus* had phenotypes agreeing to their genotype, while the remaining 35% had no gaps in their vomerine teeth.

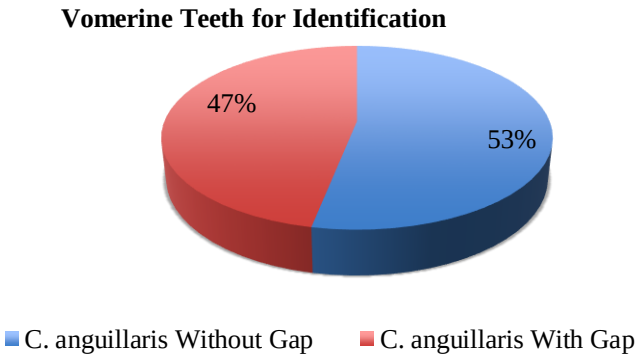

Figure 3.15. Vomerine teeth as an index of identification showing conformity or not with the genotype of *C. anguillaris* sampled from both R. Niger (n = 32; mixed samplings)

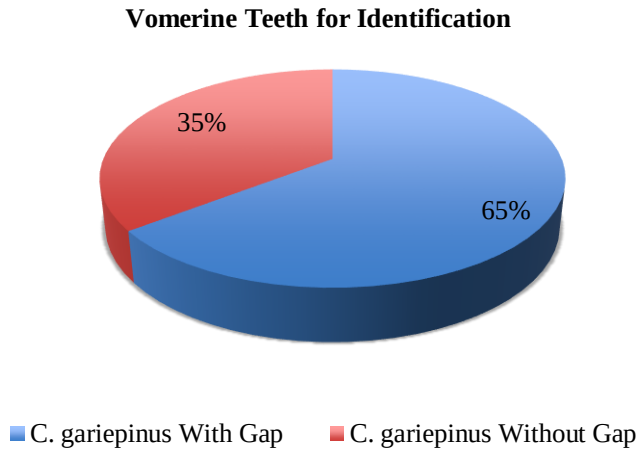

Figure 3.16. Vomerine teeth as an index of identification showing conformity or not with the genotype of *C. gariepinus* sampled from both R. Benue and Niger (n = 165 mixed samplings)

A 2 by 2 G-Test of contingency test was carried out to check relationship between genotypes and vomerine teeth. The G-values of 1.630433 obtained following the test involving genotypic *C. gariepinus* and *C. anguillaris* from lower R. Niger tested against their phenotypes **(Table 3.6.).** This value shows no significant correlation between the genotypes and phenotypes.

Table 3.6. G-Test for Catfish from Lower R. Niger

Genotyped species	No gap	Gap
Putative *C. anguillaris*	19	13
Putative *C. gariepinus*	22	27

3.4.7. Correlation of Number of Gill-Rakers vs Standard Length to Genotypes

More commonly used than the vomerine teeth across different countries, the number of gill rakers on the first branchial arch is thought to separate *C. anguillaris* from *C. gariepinus*. Results from this study show that this trait varies between the two species, water bodies and sizes of fish. **Figure 3.16** shows that all *C. anguillaris* and similarly sized *C. gariepinus* had 16 – 24 and 30 - 58 gill rakers on the first branchial arch respectively. There was a significant difference ($P < 0.05$) between the putative *C. gariepinus* from R. Benue and putative *C. anguillaris* from R. Niger and *C. gariepinus* from upper and lower R. Niger **(Figure 3.16).** Furthermore, *C. gariepinus* from lower R. Niger (Niger and Kogi states), generally (except for an individual) had a higher numbers of gill rakers (22- 73) compared those of the upper R. Niger in Kebbi (14 – 33), with a significant difference

between the two sets, while no significant difference (P > 0.05) was observed between *C. gariepinus* and *C. anguillaris* from the lower R. Niger. While there were positive correlations between standard length and number of gill rakers for the two species at specific sampling sites **(Figure 3.17),** there was no significant correlation between standard length (SL) and gill rakers (GR) for putative *C. gariepinus* from upper R. Niger, having a significantly high P values of 0.4239 and a significantly low regression coefficient of R^2 = 0.01211 **(Figure 3.17).** There was markedly reduced number of GR in larger *C. gariepinus* compared to the smaller ones in the lower R. Niger. All these separation into putative species has been based on the 8 SNPs selected and assayed.

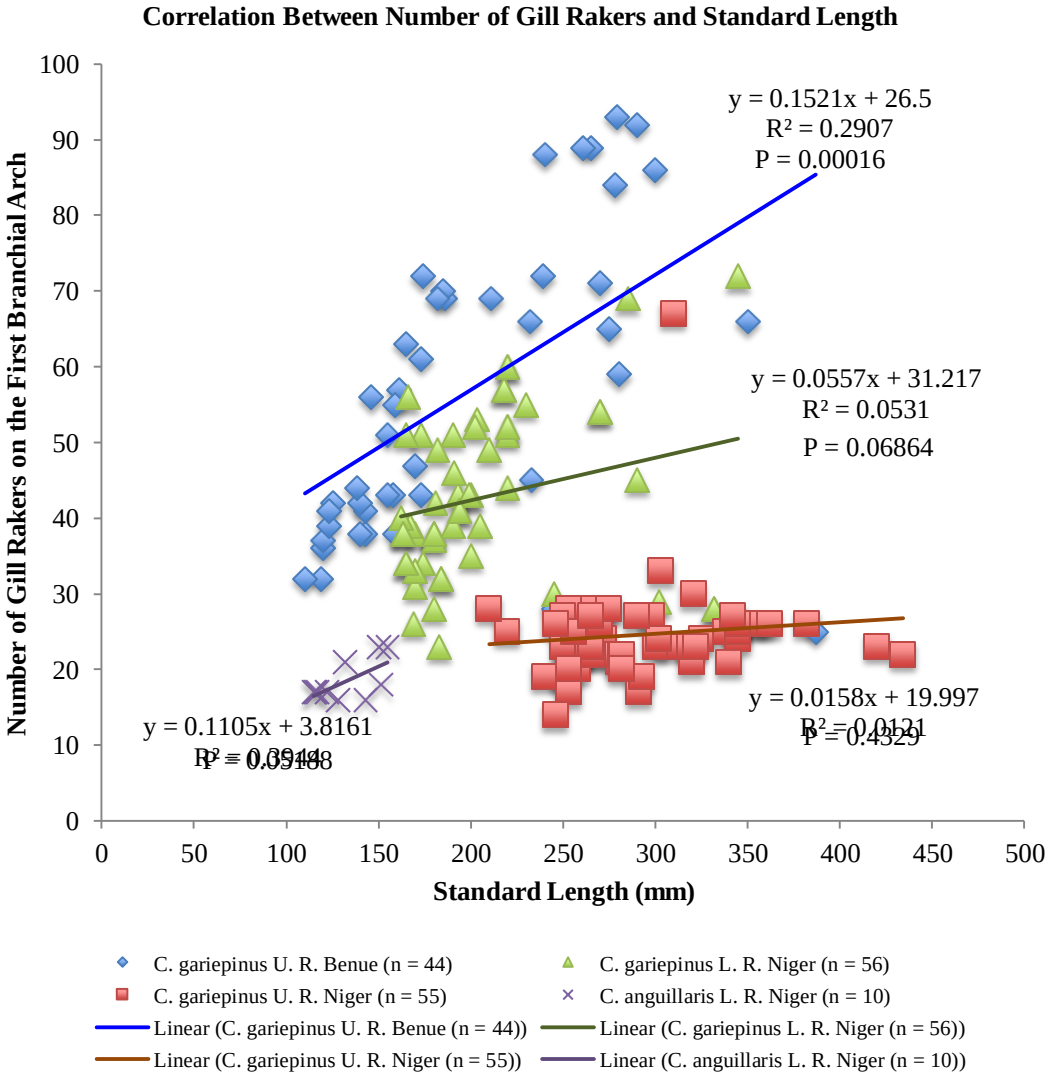

Figure 3.17. Regression equations on a graph showing levels of correlation between number of gill rakers on the first branchial arch of putative *C. gariepinus* and *C. anguillaris* with their respective standard lengths (n = 165 mixed samplings)

3.5. Discussion

The versatility and cost-effectiveness of ddRADseq is that it works with different restriction enzymes, on any species and at low cost per sample, meaning that different aquaculture species can be partially sequenced *de novo* for SNP discovery (Etter *et al.*, 2011; Davey and Blaxter, 2012; Davey *et al.*, 2012). This next-generation sequencing (NGS) platform has created impact in studies on genetic diversity and population structure in fish species such as tilapia, salmon and channel catfish (van Bers *et al.*, 2012; Johnston *et al.*, 2014; Liu *et al.*, 2016). This study represents the first attempt to use NGS in the discovery of SNPs separating *C. gariepinus* from *C. anguillaris*. Although SNP discovery for different populations require sampling of large number of fish, the high throughput of ddRADseq enables better coverage of the genome and thus, more SNPs from fewer individuals. The high number of paired-end raw reads, depth of reads, unique RAD-tags and polymorphic loci generated from this study involving 25 individuals from five different populations gave high numbers of SNPs per species. This trade-off in number of samples vs number of reads per sample was considered in selecting the sample size and number of populations to be included in this ddRAD library. In order to strengthen the analysis, samples from five geographic locations (covering three countries)**,** comprising two wild and two farmed stocks of *Clarias*, and an out-group species were included. This covered two genera (*Clarias* and *Hetrobranchus*) and two *Clarias* species (*C. anguillaris* and *C. gariepinus*), thus, offering though skewed, but broad sample set for preliminary SNP generation, species discrimination and phylogenetic analysis using ddRADseq.

3.5.1. Phylogenetic Trees

Pooling individuals from diverse populations together increases the chances of discovering relatively ancient, and thus, polymorphic SNPs (van Bers *et al.*, 2012). A PCA analysis was always conducted alongside the construction of a phylogenetic tree so as to better capture the discrimination of markers. Three main clusters (clades) were generated at every stage of the analysis, showing significant separation between the two genera and two nominal *Clarias* species. The wild population of *C. gariepinus* from R. Benue (Cga_Benue), Farmed BANAR – a Dutch strain of *C. gariepinus* in Nigeria (Cga_Baner) and the Egyptian (Cga_Egypt) populations of *C. gariepinus* all grouped together in the same clade, while all the *C. anguillaris* from R. Niger (Can_Niger) all clustered together in

a separate clade, likewise the out-group, *H. longifilis* (Hlo_Carol)**.** The three members of *C. gariepinus* from R. Benue that appear to be bit distant from the other members of the putative species, and seem to share closer ancestry with members of the outgroup and putative *C. anguillaris* could be as a result of the fact that it had evolved more recently compared to other members of its putative species. It is important to mention that this distance was only notable when many SNP markers (2587 markers for 50% coverage and up to 5 SNPs) were considered in the construct of the phylogenetic tree. When the number of markers was narrowed down (397 markers for 75% coverage and up to 2 SNPs, and further 24/25 markers for 75% coverage and up to 2 SNPs), it was more evident that they shared common ancestry with other members of putative *C. gariepinus,* and are actually distant from the putative *H. longifilis* and *C. anguillaris*, agreeing with the results of the KASP assay and PCA. The very clear distinction between the species concluded to be *C. anguillaris* and *C. gariepinus* in this study was based on high depth of reads and thousands of SNPs, and demonstrated the existence of two morphologically indistinguishable, yet genetically distinct closely related (cryptic) species in the genus *Clarias*. This agrees with the studies of Ozouf-Costaz *et al.* (1990), Teugels *et al.* (1992a), Agnese *et al.* (1997), Rognon *et al.* (1998), Agnese and Teugels (2005), Nwafili and Gao, (2007) and Nwafili (2013), involving a single mtDNA, cytochrome b, and tens of microsatellite markers and allozyme loci, although they turned out to be non-diagnostic – contrary to the findings of the present study.

Despite attempts to treat each population as a species, all *C. gariepinus* from the three distinct sources clustered together as one, while all the *C. anguillaris* (from a single population) clustered separately, evidently forming separate clades. The consistency of these clusters despite changing the condition from 2,587 shared loci with 1-5 SNPs in at least 50% of the samples to as low as 397 shared loci with 1-2 SNPs in at least 75% of the population suggest the integrity and robustness of the results**.** The further tightening of the clusters based on 25/24 species-specific SNPs/markers and the 8 SNPs selected for assay further confirms the discriminatory ability of ddRAD between *C. anguillaris* and *C. gariepinus*.

The 100% variance between *C. anguillaris*, *C. gariepinus* and *H. longifilis* as shown in depicts how genetically distinct these species are. While the variances within the species were lower, the overall cumulative variance from the bootstrap values of over 80%

indicates a high level of distinction between the species. The current study shows that *C. gariepinus* can be clearly discerned from *C. anguillaris* based on shared SNP markers and this will be very useful in studies to investigate possible introgression or hybridisation in different populations of *Clarias gariepinus*.

3.5.2. Species-Specific SNP Markers

In order to validate the SNPs as diagnostic or not for either species, assays were developed to test eight (four each for *C. anguillaris* and *C. gariepinus* respectively) of the 24 diagnostic SNP markers on the 25 individuals sequenced and 273 others from six different countries. The result shows three unique genotypes for the three species (2 *Clarias* species and 1 *Heterobranchus* species (the out-group)) in the ddRAD and a summary of all individuals assayed (25 + 273). The fact that all 25 individual were clearly distinguished based on the SNP allele frequency, and were 100% in agreement with the results from the ddRADseq strongly validates the efficacy of the SNPs as diagnostic for both species. Furthermore, all of the farmed stocks (including the one from Zambia, where there are no *C. anguillaris*) and a large group of wild stock, collected from four different location along the two main rivers (Upper R. Niger and Benue, and Lower R. Niger and Benue) conformed to one pattern of SNP genotypes, which are postulated to be *C. gariepinus*, while the other group (based on a distinctly different pattern of SNP genotypes) are therefore postulated to be *C. anguillaris*).

Since all members of the sub-genus *Clarias (Clarias)* have been reclassified and regrouped by Teugels (1986) to only two species i.e. *C. anguillaris* and *C. gariepinus*, it is therefore, only logical to conclude that these two distinct, yet very close groups are species of *C. anguillaris* and *C. gariepinus*, with *C. gariepinus* having a wider distribution across the different sampling sites in the wild and farms in the different countries.

This represents the first set of diagnostic markers developed for *C. anguillaris* and *C. gariepinus*, as previous studies involving other techniques could identify private alleles but not diagnostic markers (Ozouf-Costaz *et al.*, 1990); Teugels *et al.*, 1992a; Agnese *et al.*, 1997; Rognon *et al.*, 1998; Agnese and Teugels, 2005; Nwafili and Gao, 2007 and Nwafili, 2013). The study also shows that there are consistently two cryptic species (*C. gariepinus* and *C. anguillaris*) present in Nigeria, which are not clearly separable on the basis of morphological characters commonly used and/or suggested in the scientific

literature. The main limitation of the study is that the species concluded to be *C. anguillaris* was sampled only from one location (lower R. Niger). Future research should look at samples coming from elsewhere in the range of this species suggested in the literature. It would also be interesting to analyse farmed stocks originating from the lower R. Niger to see if these contain introgressed stocks, based on the SNP markers used here.

3.5.3. Hybridisation and Introgression between *C. gariepinus* and *C. anguillaris*

The fact that only one individual out of the 207 samples collected was found to be heterozygous for 2 of the 8 SNPs used suggests that hybridisation between these sympatrically occurring species is not common, although this is possible, as reported in the works of Agnese *et al.* (1997), who collected samples from the Senegal Basin. Only one sample was heterozygous for two of the eight SNPs tested in the current study, suggesting it is possibly an F2 hybrid, back-crossed with a *C. gariepinus* parent from R. Niger, since all the other six SNPs were of the *C. gariepinus* composite genotype.

The idea that the Dutch domesticated strain of *C. gariepinus* used in the Nigerian Aquaculture Industry contains an introgressed mixture of *C. gariepinus* and *C. anguillaris* (Nwafili and Gao 2007; Nwafili, 2013) was not supported by the findings of this study. Although the samples involved in the phylogenetic analysis were few, the PCAs and trees seem to suggest absence of hybrids in the wild populations, the Farmed Baner and Egyptian population of *C. gariepinus*. Furthermore, samples from three Dutch farms in Nigeria, three Dutch farms in The Netherlands, a source from Hungry and Poland, all presented the same genotype as the indigenous *C. gariepinus* from four distinct locations (two each) in R. Niger and R. Benue. Similarly, exactly the same genotype was found in all the *C. gariepinus* analysed from Egypt and Zambia. None of the Dutch *Clarias* presented genotypes containing alleles present in the 32 individuals from the lower R. Niger that were concluded to be *C. anguillaris* in this study, nor were there heterozygous individuals.

This study has therefore, discovered SNP markers that appear to distinguish between *C. gariepinus* and *C. anguillaris* which, with further validation, are potentially a very suitable benchmarking tool for various populations of these species, especially those under dispute. The SNPs could be useful in investigating hybridisation and introgression *C. gariepinus*, *C. anguillaris* and *H. longifilis* in both wild and aquaculture. It is worth mentioning that

although some farmed samples covered in this study were collected from the same states of the country (Lagos and Oyo states) as the previous study involving mtDNA, the absence of hybrids in this study despite the discriminatory power of the SNPs demonstrated, may suggest different possible scenarios.

- The possibility that the previous study examined stocks that have been intentionally or unintentionally bred with wild stocks of *C. anguillaris* (wrongly identified as *C. gariepinus*) in Nigeria, especially during the onset of the aquaculture revolution, when numerous hatcheries accessed only very few Dutch strains to improve or replace the wild stock of broodstock already existing on the farms. As such, proper assessment of broodstock origin and replacement plan alongside such studies is important in detecting possible sources of hybrids.
- The limitation of mtDNA in that it is not genome-wide, but only based on a single maternally inherited locus to identify and characterise an entire population, makes it not as informative and reliable as the SNPs. This limitation, coupled with the high genetic variation and distribution of *C. gariepinus,* means certain strains of same species might be picked up as second species.

3.5.4. Phenotypic-Genotypic Correlation and Diagnosis

Several authors have used phenotypic traits to describe, characterise and/or identify *C. gariepinus* and *C. anguillaris*. The use of such traits as a stand-alone index or together with a molecular technique as indices of identification has been documented (Teugels, 1982, 1986; Yisa and Olufeagba, 2003; Hanssen, 2009).

3.5.5. The Use of Vomerine Teeth for Identification of Clarias spp

Reed *et al.* (1967), Teugels (1982a; 1986; 2003), Yisa and Olufeagba (2005) and Teugels *et al.* (2007) in their respective studies reported that vomerine teeth in *C. gariepinus* were mostly conical, sub-granular, forming a crescent band, which might be slightly interrupted in the middle. Agnèse *et al.* (1997) reported that the width of the premaxillary and vomerine tooth-plates were 22.3% and 21.5% of their head length in *C. gariepinus*. The

slight interruption described as a "gap" in this study is mostly used in Nigeria, with little attention on the width of the premaxillary tooth plate, relative to the head length.

While the use of gaps or no gaps in the vomerine teeth to separate *C. gariepinus* from *C. anguillaris* is not well documented, due to its inconsistency, unreliability and inability to diagnose in very small fish (Teugels *et al.*, 2007), it is by far the most popular and practical index used to differentiate these two species in Nigeria, especially before the introduction of the Dutch strain to the aquaculture industry. For this reason, this study chose to examine the wild population of both *Clarias spp* in Nigeria, to see how diagnostic the morphology of the vomerine teeth is. The fact that only 53% and 65% of *C. anguillaris* and *C. gariepinus* respectively had vomerine teeth morphology conforming to their genotypes shows how unreliable this index is in identifying these species, thus agreeing with Teugels *et al.* (2007). A closer look at how this phenotype changes with genotype and the relationship between them shows there was no significant correlation between the genotype and phenotype (vomerine teeth). Why is this phenotype popular in Nigeria in the first place? Populations from River Niger showed more agreement for *C. gariepinus* (gap), while populations from R Niger shows more agreement for *C. anguillaris* (no – gap). This difference between populations might have been assumed for difference in species. These rivers constitute the largest water bodies in the country and are still (together with their tributaries) the main sources of wild freshwater fish, which was mostly *Clarias spp*.

It is worth mentioning that the ddRAD-seq was based on the initial sampling carried out using vomerine teeth as an index for identification. The samples analysed turned out to be perfectly distinguished i.e. genotype for *C. anguillaris* agreeing with phenotype (no gaps in the vomerine teeth) and so was the case for the wild *C. gariepinus* sequenced, having their genotype agreeing with their phenotype (present of gaps in their vomerine teeth). Since this was the basis, one will wonder which comes first? Randomly selecting samples for sequencing and genotyping before examining respective phenotypes or using phenotypes to select samples for sequencing and validating afterwards – somewhat like a chicken and egg scenario, which came first? The fact that during sampling, vomerine teeth was used does not in any way determine the eventual genotype. The teeth were used as indices because it is widely used for "distinguishing" these species in Nigeria. Their accuracy was questionable and has from this study, been confirmed inaccurate. During the initial sampling (stage 1), a total of 42 wild samples were collected comprising a minimum

of 7 samples of *C. gariepinus* (with gaps) and 7 of *C. anguillaris* (without gaps) from each river, using the vomerine teeth as an index of identification. It was out of the 42 samples that 5 samples identified as *C. anguillaris* (no gaps) from R. Niger and another 5, identified as *C. gariepinus* (with gaps) from R. Benue were used for the ddRAD-seq, along side other *C gariepinus* samples; 5 from Egypt and 5 from Barnerly. The last 2 sets were not selected or identified based on vomerine teeth; rather, they were received as farmed *C. gariepinus* and they turned out to group with the wild *C. gariepinus* from R. Benue. Furthermore, following SNP validation using KASP assay, the remaining 12 samples from R. Niger and 20 from R. Benue respectively identified based on vomerine teeth turned out not to follow similar pattern. All the 7 supposed *C. anguillaris* (with no gaps) from R. Benue turned out to be genotypically putative *C. gariepinus*, while majority of the supposed *C. gariepinus* (with gaps) from R. Niger were in fact genotypically putative *C. anguillaris*. This non-agreement prompted further sampling, thus, stage 2 from different countries and stage 3 from Rivers Niger and Benue in stage 3, noting all phenotypes and then genotyping for validation and agreement. Interestingly, all the farmed *C. gariepinus*, including those originating from Zambia (which is off the geographical range of *C. anguillaris*) turned out to be putative *C. gariepinus*. Hence, suggesting a very strong probability of coincidence that the genotypes of the initial samples sequenced matched correctly matched their phenotypes (vomerine teeth).

3.5.6. Correlation Between Standard Length and Number of Gill Rakers

This technique has been the most commonly used one for separating these species whether as a stand-alone index, in conjunction with other phenotypes or some molecular techniques (Debouche *et al.*, 1979; Teugels, 1986; Agnese *et al.*, 1997; Rongon *et al.*, 1998; Compaoré *et al.*, 2015; Zakariah *et al.*, 2016). In this study, we looked at how diagnostic the correlation between the numbers of gill rakers (GR) on the first branchial arch to standard length (SL) is, by comparing results of the correlation to the genotypes of the fish. The low number of GR relative to SL in the *C. anguillaris* samples agrees with the studies of Teugels *et al*. (1982**)**, Agnèse *et al.* (1997)**,** Teugels *et al*. (1998**)**, Rognon *et al.* (1998**)**, Wiecaszek *et al.* (2010) and Compaoré *et al.* (2015)**.** Although there were fewer and smaller samples of *C. anguillaris* relative to *C. gariepinus* in this study, it is important to state that the *C. anguillaris* had consistently lower number of GR, while there were variations in these values in *C. gariepinus*. The fact that these variations were more

evident between sampling sites than within, agreed with the study of Turan *et al.*, (2005)**,** who examined five distinct populations of *C. gariepinus* from five water bodies in Turkey. The higher number of gill rakers in *C. gariepinus* from both upper and lower River Benue and lower R Niger relative to the upper R. Niger, depicts how variable this index is for this species, and this agrees with the findings of Ola-Oladimeji *et al.* (2017)**,** who noted among 120 specimens, that the number of gill rakers was the most variable trait between the two sample sites in Ogun and Ondo states of Nigeria. The very low number of gill rakers in *C. gariepinus* from the Upper R. Niger and the lack of correlation in samples from the upper R. Niger contradicts the findings of Teugels *et al.* (1982), Agnese *et al.* (1997), Rogon *et al.* (1998) and Compaoré *et al.* (2015). Changes in the morphology of fish head in response to adaptation has been reported in the studies of Turan *et al.* (2005), who after examining 20 phenotypes, reported the highest level of variation only in the morphology of the head of *C. gariepinus*. The variation in the morphology of the head and or gill rakers in upper R. Niger might be in as a result of environmental factors such as response to adaptation, availability and type of food, feeding behaviour (more predatory on larger preys than smaller ones), water temperature, turbidity and water current (Ayinla, 1988; Admassu *et al.*, 2015).

3.5.7. Species Distribution

All samples from five out of the six countries analysed, presented consistent genotypes that were concluded to be *C. gariepinus*, while the sixth country (Nigeria) had this genotype from both farmed and wild populations and a different genotype that was concluded to be *C. anguillaris* from the wild population in the lower R. Niger. This findings agree with the studies of Johnels (1957), Lévêque *et al.* (1991), Turan *et al.* (2005) and FAO (2018b), which suggest a Pan-African distribution for *C. gariepinus* and a mainly West African location for *C. anguillaris*. Furthermore, it is surprising that not a single *C. anguillaris* was found in samples collected from R. Benue, since the earlier taxonomy of this species did extend to Cameroun, in the highlands of which the river is sourced. Also, one of R. Benue's earlier tributaries was the Chad basin, which was thought to have *C. anguillaris* (Johnels, 1957; Lévêque *et al.*, 1991; FAO, 2018b). Furthermore, the fact that both the Niger and Benue Rivers meet at the lower Niger (Lokoja) opens up the possibility of *C. anguillaris* migrating into the Benue system. It should not be suggested that *C. anguillaris* is absent from the River Benue based on the small sample

size and locations in this study. Another noteworthy finding is the fact that from the Upper R. Niger (Argungu and Yawari in Kebbi state), all the samples collected were concluded (based on SNP genotypes) to be *C. gariepinus,* and not a single *C. anguillaris* was found.

The observed variations (in vomerine teeth and gill rakers) in *C. gariepinus*, across different locations within the country, present great opportunity for aquaculture i.e. stock evaluation and improvement, e.g. through selective breeding. It is therefore important that, since diagnostic markers for this species and *C. anguillaris* have been found, studies on population structure and strains of *C. gariepinus* within and between different countries should be done. This will ensure a proper understanding of genetic structure, relatedness, potential, and the conservation needs of various populations/strains. Of particular need is for various regulatory organisations and conservationists to understand the diversity and relatedness of members of this genus (especially *C. gariepinus*), so as to properly regulate movement of this species between countries and maintain a good balance between introduction of commercially important species and conservation of biodiversity.

3.6.0. Conclusion

This study represents the first application of next generation sequencing to distinguish between *C. gariepinus* and *C. anguillaris*. Double digest RAD-seq using *Sbf*1 and *Sph*1 restriction enzymes generated over 2500 SNP markers that were used to produce a molecular phylogeny of the two species and an out-group (*H. longifilis*). Principal component analysis of this datas*et al.*so clearly separated these three species. From this dataset, 24/25 species-specific SNP markers were identified across the set of three species of *Clariidae* when 1-2 SNPs were allowed per ddRAD locus: larger numbers of species-specific markers were found when more SNPs were allowed per locus. Screening of a set of such SNPs (eight selected SNPs) using a larger test panel of *C. gariepinus* and *C. anguillaris*, showed consistent separation into two distinct genetic groups, suggesting 100% discrimination between these species. While further studies (e.g. larger sample sizes, samples of *C. anguillaris* from different locations/countries) is recommended, these markers appear to be suitable for investigating distribution, hybridisation and introgression in these apparently cryptic *Clarias* species, both in the wild and in aquaculture, and a similar approach could be applied to other species groups. Markers derived from ddRADseq such as SNPs and microsatellite markers could aid in studying the genetic

structure, relatedness, distribution and variation within *C. gariepinus* within and between countries for aquaculture and conservation purposes. All the Dutch domesticated stocks of *C. gariepinus* tested had only the *C. gariepinus* genotype, thus hybridisation/introgression of *C. gariepinus* and *C. anguillaris* was not detected. Vomerine teeth, used in identifying both species in Nigeria, have been confirmed to be inconsistent and only 64.8% reliable as a whole. Similarly, the number of gill rakers on the first branchial arch as an index of identification has been found to be not reliable. Further studies on correlation between genotypes vs morphological and meristic indices, with parallel SNP genotyping, are recommended.

CHAPTER 4. Development of Parentage Assignment and Performance Evaluation in the African Catfish (*Clarias gariepinus*)

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Experimental design, set-up and fieldwork were carried out by the author of this thesis under the supervision of David Penman, Brendan McAndrew and Almas Gheyas. The author carried out all fin clipping, tagging, measurement of phenotypes, DNA extraction and quantification. John Taggart did enrichment of microsatellite while Michael Beakert isolated other microsatellites from the ddRADseq in Chapter 4. The author optimised all the primers under the supervision of John Taggart, while the multiplex PCR optimisation was under the supervision of Sarah-Louise. The author and Sarah Louise did binning and scoring of all samples, while John Taggart ran the simulation in presence of the author. Every other content of this chapter was carried out and all the content compiled written by the author and corrected by the supervisor.

4.1. Abstract

Evaluation of the suitability of different strains or populations of fish for culture is a very important step in the development of a breeding programme. In this study, aimed at developing molecular markers for genetic management of *Clarias gariepinus* for aquaculture and using the markers to evaluate the suitability of different populations and strains for aquaculture, three farmed and two wild (first generation of domestication) populations of broodstock were sourced. Three replicates of 5 x 4 partial diallele crosses produced a total of 150 families of *C. gariepinus*.

At 8 weeks post hatch, the juveniles from each replicate were separated into three groups of a total of 250 juveniles per group and were stocked in each of the environment, noting their initial average weight and length and recording their growth and water quality parameters. DNA from 60 parents and 1200 offspring was analysed for parentage and kinship assignment. The parental DNA was badly degraded.

Four published microsatellite markers, four from sequences of ddRADseq while the remaining 4 were enriched yet from ddRAD library to make of 12 microsatellites tested and optimised for *C. gariepinus.* FAP was run for this simulated family dataset, assuming 8, 9, 10, 11 or 12 loci were available for typing. The highest assignment was using the 12 loci (95.39% to families and 96.86% to a parent), although this didn't vary significantly from using 11 or 10 loci. It is safe to conclude that using 9 of these microsatellites, over 90% of the offspring will be assigned families and parentage.

The PIC values, which were mostly above 0.5 for most of the loci, show how reliable and usable these sets of markers are. It is important to state here that the set of microsatellite markers derived from the ddRADseq showed more consistent characteristic profiles compared to those enriched or from the publication**.** This research has found suitable microsatellite markers from sequences generated from ddRADseq, thus showing the versatility of ddRADseq in the development of molecular makers.

4.2. Introduction

The importance of African catfish (*Clarias gariepinus* (Burchell, 1822)) as a species for aquaculture in Sub-Saharan Africa, some parts of North Africa, South America, Asia and Europe (FAO, 2014a) has been discussed in other parts of this thesis. By volume, its production as at 2015 was in excess of 250,000 MT, however, this has increased, with the biggest contribution coming from Nigeria, its largest producer and in itself, is the second most culture species in Africa (FAO, 2019). The annual increase in production of this species is shown in **Figure 4.1**, with most of this growth recorded in sub-Saharan Africa (Nigeria, Uganda and Kenya).

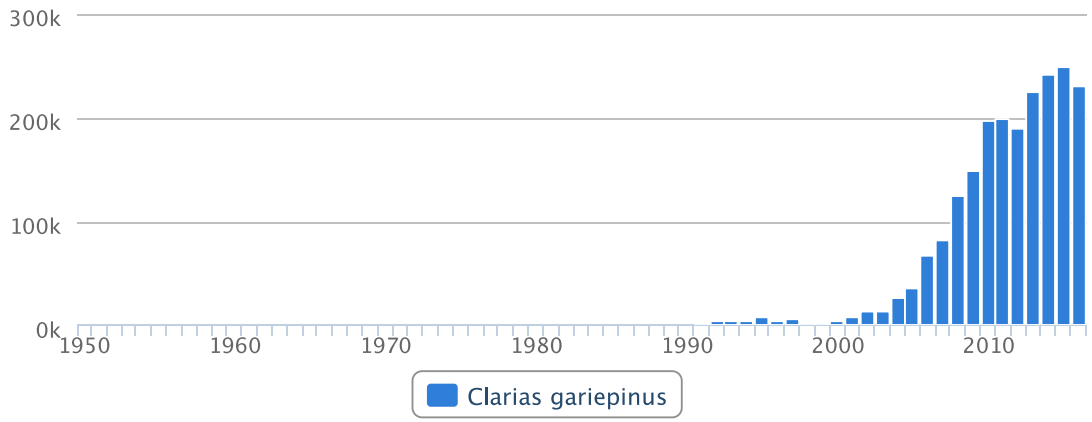

Figure 4.1. Global production of African catfish (*Clarias gariepinus*)
***Source*:** FAO, 2019

At 56% of total aquaculture production, the catfish industry in Nigeria is currently valued at over USD 800 million. The industry has reached this size, with so much more growth potential, partly driven by higher level of intensification and influx of new inexperienced farmers. To date, there is no documented evidence of planned genetic management or breeding programmes that could help to sustain the growth rate. The basic steps in developing these include assessment of the performance of available strains and development of tools such as DNA markers for parental assignment.

Evaluation of the suitability of different strains or populations of fish for culture is a very important step in the development of a breeding programme (Nguyen, 2008). Various forms of diallele crosses involving the different populations provide information on genetic variation required for selection (Das *et al.*, 1998; Nguyen,

2008). While culture systems of *Clarias* has been researched in Belgium, The Netherlands, Central Africa, Ivory Coast and South Africa (Hecht *et al*., 1996 and Miller and Atanda, 2011) with Nigeria benefiting a lot from the outcomes the focus of current research has barely shifted from catfish nutrition, endocrinology, water quality and other management issues etc. Until recently, studies on the genetic management of African catfish stocks has been minimal, and very few studies has been documented, especially using modern molecular biology techniques as has been done with tilapia, salmon, carp, etc. Presently, research aimed at setting up selective breeding programmes for *Clarias* catfish in Nigeria is still not far from the stage of evaluating the suitability of stocks. Akinwade *et al*. (2012) recorded better growth in *C. gariepinus* in a study comparing the growth performance of *C. gariepinus* and *C. anguillaris* and their reciprocal hybrids. In the same study, hybrids produced from male *C. gariepinus* and female *C. anguillaris* showed better growth than the reciprocal hybrids and even a much better growth than the pure *C. anguillaris*. Popoola *et al.* (2014) reported genetic variation between three wild and three cultured populations of *C. gariepinus,* noting more variation between the two habitats (between the wild and farmed ones) than populations within (wild or farmed) a given habitat. Other forms of research on genetics are on interspecific hybridisation (Owodeinde and Ndimele, 2010).

4.2.1. Traits of Interest for Genetic Improvement

Although less than 10% of the world's aquaculture production comes from genetically improved stock (Gjedrem, 2012; Gjedrem *et al.,* 2012), the need for genetic management in *Clarias gariepinus* has been discussed at different levels (Ponzoni and Nguyen, 2008), in response to reports of suspected inbreeding depression, questionable mortalities, uneven growth, aggression and cannibalism-induced mortalities (de Kimpe and Micha, 1974; van der Waal, 1978 and Hecht and Appelbaum, 1988; Hecth and Pienaar, 1993; Adeoye *et al.,* 2012; Igoni-Eqweke, 2018). Furthermore, findings from the review of the catfish aquaculture industry **(Chapter 1)** and results from the survey of hatchery practices in the Nigerian Aquaculture Industry **(Chapter 2)** suggest poor genetic management and replacement techniques for *C. gariepinus* broodstock, poor mating systems and the use of shooters as broodstock in most part of the industry. It has become imperative therefore, to

develop genetic management and improvement programme for this species, and especially for the Nigerian catfish aquaculture industry. Assessment of genetic variation in available stocks, and improvement of traits such as growth rate (harvest body length and weight) and survival have been identified as priorities from the previous studies. In addition, the use of shooters as broodstock is an issue to investigate. The increasing peri-urban aquaculture practice in Nigeria begs for genotype by environment studies to ascertain the performance of various populations, strains and/or crosses (e.g. hybrids) in the different rearing environments (earthen ponds, concrete tanks and plastic pools).

In order to develop effective genetic management programmes targeting improvement of such traits, it is important to evaluate the suitability of different populations for aquaculture, since there is shortage of information about the genetics of different populations of *C. gariepinus* (both wild and farmed) (Volckaert and Agnèse, 1995; Nguyen, 2008), and to assess the heritability of traits of importance.

4.2.2. Heritability of Traits

Heritability is a very important component of genetic improvement. It is a measure of the heritable proportion of phenotypic variance from one generation to another (Blonk *et al.*, 2010; Smitherman *et al.*, 1996). Phenotypic variance (V_P) is the sum of genotypic variance (V_G) and environmental variance (V_E). Heritability (h^2) indicates what proportion of the phenotypic variation is due to genetic variation, and is expressed as $h^2 = \sigma^2_A / \sigma^2_P$. In pedigreed populations, with data on phenotypes and genotypes, predicting or estimating heritability becomes possible. Heritability values differs between traits and populations and change within a given population in response to changes in population size, environmental factors or changes in gene frequencies (Oldenbroek and van der Waaij, 2015). Understanding the heritability of traits helps in genetic improvement of populations as the higher the heritability of a trait, the greater the response to selection in a breeding programme (Wang *et al.*, 2016; Volckaert and Hellemans, 1999). Therefore, it is also important to develop appropriate mating design(s) to assess heritability of traits being considered for improvement in an aquaculture situation. Factorial designs have been found to be ideal for heritability estimation (Blanc, 2003; Berg and Henryon 1998). Statistically,

analysis of variance (ANOVA), maximum likelihood (ML) and restricted maximum likelihood (REML) are methods of estimation of heritability and are computationally intensive, thus rely on specialised statistical packages like SAS, and GENSTAT.

4.2.3. Parentage Assignment

Genetic markers are very important in genetic improvement programmes, in part, due to the inability to tag fry due to their very small size, the lack of multiple culture tanks/ponds to separately grow the required number of families and the associated costs of doing so. Different polymorphic markers have successfully been used in parentage assignment. Microsatellite markers have been the most used since their introduction in the 1990s (Vandeputte and Haffray, 2014). This is due to their very high PIC (polymorphic information content) value, dispersion and abundance in the genome, and the availability of computer programmes to handle such large amount of genotyping data. Such technology now enables up to 100% of parentage assignment and increased accuracy in mating design, heritability and variation estimates for maximum genetic gain and reduced inbreeding depression in many species (Norris *et al.*, 1999; Ruzzante *et al.*, 1999; Alarcon *et al.*, 2004; Sekino *et al.*, 2002).

The advent of next-generation sequencing and rapid SNP discovery and genotyping methods has led to recent widespread use of SNPs for parentage assignment, perhaps slowing the further technological advancement of microsatellite genotyping platforms, consumables and analytical packages. It is still a very efficient method and is still in use in many pedigreed breeding programmes in aquaculture across the world.

4.2.4. Technical Tools for Parentage Assignment

Exclusion-based and likelihood-based methods of parentage assignment are the most common techniques of parentage assignment in aquaculture (Jones *et al.*, 2010). Although very sensitive to genotyping error, the exclusion-based method relies on the Mendelian principles of allele segregation, while the likelihood method is based on probabilities relying on allele frequencies, integrating genotyping error rates and therefore gives a higher but sometimes inconsistent assignment rate (Vandeputte and

Haffray, 2014). The efficiency of likelihood-based methods can be improved using sibship information (Wang and Santure, 2009). PROBMAX (Danzmann, 2012), VITASSIGN (Vandeputte *et al.*, 2006), and FAP (Taggart, 2007) are exclusion programs, while CERVUS (Kalinowski *et al.*, 2007), PAPA (Duchesne *et al.*, 2002), and PARENTE (Cercueil *et al.*, 2002) are likelihood programs used in aquaculture. The assignment power of the various packages depend on the exclusion probabilities of the markers involved, sample size (offspring) and the exponential effect of putative parents on the proportion of unassigned offspring (Vandeputte, 2012; Vandeputte and Haffray, 2014). Vandeputte *et al.* (2011) and Wang (2007) opined that overestimation of the assignment power of markers, which can be explained by Hardy–Weinberg disequilibrium, is very frequent. Other forms of errors due to null alleles, incomplete genotypes and size-shift are not uncommon (Sutton *et al.*, 2011; Yue and Xia, 2014). Using 8 – 15 microsatellite markers, up to 99% assignment power can be obtained in fish parentage assignment involving a few tens or hundreds of parents.

4.2.5. Aims and Objectives

The aim of this study is to develop molecular markers for genetic management of *Clarias gariepinus* for aquaculture. It also aims at evaluating the suitability of different populations and strains of *C. gariepinus* for aquaculture, thereby estimating variations (within and between the different populations), heritability and breeding value for individuals within these populations. The study also aims to investigate the suitability of using shooters as broodstock and develop adaptable models of breeding programmes for farmers.

Approach: A suitable facility for research was identified in Nigeria (NABDA). Experimental design (informed by the practices in the industry and literature review), sourcing of broodstock and setting up of crosses followed. Genomic DNA was extracted from the tissue of the parents and offspring and analysed. Microsatellite markers were developed and optimised at IoA and samples were sent to Queens University in Belfast for running. Scoring was done and a simulation test was carried out on the parental stock.

4.3. Methodology

4.3.1. Ethics Statement

All working procedures complied with the UK Animals Scientific Procedures Act (Parliament of the United Kingdom, 1986). This research was carried out with the approval of the University of Stirling Animal Welfare and Ethical Review Body (AWERB).

4.3.2. Choice of Stocks

Two wild stocks (each from Rivers Niger and Benue respectively), two farmed stocks and one domesticated wild stock (at least 5 generations in captivity) were the intended stocks to be used for the experiment. This was to enable studies on genetic variation between the different stocks and observe the effect of domestication on the performance and behaviour of the fish. Due to delay in release of funds and the fact that I had to construct the research facility between September, 2015 – April, 2016, there was a delay in commencement of the project, *C. gariepinus* sourced from R. Niger and Benue absorbed their eggs and it was possible to get gravid ones around March. It was also not possible to find any farm that had several generations of domesticated indigenous *C. gariepinus*. The final stocks consisted of three farmed stocks and two populations consisting of F1 (first filial) generation of domesticated wild stocks from River Benin and Lake Chad basin respectively (as described below).

4.3.3. Sampling Protocol

Three farmed and two wild (first generation of domestication) populations of broodstock were sourced from three farms and two wild locations in Nigeria **(Figure 4.2)**. ADM produces the South African strain of *C. gariepinus* (originated from South Africa) sourced in Kaduna State, Bar produces mainly the Dutch domesticated stock of *C. gariepinus* (originated from the Netherlands) sourced from Lagos State (with *H. longifilis*), while SCH produces the Dutch-domesticated strain of *C. gariepinus* (sourced from different farms in Nigeria) sourced from Nasarawa State **(Figure 4.2)**. On the other hand, the two wild stocks were originally sourced from lake Chad Basin in Borno State and River Benin in Edo State respectively **(Figure 4.2)**. For each population 7 mature males and 7 gravid females were sourced and transported to the

Aquaculture Unit of the National Biotechnology Development Agency, Abuja Nigeria. They were immediately tagged, recording individual length and weight, and collecting fin-clips under the influence of anaesthesia at a rate of 0.0625ml of clove oil/L of water. They were resuscitated in oxygenated water for 2 – 5 minutes after sampling and were left to acclimatise for 48 hours in different tanks per population before induction with 0.5 ml of ovaprim hormone per kg female. Approximately 1 cm^2 of fin was collected, water removed using filter paper and fixed in 99% ethanol. This was stored at 4°C in a refrigerator and after 24 hours, the ethanol was replaced and samples kept at the same temperature until brought to the UK for DNA extraction, sequencing, genotyping and analysis.

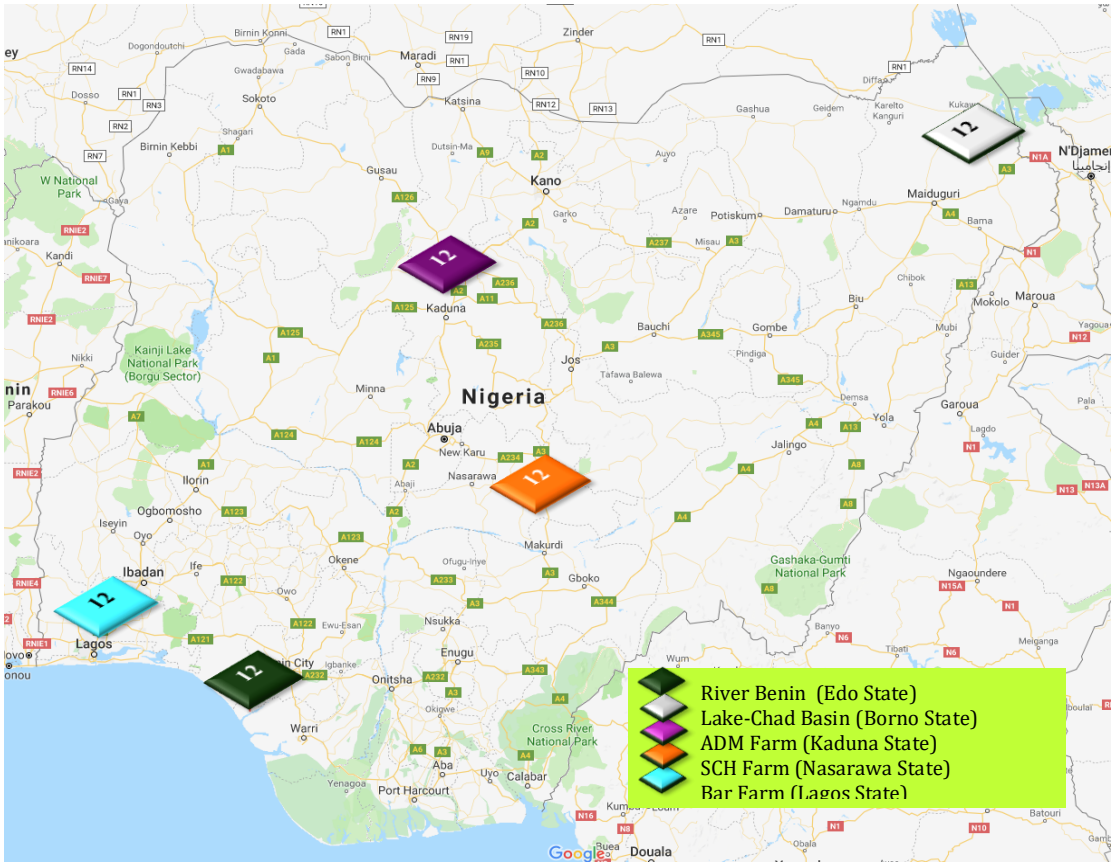

Figure 4.2. Sampling sites for wild and farmed *Clarias species* used in the study
A total of 60 broodstock were collected comprising 6 males and 6 females from each of the 5 sources. The study entailed – evaluating their suitability for aquaculture i.e.36 farmed stock and 24 wild ones.

4.3.4. Experimental and Mating Design

Three sets of mating were set at intervals of 1 month apart, with each cross involving 2 males and 2 females from each of the five populations – a 5 x 4 partial diallele

cross. The mating was such that each male fertilised 1 female from each of the 5 populations and each female was fertilised by 1 male from each of the 5 populations, in a format illustrated in **Figure 4.3**. This mating design enables even contribution of each individual and most importantly, enables accurate calculation of breeding value of each individual (average performance of half sibs). Each of the 5 x 4 partial diallele crosses produced 50 half-sib families, thus a total of 150 families were produced from the three mating sets.

	F. STRAIN	A_1	B_1	C_1	D_1	E_1	A_2	B_2	C_2	D_2	E_2	CROSSES PER M.
M. STRAIN	NO.	1	2	3	4	5	6	7	8	9	10	
A_1	1	AXA_1	BXA_1	CXA_1	DXA_1	EXA_1						ABCDE
B_1	2		BXB_1	CXB_1	DXB_1	EXB_1	AXB_1					BCDEA
C_1	3			CXC_1	DXC_1	EXC_1	AXC_1	BXC_1				CDEAB
D_1	4				DXD_1	EXD_1	AXD_1	BXD_1	CXD_1			DEABC
E_1	5					EXE_1	AXE_1	BXE_1	CXE_1	DXE_1		EABCD
A_2	6						AXA_2	BXA_2	CXA_2	DXA_2	EXA_2	ABCDE
B_2	7	AXB_2						BXB_2	CXB_2	DXB_2	EXB_2	ABCDE
C_2	8	AXC_2	BXC_2						CXC_2	DXC_2	EXC_2	ABCDE
D_2	9	AXD_2	BXD_2	CXD_2						DXD_2	EXD_2	ABCDE
E_2	10	AXE_2	BXE_2	CXE_2	DXE_2						EXE_2	ABCDE
CROSSES PER F.		ABCDE	ABCDE	ABCDE	ABCDE	ABCDE	BCDEA	CEABD	DEABC	EABCD	ABCDE	

(5x4 partial diallele cross involving 5 populations (2 wild populations and 3 farmed strains)) x 3 (entire crosses repeated three times)

5 populations (2 males and 2 females/population) x 3 replicates = 60 parents, producing 150 families

Figure 4.3. Shows the mating design and some stages of work carried out during the actual experiment. The arrow shows the sequence of the activities. The final arrow pointing back to the table shows the mating design is very important during analysis.

4.3.5. Fertilisation

Induced females were kept separately for 12 ± 3 hours in well-aerated 28^{o}C water, in a secured 100 L bowl covered with a woven mat to prevent fish from jumping out. At 8 hours post-induction, the two males from each of the populations were anaesthetised using 0.25 ml clove oil/L of water; four times the upper dose recommended for anaesthesia + recovery for this species (Hamackova *et al.*, 2006). An initial verification of inactivity following the anaesthesia was carried out before stunning the cranium (to cause brain contusion) with a blunt pestle to ensure death and then enable dissection of the ventral part of the fish to access the two lobes of testis for milt collection. The testes from each male were processed separately by maceration and addition of at least 5 ml of 0.7 g/l salt solution as an extender. Exactly 5 ml of processed milt from each male was collected in separate syringes and stored at 4 ^{o}C before fertilisation of eggs from females. At 10 hours post induction females were checked for ovulation and by 12 ± 1 hours all females had been stripped into a clean bowl, noting the weight of eggs from each female. From each female 5 g of eggs were collected and split into 5 petri dishes (1 g each). The eggs in each of the petri dishes was fertilised with milt from appropriate male, following the mating design (Figure 5.3). Fertilised eggs were incubated for 24 ± 3 hours at 28 – 30 ^{o}C in the hatchery tanks at NABDA, and at exactly 15 hrs of incubation, percentage hatchability was recorded by counting the number of dead (white) eggs and live (greenish brown) embryos.

4.3.6. Fry Rearing

At approximately 1 hour post-hatch, an equal number of hatchlings (50 fry) were collected from the incubation tanks into a single fry-rearing tank for early communal rearing to eliminate possible environment bias. This environmental bias could be due to tank effect, stocking density, light, water quality, etc. Feeding commenced at 72 hours post-hatch using shell-free artemia. Daytime feeding started 7:00 am for every 2 hours until 7 pm. This feeding regime was maintained until the 10th day post-hatch when they were weaned to micro-diet produced by Aller-Aqua, starting from 0.3 mm and size and quantities were adjusted based on growth rate and feeding regime gradually changed by increasing an hour per week. At three weeks post hatch,

shooters (fast growers) were picked out of the tank to prevent cannibalism and raised separately. Subsequently, grading was done every week to remove more shooters.

At 8 weeks post hatch, the juveniles were separated into three groups by scooping some fish into one tank after the other and repeating this until the three tanks were completely stocked (instead of completing one tank before going to the next). This was done to ensure even distribution of fish into the three groups without having the big ones in one group e.g. as a result of them remaining at the bottom of the tank. One tank was transferred into a concrete tank and the other into an earthen pond (because the pond was not ready at that time, they were held in plastic pools), while the third group remained in the collapsible tank. A total of 250 juveniles were stocked in each of the environment, noting their initial average weight and length. They were fed daily with commercial pellets, water quality monitored and grading took place at least once in two months to remove few shooters. Growth performance (length and weight) was recorded, fin-clips collected (as described above) and each of the fish were tagged at the age of 6 months post hatch under the influence of anaesthesia at rate of 0.0625ml of clove oil/L of water. They were resuscitated in oxygenated water 2 – 5 minutes after sampling and were left to continue growing.

4.3.7. Genomic DNA Extraction

Total genomic DNA was extracted using a slightly modified protein salting-out and isopropanol precipitation method (SSTNE/SDS) described by Aljanabi and Martinez (1997). This method offers good quality and quantity of genomic DNA. Each precipitated DNA sample was finally re-suspended in 5 mM Tris at pH 8.5. DNA samples were left to dissolve at 4℃ for a minimum of three days before quantification using spectrometry (NanoDrop ND 1000 Spectrophotometer, NanoDrop Technologies Inc.) and then by fluorimetry (Qubit® Fluorometer 2.0, dsDNA High Sensitivity assay kit; Invitrogen, ThermoFisher Scientific). All offspring had high molecular weight genomic DNA, with both 260/280 and 260/230 OD ratios exceeding 1.8. The integrity of each sample was checked using agarose gel (1.0%) electrophoresis. Due to a very poor quality of DNA recorded on the parental DNA, other DNA extraction techniques such as Phenol-chloroform and REAL-PURE kit were attempted with no success. Therefore, the original intention to use ddRADseq to

discover and genotype SNP markers for parental analysis was abandoned, and the focus was switched to microsatellite markers (microsatellite markers can be amplified from partially degraded DNA, while high molecular weight DNA is required for ddRADdeq). The fluorimetry measures of DNA quality was carried out only on the parental samples due to the fact that they had poor quality DNA. Based on fluorimetry values, parental samples were diluted to 2 ng/µL with 5 mM Tris, pH 8.5, while all offspring were standardised to 8 ng/µL.

4.3.8. Microsatellite Markers Development

Twelve microsatellite primers were obtained from three sources. A set of four microsatellite primers was obtained/developed from the literature (Galburosa *et al.*, 1996), from enrichment and from ddRADseq data respectively.

4.3.9. Published Microsatellite Markers

All seven microsatellite primers published by Galburosa *et al.* (1996) were screened to test amplification, polymorphism and heterozygosity on eight parental samples, and the best four were selected for use. These primers were ordered from Eurofins Genomics and dissolved in sterile TE before diluting in 90% water. They were not as polymorphic in the population tested as was reported in the author's tested population, and for the purpose of parentage assignment, more markers were required to add to the best four from this group.

4.3.10. Microsatellite Enrichment

The second set of microsatellites primers was isolated from a library of short fragments of genomic DNA of *C. gariepinus* by John Taggart, based on protocols described in Techen *et al.* (2010) and Jansson *et al.* (2016).

4.3.11. Microsatellites from ddRADseq

In order to maximise the number of highly polymorphic microsatellite markers to choose from, to complement those from the literature and microsatellite enrichment, and to explore the potential of ddRADseq in offering different types of markers for

different uses, markers were discovered from existing sequences generated from the ddRADseq library carried out for species-specific discriminatory SNPs discovery, described in **Chapter 3**. The library contained 25 samples: five *Clarias* samples from each of the four locations (Rivers Niger - putatively *C. anguillaris* - and Benue, a farm producing the Dutch-domesticated strain and the Egyptian strain – all putatively *C. gariepinus)* and five samples of *Heterobranchus longifilis* from The Netherlands as an out-group. Samples from R. Benue, Dutch-domesticated strain and the Egyptian Strain were putatively *C. gariepinus*. Microsatellites were detected using the set of shared RAD-tags from the Egyptian strain in the ddRAD experiment. The collection of reads was passed to Tandem Repeats Finder v4.09 in order to identify all potential repeats. Polymorphic loci were defined as microsatellite markers if they had simple sequence repeats (SSR), tandem arrays of short nucleotide repeats (1 – 6 bp). The repeat list was then assessed for repeat type and repeat length and primers were designed for fifteen microsatellite markers using QDD v3.1.2 (pipe3 only) and primer3 v2.3.7. These primers were ordered from Eurofins Genomics and dissolved in sterile TE before diluting an aliquot in 90% water. Aliquots of the diluted forward and reverse primers (exactly 20 µl of each) were mixed in a new tube, now containing 40 µl (a mixture of the forward and reverse primer).

4.3.12. Optimisation of Polymerase Chain Reaction (PCR)

Optimisation of PCR entails optimising the reaction parameters. Critical amongst them is the annealing temperature (T_a), which is done by testing amplification under different temperatures, usually varying by intervals of 1 ^oC or more over a range of 10 ^oC in a TProfessional Standard PCR Thermocycler. The gel image showed varying optimum annealing temperature for the same sets of samples, and the best annealing temperature for each primer over a range of samples was selected. For the reaction, 2.5 µl of Taq DNA polymerase (2x PCR mastermix ordered from Thermofisher Scientific), 0.5 µl of forward and reverse primer, 1.5 µl of water and 0.5 µl of genomic DNA made up the content of the 0.5 ml PCR tubes – a total reaction volume of 5 µl was used. **Table 4.1.** shows the PCR parameters set on the thermocycler. Temperatures were adjusted depending on the primers involved.

Table 4.1. PCR Programme optimised for *C. gariepinus* microsatellite markers

	Steps	Temperature (oC)	Time (min:sec)	Goto
	1	95.0	01:00	--
31x	2	95.0	00:15	2
	3	57.0	00:20	
	4	72.0	00:40	
	5	72.0	02:00	--
	6	20.0	00:01	--

4.3.13. Optimisation of Multiplex PCR

Depending on the fragment length and/or size of the microsatellites, several microsatellites can simultaneously be run in a single reaction (multiplexing). This was possible with the use of labels (fluorescent dye) on compatible primers (already checked using Primer Select (DNAstar Inc.), version 12.1.0 (141). 421 and verified from position of bands on the gel image). Primer Select locates pairs of primer dimers (PD) and reports their free energy value (ΔG). Lower ΔG values means higher temperature required to disrupt a secondary body (SantaLucia, 2007). As such pairs of primer dimers with lower ΔG values are selected, considering and selecting those with similar annealing temperatures and preferably different size ranges. Where size ranges overlapped, different fluorescent dyes were used. In addition to the reagents described for optimization earlier, tailed primers of separate concentrations of forward and reverse in separate tubes, and a fluorescent dye were added. A 10 µl reaction volume was made from the addition of 5 µl of Taq DNA polymerase, 0.2 µl of 1 µM tailed primer (forward primer), 0.3 µl of 10 µM pig primer (reverse), 0.3 µl of 10 µM florescent dye, 3.2 µl of water and 1.0 µl of genomic DNA. The PCR products were checked on a 1% argarose gel using 3 µl of PCR product, 1.5 µl of 6x loading dye and 0.5 µl of 100 bp ladder. The remaining PCR product was safely stored in a dark and cold environment (4^{o}C). The same PCR programme as in **Table 4.1** was used following check for optimality. Temperatures were adjusted depending on the primers involved.

4.3.14. Sample Preparation

Ready to use gels, capillaries, sample loading solution (SLS) and size standards were acquired from ABSiex, while the buffers were obtained from VWR. The fluorescent

dye was sourced from Thermofisher Scientific, and the appropriate one was added to the primer. They are cheaper than ordering large number of individually labeled primers. They are: M13R (blue), CAG (green), Godde (black) **(Table 4.2).**

Table 4.2. Fluorescent dye used in microsatellite primer optimisation

Name	Colour	Sequence	Sequence Length (bp)
M13R	Blue	GGATAACAATTTCACACAGG	20
CAGtag	Green	CAGTCGGGCGTCATCA	16
Godde	Black	CATCGCTGATTCGCACAT	18

The stored PCR product was defrosted and to 0.5 µl of the sample, 30 µl of SLS, 0.4 µl of size appropriate standard (SS-400 and SS-600) were added to a 96 well plate. The appropriate multiplexes mostly with different dyes were placed on the same lane and once complete, a drop of mineral oil was added to each well containing the multiplexes to prevent evaporation. The plate was then pulsed down before loading onto the machine. Following the manual and under supervision, the gel cartridge, capillaries used were checked to ascertain adequate content and then a sample sheet was prepared.

4.3.15. Fragment Analysis

Beckman-Coulter CEQ^{TM} 8000 Genetic Analysis System was used to run capillary electrophoresis to separate DNA fragments (ions) as they migrate through polyacrylamide gels by size exclusion effect. The migration of the DNA is triggered by application of electric field to electrolytes with the capillaries, and through fluorescence, the dye labeled DNA was detected and rendered as fragment sizes. Once a run was complete, raw data were analysed using predefined parameters and by looking at the position of the red marker (size standard). Once in appropriate location, other markers (M13R, CAG, Godde) were read individually and all observed peaks were labelled, creating locus tags, specifying size range of alleles and maximum bin width, while false peaks and "hedgehogs" were deleted. On occasions where the size standards were not in the appropriate position for all the dyes, reanalysis might fix the problem if it wasn't from the sample or faulty capillary. Reanalysis also add the created locus tags to the study.

4.3.16. Binning Setup and Scoring

Following the analysis, four polymorphic markers were selected from each of the three sources (literature, microsatellite enrichment and ddRADseq). These markers were sent to Queen's University in Belfast for validation. Upon validation, tailed primers were ordered and multiplexes developed – three multiplexes containing 3, 4 and 5 primers respectively. All the parental samples were standardised to 2 ng/µl (Q-Bit value), while all offspring were standardized to 8 ng/µl (nanodrop), checked on 1% argarose gel and sent to Belfast for genotyping. Results of genotypes were received and Bins were setup for each multiplex using Genemarker V1.85. This software was also used to score all the parents and offspring from one panel to another and from one panel (96 well plate) to another.

4.3.17. Microsatellite Marker Profiling

Profiling was done by assessing number of alleles (n), effective number of alleles (A_e), polymorphic information content (PIC), expected heterozygosity (H_e) and observed heterozygosity H_o), null allele prediction and by testing for Hardy-Weinberg equilibrium. These were calculated using GenAlEx (Peakall and Smouse 2006, 2012) and Microsatellite Toolkit (Park, Stephen. Animal Genomics Lab, University College, Dublin, Ireland).

4.3.18. Parentage Assignment

Genomic DNA of all fish (60 parents and 1,200 offspring) was extracted and genotyped. Due to the poor quality of the parental DNA, some individuals could not be genotyped; as such parentage assignment could not be carried out. However, a simulation was run using FAP (Family Analysis Package, Taggart, 2007) to check the assigning power of the microsatellite markers.

4.4. Results

Result from sampling - length, weight, relative calmness during handling, GSI (of broodstock) and fin clips were processed and stored in anticipation of success in genotyping the remaining parental fish. Unfortunately, none of the sets of diallele

crosses had all the parents genotyped. Sets 1 **(Table 4.4)** and 3 **(Table 4.6)** had more parents successfully genotyped than set 2 **(Table 4.5)**. Details of the percentage performance of each marker on the parental samples are also provided in these tables. The missing gaps are the failed samples and work is on going to attempt to extract usable DNA from the samples. All the offspring have been scored and are awaiting parentage assignment and evaluation of traits.

At the beginning of this study, there were four suitable microsatellite markers available from the literature. At this point, eight more suitable microsatellites markers have been developed and tested, bringing the number to twelve as listed in **Table 4.3.** The multiplexes created and successfully used in genotyping are shown in **Tables 4.4, 4.4** and **4.6.** Three multiplexes of 3, 4 and 5 microsatellite markers were made.

Table 4.3. List of microsatellites markers optimised for use in parentage assignment in *C. gariepinus*

Locus	Primer Sequence	Annealing Temperature (°C)	Size Range of PCR Product	Repeat Motif	Source
Cga_021_F	ATAGACTTTACATGATAAGCAGAACC	59	140	$(ATCC)_9$	ddRADseq
Cga_021_R	GAGACTTGACCAGACTATTTAACTAGT				ddRADseq
Cga_022_F	ACAAGCACCACGGGATTTCT	57	173	$(AGAT)_7$	ddRADseq
Cga_022_R	GCTACGTGTTGTTCCATGTCT				ddRADseq
Cga_026_F	GCCCATACAGAAAGCACTGA	57	160	$(AAC)_9$	ddRADseq
Cga_026_R	GCGAATGCTCCAGTACAAACC				ddRADseq
Cga_027_F	TGCAGGTAAGTACAAATGGCAA	57	215	$(AAT)_9$	ddRADseq
Cga_027_R	CATTGTCCACACCACAAGATCA				ddRADseq
Cga110_F	GCTGTAGCAAAAATGCAGATGC	58	102-138	$(GT)_2N_2(GT)_{15}$	Galbusera *et al.*, 1996
Cga110_R	TCTCCAGAGATCTAGGCTGTCC				Galbusera *et al.*, 1996
Cga202_F	TCACAGACTGATTAACTACATTCA	56	201	$(AGAT)_{20}$	Enrichment
Cga202_R	GAAGGGCTCATAAATTTCTGTTA				Enrichment
Cga203_F	CAAGCTTTTTGGAAGCAACATACTG	60	171	$(AGAT)_{16}$	Enrichment
Cga203_R	TGACAGTTAGCCACAATTGACCTATG				Enrichment
Cga204_F	CCAGGTATTCATCCATCCTCCTAT	61	181	$(ATCC)_{13}$	Enrichment
Cga204_R	TGGAAAATATAATTGCAGAAAACACA				Enrichment
Cga205_F	ACGCCCCTCGTGACCATACA	61	120	$(ATCC)_{10}$	Enrichment
Cga205_R	TTACAACCCTAGTGCCGTCATTAC				Enrichment
P01_F	GGCTAAAAGAACCCTGTCTG	57	92-104	$(GT)_{15}$	Galbusera *et al.*, 1996
P01_R	TACAGCGTCGATAAGCCAGG				Galbusera *et al.*, 1996
P03_F	CACTTCTTACATTTGTGCCC	55	142-168	$(GT)_{21}$	Galbusera *et al.*, 1996
P03_R	ACCTGTATTGATTTCTTGCC				Galbusera *et al.*, 1996
P09_F	CGTCCACTTCCCCTAGAGCG	63	180-196	$(GA)_3N_3(GT)_{11}N$	Galbusera *et al.*, 1996
P09_R	CCAGCTGCATTACCATACATGG			$(GT)_6N_2(GT)_4$	Galbusera *et al.*, 1996

Table 4.4. Scores from parents involved in the first set of mating (5x4 partial diallele cross)

	Multiplex 1						Multiplex 2								Multiplex 3										Total Scores			
Sample	**Cga_110**		**Cga_21**		**Cga_22**		**Cga202**		**Cga205**		**Cga_26**		**Cga204**		**P03**		**P09**		**Cga27**		**P01**		**Cga203**		**MP 1 (3)**	**MP 2 (4)**	**MP 3 (5)**	**TOTAL (12)**
271	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0
289	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0
277	-	-	-	-	169	169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	1
265	-	-	113	129	169	169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0	0	2
295	-	-	113	113	-	-	-	-	-	-	-	-	-	-	146	148	-	-	-	-	-	-	-	-	1	0	1	2
290	-	-	113	113	169	169	-	-	112	112	-	-	148	148	-	-	-	-	-	-	-	-	-	-	2	2	0	4
283	-	-	113	113	169	205	-	-	112	112	-	-	152	156	-	-	-	-	-	-	-	-	175	175	2	2	1	5
296	100	100	-	-	177	197	197	203	112	116	-	-	152	152	150	150	184	191	-	-	100	130	159	175	2	3	4	9
241	104	106	113	113	193	205	203	203	112	112	-	-	152	164	150	168	184	191	340	340	-	-	167	175	3	3	4	10
284	102	106	113	113	193	205	203	203	-	-	303	306	164	168	168	168	184	193	325	331	100	130	167	175	3	3	5	11
242	104	106	113	113	169	205	203	203	112	112	303	303	164	168	150	154	184	193	340	340	100	100	175	175	3	4	5	12
247	100	102	117	117	169	169	203	203	112	116	306	309	152	180	150	150	184	193	331	340	100	100	167	175	3	4	5	12
248	100	100	113	113	169	197	203	205	112	116	309	309	180	180	150	150	191	191	340	349	102	130	167	167	3	4	5	12
253	102	106	113	117	169	205	203	203	112	112	303	306	152	164	168	168	184	191	331	340	100	130	167	175	3	4	5	12
254	100	102	113	117	173	177	203	221	112	112	306	309	152	176	150	172	191	191	331	337	130	130	199	199	3	4	5	12
259	100	102	113	117	169	205	203	203	112	116	303	306	148	148	150	150	191	193	340	346	130	130	175	175	3	4	5	12
260	100	100	117	117	177	205	203	203	112	116	303	309	148	148	150	150	193	193	331	340	130	130	175	175	3	4	5	12
266	102	102	113	117	169	169	203	203	112	112	309	309	152	152	150	150	184	184	337	349	100	100	167	175	3	4	5	12
272	100	102	113	129	169	169	203	203	112	112	309	309	152	152	150	152	191	191	340	340	130	130	167	175	3	4	5	12
278	100	102	113	129	177	197	203	203	112	112	306	309	152	152	150	150	184	191	340	340	100	100	175	187	3	4	5	12
Amplified	12	12	11	11	16	16	10	10	10	10	7	7	13	13	10	10	9	9	6	6	9	9	9	9	0	0	0	0
Samples	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	0	0	0	0
% Scored	60	60	55	55	80	80	50	50	50	50	35	35	65	65	50	50	45	45	30	30	45	45	45	45	0	0	0	0

Table 4.5. Scores from parents involved in the second set of mating (5x4 partial diallele cross)

	Multiplex 1						Multiplex 2								Multiplex 3										Total Scores			
	Cga_110		Cga_21		Cga_22		Cga202		Cga205		Cga_26		Cga204		P03		P09		Cga27		P01		Cga203		MP 1 (3	MP 2 (4)	MP 3 (5)	TOTAL (12
273	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0
274	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0
297	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0
298	-	-	-	-	169	197	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	1
255	-	-	113	129	169	169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0	0	2
256	-	-	113	113	169	169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0	0	2
262	-	-	-	-	169	169	-	-	-	-	-	-	148	148	-	-	-	-	-	-	-	-	-	-	1	1	0	2
244	102	104	113	129	169	169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	0	0	3
249	100	102	117	117	177	197	203	203	-	-	-	-	180	180	-	-	-	-	-	-	-	-	-	-	3	2	0	5
267	-	-	-	-	-	-	-	-	112	116	309	309	152	180	150	168	184	191	-	-	100	110	-	-	0	3	3	6
291	102	102	-	-	169	169	-	-	112	116	-	-	148	148	-	-	-	-	-	-	130	130	175	175	2	2	2	6
279	100	102	-	-	169	169	203	203	112	116	-	-	152	152	150	150	-	-	-	-	100	100	-	-	2	3	2	7
243	100	100	-	-	169	177	199	203	112	116	-	-	152	152	150	168	191	191	-	-	100	100	175	175	2	3	4	9
285	102	104	113	117	169	193	203	203	112	112	-	-	156	168	150	154	184	193	-	-	100	130	167	175	3	3	4	10
286	102	106	113	113	193	205	203	203	-	-	303	306	164	168	150	168	184	193	325	340	-	-	167	175	3	3	4	10
261	100	102	117	117	169	205	203	203	116	116	303	306	148	148	150	168	193	193	340	340	-	-	175	175	3	4	4	11
250	100	104	117	117	169	173	195	203	116	116	309	309	152	156	150	150	191	191	337	337	100	100	167	175	3	4	5	12
268	100	102	113	117	169	169	203	203	112	116	309	309	152	180	150	168	184	191	340	349	130	130	167	167	3	4	5	12
280	102	102	113	117	169	177	203	203	112	116	306	309	152	180	150	150	191	191	331	340	100	100	175	199	3	4	5	12
292	100	102	117	117	169	205	203	203	116	116	303	309	148	148	150	150	191	193	340	346	100	100	175	175	3	4	5	12
Amplified	12	12	11	11	16	16	10	10	10	10	7	7	13	13	10	10	9	9	6	6	9	9	9	9	0	0	0	0
Samples	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	0	0	0	0
% Scored	60	60	55	55	80	80	50	50	50	50	35	35	65	65	50	50	45	45	30	30	45	45	45	45	0	0	0	0

Table 4.6. Scores from parents involved in the third set of mating (5x4 partial diallele cross)

	Multiplex 1						Multiplex 2								Multiplex 3										Total Scores			
Sample	**Cga_110**		**Cga_21**		**Cga_22**		**Cga202**		**Cga205**		**Cga_26**		**Cga204**		**P03**		**P09**		**Cga27**		**P01**		**Cga203**		**MP 1 (3)**	**MP2 (4)**	**MP3 (5)**	**TOTAL (12)**
275	-	-	113	129	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	1
257	-	-	113	129	169	169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0	0	2
264	-	-	113	129	169	169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0	0	2
281	-	-	-	-	169	169	-	-	112	112	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0	2
293	102	104	-	-	-	-	-	-	112	112	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0	2
276	102	104	-	-	169	169	-	-	112	116	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	0	3
269	-	-	113	113	-	-	-	-	116	116	-	-	152	180	150	168	187	191	-	-	100	100	167	167	1	2	4	7
294	104	106	113	113	169	193	203	203	-	-	303	303	164	168	168	168	184	191	-	-	130	130	175	175	3	3	4	10
282	102	104	129	129	169	169	203	203	112	112	303	309	148	152	150	152	191	191	-	-	100	100	175	175	3	4	4	11
299	100	102	129	129	169	177	203	203	112	112	309	309	176	176	150	170	191	191	-	-	100	100	167	187	3	4	4	11
300	100	102	113	113	173	209	199	203	116	116	309	309	152	152	150	168	184	191	-	-	100	100	175	175	3	4	4	11
245	100	100	129	129	169	169	199	203	112	112	303	309	148	152	150	152	191	191	325	340	130	130	167	175	3	4	5	12
246	100	102	129	129	169	169	203	203	112	112	303	309	148	152	152	152	191	191	325	340	100	100	175	175	3	4	5	12
251	102	102	113	113	169	169	203	203	116	116	306	306	152	180	150	150	191	191	340	349	100	100	175	199	3	4	5	12
252	100	102	113	117	169	177	199	205	112	116	309	309	152	180	150	150	184	191	337	340	100	100	167	175	3	4	5	12
258	100	102	113	117	177	205	203	203	112	116	309	309	148	148	150	168	184	193	340	340	130	130	175	175	3	4	5	12
263	102	104	113	117	193	193	203	203	112	112	303	306	152	156	168	168	184	193	325	340	100	130	167	175	3	4	5	12
270	100	100	129	129	169	169	203	203	112	112	309	309	148	152	150	150	184	191	325	340	130	130	175	175	3	4	5	12
287	102	104	113	117	193	205	203	203	112	112	303	303	156	168	150	154	184	191	325	331	130	130	167	175	3	4	5	12
288	104	106	113	117	169	205	203	203	112	112	303	303	152	164	168	168	184	193	331	340	100	130	167	175	3	4	5	12
Amplified	15	15	17	17	17	17	13	13	16	16	13	13	14	14	14	14	14	14	9	9	14	14	14	14	0	0	0	0
Samples	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	0	0	0	0
% Scored	75	75	85	85	85	85	65	65	80	80	65	65	70	70	70	70	70	70	45	45	70	70	70	70	0	0	0	0

4.4.1. Simulation and Power of Parentage Assignment

Overall the genotyping of the parental samples was sub-optimal. This was traced back to the preservative used for fin storage (it appeared not to be 99% ethanol as labelled at the point of purchase). As a result the DNA extractions were poor, the DNA being very highly degraded for all the parents **(Figure 4.4)**. On the other hand, the DNA extracted from all the offspring were of good quality **(Figure 4.5.).** They were also preserved with the same "ethanol", however, this was only for a short time before changing the ethanol upon arriving in Stirling from Nigeria. Samples from the parents had stayed for more than 8 months and as such might have degraded gradually over such a period of time compared to the offspring.

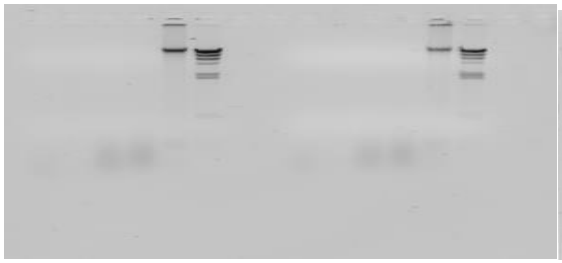

Figure 4.4. Gel image of Parental DNA of *gariepinus*

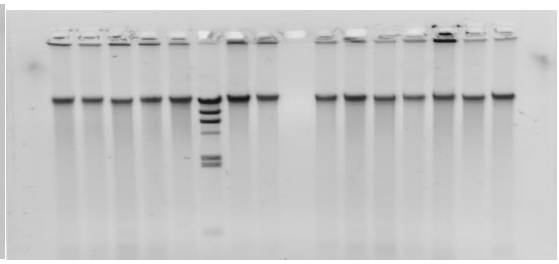

Figure 4.5. Gel image of offspring of *C.* DNA of *C. gariepinus*

The potential power of the markers could be approximately assessed however. In each tank there were the offspring from 50 families, half sib families generated from 10 sires and 10 dams. Twenty parental samples for which full scoring of all 12 loci was available were selected – divided at random into 10 males and 10 females. There were no full sets of parents available for any of the three mating sets, as shown in **Tables 4.4, 4.5 and 4.6**. To enable an approximate estimate of the potential power of the markers to assign parentage, a simulated set of tank crosses were devised. Fifty family groups were generated according to the actual breeding set up and analysed with FAP (Family Analysis Package, Taggart, 2007). Based on exclusion principles FAP predicts the resolving power of specific parental genotypic data sets, *unambiguously* discriminating among families / groups of families. This is achieved *by complete enumeration of all possible genotypic combinations*, using an efficient comparison algorithm. FAP was run for this simulated family dataset, assuming 8, 9, 10, 11 or 12 loci were available for typing. Details of the percentage of offspring assigned are shown in **Table 4.7.** The highest assignment was using the 12 loci (95.39% to families and 96.86% to a parent), although this didn't vary significantly

from using 11 or 10 loci. It is safe to conclude that using 9 of these microsatellites, over 90% of the offspring will be assigned families and parentage.

Table 4.7. Potential parentage assignment power of microsatellite markers

Number of Loci	% Assignment to Family	% Assignment to a Parent
12	95.39	96.86
11	95.22	96.83
10	92.66	94.81
9	90.46	92.85
8	79.67	83.48

4.4.2. Profile of the Microsatellite Markers

Results of profiling carried out showed (on **Table 4.8)** that the number of alleles (n) per locus ranged from 3 to 8, effective number of alleles (A_e) ranged from 1.3 to 4.24, polymorphic information content (PIC) ranged from 0.22 to 0.73, expected heterozygosity (H_e) was between 0.23 to 0.77 while observed heterozygosity (H_o) was 0.22 – 0.75.

Table 4.8. Estimation of average heterozygosity and genetic distance from a small number of individuals. Nei M (1978) Genetics, 89, 583-590.

	Cga_021	**Cga_022**	**Cga_026**	**Cga_027**	**Cga_110**	**Cga_202**	**Cga_203**	**Cga_204**	**Cga_205**	**P01**	**P03**	**P09**
Number of Samples Scored	44	48	31	27	40	36	37	42	40	35	38	36
Number of Different Alleles	3	7	3	6	4	6	5	7	2	4	8	4
Effective No. Of Alleles	2.57	2.55	2.62	3.10	3.10	1.30	2.12	4.24	1.78	2.07	2.38	2.68
Observed Heterozygosity	0.45	0.54	0.48	0.74	0.75	0.22	0.51	0.57	0.35	0.23	0.47	0.61
Expected Heterozygosity (Nei,1978-unbiased)	0.62	0.61	0.63	0.69	0.69	0.23	0.54	0.77	0.44	0.53	0.59	0.64
Polymoprhic Information Content (PIC)	0.54	0.58	0.55	0.65	0.62	0.22	0.46	0.73	0.34	0.41	0.53	0.56
Fixation Index (F_{is})	0.26	0.11	0.22	-0.09	-0.11	0.03	0.03	0.25	0.20	0.56	0.18	0.03

4.5. Discussion

The degradation of the parental samples led to sub-optimal genotyping. While this was traced back to the ethanol used and length of time taken, due to the good quality of DNA extracted from the offspring preserved in the same ethanol, but for a shorter period of time, other challenges such as inadequate power could also threaten the quality of samples preserved in absolute ethanol. Subsequent studies involving parental samples and offspring in Nigeria and/or any other part of the world with similar challenges should not only make arrangement for a reliable absolute ethanol, but also get the parental samples sent away for analysis shortly after collection of tissue, not waiting for 8 months to get those of the sibs together before sending. Delay is dangerous.

The aim of this study was to develop molecular markers for genetic management of *Clarias gariepinus* for aquaculture. It also aims at evaluating the suitability of different populations and strains of *C. gariepinus* for aquaculture, thereby estimating variations (within and between the different populations), heritability and breeding value for individuals within these populations. The study also aims to investigate the suitability of using shooters as broodstock and develop adaptable models of breeding programmes for farmers.

Due to the degradation, it was not possible to use SNPs for parentage assignment, hence the development of microsatellite markers. **A**s a result of the DNA being poor quality only 35% of parents could be confidently scored for all 12 loci. Reliable / confident assignment requires that all loci need to be scored for all parents. As this has not been possible to date, it was not possible to assign parentage to the offspring samples. It is indeed unfortunate given the amount of work put into the experiment (growth trial, lab work and analysis) and the fact that most of the objectives of this study cannot be met at this stage. Work is continuing to find an extraction methodology that will allow confident typing of the remaining parental samples. The highest assignment was using the 12 loci (95.39% to families and 96.86% to a parent), although this didn't vary significantly from using 11 or 10 loci. It is safe to conclude that using 9 of these microsatellites, over 90% of the offspring will be assigned families and parentage.

4.5.1. Profile of the Microsatellite Markers

Results of profiling carried out showed that the number of alleles (n) per locus ranged from 3 to 8, effective number of alleles (A_e) ranged from 1.3 to 4.24, polymorphic information content (PIC) ranged from 0.22 to 0.73, expected heterozygosity (H_e) was between 0.23 to 0.77, while observed heterozygosity (H_o) was 0.22 – 0.75. It is important to mention here that the lowest ranges were observed in Cga_202 - one of the enriched primers, which is likely to be a weak primer. The high observed heterozygosity in some individuals depicts a high effective number of alleles, although the uneven values of heterozygosity may result to a lower mean effective number of alleles. The large difference between the expected and observed heterozygosity in some individuals might suggest inbreeding in the populations (although very few), likely from some of the farmed populations. The PIC values, which were mostly above 0.5 for most of the loci, show how reliable and usable these sets of markers are. It is important to state here that the set of microsatellite markers derived from the ddRADseq showed more consistent characteristic profiles compared to those enriched or from the publication**.**

Based on Weir BS (2009) categories of fixation index and their interpretations, five out of the twelve loci showed very low fixation index, six were moderate and one locus had a very high value of 0.56. These values show that while some of these populations are well differentiated from others, others could be inbred. It will be interesting to see these values once all parents have been genotyped, as this will provide more information that will enable prediction of heritability of traits, growth, variation, survival and cannibalism in shooter.

4.6. Conclusions

While it was unfortunately impossible to reach conclusions about the original main objectives of this chapter (verification of parental assignment using DNA markers, evaluation of performance of catfish from different strains, analysis of origins of shooter catfish) due to the problems with the DNA from the parental fish, it can be stated that at the beginning of this research, only 4 suitable microsatellite markers were available for parentage analysis, whereas now we have a total of 12 polymorphic microsatellite markers suitable for parentage assignment (as tested by simulation analysis). Although there were challenges at every stage of this research, the main one being degradation of parental DNA, thus leading to the use of microsatellite markers, this research has found suitable

microsatellite markers from sequences generated from ddRADseq, thus showing the versatility of ddRADseq in the development of molecular makers. In countries such as Nigeria in future, care should be taken to not preserve the fin-clips in locally sourced ethanol for a long time, especially the absence of a reliable source of electricity. Rather, samples should be sent as quickly as possible for extraction.

CHAPTER 5. GENERAL DISCUSSION

5.1. General Discussion

This chapter summarises the findings from this PhD research and considers the implications for the *Clarias* catfish industry and future studies.

5.2. Main Findings

1. Practices in Nigerian catfish hatcheries were surveyed and areas of critical research, policy and resource needs have been identified. Over 90% of fish hatcheries use shooters as broodstock and 98% of farms surveyed use only farmed stocks as broodstock – neglecting the wild stock of C. gariepinus.
2. Species-diagnostic SNPs were isolated from samples of *C. gariepinus, C. anguillaris and H. longifilis* using ddRADseq. Phylogenetic studies revealed three distinct species, confirming the difference between *C. gariepinus* and *C. anguillaris*. A total of 24 of diagnostic SNPs were developed.
3. The efficacy of eight of the 24 diagnostic SNP markers was tested on over 288 samples from 6 different countries.
4. Based on results form this research, the use of vomerine teeth (a popular practice in Nigeria) and the number of gill rakers on the first gill arch for identification and differentiating between these two species were concluded to be inaccurate.
5. Based on this analysis, it appears that all hatchery populations were pure *C. gariepinus*.
6. Eight new microsatellite markers for *Clarias gariepinus* were characterised. Three multiplex PCR reactions using eight of these markers plus four from the literature were developed and optimised. Using these 12 loci, 1,220 samples were genotyped.
7. The profile of these microsatellite markers and the parentage assignment power was assessed to determine their suitability, and efficacy based on simulation. The result showed high assignment power of up to 96.86% when 12 of these loci were used.
8. The versatility of ddRADseq in enabling discovery of different kinds of molecular markers for genetic improvement was confirmed. The same library

used for SNP discovery provided four of the eight microsatellite markers described above. In addition to that multi-allelic SNP haplotypes, with high discriminatory power, were isolated from the same library (through an MSc Project – Sofolabi Sofela in 2018).

9. Growth data and genotypes from 1200 offspring were generated, awaiting completion of parental genotyping to enable parentage assignment and assessment of kinship, estimating strain genetic variation and performance and heritability of traits (growth, survival, aggression).
10. Shooters (fast growers) were isolated from the 150 families produced and their growth data and genotypes generated. Likewise, upon completion of genotyping of parents, they will be assigned parentage, noting the mean performance of members of their families, to assess their value.

5.3. Applications of ddRADseq

ddRADseq was found to be very useful in developing different molecular markers during my research. Based on one ddRADseq library, 24 SNPs were discovered that clearly distinguished between members of the *Clariidae* family – putative *C. gariepinus* and *C. anguillaris*. Thousands of other SNPs were detected which could have been used for other purposes, e.g. parentage assignment. Four of the eight microsatellite markers developed for parentage assignment were discovered from the same ddRADseq from which SNPs were discovered. Furthermore, from the same ddRADseq, multi-alleleic haplotypes with 4 - 5 alleles were discovered from multi-SNP RAD tags which are usually discarded (to focus only on RAD tags with one or two SNPs and thus simple genotypic data). Eight of the selected loci were found to be informative in species discrimination in a study involving *C. gariepinus*, where unique haplotypes were found for species of *Clarias* catfish earlier identified using SNPs, with 100% accuracy. ddRADseq (and other genotyping-by-sequencing techniques) thus present opportunities for rapid discovery of markers for a range of applications in species with little previous development of such genetic resources.

5.4. Implications on the Nigerian Catfish Aquaculture Industry

5.4.1. Identification and Management of Wild and Domesticated Stock

Wild stocks in Nigeria are currently identified to species using vomerine teeth as described in **Chapter 3.** This research has found no significant correlation between the SNP genotypes and this phenotype, and this technique was concluded to be inaccurate. This is almost not longer an important topic for discussion as results from the survey shows that only 2% of 120 hatcheries source their broodstock from the wild. The wild stocks of *C. gariepinus* in Nigeria are known to perform less than the Dutch-domesticated ones in terms of growth and fecundity. This has largely led to the neglect of the wild strains in favour of the farmed ones - largely Dutch-strain. A similar report of poor growth performance has been reported in the indigenous Egyptian strains of *Clarias* (WorldFish, 2008). This is not surprising as the Dutch-domesticated strains have been in captivity and undergone "green-fingered" selection for about four decades ((Hogendoorn and Vismans, 1980; FAO, 2014). Studies on the identification of *C. gariepinus* in **Chapter 3** of this thesis reveal some morphological differences between different populations of C. gariepinus, from two distinct locations (Upper River Niger and Upper River Benue) and even different locations on the same water body (Upper R. Niger and Lower R. Niger). Those from R. Niger had fewer numbers of gill rakers and lesser conformity with the vomerine teeth morphotype in comparison to those from River Benue. These morphological differences could extend to or be influenced by physiological differences, exhibiting high level of genetic variation, upon which selection can be based and intensified. Separate breeding programmes can be run for the wild and the farmed strains and a combination of both (as was designed in chapter 5) to allow for cross breeding e.g. to avoid inbreeding in the farmed stock of to improve the growth performance and fecundity in the wild strain. As described in **Chapter 4,** evaluation of different populations of the wild strain will now be possible using the methodology developed, and more effective due to the ability to have mating designs that enable better heritability estimates/prediction, parentage assignment and kinship test using the markers developed. Studies and genetic management of the wild and captive populations of *C. gariepinus*, *C. anguillaris* and *H. longifilis* for conservation and aquaculture purposes is also now possible with the help of the SNPs and microsatellites showing intraspecific polymorphism isolated from the ddRADseq during my PhD.

This research was partly stimulated by suspicion raised from reviewed literature that the Dutch strain may have contained some *C. anguillaris* – this research has generated evidence that this is not so. The study assessed farmed fish from Egypt, Hungary, Netherlands, Nigeria, Poland and Stirling domesticated Zambian strain, and all turned out to be (putative) *C. gariepinus*. The inclusion of the Zambian strain, from an area without any *C. anguillaris* according to the literature, was important here. Furthermore, evidence from this research suggest that these two species are rather cryptic, in the sense that while the SNP markers clearly suggest two separate species, the morphological features used to separate them (gill rakers and vomerine teeth) do not actually distinguish them.

It is interesting to know that no *C. anguillaris* was found in samples collected from all these countries. The putative *C. anguillaris* genotyped all came from the lower R. Niger, suggesting localisation of this species compared to *C. gariepinus* (Johnels, 1957; Lévêque *et al.* 1991; FAO, 2018b). All the 32 samples genotyped were less than 200 grams in weight despite collecting them over 2 sampling periods, spread almost 4 years apart. Despite their inseparable resemblance, the small sized nature of *C. anguillaris* might have been a reason for their unintentional exclusion in the first place – when wild strains of *Clarias* catfishes were collected for broodstock and for domestication in the first place.

Further studies on samples of *C. anguillaris* form other sources/countries especially from Senegal and any other West—African country is recommended, as this further will validate the efficacy of the SNP markers developed from this study. Although findings from this study has found no *C. anguillaris* in all the Dutch-domesticated strains of *C. gariepinus* collected from three farms in Netherland and three farms in Nigeria, investigating further the suggestion of possible introgression in the Dutch domesticated strain of *C. gariepinus* used in the Nigerian Aquaculture Industry is recommended. This could be by analysis of captive stocks in Nigeria that consist of Dutch stocks crossed to local stocks, or any stocks set up from wild sources, to see if there is any presence of *C. anguillaris* in captive stocks via this route. It will also be useful to research further into morphological/meristic traits not examined in this study, for possible correlation with the genotype.

5.4.2. Selective Breeding Programme for *C. gariepinus*

One of the objectives of this PhD was to evaluate suitability of stocks for aquaculture. Exploiting the use of molecular markers for this purpose necessitated the development of microsatellite markers in place of SNPs, which was aborted due to degraded DNA in the parental samples. Although this experiment is not yet concluded (still trying different methods of DNA extraction), the microsatellite markers developed, profiled and tested with over 90% assignment power **(Chapter 4)** will be useful in actual species evaluation and selective breeding programme for this species. Owing to the high GSI in *C. gariepinus* and the lack of multiple rearing facilities for different families, controlling inbreeding depression will be practically a very difficult exercise, especially in small hatcheries with very small number of broodstock. The markers developed however, will enable setting up of factorial crosses, ensuring high effective breeding number, from where mass selection is carried out based on a given growth threshold. The selected stocks are then assigned parentage to evaluate among other things, the actual contribution of each family to the selected population; comparing it to the expected maximum effective breeding number. From this sort of trial, the ideal minimum number of broodstock and mating design for medium and large sized hatcheries, with broodstock supply to smaller hatcheries (as it is unlikely that small hatcheries would have the resources or knowledge to develop this) can be derived to ensure high effective breeding number and therefore controlling inbreeding depression. *Clarias* catfish hatcheries can thus benefit from outcomes of such research as a standard to ensure sustainable broodstock management without going to the laboratory. Other traits such as growth rate (which is one of the most important thresholds for which selection is be based) - body length and weight, disease resistance, adaptation to a given environment, etc., can be evaluated with such markers. Pedigreed breeding programmes can also be carried out using these sets of markers for parentage assignment and all sorts of different breeding programmes be set up. Paralleled breeding programmes can be set up and managed separately, for different purposes, and crosses initiated between the different breeding programmes to enhance specific traits of interest. A more standard approach is to create a base population from several different strains, such as the ones designed in **Chapter 4,** but if the wild fish have poorer performance (as expected to be), they could drop out at the early stages of selection. Dutch strain appears to have good performance relative to local strains, but genetic variation in the Dutch strain and derivatives may be becoming a problem. This is especially true if the number of fish, frequency of import and genetic variation of the imported strain is small. The implication of importing only few

individuals in a strain of catfish, when compared to the size of the industry if not properly managed can result to a founder effect scenario. This happens even more when the imported strain performs better than the indigenous one and is sought for by every breeder. In such situations, the level of the industry will be dominated by somewhat a new strain with little genetic diversity and from one generation to another, heterozygousity reduces and issues related to inbreeding depression begin to arise. It is therefore important develop mechanisms and regulatory framework for genetic management of the imported strain while improving on the local one.

It is of benefit to the importers and the industry at large to have separate breeding programmes at least for these two strains (indigenous strain and the imported one (Dutch)), to enable crossing between the two to avoid inbreeding in the Dutch strain and to improve the growth performance and fecundity in the wild strain. The number of families involved and the sex ratios at times of breeding should not be skewed to ensure adequate effective population size generation after generation.

In the face of urban migration and climate change, where flooding, heat wave and drought are increasingly becoming unpredictable, partly affecting the use of earthen ponds for production, there is increase use of concrete tanks and plastic pools. In order to respond to such challenges and to create a resilient *Clarias* catfish industry in Nigeria, G x E selection, to develop strains of catfish better adapted to either or the entire different culture environment becomes a necessity.

5.4.3. Implication of Shooters

Over 90% of hatcheries surveyed used shooters as broodstock. This makes sense to the common man, especially those who have had some sort of experience with other forms of agriculture, where either the best (biggest) seeds are preserved for the next generation, or the biggest bull is mated to every female cattle in the herd to improve the growth performance. This could have been justified if the shooters were not cannibalistic or aggressive in nature (raising the potential issue of selecting for such traits) and if there was a good understanding of the implications of such practice on effective population size (in the absence of any pedigree information).

Having access to molecular markers that can assign parentage and kinship, as developed from this study, provides tools to better investigate the shooter phenomenon in catfish. Just

as was planned in this PhD, shooters from all the rearing sets (comprising 150 families) were raised and genotyped, awaiting parentage and kinship analysis. Once the relationship is established between the shooters and its family – i.e. whether or not they came from families with the best growth performance, a nested or factorial mating design between the shooters and their average sized sibs and average sized fish from another family or population will be made to produce an F_2 generation. Monitoring the growth and behaviour of the F2 and then genotyping them to see what proportion of them (F2 shooters) come from only the shooter parents in the F1.

Furthermore, in the study of fish behaviour, the type and level of cannibalism within a cohort of only full sibs, between cohorts of half sibs, unrelated mates and a combination of all, can be monitored essentially using the microsatellite markers developed during the PhD, by way of recording mortalities (if visible) and genotyping survivors. Assigning parentage and kinship will allow a walk-back to decipher the cohort related cannibalism with the help of recorded data e.g. of the initial stock. The significance of this study is that, it could influence or direct mating designs aimed at reducing cannibalism in catfish.

5.4.4. Policy Planning and Regulation

One of the findings from the survey and previous studies in Nigeria is the significant lack of knowledge on the genetic management of broodstock to ensure sustained performance. Several factors alluding to this has been discussed in **Chapter 2** of this thesis. The lack of regulation or coordinated regulation in the aquaculture industry as a whole makes it very difficult to plan polices targeting specific needs of the industry such as creating a knowledge-base on genetic management of stocks. Supposed regulatory bodies (e.g. the government) themselves sometimes encourage indiscriminate setting up of fish hatcheries by providing short courses on fish breeding to teaming unemployed youth with the hope of curbing unemployment and the mind-set that fish hatchery operation doesn't require skills beyond fertilisation and management of fry. The very big fish hatcheries, which have some knowledge on broodstock management and can afford to import broodstock from the Netherlands and South Africa, are most times not open to providing trainings or broodstock to smaller hatcheries. The shortage of information based on scientific research to support planning and implementation of such policies (e.g. genetic improvement) has also contributed to the present state of affair.

One way of providing these needed knowledge on genetic management of broodstock aside funding research and development at advanced or molecular level, diploma programmes on such courses needs to be created for hatchery operators. A quick way of disseminating finding from these advanced research and specific diploma programmes is through a licencing and certification schemes for fish hatcheries, through which outcomes of research such as this, will easily be communicated to farmers for adoption on a sustained bases. Large companies/fish farms that can operate and fund breeding programmes could benefit from such research by way of using the markers developed for parentage. In addition, due to the high cost of facilities for sequencing and genotyping, funding could be provided for a centralised breeding programme by the regulatory bodies and or organised private sector. MoU with institutions like the Institute of Aquaculture is one way of getting the breeding programme going (once other bureaucratic processes and/or logistics are taken cared of by the regulatory bodies).

One of the top problems of hatcheries surveyed was shortage supply of power (electricity). As many hatcheries are increasingly becoming peri-urban, the degree of intensification, hence need for more water and aeration increases. Shortage of power is a multi-sectorial and nationwide problem. However, clusters can be created, of fish hatcheries around cities where a stable schedule of power can be provided to farmers to enable them store, as much water for the little amount of time power is available. Solar powered fish hatcheries sounds promising, although could be beyond the financial capacity of the small-scale hatcheries, hence the need for government incentives.

5.5. Conclusions

This PhD thesis reports different studies and experiments carried out on the development of genetic improvement in *C. gariepinus*, starting with a review and survey of the industry in Nigeria and moving on to develop DNA markers and methods for evaluation of strains, breeding values, shooters, etc. Although not all objectives of the research were met, the use of molecular markers developed during the research has been explored both practically and theoretically, while future application and implications have been discussed. These molecular markers might seem advanced and expensive for the local farmers to adopt and implement, however, effective implementations and information dissemination strategy has also been discussed in the earlier part of this chapter. ddRADseq has been found to be

very useful in developing arrays of molecular markers. It is obvious that significant lack of knowledge/skills and inadequate infrastructure (technology, stable power and rearing facilities) are the current challenges facing genetic management in the *C. gariepinus* aquaculture. A sustainable approach to solving this has been discussed so as to allow the industry to benefit from the use of genetic improvement of *C. gariepinus.*

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Appendix I. Questionnaire

Institute of Aquaculture University of Stirling, Scotland, UK
Questionnaire on breeding programmes for African catfish in Nigeria

We would like to request some information from you about your breeding/management programme and your experience on African catfish (*Clarias gariepinus*) farming in Nigeria. We hope that this information will help us evaluate and improve the genetic management for existing and future hatchery managers and owners, and contribute to the development of catfish farming overall. To this end, we would be very grateful if you would take few moments of your time to complete this Questionnaire for a PhD. research project on the genetics of African Catfish in Nigeria, conducted by *Suleiman Isa Ihiabe*, a PhD student at the Institute of Aquaculture, University of Stirling, Scotland, UK, who is also a Scientific Officer at the National Biotechnology Development Agency, Abuja, and a catfish farmer (hatchery production) on the outskirts of the FCT, Abuja, Nigeria.

Note: In answering any question, you are free to choose more than one option (where applicable) e.g. in question 4, your farm may be involved in the production of *Clarias gariepinus*, *Clarias anguillaris* and also *Heterobranchus longifilis*. In such a case, you are expected to tick the three options. Space is provided in some questions for additional comments (where applicable), or where the options provided doesn't directly match your operation or intended response. Sections A and D are general questions, while B and C are specific to those involved in broodstock/fingerlings production and table-fish production respectively. Information provided will only be used for the sake of this study and individual responses will be treated as confidential: statistical analyses of the data may be published, but individual respondents will not be identified.

SECTION A: GENERAL QUESTIONS

1. **Are you a catfish farmer?**
   Yes ☐ No ☐
2. **What is the name of your farm?**
   ☐
3. **What is the address of your farm?**
   ☐
4. **What species of catfish do you deal with?**
   Clarias gariepinus ☐ *Clarias anguillaris* ☐ *Clarias lazera* ☐
   Heterobranchus bidorsalis ☐ *Heterobranchus longifilis* ☐ *Heteroclarias* ☐
   Others ☐
5. **What is/are the origin(s) of your fish?** (Please include name and location of farm or water body where
   ☐

6. **What aspect of catfish farming are you involved in?**

Hatchery ☐ Nursery ☐ Grow-out ☐ Broodstock ☐ Others ☐

7. **What year did you start this venture (fish farming)?** ☐

8. **In what form did you start the business? As a:**

Private business ☐ Partnership ☐ Family business ☐ Research Farm ☐

Hobby ☐ Others ☐

9. **What was/were the main source(s) of funds for starting up this venture?**

Private funds ☐ Bank loan ☐ Family/friend's contribution ☐

Grant from institutions ☐ Others ☐

10. **In what form is it today?**

Private business ☐ Partnership ☐ Family business ☐ Research Farm ☐

Hobby ☐ Others ☐

11. **When did you transform it to its present state?**

☐

12. **What is/are the main source(s) of fund for the venture today?**

Private funds ☐ Bank loan ☐ Family/friend's contribution ☐

Grant from institutions ☐ Others ☐

13. **How many employees do you have altogether?**

☐

14. **Haw many males and how many females?**

Males ☐ Females ☐

15. **Any special consideration on gender for particular positions to be employed into?**

Yes ☐ No ☐

16. **What sort of considerations and why?**

☐

17. **What other job(s) or business do you do aside catfish farming?**

☐

SECTION B: BROODSTOCK AND FINGERLING/JUVENILES PRODUCTION

(If you are involved in table-fish production ONLY, please go to section C)

B 1: BROODSTOCK PRODUCTION *(You may choose more than one option where necessary)*

1. **What type of broodstock culture system do you operate?** (system of growing young fish to broodstock)

   Flow-through ☐ Recirculating System ☐ Tank (outdoor) system ☐

   Pond system ☐ Others ☐

2. **What type of broodstock holding system (after maturity) do you operate?** (if different from above)

   Flow-through ☐ Recirculating System ☐ Tank (outdoor) system ☐

   Pond system ☐ Others ☐

3. **What is/are your main source(s) of water?**

   Well ☐ Borehole ☐ Stream/river/lake ☐ Pipe Borne Water ☐

4. **How many broodstock do you keep on your farm at a time?**

   Below 50 ☐ 50 - 100 ☐ 100 - 150 ☐ Above 150 ☐

5. **How many broodstock do you keep on your farm annually (if different from above)?**

   Below 100 ☐ 100 - 200 ☐ 200 - 300 ☐ Above 300 ☐

6. **How many families do they represent altogether?**

   Below 25 ☐ 25 – 50 ☐ 50 – 75 ☐ 75 – 100 ☐

   100 -125 ☐ 125 - 150 ☐ Above 150 ☐

7. **Do you have broodstock replacement programme?**

   Yes ☐ No ☐

8. **If yes, how often do you replace your broodstock?**

   Quarterly ☐ Twice a Year ☐ Once a Year ☐ Once Every 2 Years ☐

   Others ☐

9. **Why do your replace your broodstock?**

   Increased demand ☐ Aging of Broodstock ☐ Improve Quality ☐

   Others ☐

10. **What percentage of your total broodstock contribute to the replacement programme (%)?**

    Below 25 ☐ 25 - 50 ☐ 50 - 75 ☐ Above 75 ☐

11. From how many batches of hatched fish do you select fish for broodstock replacement?

0 - 2 ☐ 2 - 4 ☐ 4 - 6 ☐ Above 6 ☐

12. How old are your broodstock (months)? (Age at which they start getting used or sold as broodstock)

6-9 ☐ 9-12 ☐ Above 12 ☐

13. What is the average weight of your broodstock (kg)?

Below 0.5 ☐ 0.5-1.0 ☐ 1.0-1.5 ☐ Above 1.5 ☐

14. Are there considerable size differences between the males and the females of same age?

Yes ☐ No ☐

15. If yes, which of the sexes is bigger?

Male ☐ Female ☐

16. Are there differences in the time of maturity between the males and female?

Yes ☐ No ☐

17. If yes, which of the sexes matures earlier?

Males ☐ Females ☐

18. What is the estimated difference in time (months) of maturity between the sexes?

Below 3 ☐ 3 – 6 ☐ 6 – 9 ☐ 9 – 12 ☐ Above 12 ☐

19. What months/season do you get eggs from your broodstock?

Jan – Mar ☐ Apr – Jun ☐ Jul – Sep ☐ Oct – Dec ☐

20. If seasonal, what are the possible reasons for this (seasonal egg production)?

Availability of gravid female ☐ Temperature ☐ Market demand ☐ Rainfall ☐

Others ☐

21. If not seasonal, how do you achieve all year round egg production (e.g. raising temperature)?

☐

22. What is the average production (operating) cost (Euro) per kilogram of your broodstock?

Below € 1.00 ☐ € 1.00 – € 1.50 ☐ € 1.50 - € 2.00 ☐ Above € 2.00 ☐

23. What is the average selling price (Naira) per kilogram of your broodstock?

Below € 4.00 ☐ € 4.00 - € 8.00 ☐ € 8.00 - € 12.00 ☐ Above € 12.00 ☐

24. Where is the major market for your broodstock located? (Zone here means geopolitical zone)

Within the State ☐ Within the Zone ☐ Outside the Zone ☐ Export Market ☐

☐

Others

25. Who are your major customers?

Middle men ☐ Fish Farmers ☐ Organisations/Research Institutions ☐

Others ☐

26. Do you use female broodstock that have been used (spent) before?

Yes ☐ No ☐

27. If yes, how many times per year is same female used?

Once ☐ Twice ☐ Three times ☐ Above three times ☐

28. For how many years is a female used before discarding it?

One ☐ Two ☐ Three ☐ Above three years ☐

29. How is the milt (sperm) collected? By:-

Sacrificing the male ☐ Dissecting and Suturing ☐ Using milt from males whose pituitary have been extracted and used to induce spawning in the females ☐ Others ☐

30. How many males and how many females do you use in each of the batches from which the broodstock are selected?

1 male : 1 female ☐ 1 male : 2 females ☐ 2 males : 1 female ☐

2 males : 2 females ☐ Others ☐

31. What was the average GSI (% weight of eggs relative to body weight of the female fish (%))?

Below 15 ☐ 15 - 20 ☐ Above 20 ☐ Don't know ☐

32. What was the average percentage hatchability (%) in last 12 months?

Below 50 ☐ 50 - 75 ☐ Above 75 ☐ Don't know ☐

33. What was the percentage survival (%) from larvae to juveniles in last 12 months?

Below 25 ☐ 25 - 50 ☐ 50-75 ☐ Above 75 ☐ Don't know ☐

34. How do you replace your brood stock?

From the wild ☐ From other farms ☐ Hatching them on the farm ☐ Others ☐

35. If you source from the wild, how many different water bodies do your source from?

One ☐ Two ☐ Three ☐ Above three ☐

36. If you buy to replace broodstock, do you:-

a. Buy fingerlings/juveniles from single or different farms and raise them to broodstock age and size?

Single Farm Different Farms N/A

b. Buy table fish from a single or different farms and raise them to broodstock age and size?

Single Farm ☐ Different Farms ☐ N/A ☐

c. Buy matured broodstock from a single farm or different farms?

Single Farm ☐ Different Farms ☐ N/A ☐

d. If you buy matured broodstock, what is their age (in months) at time of purchase?

Below 6 Months ☐ 6 – 9 Months ☐ 9 – 12 Months ☐ Above 12 Months ☐

e. If you buy matured broodstock, what is their size (in kg) at time of purchase?

Below 0.5 kg ☐ 0.5 – 1.0 kg ☐ 1.0 – 1.5 kg ☐ Above 1.5 k ☐

f. What informs your choice of source of buying broodstock or potential broodstock?

Quality of Broodstock ☐ Customary Relationship ☐ Kinship (Family relationship) ☐

Price Difference ☐ Others ☐

37. If you hatch them on your farm, do you:-

a. Select for broodstock replacement at juvenile stage of the fish or at harvest (table) size?

Juvenile ☐ Table Size ☐

b. Select from a **single** batch of hatched fish from related or unrelated parents?

Related Parents ☐ Unrelated Parents ☐

c. Select from **different** batches produced from related or unrelated parents?

Related Parents ☐ Unrelated Parents ☐

d. Select from shooters (fast growers), medium growers or left over of every batch of juveniles?

Shooters ☐ Medium Growers ☐ Left-over (unsold fish) ☐

e. Select from shooters (fast growers), medium growers or left over of every batch of table fish?

Shooters ☐ Medium Growers ☐ Left-over (unsold fish) ☐

f. Select fish for broodstock e.g. from commercial fry/fingerling produced or specifically breed for production replacement broodstock etc.?

☐

g. Others ☐

38. How do you raise the recruits (future broodstock)?

a. All fish are raised together in a tank/pond etc.?

☐ ☐

Yes No

b. Fish from each batch grown separately?

Yes ☐ No ☐

c. Any attempt to identify members of the different batches at a later time?

Yes ☐ No ☐

d. If yes how? ☐

39. What type of feed is used to feed the broodstock?

Commercial Broodstock Feed ☐ Commercial Feed for Grow-out ☐ On-farm Made Broodstock Feed ☐

On-farm Feed for Grow-out ☐ Others ☐

40. What is the quantity of feed fed to the broodstock per day (% of body weight per day)?

Below 2 % ☐ 2 – 3 % ☐ 3 – 4 % ☐ 4 – 5 % ☐ Above 5 % ☐

41. What is the crude protein content of the feed fed to your broodstock?

Below 25 % ☐ 25 – 30 % ☐ 30 – 35 % ☐ 35 – 40 % ☐ Above 40 % ☐

42. How often do you check the water quality of your broodstock?

Twice daily ☐ Daily ☐ Bi-weekly ☐ Weekly ☐ Fourth Nightly ☐

Monthly ☐ Others ☐

B 2: FINGERLINGS/JUVENILES PRODUCTION *(You may choose more than one option)*

43. What type of fish seed production are you involved in?

Hatchery ☐ Nursery ☐ All of the above ☐ **If nursery ONLY go to question 54**

44. If hatchery, what type of incubation/hatching system do you operate?

Flow-through ☐ Recirculating System ☐ Aerator System ☐

Others ☐

45. Do you raise the hatchlings to fingerlings/juveniles in the same incubation/hatching unit?

Yes ☐ No ☐

46. If no, in what type of culture system do you grow them to fingerling/juveniles?

Flow-through ☐ Recirculating System ☐ Tank (outdoor) System ☐

☐ ☐

Pond System Others

47. What is your main source of water?

Well ☐ Borehole ☐ Stream/river/lake, etc. ☐ Rainfall ☐ Others ☐

48. What months/season do you breed?

Jan – Mar ☐ Apr – Jun ☐ Jul – Sep ☐ Oct – Dec ☐ All of the Above ☐

49. If seasonal, what are the possible reasons for this (seasonal breeding)?

Availability of Gravid Female ☐ Temperature ☐ Market Demand for Fingerlings ☐

Rainfall ☐ Others ☐

50. If not seasonal, how do you achieve all year round fingerling production (e.g. raising temperature)?

51. ☐

Below 0.5 ☐ 0.5 – 1.0 ☐ 1.0 - 1.5 ☐ Above 1.5 ☐

52. What is the average number of eggs per kg of female broodstock?

Below 30,000 ☐ 30,000 – 45,000 ☐ 45,000 – 60,000 ☐ Above 75,000 ☐

53. What percentage of the eggs hatch into larvae (%)?

Below 50 ☐ 50 - 75 ☐ Above 75 ☐

54. Do you deal ONLY with nursery production of catfish? (I.e. buy fry, post-fry etc. and raise them to either fingerlings juveniles or post-juveniles before sales)?

Yes ☐ No ☐ **If no, go to question 60**

55. If yes, at what stage do you buy your fish?

Fry ☐ Post-fry ☐ Fingerlings ☐ Others ☐

56. Why do you choose to buy the above size/stage (fry, post-fry, etc.) of fish?

Easy Transportation ☐ Easy Management ☐ Cheaper Price ☐ Lesser Growing Time ☐

Lower Mortality Rate ☐ Others ☐

57. What informs your choice of where to buy the above fish from?

Quality of Fry/Fingerling ☐ Customary Relationship ☐ Kinship (Family relationship) ☐

Price Difference ☐ Others ☐

58. In what type of culture system do you grow them to fingerling/juveniles?

Flow-through ☐ Recirculating System ☐ Tank (outdoor) System ☐

Pond System ☐ Others ☐

59. What is your main source of water?

Well ☐ Borehole ☐ Stream/river/lake, etc. ☐ Rainfall ☐ Others ☐

60. At what stage do you sell your fish seeds?

Fingerling (0.5g - 1.5g) ☐ Juvenile (1.5g - 2.5g and above) ☐ All of the above ☐

61. If fingerlings, what percentage of the larvae survives to fingerlings (%)?

Below 25 ☐ 25 – 50 ☐ 50 - 75 ☐ Above 75 ☐

62. What is the total length (cm) of fingerlings at time of sales?

Below 4 ☐ 4 - 5 ☐ 5 – 6 ☐ Above 6 ☐

63. What is the average weight (g) of your fingerlings at time of sales?

Below 0.5 ☐ 0.5 – 1.0 ☐ 1.0 - 1.5 ☐ Above 1.5 ☐

64. How long does it take to attain the above length and weight (weeks) from the day of hatching?

Below 3.0 ☐ 3.0 – 4.5 ☐ 4.5 – 6.0 ☐ Above 6.0 ☐ Not applicable ☐

65. How long does it take to attain the above length and weight (weeks) from the day of purchase?

Below 3.0 ☐ 3.0 – 4.5 ☐ 4.5 – 6.0 ☐ Above 6.0 ☐ Not applicable ☐

66. What is your average harvest stocking densities (fingerlings/M^3)

Below 2,000 ☐ 2,000 - 3,000 ☐ 3,000 – 4,000 ☐ Others ☐

67. What is the average production (operating) cost of fingerlings (Naira) on your farm?

Below € 0.03 ☐ € 0.03 - € 0.06 ☐ € 0.06 - € 0.08 ☐ Above € 0.08 ☐ Don't know ☐

68. What is the average selling price of fingerlings (Naira) on your farm?

Below € 0.08 ☐ € 0.08 - € 0.10 ☐ € 0.10 - € 0.12 ☐ Above € 0.12 ☐

69. How does these selling prices change during the year?

a.	**January – March**	Below € 0.08 ☐	€ 0.08 - € 0.10 ☐	€ 0.10 - € 0.12 ☐	Above € 0.12 ☐
b.	**March – June**	Below € 0.08 ☐	€ 0.08 - € 0.10 ☐	€ 0.10 - € 0.12 ☐	Above € 0.12 ☐
c.	**July – September**	Below € 0.08 ☐	€ 0.08 - € 0.10 ☐	€ 0.10 - € 0.12 ☐	Above € 0.12 ☐
d.	**Sept – December**	Below € 0.08 ☐	€ 0.08 - € 0.10 ☐	€ 0.10 - € 0.12 ☐	Above € 0.12 ☐

70. What percentage of the larvae survives to juveniles (%)?

Below 25 ☐ 25 – 50 ☐ 50 - 75 ☐ Above 75 ☐ Don't know ☐

71. What is the total length (cm) of juveniles at time of sales?

Below 6 ☐ 6.0 – 8.0 ☐ 8.0 – 10 .0 ☐ Above 10.0 ☐

72. What is the average weight (g) of your juveniles at time of sales?

Below 1.5 ☐ 1.5 – 2.0 ☐ 2.0 – 2.5 ☐ Above 2.5 ☐

73. How long does it take to attain the above length and weight (weeks) from the time of hatching?

Below 6.0 ☐ 6.0 – 8.0 ☐ 8.0 – 10 .0 ☐ Above 10.0 ☐ Not applicable ☐

74. How long does it take to attain the above length and weight (weeks) from the time of purchase

Below 6.0 ☐ 6.0 – 8.0 ☐ 8.0 – 10 .0 ☐ Above 10.0 ☐ Not applicable ☐

75. What is your average harvest stocking densities (juveniles/M^3)

Below 1000 ☐ 1,000 – 2,000 ☐ 2,000 – 3,000 ☐ Others ☐

76. What type of food/feed is used to feed your fish immediately after yolk absorption?

Shell-free Artemia ☐ Live (hatched) Artemia ☐ Zooplankton e.g. Copepods ☐

Commercial Micro-diets ☐ On-farm Micro-diets ☐ Others ☐

77. What type and or brand of feed is used to feed your fish following the end of use of live feed and or micro-die ☐

78. What is the average quantity of feed fed to the growing fingerlings (% of body weight per day)?

Below 4 % ☐ 4 – 5 % ☐ 5 – 6 % ☐ 6 – 7 % ☐ Above 7 % ☐

79. What is the crude protein content of the feed fed to your growing fingerlings (in the hatchery)?

Below 35 % ☐ 35 – 40 % ☐ 40 – 45 % ☐ 45 – 50 % ☐ Above 50 % ☐

80. How often do you check your water quality?

Twice daily ☐ Daily ☐ Bi-weekly ☐ Weekly ☐ Fourth Nightly ☐

Monthly ☐ Others ☐

81. What is the average production (operating) cost of juveniles (Naira) on your farm?

Below € 0.050 ☐ € 0.050 - € 0.075 ☐ € 0.075 – € 0.100 ☐ Above € 0.100 ☐

82. What is the average selling price of juveniles (Naira) on your farm?

Below € 0.100 ☐ € 0.100 - € 0.130 ☐ € 0.130 – € 0.170 ☐ Above € 0.170 ☐

83. How does these prices change during the year?

a.	**January – March** 0.170	Below € 0.100 ☐	€ 0.100 - € 0.130 ☐	€ 0.130 – € 0.170 ☐	Above € ☐
b.	**March – June** 0.170	Below € 0.100 ☐	€ 0.100 - € 0.130 ☐	€ 0.130 – € 0.170 ☐	Above € ☐
c.	**July – September** 0.170	Below € 0.100 ☐	€ 0.100 - € 0.130 ☐	€ 0.130 – € 0.170 ☐	Above € ☐
d.	**Sept – December** 0.170	Below € 0.100 ☐	€ 0.100 - € 0.130 ☐	€ 0.130 – € 0.170 ☐	Above € ☐

84. When is the peak fingerling/juveniles production season?

Jan – Mar ☐ Apr – Jun ☐ July – Sep ☐ Oct – Dec ☐

85. When is the peak in market demand for your catfish fingerlings/juveniles?

Jan – Mar ☐ Apr – Jun ☐ July – Sep ☐ Oct – Dec ☐

86. What was your estimated total fingerling and or juvenile production in the last 12 months?

Below 200,000 ☐ 200,000 – 400,000 ☐ 400,000 - 600,000 ☐ 600,000 – 800,000 ☐

800,000 – 1,000,0000 ☐ 1,000,0000 – 1,500,000 ☐ Above 1,500,000 ☐

87. Where is your major market for fish seed (fingerlings/juveniles) located?

Within the State ☐ Within the Zone ☐ Outside the Zone ☐ Export Market ☐

Others ☐

88. Who are your major customers?

Middle Men ☐ Independent Fish Farmers ☐ Organisations (e.g. co-operatives) ☐ Others

☐

89. What species of catfish do you deal with?

Clarias gariepinus ☐ *Clarias anguillaris* ☐ *Clarias lazera* ☐

Heterobranchus bidorsalis ☐ *Heterobranchus longifilis* ☐

Heteroclarias/Clariobranchus ☐ *Others* ☐

90. What type of culture system(s) do you use in the production of your table fish?

Flow-through ☐ Recirculating ☐ Outdoor (Tank) system ☐

Earthen pond system ☐ Others ☐

91. What is your main source of water?

Well ☐ Borehole ☐ Stream/river/lake, etc. ☐ Pipe Borne Water ☐

92. Is production seasonal on your farm?

Yes ☐ No ☐

93. What are the possible reasons for this (seasonality in table-fish production)?

Availability of Fingerlings/Juveniles ☐ Temperature ☐ Market demand ☐

Rainfall ☐ Others ☐

94. At what stage do you buy/stock your fish seeds?

Fingerling Stage ☐ Juvenile Stage ☐ If Juveniles, go to Question 11 ☐

95. What is the average total length (cm) of fingerlings at time of purchase?

Below 4 ☐ 4.0 – 5.0 ☐ 5.0 – 6.0 ☐ Above 6.0 ☐

96. What is the average weight (g) of your fingerlings at time of purchase?

Below 0.5 ☐ 0.5 – 1.0 ☐ 1.0 – 1.5 ☐ Above 1.5 ☐

97. What informs your choice of where to buy the above fish from?

Quality of Fry/Fingerling ☐ Customary Relationship ☐ Kinship (Family relationship) ☐

Price Difference ☐ Others ☐

98. What is the average market size (weight in kg) of your fish at time of sale?

Below 0.5 ☐ 0.5 - 1.0 ☐ 1.0 - 1.5 ☐ Above 1.5 ☐

99. How long does it take to attain the above market size from fingerlings stage (months)?

Below 4.0 ☐ 4.0 – 6.0 ☐ 6.0 – 8.0 ☐ Above 8.0 ☐ Go to Question 15

100. What is the total length (cm) of juveniles at time of purchase?

Below 6 ☐ 6.0 – 8.0 ☐ 8.0 – 10 .0 ☐ Above 10.0 ☐

101. What is the average weight (g) of your juveniles at time of purchase?

Below 1.5 ☐ 1.5 – 2.0 ☐ 2.0 – 2.5 ☐ Above 2.5 ☐

102. What informs your choice of where to buy the above fish from?

Quality of Fry/Fingerling ☐ Customary Relationship ☐ Kinship (Family relationship) ☐

Price Difference ☐ Others ☐

103. What is the average market size (weight in kg/fish) of your fish at time of sale?

Below 0.5 ☐ 0.5-1.0 ☐ 1.0-1.5 ☐ Above 1.5 ☐

104. How long does it take to attain the above market size from juveniles stage (months)?

Below 4.0 ☐ 4.0 – 6.0 ☐ 6.0 – 8.0 ☐ Above 8.0 ☐

105. What is the average stocking density (kg/M^3)?

Below 30 ☐ 30 - 60 ☐ 60-100 ☐ others ☐

106. What type of feed do you use to feed your table-fish?

Commercial Floating Pellets ☐ Commercial Sinking Pellets ☐ On-farm Floating Feed ☐

On-farm Sinking Pellets ☐ Others ☐

107. What is the quantity of feed fed to the broodstock per day (% of body weight per day)?

Below 2 % ☐ 2 – 3 % ☐ 3 – 4 % ☐ 4 – 5 % ☐ Above 5 % ☐

108. What is the crude protein content of the feed fed to your broodstock?

Below 30 % ☐ 30 – 35 % ☐ 35 – 40 % ☐ 40 – 45 % ☐ Above 45 % ☐

109. What is the FCR of your fish? ☐

110. How often do you check your water quality?

Twice daily ☐ Daily ☐ Bi-weekly ☐ Weekly ☐ Fourth Nightly ☐

Monthly ☐ Others ☐

111. What is the average percentage mortality (%) from stocking size to harvest?

Below 10 ☐ 10 – 20 ☐ 20 – 30 ☐ Above 30 ☐

112. Are there considerable size differences between the males and the females during harvest?

Yes ☐ No ☐

113. If yes, which of the sexes appear bigger?

Male ☐ Female ☐

114. What are the estimated average size differences (g) between the sexes at harvest?

Below 100 ☐ 100 – 200 ☐ 200 – 300 ☐ Above 300 ☐

115. What is the average production (operating) cost per kg of your table-fish (Naira)?

Below € 1.00 ☐ € 1.00 – € 1.50 ☐ € 1.50 - € 2.00 ☐ Above € 2.00 ☐

116. What is the average selling price per kg of your table-fish?

Below € 1.50 ☐ € 1.50 - € 2.00 ☐ € 2.00 - € 2.50 ☐ Above € 2.50 ☐

117. When is the peak in market demand for your catfish?

Jan – Mar ☐ Apr – Jun ☐ July – Sep ☐ Oct – Dec ☐

118. What is the estimated average annual weight (kg) of catfish you produce?

Below 10,000 ☐ 10,000 – 20,000 ☐ 20,000 - 30,000 ☐ Above 30,000 ☐

119. Where is your major market for fish seed (fingerlings/juveniles) located?

Within the state ☐ Within the Zone ☐ Outside the Zone ☐ Export Market ☐

120. Who are your major customers?

Middle men ☐ Processing Plants ☐ Consumers ☐ Others ☐

121. What type of value addition do you do (if any) on the table fish produced before sales?

Smoking ☐ Filleting ☐ Freezing ☐ Other ☐

SECTION D: GENERAL COMMENTS/OBSERVATIONS

1. What are your major challenges in hatchery production?

Diseases ☐ Poor Quality of Broodstock ☐ High Input Cost ☐

Insufficient Water ☐ Inadequate Power ☐ Marketing ☐

Others ☐

2. What are the common fish diseases that affect your catfish?

Name of Infection	Symptoms	Stage of fish life
(E.g.) Saprolegnia	Whitish patch of fungal infection, causing mortal	Larval and fry stages

3. How did you get the knowledge and skills used on your catfish farm so far?

Degree or equivalent in fisheries, aquaculture or related course ☐

Diploma in fisheries, aquaculture or related course ☐

Privately funded professional short course(s)/certificate training in fisheries, aquaculture or related course ☐

Training and empowerment from government or other institutions ☐

From family members involved in aquaculture ☐

Others ☐

4. What other form of aquaculture or aquaculture related business do you do?

Farming of other non-catfish species ☐ Commercial fish feed production ☐ Sales of fish feed ☐

Fish processing and sales ☐ Others ☐

5. If you are farming other non-catfish species, what species is it?

Tilapia ☐ *Heterotis niloticus* ☐ *African carp* ☐ *Citharinus spp* ☐

Lates niloticus ☐ Others ☐

6. **Why are you diversifying into other fish species?**

Market demand new species ☐ Decreasing demand for catfish ☐ Ease of farming of new species ☐

Hobby ☐ Others ☐

7. **How easy is it to culture the fish species when compared to your catfish?**

Relatively Easy ☐ Same Effort ☐ Relatively More Difficult ☐ Others ☐

8. **Do you think you need formal to cope with the new species?**

Yes ☐ No ☐

9. **If yes, to what extend?**

☐

10. **Any other comment**

☐

Name of Respondent ☐

Educational Qualification ☐

Discipline ☐

Contact Address of Respondent ☐

☐

Email Address ☐

Phone Number ☐

Appendix II

This appendix contains the scripts written and approach taken to identify and select SNPs from the ddRADseq of C. gariepinus, C. anguillaris and H. longifilis.

All usable common markers (1-5 SNP) coverage >= 50%

./find_pattern.pl --haplotypes denovo/batch_1.haplotypes.tsv --tag denovo/batch_1.catalog.tags.tsv --snp denovo/batch_1.catalog.snps.tsv --population clarias_species.txt -group 0 -maxsnp=5 -min=0.5 -ade > clarias.spp.pca.csv

R/adegenet population vector:

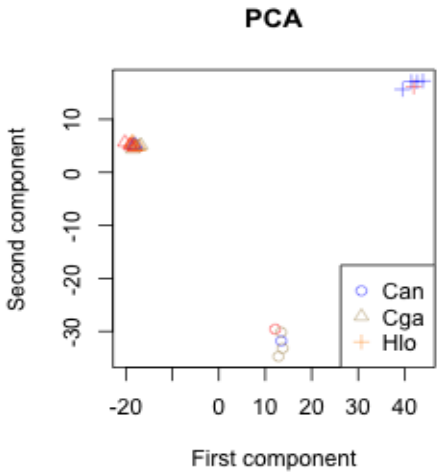
pop <- c('2','1','1','2','1','2','1','0','0','1','1','1','1','2','1','1','0','2','1','1','0','1','1','1','0');

Total markers read: 73591

Marker analysed: 2587

Marker selected: 126

SNP selected: 349



Diagnostic allele (1-2 SNP, coverage >= 75%, Fix within and between group), group specific:

./find_pattern.pl --haplotypes denovo/batch_1.haplotypes.tsv --tag denovo/batch_1.catalog.tags.tsv --snp denovo/batch_1.catalog.snps.tsv --population clarias_species.txt -group 2 -maxsnp=2 -min=0.75 -fix -fix -ade > clarias.spp.diag.csv

R/adegenet population vector:

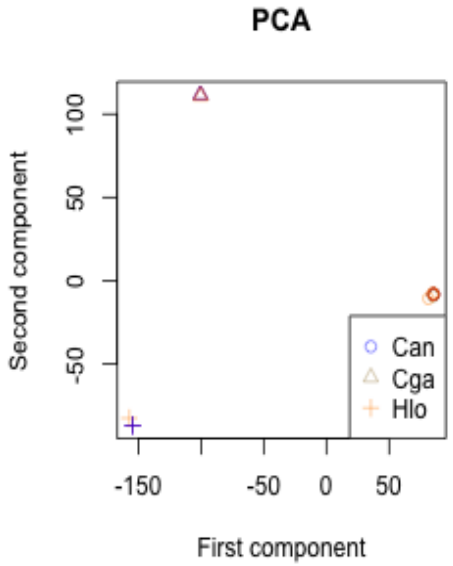
pop <- c('1','1','1','1','0','1','0','1','1','1','1','0','2','1','2','1','0','1','0','1','2','1','1','2','2');

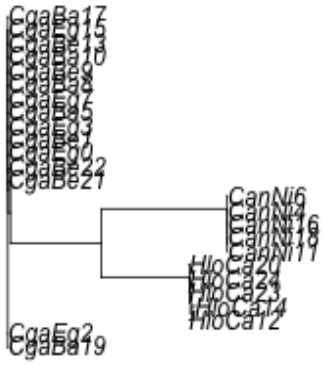
Total markers read: 73591

Marker analysed: 397

Marker selected: 24

SNP selected: 25





Recover the KASP markers

1652

2766

2995

5288

5437

5661

6243

8167

./find_pattern.pl --haplotypes denovo/batch_1.haplotypes.tsv --tag denovo/batch_1.catalog.tags.tsv --snp denovo/batch_1.catalog.snps.tsv --population clarias_species.txt -group 2 -maxsnp=2 -min=0.75 -fix -fix -ade --white kasp.list > clarias.spp.kasp.csv

R/adegenet population vector:

pop <- c('1','2','1','2','1','1','0','2','0','1','1','1','0','1','1','0','2','0','2','1','1','1','1','1','1');

Total markers read: 73591

Marker analysed: 8

Marker selected: 8

SNP selected: 9 <- select 5437_A and 5437_B

Marker_SNP Allele_Can Allele_Cga Allele_Hlo

1652_B {GG} {AA} {AA}
TGCAGGTCTAAGGTCTTACTGAATATCGAAGGCCCCATTAGTCATATATGTG[AG]AGCACTGAA
TGTGGAAGGCCTCATTAGTAATATAAGCTGCTGCCCAGGTCTTTGGTCAAATCAGACTCCTGCT
ATGAGGCCACACTC

2766_B {CC} {AA} {CC}
TGCAGGAATGTGTGTCTAAAAGCACGTTTGGCGGCGAGGCCGGGCCCAGATTACAG[AC]GCTC
TCCTGTGATCATTTGTGCATCTCCCCTGCAAGATCACAAGGCACCATCACAGAGCCAGCAGCTG
TGTCTTCAGAGCCTG

2995_B {CC} {TT} {CC}
CATGCAGCTGCGCTCTCGGGCCATGTATCCACTGTGCGGTTGTTGCTGGAAAAAGGAGCCATGG
TGGACCCTCTGGATGTGATGAAACACACTCCT[CT]TGTTCCGTGCCTGCGAGATGGGTCACCGT
GATGTCATTCTCAC

5288_A {AA} {CC} {AA}
TGCAGGACAGCGCAGGCAGCTTGCCCTCCAGTCCGTACCGGCTGGCCCAGGACGA[AC]GACGA
GTACGAGAGCACGCAGGAGTACCCGCCCTCACTGGAGCAACCAAAGAGAAGCAATGGACGCT
GGCATAGGTCCAGACTG

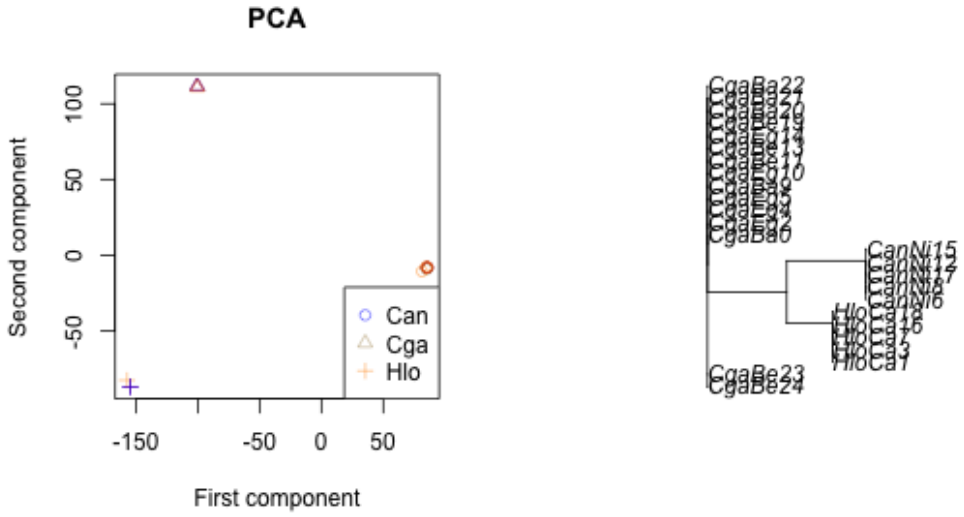
5437_A {GG} {CC} {CC}
CATGCTGTGCCAACAGATAAAGCTGAACTCACTGCCTCCACTAGCTCTGTTTGCATTTGGCATA
ATGTCCCTGTCCCACCCTTCCCCCATCCTCTACCACTCAAAAGACT[CG]CTGTCTACTTATGCTA
AGTGATTGACAGC

5437_B {GG} {GG} {AA}
CATGCTGTGCCAACAGATAAAGCTGAACTCACTGCCTCCACTAGCTCTGTTTGCATTTGGCATA
ATGTCCCTGTCCCACCCTTCCCCCATCCTCTACCACTCAAAAGACTGCTGTCTACTTAT[AG]CTA
AGTGATTGACAGC

5661_A {AA} {GG} {GG}
TGCAGGTTGCTGTAGCTGGAGGAGTGGGGCAGCCGGAATGCTGCAGGGCCAGGTGAAGGTCC[
AG]GGTGAGGTTACCGGCTCGATGGCCTCCACCACACCCATGCTGAGTGAGACCGGCAGCACGG
GCCGCTGGCACAGGCA

6243_B {GG} {CC} {CC}
CATGCTGGCGCGAAAGAGCATCATACCCGAGGAGTTCGCGCTGCCCGCGCTGGC[CG]TCGCGC
GCGCCCCGGAAGCCGGTGTTCAGGGACCGCGTGAACAAGGCGCGCTTCATTGCCAAGAGCGGC
GCGTGCAACCTGGCGC

8167_A {CC} {TT} {CC}
TGCAGGCAAAGCACACTCAGGAGGGCAGCACCTCATGGGGTGTTAACGG[CT]GAGACAGGCAC
TCTGGCAGACATGGCTGAGCTGGGAATCTGGGAGCCACTAGCTGTCAAAGCCCAAACATACAA
GACAGCAGTAGAGGTA



Diagnostic allele (1-2 SNP, coverage >= 75%, Fix within and between group), species specific:

./find_pattern.pl --haplotypes denovo/batch_1.haplotypes.tsv --tag denovo/batch_1.catalog.tags.tsv --snp denovo/batch_1.catalog.snps.tsv --population clarias_species.txt -group 1 -maxsnp=2 -min=0.75 -fix -fix -ade > clarias.spp.spdiag.csv

R/adegenet population vector:

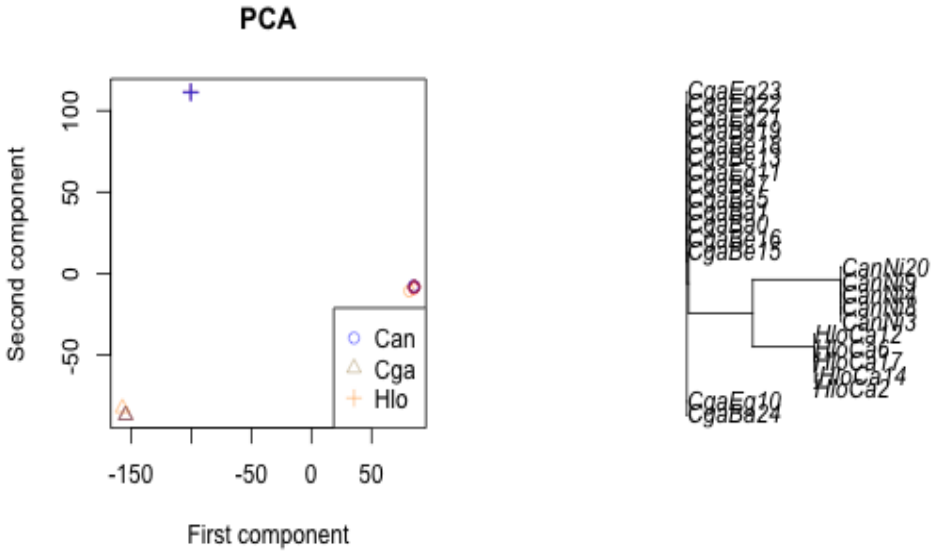
pop <- c('1','1','2','0','0','1','2','1','0','0','1','1','2','1','2','1','1','2','1','1','0','1','1','1','1');

Total markers read: 73591

Marker analysed: 397

Marker selected: 24

SNP selected: 25



Summary

The ddRAD reads were assembled *de novo* with Stacks

- 73,591 unique markers were recovered from 25 individuals / 5 species
- 2587 markers were shared by 50% of the individual with 1 to 5 SNPs per marker
- 397 markers were shared by 75% of the individual with 1 to 2 SNPs per marker
  - 24 markers (or 25 SNP) were diagnostic for a single species (E.g. Allele A in species one, allele B in every other species)