REPRODUCTIVE PERFORMANCE OF NATURALIZED RAINBOW TROUT (*Oncorhynchus mykiss,* WALBAUM 1792) FROM SAGANA COLD WATER STREAM AND IMPLICATION ON FRY PRODUCTION FOR AQUACULTURE DEVELOPMENT IN KENYA

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A THESIS SUBMITTED TO THE SCHOOL OF NATURAL RESOURCES AND ENVIRONMENTAL STUDIES IN PARTIAL FULFILLMENT FOR THE CONFERMENT OF THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE, KARATINA UNIVERSITY

OCTOBER, 2023

DECLARATION

This thesis is my original work and has not been presented for Conferment of a degree in any other University or for any other award.

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We confirm that the work reported in this thesis report was carried out by the candidate under our supervision and has been submitted with our approval as university supervisors

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DEDICATION

Efforts in this thesis are dedicated to my dearest wife and my beloved children who have been the pillar of my strength.

ACKNOWLEDGEMENT

I express my sincere appreciation to my supervisors in Karatina University, Prof. Charles Ngugi and Prof. Mucai Muchiri for their academic mentorship in the process of thesis research. I sincerely thanks the State Department of Fisheries, Aquaculture and the Blue Economy through Kenya Fisheries Service (KeFS) and Kenya Marine and Fisheries Research Institute (KMFRI) for their contribution to this study. I especially thank the Masters Class lecturers in Karatina University for their visionary guidance towards the completion of this academic work. I am also grateful to Kenya Wildlife and Fisheries Training Institute (KWFTI) and Kiganjo trout hatchery staffs for their assistance during this study.

ABSTRACT

Naturalized rainbow trout (*Oncorhynchus mykiss*) populations form important recreational fishing resource in the world. Globally, rainbow trout is among the most widely introduced fish species and has been translocated outside its native home range, into at least 99 countries including the tropical regions where it has established naturalized populations. Information on the performance of the hatchery and wild populations of *O. mykiss* following spawning interactions has not been adequately explored especially in the tropical environments. The current study aimed at evaluating the spawning interactions between naturalized and wild *O. mykiss* from the high altitude second order stream, the Sagana in Kenya. Spawning and incubation were performed under controlled conditions in a hatchery facility to assess their performance. In each spawning process, total length, weight, condition factor and fecundity of the brooders were recorded. Fertilization rate, hatchability and survival of the fry were determined. Egg diameters were modelled as a function of biometric and reproduction parameters, using generalized linear model. Total fecundity of the broodstock differed significantly among the wild strain, hatchery reared and the cross between hatchery and wild stock ($F = 8.934$, $df = 2$, $P = 0.0045$). The fecundity of the broodstock varied with the average weight of the brooders where brooders with high average weight had a high fecundity. Relative fecundity was significantly different among the three groups of fish ($F = 6.134$, $df = 2$, $P = 0.0217$) but the differences between the hatchery and cross strain of wild and hatchery fish were not significant. Fertilization rate showed significant differences among fish in the three experimental groups ($F = 10.136$, $df = 2$, $P = 0.0057$), Eyed egg survival was lowest among the wild fish but showed no significant differences between the hatchery and cross between wild and hatchery strains. The study recommends the use of hatchery and wild crossed fish to support fry production for use in subsequent restocking of all trout streams in the country. Based on this study, management strategies involving restoration of trout population that would benefit anglers without reliance on importation of eyed eggs are recommended.

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ABBREVIATION AND ACRONYMS

ATU	Accumulated Thermal Units
BOD	Biochemical Oxygen Demand
ED	Egg Diameter
EES	Eyed Egg Survival
FAO	Food and Agricultural Organisation
FR	Fertilization Rate
GLMM	General Linear Mixed Model
ICES	International Council for the exploration of the Seas
IPCC	Intergovernmental Panel on Climate Change
KeFS	Kenya Fisheries Service
KMFRI	Kenya Marine and Fisheries Research Institute
KWFTI	Kenya Wildlife and Fisheries Training Institute
LWR	Length- Weight Relationship
MT	Metric Tonnes
RF	Relative Fecundity
RRDC	River Research and Development Centre
SDGs	Sustainable Development Goals
SPSS	Statistical Package for Social Sciences
TF	Fecundity
VIF	Variation Inflation Factor

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

The declining fish stocks as a result of increased exploitation of seas and inland waters for food, commerce and recreation, have necessitated measures aimed at enhancing fish stocks (Rands *et al.*, 2010; Gebremedhin *et al.*, 2021). Aquaculture sector, being the most rapidly growing sector in the world (11% since 1984), has made important contribution to poverty alleviation, food security and improvements in fish stocks particulary in several developing countries (Kinkela *et al.*, 2019; Brugere *et al.*, 2023). In amny developing countries, there is still a considerable need for growth in aquaculture to end hunger and achieve food security as envisaged by the Sustainablle Development Goals (SDGs) (Troell *et al.*, 2023). In the curremporary world, aquaculture is going through blue revolution, refering to the massive growth aas well as intensification of aquaculture production (Ahmed and Thompson, 2019; Silvestri *et al.*, 2023). Blue revolution seek to achieve a balance between sustainability of resources, environmentally sound practices, productivity and profits in response to environmental damage resulting from the practices developed in the green revolution (Frid, 2023).

Aquaculture will continue to play a major and ever-increasing role in meeting human needs for animal source food (Garlock *et al.*, 2022; Roebuck, 2023). According to Food and Agriculture Organization (FAO), world aquaculture production achieved 87.5 million tonnes of aquatic animals (US$144.4 billion), including 66.6 million MT of food fish (US$137.7 billion) and 23.8 million MT of aquatic algae (mostly seaweeds, US$6.4 billion) (FAO United Nations, 2022). The production of farmed food fish was 42.2% of the global fish production. The present data indicate that between 2012 and 2022, world food fish from aquaculture production rose by 5.8%. This prospect of strong growth in aquaculture will persist in the foreseeable future especially in the Sub Saharan Africa (Hinrichsen *et al.*, 2022). Morever, the prospects for growth of aquaculture in the Sub Saharan Africa stems from shortfall in supply of fish from the more developed Internation Council for the exploration of the Seas (ICES) countries such as the USA, European Union and East Asian countries (Froehlich *et al.*, 2021).

Kenya has recorded tremendous growth in aquaculture since its inception believed to be in 1921 (Maar *et al.*, 1966; Ngugi *et al.*, 2007)., when the colonial administration introduced trout, common carp (*Cyprinus carpio)* and black bass (*Micropterus dolomieu)* into the country's waters with the original intent of enhancing recreational fishing. Thereafter, cultivation of these species and later of tilapia and African catfish, commenced. Hastened development of Nile tilapia and catfish aquaculture occurred in the 1960s (Aloo *et al.*, 2017). The country's aquaculture production 12,152 mt in 2010 to 22,140 mt in 2022, accounting for 12.7% of the country's total fish output (Munguti *et al.*, 2023). Sustinanance of growth in aquaculture has occurred partly due to increased public fuding in the sector (Mwamuye *et al.*, 2012; Munguti *et al.*, 2014) and owing to increased supply of seeds of Nile tilapia (*Orechromis niloticus)* and African catfish (*Clarius gariepinus)* from hatcheries (Syanya and Mathia, 2023). The country has over 1.14-million-hectare potential area suitable for fish farming with capacity to produce over 11 million MT of fish annualy.

Over the years, development of aquaculture in Kenya has faced challenges despite the public financing of the sector (Adekola *et al.*, 2022; Cheruiyot and Adhiaya, 2023).

The neglect of cold water aquaculture has resulted in its contribution of a paltry 0.5% of the total national fish production (Obwanga *et al.*, 2020). Recently, programmes aimed at boosting cold water aquaculture species have relied on compensatation for declining stocks from capture fisheries through stock enhancement programmes. The main cold water species in Kenya are the exotic rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). These species were introduced in Kenya between the year 1905 to 1920 particularly in rivers within the Mt. Kenya by the white settler for the purpose of sport fishing and other recreational needs (Copley, 1940; Seegers *et al.*, 2003). By the year 1920, most rivers originating from Mt Kenya had been stocked with trout. The naturalized population of rainbow trout was introduced to all cold-water rivers in Central and Western part of Kenya (Ngugi and Green, 2007; Weyl *et al.*, 2017). Brown trout is cultured mainly for sport fishing due to its slow growth rate and is low tolerance to desiccation, and water quality changes, therefore not preferred under commercial culture systems while rainbow trout being more tolerant to water quality and faster growth rate is preffered for aquaculture in the colder regions (Molony and Molony, 2001). Currently, the production of these trouts from aquaculture in Kenya is still too low (Olalekan *et al.*, 2022).

The declining trout stocks populations have necessitated measures aimed at enhancing their yilds, recoveri decline in stock and reduce unexpected extinction risk(s) (Rands *et al.*, 2010; Gebremedhin *et al.*, 2021). Among the measures aimed at increasing trout population owing to dwindling stock is introduction of exotic species and release into an area the species previously existed but has since been extirpated, or releasing fish into the wild to augment the viability of the extant population (Katsanevakis *et al.*, 2013; Galloway *et al.*, 2016; Bennett-Jones *et al.*, 2021). Once the fish species have been introduced to an area, they become naturalized through integration into the existing ecosystem to be able to reproduce and grow in the new environment and disseminate spontaneously (Stanković *et al.*, 2015; Estay *et al.*, 2021). The trouts in Kenya have been naturalized in many of the rivers of the highland regions through hatchery programmes, enabling it to become integrated into these aquatic systems (Ngugi and Green, 2007).

Hatcheries have played an important role in supporting the harvest and conservation of rainbow trout. Hatchery reared fish now make up large proportions of some stocks (Pulcini et al., 2014; Crichigno and Cussac, 2019). Nevertheless, hatchery use has become increasingly controversial because of the potential for negative interactions between hatchery-reared (hatchery) and naturally spawned (wild) fish (Scott *et al.*, 2015; Pinter *et al.*, 2019; Crichigno *et al.*, 2021). Stocked fish can negatively affect wild fish through genetic contamination, predation, competition, induction of premature migration, mixed-stock exploitation problems, predator attraction, and disease transmission (Fast *et al.*, 2015; Anderson *et al.*, 2020). Currently, there are a number of private and public hatcheries that have been established to enable the efficient cultivation of naturalized trouts. As a result, the viability and fitness of the wild population relative to hatchery fish may differ substantially due to adaptability in the wild and hatchery conditions. This assertion, however, needs further investigation, especially in many tropical countries where *O. mykiss* has been introduced.

Reproductive performance studies of naturalized salmonids populations are an important data source especially when determining the status of sport fishing and stream health and their integrity against a backdrop of various anthropogenic activities taking place in these watersheds (Estay *et al.*, 2021). Anglers report that rainbow trout in the high-altitude streams are in decline and the populations sizes and abundance is a concern (Okwiri *et al.,* 2019). There is a general assertion that the number of spawners has reduced and recruitment is very low. Previously streams have been stocked on a put-grow and take fishery where the eyed eggs were imported, incubated in the hatchery, and fingerlings released in the streams. Hatchery fish are reported to be poorly adapted to wild environments (Pinter *et al.*, 2019). If this is the case with cold water streams, then the abundance of wild fish can decline in a short while and reduced recruitment will result in smaller breeding population sizes and genetic diversity is lost as a result of outbreeding depression (Tsuboi *et al.*, 2019). As the genetic diversity is lost, trout populations become more susceptible to inbreeding depression and reduce survival of the young further reducing population sizes and abundance.

The lack of knowledge on spawning behaviour and survival potential of naturalized as well as hatchery reared fish that are later released into the stream limits the ability to fully assess the risks involved in maintaining a population that would support rainbow trout sport fisheries and develop a species management plan. It is important to address this issue, since this analysis may provide information for improving the management of rainbow trout in cold water streams in this country. In this study, spawning interactions between wild and hatchery-reared naturalized *O. mykiss* from River Sagana in Kenya was carried out to describe their performance and to explore the variables that could determine condition factor, fecundity, and fertilization in addition to egg survival and size.

1.2 Statement of the problem

Developing countries are currently grappling with poverty and hunger with millions of people dying from malnutrition owing to shortage of animal protein (KILIÇ, 2022). Kenya is no exception in this predicament. In many African countries, the quick production of greater amounts of animal protein by their own means is not only a central problem of the food economy, but also a prime question of economic importance (Wood and Tavan, 2022). A comparatively small effort put in fisheries development will quickly result in improved standards of living and nutrition, help secure food security for domestic consumption and even for export, if the pre-requisites are fully recognized. For sustainable development of rainbow trout farming in Kenya, the information available on rainbow trout breed improvement is limited. This study therefore proposes to investigate the status of cold-water fish aquaculture and breed improvement for rainbow trout fish in Kenya with an insight on how to improve the breed for increased production. Kenya is endowed with numerous aquatic resources with aquaculture potential (Aura *et al.*, 2022). Most of these resources are located in rural areas. Adoption of aquaculture will therefore open up new avenues for poverty alleviation in rural areas by increasing employment opportunity through provision of hired labour in aquaculture farms and help in meeting the nutritional needs of the people.

1.3 Objectives

1.3.1 General objective

The overall objective of the study was to investigate the reproductive performance of naturalized rainbow trout in cold water streams and its implication on fry production for aquaculture development in Kenya.

1.3.2 Specific objectives of the Study

1. To compare the reproductive performance of hatchery and wild naturalized brooders of rainbow trout
2. To investigate the survival rate of different populations up to fingerlings of rainbow trout.
3. To investigate the impact of change in seasons on trout fecundity of wild and hatchery populations

1.4 Hypotheses of the study

The hypotheses of the study were:

H_{01}: There is no significant difference in reproductive performance between hatchery grown brooders and wild naturalized brooders of rainbow trout.

H_{02}: There is no significant difference in the survival rate of different populations of rainbow trout.

H_{03}: There is no significant difference on trout fecundity due to change in season on both wild and hatchery populations.

1.5 Justification of the study

Rainbow trout aquaculture is a lucrative venture with data showing it to be the second most expensive fish in the Kenya after Nile perch *(Lates niloticus)* for both fresh water fish and marine fish, with one metric ton costing approximately Kshs. 225,000. Despite this, information available on rainbow trout aquaculture in Kenya is limited, there is no enough knowledge on trout reproductive performance under natural and hatachery condition. This negatively impacts investment which has a very high potential of improving the livelihoods of rural people. For this reason, a government trout farm was established in the Sagana area in year 1948 (Ngugi and Green, 2007). The development of aquaculture sub-sector will increase full use of the resources, create employment for the youth and women and also increase rural incomes. This study seeks to fill this gap in the country by investigating reproduction potential of rainbow trout and the survival rate of fingerlings.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background information on rainbow trout

Rainbow trout is cold water fish that have long been symbolic of clear, healthy mountain streams and lakes in North America (Scarnecchia *et al.*, 2022). Because of its ability to thrive in hatcheries, rainbow trout has been introduced into much of the United States and now inhabit many streams and lakes throughout the country (Graham *et al.*, 2019). The popularity of rainbow trout among anglers has placed it among the top five sport fishes in North America, and it is considered by many to be the most important game fish west of the Rocky Mountains (Arismendi *et al.*, 2019). However, reduction of good quality trout habitat due to stream bank and upland soil erosion, loss of riparian vegetation, water diversion, logging and mining activities, and point and non–point source pollution from municipal development and agriculture have significantly reduced the distribution and abundance of rainbow trout (Bunt and Jacobson, 2019; Stiling *et al.*, 2021). In addition, construction of dams, road crossings, and other structures impede the ability of rainbow trout to migrate upstream and down-stream, which is critical to successful completion of their life cycles (Cantin *et al.*, 2021; Estay *et al.*, 2021).

Implementing sound ecosystem management practices and stream and riparian improvements on private lands can help improve cold-water habitats used by rainbow trout and a host of other aquatic species (Hasegawa, 2020; Barabe, 2021). The life history requirements of the species vary tremendously depending on where the trout lives and whether it spends its life entirely in freshwater, or migrates to the sea for several years of growth before returning to its freshwater birthplace to spawn (Devaa and Ramesh, 2021; Porcel *et al.*, 2022). However, studies are limited in the tropical regions where the fish species has been introdiuced and therefore these aspects are only known for the temperate regions.

2.2 Habitat requirements for trout

Cold headwaters, creeks, small to large rivers, cool lakes, estuaries, and oceans comprise the habitats collectively used by the different populations of rainbow trout (Fetherman and Avila, 2022; Jan *et al.*, 2023). Depending on the genetic makeup of a trout population and the habitat conditions, rainbow trout will use some or all of these aquatic habitats during their lives (Collins and Baxter, 2020; Singh and Srivastava, 2023). Good trout stream habitat is complex, consisting of an array of riffles and pools, submerged wood, boulders, undercut banks and aquatic vegetation (Morrisett *et al.*, 2023). The ability to swim to and from different habitats from ocean to headwaters, or from tributary confluence to headwaters, increases the value of individual habitat components (Quinn, 2021; Hrabik *et al.*, 2023). Assuring fish passage through artificial barriers in a system of connected habitats greatly enhances the capability of an aquatic system to sustain rainbow trout populations (Kristensen *et al.*, 2019). Rainbow trout normally attains sexual maturity at an age of 3 years however, feeding strategy, temperature, light conditions significantly influence it sexual maturity (Sahashi *et al.*, 2023).

2.3 Reproductive strategies and spawning habitats

Rainbow trout spawn in main river channels and their tributaries, and inlet or outlet streams of lakes. During their spawning migrations, they are famous for their ability and tenacity to return to the streams where they hatched (Estay *et al.*, 2021).

Generally spawning in the spring and early summer, rainbow trout most commonly use stream riffles located downstream from pools as spawning areas (Kurta *et al.*, 2023). Tributaries and inlet and outlet streams containing gravels between one-half and three-inches in size are the most suitable resident trout spawning habitats (Flanagan, 2022; Nevoux *et al.*, 2022a). Using the tail, the female digs a depression in the gravel, called a *redd* and deposits her eggs, as an attending male fertilizes them (Schleppenbach, 2023). Riffle and pool tail-out habitats with well-aerated gravels free of sediment are ideal spawning habitat (Bazaz *et al.*, 2022).

Trout deposit eggs within a range of water depths and velocities that minimize the risk of desiccation as water levels recede with the seasons (Maruvada, 2019; Skoglund *et al.*, 2023). Sufficient water depth and sediment-free spawning gravels are critical to ensure that water can percolate through the spaces in the gravel, bringing oxygen to the eggs and removing metabolic wastes associated with incubation and hatching (Raine *et al.*, 2021; Fennell *et al.*, 2023). After hatching, young trout remain in the gravel until most of the yolk reserves, they are born with are used up. They emerge from the gravel as swimming fry ready to search for food (Pavel *et al.*, 2022).

2.4 Reproductive performance of naturalised rainbow trout in cold water streams

Currently, rainbow trout is the most widely introduced salmonid worldwide and one of the most widely introduced fish species in general (Hasegawa, 2020; D'Agaro *et al.*, 2022). Since 1870, when the California Acclimatization Society conducted the first artificial propagation of rainbow trout from the San Francisco Bay area (Healy *et al.*, 2020), the species has been introduced into at least 99 countries, with populations established in at least 53 of them (Hansen *et al.*, 2019). Such global success can be ascribed to a combination of factors: the importance of rainbow trout as a valued game-fish, its tolerance of relatively high temperatures, manipulation of life histories through selective breeding, rapid growth and suitability for hatchery cultivation and its economic importance in food production (Bosch *et al.*, 2019; Lobón-Cerviá *et al.*, 2019; Muhlfeld *et al.*, 2019). As a result, rainbow trout have successfully invaded many regions and, in some, even produced self-sustaining populations that can have strong impacts on local native fish populations and ecosystems (Pastorino *et al.*, 2019; Faulkner *et al.*, 2020).

2.5 Propagation of rainbow trout

Freshwater resident rainbow trout usually inhabit and spawn in small to moderately large, well-oxygenated, shallow rivers with gravel bottoms (Dieterman and Mitro, 2019). Lake resident rainbow trout are usually found in moderately deep, cool lakes with adequate shallows, and generally require access to gravelly bottomed streams for spawning (Antonov *et al.*, 2020; Borisenko *et al.*, 2022). Spawning sites are usually a bed of fine gravel in a riffle above a pool. A female trout clears a redd in the gravel by turning on her side and beating her tail up and down (Woś and Książek, 2022). Female rainbow trout usually produce 2000 to 3000 4-to-5-millimetre eggs per kilogram of weight (Källo *et al.*, 2022; Madenjian *et al.*, 2023). As eggs are released by the female, male moves alongside and deposits milt (sperm) over the eggs to fertilize them. The eggs hatch in four to seven weeks depending on temperature and the yolk sac fry or alevins commence feeding on zooplankton about two weeks after the yolk is consumed (Ferguson and Prodöhl, 2022). The growth rate of *O. mykiss* is variable and affected by area, habitat, life history and quality and quantity of food (Mundahl and Schnaser, 2023).

Trout does not spawn naturally in aquaculture systems (Weber *et al.*, 2023b), hence the need for artificial insemination so as to achieve production and ensure its continuity under culture conditions (İnanan, 2020; Judycka *et al.*, 2023). Artificial insemination (reproduction) is the collection of spermatozoa and ova and their mixing together in various media that keep spermatozoa motile (Butzge *et al.*, 2021). It is carried out in only a few species (mostly freshwater), such as salmonids, cyprinids and acipenserids. In traditional artificial insemination, the gametes are simply put together and the usual external medium (fresh or salt water) is added. The results are limited, particularly as concerns gamete economy: the sperm of a single male can fertilize only several females (Shindavina *et al.*, 2021). However, some recent studies, re-examining the concept of artificial insemination, have proposed better techniques for managing brood fish and their gametes (Beirão *et al.*, 2019; Guan *et al.*, 2019). The number of brood stock required is dependent on the number of fry or fingerlings required to meet the production schedule of the farm. The number can be back-calculated based on survival rates at the different life stages and the fecundity of the brood stock females (Figure 1).

Generally, one male to three females is deemed a satisfactory sex ratio for brood stock (D’Ambrosio *et al.*, 2020). Males and females are generally kept separate. Brood stock maintenance can be costly and labour intensive, causing some farms to purchase eyed eggs from other sources; these should be 'certified disease free', although they should be treated with iodine (100 mg/litre for 10 min) upon arrival and gradually raised to the hatchery temperature (Paul *et al.*, 2022). Brood stocks are selected for fast growth and early maturation (usually after 2 years). One frequently used management tool is the use of sex-reversed, all-female brood stock to produce all-female progeny that grow faster (Panasiak *et al.*, 2023). Functional males are produced by oral administration of the male hormone 17-methyl testosterone through starter feeds at the fry stage.

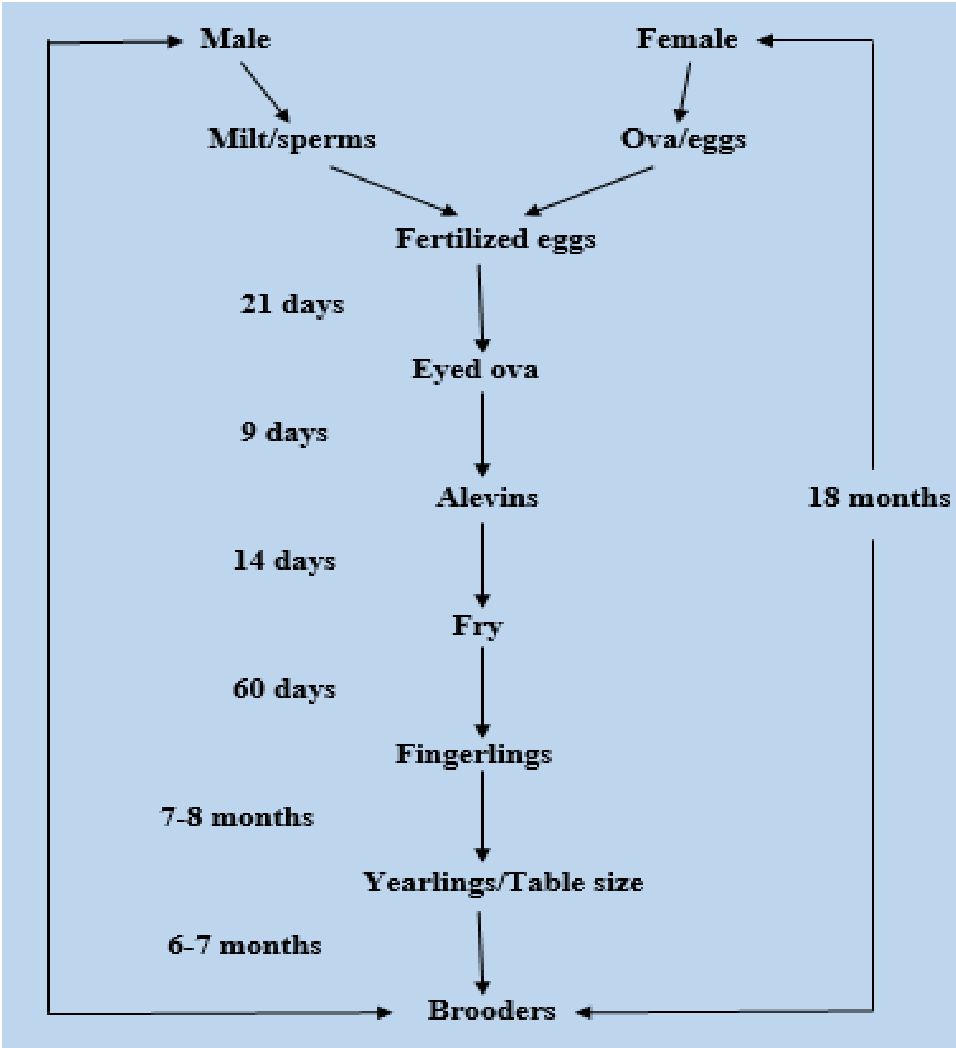

Figure 1: Life cycle of rainbow trout (source: National Trout Hatchery Annual Report, 2020)

2.6 Trout stream riparian cover

Bank structure, in-stream wood and boulders, and riparian vegetation provide protective refuge and hiding cover for rainbow trout (Caldwell *et al.*, 2020; Tamario *et al.*, 2021). Undercut banks, overhanging vegetation, turbulent or deep water, submerged or semi-submerged wood, aquatic plant beds, root masses, and large rocks also contribute to habitat diversity for rainbow trout and other aquatic life important to trout for food (Nevoux *et al.*, 2019; Richer *et al.*, 2019). Riparian vegetation (vegetation growing along a river or stream) such as trees, shrubs, grasses and forbs provide shade which moderates water temperatures and is a source of woody cover when limbs and trees fall into the stream (Wilbur *et al.*, 2020; Dauwalter *et al.*, 2022). Roots of riparian vegetation help stabilize stream banks, reducing siltation and maintaining water quality (Smialek *et al.*, 2021; Mochnacz *et al.*, 2023). Riparian plants also provide habitat for terrestrial insects that may serve as trout food (Farha *et al.*, 2020; Smith *et al.*, 2020). There is however lack of studies of these parameters in the trout aquaculture in many developing countries including trout culture in Kenyan highland areas.

2.7 Water quality parameters

Water quality is a combination of chemical, physical and biological parameters that affect the growth of cultured fish. The success of a commercial aquaculture activity depends on the optimal environmental conditions for accelerated growth at the lowest cost of resources (Boyd and Tucker, 2012; Boyd and Tucker, 2014). Water quality affects the general status of the cultured body as it determines the state of health and growth of the cultured fish (Salim *et al.*, 2016; Haq and Harigovindan, 2022). The water quality is, therefore, an essential factor to consider when planning an intensive aquaculture system suitable for trout production. Critical parameters for consideration in trout aquaculture systems are temperature, suspended solids and dissolved oxygen, nitrite, ammonia, alkalinity and carbon dioxide (Davidson *et al.*, 2013; Kocer and Sevgili, 2014).

Table 1: Water quality requirements for rainbow trout farming

Parameter	Trout Tolerance Range
Temperature (ºc)	4-20
DO (mg/l)	<2.5
pH	<4.5-9>

(Alabaster and Lloyd, 2013)

2.7.1 Temperature

Trout presence and distribution is based on water temperature, although the range of suitable temperature for its growth is quite broad (Pankhurst and King, 2010; Mugwanya *et al.*, 2022). Optimal temperatures for rainbow trout are in the range from 9 to 18°C (Ma *et al.*, 2023; Naz *et al.*, 2023). At water temperatures below 9°C and above 20°C trout metabolic rate decreases while temperatures values that are above 20°C are not suitable for trout growth and temperature above 24ºC is lethal (Yu *et al.*, 2022; Dempsey *et al.*, 2023).

2.7.2 Dissolved oxygen

Dissolved oxygen is the most important parameter, requiring continuous monitoring in aquaculture production systems, because fish aerobic metabolism requires dissolved oxygen (Caldwell and Hinshaw, 1994; Jiang *et al.*, 2021). Rainbow trout is extremely demanding on the level of dissolved oxygen in the water, the optimal concentration should not be less than 9 mg/l (Galezan *et al.*, 2020). Trout can tolerate water saturation with pure oxygen to 50 mg/l (Edsall and Smith, 1990; Waldrop *et al.*, 2020). Lethal concentration of oxygen in the water for trout is 2.5 mg/l (Nepal *et al.*, 2021). In an experiment that involved cultivation of trout in cages it shows that at high temperatures, the content of dissolved oxygen in water is not less than 9 mg/l (Devaa and Ramesh, 2021). During the whole period of cultivation of a rainbow trout especially during periods of intensive breeding, it is necessary to continuously monitor oxygen concentrations in cages because the concentration of oxygen limits the amount of fish breeding (Royer *et al.*, 2021). The concentration of oxygen at the normal growth of fish should be at the water temperature of 5°C – not less than 5.0 mg/l, at 10°C – not less than 6.0 mg/l, at 15°C – not less than 7.0 mg/l and at 20°C – not less than 8.0 mg/l (Welker *et al.*, 2019; Coutinho De Lima *et al.*, 2020).

Among the water quality parameters, dissolved oxygen is highly affected when fouling and silting occurs in cages, the oxygen for a few hours may be reduced to a critical value (6–7 mg/l) or even lethal concentration (2–3 mg/l) (Tang *et al.*, 2020; Uiuiu *et al.*, 2020). The lack of oxygen in the culture units can be judged by the behaviour of fish in water. Eliminating such a situation can be achieved by aerators, which intensively pump air or oxygen through the water environment (Pleizier *et al.*, 2021; Alimova *et al.*, 2023).

2.7.3 pH

At cultivation of rainbow trout, it is preferable to use water with pH values from 7 to 8. Water with pH within 6.5 – 8.5 is quite satisfactory for trout, but pH values lower than 4.5 and higher than 9 are lethal for trout (Kocer and Sevgili, 2014). The toxic effect of pH is increased with different content of ions of calcium, sodium and chlorine in water (Ekubo and Abowei, 2011). The presence of hydroxide ions in the water reduces the resistance of trout to low pH values. If the pH value is below 7, the ion concentration above 1.5 mg/l results in the death of trout (Boyd, 2017). In general, the growth rate of trout in acid waters is lower than in alkaline, and at constant pH within its optimum values the growth rate is higher than at varying pH values (Baldisserotto, 2011; Seanego *et al.*, 2023).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was carried out along the River Sagana and collected fish taken to Kiganjo national trout hatchery for experiments. River Sagana is a second order stream flowing from Mt. Kenya (Figure 2). The Sampling stations are labelled as S1, S2 and S3 for River Sagana. The stream originates from the South-eastern slopes of Mt. Kenya at an altitude of approximately 4000 m above sea level. The catchment stretches from latitude 0°13'S to 0°22'S to longitude 37°16'E to 37°03'E draining a watershed area of approximately 2256 km^2 (Ngugi and Green, 2000).

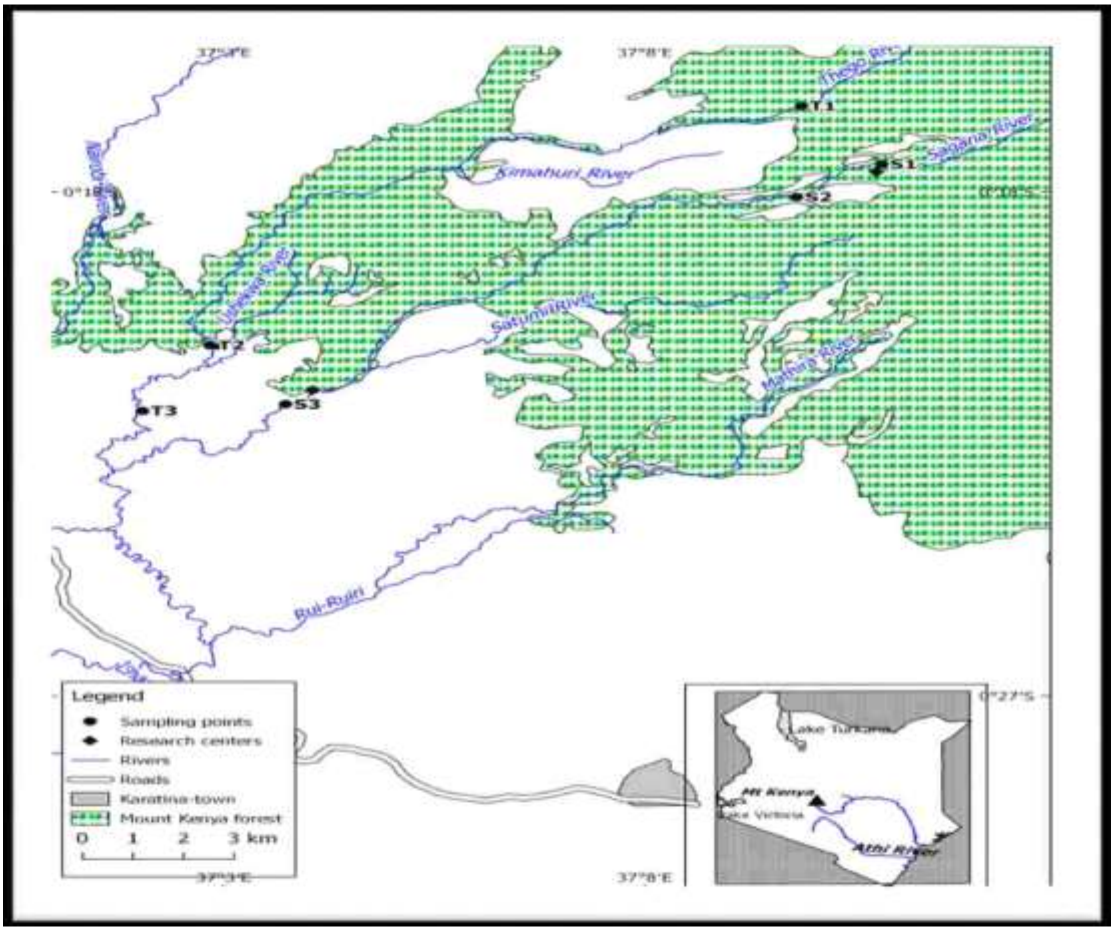

Figure 2: Location of Sagana second order stream flowing from South eastern slopes of Mt. Kenya showing the study site as S1-S3.

Kiganjo trout hatchery situated on R. Sagana was established as a River Research and Development Centre in the year 1948 (Van Someren, 1952). The station is currently mandated to develop standards and certification of fish seeds, feeds, brood stock and other cold-water fisheries products for distribution to trout fish farmers; carry out research related to cold water fisheries and to provide technical information on cold water aquaculture.

3.2 Study design

3.2.1 Fish collection

The study was conducted from March to December 2021 covering the wet and dry seasons, where wild fish were collected from T1, T2 and T3 sites along River Sagana (Figure 1). The fishing sessions were carried out using gillnets to capture breeders in the pools or riffles and the runs, where the presence of adult specimens was noted. A total of 220 fish were collected, which included 104 males and 116 females. Fish collection was done from 6 am to 11 am for the first half of February 2021. Fish were maintained in breeding ponds in Kiganjo trout hatchery, until the spawning phase. After ovulation and spawning, which were performed once, breeders were released back to the breeders' ponds no later than 24 h after their reproductive management. In some cases, fish were kept for several days in the hatchery ponds, to enable the conclusion of their maturation process and to proceed with the spawning activity.

3.2.2 Hatchery management of broodstock and artificial fertilization

Fish were monitored on a weekly basis for correct detection of ovulation and spermiation, before performing the spawning process. Females were palpated weekly to assess the distended abdomen in gravid breeders for timely detection of ovulation to ensure optimum egg quality during each spawning session. Prior to stripping, females and males were anesthetized during 2–3 min with a 40 ppm of benzocaine solution. The eggs of each female were collected in individual bowls. When egg batches presented impurities such as blood, broken eggs, and traces of yolk, the ovarian fluid was drained over a strainer to be washed using a 0.8% saline solution. Subsequently, they were returned to their bowl and the eliminated fluid was replaced by Billard's sperm diluent (Suquet *et al.*, 2000) before being fertilized.

Males were selected when milt had an appropriate external feature (intense white colour) and texture (semi-creamy and homogeneous). Fertilization was performed with 3 ml doses of pooled semen obtained from three to four males. This number of males was used in each spawning session, which ranged from five to seven during each reproductive season. It should be mentioned that all males were spawned only once; they were not reused in successive spawning sessions and were from the same origin as females. After fertilization, the eggs were rinsed several times with incubation water to remove sperm remnants and then left for 15 min to facilitate egg hardening. The eggs of each female were individually incubated to allow traceability of reproductive variables (Figure 2). The incubation process was performed in fiberglass punts with a spring water supply at a temperature of between 7ºC and 8ºC and a constant flow of 20 L/min.

A wet method of fertilization was used in this trial; where fertilization is allowed to occur in an environment that is moist. The fertilized ova were placed in incubation trays within the incubation troughs. Silt free clear cold-water flows through the troughs at a flow rate of 5 L/sec and is maintained throughout the incubation period.

The fish were held in a brooder pond, removed when ripe, and stripped. For the male, milt was collected from ripe males and eggs stripped from females. Once fertilization occurred, the eggs become fertilized ova ready for growth and maturation. Trout eggs were incubated until the “eyed” stage was reached, in hatching troughs, hatching and rearing troughs were 40-50 cm wide, 20 cm deep, and up to about 4 m in length. Two layers of eggs were placed in screened trays supported 5 cm above the bottom, and water passes through the tray (3-4 L/min). As the eggs hatched in approximately 4-14 weeks, the fry dropped through the mesh to a bottom trough. The time taken for hatching varied depending on water temperature, taking 100 days at 3.9°C and 21 days at 14.4°C (about 370-degree days). The dead (white) eggs were removed regularly to limit bacterial and fungal infections. Fungal infections were controlled using formalin (37 percent solution of formaldehyde) in the inflow water at 1:600 dilutions for 15 minutes daily, but not within 24 hours of hatching. After hatching, the trays were removed and the trough water depth kept shallow (8-10 cm) with a reduced flow until fry reached the 'swim-up' stage, the yolk sac absorbed, and active food searching began.

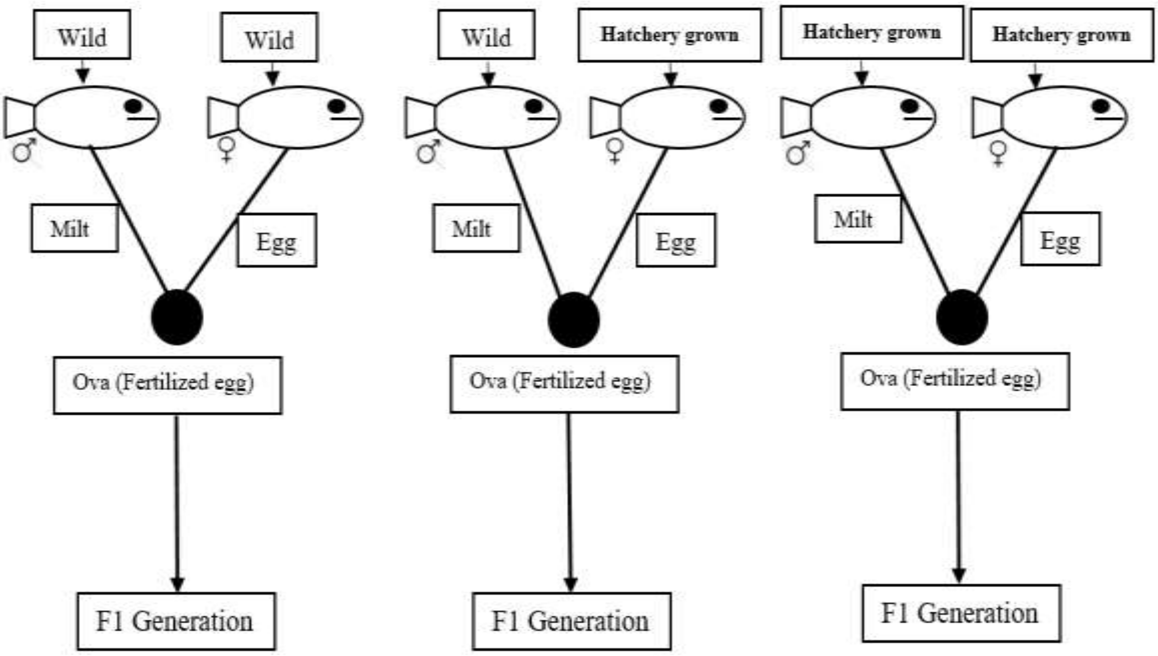

Figure 3: Experimental process of the breeding-wild with wild, wild with hatchery and hatchery with hatchery

3.3.3 Rearing of fry

Once the ova hatched to free-swimming fry, live feeds (daphnia; which is zooplankton) were offered to wean the juvenile fries for a period of two weeks after which formulated feed 50% protein was offered to the fry at a daily ration of 7% of the body weight. Survival for the ova, alevins, and free-swimming fry was recorded as growth performance progressed (Appendices). The feed pellets, made of fish meal (80 percent), fish oils, and grains, provide nutritional balance, encouraging growth and product quality, and were formulated to contain approximately 50 percent protein, 12-15 percent fat, vitamins (A, D and E), minerals (calcium, phosphorus, and sodium) and a pigment to achieve pink flesh (where desirable). When the fry was 15-25 mm long feeding commenced based on published charts, related to temperature and fish size. As growth continued, dissolved oxygen was monitored and fish moved to larger tanks to reduce density, as the fries grew to fingerlings.

3.4 Analytical techniques

3.4.1 Length-weight relationships

Length -weight relationship (LWR): the relationship between length and weight of fish was analysed by measuring fork length and wet weight of fish samples collected for the study. The statistical relationship between these parameters used the parabolic equation $W = aL^b$ (Tsoumani *et al.,* 2006). The equation was used to estimate the relationship between the weight (g) of the fish and its total length (cm). This equation was when converted into the logarithmic form gives a straight-line relationship graphically log-transformed to the equation: $\log (W) = \log a + b \log (L)$ (Morato *et al.*, 2001).Using the linear regression, parameters a and b were calculated with 'a' representing the intercept and 'b' the slope of the relationship.

In order to establish LWRs with respect to periodic variations that can affect b, fish were grouped according to the period when they were caught. If no effect was detected at the larger period classification, then b was assessed and interpreted by individual season. When applying this formula on sampled fish, b may deviate from the "ideal value" of 3 that represents an isometric growth because of certain environmental circumstances or the condition of the fish themselves. When b is less than 3, fish become slimmer with increasing length, and growth will be negatively allometric. When b is greater than 3.0, fish become heavier showing a positive allometric growth and reflecting optimum conditions for growth (Casselman, 1990).

3.4.2 Fulton's condition factor (K)

The relationship between length and weight for mean samples were used to calculate Fulton's Condition Factor Index (K) estimated using the equation $CF= (W/L^3) \times 100$

(Nash *et al.*, 2006). Condition Factor Index was established to assess the condition of the fish under study. Good growth condition of the fish is deduced when K > 1, while the fish is in poor growth condition compared to an average individual with the same length when K < 1 (Sutton *et al.,* 2000). It should be noted that the stomachs of fish were not emptied before weighing.

3.4.3 Biometry and reproductive traits

Bodyweight (g), total body length (cm), and condition factor (K) of each fish were recorded prior to gamete collection. At 100 Accumulated Thermal Units (ATUs) of incubation, a sample of 50 eggs treated with a 20% acetic acid solution was taken from each incubation to record the fertilization rate (%). Total fecundity (number of eggs/female) and relative fecundity (number of eggs/kg of female) were also recorded. At 200 ATUs, eyed egg survival (%) was determined in each incubation by subjecting eggs to a shock process to induce the mortality of the weak and infertile eggs. In contrast, viable eggs were shiny orange and translucent, with visible pigmented eyes. The sum of live and dead egg represented total fecundity per female. Fecundity and egg survival were quantified gravimetrically by weighing a sample of 500 eggs obtained from a counting paddle, on a digital balance, to determine the mean egg weight of each female. Then, the mean egg weight was used to estimate the total egg number, by dividing the total egg weight recorded in each female by the mean egg weight value. Mean egg diameter (mm) was obtained by determining the number of eggs arranged on a 300 mm ruler, and then dividing the ruler length by the number of resulting eggs.

3.5 Statistical analyses

Statistical analysis was conducted by pooling the biometric and reproductive data of breeders which, based on their common spawning period, were considered as a single stock. This criterion agrees with data available on the variation in spawning timing observed in different strains of rainbow trout, which usually associate in a given spawning period pattern with a specific strain (Bromage *et al.*, 1992). The author performed all statistical analyses using the MINITAB version 18 program (Ryan *et al.*, 2012). Prior to statistical analysis, the variables were analysed for conformance to assumptions regarding normal distribution and homogeneity of variance using the Kolmogorov–Smirnov (K-S) and Levene´s tests, respectively (Das and Imon, 2016).

To investigate the LWRs data, ANOVA was used to evaluate the statistical significance of the regression model detected at $P < 0.05$. To verify if b was significantly different from the predictions assigned for isometric growth ($b = 3$), student two sample t-test comparison was performed. While a statistically significant difference of b from 3 implies an allometric growth either positive or negative, an isometric growth is assigned when b is not statistically different from 3 (Froese, 2006). Statistical differences in b value between periods and among seasons were tested using a one-way ANOVA with P significant at < 0.05. The biometric characteristics between wild and hatchery-reared fish were compared using the Student's two sample t-test for independent samples, evaluating the statistical significance through bootstrapping with 1000 sub-sample with replacement.

Reproductive traits were modelled as a function of female breeder traits using general linear mixed models (GLMMs) (Krueger and Tian, 2004). The statistical tool was used to explore the relationships between female reproductive traits and other breeder traits (length, weight and fecundity) in order to identify all the potential combinations that could be linked to specific female reproductive traits, such as total fecundity, relative fecundity, egg diameter, fertilization rate, and eyed egg survival. Considering the fact that all fish in a year may not be independent as they have experienced similar conditions leading up to spawning, a null hypothesis that the spawning time was a random factor was tested using the Wald Z-test (Gjedrem *et al.*, 2009).

Regression models were fitted to all independent variable combinations of biometric and reproductive traits. The absence of multicollinearity between independent variables was also verified through the Variation Inflation Factor (VIF), based on the tolerance value of each variable, according to the formula VIF = 1/tolerance. When the value of the VIF was more than 5, multicollinearity was considered serious and the variable was eliminated from the analysis (Shrestha, 2020).

For this analysis, the following relationships were modelled: (a) Total fecundity (dependent variable) with the independent variables: body weight, body length, condition factor, spawning time, egg weight, and egg diameter, (b) Relative fecundity (dependent variable) with the independent variables: body weight, body length, condition factor, spawning time, egg weight and egg diameter, (c) Egg diameter (dependent variable) with the independent variables: body weight, body length, condition factor, spawning time and egg weight, (d) Fertilization rate (dependent variable) with the independent variables: body weight, body length, condition factor, spawning time, egg weight, egg diameter, total fecundity, and relative fecundity, (e) Eyed egg survival (dependent variable) with the independent variables: body weight, body length, condition factor, spawning time, egg weight, egg diameter, total fecundity, relative fecundity, and fertilization rate. Total fecundity (TF, No. of eggs/female), relative fecundity (RF, No. of eggs/kg female), fertilization rate (FR, %), egg diameter (ED, mm), and eyed egg survival (EES, %) were modelled as a function of biometric and reproductive parameters, using general linear mixed models. In the analysis, models were run using non-transformed variables.

CHAPTER FOUR

DATA ANALYSIS, PRESENTATION AND INTERPRETATION

4.1 Water quality parameters

The mean water temperature in April was 9.58 ± 0.31ºC, Dissolved Oxygen was recorded at a mean of 10.58 ± 0.39 mg/L while the water pH was 7.35 ± 0.40. The highest temperature recorded was in September at a maximum of 12.70ºC and the lowest was in June at 8.39ºC (Table 2). These water variables were, however, within the expected range for trout egg and fry development in a hatchery.

Table 2: The mean, minimum and maximum water parameters recorded in the hatchery from April to September

April 2021				
Variable	**Mean**	**StDev**	**Minimum**	**Maximum**
Temperature ^{0}C	9.58	0.31	9.01	10.11
D.O. mg/L	10.58	0.39	10.12	11.57
pH	7.35	0.40	6.66	8.05
May 2021				
Variable	**Mean**	**StDev**	**Minimum**	**Maximum**
Temperature	10.77	0.90	9.19	12.93
D.O. mg/L	10.15	0.59	8.69	10.92
pH	7.54	0.38	6.98	8.38
June 2021				
Variable	**Mean**	**StDev**	**Minimum**	**Maximum**
Temperature	9.72	0.55	8.39	10.65
D.O. mg/L	10.42	0.58	9.19	11.54
pH	7.48	0.34	6.89	8.16
July 2021				
Variable	**Mean**	**StDev**	**Minimum**	**Maximum**
Temperature	9.51	0.42	8.80	10.60
D.O. mg/L	10.85	0.46	9.99	11.71
pH	7.56	0.34	6.97	8.03
August 2021				
Variable	**Mean**	**StDev**	**Minimum**	**Maximum**
Temperature	10.85	0.64	10.00	12.20
D.O. mg/L	10.39	0.51	9.10	11.61
pH	7.52	0.38	6.73	7.99
September 2021				
Variable	**Mean**	**StDev**	**Minimum**	**Maximum**
Temperature	11.64	0.61	10.10	12.70
D.O. mg/L	9.60	0.57	8.69	10.73
pH	7.50	0.31	6.97	7.99

4.2 Biometric parameters of hatchery and wild rainbow trout brooders

During this study, measured biometric parameters for brooders of naturalized rainbow trout population from River Sagana, registered during the sampling seasons are shown in Table 3 while the statistical differences among these attributes are shown in Table 5. Significantly higher (P < 0.05) body weight, fork length, condition factor was recored in male and female wild × hatchery reared fish. Consistent with egg diameter is the egg weight which showed concomitant significant differences among the fish in the three cohorts. Eyed egg survival, was lowest among the wild fish but showed no significant differences between the hatchery and cross between wild and hatchery traits

Table 3: Biometric parameters for breeders of a naturalized rainbow trout population from River Sagana. In parenthesis, the sample size (n) is indicated

Biometric parameters	**Wild fish stock (Mean ± SD)**	**Hatchery reared (Mean ± SD)**	**Wild × Hatchery reared (Mean ± SD)**
Female body weight (g)	0.52 ± 0.04^{a} (n = 18)	0.91 ± 0.11^{b} (n = 15)	1.02 ± 0.15^{c} (n = 12)
Female Fork length (m)	0.31 ± 0.04^{a} (n = 16)	0.45 ± 0.13^{b} (n = 13)	0.46 ± 0.12^{b} (n = 18)
Female condition factor (k)	1.2 ± 0.04^{a} (n = 16)	1.4 ± 0.04^{b} (n = 12)	1.4 ± 0.04^{b} (n = 12)
Male body weight (g)	0.41 ± 0.02^{a} (n = 15)	0.40 ± 0.04^{b} (n = 15)	0.48 ± 0.04^{c} (n = 12)
Male body length (cm)	0.28 ± 0.04^{a} (n = 12)	0.40 ± 0.10^{b} (n = 13)	0.43 ± 0.11^{b} (n = 18)
Male condition factor (k)	1.1 ± 0.04^{a} (n = 12)	1.2 ± 0.04^{b} (n = 12)	1.2 ± 0.04^{b} (n = 12)

Table 4: Paired comparisons t-tests for biometric differences among female and male breeders of a naturalized rainbow trout population from River Sagana, recorded during three reproductive seasons. n=sample size

Reproductive parameters	**Wild fish stock (Mean ± SD)**	**Hatchery reared (Mean ± SD)**	**Wild × Hatchery reared (Mean ± SD)**
Body weight (g)	n = 30	n = 28	n = 30
	Trend = F > M	Trend = F > M	Trend = F > M
	t-statistics = -17.89	t-statistics = -5.81	t-statistics = -3.02
	$P < 0.0001$	$P < 0.001$	$P < 0.05$
Body length (g)	n = 30	n = 28	n = 30
	Trend = F > M	Trend = F > M	Trend = F > M
	t-statistics = -4.21	t-statistics = -5.32	t-statistics = -3.42
	$P < 0.0001$	$P < 0.001$	$P < 0.05$
Condition factor	n = 30	n = 28	n = 30
	Trend = F > M	Trend = F > M	Trend = F = M
	t-statistics = -13.89	t-statistics = -4.51	t-statistics = -1.04
	$P < 0.0001$	$P < 0.001$	$P > 0.05$ (NS)

4.3 Length weight relationships in fish species reared under different conditions

The LWR was calculated by transforming the real data to the linear equation (log Wt = log a + b log TL). The value of a = – 1.843 (wild fish), – 2.159(hatchery-reared), – 2.573(wild-hatchery) and b = 2.900(wild fish), 3.029 (hatchery-reared), 3.175 (wild-hatchery) were better fitted at r^2 = 0.95(wild fish), 0.95(hatchery-reared), 0.96(wild-hatchery) respectively as indicated in Table 5.

Table 5: Regression equations of rainbow trout from hatchery-reared, wild and crossed fish showing relationships between log weight (g) and log total length (cm)

Rainbow trout Trait	**a**	**b**	r^2	F	P
Hatchery-reared	-1.843	2.900	0.950	1799.22	0.000
Wild (from Sagana stream)	-2.159	3.029	0.953	1942.50	0.000
Wild × Hatchery-reared	-2.573	3.175	0.964	2556.94	0.000

These values were then transformed into parabolic form and the equations obtained were $W = 0.0144L^{2.900}$, $W = 0.0069L.^{3.0285}$ and $W = 0.00027L^{3.175}$ respectively (Figure 4). The graphs of the linear equation of *O. mykiss* are shown in Figure 4. The value of **a** in all shows positive allometric growth. All the **b** values were almost 3, indicating that *O. mykiss* has an isometric form of growth in weight. Furthermore, the r^2 value of LWR of *O. mykiss* was relatively high indicating a strong relationship between the length and weight for the three groups. The general parabolic equation is Y = a + bX and the Regression equations for LLR were as follows: SL = 1.106 TL + 1.667, CFL = 1.059 TL + 0.815 and CFL = 0.903 SL + 0.363. Results for LLR indicated that the value of correlation coefficient (r^2) were highly correlated, $r^2 > 0.9$ at $P < 0.05$. The value for the correlation coefficient (r^2) were 0.950, 0.953, and 0.964 respectively and are shown in Figure 5.

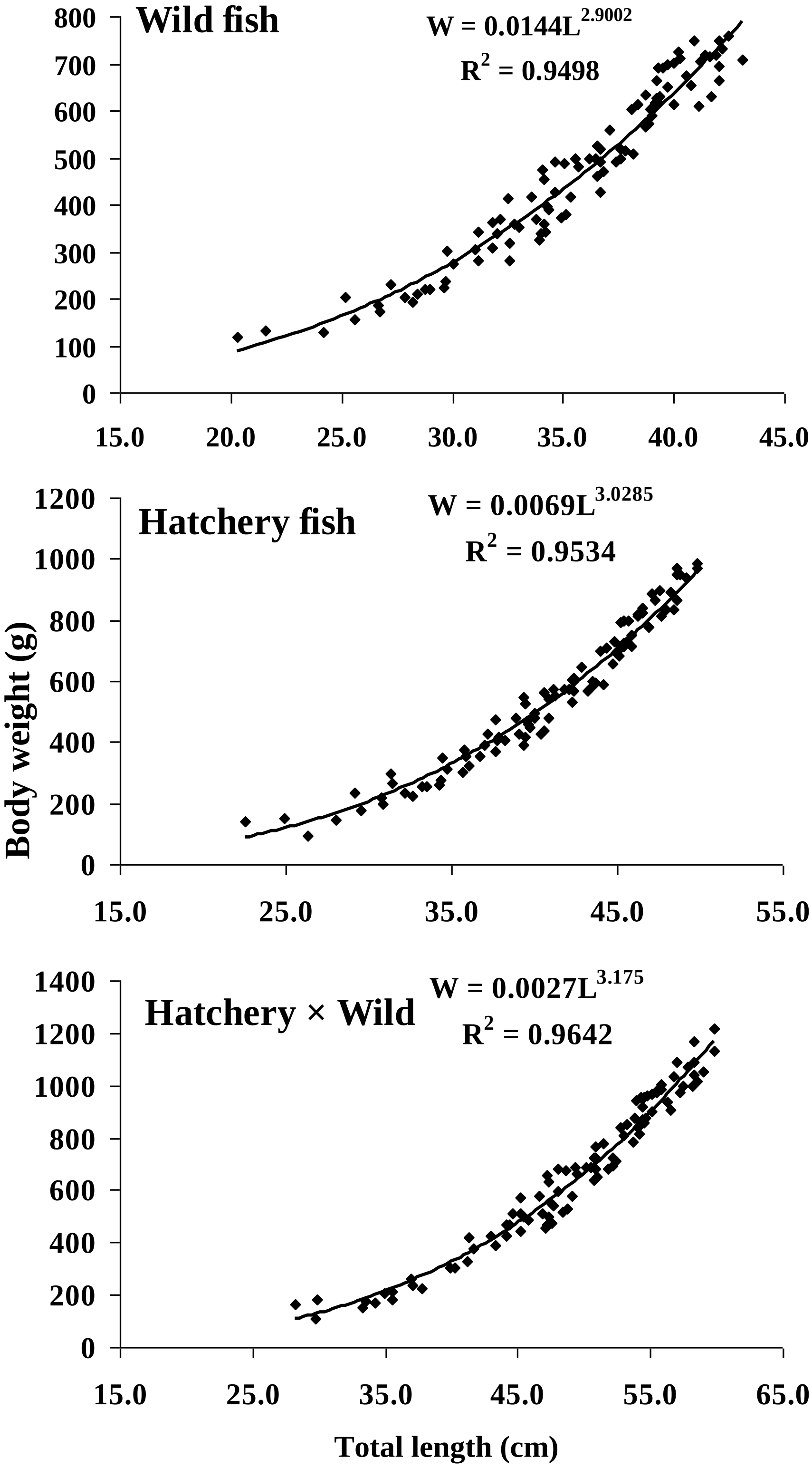

Figure 4: The length-weight relationship among rainbow trout under different breeding conditions

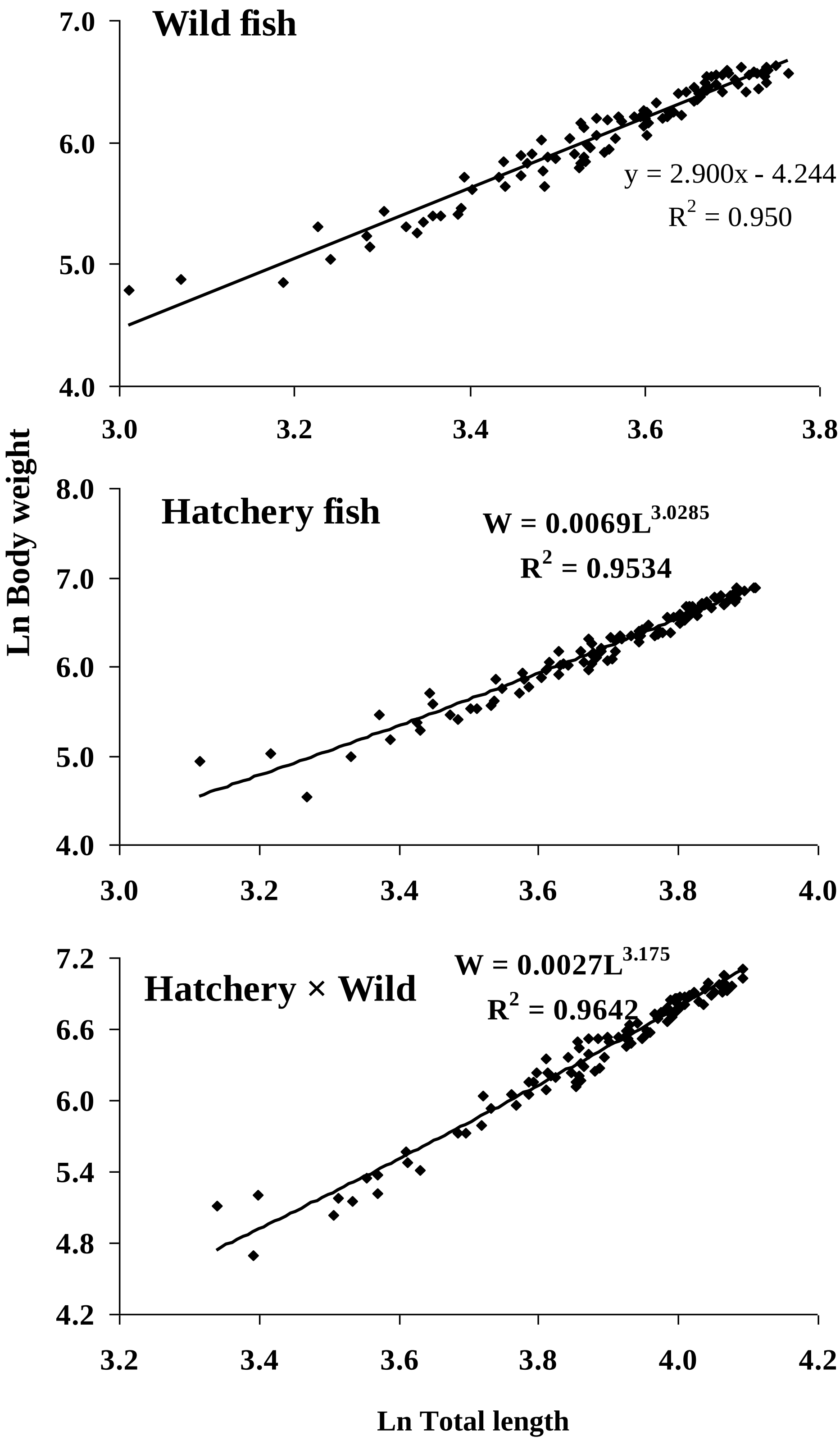

Figure 5: Log-transformed length-weight relationship among traits under different breeding conditions

4.3 Reproductive traits among hatchery, wild, and crossed rainbow trout

The reproductive traits between the hatchery and wild rainbow trout brooders are shown in Table 6. Total fecundity of the broodstock differs significantly between the wild strain, hatchery reared, and the cross between hatchery and wild stock (One-Way ANOVA; $F = 8.934$, $df = 2$, $P = 0.0045$). Moreover, the fecundity of the broodstock was consistent with the average weight of the brooders where the cross between the wild and hatchery-reared fish provided superior performance. Meanwhile, Relative fecundity was significantly different among the three groups of fish (One-Way ANOVA; $F = 6.134$, $df = 2$, $P = 0.0217$) but the differences between the hatchery and cross strain of wild and hatchery fish showed no significant differences. The fertilization rate showed significant differences among fish in the three experimental groups (One-Way ANOVA; $F = 10.136$, $df = 2$, $P = 0.0057$), with no discernible difference observed between the hatchery and cross strain of wild and hatchery fish. Meanwhile, egg diameter showed consistent significant differences among fish in the three experimental fish groups (One-Way ANOVA; $F = 23.338$, $df = 2$, $P = 0.0005$). Table 2 below shows the mean and standard deviation of the reproductive parameters of the three population of fish. The fertilization rate differed for the three population with wild spawned fish having the lowest fertilization rate. Survival rate was lowest on wild spawning fish and highest on crossed spawned fish between wild and hatchery brooders.

Table 6: Reproductive parameters for female breeders of naturalized wild and hatchery reared rainbow trout populations during sampling period in 2021. In parenthesis, the sample size (n) is indicated

Reproductive parameters	**Wild fish stock (Mean ± SD)**	**Hatchery-reared (Mean ± SD)**	**Wild × Hatchery-reared (Mean ± SD)**
Total fecundity (No. of eggs/female)	2134 ± 335^{a} (n = 42)	3245± 234^{b} (n = 45)	3745± 316^{c} (n = 40)
Relative fecundity (No of eggs per/kg female)	6275± 876^{a} (n = 42)	7211± 925^{b} (n = 45)	7201± 1005^{b} (n = 40)
Fertilization rate (%)	82.2 ± 6.7^{a} (n = 42)	93.4 ± 4.5^{b} (n = 45)	94.9 ± 4.1^{b} (n = 40)
Egg diameter (mm)	5.1 ± 0.13^{a} (n = 24)	5.6 ± 0.12^{b} (n = 20)	5.9 ± 0.14^{c} (n = 25)
Egg weight (mg)	74.2 ± 3.4^{a} (n = 24)	81.3 ± 3.1^{b} (n = 20)	88.4 ± 2.7^{c} (n = 25)
Survival of fry (%)	61.4 ± 10.2	84.5 ± 5.6	86.7 ± 5.9
Eyed egg survival, EES (%)	78.3 ± 5.6^{a}	87.4 ± 7.8^{b}	87.1 ± 6.4^{b}

4.4 Relationships between biometric and reproductive parameters of hatchery and wild trout brooders

Coefficients for the regression of total fecundity, relative fecundity, egg diameter and eyed egg survival on reproductive variables for the naturalized rainbow trout of River Sagana are shown in Table 6. The variance component for the spawning year was low, accounting for only between 0.34% and 14.82% of the total variance; therefore, this factor did not represent a significant effect (Wald Z-test: P = 0.37) to be considered a random factor in the GLMM analysis. Thus, the models were fitted considering a sample size of the full data set (n = 133). There was an absence of multicollinearity between the independent variables given that the VIF was below 5 (VIF range = 1.01–1.33). Thus, this factor did not affect model estimations.

TF showed a significant positive correlation with FBW and a significant negative correlation with EW (intercept = 2579. 69; FBW slope = 1.46, t-test: t = 12.19, df =

56.95, $P < 0.001$; EW slope = -26.44, t-test: t = -7.47, df = 111.82, $P < 0.001$) (Table 6 and Fig. 3). FBL and EW were significantly negatively associated with RF (intercept = 4667. 66; FBL slope = -32.25, t-test: t = -4.82, df = 117, $P < 0.001$; EW slope = -14.72, t-test: t = -7.04, df = 117, $P < 0.001$) (Table 7). Models with ED as a response variable ranked highest.

Table 7: Coefficients for the regression of total fecundity, relative fecundity, egg diameter and eyed egg survival on reproductive variables for the naturalized rainbow trout of River Sagana.

Dependent variable	**Intercept**	***P*-value**	**Slope (1st variable)**	***P*-value**	**Slope (2nd variable)**	**P-value**
Total fecundity	2001	< 0.0001	1.37	< 0.0001	-26.3	< 0.0001
Relative fecundity	4275	< 0.0001	-27.5	< 0.0001	-16.5	< 0.0001
Egg diameter	4.2	< 0.0001	0.02	< 0.0001	1.12	< 0.0001
Egg survival	56.3	< 0.0001	0.44	0.002	-0.02	0.012

Bivariate linear regression between total fecundity and female body weight in wild, hatchery reared and naturalized rainbow trout from River Sagana is provided in Figure 6. Regression lines (solid line) fitted by ordinary least-squares are shown. There was significant ($P < 0.05$) positive correlation between total fecundity and female body weight for only the wild × hatchery naturalized population, such correlations were not significant ($P > 0.05$) for the wild fish as well as hatchery reared fish. The total number of eggs spawned was not significantly ($P > 0.05$) affected by female body weight for the hatchery reared fish.

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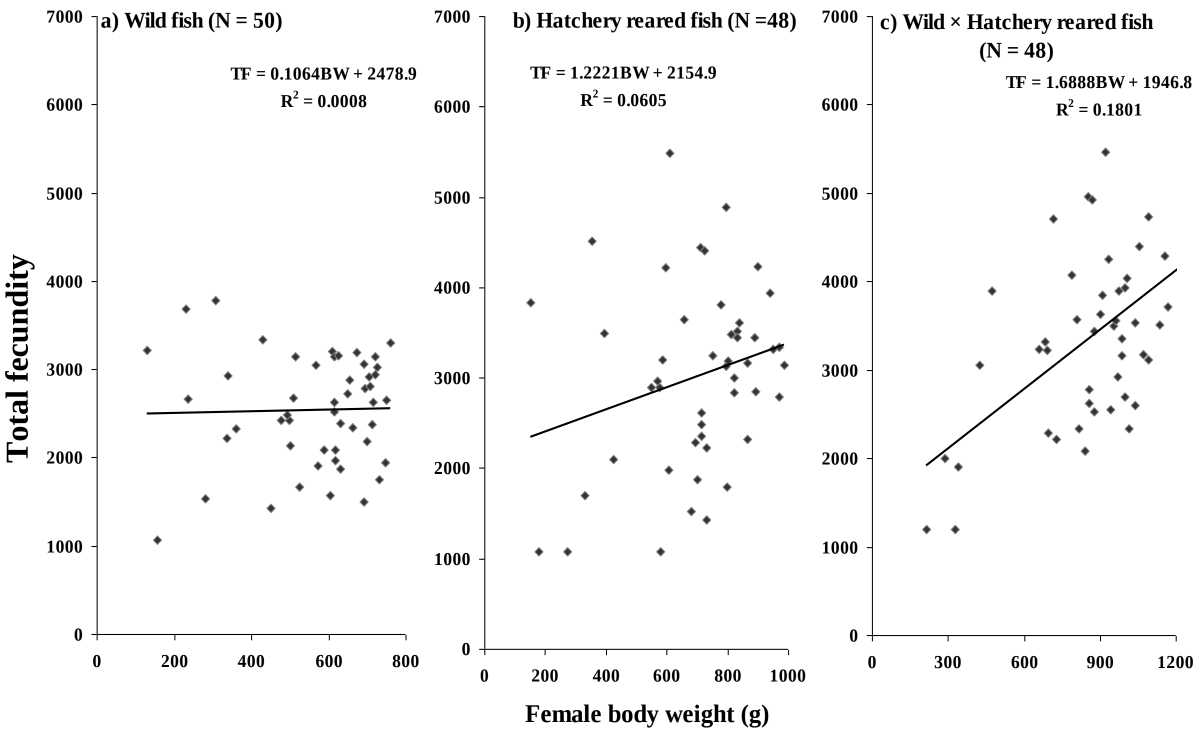

Figure 6: Bivariate linear regression between Total fecundity (No. of eggs/female) against female body weight in (a) wild population, (b) hatchery reared population and (c) wild × hatchery reared population of rainbow trout in River Sagana

Simple linear regression between total fecundity and egg weight in wild, hatchery reared and naturalized rainbow trout from River Sagana is provided in Figure 7. Regression lines (solid line) fitted by ordinary least-squares are shown. There was a negative significant correlation between total fecundity and egg weight in wild fish while for the wild × hatchery naturalized population the relationship was positive and significant ($P < 0.05$). The total number of eggs spawned was not significantly ($P > 0.05$) affected by egg weight for the hatchery reared fish.

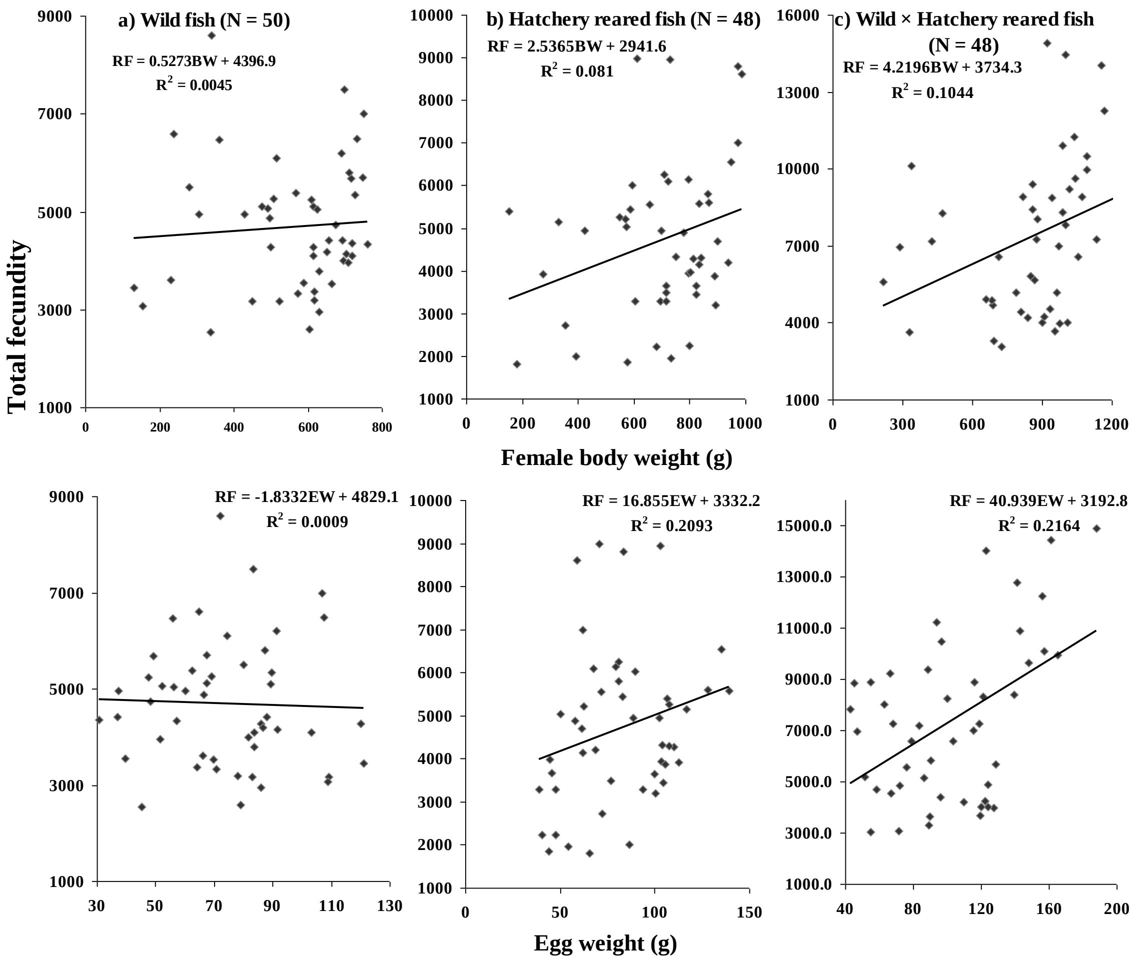

Figure 7: Bivariate linear regression between Total fecundity (No. of eggs/female) against egg weight (g) in (a) wild population, (b) hatchery reared population and (c) wild × hatchery reared population of rainbow trout in River Sagana

Bivariate linear regression between relative fecundity and body weight in wild, hatchery reared and naturalized rainbow trout from River Sagana are shown in Figure 8. Regression lines fitted by ordinary least-squares are shown. There were significant ($P < 0.05$) positive correlations between relative fecundity and female body weight for the hatchery reared and wild × hatchery naturalized population.

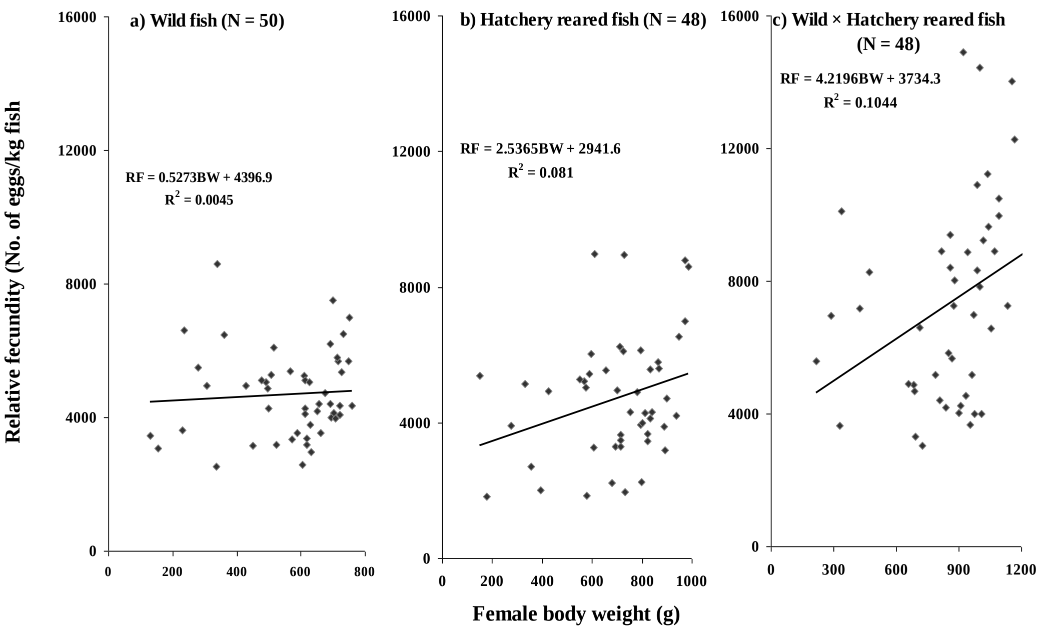

Figure 8. Bivariate linear regression between Relative fecundity (No. of eggs/kg fish) against female body weight and egg weight (g) in (a) wild population, (b) hatchery reared population and (c) wild × hatchery reared population of rainbow trout in Sagana

Bivariate linear regression between relative fecundity and egg weight in wild, hatchery reared and naturalized rainbow trout from River Sagana are shown in Figure 9. Regression lines fitted by ordinary least-squares are shown, Relative fecundity was also positively correlated with egg weight in hatchery reared and wild × hatchery naturalized rainbow trout fish ($P < 0.05$).

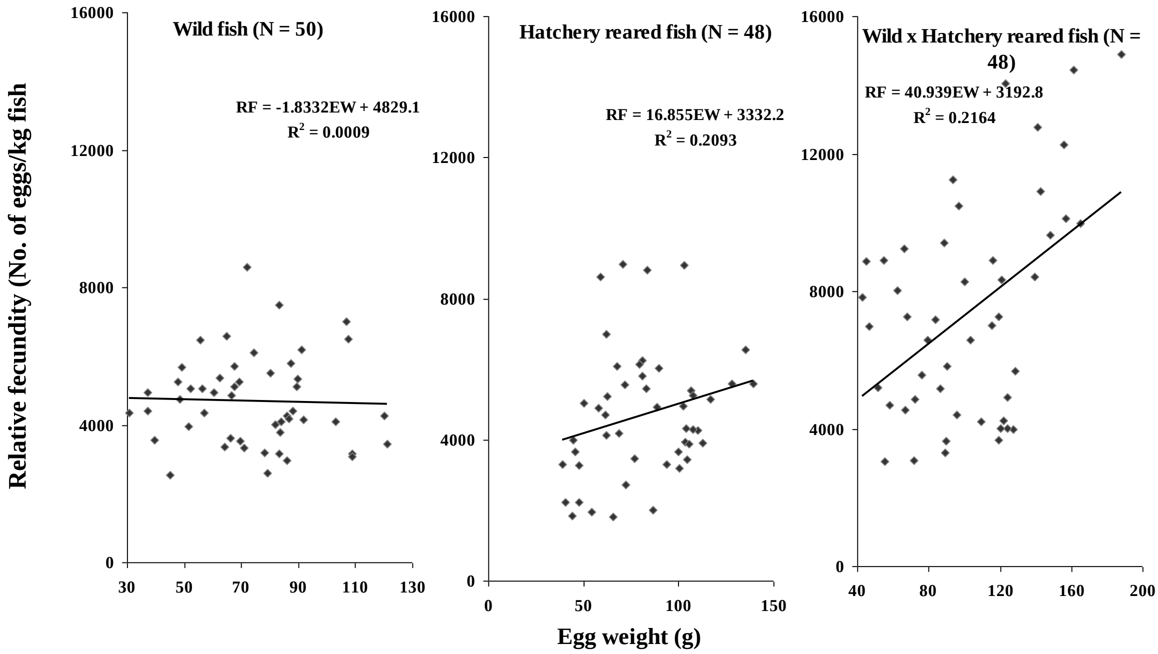

Figure 9: Bivariate linear regression between Relative fecundity (No. of eggs/kg fish) against egg weight (g) in (a) wild population, (b) hatchery reared population and (c) wild × hatchery reared population of rainbow trout in Sagana

Bivariate linear regression between LN (eyed egg survival (%) and fertilization rates in wild, hatchery reared and naturalized rainbow trout from River Sagana are shown in Figure 10. Regression lines fitted by ordinary least-squares are shown. Survival of the eyed eggs was significantly affected positively by fertilization rates in the hatchery raised and wild × hatchery naturalized rainbow trout but not in the wild stock.

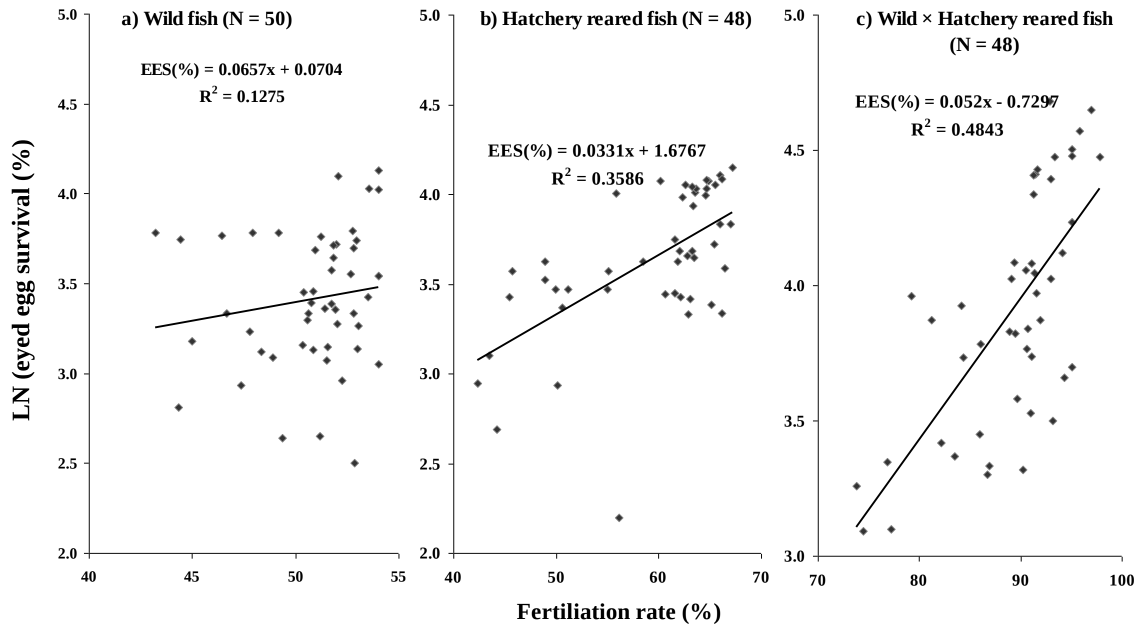

Figure 10. Bivariate linear regression between LN of eyed egg survival (%) against fertilization rates (%) in (a) wild population, (b) hatchery reared population and (c) wild × hatchery reared population of rainbow trout in Sagana. Eyed egg survival data was log-transformed based on the equation y = LN(y/(100-y). Regression lines fitted by ordinary least-squares are shown.

Bivariate linear regression between LN (eyed egg survival (%) and relative fecundity (No. of eggs/kg fish) in wild, hatchery reared and naturalized rainbow trout from River Sagana are shown in Figure 11. Regression lines fitted by ordinary least-squares are shown. Survival of the eyed eggs was significantly ($P < 0.05$) affected positively by number of eggs in the hatchery raised and wild × hatchery naturalized rainbow trout but not in the wild stock.

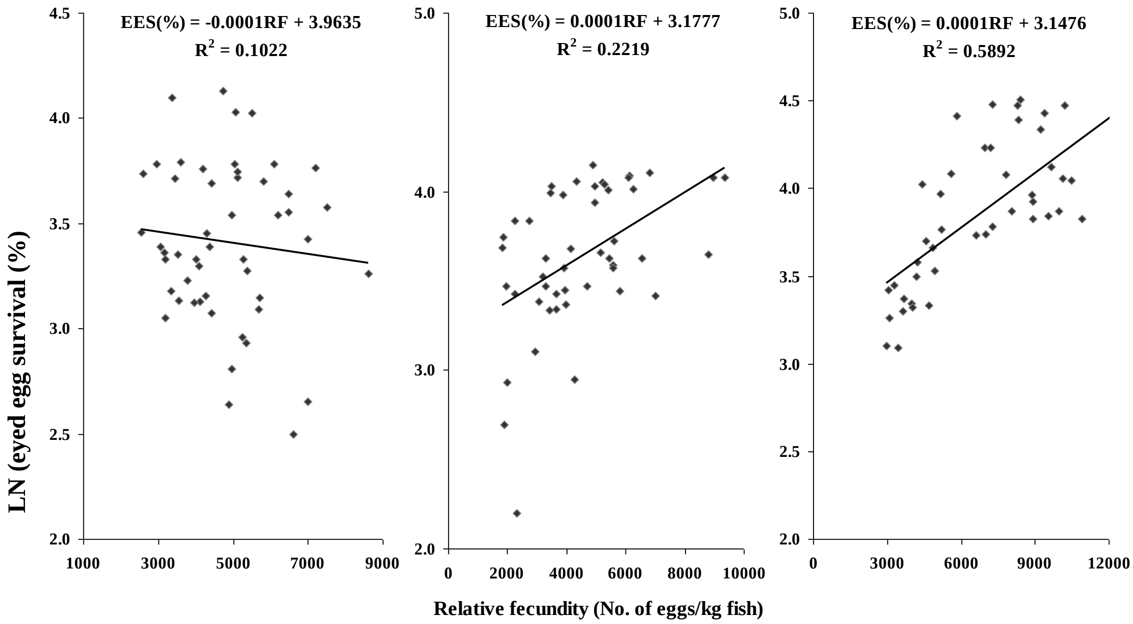

Figure 11. Bivariate linear regression between LN of eyed egg survival (%) against fertilization rates (%) in (a) wild population, (b) hatchery reared population and (c) wild × hatchery reared population of rainbow trout in Sagana. Eyed egg survival data was log-transformed based on the equation y = LN(y/(100-y)). Regression lines fitted by ordinary least-squares are shown.

CHAPTER FIVE

SUMMARY OF FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary of findings

During this study, the water variations were observed where water temperature in April was 9.58 ± 0.31ºC, Dissolved Oxygen was recorded at a mean of 10.58 ± 0.39 mg/L while the water pH was 7.35 ± 0.40. The highest temperature recorded was in September at a maximum of 12.70ºC and the lowest was in June at 8.39ºC due to seasonal variations within the study regions. The region experience highland equitorial climate where seasons occur due to titlting angle of the earth either southward (in March) or northwards (September) causing slight variations in temperature over the area (Williams and Pollard, 2003). Variation in the climate likely to cause changes in dissolved oxygen levels within the water in the region. However, water quality variations were not in ranges that affected the growth of trout (MacIntyre *et al.*, 2008; Person-Le Ruyet *et al.*, 2008).

During the stury, significantly higher ($P < 0.05$) body weight, fork length, condition factor was recored in male and female wild × hatchery reared fish. This may suggest that wild × hatchery raised fish grew better. It is widely believed that the goals of breeding or breeding programmes is yo allow selection of the most essential traits which determines the direction and extent of genetic trends in the population under selective breeding (D’Ambrosio *et al.*, 2020). These traits can be developeeed over a period of time when the conditions are ideal for such developments. Therefore, pending further investigations, there is tendency of wild × hatchery reared fish to develop genetic structure thatallows them to have better biometric parameters.

The better growth of wild × hatchery reared fish was confirmed using the LWRs where they were found to exhibit positive allometric growth (b > 3.0) were established and their coefficient of determination r^2 values varied between 0.950 and 0.964. This high coefficient of determination values obtained in the assessment of LWRs indicates a good quality of the prediction of linear regression for the analysed fish. A significant correlation ($P < 0.05$) was observed for all tested samples. The negative allometric growth deduced for wild analysed fish (b < 3, t-test, $P < 0.05$) suggested that trout has a relatively low growth rate and tend to be slim. With b fluctuating between 2.900 and 3.175, positive allometry was detected for the hatchery raised fish.

In determing the reproductive performance of the fish using battery of measures, several observations were made. Fecundity also referred to as total or absolute fecundity is concerned with total number of oocytes possibly laid by an individual brood fish during its breeding period (Ganias and Lowerre‐Barbieri, 2018). Meanwhile relative fecundity is the number of mature oocytes in a female divided by the total weight of that female (Armstrong and Witthames, 2012). The egg productivity of rainbow trout in hatcheries has so far received far little attention in commercial breeding programmes because its high relative fecundity (1500 to 2000 eggs/kg of body weight) (D’Ambrosio *et al.*, 2020). In this study, total and relative fecundity significantly varied due to differences in female body weight and egg weight. This trend indicates that the population analysed fits well to the general reproductive trade-off pattern between egg number and fish size described for the species. The total fecundity and relative fecundity fall within the range of, or were close to, data reported from other tropical riverine environments (Bromage *et al.*, 1992; Bazaz *et al.*, 2022; Nevoux *et al.*, 2022b). However, regression equations output suggests that the reproductive performance of the River Sagana population has a more reduced fecundity in comparison either with naturalized populations or with cultured stocks of trout species.

In this study, total and relative fecundity was largely affected by female body weight in the naturalized population, but not in the wild fish population. Total fecundity increased with increasing body size of female broodstock is a common phenomenon in Nile tilapia, *Oreochromis niloticus* (Duponchelle and Legendre, 2000; Shoko *et al.*, 2015; Wagaw *et al.*, 2022), and has been reported in other species such as river catfish (*Clupisoma garua*), Gagora catfish (Arifur *et al.*, 2020), Manyas shemaya, *Alburnus carinatus* (Gülşah and Gaygusuz, 2020), and grey mullet *Liza parsia* (Rheman *et al.*, 2002), *Puntius ticto* (Ticto, 2012) among others. This has also been reported in species of trouts (Alp *et al.*, 2003) including rainbow trout (Chandra *et al.*, 2018; Cakmak *et al.*, 2019; Yousuf and Razak, 2022). Nevertheless, negative relative fecundity has also been reported with respect to body mass for the wild anadromous brown trout *Salmo trutta* (Rinaldo, 2020).

The fundamental fecundity-weight relationship for could not explain the link between fecundity (total and relative) and body weight in wild trout population in the current study. Presence of varying selective pressures in the wild (Hutchings and Ferguson, 1992; Knudsen *et al.*, 2003) may be one of the factors responsible for the distortion of fecundity-weight relationships which, suggest presence of negative environmental factors that may distort the reproductive performance of wild population of rainbow trout. We observed massive human activities along the river ecotones and therefore their impacts on reproductive biology need further confirmation.

The total fecundity was also affected by egg weight in the naturalized population which agrees with studies in other fish species such as the pool barb, *Puntius sophore* (Kant *et al.*, 2016), zig-zag eels, and *Mastacembelus armatus* (Rashid and Dobriyal, 2020). In many studies, the positive correlation between total fecundity and egg weight seems to be the rule, rather than the exception. A puzzling observation however was that the number of eggs spawned was negatively correlated with egg weight in wild fish populations. Then, the question is; why should increased fecundity result in reduced egg weight in wild fish populations? Firstly, it is speculated that if wild trout undergo ontogenetic habitat shifts in spawning habitat, then egg size may be affected or even decreased according to abiotic conditions (Winemiller and Rose, 1992; Armstrong and Nislow, 2006). In addition, studies have reported tradeoffs in some populations between fecundity and egg weight; where fish in some populations produced fewer but heavier eggs (Jonsson and Jonsson, 1999) and in others, females produced more eggs but of a smaller size (Lobon-Cervia *et al.*, 1997; Nicola and Almodóvar, 2002; Jensen *et al.*, 2019); Klemetsen et al. 2003; Olsen and Vøllestad 2003). The tradeoffs occur due to trout growth as a function of environmental conditions (Braun *et al.*, 2013). Secondly, the reproductive strategy could shift from one strategy for small individuals to another strategy for large individuals (Nevoux *et al.*, 2019), which support the idea that small individuals fish populations have low fecundity compared to their large conspecifics and will strive to gain higher fitness on numerous small eggs and favourable environmental conditions. In this study the hatchery-reared and naturalize hatchery × wild population appeared to invest on more eggs wiled the wild population appeared to invest their energy on larger egg size rather than numbers which has higher chances of survival. This may be a phenotype-response to the environment caused by resource availability and homogeneity (Rinaldo, 2020), which may be supported by the idea that egg size in trouts is partly plastic and may develop in response to maternal growth or spawning habitat (Nevoux *et al.*, 2019).

This study revealed that the significant positive effect of fertilization rate and relative fecundity. The positive effect of fertilization rate on embryo survival is consistent with data available for cultured stocks of rainbow trout available at national trout farm (National trout hatchery annual report, 2020). However, the positive effect of relative fecundity on egg survival is an unexpected result, given that insignificant correlations between these variables have been found at least in cultured stocks of this species. This result suggests that rainbow trout females of River Sagana that yield large egg numbers have an increasing trend towards better egg viability. This reproduction pattern could be related to a particular reproduction strategy of the rainbow trout population in the River Sagana, to improve its viability in the habitat that is a characteristic of the riverine environment.

Egg diameter showed a positive relationship with egg weight, and also with body length and body weight, egg weight was measure using gravimetric method. Regression equation predicts that females with a body length of 40 cm and 50 cm, will present an egg size of 4.97 mm and 5.24 mm, respectively. The positive correlation between egg size and body length coincides with previous reports of other salmonids, such as brown trout (Estay *et al.*, 1994; Weber *et al.*, 2023a) where egg size typically increases with female size, in a strong and consistent relationship. In naturalized rainbow trout from the Great Lakes in North America a positive association between both female length and egg size has also been recorded (Johnston *et al.*, 2016). Therefore, this result is in accordance with observations reported for different salmonid species in which larger females usually produce larger eggs. Although egg size has been related to embryo survival, no consistent relationship has been found. In this case, the reproductive parameter was mostly affected by the combination of fertilization rate and relative fecundity, which ranked highest in the model. This result is consistent with available data indicating that more than egg size, egg content, such as lipid and essential fatty acid content, seems to play an important role in embryo survival, both showing an ontogenetic trend (Jonsson and Jonsson, 1999; Johnston *et al.*, 2016; Kant *et al.*, 2016; Källo *et al.*, 2022; Judycka *et al.*, 2023).

The survival of the eyed eggs was positively affected by female body weight and egg weight in the hatchery raised and wild × hatchery naturalized rainbow trout since the eggs size in the hatchery was somewhat larger than egg size in the wild and naturally larger egg sizes can survive much longer (Leblanc *et al.*, 2023). The survival of the eyed eggs at higher body weight of the broodstock may be attributed to the maternal fitness which enable them to spawn healthier and larger eggs that survive much longer. In the wild survival of the eyed stage may be limited by their interactions with environmental cues which lower reproductive fitness (Alix *et al.*, 2020). Therefore egg survival appears to be controlled by female body weight and egg size. Models for egg size reveal that this variable is affected by egg weight and also by body length or body weight, all with positive correlations. This result concurs with observations reported for other naturalized populations of rainbow trout, and with other salmonids, further supporting evidence that larger females of this species usually produce larger eggs than smaller females.

Most studies of interactions between captive-bred and wild fish have focused on hatchery populations stocked into areas containing native populations of the same species, particularly for the commonly artificially propagated species of the family Salmonidae (Bruce *et al.*, 2020; O'Sullivan *et al.*, 2020; Prunier *et al.*, 2022). The study found out that biometric traits vary as a function of sex and that there are various types of relationship trends that may explain the variation of reproductive traits across experimental units.

Fish length or weight is positively correlated with egg size and total fecundity, but relative fecundity showed an opposite trend. Results from this study support these expectations in all experimental units analyzed. The highest-ranking models for total fecundity included the predictor variable female body weight, with a positive effect, combined with egg weight with a negative effect. In the case of relative fecundity, top-ranking models included negative significant relationships with female body length along with egg weight. Thus, results in this study indicates that, in the River Sagana population of rainbow trout, there is a positive correlation between female body weight and total fecundity and an inverse association with relative fecundity as shown elsewhere (Dürranİ, 2023).

Total fecundity increasing with body weight in naturalized females of rainbow trout is not an unexpected result, since this trend has been observed in several naturalized populations of the Northern Hemisphere (Estay *et al.*, 2021; Dedual, 2023). This trend is also in accordance with data reported in cultured strains of rainbow trout (Dedual, 2023). This study indicates that under natural conditions, the introduced populations of rainbow trout from the River Sagana follow a general reproductive pattern. However, when using the regression equation obtained in this study analysis to calculate expected total and relative fecundity at specific body length and weight, including the egg weight. River Sagana population presents lower values for these reproductive parameters than other naturalized populations from the Northern Hemisphere. For example, females measuring 63.9 and 59.1 cm body length and with a mean egg weight of 63 mg, were reported relative fecundity values of 2195 and 2414, respectively (DuBois and Dubielzig, 2004). In contrast, this analysis indicates only 1680.0 and 1834.7 eggs/kg female, respectively, using the regression equation for relative fecundity. The same pattern is observed when the expected total fecundity as a function of female body weight is compared to the reproductive performance of cultured stocks. For example, it has been observed that a mean number of 3483 and 5530 eggs/female for individuals, respectively, with a bodyweight of 1720 g (mean egg weight = 64.4 mg) and 2784 g (Kanyılmaz *et al.*, 2016). In this case, however, the regression equation indicates a reduction in the total fecundity, since numbers of 3382.9 and 5073.3 eggs/female are expected, respectively, based on the regression equation for total fecundity. This result suggests reduced fecundity in the naturalized rainbow trout of River Sagana in comparison with naturalized populations from the northern hemisphere or with cultured stocks of this species.

Based on early egg survival analysis of the River Sagana population, the author was able to estimate the level of embryo viability in a self-sustaining population of an introduced species. This data, collected under controlled conditions in a hatchery facility, has not been obtained for other naturalized rainbow trout populations in River Sagana. In the Northern Hemisphere, this approach has been applied to analyse survival at the hatching stage in pure and hybrid trout crosses of naturalized strains (Poulos, 2019).

Other reproductive parameters such as fertilization rate, egg diameter, egg weight, and eyed egg survival showed significant differences among fish in the three experimental groups, with wild traits having inferior traits while hatchery-raised and cross strain between wild and hatchery-raised fish showing somewhat similarity in their traits. Fingerlings from wild-hatchery fish had superior trait than those from wild-wild fish. These comparisons support the proposal that the River Sagana population has a higher survival performance at the early development than cultured stocks. Models relating eyed egg survival to reproductive traits indicate the effect of fertilization rate and relative fecundity, both with a positive effect on the response variable. Also, of interest was the significant positive effect of relative fecundity on egg survival. The result suggests that rainbow trout females in River Sagana yielding large egg numbers will have increasing egg viability.

The total fecundity parameter fell within the range reported for naturalized rainbow trout populations in other regions of the world (TF = 2170–3195) (Blanchfield et al., 2009). The study established that the total and relative fecundity of the broodstock was better for the fish where a cross was done between hatchery and wild rainbow trout. Hatchery-raised rainbow trout typically have a morphology that is quite distinct from their wild counterparts (Krueger and Tian, 2004; Kristensen *et al.*, 2019; Kurta *et al.*, 2023). However, some phenotypic traits associated with hatchery-raised genotypes and a captive-rearing environment can be lost following release into the wild (Miller *et al.*, 2004). It is likely that crossed fish between hatchery and wild fish acquiring superior reproductive traits is higher through consistent breeding programmes in the hatchery.

5.2 CONCLUSIONS

This study provides new information on how biometric and reproductive traits of rainbow trout are linked with fecundity and early life-history characteristics. Wild-hatchery spawned fingerlings shown more superior traits than hatchery-hatchery and wild-wild spawned fingerlings. Wild brooders have the lower fecundity, lower fish weight and lower egg weight compared to hatchery brooders, this is due to the low availability of food and the variations of conditions such as temperature and pH in the wild compared to hatchery.

This study provides new information on survival rate of fingerlings from brooders of different environmental origins and how seasonal changes have effect on breeding of trout.

5.3 RECOMMENDATIONS

5.3.1 The sustainability of trout fisheries

The study recommends the use of hatchery and wild crossed fish to support fry production for use in subsequent restocking of all trout streams in the country.

5.3.2 Suggestions for further research

This study found some gaps in areas such as estimating the survival of trout in the wild environment and whether fecundity variations are associated with egg weight.

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APPENDICES

Appendices: a) Attending to the ova, whereby the dead ones are removed to maintain the high water quality and to prevent infection to the live ova by microbes. **(b)** Eyed ova whereby the ova show to have two dark dots which are the eyes. How quickly the eggs hatch depends on the water temperature.

Appendices 2 : Research Permit

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NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

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