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Characterization of Circular RNAs in Nile Tilapia Muscle and Thermal Modulation of their Expression in Relation to Growth

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Md. Golam Rbbani

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Preface

This dissertation is submitted to fulfil the requirements for the degree of Philosophiae Doctor (PhD) at the Faculty of Biosciences and Aquaculture (FBA), Nord University, Bodø, Norway. The research articles and manuscript included in this dissertation present original research conducted over a period of 4 years. This PhD research was carried out as part of the EPIFISH project, which received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement nos. 683210), the Research Council of Norway under the Toppforsk programme (grant agreement no. 250548), and Nord University (Norway).

The PhD project team consisted of the following members:

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Jorge Fernandes, Professor, FBA, Nord University: primary supervisor

Artem Nedoluzhko, Professor, European University at St. Petersburg: co-supervisor

Joost Andre M Raeymaekers, Professor, FBA, Nord University: co-supervisor

Jorge Galindo-Villegas, Researcher, FBA, Nord University: co-supervisor

Md. Golam Rbbani,

Bodø, August 2023

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I was interviewed for this PhD position on the second day after my Master's thesis defense. I felt incredibly excited when I received an unofficial confirmation from Prof. Jorge Fernandes. I immediately informed my then supervisor, Prof. Xiwu Yan, and packed my bags to embark on this journey. Since then, it has been a roller coaster ride of learning and personal growth as a researcher and individual. This journey has been anything but smooth, with numerous obstacles and emotional ups and downs, but I have received incredible support from my team and friends. I would like to take this opportunity to express my heartfelt gratitude to them.

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I would also like to express my gratitude to Nord University and the funding agencies that funded this research. Your support made it possible for me to carry out this study and contribute to the field of circRNA research in teleost muscle growth.

Last but not least, I am thankful to my parents, brother and sister for their unwavering encouragement and belief in my abilities. Your love and support have been my motivation and strength throughout this journey.

Although this PhD journey has had its challenges, I am proud of the work accomplished and the knowledge gained. I look forward to continuing my research career and contributing to the scientific community in the future.

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List of abbreviations

5hmC	5-hydroxymethylcytosine
5mC	5-methylcytosine
AKT	Protein kinase B
asb15	Ankyrin repeat and SOCS box containing 15
AS-circRNA	Antisense circRNA
BSJ	Backsplicing junction
capn9	Calpain 9
cdnk3	Cyclin dependent kinase inhibitor 3
ceRNAs	Competing endogenous RNAs
CircRNAs	Circular RNAs
ciRNA	Intronic circRNA
creb5	cAMP responsive element binding protein 5
DEcircRNAs	Differentially expressed circRNAs
dnmt1	DNA methyltransferase 1
ecircRNA	Exonic circRNA
EIcircRNA	Exon-intron circRNA
eif3j	Eukaryotic Translation Initiation Factor 3 Subunit J
ERK	Extracellular signal-regulated kinase
FAO	Food and agriculture organization
FaST	Freshwater Aquaculture Center Selected Tilapia
fgf13	Fibroblast growth factor 13
fgf14	Fibroblast growth factor 14
fgf164	Fibroblast growth factor 164
FGFRs	Fibroblast growth factor receptors
GHR	Growth hormone receptor
GIFT	Genetically Improved Farmed Tilapia
GST	GenoMar Supreme Tilapia

hdac4	Histone deacetylase 4
IGF	Insulin-like growth factor
igfbp2	Insulin-like growth factor-binding protein-2
IGFBPs	Insulin-like growth factor binding proteins
IGFPB1a	Insulin-like growth factor binding protein 1a
il-4	Interleukin 4
IPA	Integrated prediction algorithms
IRESs	Internal ribosome entry sites
JAK	Janus kinase
lncRNAs	Long non-coding RNAs
m^6A	N6 methyladenosine
map2k4	Mitogen-activated protein kinase kinase 4
MAPK	Mitogen-activated protein kinase
mapk15	Mitogen-Activated Protein Kinase 15
mapk3k2	Mitogen-activated protein kinase kinase kinase 2
mef2c	Myocyte-specific enhancer factor 2C
miRNAs	microRNAs
ML	Machine learning-based method
mrf4	Myogenic factor 6
MRFs	Myogenic regulatory factors
mRNA	messenger RNA
mstn	Myostatin
myf5	Myogenic factor 5
myog	Myogenin
myhc	Myosin heavy chain
MyoD	Myoblast determination protein 1
myod1	Myogenic differentiation 1
NCBI	National Center for Biotechnology Information
ncRNAs	Non-coding RNAs

nfatc2	Nuclear factor of activated T cells 2
nt	Nucleotides
ORF	Open reading frame
PAIP2	Poly (A) binding protein interacting protein 2
PCR	Polymerase chain reaction
PGF2	Prostaglandin F2-alpha
PI3K	Phosphoinositide 3-kinase
piRNAs	Piwi-interacting RNAs
Pol II	RNA polymerase II
QTL	Quantitative trait locus
RAS	Recirculatory aquaculture system
RBPs	RNA binding proteins
RISC	RNA-induced silencing complex
RNase R	Ribonuclease R
RNA-Seq	RNA sequencing
rpl7	Ribosomal protein L7
rRNA	Ribosomal RNA
RT-qPCR	Reverse transcription quantitative PCR
siRNAs	Short interfering RNAs
snoRNAs	Small nucleolar RNAs
SNPs	Single-nucleotide polymorphisms
sox6	SRY-box transcription factor 6
Sry	Sex-determining region Y
STAT	Signal transducer and activator of transcription
tet1	Ten-eleven translocation 1
tgfbi	Transforming growth factor, beta-induced
TGFBR2	Transforming growth factor beta receptor II
TGF-β	Transforming growth factor beta
tnfsf12	TNF superfamily member 12

TOR	Mammalian target of rapamycin
tricircRNA	tRNA intronic circRNA
UTRs	Untranslated regions
YTHDF3	YTH N6-Methyladenosine RNA Binding Protein F3

Abstract

Circular RNA (circRNA) molecules, characterized by their closed-loop structure, have emerged as intriguing byproducts of pre-mRNA processing, orchestrated by the spliceosome machinery. They are versatile and have a multifaceted nature by being intricately intertwined with fundamental biological functions, including growth, development, and immune responses. Notably, circRNAs showcase tissue-specific expression patterns, underscoring their regulatory role in diverse physiological processes. Considering the dynamic environmental fluctuations experienced by teleost fish throughout their lives, the combined impact of circRNAs and environmental factors on growth is plausible. The hypothesis underlying this thesis is that circRNA expression in fast muscle modulates muscle growth and is influenced by developmental temperature.

We have identified a vast number of circRNAs in fast muscle tissue of Nile tilapia. Their presence must be validated, since linear transcripts can produce false-positive circRNA signals in RNA sequencing data. To address this challenge, we developed CircPrime, a user-friendly web platform simplifying primer design for circRNA validation via routine PCR methods. CircPrime is notably versatile and can accommodate diverse organisms, proving invaluable for researchers studying non-model species. Additionally, CircPrime identifies optimized thermocycling conditions, aiding in precise experiment planning and efficient validation of backsplicing events.

Expanding our investigation, we delved into the possible regulatory role of circRNAs in teleost muscle growth. Through an integrative omics approach, we successfully identified 1947 differentially expressed mRNAs, 9 miRNAs, and 4 circRNAs between fast- and slow-growing fish. Among these, several muscle-associated genes, including *mapk15, igfbp2, creb5, tnfsf12, fgf16, tgfbi, capn9, myf5, fgf13, fgf14, myod1, map2k4, map3k2, cdkn3, and asb15*, were identified as contributors to substantial shifts in muscle development. Particularly intriguing, the miRNAs that we discovered have the

potential to regulate myogenic genes and harbored binding sites for novel circRNAs—namely, circMef2c and circLaminA/C. Our findings suggest that intricate RNA interaction networks, including circMef2c-miR-202-fgf14, circMef2c-miR-202-tet3, circLaminA/C-miR-34-igfbp2, and circLaminA/C-miR-10819-map3k5-igfbp2, participate in the orchestration of growth-related processes.

Furthermore, we assessed circRNA expression in the muscle tissue of Nile tilapia exposed to different temperatures during embryonic development. Interestingly, a higher abundance of circRNAs was observed in the control group (28 °C) compared to both lower (24 °C) and higher (32 °C) temperature groups. This observation suggests that deviations in thermal conditions from an optimum value could potentially impede the complex process of circRNA biogenesis within Nile tilapia muscle tissue. Notably, circRNAs such as circNexn, circTTN, and CirTTN_b displayed potential binding sites with multiple miRNAs, including miR-1, miR-7, miR-27, miR-181, miR-140, miR-144b, miR-206, miR-214 and miR-221, which are involved in muscle hypertrophy. This phenomenon reflects the adaptive response of fish to varying temperature conditions, optimizing physiological and developmental processes to ensure survival and growth.

In summary, this thesis significantly advances our understanding of complex muscle growth by revealing the circRNA-miRNA-mRNA network. More importantly, it breaks new ground by elucidating the intricate landscape of circRNAs in teleost muscle growth and their remarkable plasticity to temperature fluctuations during embryonic development, which is highly relevant in the context of climate change. In addition, these findings bear great potential for application in aquaculture practices. The identification of novel growth-related circRNAs lays the groundwork for strategies aimed at optimizing growth and resilience in farmed fish populations, namely the use of circRNAs as biomarkers in selective breeding programmes. It also directly addresses challenges stemming from shifting environmental conditions, thus contributing significantly to the sustainability and productivity of aquaculture systems.

Sammendrag på Norsk

Circular RNA (circRNA)-molekyler, kjennetegnet av sin lukkede løkkestruktur, har dukket opp som fascinerende biprodukter av pre-mRNA-prosessering, orkestrert av spliceosommaskineriet. De er allsidige og har en mangefasettert karakter ved å være intrikat sammenflettet med grunnleggende biologiske funksjoner, inkludert vekst, utvikling og immunrespons. Særlig viser circRNAer vevsspesifikke uttrykksmønstre, noe som understreker deres regulatoriske rolle i ulike fysiologiske prosesser. Med tanke på de dynamiske miljøfluktuasjonene som teleostfisk opplever gjennom livet, er den kombinerte påvirkningen av circRNAer og miljøfaktorer på vekst plausibel. Hypotesen som ligger til grunn for denne avhandlingen, er at circRNA-uttrykk i hurtigmuskulatur modulerer muskelvekst og påvirkes av utviklingstemperatur.

Vi har identifisert et stort antall circRNAer i hurtigmuskulaturen til Nile-tilapia. Deres tilstedeværelse må bekreftes, siden lineære transkripter kan produsere falske positive circRNA-signaler i RNA-sekvensdata. For å håndtere denne utfordringen utviklet vi CircPrime, en brukervennlig webplattform som forenkler primerdesign for circRNA-validering via rutinemessige PCR-metoder. CircPrime er bemerkelsesverdig allsidig og kan tilpasses ulike organismer, og den er uvurderlig for forskere som studerer ikke-modellarter. I tillegg identifiserer CircPrime optimaliserte termocyklingsbetingelser, noe som hjelper med presis eksperimentplanlegging og effektiv validering av baksplicing-hendelser.

Videre dykket vi ned i den mulige regulatoriske rollen til circRNAer i teleost muskelvekst. Gjennom en integrert omisk tilnærming identifiserte vi vellykket 1947 differentielt uttrykte mRNA-er, 9 miRNA-er og 4 circRNAer mellom raskt- og saktevoksende fisk. Blant disse ble flere muskelrelaterte gener, inkludert *mapk15, igfbp2, creb5, tnfsf12, fgf16, tgfbi, capn9, myf5, fgf13, fgf14, myod1, map2k4, map3k2, cdkn3* og *asb15*, identifisert som bidragsytere til betydelige endringer i muskelutviklingen. Spesielt interessant var miRNAene vi oppdaget, som har potensial

til å regulere myogene gener og har bindingssider for nye circRNAer, nemlig circMef2c og circLaminA/C. Våre funn antyder at intrikate RNA-interaksjonsnettverk, inkludert circMef2c-miR-202-fgf14, circMef2c-miR-202-tet3, circLaminA/C-miR-34-igfbp2 og circLaminA/C-miR-10819-map3k5-igfbp2, deltar i koordineringen av vekstrelaterte prosesser.

Videre vurderte vi circRNA-uttrykk i muskelvevet til Nile-tilapia utsatt for ulike temperaturer under embryonal utvikling. Interessant nok ble en høyere mengde circRNAer observert i kontrollgruppen (28 °C) sammenlignet med både lavere (24 °C) og høyere (32 °C) temperaturgrupper. Denne observasjonen antyder at avvik i termiske forhold fra en optimal verdi potensielt kan hindre den komplekse prosessen med circRNA-biogenese innenfor muskelvevet til Nile-tilapia. Bemerkelsesverdig har circRNAer som circNexn, circTTN og circTTN_b vist potensielle bindingssider med flere miRNAer, inkludert miR-1, miR-7, miR-27, miR-181, miR-140, miR-144b, miR-206, miR-214 og miR-221, som er involvert i muskelhypertrofi. Dette fenomenet reflekterer tilpasningsdyktig respons på varierende temperaturforhold hos fisk, noe som optimaliserer fysiologiske og utviklingsmessige prosesser for å sikre overlevelse og vekst.

Oppsummert bidrar denne avhandlingen betydelig til vår forståelse av kompleks muskelvekst ved å avdekke circRNA-miRNA-mRNA-nettverket. Enda viktigere, den baner vei for å avdekke det intrikate landskapet av circRNAer i teleost muskelvekst og deres bemerkelsesverdige plastisitet overfor temperatursvingninger under embryonal utvikling, noe som er svært relevant i forbindelse med klimaendringer. I tillegg har disse funnene stort potensial for anvendelse i akvakulturpraksis. Identifiseringen av nye vekstrelaterte circRNAer legger grunnlaget for strategier som tar sikte på å optimalisere vekst og motstandskraft i oppdrettsfiskpopulasjoner, spesielt bruken av circRNAer som biomarkører i selektive avlsprogrammer. Det adresserer også direkte utfordringer som følger av skiftende miljøforhold, og bidrar dermed betydelig til bærekraft og produktivitet av akvakultursystemer.

1 Introduction

1.1 Aquaculture and its current status

Aquaculture refers to the farming of aquatic organisms such as fish, mollusks, crustaceans and aquatic plants (FAO). Approximately 10,000 years after land-based agriculture and animal farming began, the first attempts at fish farming for human consumption started 8,000 years ago in China with the controlled aquaculture of common carp (*Cyprinus carpio*) (2019, Wang and Lu, 2016). The discovery of Nile tilapia (*Oreochromis niloticus*) paintings on Theban tombs (Figure 1) suggests some level of control over fish reproduction (Teletchea, 2021). A few centuries later, the farming of the silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Hypophthalmichthys nobilis*) and grass carp (*Ctenopharyngodon idella*) started in China (Balon, 2004). Approximately 2000 years ago, during the Roman Empire, wild stocks of common carp were abundant in the Danube River, and their cultivation reached its peak in Europe (Balon, 2004). Furthermore, aquaculture development in North America, Australia and a number of Pacific island states represent more recent events, dating back to the 19th century. Slow progress in the development of aquaculture can be attributed to the unique characteristics of the aquatic environment. The lack of proper understanding of aquatic ecosystems hindered our ability to comprehend the behavior and biology of many fishes for a long time. Over the past few decades, researchers have made significant progress in understanding aquatic ecosystems, species biology, and species behavior, which has truly improved the aquaculture industry.

Currently, aquaculture is the fastest-growing farm food sector, producing more than half of all fish destined for human consumption worldwide (FAO, 2022, FAO, 2019). The total number of cultured freshwater and marine species is approximately 425 (FAO, 2022). Based on the time series data of major aquaculture species, common carp, roho labeo (*Labeo rohita*), Nile tilapia, Atlantic salmon (*Salmo salar*), giant tiger prawn (*Penaeus monodon*) and Eurasian perch (*Perca fluviatilis*) dominate production. Global

fisheries and aquaculture production reached 214 million tonnes in 2020 (FAO, 2022). According to the FAO (2022), 89% (157 million tonnes) of aquaculture product (excluding algae) are used for direct human consumption, compared to 67% in the 1960s. Consequently, the aquaculture production of farmed aquatic animals has increased by an average of 4.6% per year from 2010 to 2018. China is the largest fish producer, accounting for 35% of global production in 2020. Egypt, Chile, India, Indonesia, Vietnam, Bangladesh and Norway, among other key producing countries, have consolidated their regional or global production shares to varying degrees over the last two decades.

Figure 1. Central Garden Pool in the Garden of Nebamun's Tomb Painting, late 18th dynasty, circa 1350 BCE (British Museum). The pool contains Nile tilapia, other fish and swimming fowl (photo in the public domain).

It is estimated that over 90% of global aquaculture occurs in low- and middle-income countries, contributing to sustainable development directly through human consumption or indirectly through economic growth (Houston et al., 2020). Coastal and marine aquaculture is vital to the livelihoods, employment and local economic growth of coastal people in many developing countries. In 2020, marine and coastal aquaculture yielded 33.1 million tonnes of aquatic animals worth approximately USD 84.1 billion (FAO, 2022). Anchovies (*Engraulis sp)* and Alaska pollock (*Gadus*

chalcogrammus) were the most abundant catches, with more than 10.5 million tonnes being captured annually. Despite technological advancements in marine fish farming, mollusks continue to outnumber finfish and crustaceans in marine and coastal aquaculture. Aquaculture techniques vary widely in terms of methods, practices, equipment and integration with other agricultural activities. Earthen ponds remain the most commonly used resource for inland aquaculture production. Raceway tanks, aboveground tanks, pens and cages are also regularly used in inland aquaculture systems. Although rice-fish culture remains important in many places, integrated inland aquaculture systems have made significant advancements in recent years. Whilst aquaculture practices vary widely between geographical regions, developing countries such as China, India, Indonesia and Bangladesh dominate inland aquaculture production. In 2020, inland aquaculture produced 54.4 million tonnes of aquatic animals, accounting for more than 62.2% of global farmed food fish production (Figure 2). In many countries, producers are given greater control over the practices and species to be produced (Naylor et al., 2021). As a result, deeper lakes and reservoirs are used for cage-based production, and many shallow lakes are converted for aquaculture either by segregating, using dykes, or creating pens with stakes and netting (Newton et al., 2021). Inland aquaculture is widely recognized for the production of grass carp, tilapia, shrimp, common carp, silver carp, bighead carp and black carp throughout the world. Focusing on 'the four domesticated fish' (i.e., black carp, grass carp, silver carp and bighead carp), pond and pen systems relied on complex polycultures with up to nine species filling different niches within a synergistic system (Wang et al., 2015). Also, these fish were frequently integrated with other livestock (e.g., pigs and ducks). Freshwater species represent approximately 90% of the ornamental fish in 125 countries, with their trade being worth an estimated global export value of USD 15–30 billion annually (EversPinnegar and Taylor, 2019). The overall expansion of inland/freshwater aquaculture in different parts of the world is motivated by the decline in wild inland fisheries that once supported urban demands for livelihood and food security.

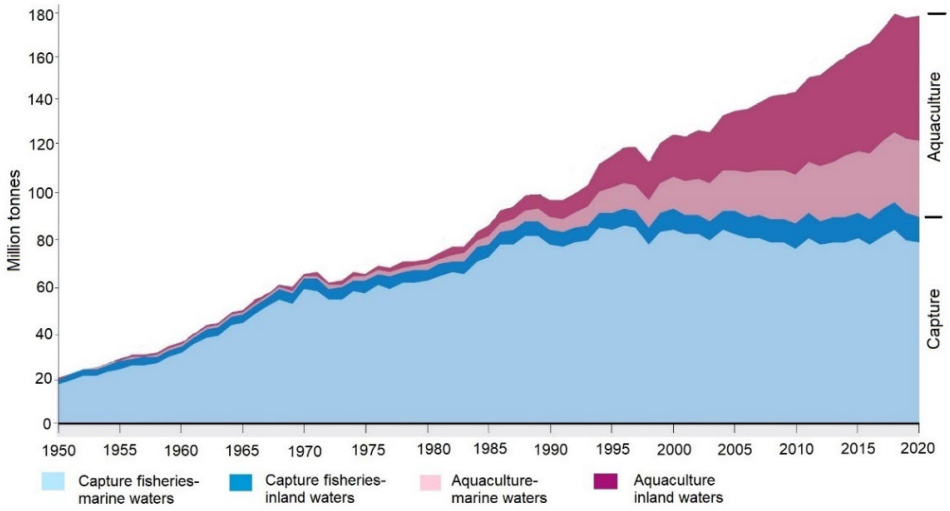

Figure 2. Fisheries and aquaculture production from 1950 to 2020 (FAO, 2022).

1.2 Nile tilapia biology and its relevance in aquaculture

Tilapia is a generic name for the cichlid group that belongs to the tilapiine cichlid tribe, which comprises hundreds of species, including the economically valuable genera *Oreochromis*, *Tilapia* and *Sarotherodon*. Nile tilapia, one of the earliest fish species cultured over 4,000 years ago, is a freshwater fish that inhabits ponds, rivers, lakes and shallow streams, with fewer inhabiting brackish water. Nile tilapia is indigenous to 22 countries, and its natural habitats primarily include the Nile Basin, several river basins in West Africa, various water bodies in the East African Rift Valley, Lake Tana in Ethiopia and the Yarkon Basin in Israel (Trewavas, 1983). Nile tilapia have a laterally compressed body covered with cycloid scales and an interrupted lateral line (Figure 3). The mouth protrudes and is usually bordered with wide, often swollen lips. The jawline contains three to seven series of teeth, making it an efficient feeder capable of capturing and processing a wide variety of food items. Nile tilapia are omnivorous, primarily feeding on detritus, blue-green or green algae, diatoms, macrophytes and bacteria (El-Naggar et al., 2019). Additionally, they feed on small arthropods and may even prey on their

fry in the absence of adequate food. In a suitable culture environment, Nile tilapia can grow up to 60 cm in length and exceed 5 kg in weight. Typically, males are larger and grow faster than females. Although their dorsal, pelvic and anal fins are very spiny, their caudal fin is truncated and contains a pinkish-red posterior margin. Nile tilapia have three color variants: wild type, red and blonde, but the body color also depends on environmental, physiological and dietary factors. During the breeding season, mature female tilapia usually develop a pink coloration in their throats and fins. The male genital papilla has one opening (the urinary pore of the ureter) through which both milt and urine flow. Females have a separate oviduct for the passage of eggs and a urinary pore for urination. In the wild, *O. niloticus* attains sexual maturity at 3–6 months, reaching a total length of 20–30 cm (weight: 150–250 g) (Lowe-McConnell 1958; Gwahaba 1973). However, differences in age at maturity are influenced by the abundance and seasonal variation of food, temperature, photoperiod and many other environmental factors. Nile tilapia in their natural habitat experience a broad spectrum of temperatures, including pronounced seasonal fluctuations from summer periods (28–34°C) to winter seasons (22–26°C). They can also inhabit extreme environments like high-altitude lakes with consistently lower temperatures (17–24°C) or hydrothermal hot springs where temperatures exceed 40°C. The upper and lower lethal temperature for Nile tilapia is 10 °C and 42 °C, respectively (Bezault et al., 2007, Nivelle et al., 2019). As mouth brooders, females pick up eggs in their mouths immediately after fertilization and incubate them for up to several days after hatching. The suitable breeding temperature for Nile tilapia is between 20 and 35 °C (Chervinski 1982). Nile tilapia spawn at least twice during the breeding season, producing an average of 1000–1500 offspring per female. All of these attributes—including a wide range of diets, environmental adaptability, and resilience to pollution—make Nile tilapia one of the leading farmed fish worldwide.

Figure 3. An adult female Nile tilapia (Oreochromis niloticus), aged 1.5 years, from our recirculating aquaculture system at Nord University. Photo by Artem Nedoluzhko (June 2022). The scale bar indicates 2 cm.

The earliest evidence of Nile tilapia cultivation was found in irrigation ponds painted on Theban tomb bas-reliefs (Harache, 2002). Illustrations from the tombs indicate that Nile tilapia were cultured more than 3,500 years ago (Figure 1). Currently, Tilapia is farmed in 145 countries, and its production has exceeded 5.4 million tonnes, which accounts for 8% of total finfish production (FAO, 2022). Tilapia aquaculture began in China during the early 1960s and greatly expanded to became the world's largest tilapia producer, accounting for one-third (1.6 million tonnes) of global production in 2020 (FAO, 2022). Indonesia is the second-largest tilapia producer (20.27%), surpassing Egypt (17.43%) in 2013. Other significant producers include the Philippines, Thailand, Vietnam, Bangladesh and Brazil, which also significantly contribute to the global market supply of tilapia.

The global production of Nile tilapia has surpassed 4.5 million tonnes (as depicted in Figure 4), with an estimated value projected at USD 8.3 billion. This value accounts for approximately 3.3% of the total worth of all aquacultured animals, as highlighted by recent studies (Miao and Wang, 2020, El- Sayed and Fitzsimmons, 2023). While Asia is by far the dominant producer, Africa and America have successfully managed to increase global Nile tilapia production shares to 21.8 and 9.3%, respectively. Nile tilapia culture practices are diverse and include intensive monoculture, extensive or semi-

intensive monoculture, and polyculture. Many countries practice intensive or semi-intensive tilapia farming, which is characterized by high density, selectively improved strains in ponds, and intensive monoculture in floating cages, pens, tanks or recirculated systems (Fabrice, 2018, Barbosa et al., 2022, da Silva Cachode Moura and Henry-Silva, 2020). Nile tilapia culture gained popularity in the 1980s due to the successful control of reproduction in captivity, hybridization and monosex farming.

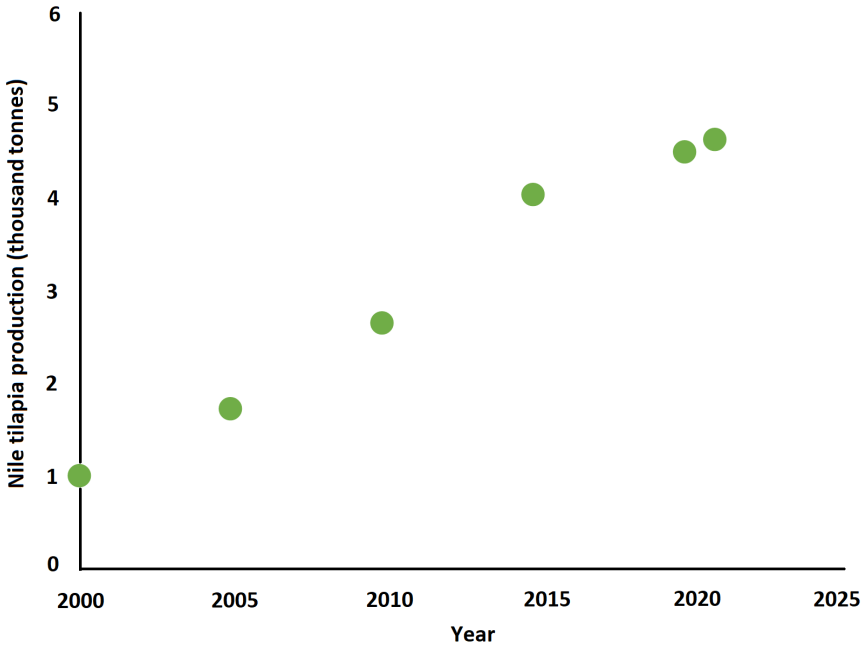

Figure 4. Global Nile tilapia production (both farming and capture fisheries) from 2000 to 2022 (FAO, 2022).

Later in the 1990s to 2000s, male tilapia populations were produced using the androgen 17-α-methyltestosterone to increase productivity in tilapia growth per unit of time (Kumar and Engle, 2016). Monosex populations of tilapia are preferable due to the growth differences between sexes, where males grow faster than females. Moreover, sex reversal prevents issues such as undesirable reproduction, overcrowding and the harvesting of unmarketable fish. In some countries, the

prevalence of monosex culture contributed to the early expansion of the tilapia industry. The Genetically Improved Farmed Tilapia (GIFT) project, which started in 1987 to improve tilapia seed quality and diversify its production, has become the most successful tilapia breeding program worldwide. Eight African and Asian tilapia founder populations were used in the GIFT program. Since then, GIFT strains have been introduced to over 11 countries, such as China, Thailand, Malaysia and Indonesia, and their adaptability has led to the rapid growth of the tilapia industry.

Nile tilapia has the potential to become the most important farmed fish species, thereby contributing to future global food security (AnsahFrimpong and Hallerman, 2014, Moses et al., 2021). However, the Nile tilapia industry is far from reaching its full potential in terms of value. Therefore, targeted transformative changes are required in culture systems, together with molecular tools and improved breeding and marketing strategies.

1.3 Growth improvement and selective breeding in aquaculture

Growth traits (body weight and length) are the most desirable traits in the aquaculture industry. Asia dominates the aquaculture industry due to the introduction of improved strains in farms, some of which have a wide range of food adaptability, better feed conversion efficiency, tolerance to salinity and disease, the potential for high stocking density, and rapid growth rate. Fish are excellent models for implementing genetic improvement techniques such as hybridization and crossbreeding, chromosome manipulation, sex control, transgenesis and selective breeding. To date, a number of breeding programs have utilized hybridization between different species and selective strains to improve fish growth rates.

Hybridization is a technique by which genetically differentiated individuals or groups are bred within species strains or between separate species. Hybridization has been widely used to transfer desirable traits (e.g., growth, improved flesh quality, environment tolerance disease resistance) from one group or species to another or

combine valuable traits from two species into a single group. Since the 1980s, the hybridization technique has been implemented in aquatic organisms with specific desirable traits for performance improvement. The introduction of faster-growing and slowly maturing Nile tilapia in the early 1980s led to the development of several interspecific hybrids. Taiwanese red hybrid tilapia (a mutant reddish-orange female Mozambique tilapia crossed with a normal male Nile tilapia) and Israeli red hybrid tilapia (a cross of Egyptian Nile tilapia and wild blue tilapia (*O. aureus*)) are the most prominent strains, with superior growth, greater salinity tolerance and a distinctive red color (Galman and Avtalion, 1983, Rosenstein and Hulata, 1994). Since males grow faster and more uniformly than females, hybrid tilapia have been adopted in breeding programs to produce all-male tilapia. Mtaki et al. (2022) have shown that the crossing of *O. niloticus* (Z) and *O. urolepis urolepis* (H) results in 100% male hybrids with faster growth performance. The success of obtaining male progeny by interspecific hybridization relies on the determinant locus, polygenic component and the temperature during the early growth stage (BaroillerD'Cotta and Saillant, 2009, El-Zaeem and Salam, 2013).

The selective breeding of tilapia populations started in the early 1990s. This has boosted Nile tilapia culture and significantly enhanced production over the last three decades. Even though Africa has abundant tilapia genetic resources, Nile tilapia aquaculture has not grown considerably in Africa. According to various published reports, the GIFT, Genetically Enhanced Tilapia - EXCEL (GET-EXCEL), Freshwater Aquaculture Center Selected Tilapia (FaST), GenoMar Supreme Tilapia (GST), and Hainan Progift are among the most important selective breeding programs implemented in various Asian countries (Ponzoni et al., 2011). The most successful selective breeding program is the GIFT developed by the World Fish Center. The GIFT Project partners (WorldFish Center, formerly known as the International Center for Living Aquatic Resources Management, ICLARM and the Institute of Aquaculture Research, Norway) collected wild Nile tilapia from Egypt, Ghana, Senegal and Kenya and a commercial strain from the Philippines to produce a couple of hundred full-sib

families in each generation (Gjedrem, 2012). Over five generations, the genetic gain per generation from selection for growth performance was between 12 and 17% (Ponzoni et al., 2011). Currently, the selection program of the GIFT strain at the WorldFish Centre is in its 20th generation, and descendants of this strain are being cultured in more than 87 countries in Africa, Asia and Latin America. GST, a commercial strain of the GIFT (started breeding after the 10th generation), is in its 29th generation of a genetic selection program, primarily for growth, fillet yield, resistance to *Streptococcus agalactiae* and *Flavobacterium columnare*, and survival. (YanezJoshi and Yoshida, 2020). Although genetic studies on disease resistance in Nile tilapia are limited, some studies have reported a heritability value for *S. agalactiae* resistance ranging from 0.22 to 0.38 (Suebsong et al., 2019, Sukhavachana et al., 2019).

Various molecular approaches have been employed in research to evaluate genetics as an alternative to traditional methods relying on pedigree and phenotype information. Next-generation sequencing and high-performance genotyping techniques (e.g., genomics technologies) have allowed for a better understanding of the genetic basis and improvement of genetic evaluation methods for important traits that are difficult to measure in tilapia breeding programs. Advancements in the genome sequences of Nile tilapia and four other cichlid species provide the necessary tools to identify genome-wide genetic variations that contribute to phenotypic diversity among/within tilapia lines and to detect the genome's response to artificial selection, diverse environmental factors and diseases. Thousands of single-nucleotide polymorphisms (SNPs) have been identified in Nile tilapia through next-generation DNA sequencing data, along with numerous microsatellites for *O. aureus*, *O. niloticus* and *O. mossambicus* (Liu et al., 2013, McConnell et al., 2000, Van Bers et al., 2012). Gene-associated SNPs, linkage maps and quantitative trait loci (QTL) play a crucial role in understanding important traits (e.g., growth and sex traits). Liu et al. (2014) found a significant QTL for growth and the sex-determining gene on chromosome LG1, which explains more than 65% of the phenotypic variance in a hybrid population of Mozambique and red tilapia. By QTL mapping for growth traits in saline conditions, Lin

et al. (2016) revealed that body weight, body length and body thickness are associated with LG12, LG20 and LG2, accounting for less than 3% of phenotypic variation across all individuals. RNA sequencing (RNA-Seq) technology has also been used to identify gene transcripts that are involved in desirable traits in response to infections and interactions with environmental factors, such as temperature, hypoxia, salinity and alkaline conditions (YanezJoshi and Yoshida, 2020, LiLin and Xia, 2017, Li et al., 2014, Tao et al., 2016, Wessels et al., 2014, Xu et al., 2015). Additionally, methods for gene editing have been developed for Nile tilapia, and the use of RNA-Seq and targeted mutagenesis with CRISPR/Cas9 technology is expected to aid in understanding the genetic basis of growth in this species (Li et al., 2014).

1.4 Muscle growth in teleosts

Muscle growth can be defined as an increase in the size and number of muscle cells or muscle fibers. There are numerous ways to define growth in fish, such as relative growth, instantaneous growth and size-specific growth; however, gathering such information requires the size of the fish at two or more points in time, either directly or indirectly through measurement (Lugert et al., 2016, CraneOgle and Shoup, 2020). Teleosts are unique among vertebrates inasmuch as they have indeterminate growth, with body size and muscle mass increasing until death or senescence. In teleosts, (fast) muscle constitutes 40–70% of the organism's body mass; therefore, muscle mass gain throughout the lifespan accounts for most of the growth. On the other hand, slow muscle constitutes a smaller fraction, generally less than 10%, of the myotomal musculature. Slow muscle is distinguished by its strong capillary network, abundant mitochondria, conspicuous lipid droplets, and substantial glycogen storage. Collectively, the muscle serves essential roles in movement, locomotion, metabolism and homeostasis (Goody and Henry, 2018).

Myogenesis is the common process by which animals form muscle tissue, which involves incorporating complex molecular events such as the specification, proliferation, differentiation, migration and fusion of precursor cells. Several studies

have described three phases of muscle formation in teleost fish (Johnston, 1999, Karkach, 2006, Valente et al., 2013). The first phase of myogenesis starts at the early embryonic stage, during which adaxial and posterior somatic cells give rise to the formation of the primary myotome. During the second phase, myoblast proliferation occurs at the dorsal and ventral apices of the myotomes of yolk-sac larvae. In the third and final phase, myoblasts on the surface of embryonic muscle fibers are activated, and this process continues throughout much of juvenile and adult stages.

Muscle formation is initiated before the development of all somites in the trunk and proceeds in a rostral to caudal direction. Paraxial mesoderm cells adjacent to the notochord express the myogenic regulatory factors (MRFs) *myoD*, *mf5*, *myogenin*, and *MRF4 (myf*6). MRFs have unique spatial and temporal expression patterns with specific and redundant functions (PownallGustafsson and Emerson, 2002). They induce the expression of both slow and fast forms of *myosin heavy chain* (*myhc*) proteins, leading to commitment toward the myogenic cell lineage (Bryson-Richardson et al., 2005). Once committed, cells form a monolayer against the notochord, which is activated by Hedgehog signaling - a critical component that serves a variety of developmental roles. Monolayer cells, also known as adaxial cells, form the precursors of slow muscle fiber (Fernandes et al., 2005, Rescan, 2008, Nguyen-Chi et al., 2012). Shortly after somite segmentation, adaxial cells start expressing the contractile proteins (myosin) of both slow and fast muscle fibers (Valente et al., 2013). Adaxial cells located in the dorsal and ventral positions of somites migrate outward through the somite to a lateral position, thus forming a layer of superficial slow fibers on the external surface of the myotome. The remaining cells from the dorsoventral midline differentiate and give rise to muscle pioneer cells that differentiate into slow-twitch muscle fibers (Keenan and Currie, 2019, Nguyen-Chi et al., 2012). Conversely, differentiating fast precursors are distinct from slow precursors because they originate from the lateral somite and fuse to generate multinucleated fibers expressing fast MyHC. A small subpopulation of these fibers is called medial fast fibers. It is said that the migration of slow muscle fibers through the

somite creates a morphogenetic signal that patterns fast muscle fibers (Henry and Amacher, 2004, Nguyen-Chi et al., 2012).

After the embryonic phase, muscle growth occurs through a combination of two strategies: hyperplasia and hypertrophy (Rowlerson and Veggetti, 2001). Hyperplastic growth involves the generation of new muscle fibers through the differentiation of muscle stem cells, while hypertrophic muscle growth involves the expansion of pre-existing muscle fibers. Teleosts have two types of hyperplasia -stratified and mosaic- that differ primarily in the location of the myotome where they originate (Rescan, 2008). Stratified hyperplasia generates new muscle fibers at the dorsal and ventral extremes of the myotome, at the junction between slow and fast fibers, and laterally to the embryonic slow fiber layer adjacent to the horizontal septum (Rescan, 2008, Steinbacher et al., 2006). Mosaic hyperplasia, which occurs well into adulthood, generates new muscle fibers throughout the entire myotome, giving a characteristic mosaic appearance to a muscle cross-section. Postnatal muscle growth involves the hypertrophy of fibers mainly formed during early embryonic and juvenile stages (KiesslingRuohonen and Bjørnevik, 2006, Rowlerson and Veggetti, 2001). The number of fibers increases until it reaches a genetically predetermined body length, and then the fibers enlarge through hypertrophy until they reach their maximum functional diameter. The maximum diameter of white muscle fibers was reported to be in the range of 100–300 µm (Rowlerson and Veggetti, 2001). Fibers often become larger when myofibrils are packed into them. Moreover, additional nuclei are also added to fibers as the expansion continues. New nuclei are supplied by a population of satellite cells (muscle stem cells) enclosed in the basal lamina of muscle fiber capable of proliferation (JohnstonBower and Macqueen, 2011, Fernandes et al., 2005).

Fish muscle growth is a highly dynamic and complex process that is influenced by several internal and external factors. Understanding the mechanisms underlying fish muscle growth is important for improving aquaculture practices and enhancing the production of high-quality fish products for human consumption.

1.5 Environmental plasticity of muscle growth

Muscle plasticity refers to the ability of the muscle to adapt to environmental changes over a prolonged period. Fish muscle undergoes structural and functional changes in response to environmental variations via a complex interplay of physiological, cellular and molecular properties, including the signaling pathways, protein synthesis, protein degradation and gene expression patterns involved in muscle plasticity (Pittman et al., 2013, JohnstonBower and Macqueen, 2011, Campos et al., 2013). The ability of the muscle to undergo plastic changes is essential for its role in movement, metabolism and homeostasis, and is important for maintaining overall health and fitness throughout the lifespan. Understanding fish muscle plasticity is critical for improving fish growth and quality, developing aquaculture practices and studying the evolution of fish locomotion and ecology.

Recent genome-wide analyses of domesticated teleost species have identified several independent genomic regions responsible for many phenotypic attributes, including faster growth and greater disease resistance. These selected traits have resulted in differences in muscle and skeletal structure, as well as nutritional requirements when compared to their wild counterparts (Gjedrem, 2012, Christie et al., 2016). For example, in steelhead trout (*Oncorhynchus mykiss gairdneri*), 723 differentially expressed genes were identified between wild and first-generation hatchery fish (Christie et al., 2016). Similarly, Konstantinidis et al. (2020) found that the muscle transcriptome of Nile tilapia changed after a single generation of domestication, with the upregulation of 2015 genes in captive fish compared to wild progenitors. Moreover, environmental factors such as temperature, food availability, pH, photoperiod, salinity and dissolved oxygen levels can significantly impact the growth rates of fish species (Figure 5). The muscle phenotypic responses to these changes vary depending on the species and developmental stage. Many studies have shown that early embryonic muscle formation is most vulnerable to the culture environment and can continue to affect the fish throughout their life (Dubinska-Magiera et al., 2016, Melo et al., 2019, Shulgina

et al., 2021, Steinbacher et al., 2011, Campos et al., 2013). The changes in muscle development persisted into adulthood and had a significant effect on the swimming performance and survival of the fish (Jonsson and Jonsson, 2014). However, several studies have reported that changes in some parameters, such as temperature, dissolved oxygen concentration and daylight length within the acceptable range, produce plastic effects on muscle growth (Johnston, 2006, JohnstonBower and Macqueen, 2011, Valente et al., 2013).

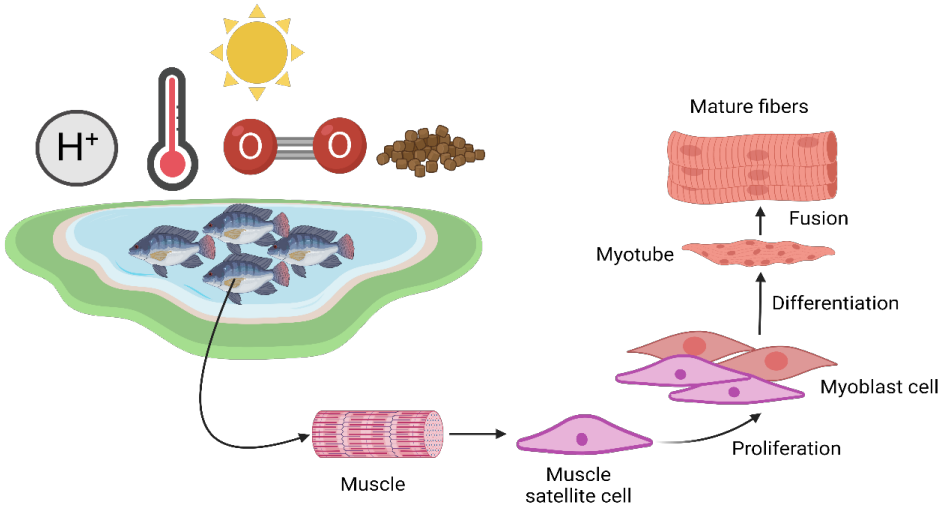

Figure 5. Environmental factors that alter muscle physiology in teleosts. Temperature, oxygen, pH, photoperiod and nutrition trigger physiological changes by activating muscle satellite cells. Subsequently, muscle cells undergo processes of proliferation, differentiation, and fusion, ultimately leading to the production of muscle fibers. Figure designed with BioRender.

In the case of temperature, even small differences can affect the distribution of muscle fiber types in larval and juvenile fish. Scott et al. (2012) found that zebrafish (*Danio rerio*) exposed to variable embryonic temperatures had wider slow muscle areas and a higher number of fast muscle fibers in cold water acclimation. Cold water exposure causes an expansion of the mitochondrial compartment to counterbalance the effect. On the other hand, short-term exposure to high temperatures leads to immediate adjustments in metabolic pathways and fiber-type distribution in muscle tissue. For

example, high temperatures have been shown to increase the mitochondrial content of embryonic slow and fast muscle fibers but reduce myofibrillar volume density in Atlantic herring (*Clupea harengus*) (JohnstonBower and Macqueen, 2011, Pittman et al., 2013). However, Macqueen et al. (2008) observed compensatory growth after transferring salmon larvae to ambient temperature. Fish treated at 2 and 5 °C were smaller than those treated at 8 and 10 °C in the beginning; however, no significant differences were detected between 5, 8 and 10 °C treatments at the end of the experiment. Similarly, blackspot seabream (*Pagellus bogaraveo*) kept at higher temperatures showed an increase in the number of white muscle fibers and the total cross-sectional area of red muscle at the post-anal level. Moreover, the red muscle fiber number in the larvae also increased with higher temperature during embryonic development (Silva et al., 2011).

Dissolved oxygen concentration also has a significant effect on fish growth. Due to eutrophication and pollution, the dissolved oxygen content in coastal and inland waters has dramatically reduced. Low dissolved oxygen (hypoxia) is critically associated with the metabolic demands of fish at different times. Several studies have found a reduction in muscle mass during the embryonic and larval stages when treated with distinct oxygen levels (CrockerChapman and Martinez, 2013, Sänger, 1993, Jew et al., 2013, RossiTurko and Wright, 2018). The early embryonic stages of development require a relatively high oxygen demand due to a higher metabolic rate. As the fish embryo undergoes rapid cell division and growth during this stage, a lot of energy is required, obtained through nutrient breakdown. This process, in turn, demands more oxygen to fuel the metabolic processes involved. Thorarensen et al. (2010) have shown the progressive growth of halibut when oxygen saturation was increased from 57 to 100%. Furthermore, oxygen can influence cellular responses through the insulin-like growth factor (IGF) signaling pathway. For instance, early hypoxia during zebrafish development upregulated the expression of *igfpb1a* in muscle, thereby enhancing the growth-inhibitory effects of *igfbp1* paralogs (Shimizu and Dickhoff, 2017).

Dietary sources and nutrients are primary contributors to growth performance and muscle cellularity, with hypertrophy and hyperplasia being largely influenced by the nutrient content of the diet. The effectiveness of dietary supplements with various growth promoters has been demonstrated in many aquacultured species (Zhao et al., 2018, Bjørnevik et al., 2003, Valente et al., 2016, Skjaerven et al., 2016, Adam et al., 2022, Canada et al., 2017, Lanes et al., 2012, Peruzzi et al., 2018). Among the various components of the diet, protein and their amino acid composition are the most crucial for fish growth. Furthermore, the micronutrient status of the parents' generation can have an impact on the health of their offspring. Skjærven et al. (2016) showed that the deficiency of one carbon micronutrient in zebrafish parents had a negative effect on the overall growth of their offspring, the general health of sperm and oocytes, as well as the particular deposition of nutrients and mRNA in the oocytes. The F0 generation of zebrafish showed a reduction in overall growth, and a significant effect on fecundity was observed in the F1 generation. Salmon, cod, tilapia and carp have been reported to exhibit rapid growth rates with increased dietary protein levels. For example, Bjørnevik et al. (2003) reported that salmon fed with a high-protein (50%) diet at an early stage showed significantly higher muscle cross-section area, white muscle diameter and fiber number when compared to those fed the corresponding low-protein (45%) diet.

Photoperiod is a key factor influencing somatic growth plasticity in fish. Extended photoperiods have been shown to stimulate the release of the hormones, growth factors and genes that control maturation and final growth, resulting in long-term growth changes (TriantaphyllopoulosCartas and Miliou, 2020, Shulgina et al., 2021). Photoperiod is believed to be the primary proximal cue for muscle growth in salmon, with continuous light treatment in the winter and spring resulting in increased fiber quantity coinciding with smoltification (Johnston et al., 2000, Johnston et al., 2003). Photoperiod also affects muscle structure differentiation in other fish species. For instance, Ayala et al. (2013) showed that the most pronounced white muscle hyperplasia in shi drum (*Umbrina cirrosa*) larvae was observed under a 24-hour light

regime. In Atlantic cod (*Gadus morhua*), continuous light resulted in 13.3% and 10.5% higher sizes at days 120 and 180, respectively, than those found in the natural photoperiod group (NagasawaGiannetto and Fernandes, 2012). Additionally, photoperiod significantly affects the growth of Nile tilapia, with the highest specific growth rate and average daily gain achieved under 18L:6D and 24L:0D photoperiods compared to 12L:12D and 6L:18D (El- Sayed and Kawanna, 2007).

Water pH is a crucial factor that influences stress response and fish development. Low water pH has the potential to boost fish growth by chemically shifting fish metabolites toward less hazardous compounds and altering the structure and activity of the accumulated molecules (Martins et al., 2009). For example, low pH in a recirculatory aquaculture system (RAS) can enhance carbon dioxide removal from biofilters, as shown by studies on several fish species, including Nile tilapia, African catfish (*Clarias gariepinus*) and black sole (*Solea solea*) (Mota et al., 2018, Mota et al., 2014). However, fish exposed to pH levels beyond their optimal range may devote more energy to organs and priority processes, thereby diminishing their contribution to long-term anabolic events. Studies have demonstrated that extreme pH levels can severely affect fish growth and reproduction, even leading to mass mortality in species such as turbot (*Psetta maxima*) (Mota et al., 2018), common carp (SapkaleSingh and Desai, 2011) and other species (Abbink et al., 2012, Zaniboni-Filho et al., 2009). However, Nile tilapia is highly tolerant to variable pH and can adapt and survive in acidified water as low as pH 4.0 (Van Ginneken et al., 1997). Nevertheless, a gradual increase in water acidification up to pH 4.0 showed no adverse effect on physiological properties and growth performance in Nile tilapia (Rebouças et al., 2015, Van Ginneken et al., 1997).

Salinity serves a critical role in fish development and physiology, with changes in salinity levels impacting their ability to maintain a consistent internal environment. While salt (NaCl) is often used in hatcheries to improve the survival and growth of fish in their early stages (Shahjahan et al., 2017, dos Santos and Luz, 2009, Luz and dos Santos, 2010), excessive changes in salinity can lead to stress and decreased efficiency

in transforming food energy into net energy, with consequences for muscle mass and growth performance. For instance, pacamã (*Lophiosilurus alexandri*) juveniles reared in salinities of up to 2.5 ppt had no alterations to muscle cellularity, whereas salinities greater than 5 ppt for long periods resulted in impaired osmoregulation along with changes in muscle cellularity (Takata et al., 2021). Fluctuations in salinity have also been reported to greatly affect the growth performance of African catfish (Saravanan et al., 2018, Sarma et al., 2013).

1.6 Molecular basis of muscle plasticity with focus on non-coding RNAs

Muscle formation is regulated by an array of molecules, including transcription factors, signaling components, hormones, long non-coding RNAs, microRNAs, and circular RNAs. MRFs, which include *myod*, *myf5*, *mrf4* and *myog*, are highly conserved transcription factors that bind to the promoter regions of muscle-specific genes and regulate their expression (Rescan, 2001). Other genes, such as *caveolin-3*, *IL-4*, *PGF2*, *calpain*, *calpastatin*, *nfatc2* and *transmembrane semaphorin sema4c*, are also involved in regulating myoblast fusion and muscle diameter (JohnstonBower and Macqueen, 2011, Horsley and Pavlath, 2004). Satellite cells, which are a population of muscle precursor cells, remain under the basal lamina and respond to environmental stimuli through signaling pathways. Environmental stimuli are sensed at the molecular level and transduced to the nucleus through signaling pathways. Signaling molecules precisely regulate MRF expression to modify the skeletal muscle phenotype (Vélez et al., 2017, Fuentes et al., 2013), and transforming growth factor beta (TGF-β), IGFs and growth hormones are at the center of initiating a cascade of reactions that control muscle regeneration, proliferation and differentiation.

In vertebrates, muscle is one of the main target tissues of IGFs, which directly stimulate hypertrophy and inhibit muscle atrophy. IGFs, comprising IGF1 and IGF2, are peptides with a structure similar to insulin and have complementary functions (VongChan and Cheng, 2003, DuanRen and Gao, 2010, Fuentes et al., 2013). To initiate a cascade of

signaling events, fish possess their own insulin-like growth factor receptors and several insulin-like growth factor binding proteins (IGFBPs). Macqueen et al. (2013) discovered 19 different *igfbp* genes in salmonid fishes—which is 13 more than in humans—using a phylogenetic approach. When triggered, IGF-ligand binds to IGF-1R, resulting in the activation of two major pathways, namely mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/TOR) (Fuentes et al., 2013). Initiation of the MAPK/ERK pathway leads to the activation of downstream targets such as transcription factors, cytoskeletal proteins and cell cycle regulators. In fish, activation of the MAPK/ERK stimulates myogenic cell proliferation, differentiations and protein synthesis (Fuentes et al., 2011, Fuentes et al., 2013). On the other hand, the PI3K/AKT pathway stimulates the entrance of amino acids and glucose into the cell, which provide substrates and energy for protein synthesis and cell proliferation via TOR signaling (Vélez et al., 2017). Using diets with different levels of isoleucine, Jiang et al. (2021) have shown that hybrid bagrid catfish (*Pelteobagrus vachelli* Z x *Leiocassis longirostris* H) increase muscle protein deposition by activating the AKT-TOR-S6K1 and AKT-FOXO3a signaling pathways. Additionally, the TGF-β pathway and its downstream components are responsible for inducing the *mstn* gene and regulatory follistatin protein. TGF-β signaling activation starts with binding ligands to TGF-β receptor1 and 2, which later initiates a phosphorylation cascade. TGFBR2 activation in C2C12 cells was linked to myogenesis regulation by downregulating *myod* and *myog* (Ding et al., 2020). In rainbow trout (*Oncorhynchus mykiss*), it has been shown that *follistatin* and *activin type IIB* receptors have an inhibitory effect on TGF-β signaling that subsequently alters muscle morphology (PhelpsJaffe and Bradley, 2013).

Growth hormone (GH) signaling is another important pathway in fish that is involved in the regulation of growth and development, metabolism, protein degradation and muscle atrophy. GH is primarily produced and secreted by the pituitary gland and acts on target tissues through the growth hormone receptor (GHR). The binding of GH to the GHR activates several signaling pathways, including the Janus kinase/signal

transducer and activator of transcription (JAK/STAT) and MAPK pathways. Using zebrafish, Silva et al. (2015) have shown that double transgenics (carrying both GH and GHR genes) displayed imbalanced growth, leading to notably reduced weight in comparison to GH transgenics. In addition, the overexpression of GH and GHR resulted in a strong decrease in somatotrophic axis intracellular signaling by diminishing its signal transducer (STAT5.1), thereby reducing muscle growth.

There is a growing body of evidence that highlights the crucial role of non-coding RNAs (ncRNAs) in regulating growth and development in fish by controlling gene expression at different levels, including signaling molecules, chromatin modification, transcription, and post-transcriptional processing (Solís et al., 2022, Deng et al., 2022). ncRNAs are commonly known as transcripts or transcript segments that do not translate into protein. Transfer RNA and ribosomal RNA were the first ncRNAs described during the 1950s, with both being involved in protein synthesis (Cech and Steitz, 2014, Huang and Zhang, 2014). The last few decades have seen an increasing number of studies on ncRNAs due to their roles in diverse biological processes, such as development, differentiation and epigenetic regulation. The rapid advancement of high-throughput sequencing technology has enabled researchers to determine that approximately 96–99% of all transcriptional output in an organism is linked to ncRNAs. To date, a number of ncRNAs, including microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), PIWI-interacting RNAs (piRNAs), lncRNAs and circular RNAs, have been discovered. Based on their size, architecture and mechanisms of action, ncRNAs are divided into small ncRNAs (<200 nucleotides) and long ncRNAs (>200 nucleotides) (Sun and Chen, 2020). However, some ncRNAs with varying lengths, such as circRNAs, enhancer RNAs and promoter-associated transcripts, may simultaneously fall under both categories. The expression patterns of these ncRNAs are believed to be under precise regulatory control or specific to certain types of tissues. Evidence has shown that snRNAs perform splicing processes for mRNAs, whereas tRNAs specifically recognize three-nucleotide mRNA sequences and recruit the appropriate amino acids to the ribosome to decode the mRNA sequence into a peptide or protein (Maniatis and

Reed, 1987, Cech and Steitz, 2014). Regulatory RNAs such as interfering RNAs (siRNAs), miRNAs and circRNA are also found in a multitude of organisms and play a role in silencing or regulating the expression of target mRNAs.(Huang and Zhang, 2014)

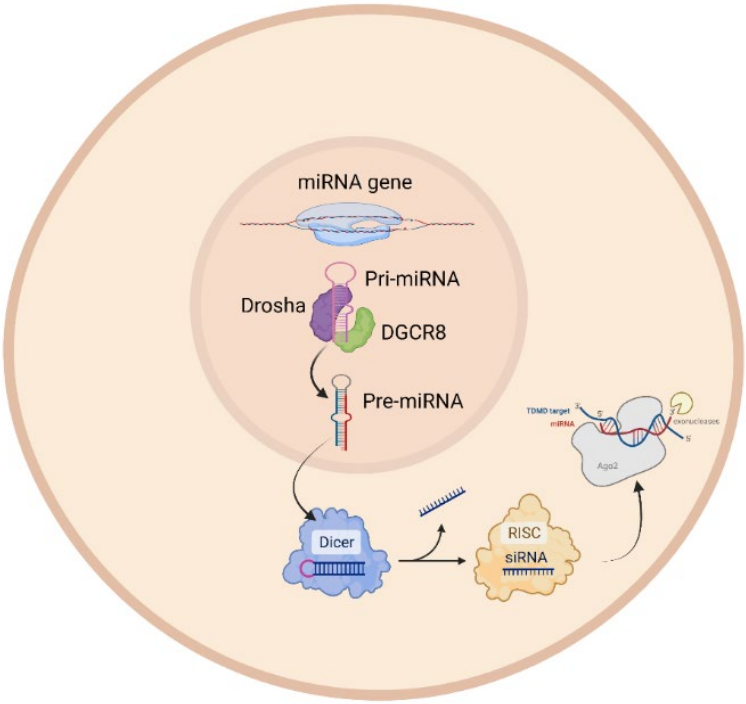

Figure 6. Schematic diagram of miRNA biogenesis and its mode of action. mRNA genes are transcribed to pri-miRNAs, which are processed in the nucleus by Drosha to produce pre-miRNAs. Dicer further removes a strand, and the mature miRNAs incorporate with the RNA-induced silencing complex (RISC) to bind the 3' UTR region of their target gene. Figure generated with BioRender.

miRNAs are small RNA molecules consisting of approximately 18-30 nucleotides that regulate gene expression by binding to the 3' untranslated regions (UTRs) of target mRNAs. They were first identified in the *lin-4* gene of roundworm (*Caenorhabditis elegans)* (LeeFeinbaum and Ambros, 1993). The biogenesis of miRNAs starts with the transcription of primary mRNA transcripts by RNA polymerase II in the cell nucleus (Figure 6). Drosha and Dicer process primary mRNA transcripts into a double-stranded

miRNA duplex with approximately 22 nucleotides, which integrates into the RNA-induced silencing complex. Removing one strand forms a mature single-stranded miRNA that acts as a guide to complementary sequences primarily located within the 3'-UTR of target mRNAs. Recent research suggests that mature miRNAs can enter the nucleus and suppress ncRNAs.

miRNAs are evolutionarily conserved across vertebrates and regulate up to 60% of all mRNAs in mammals (Friedman et al., 2009). The genome harbors hundreds of distinct miRNA genes, each of which can putatively regulate hundreds of target genes (Zhao and Srivastava, 2007). The expression of miRNAs is involved in several physiological processes, such as stem cell differentiation, cell lineage specification, myogenesis and immune response (ZhaoSamal and Srivastava, 2005, Zhao and Srivastava, 2007, Wang et al., 2018). For example, miR-34c expression in zygotes initiates first-cell division through modulation of the *bcl2* gene. In teleosts, miRNAs were first described in zebrafish with a spatio-temporal expression pattern. Since then, hundreds of miRNAs have been described in several teleost species, including common carp, pufferfish (*Takifugu rubripes*), Atlantic halibut (*Hippoglossus hippoglossus*), Senegalese sole and Nile tilapia (Bizuayehu and Babiak, 2014, Yan et al., 2013, Bizuayehu et al., 2013, Campos et al., 2014).

The number of miRNAs present at a particular time tightly regulates both muscle development and growth. Johnston et al. (2009) found an association of miRNA expression with muscle plasticity under various embryonic temperatures, observing 14 upregulated and 15 downregulated miRNAs (including let-7e, miR-365, miR-1 and miR-206) during the embryonic period, which presumably serve a role in the transition from hyperplasia to hypertrophy. miR-1 regulates the expression of transcription factor *histone deacetylase 4* (*hdac4*) during early myogenesis, whilst the downregulation of *hadc4* inhibits muscle cell differentiation by repressing *mef2c* (Chen et al., 2006). MiR-1 can also inhibit the IGF-AKT signaling pathway by targeting *igf1* and *igf1r* genes (HorakNovak and Bienertova-Vasku, 2016). Additionally, miR-206 modulates myotube

formation by regulating the production of connexin43, a gap junctional protein necessary for myoblast fusion (AndersonCatoe and Werner, 2006). In Senegalese sole larvae, Campos et al. (2014) reported that upregulation of miR-206 during early development at a temperature of 21 °C could result in the downregulation of myogenic inhibitors such as *sestrin1* and *sestrin3*, promoting growth by inhibiting mTOR signaling pathway.

Several studies have shown that microRNAs can selectively regulate genes specific to muscle development. MiR-203b was also found to regulate *myod*, an essential myogenic regulatory factor that modulates myoblast activation in Nile tilapia (Yan et al., 2013). Another muscle-specific gene, *mstn*, is also regulated by miR-2014-5p, miR-1232-5p and miR-181b-5p in fish (Lou et al., 2021, Zhao et al., 2019). In large yellow croaker (*Larimichthys crocea*), miR-2014-5p and miR-1232-5p regulate *mstn* expression at different stages of starvation (Lou et al., 2021). Therefore, the regulation of miRNAs in fish muscle is a complex process involving multiple factors and genes.

In addition to their role in growth and development, miRNAs are also involved in fiber type specification and phenotype determination in fish muscle (Wang et al., 2011, Nachtigall et al., 2015). For instance, miR-499 represses the translation of the *sox6* gene in muscle fibers. *sox6* transcripts are usually expressed at a higher rate in fast-twitch fibers, whereas slow-twitch muscle-specific genes—including slow *myosin heavy chain 1* and slow *troponin* inhibition—are among the main functions of *sox6* (Von Hofsten et al., 2008). Thus, higher expression of miR-499 defines slow-twitch muscle in teleosts.

Variations in miRNA expression have the potential to exert a substantial impact on the overall size and mass of an organism. Numerous studies have underscored the importance of temporal and tissue-specific miRNAs in developmental processes and their contribution to somatic growth. For example, miRNAs such as let-7j, miR-140, miR-192, miR-204, miR-218a, miR-218b, miR-301c, miR-460 and miR-34 have been associated with somatic growth in tilapia (Huang et al., 2012). Furthermore, the

utilization of fasting-refeeding models has provided insights into miRNA expression and its role in fish muscle growth, particularly during the transition from catabolic to anabolic states. For instance, in Chinese perch (*Siniperca chuatsi*), miR-10c, miR-107a, miR-133a-3p, miR-140-3p, miR-181a-5p, miR-206 and miR-214 have shown elevated expression within 1 hour after refeeding, among which miR-10c, miR-107a, miR-140-3p and miR-181a-5 contain a target site for *mstn* gene, which is a negative regulator of muscle growth (Zhu et al., 2015). Similarly, in grass carp, miR-181a-5p and miR-206 were sharply upregulated within 1 or 3 hours after refeeding (Xin et al., 2014). Moreover, specific nutrients can alter miRNA expression in muscle satellite cells. The coregulation of miRNAs and mRNAs defines cell differentiation arrest and resumption in rainbow trout (Latimer et al., 2017). Thus, these findings indicate that miRNA regulates muscle-specific genes in the resumption of myogenesis with feeding.

While miRNAs have been extensively studied as a regulator of muscle-specific genes, recent studies have shown that circRNAs can act as competing endogenous RNAs (ceRNAs) and regulate miRNA activity by sequestering miRNAs and preventing them from binding to their target mRNAs (Peng et al., 2019, Zhang et al., 2022, Li et al., 2018). Therefore, circRNAs may play a crucial role in regulating miRNA-mediated gene expression in growth. In the next section, we will explore the circRNA features and functions, and delve deeper into their emerging role in muscle growth.

1.7 Circular RNAs (circRNAs)

circRNAs are a type of ncRNA that form a covalently closed continuous loop structure. They are generated by a backsplicing event in which a downstream splice donor site is joined to an upstream splice acceptor site, resulting in the formation of a circRNA molecule. They were initially discovered in plant viroids and hepatitis delta virus in the 1970s (Kos et al., 1986, Sanger et al., 1976) and later in yeast mitochondrial RNAs (Arnberg et al., 1980). However, due to their low expression levels and lack of functional evidence, they were not extensively studied for several decades. In the 1990s, the discovery of self-splicing introns led to the identification of circRNAs in

various species; however, they were deemed to be the by-products of irregular splicing (Capel et al., 1993, Cocquerelle et al., 1992, Cocquerelle et al., 1993, Puttaraju and Been, 1992). In recent years, the emergence of RNA-Seq data and improved biochemical enrichment, next-generation sequencing, and computational methods have led to the widespread expression of circRNAs being recognized (Westholm et al., 2014, Salzman et al., 2012, Hansen et al., 2016) along with their potential regulatory functions (Rbbani et al., 2021, Memczak et al., 2013).

1.7.1 CircRNA biogenesis

CircRNAs are classified into five categories according to their origin: exonic circRNA (ecircRNA), intronic circRNA (ciRNA), exon-intron circRNA (EIcircRNA), intergenic circRNAs, and tRNA intronic circRNA (tricircRNA) (Tang et al., 2021, Dong et al., 2017).

The biogenesis of circRNAs is a multi-step process involving pre-mRNA splicing, reverse splicing and lariat debranching (Li et al., 2015, Zhang et al., 2016). Exonic and exon-intronic circRNAs are generally produced by non-canonical splicing, widely regarded as backsplicing. Unlike conventional splicing, the 3′ end of a downstream splice acceptor site binds to the 5′ end of an upstream splice donor site to form a circular structure (Figure 7). The canonical splicing of pre-mRNA involves the removal of introns and the ligation of exons in a linear order. The spliceosome leads removal of the intron via recognition of the flanking intron section at both ends, thereby facilitating mature RNA formation (Moore and Proudfoot, 2009). However, this process also produces circRNA from the same transcript by competing with mature RNA production. According to the literature, more than 80% of circRNAs fall under the exonic circRNA category (Tang et al., 2021). Highly expressed circRNAs are mainly composed of multiple exons and few introns, indicating the significance of spliceosomes in circRNA formation.

Intronic circRNAs are generated via a lariat-derived mechanism, where a lariat structure is formed during pre-RNA splicing (Figure 7). RNA motifs containing a GU-rich region close to the 5' splice site and a C-rich sequence close to the branch point help

the lariat to escape debranching and retain a circular form known as ciRNA (Chen, 2020, Ward et al., 2021). In addition, tRNA driving circRNA biogenesis occurs during the intron removal of pre-tRNA, where the introns derived from elongator tRNA undergo end-joining to form tcircRNA, and the exon halves ligate together to produce a mature tRNA (NotoSchmidt and Matera, 2017, Schmidt et al., 2019).

The production of circRNAs can be influenced by various factors, including RNA binding proteins (RBPs), complementary sequences in the flanking intron, transcription elongation rates, RNA editing, secondary structures and intermediate lariat structures. RBPs can interact with exon-bordering introns to reduce the distance between the receptor and donor sites. Quaking, muscleblind, and fused in sarcoma (FUS) are among the RBPs that act as transacting factors and enhance circularisation (Tang et al., 2021, Ward et al., 2021). They can reduce the distance between the receptor and donor sites through interactions with exon-bordering introns (Figure 7).

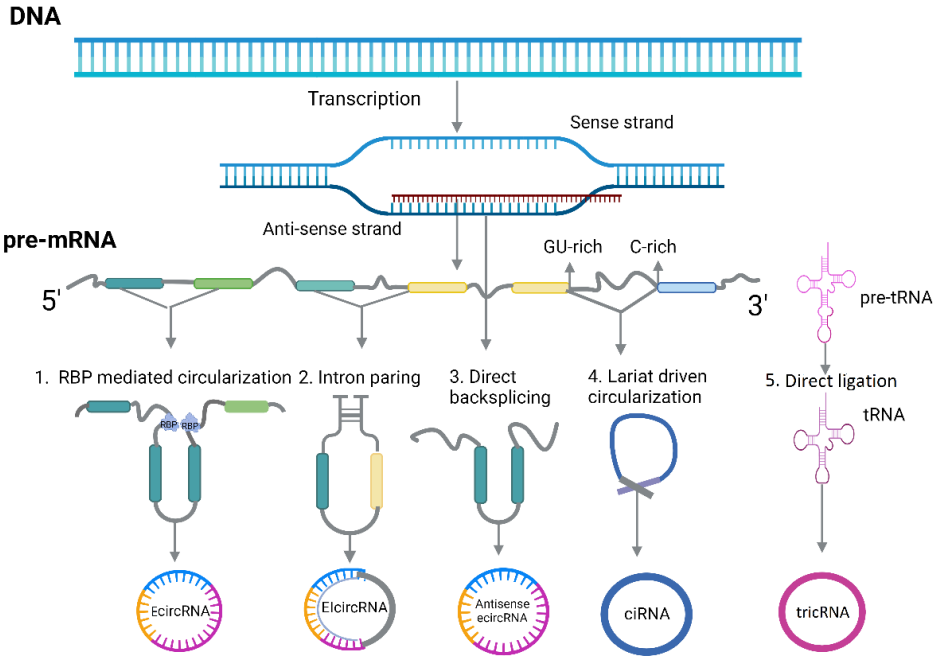

Figure 7. CircRNA biogenesis and types. Several mechanisms are involved in this process: 1) RBP-mediated circRNA formation by pre-miRNA folding (exonic circRNA or EcircRNA). 2) Pairing between the 2 introns flanking of exons (exon-intron circRNA or EIcircRNA). 3) Back-splicing sites promoting the ligation of downstream 5'donor sites with upstream 3'acceptor sites (antisense circRNA or AS-circRNA). 4) Intron lariat-derived circularization (intronic circRNA or ciRNA). 5) tricRNA exon termini linking to each other to form mature tRNA and intron termini are ligated together to form tricRNA (tRNA intronic circRNA or tricRNA). Figure generated with BioRender.

1.7.2 Characteristics and function of circRNAs

Although circRNAs are primarily produced in the nucleus, most exonic circRNAs are localized in the cytoplasm. However, other circRNAs with introns are retained in the nucleus and can function as regulatory molecules by interacting with other RNAs, proteins or cellular structures. It has been suggested that circRNA transport within cells is predominantly dependent on their length. By knocking down several proteins involved in RNA transport, Li et al. (2019) showed that AP56/DDX39B dictates the export of long circRNAs, while URH49/DDX39A is responsible for short circRNAs. The

abundance of circRNAs is especially high in proliferative tissues, although they are distributed across different tissues. CircRNAs have unique properties compared to their linear isoforms, such as being highly stable, resistant to exonucleases and having the ability to accumulate to high levels in cells (Vicens and Westhof, 2014). Several studies have shown that circRNAs show distinct temporal and spatial expression profiles in various tissues and that a small portion of them is evolutionarily conserved (Jeck et al., 2013, Rybak-Wolf et al., 2015). For example, Xia et al. (2017) have reported that 92.6, 3.9, 2.3, 1 and 0.1% of circRNAs are conserved in the brain, heart, liver, skin, and lungs, respectively. The stability, expression pattern and evolutionary conservation of circRNAs suggest that they could serve important regulatory roles in physiological and molecular processes (Huang et al., 2017, Greco et al., 2018, Ouyang et al., 2018), although many of the identified circRNAs have yet to be functionally characterised. CircRNAs have recently been gaining attention due to their gene regulatory potential. They are known to play a wide range of functions, including serving roles in microRNA sponging, protein sponging, protein translation, transcription regulation and splicing regulation (Figure 8).

The most studied function of circRNAs is their role as miRNA sponges. CircRNAs contain multiple miRNA response elements to sequester miRNAs, thereby preventing them from binding to and suppressing their target mRNAs. The phenomenon of circRNAs and mRNAs competing for shared miRNAs is known as competing endogenous RNAs (ceRNAs). In 2013, Hansen et al. (2013) identified the circRNA ciRS-7 (also known as CDR1as) as a highly expressed circRNA in the human brain that contains more than 70 binding sites for miR-7. They demonstrated that ciRS-7 acts as a sponge for miR-7, leading to the increased expression of miR-7 target genes. In zebrafish, miR-7 is crucial for midbrain development, whilst the expression of circCDR1as reduces midbrain size, similar to miRNA-7 (Memczak et al., 2013).

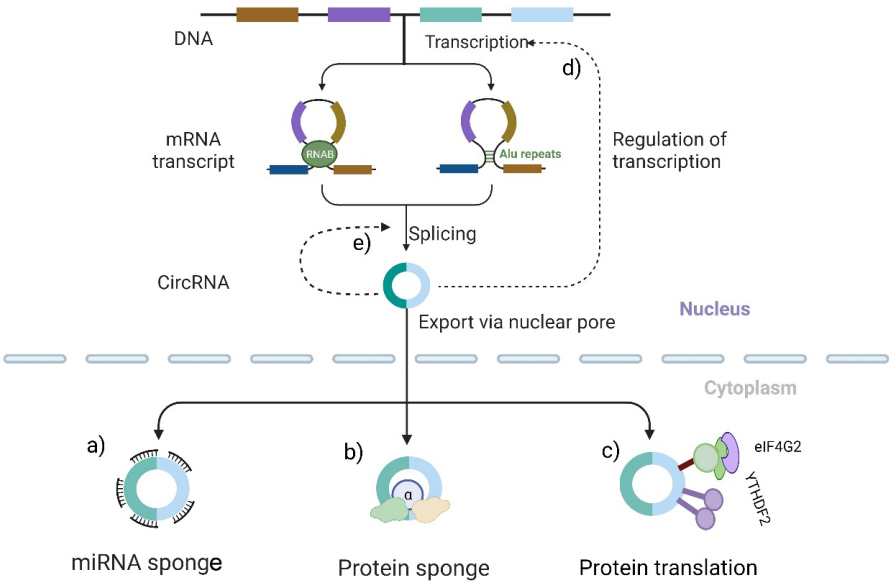

Figure 8. Functional roles of circRNAs in eukaryotes. Cytoplasmic circRNAs exhibit diverse functions, including acting as miRNA sponges (a), protein sponges (b), and facilitators of protein translation (c). Meanwhile, nuclear circRNAs are involved in regulating mature RNA formation (d) and pre-mRNA splicing (e). Figure designed with BioRender.

Furthermore, the circSry transcript from the *sex-determining region Y* (*Sry*) gene in mice contains 16 target sites for miR-138 and influences testis development (Hansen et al., 2013). Recently, a number of studies have demonstrated the ability of circRNAs to exert biological functions through miRNA sponging. For instance, circHIPK3 has been reported to soak up multiple miRNAs, including miR-379, miR-4288, miR-558 and miR-7, thereby regulating cell growth (Tang et al., 2021, Zheng et al., 2016).

CircRNAs can also regulate transcriptional activity in the nucleus through interaction with RNA polymerase II and U1 small nuclear ribonucleoprotein at the promoters of their parent genes (Li et al., 2015). CircEIF3J and circPAIP2 have been shown to interact with U1A and U1C proteins, as well as U1 small nuclear RNA, and together bind with the promoter region of the *eif3j* and *paip2* genes, thus elevating their expression. Similarly, Ci-ankrd52 (also known as ANKRD52) functions as a positive regulator of RNA polymerase II (Pol II) when present in the nucleus. It assembles the transcription site

of the ANKRD52 gene and interacts with the elongation machinery of Pol II, promoting transcriptional elongation and potentially recruiting additional factors to further enhance gene expression (Zhang et al., 2013). Many circRNAs have the potential to bind and store RBPs to particular subcellular locations, similar to their known miRNA sponging activity. For example, circ-Mbl contains several binding sites for the MBL protein, thereby regulating both circ-Mbl and MBL biogenesis (Huang et al., 2020). CircFOXO3 is another classic example of a protein interacting with circRNA. CircFOXO3 induces apoptosis by promoting interaction between MDM2 and P53 (Du et al., 2017). Moreover, Wang et al. (2015) have shown that circRNAs may translate into proteins through insertion into an internal ribosome entry site. Circ-ZNF609 is one of the special circRNAs containing an open reading frame (ORF) spanning from the start codon to an in-frame stop codon (Legnini et al., 2017). Legnini et al. (2017)discovered that circ-ZNF609 is associated with heavy polysomes that translated into a protein in a splicing-dependent and cap-independent manner. Cap-independent translation through internal ribosome entry sites (IRESs) has been suggested to be elevated in malignancies to encourage the translation of genes that are crucial for stress responses, apoptosis and cell cycle regulation (Leprivier et al., 2015). It has also been suggested that N^6 methyladenosine (also known as m^6A) motifs are enriched in circRNAs, with a single m^6A site being sufficient to drive translation initiation (Yang et al., 2017). m^6A is the most abundant modification in RNA and it regulates post-transcriptional gene regulation. *YTHDF3*, known as the m^6A reader, recruits the eukaryotic translation initiator eIF4 to the m^6A-containing circRNAs. m^6A modification near the 3' UTRs region was found to increase translation, whereas m^6A in 5′ UTRs promoted cap-independent translation through a YTHDF2-protection mechanism. Yang et al. (2017) have shown that the circRNAs may contain multiple m^6A motifs and can effectively translate into protein. The authors further suggested that the peptides produced by circRNA are unique and only represent a small fraction of the circRNA-encoded proteome.

Recently, the function of circRNAs in epigenetic regulation has been uncovered. Gene expression linked to DNA methylation and histone modification is important in growth

and cancer biology. Two essential genes in the regulation of DNA methylation are DNA *methyltransferase* 1 (*dnmt1*) and *ten-eleven translocation 1 (tet1*). The *ten-eleven translocation* (TET) family can convert 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC), 5-formylcytosine and 5-carboxylcytosine, as well as further induce passive or active DNA demethylation, whereas the dnmt family regulates *de novo* DNA methylation and is faithfully maintained during DNA replication (Zhang et al., 2022). According to Ferreira et al. (2018), when CpG island methylation in the promoter regions of corresponding genes is increased, the expression of linear and circRNA transcripts is significantly downregulated. CircFECR1 induces extensive CpG DNA demethylation by downregulating the transcription of dnmt1 (Chen et al., 2018). It was further demonstrated that CircFECR1 interacts with the FLI1 promoter in cis and recruits *tet1* demethylase. By coordinating the regulation of DNA-methylating and -demethylating enzymes, circRNA functions as an upstream regulator to control the formation of breast cancer tumors.

1.7.3 Role of circRNAs in muscle growth

Over the past few years, circRNAs have been extensively studied in farmed animals (e.g., cattle, goats, chickens and to limited extent fish) to shed light on the role of individual circRNAs in muscle growth. To date, it has been documented that both skeletal and cardiac muscles are capable of producing a range of circRNAs, with reported counts between 622 to 38,000 (Rbbani et al., 2021). In teleosts, largemouth bass (*Micropterus salmoides*) and blunt snout bream (*Megalobrama amblycephala*) express a total of 823 and 445 circRNAs in muscle tissue, respectively. Most muscle circRNAs are derived from the exonic region. Ling et al. (2020) reported that 92.4% of exonic circRNAs in goats are indicative of their regulatory roles at the post-transcriptional level. CircRNA functional clustering has revealed their association with various important signaling pathways, such as Wnt/PI3K-Akt, which is required for muscle development. However, the primary mechanism through which circRNAs regulate muscle growth is the miRNA sponge function (Hall et al., 2019). In a murine muscle cell line, circRNAs generally harbor one or more miRNA binding sites (Zhang et

al., 2018). Target prediction analysis revealed that circRNA-4297 and circRNA-34742 had 29 and 25 miRNA binding sites, respectively. Several of these miRNAs, such as miR-133, miR-24 and miR-23a, were previously known for regulating myoblast proliferation and differentiation processes.

Accumulating evidence suggests that different circRNAs in muscle can positively or negatively impact myoblast proliferation and differentiation. Among them, circTTN shows high expression both in embryonic and adult stages and regulates gene expression at the post-transcriptional level (Wang et al., 2019). CircTTN functions as a competing endogenous RNA of miR-432, leading to the activation of the *IGF2* gene expression. *IGF2* induces the autophosphorylation of tyrosine residues through binding to its receptor, which, in turn, activates the PI3K/AKT cascade, triggering myoblast proliferation (Liu and Zhang, 2011). On the contrary, circFUT10 plays a crucial role in myoblast inhibition and apoptosis. Le et al. (2018) have shown that a ceRNA regulatory network, circFUT10/miR-133a/SRF, impedes myoblast proliferation at the embryonic and adult stages. In addition, circEDC3 in chicken has the potential to translate protein through IRESs and m^6A motifs and negatively regulate muscle growth (Wei et al., 2022).

It has been frequently reported that the circRNAs expressed in myoblasts also regulate the cell cycle and muscle differentiation (Di Agostino et al., 2020). In higher vertebrates, circRNAs such as circSNX29, circHUWE1, circMYBPC1, circUSP13 and circEch1 regulate myoblast differentiation in skeletal muscle through several gene regulatory mechanisms during ontogeny (Wang and Wang, 2015). For example, circSNX29 in Qinchuan cattle showed significant downregulation at the embryonic stage (Peng et al., 2019). By functioning as a ceRNA, circSNX29 facilitated myoblast differentiation and inhibited cell proliferation. Sequestered miR-744 can induce myoblast apoptosis through the increased expression of caspase-9 in a *bcl2*-dependent manner. In addition, miR-744 targets the Wnt5a and CaMKIId genes, and their expression activate the Wnt5a/Ca2+/CaMKIId pathway to regulate myoblast differentiation. In bovine primary myoblasts, circMYBPC1 acted as a ceRNA by sponging miR-23a to rescue its target

myhc gene, and it directly bound to MyHC protein to increase *myhc* gene expression, thereby regulating mRNA and protein levels to promote muscle growth (Chen et al., 2021). In chicken, circHIPK3 expression varied during embryonic development and post-hatch, and it interacted with miR-30a-3p to promote myoblast differentiation (Zeng et al., 2018). Similarly, circUSP13 promoted differentiation and inhibited apoptosis in goat primary myoblasts by sponging miR-29c, which led to the upregulated expression of *myog* and *myhc* and increased muscle cell growth in growing lambs (Zhang et al., 2022).

CircRNA research in teleosts remains in its infancy, particularly with regard to muscle growth. The first few studies on this topic, mostly in zebrafish, have described their features and distribution in different tissues. Sharma et al. (2019) reported 1014 and 686 circRNAs in cardiac and skeletal muscle tissue, respectively. Circ_ttnb1 showed high expression in muscle tissue, while circ_furina exhibited high expression in heart muscle tissue but low expression in skeletal muscle tissue. Comparing fast- and slow-growing blunt snout bream, Liu et al. (2021) identified a total of 445 circRNAs, including 42 differentially expressed circRNAs between the groups. Aminoglycan biosynthetic and metabolic processes, cytokinetic processes and the adherens junction pathway were among the top pathways related to differentially expressed circRNAs. As with other vertebrates, the circRNA-miRNA-mRNA network is also prominent in teleosts (Guo et al., 2023, Rbbani et al., 2021). CircRNA-miRNA-mRNA networks consisting of 15 circRNAs, 14 miRNAs and 27 mRNAs were observed in blunt snout bream (Liu et al., 2021). Novel_circ_0001608 was suggested to act as a sponge of dre-miR-153b-5p and promote muscle growth by elevating the expression of the target gene MamblycephalaGene14755 (*pik3r1*). Similarly, novel_circ_0002886, dre-miR-124-6-5p and *apip* compete with each other for binding and act as ceRNAs. In seabass, circRNA389 and circRNA399 harbor 30 miRNAs and significantly regulate growth genes (Bai et al., 2022). Furthermore, a number of circRNAs were reported to be produced from genes, including *muscleblind-like protein 1*, *myocyte-specific enhancer factor 2a*, *supervillin-like protein*, *myosin heavy chain* and *insulin-like growth factor binding*

protein 3, indicating their influence on muscle growth. Nedoluzhko et al. (2020) also reported a number of circRNA host genes predicted in Nile tilapia that were related to muscle functions, including *calcium/calmodulin-dependent protein kinase*, *troponin T3* and *myocyte-specific enhancer factor 2C*.

1.7.4 Sequencing and bioinformatic approaches to identify circRNAs

During the early stages of sequencing technology, knowledge of circRNAs was limited and traditional sequencing methods posed challenges in studying them. RNA-Seq, a deep-sequencing-based method for transcriptome profiling, has provided more precise measurements of transcript levels and isoforms (WangGerstein and Snyder, 2009). However, mRNA sequencing technology uses poly A enrichment to detect expressed transcripts, leaving out non-canonical transcripts. Additionally, the unique circular shape of circRNAs makes them difficult to separate from regular RNA transcripts through size selection. Salzman et al. (2012) employed the ribo-minus RNA sequencing technique to find rearranged transcripts produced by ordinary splicing and discovered hundreds of transcripts in which exon order was a circular permutation of the order encoded in the genome. This expanded the scope of investigating circRNAs across species and identifying their functional relevance. Using ribosome-depleted RNA with ribonuclease R (RNase R) (Circleseq), Jeck et al. (2013) provided greater confidence in circRNA identification than unenriched sequencing alone. Several technical variations in circRNA enrichment have since been used, including rRNA-, polyA+RNase R, rRNA-+polyA+RNase R and polyA+RNase R+rRNA-. A recent comparison among these methods showed that polyA+RNase R+rRNA- is preferable for identifying a large number of circRNAs, while polyA+RNase R is better suited to identifying circRNAs that require a higher degree of precision (Shi et al., 2022).

Several computational tools have been developed to identify circRNAs in eukaryotic organisms using different types of circRNA sequencing, such as single-end or paired-end sequencing reads. Although poly(A) selection libraries theoretically do not contain circRNA reads, circRNA sequencing outputs may still contain a small proportion of

circRNA due to less accurate circRNA selection and the specificity of identification tools. To avoid such issues, it is recommended to use paired-end read sequencing and strict cut-offs for read counts during identification.

One of the crucial steps in discovering backsplicing junction (BSJ) reads is termed remapping (Szabo and Salzman, 2016). All available tools, except for machine learning, use different algorithms to identify backsplice junction reads. External aligners such as Bowtie, BWA, STAR, TopHat and minimap2 are commonly used for mapping data with reference genomes. Below is a list of circRNA-finding tools:

CIRI, find_circ, CIRCexplorer, KNIFE, CircCode, FUCHS and DCC are commonly used tools for identifying backsplicing events using splicing algorithms (ChengMetge and Dieterich, 2016, Metge et al., 2017, Sun and Li, 2019, Szabo et al., 2015, Zhang et al., 2014, GaoWang and Zhao, 2015, Song et al., 2016, Memczak et al., 2013). Some of these tools allow a combination of mapped and unmapped reads (Table 1). Find_circ was the first RNA-Seq-based circRNA prediction tool to use backspliced sequencing read identification. Later, in 2018, Gao et al. (2018) developed a powerful tool (CIRI2) that analyses sequence data to find junction reads and employs various filtration techniques to eliminate false positives. CIRCexplorer facilitates *de novo* assembly for backsplicing transcripts and identifies circRNA by recognizing both alternative and backsplicing signals. The most sensitive tool in terms of true circRNA identification (98% specificity and 96% sensitivity) is MapSplice (Wang et al., 2010). It assigns a single best alignment for each read using a composite score based on mismatches, base call quality and junction score. Tools such as UROBORUS and Ularcirc are more restricted in identifying circRNAs, with low expression levels in RNA-Seq datasets without RNase R treatment (Humphreys et al., 2019, Song et al., 2016). Some tools, such as ACFS and NCLscan, have been developed to overcome specific issues, including the presence of fusion and trans-splicing events (Chuang et al., 2016, You and Conrad, 2016). CircRNA_finder can identify circRNAs without the need for gene annotations or exon-intron structure, making it more suitable for species with no available annotation data.

The host genes of circRNAs are crucial for understanding their function. CircParser has been developed to identify host genes, circRNA types and their minimum and maximum base pair sizes (Nedoluzhko et al., 2020). In addition, circView can quantify the number of samples expressing the predicted circRNAs and visualize them (Feng et al., 2019).

Despite the availability of many tools, the benchmarking of accurate or low false positive splice site identification is still required in this field. It has been suggested that algorithm-specific false positives or low-abundance circRNAs can be eliminated by combining the shared output from two or more algorithms; however, this can often lead to variable outcomes due to filtering criteria. Szabo et al. (2016) reported that the length and read count of detected circRNAs might vary based on the algorithm. For example, the median lengths of circRNAs from H9 cells were 353 nt and 260 nt in CircExplorer and KNIFE, respectively. In addition, most of the algorithms detect circRNA without providing their full-length sequence. The degenerate sequence motifs at exon boundaries imply that a convolution of homology and sequencing errors can lead to -false positive alignments (Szabo and Salzman, 2016). Moreover, Zeng et al. (2017) reported that circRNA prediction tools are vulnerable to sequencing errors and strongly affected by sequencing depth.

Table 1. List of circRNA identification tools.

Tool name	Tool type	Aligner, tool or method required	Algorithm category	Programming language	Reference
CirComPara2	Annotation	STAR, Hisat2, BWA	BSJ	Python	(Gaffo et al., 2022)
Circfull	*De novo*	minimap2	BSJ	Python	(Liu et al., 2021)
CircCode	Annotation	STAR	BSJ	Python	(Sun and Li, 2019)
Circall	Annotation		BSJ	C++	(Nguyen et al., 2021)
seekCRIT	Annotation	STAR	BSJ	Python	(Chaabane et al., 2020)
CircMiner	Annotation	*k*-mer	BSJ	C++	(Asghari et al., 2020)
CircAST	Annotation	Tophat2	BSJ	Python	(Wu et al., 2019)
CIRCexplorer	*De novo*; annotation	STAR, BWA	BSJ	Python	(Ma et al., 2021)
circRNA_finder	*De novo*	STAR	BS	Perl, AWK	(Westholm et al., 2014)
CircRNAFisher	*De novo*	Bowtie2	BSJ	Perl	(Jia et al., 2019)
ACValidator	Annotation	BWA, Bowtie2	BSJ	Python, Shell	(Sekar et al., 2020)
ANNOgesic	Annotation	segemehl	BSJ	Python	(YuVogel and Förstner, 2018)
BIQ	Annotation	k-mer**	BSJ	C++, Perl, JavaScript	(Menzel and Meyer, 2019)
CircDBG	Annotation	k-mer	BSJ	C++	(Li and Wu, 2020)
CircMarker	Annotation	k-mer	BSJ	C++	(Li et al., 2018)
circtools	Annotation	DCC, FUCHS	BSJ	Python, R	(JakobiUvarovskii and Dieterich, 2019)
DCC and CircTest	Annotation	STAR	BSJ	Python, R	(ChengMetge and Dieterich, 2016)
NCLcomparator	Annotation	STAR, BLAT	BSJ	Shell	(Chen and Chuang, 2019)
PRAPI	Annotation	CIRIexplorer	BSJ	Python, R	(Gao et al., 2018)
PTESFinder	Annotation	Bowtie	BSJ	Java, Shell	(Izuogu et al., 2016)
Ularcirc	Annotation	STAR	BSJ	R	(Humphreys et al., 2019)
CirComPara	Annotation	-	IPA	Python, R	(Gaffo et al., 2017)
CircRNAwrap	Annotation	-	IPA	Perl, Shell, R	(LiBu and Zhao, 2019)
DeepCirCode	*De novo*; annotation	-	ML	R	(Wang and Wang, 2019)
miARma	*De novo*	BWA (CIRI)	BSJ	Perl, Python, R	(Andrés-León and Rojas, 2019)
AutoCirc	Annotation	Bowtie2	BSJ	C++, Perl	(Zhou et al., 2017)
hppRNA	Annotation	STAR (DCC and CircTest)	BSJ	Perl, R	(Wang, 2018)

Identify circular RNA reads	Annotation	STAR	BSJ	Perl	(Smid et al., 2019)
ROP	Annotation	TopHat-Fusion, CIRCexplorer2	BSJ	Shell	(Mangul et al., 2018)
segemehl	Annotation	segemehl	BSJ	C++	(Hoffmann et al., 2009)
STARChip	Annotation	STAR	BSJ	Perl, R, Shell	(AkersSchadt and Losic, 2018)
UROBORUS	Annotation	Bowtie	BSJ	Perl	(Song et al., 2016)
circ_battle	*De novo*, annotation	-	IPA	-	(Hansen, 2018)
WebCircRNA	*De novo*; annotation	-	ML	Python	(Pan et al., 2018)
CircPro	*De novo*; annotation	BWA (CIRI2)	BSJ	Perl	(Meng et al., 2017)
CIRI	*De novo*	BWA	BSJ	Perl	(GaoWang and Zhao, 2015)
circScan	Annotation	Bowtie2	BSJ	C++, Perl	(Li et al., 2017)
circTools (starBase)	Annotation	Bowtie2	BSJ	C++	(Zhang and Yang, 2018)
RAISE	Annotation	-	IPA	Shell	(Li et al., 2017)
PredcircRNA	*De novo*; annotation	-	ML	Python	(Pan and Xiong, 2015)
CPSS 2.0	annotation	-		Perl, PHP, R	(Wan et al., 2017)
MapSplice	*De novo*; annotation	Bowtie	BSJ	Python	(Wang et al., 2010)
ACFS	*De novo*	BWA, BLAT	BSJ	Perl	(You and Conrad, 2016)
circseq-cup	Annotation	TopHat, STAR, segemehl	BSJ	Perl, Python	(Ye et al., 2017)
exceRpt	Annotation	STAR, Bowtie2	BSJ	R, Sell, Perl	(Rozowsky et al., 2019)
KNIFE	Annotation	Bowtie2, Bowtie	BSJ	Python, R, Perl, Shell	(Szabo et al., 2015)
NCLscan	Annotation	BWA, BLAT	BSJ	Python, C++	(Chen and Chuang, 2019)
PcircRNA_finder	Annotation	-	IPA	Perl, Python	(Chen et al., 2016)
PredicircRNATool	annotation	-	ML	MATLAB	(Liu et al., 2016)
find_circ	*De novo*	Bowtie2	BSJ	Python	(Memczak et al., 2013)
CircularRNAPipeline	Annotation	CIRCExplorer, TopHat-Fusion	BSJ	Python	(Fan et al., 2015)

*Abbreviations: BSJ, back-splice-junction-based method; IPA, integrated prediction algorithms; ML, machine learning-based method. ** k-mer method does not require aligner

Recently, machine learning has made significant advances in bioinformatics, and several machine learning methods are now used to differentiate circRNAs based on their sequence characteristics without relying on sequencing data. The primary advantage of using machine learning is the ability to combine several stages of information processing, including feature selection and classification (Emmert-Streib et al., 2020). Machine learning approaches have been successful in solving numerous biological problems, particularly in genetics and genomics, such as identifying transcription start sites, identifying alternative splicing sites and assigning functional annotation to genes. PredcircRNA is the first machine learning tool that classifies circRNAs using multiple kernel learning frameworks and integrates various features, including ALU and tandem repeats, SNP densities and ORFs from transcripts (Pan and Xiong, 2015). CircLGB differentiates circRNAs from other lncRNAs using three distinctive features: A-to-I deamination, A-to-I density and the internal ribosome entry site (Zhang et al., 2020). DeepCirCode is the first deep learning model built using a convolutional neural network that automatically extracts more than flanking 50-nt sequences of the backsplicing site to predict circRNAs (Wang and Wang, 2019). CircCNN is fundamentally similar to DeepCirCode but converts position probability matrix features to motifs to predict pre-mRNA backsplicing sites (Shen et al., 2022). Furthermore, machine learning models have been employed to predict gene ontology functions of circRNAs. For example, DeepciRGO uses a novel representation learning approach called HIN2Vec to calculate the topology features of both proteins and circRNAs across a heterogeneous network. Using the topology features, a deep multi-label hierarchical classification model predicts the biological process function for each circRNA in the gene ontology (Deng et al., 2020).

As discussed in the previous section, the detection and quantification of circRNAs rely on the presence of their characteristic backsplice junction. The reported number of circRNAs across different species has increased significantly and has exceeded thousands. To manage the increasing number of identified circRNAs, various databases have been established using various bioinformatic tools (GlazarPapavasileiou and

Rajewsky, 2014, Meng et al., 2019, Xia et al., 2018, Chu et al., 2017). For example, CircBase has standardized multiple circRNA datasets into a single database, enabling users to search, browse and download the genomic annotations of circRNAs (GlazarPapavasileiou and Rajewsky, 2014).

Despite the increasing number of identified circRNAs, only a few of them have been validated using experimental techniques, such as polymerase chain reaction (PCR), Northern blot, *in situ* hybridization, gel trap electrophoresis and the RNase H degradation assay (Jeck and Sharpless, 2014, Panda and Gorospe, 2018, SchindlerKrolewski and Rush, 1982, Schneider et al., 2018, You et al., 2015). Among these techniques, the most popular method for validating circRNAs is the reverse transcription (RT) of total RNA followed by PCR amplification (Figure 9). Reverse transcription quantitative PCR (RT-qPCR) is a highly reliable, accurate and quantitative technique for circRNA expression quantification. To perform PCR or qPCR assays, backsplice junction-specific divergent primer pairs are required (Hansen et al., 2011, Panda and Gorospe, 2018). Recently, various primer tools have been developed to design circRNA primers. One of the earliest backsplice junction-specific primer design tools is CircInteractome (web-based), which uses the template sequence around the circRNA junction to design primers for circRNA amplification (Dudekula et al., 2016). Later, Zhong et al. (2018) introduced circPrimer, which can annotate and extract spliced sequences for circRNAs and design primers with one primer spanning the spliced junction. However, circPrimer searches the RefSeq GTF files for transcripts with a genomic location of circRNA, which may not be effective in some teleost species due to the lack of well-annotated GTF files in the National Center for Biotechnology Information (NCBI) database. CIRCprimerXL is another web-based tool that supports circRNA primer design for specific species, such as humans, mice, rats, zebrafish, western clawed frog *(Xenopus tropicalis)* and roundworms (Vromman et al., 2022). This tool considers sequence regions with secondary structures, as well as common SNPs, when selecting primer pairs. This feature makes the use of CIRCprimerXL advantageous over other primer design tools.

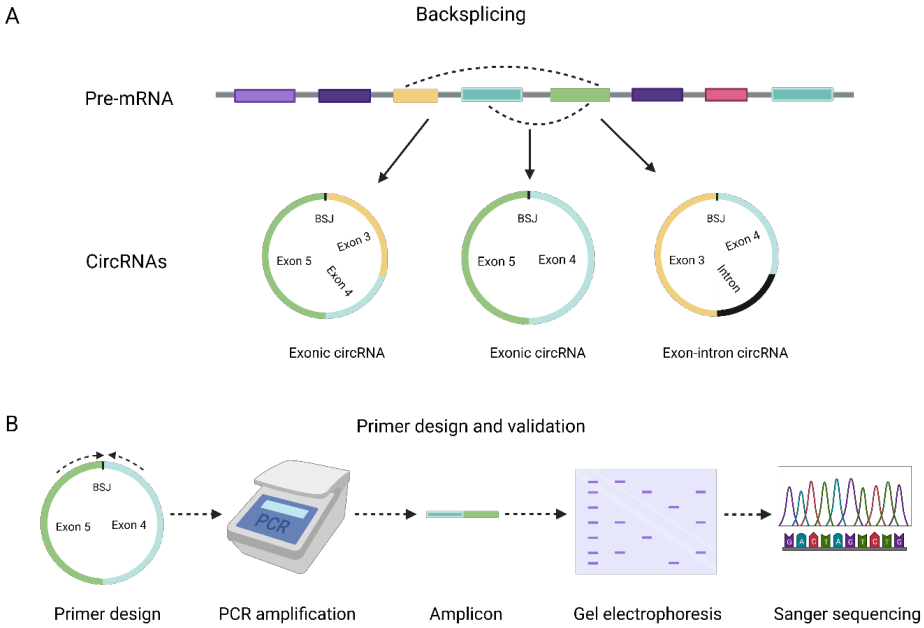

Figure 9. CircRNA backsplicing, primer design and validation: A) production of multiple circRNAs from a single gene; B) Backsplice junction (BSJ)-specific primer design and circRNA detection. Figure prepared with BioRender.

However, many researchers are skeptical of an accurate real-time quantification of circRNAs without digesting the corresponding linear transcripts (Panda and Gorospe, 2018, Vromman et al., 2021). Linear transcripts identical to the sequence surrounding the circRNA BSJ can lead to false positive circRNA signals in RT-qPCR experiments. Therefore, some studies have recommended using RNase R before quantifying circRNA via RT-qPCR; however, RNase R protocols differ in terms of their RNA input amount, enzyme concentration, incubation times and cleaning method (Vromman et al., 2021). It is crucial to check whether the primers produce multiple bands in gel or melting curves after running the RT-qPCR cycles.

As the circRNA research field grows, the bioinformatics toolbox for their identification is also expanding, which has allowed us to detect circRNAs and comprehend more

features and functions. However, circRNA annotation remains understudied and it is necessary to develop a reliable annotation tool and additional functional databases to understand the precise molecular and biochemical functions of circRNAs.

2 Objectives

Muscle growth in teleosts is a very complex process regulated by a multitude of genes and environmental factors. CircRNAs are now recognized as key regulators of gene expression in various taxa but their involvement in regulation of muscle growth in teleosts was unknown at the start of this PhD thesis.

My hypothesis is that circRNA expression in fast muscle is related to growth rate and influenced by developmental temperature, leading to long-term effects on muscle growth.

The overarching objective of this thesis is to gain a better understanding of circRNA-mediated ceRNA networks in teleost muscle growth regulation and investigate the relationship between the thermal developmental plasticity of circRNA expression and muscle growth. Nile tilapia was selected as the target species, since it is one of the most economically important aquacultured fish globally and has a fast growth rate, adaptability to different environments, and relatively short lifecycle. Additionally, it is an ideal model organism for studying teleost muscle growth regulation due to its well-documented growth patterns at different stages and availability of genomic information.

Specific objectives are:

1) To provide a user-friendly solution for DNA primer design and thermocycling conditions for circRNA identification using standard PCR methods. (paper I)
2) To gain insight into the circRNAs and competing endogenous networks involved in regulation of fish muscle growth. (paper II)
3) To investigate the thermal developmental plasticity of circRNA expression and its long-term effects on muscle growth in teleosts. (paper III)

Overall, this research contributes to a better understanding of the regulatory mechanisms underlying teleost muscle growth and provides insights into potential targets for improving aquaculture production of Nile tilapia.

3 Discussion

The aquaculture sector has undergone significant advancements over the past few decades, successfully achieving significant economic and societal sustainability goals, such as reducing the negative effects of overfishing and supplying essential nutrients to populations worldwide.

However, the aquaculture industry still faces challenges that require prompt attention to sustain its growth and efficiency. One of the most significant challenges is the culture environment, which may impose strong selection and have significant implications for the domestication of fish species. Environmental factors, such as water temperature, can significantly influence embryonic development and pose challenges in maintaining optimal growth conditions (Ayala et al., 2000, Campos et al., 2013, Campos et al., 2013). Additionally, early life experiences leave a lasting physiological memory that affects both postnatal growth and physiological function in organisms, and different coding and non-coding RNAs, and molecular signaling pathways associated with responses to environmental stimuli play significant roles in this process (CamposValente and Fernandes, 2012, Jonsson and Jonsson, 2014). Therefore, it is essential to understand circRNA expression and co-regulation mechanisms with other RNAs in fish muscle growth to improve production efficiency and profitability.

Here, we have introduced a versatile circRNA primer design tool to enhance the validation process of circular RNAs, thereby elevating the precision of circRNA-related research **(Paper I)**. Our investigative efforts have led to an extensive characterization of circRNAs within the muscle tissue of Nile tilapia, providing the first evidence of probable ceRNA networks and miRNA sponge interactions in orchestrating the regulation of muscle growth in Nile tilapia **(Paper II)**. In addition, we also showed the long-term impact of developmental temperature on muscle growth and circRNA expression in Nile tilapia (**Paper III**).

3.1 Validation of circular junctions of novel circRNAs in model and non-model organisms

CircRNA molecules have been found to play crucial roles within the cell machinery rather than being mere transcriptional byproducts. Though circRNA research in mammals has rapidly advanced and reached functional levels, studies on farmed animals have primarily focused on identifying and analyzing the expression variations associated with different biological events. Current detection studies on circRNAs are based on RNA sequencing technology and with the advancement of modern sequencing tools (including short and long-read technologies), circRNAs may now be identified in a wide range of eukaryotic species, including teleosts. However, several challenges still need to be addressed in sequencing strategies and validation processes to further our understanding of circRNAs and their roles in cellular biology.

Identifying and quantifying circRNAs can be challenging due to their low abundance and structural complexity. Since circRNAs lack free ends, the most common methods involve fragmenting circular forms for amplification. Given that circRNAs are derived from the same precursor RNA as linear mRNAs, selectively enriching circRNAs from total RNA is crucial during library preparation. Additionally, the majority of total RNA is composed of ribosomal RNA (80-90%), which can interfere with circRNA detection. Therefore, it is essential to use appropriate methods to enrich circRNA and reduce ribosomal RNA contamination. To date, various approaches have been used to prepare circRNA libraries, including, including ribosomal RNA depletion, ribosomal RNA depletion + Rnase R digestion, and ribosomal RNA depletion + RNAse R depletion + polyA depletion (Liu et al., 2021, Rybak-Wolf et al., 2015, Shi et al., 2022). This study used ribosomal RNA-depleted RNA treated with RNaseR for library preparation (**papers I, II and III**). Ribosomal RNA depletion leaves all mRNA and noncoding RNA due to sequence-specific hybridization and depletion. Further, linear RNA depletion with RNase R made it easier to detect expressed circRNA. It is worth noting that teleost species have undergone specific gene duplications, which can complicate RNA

sequencing analysis. Therefore, the use of RNase R for removing linear RNA is particularly recommended for any teleost species, as it helps to reduce the complexity of the RNA population and enhances the detection of circRNAs.

Sequencing depth and length are crucial factors in the analysis of circRNA (NguyenNguyen and Vu, 2022, GaoWang and Zhao, 2015). As circRNA expression levels are low and tissue-specific, they may be missed due to detection sensitivity, and therefore, bioinformatic tools have been developed to overcome these issues. These tools can utilize both single-end and paired-end sequencing data, with a minimum read length of 40 bp (GaoWang and Zhao, 2015). However, the required read depth for circRNA detection and quantification can vary and is dependent on the species and conditions under investigation. Studies have shown that 20-40 million reads per sample are sufficient to capture circRNA variations, but higher depths are required for better results (Robic et al., 2021, Zhang et al., 2022, GaoWang and Zhao, 2015, Szabo and Salzman, 2016, Legnini et al., 2017). In **Papers I and II**, we used a 150 bp paired-end sequencing with a depth of ~25 million reads per sample, which is well within the recommended range. Nevertheless, we used 100 bp reads with ~80 million reads per sample for **Paper III**, as we found that statistical algorithms perform better with higher depth.

Furthermore, the choice of bioinformatic tool is also critical for accurate circRNA analysis. Although different tools can yield variable results even on the same dataset, sensitivity tests have shown that CIRI2 is the most efficient tool with high sensitivity and low false discovery rate (Wang et al., 2017, Hansen et al., 2016, GaoZhang and Zhao, 2018). CIRI2 and CIRIquant (which utilizes CIRI2 for circRNA detection) are highly efficient for reads ranging from 60 to 100 bp (Zhang et al., 2020, GaoZhang and Zhao, 2018). CIRI2 can also identify circRNA *de novo*, making it a valuable tool for studying circRNAs in teleost species. Therefore, we have used CIRI2 (**Papers I and II**) and CIRIquant (**Paper III**) in our analysis to achieve higher accuracy in circRNA detection.

Validation of circRNAs is necessary as linear transcripts may lead to false-positive circRNA signals in RNA sequencing. Among the different validation methods, the highest-regarded benchmarks for identifying circRNA expression in cells and tissues are reverse transcription coupled with quantitative PCR (qPCR) (Vromman et al., 2021, Zhang et al., 2020). Sanger sequencing further confirms the amplification of the junction point. Divergent primers are used to amplify the circRNA transcripts. These primer pairs amplify in opposite directions regarding the genomic orientation, thereby amplifying a circRNA transcript without amplifying linear transcripts from that same genomic region (Vromman et al., 2021, Zhang et al., 2020). Several primer design tools, such as CircInteractome, circtools, CIRCprimerXL, CircPrimer, and CircPrimer 2.0, have been developed for specific model organisms (Dudekula et al., 2016, JakobiUvarovskii and Dieterich, 2019, Vromman et al., 2022, Zhong et al., 2018, Zhong and Feng, 2022). However, these tools differ significantly in terms of their interface and functionality. For instance, CircPrimer and CircPrimer 2.0 are convenient tools for designing junction-specific primers that are implemented as a graphical interface and a command-line interface, respectively (Zhong and Feng, 2022, Zhong et al., 2018). Since many of these tools are designed only for specific organisms, and researchers working with non-model organisms, such as teleosts and plants, struggle to find suitable primer design tools. Therefore, a user-friendly primer designing tool that can work with a wide range of organisms was necessary.

In **Paper I**, we present CircPrime, a web-based platform that provides an easier solution for DNA primer design and thermocycling conditions for circRNA identification with routine PCR methods. The web interface of CircPrime determines the genome by taxonomic ID using the NCBI database and conveniently designs primers. This tool is also useful for designing primers for circRNA quantification with qRT-PCR (**Paper II**). Compared to other primer design tools, CircPrime has several advantages. It is a web-based platform, so users do not need to install any software on their local machines. Moreover, it can accommodate a wide range of organisms, making it particularly useful for researchers studying non-model organisms. Additionally, CircPrime provides

thermocycling conditions easily accessible to researchers with varying levels of expertise in PCR methods.

Taken together, circRNA identification, junction validation and quantification are necessary for selecting effective candidate biomarkers in teleosts. Given the emerging nature of the circRNA field in teleost species, circPrime will help future researchers in effectively experimental planning and validation of circRNA-seq data. This includes utilizing techniques like qPCR and Sanger sequencing to confirm backsplicing events, facilitating a robust understanding of circRNA dynamics in future studies.

3.2 CircRNAs are abundantly expressed in fast muscle, indicating their potential role in muscle development and growth in teleosts

Tissue-specific expression is a prominent characteristic of circRNAs, and they have been implicated in regulating various biological processes, including proliferation and differentiation, by co-regulating different molecular factors. Several studies have investigated the presence and abundance of circRNAs in muscle tissues, encompassing both skeletal and cardiac muscle, across diverse species (Greco et al., 2018, YangHe and Chen, 2021). However, our knowledge of muscle circRNAs in teleost species is very limited. In zebrafish, a well-studied teleost model organism, a comprehensive genome-wide analysis identified a significant number of circRNAs in muscle, indicating their integral role within the muscle transcriptome (Sharma et al., 2019). These findings support the notion that circRNAs likely possess functional implications in muscle-related processes, including muscle development, growth, and homeostasis.

In our study in Nile tilapia, we aimed to explore the landscape of circRNAs in fast muscle tissue and their potential role in growth. The results revealed a vast number of circRNAs originating from exons, introns, intergenic regions, and the antisense strand, with a total of 5141 circRNAs identified in fast muscle tissue (**Paper III**). This extensive catalogue of circRNAs highlights the complexity and diversity of circRNAs in Nile tilapia.

Furthermore, we observed a significant discrepancy in the count of fast muscle circRNAs between fast- and slow-growing tilapia , with numbers varying from 62 to 562 (**Paper II**). These findings underscore the potential relevance of circRNAs in the realm of muscle biology, substantiating their involvement in the intricate processes of muscle growth. It is noteworthy that this variation in circRNA expression aligns with our previous experimental results contrasting wild Nile tilapia and the first generation of domesticated Nile tilapia (Nedoluzhko et al., 2020). In addition, recent studies in other fish species, such as blunt snout bream and largemouth seabass (*Micropterus salmoides*), identified a substantial number of circRNAs in muscle tissues (Bai et al., 2022, Liu et al., 2021). While the emergence of circRNAs as pivotal players in gene regulation is currently acknowledged, the exact roles and mechanisms they undertake in fish muscle remain largely unexplored. A significant hurdle arises from the intricate nature of conducting functional assays, particularly within non-model species. The conventional methods designed for linear RNAs often prove inadequate for circRNAs due to their distinct circular structure. As a result, the task of designing experiments to precisely unravel circRNA functions becomes complex. Despite the challenges presented, the observed variations in circRNA expression under different experimental conditions and the intricate interactions between circRNAs, microRNAs and proteins present compelling avenues for investigating their roles and functions.

The abundance of circRNAs in fast muscle that we found in our study (**papers I, II, and III**) can be attributed at least in part to the enrichment of the spliceosome pathway (**Paper II**). The spliceosome primarily functions by removing introns from pre-mRNA transcripts, but it also plays a role in circRNA biogenesis (Zhang et al., 2016). During the backsplicing process, the spliceosome deviates from the conventional splicing pathway, skipping the downstream splice acceptor site and joining the upstream splice donor site with the upstream splice acceptor site to form circRNAs. Several studies have provided evidence for this mechanism, showing that the spliceosome can catalyze backsplicing to generate circRNAs (Lasda and Parker, 2014, ShiLi and Zhao, 2021, Starke et al., 2015). Wang et al. (2019) identified a large number of highly expressed circRNAs

and a few lowly expressed circRNAs upon spliceosome inhibition, while Shi et al. (2021) demonstrated the presence of back-splicing products and splicing intermediates in spliceosomal complexes. These findings are in accordance with our data and support the high abundance of circRNA in Nile tilapia fast muscle.

We also observed that the majority of circRNAs in Nile tilapia muscle are derived from exonic regions (**Papers II and III**), consistent with previous findings in skeletal muscle tissues of blunt snout bream (Liu et al., 2021). This prevalence of exon-derived circRNAs suggests their potential involvement in post-transcriptional regulation and further supports the notion of circRNAs playing a regulatory role in muscle-related processes. Additionally, the GO term analysis of circRNA host genes revealed their association with developmental processes, metabolic processes, cell differentiation, muscle structure development, muscle cell development, protein binding, transcription factor binding, and transcription coregulator activity (**Paper III**).

In **Paper II**, we specifically compared the expression of circRNAs between fast and slow-growing fish and found four differentially expressed circRNAs (DEcircRNAs). This significant finding underscores the dynamic nature of circRNA expression and indicates their potential impact on the growth rate of teleosts.

In **Paper III**, our study focused on investigating the expression patterns of circRNAs in fish with different growth rates that were exposed to varying embryonic temperatures. We identified three DEcircRNAs derived from genes such as *nexilin, and titin* which are known to be associated with muscle growth. The observed differential expression of circRNAs suggests their potential role in reflecting phenotypic differences, particularly in terms of growth and muscle fiber size.

Overall, these findings highlight the dynamic nature of circRNA expression and its significant impact on muscle growth. Identification of specific DEcircRNAs associated with muscle growth in **Paper II and III** suggests their potential involvement in modulating muscle growth and influencing phenotypic differences.

3.3 Competing endogenous RNA networks (ceRNAs) and miRNA sponges may modulate teleost muscle growth

Regulation of gene expression involves diverse mechanisms, such as cis-regulation mediated by enhancer sequences and trans-regulation mediated by transcription factors and RNA binding proteins (Casamassimi and Ciccodicola, 2019, RemenyiScholer and Wilmanns, 2004). While coding RNAs have long been recognized as the primary mediators of protein synthesis, noncoding RNAs, including circRNAs, have emerged as important players in gene expression regulation (Mattick et al., 2023, YangWilusz and Chen, 2022).

We identified several muscle-associated genes, including *mapk15, igfbp2, creb5, tnfsf12, fgf16, tgfbi, capn9, myf5, fgf13, fgf14, myod1, map2k4, map3k2, cdkn3, and asb15* could contribute to significant changes in muscle development (**Paper II**). The differential expression of these muscle-associated genes suggests that they may be regulated by specific factors or pathways that are differentially active in fish with distinct growth rates. This regulation can occur at the transcriptional level through the binding of transcription factors to regulatory elements in the promoter regions and it can also involve post-transcriptional regulation, such as the interaction of RNA-binding proteins with the mRNA molecules of these genes.

In recent years, Increased exploration of the role of circRNAs and miRNAs has revealed their function in post-transcriptional regulation. These noncoding RNAs participate in ceRNA networks, where circRNAs compete with mRNAs for binding to shared microRNA response elements. One such study conducted by Lei et al. (2022) focused on chicken muscle and identified 361 miRNAs, 68 circRNAs, 599 mRNAs, and 31,063 interacting pairs through *in silico* analysis of differentially expressed circRNAs and mRNAs. Furthermore, functional studies have confirmed the presence of ceRNA network in muscle tissue, highlighting the relevance in gene expression regulation.

Through a comparison of transcriptomes between fast- and slow-growing Nile tilapia (**Paper II**), we identified differential expression of 1947 mRNAs, nine miRNAs, and four circRNAs. Notably, circMef2c (derived from the *mef2c* gene) and circLaminA/C (derived from the *laminA/C* gene) were upregulated in fast-growing fish. Through *in silico* analysis, we found that *circMef2c* exhibited target correlations with oni-miR-34, oni-miR-130b-5p, and oni-miR-202, while circLaminA/C showed target correlations with oni-miR-34, oni-miR-130b-5p, and oni-miR10819 (Figure 10). These findings provide insights into specific circRNAs and miRNAs involved in muscle growth regulation.

In addition to their interactions with circRNAs, the miRNAs oni-miR-34, oni-miR-130b-5p, and oni-miR-202 were found to have their own target mRNAs. The predicted targets of oni-miR-34 and miR-202 were *igfbp2, rpl7* and *fgf14, tet3*, respectively (paper II). GFBP2 is known to function as a carrier protein for IGF1 in fish and shellfish, regulating the availability and activity of IGF1 (Chandhini et al., 2021). This interaction allows IGFBP2 to transport IGF1 to target cells, influencing processes related to cell growth, proliferation, and differentiation (Pozios et al., 2001).

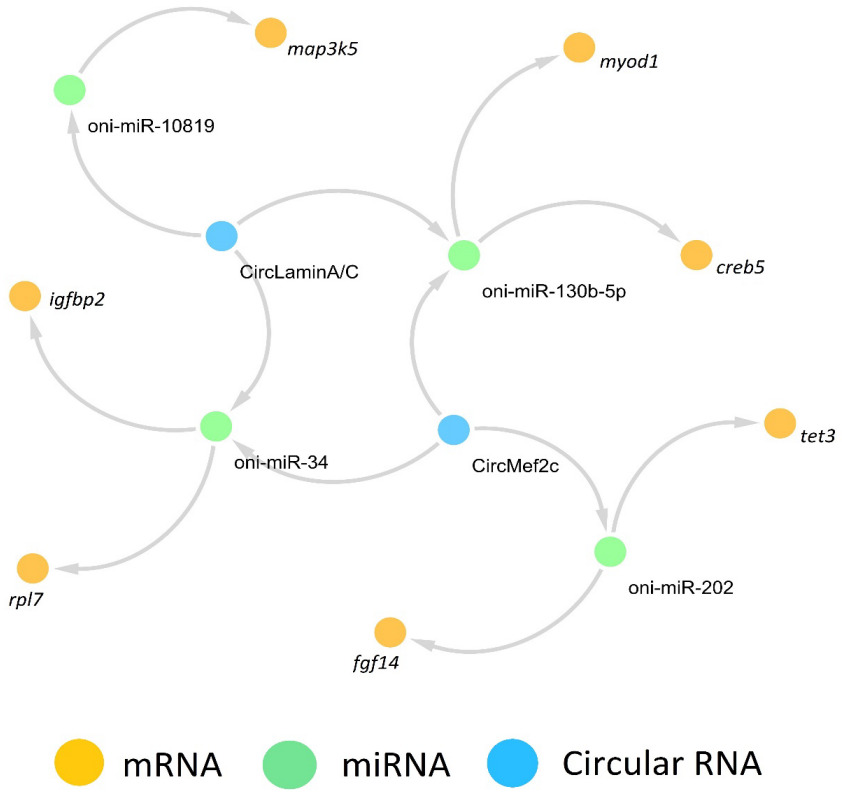

Figure 10. Simplified competing endogenous RNA (ceRNA) network in Nile tilapia muscle. This network involves 7 mRNA, 4 miRNA, and 2 circRNA components. CircRNAs are depicted as blue nodes, miRNAs as green nodes, and mRNAs as yellow nodes.

On the other hand, *rpl7* (*ribosomal protein L7*), a component of the 60s subunit of the ribosome, plays a crucial role in myoblast proliferation. Knockdown of *rpl7* has been shown to significantly increase the expression of differentiation markers such as *myogenin* and MyHC1 at both the mRNA and protein levels (Yan et al., 2021). This knockdown also leads to an accelerated formation of myotubes, indicating that *rpl7* promotes myoblast proliferation but inhibits myoblast differentiation.

fgf14 is an important gene involved in somatic growth in Nile tilapia muscle. Recent research has demonstrated that overexpression of *fgf14* affects cell proliferation and induces cell apoptosis by modulating the PI3K/AKT/mTOR pathway (Su et al., 2020). On

the other hand, *tet3*, which is a target gene for miR-202, is involved in epigenetic regulation. *tet3* is responsible for removing methyl groups from DNA molecules, a process known as DNA demethylation. Demethylation has the potential to influence gene expression and cellular differentiation. In a comparative study between wild and domesticated Nile tilapia, an upregulation of *tet3* was observed in wild tilapia, coinciding with a comprehensive demethylation process in muscle cells (Konstantinidis et al., 2020). It highlights the significant impact of *tet3* on gene transcription and the potential role of epigenetic regulation in somatic growth.

The specific roles of oni-miR-130b-5p and oni-miR-10819 in muscle proliferation, differentiation, and autophagy are not well understood. However, it has been observed that these miRNAs are expressed at higher levels in slow-growing fish compared to the fast-growing group. The biological function of miR-130b-5p depends mostly on its target genes. Huang et al. (2021) have demonstrated that the overexpression of miR-130b-5p or silencing of its target gene suppresses self-renewal ability, proliferation, migration, and invasion in both *in vitro* and *in vivo*. In addition, miR-130b-5p targets RASAL1 to promote proliferation, migration, and invasion in various contexts (Chen et al., 2018). This dual role of miR-130b-5p in different cellular contexts may be attributed to its influence on apoptosis and cell cycle arrest. Bioinformatic analysis has revealed that oni-miR-130b-5p targets and downregulates *myod1* (also known as *myod) and creb5* (**Paper II**). Downregulation of *myod1* can negatively regulate the proliferation of self-renewing myogenic stem cell populations, leading to a reduction in post-natal growth (JohnstonBower and Macqueen, 2011, Lahmann et al., 2019). Additionally, *creb5* is known as a substrate for Akt/PKB and is involved in cell processes. Previous studies in Nile tilapia have suggested its role in hydroxymethylation, an epigenetic modification (Konstantinidis et al., 2020). These findings highlight the regulatory role of miR-130b-5p and its target genes (*myod1* and *creb5*) in controlling cell survival, proliferation, and epigenetic regulation of growth.

On the other hand, oni-miR-10819 has a binding site for the *map3k5* gene, which is prominently expressed in muscle tissue and plays important roles in oxidative stress, cellular proliferation, differentiation, and immune responses (Pu et al., 2016, Yang et al., 2023, Chen et al., 2019). Silencing of *map3k5* has been linked to overall body size traits such as height and length (Yang et al., 2023).

Based on these observations, we propose that the circRNA-mediated ceRNA network, specifically involving circMef2c and circLaminA/C, may play a significant role in regulating muscle cell proliferation and differentiation during fast muscle growth in Nile tilapia.

Extensive research conducted in humans, mice, cows, goats, and chickens has highlighted the sponge function of circRNAs in regulating a wide range of developmental processes, including growth and disease. A sponge RNA must have several binding sites for miRNAs to be considered true sponges for miRNAs (Panda, 2018). For example, circFGFR4, which contains eighteen binding sites for miR-107, has been shown to promote myoblast differentiation and protect cells from apoptosis when overexpressed (Li et al., 2018). Through luciferase screening and RNA pull-down assays, authors have confirmed that circFGFR4 acts as a sponge for miR-107, further supporting its role in muscle regulation. Furthermore, circSNX29 functions as a miR-744 sponge, leading to the upregulation of CaMKIId and Wnt5a expression through activation of the Wnt pathway, thereby promoting myoblast differentiation (Peng et al., 2019).

Similarly, we have identified circNexn, circTTN, and circTTN_b as potential miRNA sponges (**Paper III**). Our analysis revealed that these circRNAs have the ability to bind to a total of 314 miRNAs. Notably, miR-27, miR-34, miR-133, miR-206, and miR-218a were detected in Nile tilapia muscle and had their own binding sites within the 3' untranslated region (UTR) of muscle-specific genes. This suggests the involvement of circRNAs in regulating muscle cell proliferation and differentiation processes.

Furthermore, we also observed that some of these miRNAs, including oni-miR-9a, oni-miR-221, oni-miR-1, and oni-miR-181b, have multiple binding sites within circTTN and circTTN_b. This indicates that circRNAs in Nile tilapia muscle have the potential to efficiently sequester multiple copies of the same or different miRNAs, making them potent miRNA sponges. By sequestering miRNAs, these circRNAs can influence the availability and activity of these regulatory molecules, ultimately impacting gene expression and contributing to the complex regulatory networks involved in muscle-related processes.

Based on the above finding, we conclude that the circRNAs identified in Nile tilapia have the potential to act as miRNA sponges and participate in ceRNA network that regulates muscle development. The ability of circRNAs to interact with multiple miRNAs, particularly those known to be involved in muscle growth and development, suggests that they may play a role in the long-term regulation of muscle growth in Nile tilapia through the combined mechanisms. These results highlight the intricate regulatory interactions between circRNAs, miRNAs, and muscle-related processes in Nile tilapia.

3.4 Regulation of circRNA expression in teleosts by developmental temperature

CircRNAs have been recognized as important regulators of embryonic development across various species. Studies in zebrafish have shown abundant expression of circRNAs at different time points during development, with differential expression patterns between different stages (Liu et al., 2019).

Given that environmental conditions can profoundly influence mRNA and miRNA expression, they are also likely to impact circRNA expression. In the case of Nile tilapia, temporal expression of circRNAs was observed under variable temperature conditions comparing fish of the same age (**Paper III**). We demonstrated that temperature differences during embryonic development could exert lasting effects on growth

patterns for up to three months post-fertilization, even when the fish were reared at a uniform temperature following the pharyngula stage. Concomitant changes in circRNA expression were observed among fish reared at different temperatures. These differences in both growth and circRNA expression profiles are undoubtedly rooted in the lasting impacts of temperature fluctuations on the intricate course of fish development. A particularly intriguing observation is the higher number of circRNAs in the control temperature group (28 °C) compared to the lower (24 °C) and higher (32 °C) temperature groups. This observation suggests that daviations in thermal conditions from an optimum value could potentially impede the complex process of circRNA biogenesis within Nile tilapia muscle tissue. This unique insight adds an intriguing layer of novelty to our study, shedding light on the nuanced interplay between circRNA expression and temperature fluctuations. Furthermore, differentially expressed circRNAs, as elucidated in **Paper III**, underscore their potential as key orchestrators of miRNAs molecules involved in temperature-responsive pathways, allowing the fish to adjust their physiological and developmental processes to optimize their survival and growth under different temperature conditions. This novel findings lends to the notion that circRNAs in fish muscle play an integral role in finely regulating gene expression and orchestrating intricate molecular responses to temperature dynamics. As a result, our research not only contributes to the growing body of knowledge on circRNAs but also unveils their potential involvement in coordinating growth responses to environmental changes, particularly in the context of temperature fluctuations.

Previous studies in rat, sows and fish have reported a significant number of circRNA expressed at heat stress condition and many of them are connected to the different biological function including metabolic, immune, and reproduction (Fan et al., 2019, Quan et al., 2021, Zhang et al., 2019). Notably, a study conducted by Quan et al. (2021) meticulously identified 324 differentially expressed circRNAs within the liver in response to heightened heat stress conditions. Furthermore, Fan et al. (2019) reported 53 differentially expressed circRNAs in heat-acclimated rats, playing roles in various biological processes.

In natural environments, water temperature undergoes seasonal variations and can be influenced by a range of anthropogenic factors, including climate change, pollution, and habitat alteration (WoodwardPerkins and Brown, 2010, Huang et al., 2021, Yuan et al., 2015). These fluctuations in temperature have significant implications for fish throughout their life stages, impacting their growth, development, and overall fitness. Extensive research has demonstrated that the effects of developmental temperature on fish growth are multifaceted and involve intricate molecular processes (Zhang et al., 2018, Campos et al., 2013, Campos et al., 2013, Campos et al., 2014). In the case of Nile tilapia, developmental temperature has been found to exert a considerable influence on various aspects of growth, particularly muscle cellularity and size (Abd El-Hack et al., 2022, Mirea et al., 2013, Santo et al., 2020). At the molecular level, temperature plays a crucial role in modulating gene expression patterns, protein synthesis, enzyme activity, hormone production, and other cellular processes vital for growth and development (Campos et al., 2013, Mathers et al., 1993, Deane and Woo, 2009, Campos et al., 2013). It has been reported that circRNAs display stress-specific expression patterns in various organisms (Li et al., 2020). For instance, novel_circ_003889, novel_circ_002325 and novel_circ_002446 modulate several genes (e.g., *hsp70, hspa13, hsp90ab1*) involved in metabolic processes, estrogen signalling pathways, and the HIF-1 signalling pathway (Quan et al., 2021). In addition, rno_circRNA_014301 and rno_circRNA_010393, along with their interactions with miRNAs and target genes (e.g., *hif-1α, lppr4, mfap4*) contribute to pathways related to hypoxia, substance/energy metabolism, and inflammatory responses (Fan et al., 2019). Specifically, circRNAs like rno_circRNA_014301 and rno_circRNA_010393, along with their interactions with miRNAs and target genes (e.g., *hif-1α, lppr4, mfap4*), play roles in these pathways. Thus, the differential expression of circNexn, circTTN, and circTTN_b in our study highlights the dynamic and adaptive nature of circRNA regulation in response to developmental temperature changes. This regulation of expression is likely to have implications on various biological and molecular functions.

The analysis of circRNA expression patterns in Nile tilapia under different developmental temperatures yields valuable new insights into the impact of temperature on circRNA regulation. By unravelling the functional roles and molecular interactions of temperature-responsive circRNAs, we can deepen our understanding of the mechanisms underlying fish growth and their ability to adapt to changing environmental conditions. This knowledge contributes to our broader comprehension of the intricate relationship between temperature, circRNAs, and developmental processes in teleosts.

3.5 Plasticity of circRNA expression and its potential correlation with long-term growth in teleost fish

If circRNAs play a role in regulating muscle function in response to temperature, it is expected that their expression would be influenced by temperature variations. As discussed in the previous section, our study has provided evidence suggesting that circRNA expression is influenced by early developmental temperature and potentially correlated with fish growth (**Paper III**).

In addition to investigating circRNA expression, we also examined the growth rate of Nile tilapia exposed to different temperatures during embryonic development. This dual approach provided us with a holistic understanding of the intricate relationship between temperature, circRNA expression and growth patterns. Our observations demonstrated that embryos exposed to 32 °C exhibited a faster somatic growth compared to those at 28 °C and 24 °C. Importantly, this difference in size (i.e., weight) persisted remarkably even four months after the initial fertilization event (**Paper III**).

The phenomenon we observed in Nile tilapia is emblematic of a broader trend in fish biology. Various fish species have displayed varying responses to temperature changes, often exhibiting medium to long-term effects on their growth patterns. For instance, studies focused on Senegalese sole showed compelling correlation between elevated temperatures and accelerated growth rates, resulting in increased body weight. In a

notable study, embryos exposed to distinct incubation temperatures—15 °C, 18 °C, and 21 °C —followed by larval rearing at 21 °C, exhibited a remarkable enhancement in somatic growth 30 days post-hatch (Campos et al., 2013). Intriguingly, the 18 °C and 21 °C groups displayed weight increments of 25 % and 27 %, respectively, compared to the 15 °C group. Similarly, zebrafish also exhibited intricate temperature-dependent growth responses. Varied temperature during early developmental stages can lead to altered growth trajectories, impacting body size and proportions even in adulthood (SchnurrYin and Scott, 2014).

Considering the complex nature of fish growth involving hyperplasia and hypertrophy during the post-embryonic growth phase, the disparities in myofiber number and size across temperature groups assume significant relevance (**Paper III**). Specifically, the 32 °C group exhibited a significantly higher number of muscle fibers, indicating increased hyperplasia. However, the size of the fish showed an opposite trend, suggesting decreased hypertrophy in the same group. Importantly, earlier research involving different strains of Nile tilapia have also demonstrated hypertrophic growth in lower temperature in comparison with the high temperature group (SalomãoSantos and Mareco, 2018). Additionally, the distinct fiber number disparities that emerge during early developmental stages bear significance over the majority of growth in adulthood (Lima et al., 2017). The orchestration of this growth process may not be solely regulated by temperature and circRNA, but also by the targeted miRNAs of differentially expressed circRNAs, as highlighted in **Paper III**. Prominent among these are miR-1, miR-7, miR-27, miR-181, miR-140, miR-144b, miR-206, miR-214 and miR-221 (**Paper III**), known for their pivotal roles in driving hypertrophy and hyperplasia during muscle growth (Hitachi and Tsuchida, 2013). In view of this multifaceted interaction, we suggest that circRNAs contribute to the fine-tuned orchestration of hyperplasia and hypertrophy events that govern Nile tilapia muscle growth.This has also been observed in a few other fish species, such as largemouth bass and blunt snout bream, highlighting the potential role of circRNAs in regulating muscle growth across diverse teleost taxa (Bai et al., 2022, Liu et al., 2021). In particular, the study focusing on skeletal muscle in

largemouth bass identified 44 circRNAs with differential expression patterns between juvenile and adult, indicating their potential involvement in muscle growth and development (Bai et al., 2022). Moreover, previous research has demonstrated a direct correlation between specific circRNAs, such as circEsyt2, circFgfr2, circMYBPC1, circRNA388, circRNA399, novel_circ_0000777, novel_circ_0001407, novel_circ_0002704, and muscle development and growth in various contexts, including fish species (Bai et al., 2022, Liu et al., 2021, Yan et al., 2022, Chen et al., 2021, Gong et al., 2021). Collectively, these findings suggest a conserved mechanism of circRNA-mediated regulation of muscle growth across different species. Taken together, our study supports the notion that circRNA expression is influenced by temperature variations during early ontogeny and may have functional implications for fish growth. The observed differences in both the number and size of muscle fibers among the temperature groups suggests that circRNAs may be involved in the observed long-term effect of embryonic temperature on muscle growth in teleosts. Further research is needed to unravel the specific mechanisms underlying these relationships and to elucidate the roles of circRNAs in regulating the phenotypic plasticity of muscle growth in fish.

4 Conclusions

This thesis provides a comprehensive characterization of circRNAs and their potential regulatory role in teleost muscle growth, pioneering the field of circRNA research in this context. Throughout our research, we discovered a vast number of circRNAs distributed across the genome, shedding light on their diverse origins and distribution patterns. While the majority of circRNAs were found to originate from the sense strand, it is notable that a significant proportion also derived from the antisense strand in the Nile tilapia genome in muscle. The differentially expressed circRNAs identified in fast muscle exhibit great potential in regulating various aspects of muscle growth. Notably, these muscle-derived circRNAs have the potential to participate in ceRNA networks and act as miRNA sponges, suggesting their involvement in the regulation of hypertrophy and hyperplasia (Figure 11). This highlights the functional relevance of circRNAs in the context of muscle development and growth.

One major challenge during our study was adopting an appropriate method for identifying circRNA and their subsequent validation. We showed that ribosomal RNA-depleted RNA treated with RNase R helps to reduce the complexity of the RNA population and enhance detection efficiency. Additionally, we successfully validated the circRNA junctions using primer design facilitated by CircPrime, a valuable platform that provides researchers with a reliable approach for circRNA validation (Figure 11).

Our research extended to temperature-related expression patterns of circRNAs in Nile tilapia muscle. Remarkably, some circRNAs exhibited expression dynamics linked to effects on long-term growth, indicating their potential as molecular markers for growth assessment. Noteworthy among these are circNexn, circTTN, and CirTTN_b boasting multiple binding sites for specific or diverse miRNAs, suggesting their pivotal roles as miRNA sponges (Figure 11). We also highlighted highlighted the co-regulation of circRNAs, miRNAs, and mRNAs within ceRNA networks with specific circRNAs, such as

circMef2c and circLaminA/C, potentially playing significant roles in muscle cell proliferation and differentiation during fast muscle growth in Nile tilapia.

Overall, this thesis significantly advances our understanding of the molecular mechanisms underlying muscle growth in Nile tilapia, with a specific focus on circRNAs. It lays a strong foundation for future research on muscle development, aiming to identify novel markers associated with muscle growth. The insights gained from this research are particularly important in the context of aquaculture and the challenges posed by global warming. Understanding the molecular mechanisms in relation to temperature variation is crucial for developing targeted strategies to optimize muscle growth and improve fish quality in aquaculture settings. The potential of circRNAs as molecular markers offers a promising avenue for assessing and monitoring the response of fish to temperature changes. By utilizing circRNAs as indicators, aquaculture practitioners can make informed decisions to enhance productivity and adapt to changing environmental conditions.

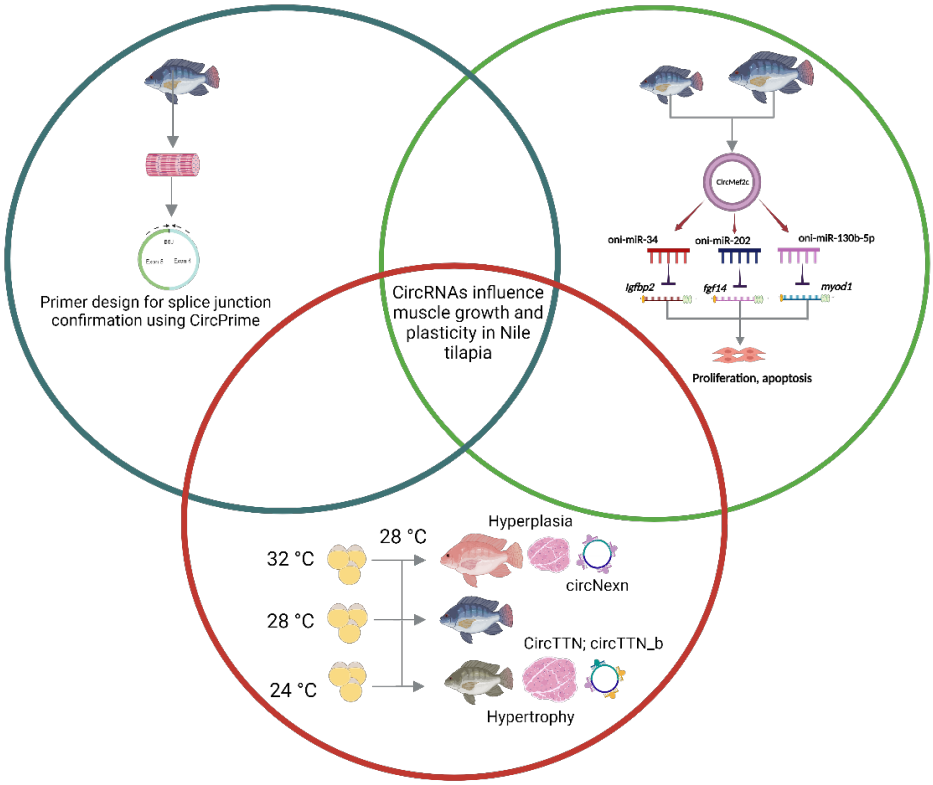

Figure 11. Key revealation of the PhD thesis. The figure summarizes the primary findings and contributions, focusing on circRNA-mediated regulatory networks in teleost muscle growth and their correlation with thermal developmental plasticity.

5 Future perspectives

The results from the two experiments conducted in Nile tilapia have identified several circRNAs with the potential to act as regulatory molecules in muscle growth and response to temperature variation. Expanding the investigation of circRNAs to other teleost fish species can provide a broader understanding of their roles in muscle growth and development. Comparative studies across different tissues, strains, and populations can uncover conserved circRNAs and regulatory mechanisms, contributing to a more comprehensive understanding of circRNA biology in fish.

In the cytoplasm, circRNAs emerge as noteworthy epigenetic regulators, particularly through their interactions with microRNAs. Adding to their functional complexity, circRNAs themselves undergo intriguing modifications. Of particular interest are N6-methyladenosine (m^6A) and N1-methyladenosine (m1A). N6-methyladenosine, found abundantly in eukaryotic mRNAs and non-coding RNAs, plays a pivotal role in post-transcriptional regulation. However, despite a few reports suggesting the involvement of m^6A and m1A modifications in circRNA stability and degradation, the role of these epigenetic modifications on muscle development and growth remains elusive (BooHa and Kim, 2022, Ren et al., 2022). Thus, there lies a compelling opportunity to explore the captivating realm of circRNA biology further. Unraveling the intricacies of their degradation pathways and uncovering potential roles of epigenetic modifications like m^6A and m1A holds the promise of unveiling novel insights into post-transcriptional gene regulation and the dynamics of cellular processes.

Furthermore, some studies have suggested that circRNAs could serve as biomarkers for economically important traits in livestock (YangHe and Chen, 2021). To validate the identified circRNAs as molecular markers for muscle growth, it is crucial to investigate their interaction mechanisms and individual effects. Future research should focus on validating these circRNAs as markers in larger populations of Nile tilapia and other commercially important fish species. Additionally, exploring the use of circRNA markers

in selective breeding programs and monitoring fish response to environmental changes can further enhance aquaculture practices. Integrating circRNA data with other omics datasets, such as proteomics and metabolomics, can provide a comprehensive view of the molecular processes involved in muscle growth, enabling the discovery of complex regulatory networks and key pathways.

Furthermore, it has been observed that circRNAs interact with chromatin-modifying enzymes, including histone methyltransferases, demethylases, acetyltransferases, deacetylases, and DNA methyltransferases (Zhang et al., 2022, Zhou et al., 2020). For instance, in the silkworm Bombyx mori, the circRNA circEgg inhibits histone H3 lysine 9 methylation by encoding the circEgg P122 protein (Wang et al., 2020). Although these mechanisms have not been reported in teleost species, it is worth exploring them. Nuclear circRNAs can also influence alternative splicing, an essential mechanism of gene regulation. Their interactions with transcription factors and RNA polymerase-associated RNA-binding proteins, pivotal components of spliceosome assembly and transcriptional regulation, underscore their intricate involvement in these processes. A promising avenue for future investigation lies in unraveling the intricate mechanism by which circRNAs serve as scaffolds, orchestrating the recruitment of chromatin-modifying complexes. Such process could potentially induce alterations in chromatin structure, consequently modulating gene transcription dynamics. Furthermore, circRNAs have been shown to shuttle between the nucleus and cytoplasm, participating in nucleocytoplasmic transport. Exploring the regulatory factors that control the nucleocytoplasmic shuttling of circRNAs is a promising direction. Investigating the protein partners that facilitate or restrict their transport, as well as the signals that govern their localization, will provide insights into the dynamic interplay between the nucleus and cytoplasm.

It is important to acknowledge that despite the identification of numerous circRNAs, there may be additional circRNAs that were not captured in the present study. The latest sequencing technologies such as Nanopore, SMRT (Single-Molecule Real-Time),

and single-cell sequencing, coupled with the appropriate bioinformatics approaches, can facilitate the discovery of novel full-length circRNAs, expanding our knowledge of their diversity and functions.

References

Abbink W, Garcia AB, Roques JAC, Partridge GJ, Kloet K & Schneider O. (2012). The effect of temperature and pH on the growth and physiological response of juvenile yellowtail kingfish *Seriola lalandi* in recirculating aquaculture systems. *Aquaculture* 330**:** 130-135.

Abd El-Hack ME, El-Saadony MT, Nader MM, Salem HM, El-Tahan AM, Soliman SM, et al. (2022). Effect of environmental factors on growth performance of Nile tilapia (*Oreochromis niloticus*). *Int J Biometeorol* 66**:** 2183-2194.

Adam AC, Saito T, Espe M, Whatmore P, Fernandes JMO, Vikesa V, et al. (2022). Metabolic and molecular signatures of improved growth in Atlantic salmon (*Salmo salar*) fed surplus levels of methionine, folic acid, vitamin B(6) and B(12) throughout smoltification. *Br J Nutr* 127**:** 1289-1302.

Akers NK, Schadt EE & Losic B. (2018). STAR Chimeric Post for rapid detection of circular RNA and fusion transcripts. *Bioinformatics* 34**:** 2364-2370.

Anderson C, Catoe H & Werner R. (2006). MIR-206 regulates connexin43 expression during skeletal muscle development. *Nucleic Acids Res* 34**:** 5863-71.

Andrés-León E & Rojas AM. (2019). miARma-Seq, a comprehensive pipeline for the simultaneous study and integration of miRNA and mRNA expression data. *Methods* 152**:** 31-40.

Ansah YB, Frimpong EA & Hallerman EM. (2014). Genetically-Improved Tilapia Strains in Africa: Potential Benefits and Negative Impacts. *Sustainability* 6**:** 3697-3721.

Arnberg A, Van Ommen G-J, Grivell L, Van Bruggen E & Borst P. (1980). Some yeast mitochondrial RNAs are circular. *Cell* 19**:** 313-319.

Asghari H, Lin Y-Y, Xu Y, Haghshenas E, Collins CC & Hach F. (2020). CircMiner: accurate and rapid detection of circular RNA through splice-aware pseudo-alignment scheme. *Bioinformatics* 36**:** 3703-3711.

Ayala MD, Abellan E, Arizcun M, Garcia-Alcazar A, Navarro F, Blanco A, et al. (2013). Muscle development and body growth in larvae and early post-larvae of shi drum, *Umbrina cirrosa* L., reared under different larval photoperiod: muscle structural and ultrastructural study. *Fish Physiol Biochem* 39**:** 807-27.

Ayala MD, Lopez-Albors O, Gil F, Latorre R, Vazquez JM, Garcia-Alcazar A, et al. (2000). Temperature effect on muscle growth of the axial musculature of the sea bass (*Dicentrarchus labrax* L.). *Anat Histol Embryol* 29**:** 235-41.

Bai Y, Ding X, Liu Z, Shen J & Huang Y. (2022). Identification and functional analysis of circRNAs in the skeletal muscle of juvenile and adult largemouth bass (*Micropterus salmoides*). *Comp Biochem Physiol Part D Genomics Proteomics* 42**:** 100969.

Balon EK. (2004). About the oldest domesticates among fishes. *J Fish Biol* 65**:** 1-27.

Barbosa PTL, Povh JA, Farias KNN, Da Silva TV, Teodoro GC, Ribeiro JS, et al. (2022). Nile tilapia production in polyculture with freshwater shrimp using an aquaponic system and biofloc technology. *Aquaculture* 551**:** 737916.

Baroiller JF, D'cotta H & Saillant E. (2009). Environmental effects on fish sex determination and differentiation. *Sex Dev* 3**:** 118-35.

Bezault E, Clota F, Derivaz M, Chevassus B & Baroiller J-F. (2007). Sex determination and temperature-induced sex differentiation in three natural populations of Nile tilapia (*Oreochromis niloticus*) adapted to extreme temperature conditions. *Aquaculture* 272**:** S3-S16.

Bizuayehu TT & Babiak I. (2014). MicroRNA in teleost fish. *Genome Biol Evol* 6**:** 1911-37.

Bizuayehu TT, Fernandes JM, Johansen SD & Babiak I. (2013). Characterization of novel precursor miRNAs using next generation sequencing and prediction of miRNA targets in Atlantic halibut. *PloS one* 8**:** e61378.

Bjørnevik M, Beattie C, Hansen T & Kiessling A. (2003). Muscle growth in juvenile Atlantic salmon as influenced by temperature in the egg and yolk sac stages and diet protein level. *J Fish Biol* 62**:** 1159-1175.

Boo SH, Ha H & Kim YK. (2022). m1A and m6A modifications function cooperatively to facilitate rapid mRNA degradation. *Cell Rep* 40**:** 111317.

Bryson-Richardson RJ, Daggett DF, Cortes F, Neyt C, Keenan DG & Currie PD. (2005). Myosin heavy chain expression in zebrafish and slow muscle composition. *Dev Dyn* 233**:** 1018-22.

Campos C, Fernandes JMO, Conceicao LEC, Engrola S, Sousa V & Valente LMP. (2013). Thermal conditions during larval pelagic phase influence subsequent somatic growth of Senegalese sole by modulating gene expression and muscle growth dynamics. *Aquaculture* 414**:** 46-55.

Campos C, Sundaram AY, Valente LM, Conceicao LE, Engrola S & Fernandes JM. (2014). Thermal plasticity of the miRNA transcriptome during Senegalese sole development. *BMC Genomics* 15**:** 525.

Campos C, Valente LM, Conceicao LE, Engrola S & Fernandes JM. (2013). Temperature affects methylation of the myogenin putative promoter, its expression and muscle cellularity in Senegalese sole larvae. *Epigenetics* 8**:** 389-97.

Campos C, Valente LM, Conceicao LE, Engrola S, Sousa V, Rocha E, et al. (2013). Incubation temperature induces changes in muscle cellularity and gene expression in Senegalese sole (*Solea senegalensis*). *Gene* 516**:** 209-17.

Campos C, Valente LM & Fernandes JM. (2012). Molecular evolution of zebrafish dnmt3 genes and thermal plasticity of their expression during embryonic development. *Gene* 500**:** 93-100.

Campos C, Valente LMP, Conceicao LEC, Engrola S & Fernandes JMO. (2014). Molecular regulation of muscle development and growth in Senegalese sole larvae exposed to temperature fluctuations. *Aquaculture* 432**:** 418-425.

Canada P, Conceicao LEC, Mira S, Teodosio R, Fernandes JMO, Barrios C, et al. (2017). Dietary protein complexity modulates growth, protein utilisation and the expression of protein digestion-related genes in Senegalese sole larvae. *Aquaculture* 479**:** 273-284.

Capel B, Swain A, Nicolis S, Hacker A, Walter M, Koopman P, et al. (1993). Circular transcripts of the testis-determining gene Sry in adult mouse testis. *Cell* 73**:** 1019-1030.

Casamassimi A & Ciccodicola A. (2019). Transcriptional Regulation: Molecules, Involved Mechanisms, and Misregulation. *Int J Mol Sci* 20**:** 1281.

Cech TR & Steitz JA. (2014). The noncoding RNA revolution-trashing old rules to forge new ones. *Cell* 157**:** 77-94.

Chaabane M, Andreeva K, Hwang JY, Kook TL, Park JW & Cooper NG. (2020). seekCRIT: detecting and characterizing differentially expressed circular RNAs using high-throughput sequencing data. *PLoS Comput Biol* 16**:** e1008338.

Chandhini S, Trumboo B, Jose S, Varghese T, Rajesh M & Kumar VJR. (2021). Insulin-like growth factor signalling and its significance as a biomarker in fish and shellfish research. *Fish Physiol Biochem* 47**:** 1011-1031.

Chen C-Y & Chuang T-J. (2019). NCLcomparator: systematically post-screening non-co-linear transcripts (circular, trans-spliced, or fusion RNAs) identified from various detectors. *BMC Bioinform* 20**:** 1-11.

Chen C, Nelson LJ, Avila MA & Cubero FJ. (2019). Mitogen-Activated Protein Kinases (MAPKs) and Cholangiocarcinoma: The Missing Link. *Cells* 8**:** 1172.

Chen H, Yang Y, Wang J, Shen D, Zhao J & Yu Q. (2018). miR-130b-5p promotes proliferation, migration and invasion of gastric cancer cells via targeting RASAL1. *Oncol Lett* 15**:** 6361-6367.

Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, et al. (2006). The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat Genet* 38**:** 228-33.

Chen L, Yu Y, Zhang X, Liu C, Ye C & Fan L. (2016). PcircRNA_finder: a software for circRNA prediction in plants. *Bioinformatics* 32**:** 3528-3529.

Chen LL. (2020). The expanding regulatory mechanisms and cellular functions of circular RNAs. *Nat Rev Mol Cell Biol* 21**:** 475-490.

Chen M, Wei X, Song M, Jiang R, Huang K, Deng Y, et al. (2021). Circular RNA circMYBPC1 promotes skeletal muscle differentiation by targeting MyHC. *Mol Ther Nucleic Acids* 24**:** 352-368.

Chen N, Zhao G, Yan X, Lv Z, Yin H, Zhang S, et al. (2018). A novel FLI1 exonic circular RNA promotes metastasis in breast cancer by coordinately regulating TET1 and DNMT1. *Genome Biol* 19**:** 218.

Cheng J, Metge F & Dieterich C. (2016). Specific identification and quantification of circular RNAs from sequencing data. *Bioinformatics* 32**:** 1094-1096.

Christie MR, Marine ML, Fox SE, French RA & Blouin MS. (2016). A single generation of domestication heritably alters the expression of hundreds of genes. *Nat Commun* 7**:** 10676.

Chu Q, Zhang X, Zhu X, Liu C, Mao L, Ye C, et al. (2017). PlantcircBase: A Database for Plant Circular RNAs. *Mol Plant* 10**:** 1126-1128.

Chuang TJ, Wu CS, Chen CY, Hung LY, Chiang TW & Yang MY. (2016). NCLscan: accurate identification of non-co-linear transcripts (fusion, trans-splicing and circular RNA) with a good balance between sensitivity and precision. *Nucleic Acids Res* 44**:** e29.

Cocquerelle C, Daubersies P, Majerus M-A, Kerckaert J-P & Bailleul B. (1992). Splicing with inverted order of exons occurs proximal to large introns. *EMBO j* 11**:** 1095-1098.

Cocquerelle C, Mascrez B, Hétuin D & Bailleul B. (1993). Mis-splicing yields circular RNA molecules. *The FASEB Journal* 7**:** 155-160.

Crane DP, Ogle DH & Shoup DE. (2020). Use and misuse of a common growth metric: guidance for appropriately calculating and reporting specific growth rate. *Rev Aquac* 12**:** 1542-1547.

Crocker CD, Chapman LJ & Martinez ML. (2013). Hypoxia-induced plasticity in the metabolic response of a widespread cichlid. *Comp Biochem Physiol B Biochem Mol Biol* 166**:** 141-7.

Da Silva Cacho JC, De Moura RST & Henry-Silva GG. (2020). Influence of Nile tilapia (*Oreochromis niloticus*) fish farming in net cages on the nutrient and particulate matter sedimentation rates in Umari reservoir, Brazilian semi-arid. *Aquac Rep* 17**:** 100358.

Deane EE & Woo NYS. (2009). Modulation of fish growth hormone levels by salinity, temperature, pollutants and aquaculture related stress: a review. *Rev Fish Biol Fish* 19**:** 97-120.

Deng L, Lin W, Wang J & Zhang J. (2020). DeepciRGO: functional prediction of circular RNAs through hierarchical deep neural networks using heterogeneous network features. *BMC Bioinform* 21**:** 519.

Deng QX, Zhao N, Zhu CH & Zhang B. (2022). Long non-coding RNAs in the physiology of aquaculture animals: a perspective update. *Rev Fish Biol Fish* 32**:** 1103-1122.

Di Agostino S, Riccioli A, De Cesaris P, Fontemaggi G, Blandino G, Filippini A, et al. (2020). Circular RNAs in Embryogenesis and Cell Differentiation With a Focus on Cancer Development. *Front Cell Dev Biol* 8**:** 389.

Ding Z, Lin J, Sun Y, Cong S, Liu S, Zhang Y, et al. (2020). miR-122-5p negatively regulates the transforming growth factor-beta/Smad signaling pathway in skeletal muscle myogenesis. *Cell Biochem Funct* 38**:** 231-238.

Dong Y, He D, Peng Z, Peng W, Shi W, Wang J, et al. (2017). Circular RNAs in cancer: an emerging key player. *J Hematol Oncol* 10**:** 1-8.

Dos Santos JCE & Luz RK. (2009). Effect of salinity and prey concentrations on *Pseudoplatystoma corruscans*, *Prochilodus costatus* and *Lophiosilurus alexandri* larviculture. *Aquaculture* 287**:** 324-328.

Du WW, Fang L, Yang W, Wu N, Awan FM, Yang Z, et al. (2017). Induction of tumor apoptosis through a circular RNA enhancing Foxo3 activity. *Cell Death Differ* 24**:** 357-370.

Duan C, Ren H & Gao S. (2010). Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation. *Gen Comp Endocrinol* 167**:** 344-51.

Dubinska-Magiera M, Daczewska M, Lewicka A, Migocka-Patrzalek M, Niedbalska-Tarnowska J & Jagla K. (2016). Zebrafish: A Model for the Study of Toxicants Affecting Muscle Development and Function. *Int J Mol Sci* 17**:** 1941.

Dudekula DB, Panda AC, Grammatikakis I, De S, Abdelmohsen K & Gorospe M. (2016). CircInteractome: a web tool for exploring circular RNAs and their interacting proteins and microRNAs. *RNA Biol* 13**:** 34-42.

El-Naggar HA, Allah HMMK, Masood MF, Shaban WM & Bashar MAE. (2019). Food and feeding habits of some Nile River fish and their relationship to the availability of natural food resources. *Egypt J Aquat Res* 45**:** 273-280.

El-Zaeem SY & Salam GM. (2013). Production of genetically male tilapia through interspecific hybridization between *Oreochromis niloticus* and *O-aureus*. *Iran J Fish Sci* 12**:** 802-812.

El-Sayed AFM & Fitzsimmons K. (2023). From Africa to the world—The journey of Nile tilapia. *Rev Aquac* 15**:** 6-21.

El-Sayed AFM & Kawanna M. (2007). Effects of photoperiod on growth and spawning efficiency of Nile tilapia (*Oreochromis niloticus* L.) broodstock in a recycling system. *Aquac Res* 38**:** 1242-1247.

Emmert-Streib F, Yang Z, Feng H, Tripathi S & Dehmer M. (2020). An Introductory Review of Deep Learning for Prediction Models With Big Data. *Front Artif Intell* 3**:** 4.

Evers HG, Pinnegar JK & Taylor MI. (2019). Where are they all from?–sources and sustainability in the ornamental freshwater fish trade. *J Fish Biol* 94**:** 909-916.

Fabrice T. (2018). Fish domestication: an overview. *Animal domestication*.

Fan L, An G, Wang S, Chen X, Liu Y, Liu Z, et al. (2019). Circular RNA Expression Profiling and Selection of Key Circular RNAs in the Hypothalamus of Heat-Acclimated Rats. *Front Physiol* 10**:** 1112.

Fan X, Zhang X, Wu X, Guo H, Hu Y, Tang F, et al. (2015). Single-cell RNA-seq transcriptome analysis of linear and circular RNAs in mouse preimplantation embryos. *Genome Biol* 16**:** 1-17.

Fao. (2019). Food and Agricultural Organization. Yearbook of Fishery and Aquaculture Statistics 2017.

Fao. (2022). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome.

Feng J, Xiang Y, Xia S, Liu H, Wang J, Ozguc FM, et al. (2019). CircView: a visualization and exploration tool for circular RNAs. *Briefings Bioinf* 20**:** 745-751.

Fernandes JM, Mackenzie MG, Elgar G, Suzuki Y, Watabe S, Kinghorn JR, et al. (2005). A genomic approach to reveal novel genes associated with myotube formation in the model teleost, *Takifugu rubripes*. *Physiol Genomics* 22**:** 327-38.

Ferreira HJ, Davalos V, De Moura MC, Soler M, Perez-Salvia M, Bueno-Costa A, et al. (2018). Circular RNA CpG island hypermethylation-associated silencing in human cancer. *Oncotarget* 9**:** 29208-29219.

Friedman RC, Farh KK, Burge CB & Bartel DP. (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 19**:** 92-105.

Fuentes EN, Bjornsson BT, Valdes JA, Einarsdottir IE, Lorca B, Alvarez M, et al. (2011). IGF-I/PI3K/Akt and IGF-I/MAPK/ERK pathways in vivo in skeletal muscle are regulated by nutrition and contribute to somatic growth in the fine flounder. *Am J Physiol Regul Integr Comp Physiol* 300**:** R1532-42.

Fuentes EN, Valdes JA, Molina A & Bjornsson BT. (2013). Regulation of skeletal muscle growth in fish by the growth hormone--insulin-like growth factor system. *Gen Comp Endocrinol* 192**:** 136-48.

Gaffo E, Bonizzato A, Te Kronnie G & Bortoluzzi S. (2017). CirComPara: a multi-method comparative bioinformatics pipeline to detect and study circRNAs from RNA-seq data. *Non-coding RNA* 3**:** 8.

Gaffo E, Buratin A, Dal Molin A & Bortoluzzi S. (2022). Sensitive, reliable and robust circRNA detection from RNA-seq with CirComPara2. *Brief Bioinformatics* 23**:** bbab418.

Galman O & Avtalion R (1983). A preliminary investigation of the characteristics of red tilapias from the Philippines and Taiwan. *Proceedings of the International Symposium on Tilapia in Aquaculture.* Tel-Aviv Univ. Press Tel-Aviv.

Gao Y, Wang H, Zhang H, Wang Y, Chen J & Gu L. (2018). PRAPI: post-transcriptional regulation analysis pipeline for Iso-Seq. *Bioinformatics* 34**:** 1580-1582.

Gao Y, Wang J & Zhao F. (2015). CIRI: an efficient and unbiased algorithm for de novo circular RNA identification. *Genome Biol* 16**:** 4.

Gao Y, Zhang J & Zhao F. (2018). Circular RNA identification based on multiple seed matching. *Briefings Bioinf* 19**:** 803-810.

Gjedrem T. (2012). Genetic improvement for the development of efficient global aquaculture: A personal opinion review. *Aquaculture* 344**:** 12-22.

Glazar P, Papavasileiou P & Rajewsky N. (2014). circBase: a database for circular RNAs. *RNA* 20**:** 1666-70.

Gong X, Tian M, Cao N, Yang P, Xu Z, Zheng S, et al. (2021). Circular RNA circEsyt2 regulates vascular smooth muscle cell remodeling via splicing regulation. *J Clin Invest* 131.

Goody MF & Henry CA. (2018). A need for NAD+ in muscle development, homeostasis, and aging. *Skelet Muscle* 8**:** 9.

Greco S, Cardinali B, Falcone G & Martelli F. (2018). Circular RNAs in muscle function and disease. *Int J Mol Sci* 19**:** 3454.

Guo M, Wang Y, Fu X, Tao W & Li C. (2023). circRNA1149 from Apostichopus japonicus suppresses coelomocyte apoptosis act as miR-92a sponge to regulate Bax expression in response to *Vibrio splendidus* infection. *Aquaculture* 562**:** 738812.

Hall IF, Climent M, Quintavalle M, Farina FM, Schorn T, Zani S, et al. (2019). Circ_Lrp6, a circular RNA enriched in vascular smooth muscle cells, acts as a sponge regulating miRNA-145 function. *Circ Res* 124**:** 498-510.

Hansen TB. (2018). Improved circRNA identification by combining prediction algorithms. *Front Cell Dev Biol* 6**:** 20.

Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, et al. (2013). Natural RNA circles function as efficient microRNA sponges. *Nature* 495**:** 384-388.

Hansen TB, Venø MT, Damgaard CK & Kjems J. (2016). Comparison of circular RNA prediction tools. *Nucleic Acids Res* 44**:** e58-e58.

Hansen TB, Wiklund ED, Bramsen JB, Villadsen SB, Statham AL, Clark SJ, et al. (2011). miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA. *EMBO J* 30**:** 4414-22.

Harache Y. (2002). Development and diversification issues in aquaculture. A historical and dynamic view of fish culture diversification. *Cah Options Mediterr* 59**:** 15-23.

Henry CA & Amacher SL. (2004). Zebrafish slow muscle cell migration induces a wave of fast muscle morphogenesis. *Dev Cell* 7**:** 917-923.

Hitachi K & Tsuchida K. (2013). Role of microRNAs in skeletal muscle hypertrophy. *Front Physiol* 4**:** 408.

Hoffmann S, Otto C, Kurtz S, Sharma CM, Khaitovich P, Vogel J, et al. (2009). Fast mapping of short sequences with mismatches, insertions and deletions using index structures. *PLoS Comput Biol* 5**:** e1000502.

Horak M, Novak J & Bienertova-Vasku J. (2016). Muscle-specific microRNAs in skeletal muscle development. *Dev Biol* 410**:** 1-13.

Horsley V & Pavlath GK. (2004). Forming a multinucleated cell: molecules that regulate myoblast fusion. *Cells Tissues Organs* 176**:** 67-78.

Houston RD, Bean TP, Macqueen DJ, Gundappa MK, Jin YH, Jenkins TL, et al. (2020). Harnessing genomics to fast-track genetic improvement in aquaculture. *Nat Rev Genet* 21**:** 389-409.

Huang A, Zheng H, Wu Z, Chen M & Huang Y. (2020). Circular RNA-protein interactions: functions, mechanisms, and identification. *Theranostics* 10**:** 3503.

Huang B & Zhang R. (2014). Regulatory non-coding RNAs: revolutionizing the RNA world. *Mol Biol Rep* 41**:** 3915-23.

Huang C, Li Y, Hu S, Chi J, Lin G, Lin C, et al. (2012). Differential expression patterns of growth-related microRNAs in the skeletal muscle of Nile tilapia (*Oreochromis niloticus*). *J Anim Sci* 90**:** 4266-4279.

Huang MR, Ding LY, Wang J, Ding CZ & Tao J. (2021). The impacts of climate change on fish growth: A summary of conducted studies and current knowledge. *Ecol Indic* 121**:** 106976.

Huang S, Yang B, Chen B, Bliim N, Ueberham U, Arendt T, et al. (2017). The emerging role of circular RNAs in transcriptome regulation. *Genomics* 109**:** 401-407.

Huang Y & Luo F. (2021). Elevated microRNA-130b-5p or silenced ELK1 inhibits self-renewal ability, proliferation, migration, and invasion abilities, and promotes apoptosis of cervical cancer stem cells. *IUBMB Life* 73**:** 118-129.

Humphreys DT, Fossat N, Demuth M, Tam PP & Ho JW. (2019). Ularcirc: visualization and enhanced analysis of circular RNAs via back and canonical forward splicing. *Nucleic Acids Res* 47**:** e123-e123.

Izuogu OG, Alhasan AA, Alafghani HM, Santibanez-Koref M, Elliott DJ & Jackson MS. (2016). PTESFinder: a computational method to identify post-transcriptional exon shuffling (PTES) events. *BMC Bioinform* 17**:** 1-11.

Jakobi T, Uvarovskii A & Dieterich C. (2019). circtools-a one-stop software solution for circular RNA research. *Bioinformatics* 35**:** 2326-2328.

Jeck WR & Sharpless NE. (2014). Detecting and characterizing circular RNAs. *Nat Biotechnol* 32**:** 453-61.

Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, et al. (2013). Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 19**:** 141-57.

Jew CJ, Wegner NC, Yanagitsuru Y, Tresguerres M & Graham JB (2013). Atmospheric oxygen levels affect mudskipper terrestrial performance: implications for early tetrapods. Oxford University Press.

Jia G-y, Wang D-l, Xue M-z, Liu Y-w, Pei Y-c, Yang Y-q, et al. (2019). CircRNAFisher: a systematic computational approach for de novo circular RNA identification. *Acta Pharmacol Sin* 40**:** 55-63.

Jiang Q, Yan M, Zhao Y, Zhou X, Yin L, Feng L, et al. (2021). Dietary isoleucine improved flesh quality, muscle antioxidant capacity, and muscle growth associated with AKT/TOR/S6K1 and AKT/FOXO3a signaling in hybrid bagrid catfish (*Pelteobagrus vachelli*female symbol x *Leiocassis longirostris*male symbol). *J Anim Sci Biotechnol* 12**:** 53.

Johnston IA. (1999). Muscle development and growth: potential implications for flesh quality in fish. *Aquaculture* 177**:** 99-115.

Johnston IA. (2006). Environment and plasticity of myogenesis in teleost fish. *J Exp Biol* 209**:** 2249-2264.

Johnston IA, Bower NI & Macqueen DJ. (2011). Growth and the regulation of myotomal muscle mass in teleost fish. *J Exp Biol* 214**:** 1617-1628.

Johnston IA, Lee HT, Macqueen DJ, Paranthaman K, Kawashima C, Anwar A, et al. (2009). Embryonic temperature affects muscle fibre recruitment in adult zebrafish: genome-wide changes in gene and microRNA expression associated with the transition from hyperplastic to hypertrophic growth phenotypes. *J Exp Biol* 212**:** 1781-93.

Johnston IA, Manthri S, Robertson B, Campbell P, Mitchell D & Alderson R. (2000). Family and population differences in muscle fibre recruitment in farmed Atlantic salmon (*Salmo salar*). *BAM-PADOVA*- 10**:** 291-296.

Johnston IA, Manthri S, Smart A, Campbell P, Nickell D & Alderson R. (2003). Plasticity of muscle fibre number in seawater stages of Atlantic salmon in response to photoperiod manipulation. *J Exp Biol* 206**:** 3425-35.

Jonsson B & Jonsson N. (2014). Early environment influences later performance in fishes. *J Fish Biol* 85**:** 151-88.

Karkach AS. (2006). Trajectories and models of individual growth. *Demographic Res* 15**:** 348-+.

Keenan SR & Currie PD. (2019). The Developmental Phases of Zebrafish Myogenesis. *J Dev Biol* 7**:** 12.

Kiessling A, Ruohonen K & Bjørnevik M. (2006). Muscle fibre growth and quality in fish. *Arch Anim Breed* 49**:** 137-146.

Konstantinidis I, Saetrom P, Mjelle R, Nedoluzhko AV, Robledo D & Fernandes JMO. (2020). Major gene expression changes and epigenetic remodelling in Nile tilapia muscle after just one generation of domestication. *Epigenetics* 15**:** 1052-1067.

Kos A, Dijkema R, Arnberg AC, Van Der Meide PH & Schellekens H. (1986). The hepatitis delta (delta) virus possesses a circular RNA. *Nature* 323**:** 558-60.

Kumar G & Engle CR. (2016). Technological Advances that Led to Growth of Shrimp, Salmon, and Tilapia Farming. *Rev Fish Sci Aquac* 24**:** 136-152.

Lahmann I, Bröhl D, Zyrianova T, Isomura A, Czajkowski MT, Kapoor V, et al. (2019). Oscillations of MyoD and Hes1 proteins regulate the maintenance of activated muscle stem cells. *Genes Dev* 33**:** 524-535.

Lanes CF, Bolla S, Fernandes JM, Nicolaisen O, Kiron V & Babiak I. (2012). Nucleotide enrichment of live feed: a promising protocol for rearing of Atlantic cod *Gadus morhua* larvae. *Mar Biotechnol (NY)* 14**:** 544-58.

Lasda E & Parker R. (2014). Circular RNAs: diversity of form and function. *RNA* 20**:** 1829-1842.

Latimer M, Sabin N, Le Cam A, Seiliez I, Biga P & Gabillard JC. (2017). miR-210 expression is associated with methionine-induced differentiation of trout satellite cells. *J Exp Biol* 220**:** 2932-2938.

Lee RC, Feinbaum RL & Ambros V. (1993). The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 75**:** 843-54.

Legnini I, Di Timoteo G, Rossi F, Morlando M, Briganti F, Sthandier O, et al. (2017). Circ-ZNF609 Is a Circular RNA that Can Be Translated and Functions in Myogenesis. *Mol Cell* 66**:** 22-37 e9.

Lei Q, Hu X, Han H, Wang J, Liu W, Zhou Y, et al. (2022). Integrative analysis of circRNA, miRNA, and mRNA profiles to reveal ceRNA regulation in chicken muscle development from the embryonic to post-hatching periods. *BMC Genom* 23**:** 342.

Leprivier G, Rotblat B, Khan D, Jan E & Sorensen PH. (2015). Stress-mediated translational control in cancer cells. *Biochim Biophys Acta* 1849**:** 845-60.

Li B, Zhang X-Q, Liu S-R, Liu S, Sun W-J, Lin Q, et al. (2017). Discovering the Interactions between Circular RNAs and RNA-binding Proteins from CLIP-seq Data using circScan. *BioRxiv***:** 115980.

Li D, Li Z, Yang Y, Zeng X, Li Y, Du X, et al. (2020). Circular RNAs as biomarkers and therapeutic targets in environmental chemical exposure-related diseases. *Environ Res* 180**:** 108825.

Li H, Wei X, Yang J, Dong D, Hao D, Huang Y, et al. (2018). circFGFR4 Promotes Differentiation of Myoblasts via Binding miR-107 to Relieve Its Inhibition of Wnt3a. *Mol Ther Nucleic Acids* 11**:** 272-283.

Li H, Yang J, Wei X, Song C, Dong D, Huang Y, et al. (2018). CircFUT10 reduces proliferation and facilitates differentiation of myoblasts by sponging miR-133a. *J Cell Physiol* 233**:** 4643-4651.

Li HL, Lin HR & Xia JH. (2017). Differential Gene Expression Profiles and Alternative Isoform Regulations in Gill of Nile Tilapia in Response to Acute Hypoxia. *Mar Biotechnol (NY)* 19**:** 551-562.

Li L, Bu D & Zhao Y. (2019). Circ RNA wrap–a flexible pipeline for circ RNA identification, transcript prediction, and abundance estimation. *FEBS Lett* 593**:** 1179-1189.

Li L, Zheng Y-C, Kayani MUR, Xu W, Wang G-Q, Sun P, et al. (2017). Comprehensive analysis of circRNA expression profiles in humans by RAISE. *Int J Oncol* 51**:** 1625-1638.

Li M, Yang H, Zhao J, Fang L, Shi H, Li M, et al. (2014). Efficient and heritable gene targeting in tilapia by CRISPR/Cas9. *Genetics* 197**:** 591-9.

Li X, Chu C, Pei J, Măndoiu I & Wu Y. (2018). CircMarker: a fast and accurate algorithm for circular RNA detection. *BMC Genom* 19**:** 79-87.

Li X & Wu Y. (2020). Detecting circular RNA from high-throughput sequence data with de Bruijn graph. *BMC Genom* 21**:** 1-11.

Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, et al. (2015). Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol* 22**:** 256.

Li Z, Kearse MG & Huang C. (2019). The nuclear export of circular RNAs is primarily defined by their length. *RNA Biol* 16**:** 1-4.

Lima EC, Povh JA, Otonel RA, Leonhardt JH, Alfieri AA, Headley SA, et al. (2017). Morphology and muscle gene expression in GIFT and Supreme Nile tilapia varieties reared in two cultivation systems. *Genet Mol Res* 16**:** 1-15.

Lin G, Chua E, Orban L & Yue GH. (2016). Mapping QTL for Sex and Growth Traits in Salt-Tolerant Tilapia (*Oreochromis spp*. X*O. mossambicus*). *PloS one* 11**:** e0166723.

Ling Y, Zheng Q, Zhu L, Xu L, Sui M, Zhang Y, et al. (2020). Trend analysis of the role of circular RNA in goat skeletal muscle development. *BMC Genom* 21**:** 220.

Liu F, Sun F, Li J, Xia JH, Lin G, Tu RJ, et al. (2013). A microsatellite-based linkage map of salt tolerant tilapia (*Oreochromis mossambicus* x *Oreochromis spp*.) and mapping of sex-determining loci. *BMC Genom* 14**:** 58.

Liu F, Sun F, Xia JH, Li J, Fu GH, Lin G, et al. (2014). A genome scan revealed significant associations of growth traits with a major QTL and GHR2 in tilapia. *Sci Rep* 4**:** 7256.

Liu H, Hu Y, Yin J, Yan XY, Chen WJ, Jiang CY, et al. (2019). Profiles analysis reveals circular RNAs involving zebrafish physiological development. *J Cell Physiol* 234**:** 15922-15933.

Liu L, Chen Y, Diao J, Luo L & Gao Z. (2021). Identification and Characterization of Novel circRNAs Involved in Muscle Growth of Blunt Snout Bream (*Megalobrama amblycephala*). *Int J Mol Sci* 22**:** 10056.

Liu M & Zhang S. (2011). Amphioxus IGF-like peptide induces mouse muscle cell development via binding to IGF receptors and activating MAPK and PI3K/Akt signaling pathways. *Mol Cell Endocrinol* 343**:** 45-54.

Liu Z, Han J, Lv H, Liu J & Liu R. (2016). Computational identification of circular RNAs based on conformational and thermodynamic properties in the flanking introns. *Comput Biol Chem* 61**:** 221-225.

Liu Z, Tao C, Li S, Du M, Bai Y, Hu X, et al. (2021). circFL-seq reveals full-length circular RNAs with rolling circular reverse transcription and nanopore sequencing. *Elife* 10**:** e69457.

Lou Z, Zhao Y, Zhang Y, Zheng B, Feng H, Hosain MA, et al. (2021). MiR-2014-5p and miR-1231-5p regulate muscle growth of Larimichthys crocea by targeting MSTN gene. *Comp Biochem Physiol B Biochem Mol Biol* 252**:** 110535.

Lugert V, Thaller G, Tetens J, Schulz C & Krieter J. (2016). A review on fish growth calculation: multiple functions in fish production and their specific application. *Rev Aquac* 8**:** 30-42.

Luz RK & Dos Santos JCE. (2010). Effect of salt addition and feeding frequency on cascudo preto *Rhinelepis aspera* (Pisces: Loricariidae) larviculture. *J Appl Ichthyol* 26**:** 453-455.

Ma X-K, Xue W, Chen L-L & Yang L. (2021). CIRCexplorer pipelines for circRNA annotation and quantification from non-polyadenylated RNA-seq datasets. *Methods* 196**:** 3-10.

Macqueen DJ, Garcia De La Serrana D & Johnston IA. (2013). Evolution of ancient functions in the vertebrate insulin-like growth factor system uncovered by study of duplicated salmonid fish genomes. *Mol Biol Evol* 30**:** 1060-76.

Macqueen DJ & Johnston IA. (2008). Evolution of follistatin in teleosts revealed through phylogenetic, genomic and expression analyses. *Dev Genes Evol* 218**:** 1-14.

Mangul S, Yang HT, Strauli N, Gruhl F, Porath HT, Hsieh K, et al. (2018). ROP: dumpster diving in RNA-sequencing to find the source of 1 trillion reads across diverse adult human tissues. *Genome Biol* 19**:** 1-12.

Maniatis T & Reed R. (1987). The role of small nuclear ribonucleoprotein particles in pre-mRNA splicing. *Nature* 325**:** 673-678.

Martins CI, Ochola D, Ende SS, Eding EH & Verreth JA. (2009). Is growth retardation present in Nile tilapia *Oreochromis niloticus* cultured in low water exchange recirculating aquaculture systems? *Aquaculture* 298**:** 43-50.

Mathers EM, Houlihan DF, Mccarthy ID & Burren LJ. (1993). Rates of Growth and Protein-Synthesis Correlated with Nucleic-Acid Content in Fry of Rainbow-Trout, *Oncorhynchus-Mykiss* - Effects of Age and Temperature. *J Fish Biol* 43**:** 245-263.

Mattick JS, Amaral PP, Carninci P, Carpenter S, Chang HY, Chen LL, et al. (2023). Long non-coding RNAs: definitions, functions, challenges and recommendations. *Nat Rev Mol Cell Biol* 24**:** 430-447.

Mcconnell SK, Beynon C, Leamon J & Skibinski DO. (2000). Microsatellite marker based genetic linkage maps of *Oreochromis aureus* and *O. niloticus* (Cichlidae): extensive linkage group segment homologies revealed. *Anim Genet* 31**:** 214-8.

Melo LH, Martins YS, Melo RMC, Prado PS, Luz RK, Bazzoli N, et al. (2019). Low salinity negatively affects early larval development of Nile tilapia, *Oreochromis niloticus*: insights from skeletal muscle and molecular biomarkers. *Zygote* 27**:** 375-381.

Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, et al. (2013). Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 495**:** 333-8.

Meng X, Chen Q, Zhang P & Chen M. (2017). CircPro: an integrated tool for the identification of circRNAs with protein-coding potential. *Bioinformatics* 33**:** 3314-3316.

Meng X, Hu D, Zhang P, Chen Q & Chen M. (2019). CircFunBase: a database for functional circular RNAs. *Database (Oxford)* 2019.

Menzel P & Meyer IM. (2019). BIQ: A method for searching circular RNAs in transcriptome databases by indexing backsplice junctions. *bioRxiv***:** 556993.

Metge F, Czaja-Hasse LF, Reinhardt R & Dieterich C. (2017). FUCHS—towards full circular RNA characterization using RNAseq. *PeerJ* 5**:** e2934.

Miao W & Wang W. (2020). Trends of aquaculture production and trade: Carp, tilapia, and shrimp. *Asian Fish Sci* 33**:** 1-10.

Mirea C, Cristea V, Grecu IR & Dediu L. (2013). Influence of different water temperature on intensive growth performance of Nile tilapia *Oreochromis niloticus* Linnaeus 1758 in a recirculating aquaculture system. *Lucr Ştiinţifice-Seria Zooteh* 60**:** 227-231.

Moore MJ & Proudfoot NJ. (2009). Pre-mRNA processing reaches back totranscription and ahead to translation. *Cell* 136**:** 688-700.

Moses M, Chauka LJ, De Koning DJ, Palaiokostas C & Mtolera MSP. (2021). Growth performance of five different strains of Nile tilapia (*Oreochromis niloticus*) introduced to Tanzania reared in fresh and brackish waters. *Sci Rep* 11**:** 11147.

Mota VC, Hop J, Sampaio LA, Heinsbroek LTN, Verdegem MCJ, Eding EH, et al. (2018). The effect of low pH on physiology, stress status and growth performance of turbot (*Psetta maxima* L.) cultured in recirculating aquaculture systems. *Aquac Res* 49**:** 3456-3467.

Mota VC, Martins CIM, Eding EH, Canario AVM & Verreth JAJ. (2014). Steroids accumulate in the rearing water of commercial recirculating aquaculture systems. *Aquac Eng* 62**:** 9-16.

Mtaki K, Limbu SM, Mmochi AJ & Mtolera MS. (2022). Hybrids production as a potential method to control prolific breeding in tilapia and adaptation to aquaculture climate-induced drought. *Fish aquac* 7**:** 647-652.

Nachtigall PG, Dias MC, Carvalho RF, Martins C & Pinhal D. (2015). MicroRNA-499 expression distinctively correlates to target genes sox6 and rod1 profiles to resolve the skeletal muscle phenotype in Nile tilapia. *PLoS One* 10**:** e0119804.

Nagasawa K, Giannetto A & Fernandes JM. (2012). Photoperiod influences growth and mll (mixed-lineage leukaemia) expression in Atlantic cod. *PLoS One* 7**:** e36908.

Nakajima T, Hudson MJ, Uchiyama J, Makibayashi K & Zhang J. (2019). Common carp aquaculture in Neolithic China dates back 8,000 years. *Nat Ecol Evol* 3**:** 1415-1418.

Naylor RL, Hardy RW, Buschmann AH, Bush SR, Cao L, Klinger DH, et al. (2021). A 20-year retrospective review of global aquaculture. *Nature* 591**:** 551-563.

Nedoluzhko A, Sharko F, Rbbani MG, Teslyuk A, Konstantinidis I & Fernandes JM. (2020). CircParser: a novel streamlined pipeline for circular RNA structure and host gene prediction in non-model organisms. *PeerJ* 8**:** e8757.

Newton R, Zhang W, Xian Z, Mcadam B & Little DC. (2021). Intensification, regulation and diversification: The changing face of inland aquaculture in China. *Ambio* 50**:** 1739-1756.

Nguyen-Chi ME, Bryson-Richardson R, Sonntag C, Hall TE, Gibson A, Sztal T, et al. (2012). Morphogenesis and cell fate determination within the adaxial cell equivalence group of the zebrafish myotome. *PLoS Genet* 8**:** e1003014.

Nguyen DT, Trac QT, Nguyen T-H, Nguyen H-N, Ohad N, Pawitan Y, et al. (2021). Circall: fast and accurate methodology for discovery of circular RNAs from paired-end RNA-sequencing data. *BMC Bioinform* 22**:** 1-18.

Nguyen MH, Nguyen HN & Vu TN. (2022). Evaluation of methods to detect circular RNAs from single-end RNA-sequencing data. *BMC Genom* 23**:** 106.

Nivelle R, Gennotte V, Kalala EJK, Ngoc NB, Muller M, Melard C, et al. (2019). Temperature preference of Nile tilapia (*Oreochromis niloticus*) juveniles induces spontaneous sex reversal. *PLoS One* 14**:** e0212504.

Noto JJ, Schmidt CA & Matera AG. (2017). Engineering and expressing circular RNAs via tRNA splicing. *RNA Biol* 14**:** 978-984.

Ouyang H, Chen X, Wang Z, Yu J, Jia X, Li Z, et al. (2018). Circular RNAs are abundant and dynamically expressed during embryonic muscle development in chickens. *DNA Res* 25**:** 71-86.

Pan X & Xiong K. (2015). PredcircRNA: computational classification of circular RNA from other long non-coding RNA using hybrid features. *Mol Biosyst* 11**:** 2219-26.

Pan X, Xiong K, Anthon C, Hyttel P, Freude KK, Jensen LJ, et al. (2018). WebCircRNA: classifying the circular RNA potential of coding and noncoding RNA. *Genes* 9**:** 536.

Panda AC (2018). Circular RNAs act as miRNA sponges. *Circular RNAs.* Springer.

Panda AC & Gorospe M. (2018). Detection and analysis of circular RNAs by RT-PCR. *Bio-protocol* 8**:** e2775-e2775.

Peng S, Song C, Li H, Cao X, Ma Y, Wang X, et al. (2019). Circular RNA SNX29 Sponges miR-744 to Regulate Proliferation and Differentiation of Myoblasts by Activating the Wnt5a/Ca2+ Signaling Pathway. *Mol Ther Nucleic Acids* 16**:** 481-493.

Peruzzi S, Puvanendran V, Riesen G, Seim RR, Hagen O, Martinez-Llorens S, et al. (2018). Growth and development of skeletal anomalies in diploid and triploid Atlantic salmon (*Salmo salar*) fed phosphorus-rich diets with fish meal and hydrolyzed fish protein. *PLoS One* 13**:** e0194340.

Phelps MP, Jaffe IM & Bradley TM. (2013). Muscle growth in teleost fish is regulated by factors utilizing the activin II B receptor. *J Exp Biol* 216**:** 3742-50.

Pittman K, Yufera M, Pavlidis M, Geffen AJ, Koven W, Ribeiro L, et al. (2013). Fantastically plastic: fish larvae equipped for a new world. *Rev Aquac* 5**:** S224-S267.

Ponzoni RW, Nguyen NH, Khaw HL, Hamzah A, Bakar KRA & Yee HY. (2011). Genetic improvement of Nile tilapia (*Oreochromis niloticus*) with special reference to the work conducted by the WorldFish Center with the GIFT strain. *Reviews in Aquaculture* 3**:** 27-41.

Pownall ME, Gustafsson MK & Emerson CP, Jr. (2002). Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. *Annu Rev Cell Dev Biol* 18**:** 747-83.

Pozios KC, Ding J, Degger B, Upton Z & Duan C. (2001). IGFs stimulate zebrafish cell proliferation by activating MAP kinase and PI3-kinase-signaling pathways. *Am J Physiol Regul Integr Comp Physiol* 280**:** R1230-9.

Pu L, Zhang LC, Zhang JS, Song X, Wang LG, Liang J, et al. (2016). Porcine MAP3K5 analysis: molecular cloning, characterization, tissue expression pattern, and copy number variations associated with residual feed intake. *Genet Mol Res* 15.

Puttaraju M & Been M. (1992). Group I permuted intron-exon (PIE) sequences self-splice to produce circular exons. *Nucleic Acids Res* 20**:** 5357-5364.

Quan J, Kang Y, Luo Z, Zhao G, Li L & Liu Z. (2021). Integrated analysis of the responses of a circRNA-miRNA-mRNA ceRNA network to heat stress in rainbow trout (*Oncorhynchus mykiss*) liver. *BMC Genom* 22**:** 48.

Rbbani G, Nedoluzhko A, Galindo-Villegas J & Fernandes JMO. (2021). Function of Circular RNAs in Fish and Their Potential Application as Biomarkers. *Int J Mol Sci* 22**:** 7119.

Rebouças VT, Lima FRdS, Cavalcante DdH & Sá MVdC. (2015). Tolerance of Nile tilapia juveniles to highly acidic rearing water. *Acta Sci - Anim Sci* 37**:** 227-233.

Remenyi A, Scholer HR & Wilmanns M. (2004). Combinatorial control of gene expression. *Nat Struct Mol Biol* 11**:** 812-5.

Ren L, Jiang Q, Mo L, Tan L, Dong Q, Meng L, et al. (2022). Mechanisms of circular RNA degradation. *Commun Biol* 5**:** 1355.

Rescan PY. (2001). Regulation and functions of myogenic regulatory factors in lower vertebrates. *Comp Biochem Physiol B Biochem Mol Biol* 130**:** 1-12.

Rescan PY. (2008). New insights into skeletal muscle development and growth in teleost fishes. *J Exp Zool B Mol Dev Evol* 310**:** 541-8.

Robic A, Cerutti C, Kuhn C & Faraut T. (2021). Comparative Analysis of the Circular Transcriptome in Muscle, Liver, and Testis in Three Livestock Species. *Front Genet* 12**:** 665153.

Rosenstein S & Hulata G. (1994). Sex reversal in the genus Oreochromis: optimization of feminization protocol. *Aquac Res* 25**:** 329-339.

Rossi GS, Turko AJ & Wright PA. (2018). Oxygen drives skeletal muscle remodeling in an amphibious fish out of water. *J Exp Biol* 221**:** jeb180257.

Rowlerson A & Veggetti A (2001). Cellular mechanisms of post-embryonic muscle growth in aquaculture species. *Muscle development and growth.* Academic Press.

Rozowsky J, Kitchen RR, Park JJ, Galeev TR, Diao J, Warrell J, et al. (2019). exceRpt: a comprehensive analytic platform for extracellular RNA profiling. *Cell Syst* 8**:** 352-357. e3.

Rybak-Wolf A, Stottmeister C, Glazar P, Jens M, Pino N, Giusti S, et al. (2015). Circular RNAs in the Mammalian Brain Are Highly Abundant, Conserved, and Dynamically Expressed. *Mol Cell* 58**:** 870-85.

Salomão RAS, Santos VBd & Mareco EA. (2018). Influence of rearing temperature on muscle growth and adipose tissue in Nile tilapia (*Oreochromis niloticus*) strains. *Acta Sci - Anim Sci* 40.

Salzman J, Gawad C, Wang PL, Lacayo N & Brown PO. (2012). Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS One* 7**:** e30733.

Sänger AM. (1993). Limits to the acclimation of fish muscle. *Rev Fish Biol Fish* 3**:** 1-15.

Sanger HL, Klotz G, Riesner D, Gross HJ & Kleinschmidt AK. (1976). Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. *PNAS* 73**:** 3852-6.

Santo AHE, De Alba G, Reis YD, Costa LS, Sanchez-Vazquez FJ, Luz RK, et al. (2020). Effects of temperature regime on growth and daily rhythms of digestive factors in Nile tilapia (*Oreochromis niloticus*) larvae. *Aquaculture* 528**:** 735545.

Sapkale PH, Singh RK & Desai AS. (2011). Optimal water temperature and pH for development of eggs and growth of spawn of common carp (*Cyprinus carpio*). *J Appl Anim Res* 39**:** 339-345.

Saravanan M, Ramesh M, Petkam R & Poopal RK. (2018). Influence of environmental salinity and cortisol pretreatment on gill Na+/K+ - ATPase activity and survival and growth rates in Cyprinus carpio. *Aquac Rep* 11**:** 1-7.

Sarma K, Prabakaran K, Krishnan P, Grinson G & Kumar AA. (2013). Response of a freshwater air-breathing fish, Clarias batrachus to salinity stress: an experimental case for their farming in brackishwater areas in Andaman, India. *Aquac Res* 21**:** 183-196.

Schindler CW, Krolewski JJ & Rush MG. (1982). Selective trapping of circular double-stranded DNA molecules in solidifying agarose. *Plasmid* 7**:** 263-70.

Schmidt CA, Giusto JD, Bao A, Hopper AK & Matera AG. (2019). Molecular determinants of metazoan tricRNA biogenesis. *Nucleic Acids Res* 47**:** 6452-6465.

Schneider T, Schreiner S, Preusser C, Bindereif A & Rossbach O. (2018). Northern Blot Analysis of Circular RNAs. *Methods Mol Biol* 1724**:** 119-133.

Schnurr ME, Yin Y & Scott GR. (2014). Temperature during embryonic development has persistent effects on metabolic enzymes in the muscle of zebrafish. *J Exp Biol* 217**:** 1370-1380.

Scott GR & Johnston IA. (2012). Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *PNAS* 109**:** 14247-52.

Sekar S, Geiger P, Adkins J, Tassone E, Serrano G, Beach TG, et al. (2020). ACValidator: a novel assembly-based approach for in silico verification of circular RNAs. *Biol Methods Protoc* 5**:** bpaa010.

Shahjahan M, Kabir M, Sumon KA, Bhowmik LR & Rashid H. (2017). Toxicity of organophosphorus pesticide sumithion on larval stages of stinging catfish Heteropneustes fossilis. *Chin J Oceanol* 35**:** 109-114.

Sharma D, Sehgal P, Mathew S, Vellarikkal SK, Singh AR, Kapoor S, et al. (2019). A genome-wide map of circular RNAs in adult zebrafish. *Sci Rep* 9**:** 1-11.

Shen Z, Shao YL, Liu W, Zhang Q & Yuan L. (2022). Prediction of Back-splicing sites for CircRNA formation based on convolutional neural networks. *BMC Genom* 23**:** 581.

Shi H, Zhou Y, Jia E, Liu Z, Pan M, Bai Y, et al. (2022). Comparative analysis of circular RNA enrichment methods. *RNA Biol* 19**:** 55-67.

Shi S, Li X & Zhao R. (2021). Detecting circRNA in purified spliceosomal P complex. *Methods* 196**:** 30-35.

Shimizu M & Dickhoff WW. (2017). Circulating insulin-like growth factor binding proteins in fish: Their identities and physiological regulation. *Gen Comp Endocrinol* 252**:** 150-161.

Shulgina NS, Churova MV, Murzina SA, Krupnova MY & Nemova NN. (2021). The Effect of Continuous Light on Growth and Muscle-Specific Gene Expression in Atlantic Salmon (*Salmo salar* L.) Yearlings. *Life* 11**:** 328.

Silva AC, Almeida DV, Nornberg BF, Figueiredo MA, Romano LA & Marins LF. (2015). Effects of Double Transgenesis of Somatotrophic Axis (GH/GHR) on Skeletal Muscle Growth of Zebrafish (*Danio rerio*). *Zebrafish* 12**:** 408-13.

Silva P, Valente LMP, Olmedo M, Alvarez-Blazquez B, Galante MH, Monteiro RAF, et al. (2011). Influence of temperature on muscle fibre hyperplasia and hypertrophy in larvae of blackspot seabream, *Pagellus bogaraveo*. *Aquac Res* 42**:** 331-340.

Skjaerven KH, Jakt LM, Dahl JA, Espe M, Aanes H, Hamre K, et al. (2016). Parental vitamin deficiency affects the embryonic gene expression of immune-, lipid transport- and apolipoprotein genes. *Sci Rep* 6**:** 34535.

Smid M, Wilting SM, Uhr K, Rodríguez-González FG, De Weerd V, Prager-Van Der Smissen WJ, et al. (2019). The circular RNome of primary breast cancer. *Genome Res* 29**:** 356-366.

Solís FJT, Fernandes JM, Sarropoulou E & Monzón IF (2022). Noncoding RNAs in fish physiology and development: miRNAs as a cornerstone in gene networks. *Cellular and Molecular Approaches in Fish Biology.* Elsevier.

Song X, Zhang N, Han P, Moon BS, Lai RK, Wang K, et al. (2016). Circular RNA profile in gliomas revealed by identification tool UROBORUS. *Nucleic Acids Res* 44**:** e87.

Starke S, Jost I, Rossbach O, Schneider T, Schreiner S, Hung L-H, et al. (2015). Exon circularization requires canonical splice signals. *Cell Rep* 10**:** 103-111.

Steinbacher P, Haslett JR, Six M, Gollmann HP, Sanger AM & Stoiber W. (2006). Phases of myogenic cell activation and possible role of dermomyotome cells in teleost muscle formation. *Dev Dyn* 235**:** 3132-43.

Steinbacher P, Marschallinger J, Obermayer A, Neuhofer A, Sanger AM & Stoiber W. (2011). Temperature-dependent modification of muscle precursor cell behaviour is an underlying reason for lasting effects on muscle cellularity and body growth of teleost fish. *J Exp Biol* 214**:** 1791-801.

Su T, Huang L, Zhang N, Peng S, Li X, Wei G, et al. (2020). FGF14 Functions as a Tumor Suppressor through Inhibiting PI3K/AKT/mTOR Pathway in Colorectal Cancer. *J Cancer* 11**:** 819-825.

Suebsong W, Poompuang S, Srisapoome P, Koonawootrittriron S, Luengnaruemitchai A, Johansen H, et al. (2019). Selection response for *Streptococcus agalactiae* resistance in Nile tilapia *Oreochromis niloticus*. *J Fish Dis* 42**:** 1553-1562.

Sukhavachana S, Poompuang S, Onming S & Luengnaruemitchai A. (2019). Heritability estimates and selection response for resistance to *Streptococcus agalactiae* in red tilapia *Oreochromis spp*. *Aquaculture* 502**:** 384-390.

Sun P & Li G. (2019). CircCode: a powerful tool for identifying circRNA coding ability. *Front Genet* 10**:** 981.

Sun P & Li G. (2019). CircCode: a powerful tool for identifying circRNA coding ability. *Front Genet* 10**:** 981.

Sun YM & Chen YQ. (2020). Principles and innovative technologies for decrypting noncoding RNAs: from discovery and functional prediction to clinical application. *J Hematol Oncol* 13**:** 109.

Szabo L, Morey R, Palpant NJ, Wang PL, Afari N, Jiang C, et al. (2015). Statistically based splicing detection reveals neural enrichment and tissue-specific induction of circular RNA during human fetal development. *Genome Biol* 16**:** 126.

Szabo L & Salzman J. (2016). Detecting circular RNAs: bioinformatic and experimental challenges. *Nat Rev Genet* 17**:** 679-692.

Takata R, Mattioli CC, Bazzoli N, Correa JD & Luz RK. (2021). The effects of salinity on growth, gill tissue and muscle cellularity in *Lophiosilurus alexandri* juvenile, a Neotropical freshwater catfish. *Aquac Res* 52**:** 4064-4075.

Tang X, Ren H, Guo M, Qian J, Yang Y & Gu C. (2021). Review on circular RNAs and new insights into their roles in cancer. *Comput Struct Biotechnol J* 19**:** 910-928.

Tao W, Sun L, Shi H, Cheng Y, Jiang D, Fu B, et al. (2016). Integrated analysis of miRNA and mRNA expression profiles in tilapia gonads at an early stage of sex differentiation. *BMC Genom* 17**:** 328.

Teletchea F. (2021). Fish domestication in aquaculture: 10 unanswered questions. *Anim Front* 11**:** 87-91.

Thorarensen H, Gustavsson A, Mallya Y, Gunnarsson S, Arnason J, Arnarson I, et al. (2010). The effect of oxygen saturation on the growth and feed conversion of Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture* 309**:** 96-102.

Trewavas E. (1983). Tilapiine fishes of the genera Sarotherodon, Oreochromis and Danakilia.

Triantaphyllopoulos KA, Cartas D & Miliou H. (2020). Factors influencing GH and IGF-I gene expression on growth in teleost fish: how can aquaculture industry benefit? *Rev Aquac* 12**:** 1637-1662.

Valente LM, Moutou KA, Conceicao LE, Engrola S, Fernandes JM & Johnston IA. (2013). What determines growth potential and juvenile quality of farmed fish species? *Rev Aquacult* 5**:** S168-S193.

Valente LMP, Cabral EM, Sousa V, Cunha L & Fernandes JMO. (2016). Plant protein blends in diets for Senegalese sole affect skeletal muscle growth, flesh texture and the expression of related genes. *Aquaculture* 453**:** 77-85.

Van Bers NE, Crooijmans RP, Groenen MA, Dibbits BW & Komen J. (2012). SNP marker detection and genotyping in tilapia. *Mol Ecol Resour* 12**:** 932-41.

Van Ginneken V, Van Eersel R, Balm P, Nieveen M & Van Den Thillart G. (1997). Tilapia are able to withstand long-term exposure to low environmental pH, judged by their energy status, ionic balance and plasma cortisol. *J Fish Biol* 51**:** 795-806.

Vélez E, Lutfi E, Azizi S, Perelló M, Salmerón C, Riera-Codina M, et al. (2017). Understanding fish muscle growth regulation to optimize aquaculture production. *Aquaculture* 467**:** 28-40.

Vicens Q & Westhof E. (2014). Biogenesis of circular RNAs. *Cell* 159**:** 13-14.

Von Hofsten J, Elworthy S, Gilchrist MJ, Smith JC, Wardle FC & Ingham PW. (2008). Prdm1-and Sox6-mediated transcriptional repression specifies muscle fibre type in the zebrafish embryo. *EMBO Rep* 9**:** 683-689.

Vong QP, Chan KM & Cheng CH. (2003). Quantification of common carp (Cyprinus carpio) IGF-I and IGF-II mRNA by real-time PCR: differential regulation of expression by GH. *J Endocrinol* 178**:** 513-21.

Vromman M, Anckaert J, Vandesompele J & Volders PJ. (2022). CIRCprimerXL: Convenient and High-Throughput PCR Primer Design for Circular RNA Quantification. *Front Bioinform* 2**:** 834655.

Vromman M, Yigit N, Verniers K, Lefever S, Vandesompele J & Volders PJ. (2021). Validation of Circular RNAs Using RT-qPCR After Effective Removal of Linear RNAs by Ribonuclease R. *Curr Protoc* 1**:** e181.

Wan C, Gao J, Zhang H, Jiang X, Zang Q, Ban R, et al. (2017). CPSS 2.0: a computational platform update for the analysis of small RNA sequencing data. *Bioinformatics* 33**:** 3289-3291.

Wang D. (2018). hppRNA—a Snakemake-based handy parameter-free pipeline for RNA-Seq analysis of numerous samples. *Brief Bioinform* 19**:** 622-626.

Wang J, Liu K, Liu Y, Lv Q, Zhang F & Wang H. (2017). Evaluating the bias of circRNA predictions from total RNA-Seq data. *Oncotarget* 8**:** 110914-110921.

Wang J & Wang L. (2019). Deep learning of the back-splicing code for circular RNA formation. *Bioinformatics* 35**:** 5235-5242.

Wang K, Singh D, Zeng Z, Coleman SJ, Huang Y, Savich GL, et al. (2010). MapSplice: accurate mapping of RNA-seq reads for splice junction discovery. *Nucleic Acids Res* 38**:** e178.

Wang M, Hou J, Muller-Mcnicoll M, Chen W & Schuman EM. (2019). Long and Repeat-Rich Intronic Sequences Favor Circular RNA Formation under Conditions of Reduced Spliceosome Activity. *iScience* 20**:** 237-247.

Wang M, Jiang S, Wu W, Yu F, Chang W, Li P, et al. (2018). Non-coding RNAs Function as Immune Regulators in Teleost Fish. *Front Immunol* 9**:** 2801.

Wang M & Lu M. (2016). Tilapia polyculture: a global review. *Aquac Res* 47**:** 2363-2374.

Wang Q, Cheng L, Liu J, Li Z, Xie S & De Silva SS. (2015). Freshwater aquaculture in PR C hina: trends and prospects. *Rev Aquac* 7**:** 283-302.

Wang X, Cao X, Dong D, Shen X, Cheng J, Jiang R, et al. (2019). Circular RNA TTN acts as a miR-432 sponge to facilitate proliferation and differentiation of myoblasts via the IGF2/PI3K/AKT signaling pathway. *Mol Ther Nucleic Acids* 18**:** 966-980.

Wang X, Ono Y, Tan SC, Chai RJ, Parkin C & Ingham PW. (2011). Prdm1a and miR-499 act sequentially to restrict Sox6 activity to the fast-twitch muscle lineage in the zebrafish embryo. *Development* 138**:** 4399-404.

Wang Y & Wang Z. (2015). Efficient backsplicing produces translatable circular mRNAs. *RNA* 21**:** 172-179.

Wang Z, Gerstein M & Snyder M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 10**:** 57-63.

Wang Z, Zhang Y, Dai K, Liang Z, Zhu M, Zhang M, et al. (2020). circEgg regulates histone H3K9me3 by sponging bmo-miR-3391-5p and encoding circEgg-P122 protein in the silkworm, Bombyx mori. *Insect Biochem Mol Biol* 124**:** 103430.

Ward Z, Pearson J, Schmeier S, Cameron V & Pilbrow A. (2021). Insights into circular RNAs: their biogenesis, detection, and emerging role in cardiovascular disease. *RNA Biol* 18**:** 2055-2072.

Wei YH, Zhao XY, Shen XX, Ye L, Zhang Y, Wang Y, et al. (2022). The expression, function, and coding potential of circular RNA circEDC3 in chicken skeletal muscle development. *J Integr Agric* 21**:** 1444-1456.

Wessels S, Sharifi RA, Luehmann LM, Rueangsri S, Krause I, Hoerstgen-Schwark G, et al. (2014). Allelic variant in the anti-Mullerian hormone gene leads to autosomal and temperature-dependent sex reversal in a selected Nile tilapia line. *PLoS One* 9**:** e104795.

Westholm JO, Miura P, Olson S, Shenker S, Joseph B, Sanfilippo P, et al. (2014). Genome-wide analysis of drosophila circular RNAs reveals their structural and sequence properties and age-dependent neural accumulation. *Cell Rep* 9**:** 1966-1980.

Woodward G, Perkins DM & Brown LE. (2010). Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philos Trans R Soc Lond B Biol Sci* 365**:** 2093-106.

Wu J, Li Y, Wang C, Cui Y, Xu T, Wang C, et al. (2019). CircAST: full-length assembly and quantification of alternatively spliced isoforms in circular RNAs. *Genom Proteom Bioinform* 17**:** 522-534.

Xia S, Feng J, Chen K, Ma Y, Gong J, Cai F, et al. (2018). CSCD: a database for cancer-specific circular RNAs. *Nucleic Acids Res* 46**:** D925-D929.

Xia S, Feng J, Lei L, Hu J, Xia L, Wang J, et al. (2017). Comprehensive characterization of tissue-specific circular RNAs in the human and mouse genomes. *Briefings Bioinf* 18**:** 984-992.

Xin Z, Wu-Ying C, Ping W, Tan Y, Tao C & Zhang J-S. (2014). MicroRNA signature in response to nutrient restriction and re-feeding in fast skeletal muscle of grass carp (*Ctenopharyngodon idella*). *Zool Res* 35**:** 404.

Xu Z, Gan L, Li T, Xu C, Chen K, Wang X, et al. (2015). Transcriptome Profiling and Molecular Pathway Analysis of Genes in Association with Salinity Adaptation in Nile Tilapia Oreochromis niloticus. *PLoS One* 10**:** e0136506.

Yan B, Guo JT, Zhu CD, Zhao LH & Zhao JL. (2013). miR-203b: a novel regulator of MyoD expression in tilapia skeletal muscle. *J Exp Biol* 216**:** 447-51.

Yan B, Zhu CD, Guo JT, Zhao LH & Zhao JL. (2013). miR-206 regulates the growth of the teleost tilapia (*Oreochromis niloticus*) through the modulation of IGF-1 gene expression. *J Exp Biol* 216**:** 1265-9.

Yan J, Yang Y, Fan X, Liang G, Wang Z, Li J, et al. (2022). circRNAome profiling reveals circFgfr2 regulates myogenesis and muscle regeneration via a feedback loop. *J Cachexia Sarcopenia Muscle* 13**:** 696-712.

Yan J, Yang Y, Fan X, Tang Y & Tang Z. (2021). Sp1-Mediated circRNA circHipk2 Regulates Myogenesis by Targeting Ribosomal Protein Rpl7. *Genes (Basel)* 12**:** 696.

Yanez JM, Joshi R & Yoshida GM. (2020). Genomics to accelerate genetic improvement in tilapia. *Anim Genet* 51**:** 658-674.

Yang L, Wilusz JE & Chen LL. (2022). Biogenesis and Regulatory Roles of Circular RNAs. *Annu Rev Cell Dev Biol* 38**:** 263-289.

Yang XB, Wang WM, Zhang DY, Li XL, Zhang YK, Zhao Y, et al. (2023). Genetic polymorphism of the ovine MAP3K5 gene and its association with body size traits in Hu sheep of China. *Arch Anim Breed* 66**:** 71-79.

Yang Y, Fan X, Mao M, Song X, Wu P, Zhang Y, et al. (2017). Extensive translation of circular RNAs driven by N 6-methyladenosine. *Cell Res* 27**:** 626-641.

Yang Z, He T & Chen Q. (2021). The Roles of CircRNAs in Regulating Muscle Development of Livestock Animals. *Front Cell Dev Biol* 9**:** 619329.

Ye C-Y, Zhang X, Chu Q, Liu C, Yu Y, Jiang W, et al. (2017). Full-length sequence assembly reveals circular RNAs with diverse non-GT/AG splicing signals in rice. *RNA Biol* 14**:** 1055-1063.

You X & Conrad TO. (2016). Acfs: accurate circRNA identification and quantification from RNA-Seq data. *Sci Rep* 6**:** 1-11.

You X, Vlatkovic I, Babic A, Will T, Epstein I, Tushev G, et al. (2015). Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. *Nat Neurosci* 18**:** 603-610.

Yu S-H, Vogel J & Förstner KU. (2018). ANNOgesic: a Swiss army knife for the RNA-seq based annotation of bacterial/archaeal genomes. *Gigascience* 7**:** giy096.

Yuan YJ, Zeng GM, Liang J, Huang L, Hua SS, Li F, et al. (2015). Variation of water level in Dongting Lake over a 50-year period: Implications for the impacts of anthropogenic and climatic factors. *J Hydrol* 525**:** 450-456.

Zaniboni-Filho E, Nuner AP, Reynalte-Tataje DA & Serafini RL. (2009). Water pH and Prochilodus lineatus larvae survival. *Fish Physiol Biochem* 35**:** 151-5.

Zeng K, Chen X, Xu M, Liu X, Hu X, Xu T, et al. (2018). CircHIPK3 promotes colorectal cancer growth and metastasis by sponging miR-7. *Cell Death Dis* 9**:** 1-15.

Zeng X, Lin W, Guo M & Zou Q. (2017). A comprehensive overview and evaluation of circular RNA detection tools. *PLoS Comput Biol* 13**:** e1005420.

Zhang C, Cui H, Huang C, Kong F, Yang Q, Miao P, et al. (2022). Interactions of circRNAs with methylation: An important aspect of circRNA biogenesis and function (Review). *Mol Med Rep* 25**:** 1-15.

Zhang D, Zhang X, Li F, Li X, Zhao Y, Zhang Y, et al. (2022). Identification and characterization of circular RNAs in association with the feed efficiency in Hu lambs. *BMC Genom* 23**:** 1-12.

Zhang G, Deng Y, Liu Q, Ye B, Dai Z, Chen Y, et al. (2020). Identifying circular RNA and predicting its regulatory interactions by machine learning. *Front Genet* 11**:** 655.

Zhang H, Hu B, Xiong J, Chen T, Xi Q, Luo J, et al. (2019). Genomewide analysis of circular RNA in pituitaries of normal and heat-stressed sows. *BMC Genom* 20**:** 1013.

Zhang J, Chen S, Yang J & Zhao F. (2020). Accurate quantification of circular RNAs identifies extensive circular isoform switching events. *Nat Commun* 11**:** 1-14.

Zhang P, Guo N, Gao K, Su F, Wang F & Li Z. (2020). Direct recognition and sensitive detection of circular RNA with ligation-based PCR. *Org Biomol Chem* 18**:** 3269-3273.

Zhang P, Xu H, Li R, Wu W, Chao Z, Li C, et al. (2018). Assessment of myoblast circular RNA dynamics and its correlation with miRNA during myogenic differentiation. *Int J Biochem Cell Biol* 99**:** 211-218.

Zhang Q, Kopp M, Babiak I & Fernandes JMO. (2018). Low incubation temperature during early development negatively affects survival and related innate immune processes in zebrafish larvae exposed to lipopolysaccharide. *Sci Rep* 8**:** 4142.

Zhang X-O, Wang H-B, Zhang Y, Lu X, Chen L-L & Yang L. (2014). Complementary sequence-mediated exon circularization. *Cell* 159**:** 134-147.

Zhang X-Q & Yang J-H. (2018). Discovering circRNA-microRNA interactions from CLIP-Seq data. *Methods Mol Biol* 1724**:** 193-207.

Zhang Y, Xue W, Li X, Zhang J, Chen S, Zhang JL, et al. (2016). The Biogenesis of Nascent Circular RNAs. *Cell Rep* 15**:** 611-624.

Zhang Y, Zhang XO, Chen T, Xiang JF, Yin QF, Xing YH, et al. (2013). Circular intronic long noncoding RNAs. *Mol Cell* 51**:** 792-806.

Zhang Z, Fan Y, Deng K, Liang Y, Zhang G, Gao X, et al. (2022). Circular RNA circUSP13 sponges miR-29c to promote differentiation and inhibit apoptosis of goat myoblasts by targeting IGF1. *FASEB J* 36**:** e22097.

Zhao H, Xia J, Zhang X, He X, Li L, Tang R, et al. (2018). Diet Affects Muscle Quality and Growth Traits of Grass Carp (*Ctenopharyngodon idellus*): A Comparison Between Grass and Artificial Feed. *Front Physiol* 9**:** 283.

Zhao Y, Samal E & Srivastava D. (2005). Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature* 436**:** 214-220.

Zhao Y & Srivastava D. (2007). A developmental view of microRNA function. *Trends Biochem Sci* 32**:** 189-97.

Zhao Z, Yu X, Jia J, Yang G, Sun C & Li W. (2019). miR-181b-5p May Regulate Muscle Growth in Tilapia by Targeting Myostatin b. *Front Endocrinol* 10**:** 812.

Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, et al. (2016). Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat Commun* 7**:** 11215.

Zhong S & Feng J. (2022). CircPrimer 2.0: a software for annotating circRNAs and predicting translation potential of circRNAs. *BMC Bioinform* 23**:** 215.

Zhong S, Wang J, Zhang Q, Xu H & Feng J. (2018). CircPrimer: a software for annotating circRNAs and determining the specificity of circRNA primers. *BMC Bioinform* 19**:** 292.

Zhou C, Molinie B, Daneshvar K, Pondick JV, Wang J, Van Wittenberghe N, et al. (2017). Genome-wide maps of m6A circRNAs identify widespread and cell-type-specific methylation patterns that are distinct from mRNAs. *Cell Rep* 20**:** 2262-2276.

Zhou WY, Cai ZR, Liu J, Wang DS, Ju HQ & Xu RH. (2020). Circular RNA: metabolism, functions and interactions with proteins. *Mol Cancer* 19**:** 172.

Zhu X, Chen D, Hu Y, Wu P, Wang K, Zhang J, et al. (2015). The microRNA signature in response to nutrient restriction and refeeding in skeletal muscle of Chinese perch (*Siniperca chuatsi*). *Mar Biotechnol* 17**:** 180-9.

Paper I

BMC Bioinformatics

SOFTWARE

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CircPrime: a web-based platform for design of specific circular RNA primers

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Abstract

Background: Circular RNAs (circRNAs) are covalently closed-loop RNAs with critical regulatory roles in cells. Tens of thousands of circRNAs have been unveiled due to the recent advances in high throughput RNA sequencing technologies and bioinformatic tools development. At the same time, polymerase chain reaction (PCR) cross-validation for circRNAs predicted by bioinformatic tools remains an essential part of any circRNA study before publication.

Results: Here, we present the CircPrime web-based platform, providing a user-friendly solution for DNA primer design and thermocycling conditions for circRNA identification with routine PCR methods.

Conclusions: User-friendly CircPrime web platform (http://circprime.elgene.net/) works with outputs of the most popular bioinformatic predictors of circRNAs to design specific circular RNA primers. CircPrime works with circRNA coordinates and any reference genome from the National Center for Biotechnology Information database).

Keywords: Circular RNAs, RNA-sequencing, circRNAs, Primer design, RT-PCR, qPCR, Validation, PCR, Web platform, Prediction

Background

In recent years, there is a marked increase in the number of circular RNA (circRNA)-related studies (Fig. 1). CircRNAs have become a main focus of non-coding RNA biology research because they affect many genetic regulatory networks [15]. These covalently closed-loop RNA molecules are an integral part of the cell regulome and interact with RNA-binding proteins [13, 24]. They can modulate microRNA expression [10, 32] and indirectly affect gene expression [1, 23]. In addition, some of them contain exon parts and can thus be translated into proteins [17, 19]. Recent efforts show that artificial circRNA molecules can be used to enhance peptide production [4].

Modern sequencing technologies make it now possible to identify hundreds of circRNAs that may be used as growth markers for aquaculture [22], as biomarkers and therapeutic targets of human diseases like cancer [16], cardiovascular diseases [2] or brain disorders [7, 20]. However, in silico prediction of circRNAs leads to numerous

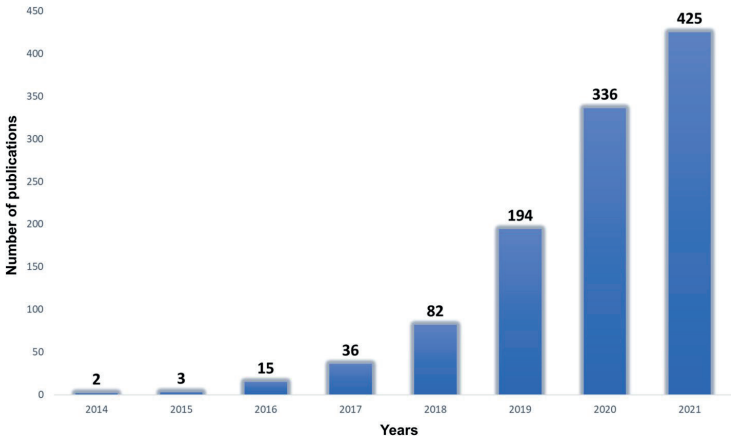

Fig. 1 Exponential increase in the number of publications mentioning "circRNA" in their title. X-axis shows years from 2014 until 2021; Y-axis represents the number of publications. *Source*: Web of Science, accessed 6 October 2022

false-positives [11], as well as inconsistencies among different bioinformatic pipelines [21]. As a result, cross-checking and validation of circRNAs is an essential component of any circRNA study [6].

Reverse transcription PCR (RT-PCR) and quantitative PCR (qPCR) are considered the gold standards for identification of circRNA expression in cells [6]. At the same time, primer design for the circRNAs validation differs from the design for the their linear host genes [28]. To date, only a few tools have been published that allow the development of primers for validation of circRNAs. At the same time, they require additional software to be installed in different operating systems—CircPrimer [35], CircPrimer2.0 [34] and circtools [12], or work as a web tool with already known circRNAs of model organisms, namely human [8] or novel circRNAs for limited number of animal species [28]. Here, to overcome these previous constraints and facilitate the circRNA studies, we present the user-friendly CircPrime web platform (http://circprime.elgene.net/). This tool works with outputs of the most popular bioinformatic predictors of circRNAs, such as CIRI2 [9], KNIFE [26], CIRCexplorer2 [33], find_circ [18], circRNA_finder [30], DCC [5], mapsplice [29] and common BED files. Importantly, CircPrime is suitable for all organisms that have reference genome assemblies in the National Center for Biotechnology Information database (NCBI).

Implementation

To date, there are several methods for PCR-based identification of different circRNAs types (Fig. 2A). One of them is based on rolling circle amplification (RCA). This method avoids deep RNA sequencing and bioinformatic analysis, but is only capable of identifying a limited number of circRNA types [3]. The other most commonly used method assumes a longer workflow, which comprises circRNA enrichment, circRNA-library construction, deep sequencing, circRNA prediction, and finally RT-PCR/qPCR

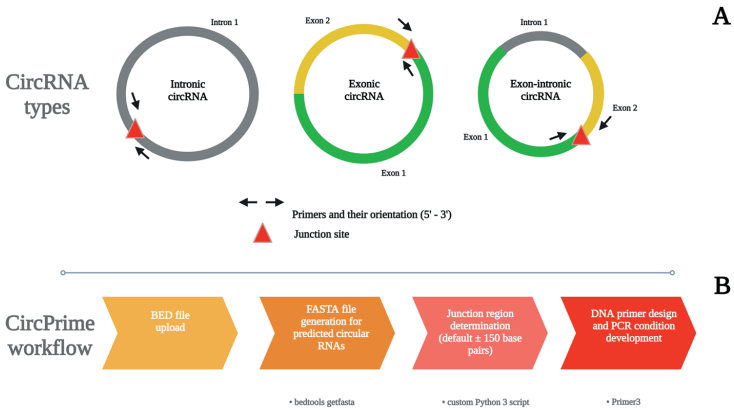

Fig. 2 Circular RNA types and overview of the CircPrime pipeline. **A** Possible structural variants of circular RNA for primer design for validation. **B** The main steps of CircPrime pipeline and tools combined in it

Table 1 Required and default parameters for CircPrime web platform usage

CircPrime parameter	Parameter description
CircRNA BED file	Required
Shift range left	Default 150 nucleotides
Shift range right	Default 150 nucleotides
Optimum length of a primer	Default 19 nucleotides
Minimum acceptable length of a primer	Default 15 nucleotides
Maximum acceptable length of a primer	Default 20 nucleotides
PCR product size	Default 150–200 nucleotides
Optimum melting temperature for a primer	In Celsius
Minimum acceptable melting temperature for a primer	In Celsius
Maximum acceptable melting temperature for a primer	In Celsius
Number of primer sets	Default 4 sets

validation of bioinformatically predicted circRNAs [25]. PCR primers for this validation are designed to target the circRNA fragment overlapping a junction (back-splice) site of a specific circRNA (Fig. 2A).

We developed CircPrime, as a streamlined pipeline in Python 3 and web platform, which makes use of output files from the most popular circRNAs in silico predictors. The web application uses the flask framework (https://github.com/pallets/flask). The representative state transfer (REST) was chosen as the architecture for the design of the web platform [31], which fully complies with the HTTP protocol. The CircPrime script implemented into the web platform currently contains the four main modules shown in Fig. 2B and works under the parameters presented in Table 1.

After the first step, which includes BED file uploading, CircPrime generates FASTA files using circRNA coordinates and reference genome from the NCBI. Then CircPrime extracts junction regions from the uploaded BED file and develops primer sets with the recommended melting temperature (Tm) for each circRNA in the list (up to 100) using

Primer3 (Fig. 2B) [14, 27]. An example of the CircPrime output is presented as Supplementary Dataset 2.

Results

The novel CircPrime web-based platform was evaluated to design primer sets for RT-PCR validation of circRNA expression in the muscle transcriptome of a teleost, the Nile tilapia (*Oreochromis niloticus*). Successfully, we showed that CircPrime significantly simplifies the primer design process for bioinformatically predicted circRNAs without the need to upload a reference genome of the organism studied. In this study, we applied circRNA transcriptome sequencing and circRNA prediction by CIRI2 [9] in four Nile tilapia skeletal muscle tissue specimens for successful validation of the novel CircPrime web platform. This tool designed circRNA primer pairs, which were used to validate their RT-PCR efficiency. The genome coordinates for 10 circular RNAs expressed in all muscle samples were used for primer design. CircPrime was able to design primer sets for 9 of the 10 circRNAs (Supplementary Dataset 2). Subsequently, four of them (Table S1) were validated using PCR (see details in Supplementary Material).

At present, circPrimer [35], circPrimer2.0 [34], and circtools [12] are mainly used for primer design, and all of them cope well with their main task. At the same time, these tools have a number of significant differences in the interface and functionality, e.g., circtools [12] is a modular platform based on the Python3, which combines several functions in a single script managed from the command line. Circtools has numerous parameters and allows to choose various options for primer design. CircPrimer [35] and its updated version, CircPrimer2 [34], are convenient tools for circRNA research that are implemented as a graphical interface and a command-line interface. CircPrimer2 allows users to search, annotate, and visualize circRNAs and also helps users develop primers for circRNAs and verify the specificity of circRNA primers. Unlike circPrimer [35], circPrimer 2.0 [34], and circtools [12], which are pre-installed to work only with the mouse and human genomes, CircPrime itself determines the genome by ID using the NCBI database, and its web interface does not need to be installed and is immediately ready for convenient and fast use.

We expect that this bioinformatic tool will play a relevant role on varied studies describing circRNAs expression and their possible functionality. CircPrime is applicable for any organism, including even those with a relatively poorly annotated genome assembly, such as Nile tilapia (NCBI accession: GCF_001858045.2_O_niloticus_UMD_NMBU).

Conclusions

Herein, we present a Circprime web platform (http://circprime.elgene.net/) for PCR primer design and PCR conditions development for validation of circRNAs predicted based on RNA-sequencing data using different types of bioinformatics tools. We expect that this web tool will be convenient for users who intend to analyze the expression of circRNAs in animal and plant transcriptomes.

Availability and requirements

Project name: CircPrime. Project home page: http://circprime.elgene.net/. CircPrime documentation: https://circprime.readthedocs.io/. Operating system(s): Platform independent.

Programming language: Python 3.10. Other requirements: None. License: GNU GPL Version 3.

Abbreviations

PCR	Polymerase chain reaction
CircRNA	Circular RNA
RT-PCR	Reverse transcription PCR
qPCR	Quantitative PCR
NCBI	National Center for Biotechnology Information database
RCA	Rolling circle amplification

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12859-023-05331-y.

Additional file 1. Supplementary Dataset 1: CIRI2 output files for four Nile tilapia circRNA-Seq datasets. Overlapped circRNAs. Overlapped and overrepresented circRNAs of Nile tilapia muscle, which were used for CircPrime web-based platform validation.

Additional file 2. Supplementary Dataset 2: An example of CircPrime output: primer pairs and PCR conditions for them, which were developed for bioinformatically predicted circRNAs.

Additional file 3. Supplementary File: Additional material that supports the main manuscript. Extended Methods and Results sections.

Acknowledgements
Not applicable.

Author contributions
F.S.—wrote tool script and implemented it on a web-based platform, wrote and approved the final draft; G.R.—performed the experiments, prepared figures and/or tables, and approved the final draft; P.S.—performed the experiments, prepared figures and/or tables, and approved the final draft; J.R.—conceived and designed the experiments and approved the final draft; J.G.—conceived and designed the experiments and approved the final draft; A.N.—conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft. J.M.O.F.—conceived and designed the experiments, authored or reviewed drafts of the paper, approved the final draft, administrated project and acquisited funding. All authors read and approved the final manuscript.

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Availability of data and materials
The user-friendly CircPrime tool for circular RNA primer development is written in Python 3 and implemented on a web-based platform. It is freely available online at http://circprime.elgene.net/. The RNA-seq dataset generated and analysed during the current study is available in the GEO (NCBI) repository, under the accession number PRJNA826285: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA826285 (accessed on 05 May 2023). CircPrime documentation is available at https://circprime.readthedocs.io/.

Declarations

Ethics approval and consent to participate
This research was approved by the Nord University (Bodø, Norway) ethical committee. The experimental procedures involving animals were performed in accordance with the regulation and instructions of the Norwegian Animal Research Authority (FOTS ID 1042). All procedures involving animals were conducted according to the EU Directive 2010/63 on the use of animals for scientific purposes.

Consent for publication
Not applicable.

Competing interests
The authors declare there are no competing interests.

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References
1. Ashwal-Fluss R, et al. circRNA biogenesis competes with pre-mRNA splicing. Mol Cell. 2014;56(1):55–66.
2. Aufiero S, et al. Circular RNAs open a new chapter in cardiovascular biology. Nat Rev Cardiol. 2019;16(8):503–14.
3. Boss M, Arenz C. A fast and easy method for specific detection of circular RNA by rolling-circle amplification. ChemBioChem. 2020;21(6):793–6.
4. Chen R, et al. Engineering circular RNA for enhanced protein production. Nat Biotechnol. 2023;41(2):262–72.
5. Cheng J, Metge F, Dieterich C. Specific identification and quantification of circular RNAs from sequencing data. Bioinformatics. 2016;32(7):1094–6.
6. Das A, Das D, Panda AC, Validation of circular RNAs by PCR. In: Basu C editor, PCR primer design. New York: Springer US; 2022. p. 103–14.
7. Dube U, et al. An atlas of cortical circular RNA expression in Alzheimer disease brains demonstrates clinical and pathological associations. Nat Neurosci. 2019;22(11):1903–12.
8. Dudekula DB, et al. CircInteractome: a web tool for exploring circular RNAs and their interacting proteins and microRNAs. RNA Biol. 2016;13(1):34–42.
9. Gao Y, Zhang J, Zhao F. Circular RNA identification based on multiple seed matching. Brief Bioinform. 2018;19(5):803–10.
10. Hansen TB, et al. Natural RNA circles function as efficient microRNA sponges. Nature. 2013;495(7441):384–8.
11. Hansen TB, et al. Comparison of circular RNA prediction tools. Nucleic Acids Res. 2016;44(6): e58.
12. Jakobi T, Uvarovskii A, Dieterich C. circtools-a one-stop software solution for circular RNA research. Bioinformatics. 2019;35(13):2326–8.
13. Ji F, et al. IGF2BP2-modified circular RNA circARHGAP12 promotes cervical cancer progression by interacting m(6)A/FOXM1 manner. Cell Death Discov. 2021;7(1):215.
14. Koressaar T, Remm M. Enhancements and modifications of primer design program Primer3. Bioinformatics. 2007;23(10):1289–91.
15. Kristensen LS, et al. The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet. 2019;20(11):675–91.
16. Kristensen LS, et al. Circular RNAs in cancer: opportunities and challenges in the field. Oncogene. 2018;37(5):555–65.
17. Legnini I, et al. Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis. Mol Cell. 2017;66(1):22-37.e29.
18. Memczak S, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013;495(7441):333–8.
19. Mookherjee T, et al. Characterizations of a novel peptide encoded by a circular RNA using in-silico analyses. Biochem Biophys Res Commun. 2022;630:36–40.
20. Nedoluzhko A, et al. The biomarker and therapeutic potential of circular Rnas in Schizophrenia. Cells. 2020;9(10):66.
21. Nedoluzhko A, et al. CircParser: a novel streamlined pipeline for circular RNA structure and host gene prediction in non-model organisms. PeerJ. 2020;8: e8757.
22. Rbbani G, et al. Function of circular RNAs in fish and their potential application as biomarkers. Int J Mol Sci. 2021;22(13):66.
23. Rbbani G, et al. The novel circular RNA CircMef2c is positively associated with muscle growth in Nile tilapia. Genomics. 2023;115(3): 110598.
24. Shao M, et al. CRIT: Identifying RNA-binding protein regulator in circRNA life cycle via non-negative matrix factorization. Mol Ther Nucleic Acids. 2022;30:398–406.
25. Shi H, et al. Comparative analysis of circular RNA enrichment methods. RNA Biol. 2022;19(1):55–67.
26. Szabo L, et al. Statistically based splicing detection reveals neural enrichment and tissue-specific induction of circular RNA during human fetal development. Genome Biol. 2015;16(1):126.
27. Untergasser A, et al. Primer3–new capabilities and interfaces. Nucleic Acids Res. 2012;40(15): e115.
28. Vromman M, et al. CIRCprimerXL: convenient and high-throughput PCR primer design for circular RNA quantification. Front Bioinform. 2022;2: 834655.
29. Wang K, et al. MapSplice: accurate mapping of RNA-seq reads for splice junction discovery. Nucleic Acids Res. 2010;38(18): e178.
30. Westholm JO, et al. Genome-wide analysis of drosophila circular RNAs reveals their structural and sequence properties and age-dependent neural accumulation. Cell Rep. 2014;9(5):1966–80.
31. Wilde E, Pautasso C, Alarcón R. REST: advanced research topics and practical applications; 2014.
32. Zhang L, et al. Circular RNA circUBE2J2 acts as the sponge of microRNA-370-5P to suppress hepatocellular carcinoma progression. Cell Death Dis. 2021;12(11):985.
33. Zhang XO, et al. Diverse alternative back-splicing and alternative splicing landscape of circular RNAs. Genome Res. 2016;26(9):1277–87.
34. Zhong S, Feng J. CircPrimer 2.0: a software for annotating circRNAs and predicting translation potential of circRNAs. BMC Bioinform. 2022;23(1):215.
35. Zhong S, et al. CircPrimer: a software for annotating circRNAs and determining the specificity of circRNA primers. BMC Bioinform. 2018;19(1):292.

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The novel circular RNA CircMef2c is positively associated with muscle growth in Nile tilapia

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ABSTRACT

Muscle growth in teleosts is a complex biological process orchestrated by numerous protein-coding genes and non-coding RNAs. A few recent studies suggest that circRNAs are involved in teleost myogenesis, but the molecular networks involved remain poorly understood. In this study, an integrative omics approach was used to determine myogenic circRNAs in Nile tilapia by quantifying and comparing the expression profile of mRNAs, miRNAs, and circRNAs in fast muscle from full-sib fish with distinct growth rates. There were 1947 mRNAs, 9 miRNAs, and 4 circRNAs differentially expressed between fast- and slow-growing individuals. These miRNAs can regulate myogenic genes and have binding sites for the novel circRNA circMef2c. Our data indicate that circMef2c may interact with three miRNAs and 65 differentially expressed mRNAs to form multiple competing endogenous RNA networks that regulate growth, thus providing novel insights into the role of circRNAs in the regulation of muscle growth in teleosts.

1. Introduction

Aquaculture is a rapidly developing industry that plays a vital role in securing food for the increasing human population. According to recent global statistics, world aquaculture production reached another all-time high of 82 million tons in 2018 [1]. Growth is one of the most important traits in aquaculture, which directly benefit farmers and has been the key trait of selection for all the breeding programs across the species [2]. Fish growth is largely associated with muscle tissue, which constitutes around 40–60% of the edible body mass [3]. Fish muscle formation and growth include some fundamental events, namely the early muscle cell commitment, the development of different proportions of slow and fast muscle fibers, and hyperplasia (increase in fiber number) and hypertrophy (increase in fiber size) throughout much of ontogeny [3,4]. These processes require a coordinated expression of many genes and diverse molecular pathways. In recent years, an increasing number of studies have suggested the importance of epigenetic mechanisms, including non-coding RNAs, in fish muscle development [5–10]. In-depth knowledge about regulatory mechanisms involved in fish growth is essential to explore the observed phenotypic variation in aquaculture conditions. Yet, our understanding of the complex regulatory networks involved in controlling fish muscle growth is still limited.

Living organisms transcribe thousands of non-coding RNAs, including microRNAs (miRNAs), small interfering RNAs (siRNAs), piwi-interacting RNAs (piRNAs), and long non-coding RNAs (lncRNAs) [11]. Together, they regulate gene expression at transcriptional and post-transcriptional levels. miRNAs inhibit gene expression mainly by pairing with complementary sequences in the 3'-UTR of their target mRNAs, thus inducing their degradation or translational repression. The role of miRNAs in muscle development, growth, and regeneration, as well as conditions such as atrophy, has been reported in teleosts [6,12,13]. More recently, it has been discovered that circRNAs alter both miRNA and mRNA expression, and play a crucial role in various physiological, biological, and molecular processes [14–16]. Exonic and exonic-intronic circular RNAs (circRNAs) are uncapped, non-polyadenylated, endogenous non-coding RNAs formed by back-splicing of precursor mRNAs. In contrast, intronic circRNAs are typically derived from spliced introns (lariats) that escape debranching and are circularized by 2′-5′

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phosphodiester bonds. circRNAs are relatively resistant to exonucleases, making them more stable than the traditional linear mRNA counterparts.

Although the majority of circRNAs identified in different species still lack functional annotation, there is growing evidence that circRNAs participate in epigenetic, transcriptional, and post-transcriptional regulation of gene expression [14,17,18]. CircRNAs also act as protein sponges and competing endogenous RNA (ceRNA) in various organisms, thus regulating phenotypic expression. ceRNA is a post-transcriptional gene regulation mechanism in which circRNAs and mRNAs crosstalk and compete for shared target miRNAs. CircRNAs usually contain microRNA-responsive elements by which they modulate miRNA activity, thereby reducing their availability to bind to the targeted protein-coding transcripts. Moreover, some circRNAs contain binding sites for multiple miRNAs or multiple sites for a single miRNA type/family, often referred to as a miRNA sponge; therefore, circRNA-miRNA-mRNA (ceRNA) networks may influence various biological pathways and the expression of many genes [19]. The role of circRNAs in muscle growth has been described in different livestock species reviewed by [3]. For example, circSNX29 in bovine skeletal muscle serves as an endogenous miR-744 sponge and promotes myoblast differentiation [20]. In contrast, there is a single report about circRNAs in teleost muscle, suggesting that a novel_circ_0002886 in snout bream (*Megalobrama amblycephala)* may act as ceRNA and inhibit the apoptosis of skeletal muscle cells [21]. Therefore, there is a need to characterize ceRNA networks to understand the processes regulating muscle growth in teleosts.

Nile tilapia (*Oreochromis niloticus*) is among the most studied teleosts, since it is the second most important farmed fish worldwide [1]. Nowadays, selective breeding programs for various tilapia species and their hybrids are undergoing to improve physiological and development processes, such as growth and disease resistance [2,22]. Thus, understanding the molecular mechanisms underlying different biochemical and physiological processes affecting muscle growth is one of the central themes of genetic improvement; and the paper aims to investigate the potential regulatory mechanisms of circRNAome in muscle. Our results provide the first overview of circRNA, miRNA, and mRNA co-expression in a regulatory network with possible functional implications for the regulation of muscle growth in teleosts.

2. Results

2.1. Experimental groups and summary of sequencing data

The growth performance of the Nile tilapia full-sibs is shown in Fig. 1. The mean total length and mean body weight of BM (Big male, i.e., fast-growing) were 2.1- and 10.0-fold higher than their SM (Small male, i.e., slow-growing) counterparts, respectively. Individual libraries of BM and SM groups were sequenced (Tables S1-S3 in Supplementary Material), and a total of 964,592,220 read pairs, 660,513,692 read pairs, and 319,445,174 single reads were generated from mRNA-seq, circRNA-seq and miRNA-seq data, respectively (Table 1). Trimming of adapter and low-quality reads yielded 938,496,548 mRNA read pairs, 623,923,612 circRNA read pairs, and 276,419,289 miRNA read pairs with quality > Q20. The average mapping rate of clean reads to the Nile tilapia reference genome was 93.9% mRNA, 91.3% circRNA, and 58.9% miRNA. Detailed analysis showed that around 73% and 92.5% of transcripts are mapped in the correct position and orientation (i.e., both mates of a read pair map to the same chromosome, oriented towards each other) for mRNA and circRNA, respectively. Mapped read counts were used in further differential expression analyses.

2.2. Identification of differentially expressed mRNAs (DE-mRNAs) between slow- and fast-growing males

We retrieved a total of 1947 differentially expressed mRNAs (DE-mRNAs) with |Log_2fold change| ≥1 and *p-adjusted value* ≤ 0.05. The complete list of the identified genes is reported in Supplementary Table S4. Among the 1947 DE-mRNAs, 1002 were up-regulated, and 945 were down-regulated in SM group compared to BM. The volcano plot illustrates the changes in mRNA expression (Supplementary Fig. S1). To confirm if the transcriptome diversity described above reflects genuine expression differences in muscle or not, we performed a principal component analysis. The first principal component (PC1) accounts for 33% of the variance in our data and separates samples into two groups (Supplementary Fig. S2). It also reflects that fast-growing fish have more individual differences than their slow-growing counterparts. Further, hierarchical clustering of DE-mRNAs provided an overview of the expression patterns and showed a clear separation between groups (Fig. 2). Several growth-associated protein-coding genes, including *igf2bp2, tgfbi, myod1, myf5, tnfsf12, adamts16, asb2, tmod4,* and *hacd1*, were significantly less expressed in the slow-growing than in the fast-growing group.

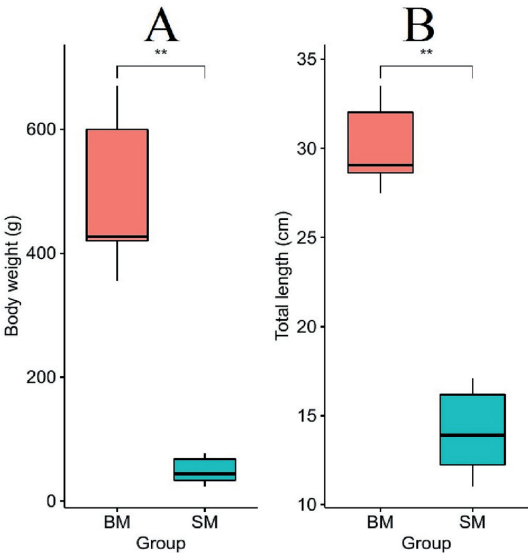

Fig. 1. Length and weight of slow- and fast-growing fish. Difference in body weight and total length at 9 months between slow- (SM) and fast-growing (BM) groups of Nile tilapia used in this study. BM group differences are shown by red boxplot, SM group differences are marked in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Genes up-regulated in slow-growing Nile tilapia are enriched in splice-related GO terms and the spliceosome pathway

Gene ontology (GO) analysis of differentially expressed mRNA, which includes biological process, molecular function, and cellular component, was conducted to understand the potential involvement of DE mRNAs in molecular pathways. The result demonstrated that the down-regulated genes in the SM group are significantly enriched in some important functional terms, including regulation of muscle cell differentiation, myoblast and myotube differentiation, positive regulation of striated muscle cell differentiation, ribonucleoprotein complex ribosome (Fig. 3). Conversely, up-regulated genes in the SM group are enriched in mRNA splicing via spliceosome, spliceosomal small nuclear ribonucleoproteins (snRNPs) complex, spliceosomal tri-snRNP complex, transcription initiation from RNA polymerase II promoter,

Table 1
Summary of RNA sequencing and mapping statistics for combined Nile tilapia muscle datasets.

Library type	Total reads	Clean reads	Clean reads >Q20 (%)	Mapping rate (%)	Pairs (%)	Singletons (%)
circRNA	660,513,692	623,923,612	94.4	91.3	92.7	1.2
mRNA	964,592,220	938,496,548	97.2	93.9	70.5	2.5
miRNA	319,445,174	276,419,289	86.5	58.9	–	–

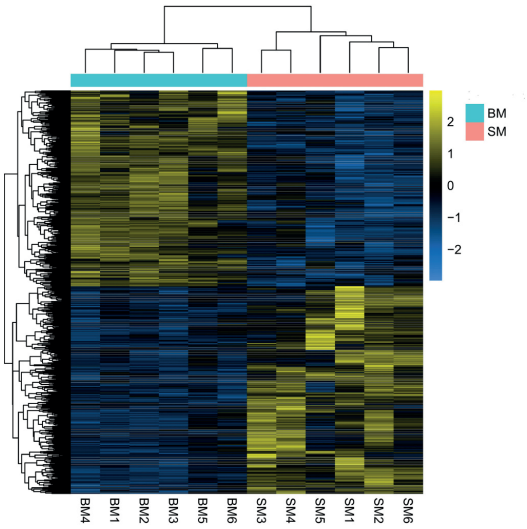

Fig. 2. Muscle transcriptome differences between fast-growing (BM) and slow-growing fish (SM). Heatmap of differentially expressed mRNAs in Nile tilapia fast muscle (BM-fast growing, SM-Slow growing). The colour scale represents the difference in expression with an adjusted *p-value* ≤ 0.05 and |Log_2fold change| ≥1.

autophagosome, proteasome complex (Fig. 4). Multiple splice-related GO terms imply shifts in the splicing pattern of splicing-related genes that encode the spliceosome and its accessory proteins. Detailed analysis of the different terms revealed several genes associated with multiple GO terms, namely *myf5, moyd1, trmt1l, rpl9, fxr1, sf3a3, prpf6, txnl4a, optn* (Fig. S3A and S4B in Supplementary Material).

KEGG analysis revealed several significantly changed pathways between the groups with an adjusted *p-value* ≤ 0.05 (Fig. 5). Among the top enriched pathways, spliceosome, protein processing in the endoplasmic reticulum, mitophagy (Fig. 6A), and proteosome suggest that these genes may play a vital role in muscle growth through their involvement in myofiber degeneration, specific circRNA/gene or protein production and degradation. The spliceosome (Fig. 6B) contributes to splicing regulation by modulating splice site choice from pre-mRNA transcripts. The differentially expressed genes *snu114, sf3a,* and *u1a* are essential components for snRNPs suggesting a significant impact on pre-mRNA splicing process.

2.4. Multiple splice variants are expressed in Nile tilapia muscle

An average of 85.5% concordant read pairs were uniquely mapped and used for subsequent analysis. Expression levels of different isoforms were estimated based on Fragments Per Kilobase of transcript per Million (FPKM) mapped reads (Supplementary Fig. S4). We were able to detect a total of 59,119 splicing events from 42,621 genes. IGV-Sashimi plot shows the genomic region containing the alternative exons of *dusp22* gene (NC_031981.2:27,054,572-27,060,684) (Fig. 7A). Besides, 845 transcription start sites were significantly differentially expressed, describing the transcription initiation and regulation variability at the transcriptional level. A total of 113,949 splice variants were expressed in at least one of the 12 samples (arbitrary cutoff with reads per kilobase of exon per million reads mapped ≥0.1), and among them, 423 were significantly differentially expressed between BM and SM groups (Fig. 7B). For example, *znf414, mapk6, moyd1, sparc,* and *tgfbi* were among the genes producing isoforms related to muscle growth. Moreover, 585 differentially expressed coding sequences were found between the groups from all isoforms of the expressed gene attributes.

2.5. Skeletal muscle myomiRs are expressed at different levels between slow- and fast-growing Nile tilapia

The majority of small RNA reads in each library were between 17 and 33 nucleotides (nt) long (Fig. S5 in Supplementary Material), with a marked peak at 22 nt. The average mapping efficiency of miRNA reads was 58.9% (Table 1). Subsequently, the expression levels of miRNAs were calculated based on the read count and were normalized to identify differentially expressed miRNA. As shown in Fig. 8, a total of 9 miRNAs significantly differentially expressed miRNAs were identified with a stringent threshold (q-value ≤0.05). The statistical significance of miRNA expression ratios against their q-values is shown in Supplementary Fig. S6. Furthermore, PCA analysis confirms the clustering of BM and SM groups, where the first principal component (PC1), explained 34% variability in the data (Supplementary Fig. S7). Interestingly, muscle-related oni-miR-202, oni-miR-21, oni-miR-217, oni-miR-34, oni-miR-731 and oni-miR-99b were significantly differentially expressed between the groups analyzed.

2.6. Four novel circRNAs are differentially expressed with size in Nile tilapia fast muscle

The number of distinct circRNAs identified in each library varied between 62 and 470 (Fig. S8 in Supplementary Material). SM had a comparatively higher number of circRNAs than BM, indicating different circRNAomes between groups. However, these circRNAs were derived from both sense- and anti-sense strands. The number of circRNAs was not uniformly distributed across all the linkage groups (Fig. 9A). The highest number of circRNAs was produced from LG16 and LG7, whereas LG2, LG19, and the mitochondrial genome produced the least number of circRNAs. The size distribution of circRNAs ranged from 45 to over 2000 nt; 51.5% of circRNAs had a predicted spliced length of <2000 nt; 14.9% had a length 1000–2000 nt, and 36.5% had a length under 1000 nt (Fig. 9B). Host gene annotations results showed that most genes produce one circRNA rather than producing more than one circRNA. Alternative splicing events were observed in the muscle circRNAs. For example, two circRNAs derived from LG11 started at position 30,158,064 that ended at positions 30,166,226 and 30,171,209.

We conducted a principal component analysis to confirm the circRNA diversity between BM and SM groups. The first (PC1) and second (PC2) principal components account for 19% and 16% of the variance in our data, respectively, and separate the data in two groups (Supplementary Fig. S9). A total of 4 circRNAs were differentially expressed between BM and SM groups with padj-value ≤0.05 and |Log_2fold change| ≥ 1 as the threshold. Among the DE-circRNAs, two

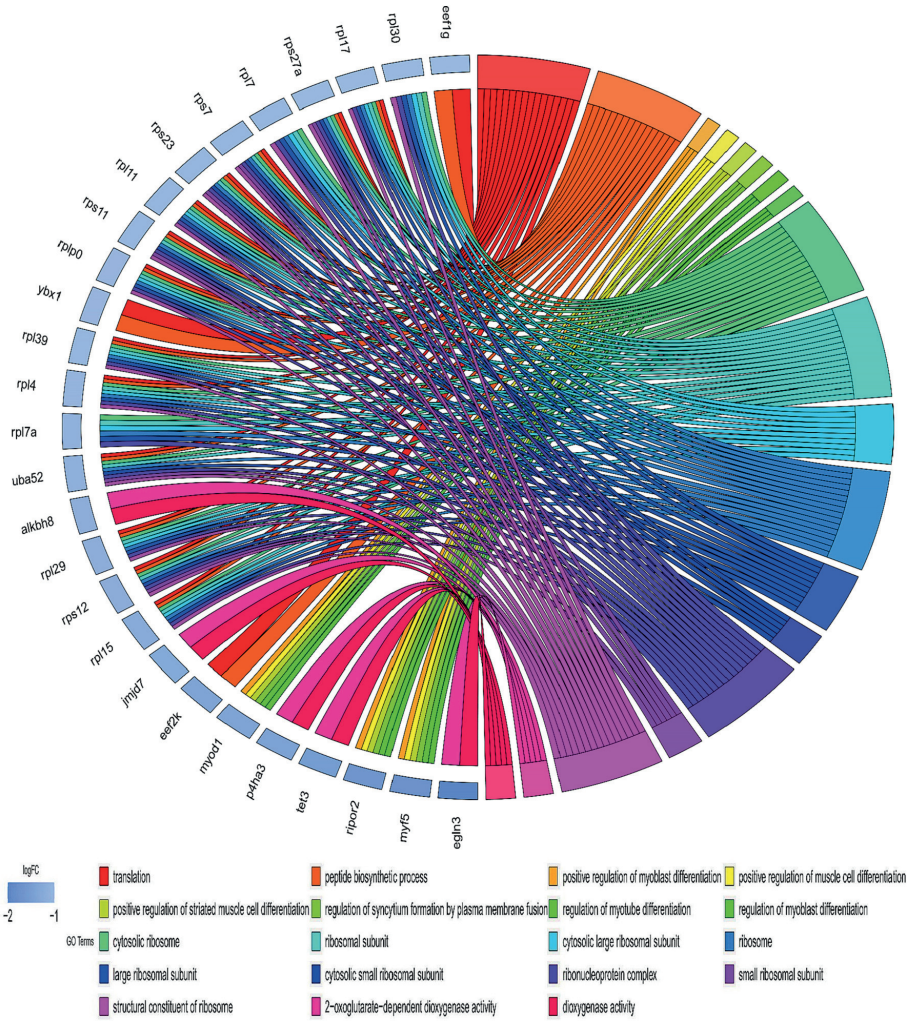

Fig. 3. Gene ontology (GO) enrichment of down-regulated mRNA. The chord diagram shows the relationship between some enriched terms and the $\log_2$ fold change. The gradient colour bar represents the Log_2 fold change (adjusted *p-value* $\leq$ *0.05* and $|\text{Log}_2 \text{ fold change}| \geq 1$).

were significantly down-regulated, and two were statistically up-regulated in SM group. The distribution of differentially expressed circRNAs is shown in a volcano plot (Fig. 9C). These DE-circRNAs were further classified into exonic and exon-intronic circRNAs. The detailed features and structural patterns of differentially expressed circRNAs between BM and SM groups are summarized in Table 2. Circ_NC_031972.2:9033630–9,035,959 produced from *myocyte-specific enhancer factor 2C-* was down-regulated by 1.2-fold, whereas circRNA NC_031972.2:48086230–48,086,927 associated with *troponin T* was up-regulated by 4.7-fold in slow-growing fish compared to their fast-growing counterparts. These circRNAs might play an important role in muscle growth in Nile tilapia.

2.7. Validation of circRNA, mRNA, and miRNA expression by qPCR

All the differentially expressed circRNAs were quantified using qRT-PCR. Divergent primers of length ~ 20 nucleotides were designed to amplify exclusively the circRNA isoform (Table 3). The relative expression patterns of four circRNAs were consistent with the trends obtained from circRNA sequencing data (Fig. 9D and E). Furthermore, we used PCR and Sanger sequencing to confirm the back-splice junctions of circMef2c (Fig. S10 in Supplementary Material). The circular isoforms for all the Nile tilapia fast muscle samples tested could be amplified with these divergent primers, resulting in a ~ 300 bp amplicon, whereas no product was obtained in the negative control (RNAse-free water) (Supplementary Fig. S11).

Similarly, the expression analyses performed on the 15 selected mRNAs and 8 miRNAs yielded results that followed the trends in transcriptomic data (Supplementary Figs. S12 and S13).

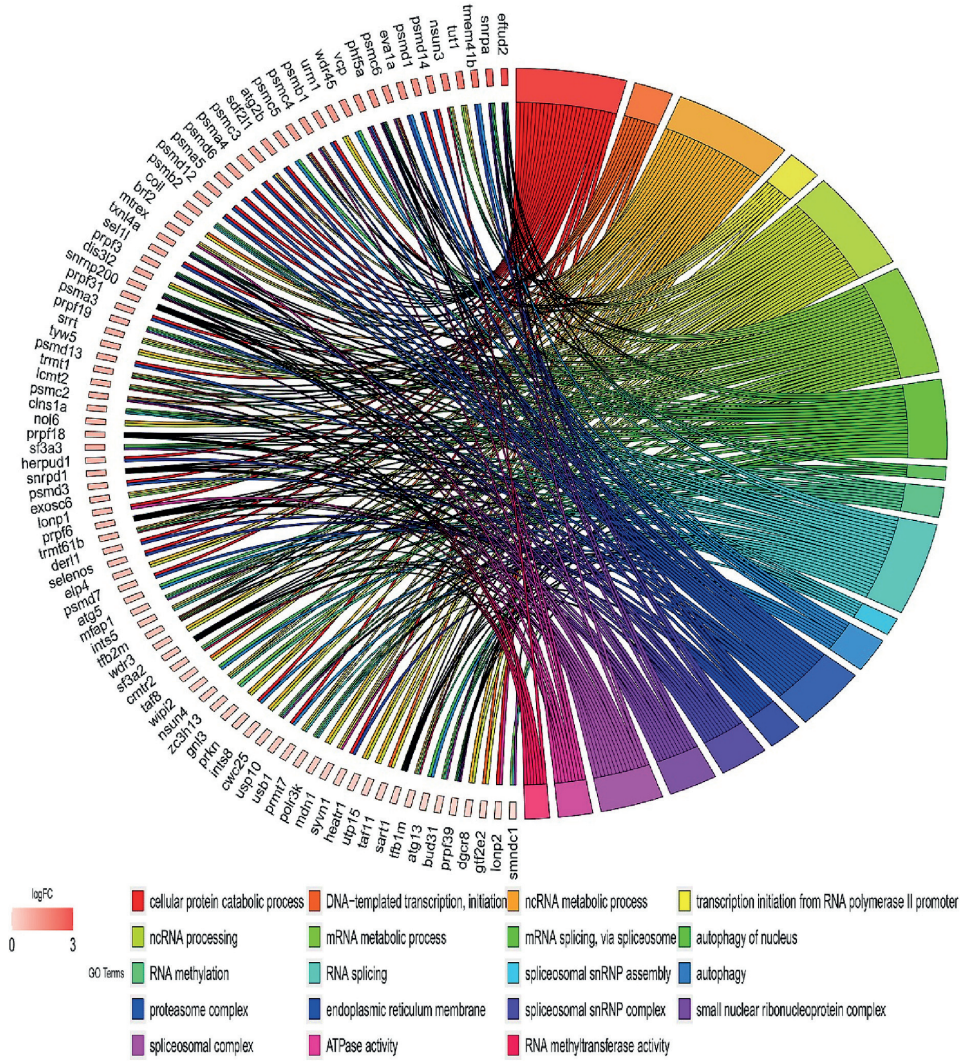

Fig. 4. Gene ontology (GO) enrichment of up-regulated mRNA. The chord diagram shows the relationship between significantly enriched terms and the $\log_2$ fold change. The gradient colour bar represents the Log_2 fold change (adjusted *p-value* $\leq$ *0.05* and $|\text{Log}_2 \text{ fold change}| \geq 1$).

2.8. Genes differentially expressed with size may form a circRNA–miRNA–mRNA competitive network involved in muscle growth

An integrative analysis of the interplay among the three classes of RNA was performed based on differential expression analysis to elucidate their functional connections in Nile tilapia muscle growth. The putative target interactions between DE-circRNA, DE-miRNA, and DE-miRNA-DE-miRNA were predicted. Down-regulated genes correlate with up-regulated miRNA and down-regulated circRNAs, whereas up-regulated genes correlate with downstream miRNA and upstream circRNAs. The circRNA–miRNA–mRNA competitive network (ceRNAs) was constructed by combining circRNA-miRNA pairs with miRNA-mRNA interactions (Fig. 10). The network contains one circRNA, 3 miRNAs, and 65 mRNAs, providing a comprehensive perspective into the links between circRNA, miRNA, and mRNAs in muscle growth.

3. Discussion

With the recent development of transcriptome sequencing technology, circRNAs have been found with different expression patterns in multiple tissues to exert specific roles in various bioprocesses, including embryonic development and heat stress response. To the best of our knowledge, there is no comprehensive study to describe the complex regulatory role of circRNAs in teleost muscle. This study, for the first time, identifies and characterizes the expression pattern of circRNAs in Nile tilapia muscle by high-throughput sequencing and their regulatory networks with miRNA and mRNA as ceRNAs.

Systematical analysis of circRNA transcriptome in Nile tilapia

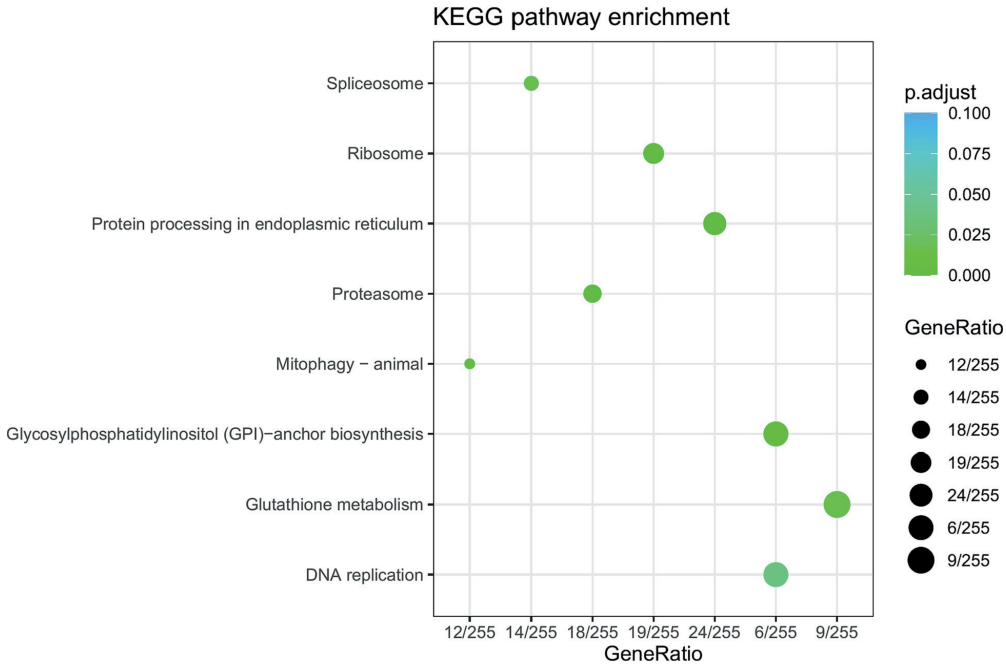

Fig. 5. KEGG pathways altered between fast-growing (BM) and slow-growing fish (SM). The scatter diagram shows significant pathway enrichments for DE-mRNAs. Gene ratio is the number of DE-mRNAs in this pathway to all the genes in this pathway. The X-axis corresponds to the gene ratio of pathway, and the Y-axis represents a different pathway. The colour intensity of the nodes shows the degree of enrichment, dot size represents the count of genes in a pathway and the q-value is colour-coded.

enabled us to detect specific features and relative abundances of different circRNAs, and RT-qPCR validation has confirmed their expression (Fig. 9D). We found a total of 2949 novel circRNAs in slow- and fast-growing full-sib individuals. These results agree with the scope and abundance of circRNAs that have been identified in fish. Nedoluzhko et al. [23] previously reported the presence of 446–928 circRNAs in white muscle tissue of wild and first-generation domesticated Nile tilapia. In a different study, Liu. et al. [21] have identified 445 circRNA in blunt snout bream muscle. When comparing the expression profile of RNAs between slow and fast-growing Nile tilapia groups, we found significant changes in circRNA expression levels accompanied by the size of the fish. A total of 4 differentially expressed circRNAs were identified between fast-growing and slow-growing groups. Of these, two circRNAs were up-regulated, and two were down-regulated. Circ_NC_031972.2:9033630–9,035,959 (CircMef2C), located in LG7, is one of the down-regulated circRNAs in SM group. It is derived from the combining exon and intron region of the *myocyte-specific enhancer factor 2C-like (mef2c)* gene. The host genes of circRNAs are an important source for understanding the biological function of circRNAs. Ashwal-Fluss et al. [24] described that the relationship between circRNAs and their host genes helps to predict the role of circRNAs in organisms. *mef2c* is known to play a crucial role in sarcomeric gene expression, fiber type control, and regulation of metabolism, thus controlling overall body size [25]. Down-regulation of circMef2c in the slow-growing male group suggests its involvement in Nile tilapia muscle growth. Several studies in livestock animals showed that circRNA produced from muscle-associated genes significantly influences muscle development. For example, circRNA produced from the *huwe1* gene, responsible for ubiquitination and proteasomal degradation of *myoD*, promotes proliferation and differentiation of myoblasts, inhibiting apoptosis [26]. Likewise, circMYBPC1 produced from the *mybpc1* gene promotes the differentiation of myoblasts and skeletal muscle regeneration [27]. In addition, a significant number of mRNAs had a similar trend of expression compared to circRNA expression in the slow-growing group but an opposite pattern from miRNA, which suggests the possibility of ceRNA network in muscle growth of Nile tilapia.

Using high-throughput RNA-seq analysis, we identified 1947 DE mRNAs, including 1002 up-regulated mRNAs and 945 down-regulated mRNAs in the slow-growing group. Among these down-regulated mRNAs, a total of 52 mRNAs, including *mapk15, igfbp2, creb5, adamts16, tnfsf12, fgf16, tgfbi, capn9, myf5, fgf13, fgf14, myod1, map2k4, map3k2, cdkn3, asb15* are associated with muscle growth which suggests a massive change in muscle development pattern at molecular levels. A recent approach to finding molecular differences in muscle growth of Chinese perch (*Siniperca chuatsi*) and large-scale loach (*Paramisgurnus dabryanus*) reported 8942 and 320 DE mRNAs between large and small individuals, respectively [28,29]. Our results are in line with these findings that describe the significant impact of *myf5, igf, and igfbp* in the accumulation of muscle mass [30,31].

GO term annotation of differentially expressed mRNAs were assembled into glycerophospholipid metabolic process, muscle fiber development, striated muscle cell development, spliceosomal complex, mRNA splicing via spliceosome, spliceosomal tri-snRNP complex, spliceosomal snRNP complex, autophagy, process utilizing autophagic mechanism. Furthermore, in KEGG pathway enrichment analysis, we found that DE-mRNAs were associated with spliceosome, mitophagy, and ribosome proteasome. Mitochondria are essential energy-providing organelles that are required in the maintenance of muscle tissue, and mitophagy is a necessary pathway to maintain mitochondrial quality in muscle homeostasis [32,33]. The genes *bnip3, pgam5, park7, and optn* are

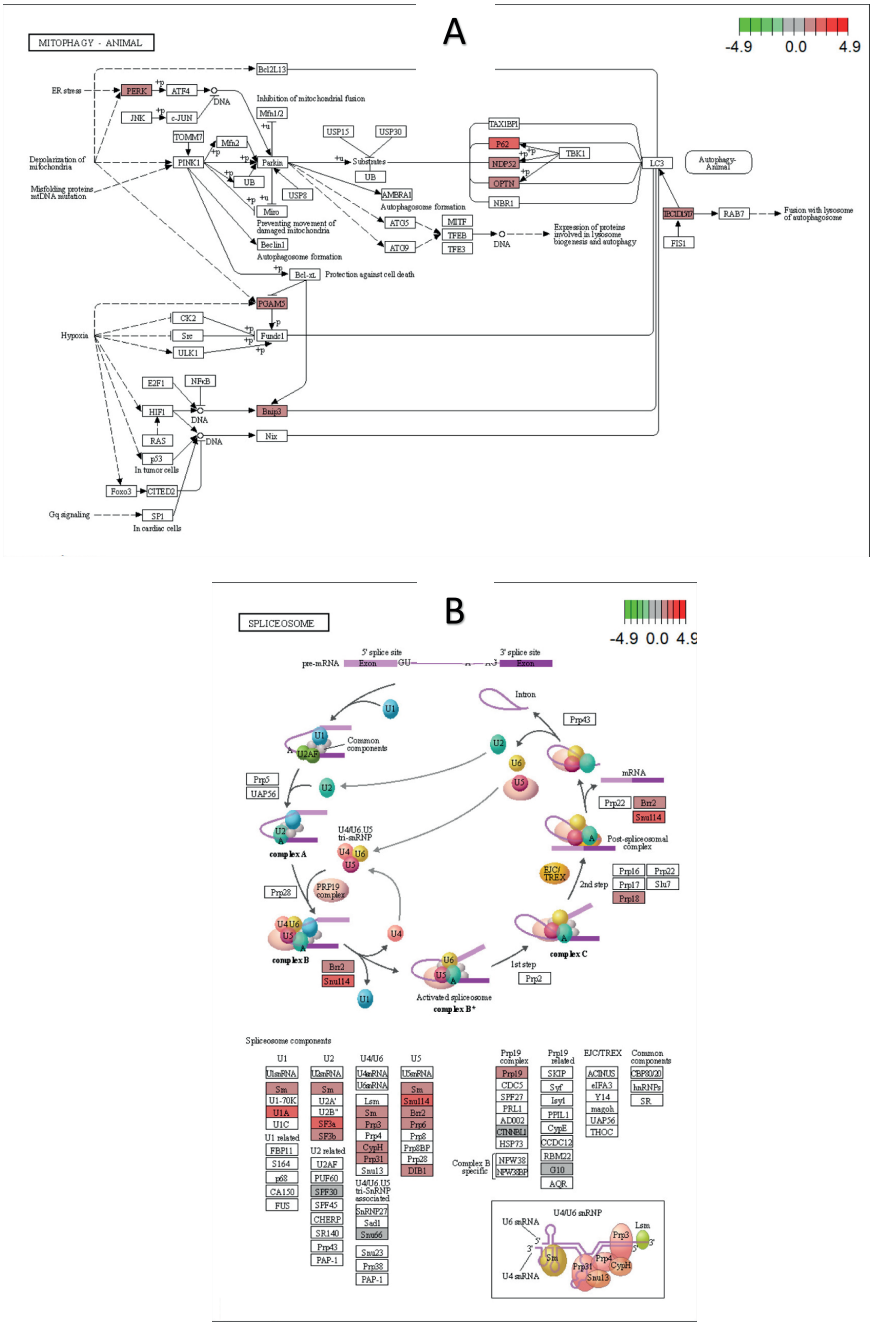

Fig. 6. Graphical representation of (A) spliceosome and (B) mitophagy KEGG pathways. The boxes represent mRNAs in the pathway, with those in red being differentially expressed between BM and SM groups. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

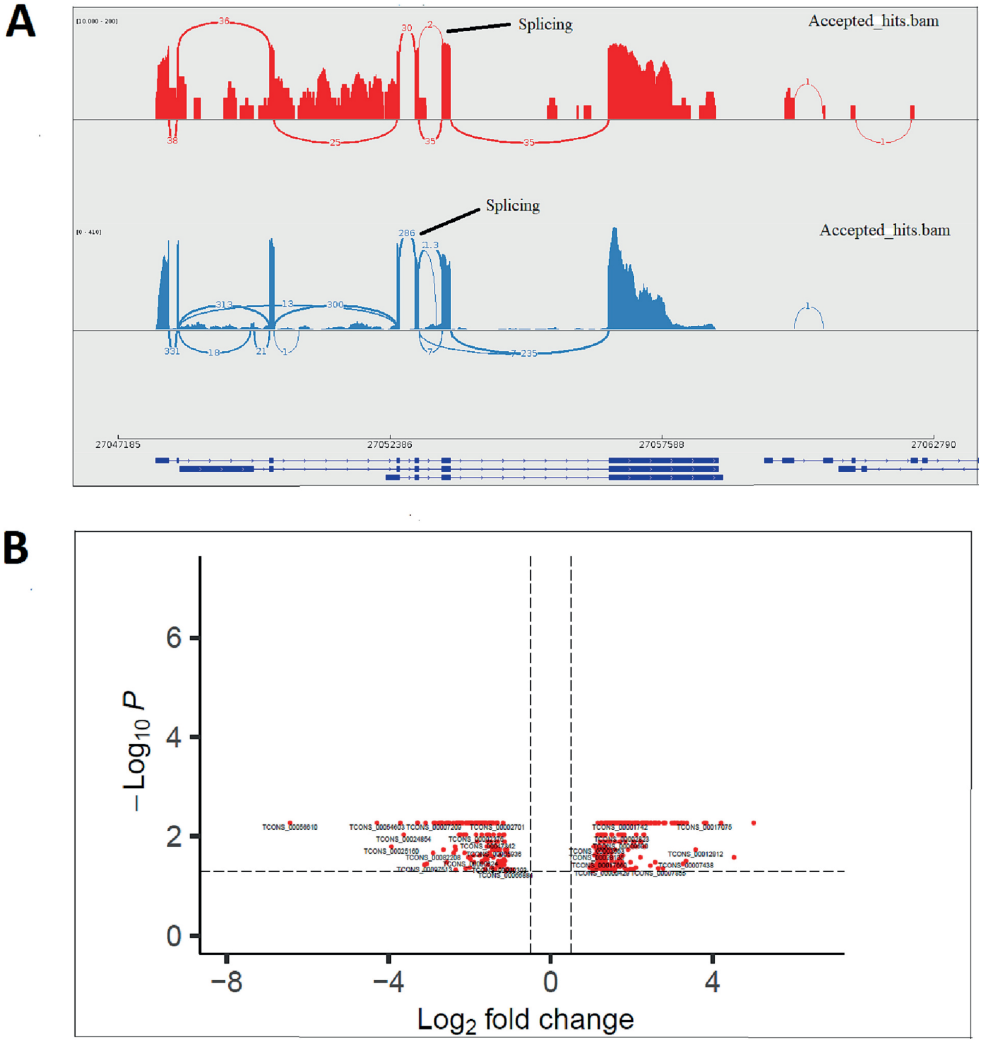

Fig. 7. Comparison of alternative splicing between fast-growing (BM) and slow-growing groups. A) IGV-Sashimi plot for alternatively spliced exon and flanking exons of *dusp22* in two samples (blue (SM) and red (BM)). Regions in genomic coordinates are plotted on the x-axis, and read density (whose value is configurable via IGV) on the y-axis. B) Volcano plot of expressed gene isoforms in Nile tilapia fast muscle. Significant (p-*value* ≤ 0.05) and non-significant isoforms are marked with red and black dots, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly enriched in this pathway; *park7* can activate the transcription of autophagy genes induced by endoplasmic reticulum stress [34]. Besides, *bnip3* - a member of the *bcl-2* family of cell death-regulating factors - mediates mPTP opening, mitochondrial potential, mitochondrial respiratory collapse, and ATP shortage of mitochondria from multiple cells [35]. Thus, impaired mitophagy suggests selective autophagy of damaged or unnecessary mitochondria could lead to loss of muscle mass and function in adult fish.

The spliceosome is a large RNP complex comprising U1, U2, U4/6, and U5 small nuclear RNPs (snRNPs) and several hundred protein factors. Core protein components of the spliceosome contribute to splicing regulation by modulating splice site choice. The spliceosome recognizes splicing signals located both at exon-intron boundaries and numerous *cis*-regulatory sequences that act as splicing enhancers or silencers [36]. It has been shown that spliceosomal complex-dependent alternative splicing produces multiple mRNA variants in muscle [28]. The spliceosome is also involved in circRNA production in addition to its role in constitutive and alternative linear RNA splicing [37]. The requirement of the spliceosome in circRNA formation has been investigated by inhibiting the canonical spliceosome using a pre-mRNA splicing inhibitor [38]. The authors showed that isoginkgetin, which blocks spliceosome assembly at the stage of U4/U5/U6-tri-snRNP, drastically reduces the turnover of natural circRNAs in HeLa cells. In addition, Liang et al. [39] have found that circular RNAs become the preferred output when

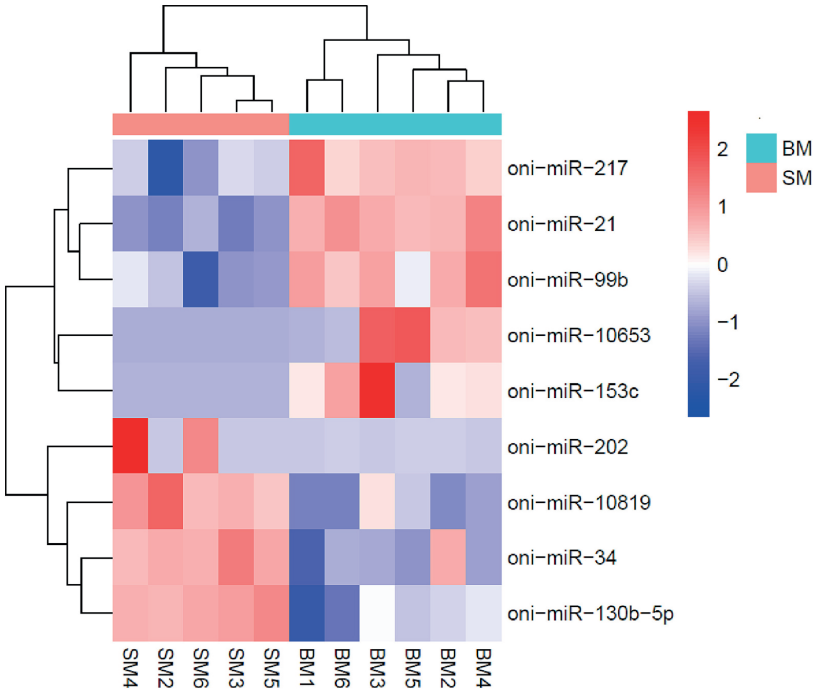

Fig. 8. Heatmap of differentially expressed miRNAs in Nile tilapia fast muscle. The change in colour represents the difference in expression between slow- (SM) and fast-growing (BM) fish, with an adjusted *p-value* ≤ 0.05 and $|\text{Log}_2\text{fold change}| \geq 1$.

core spliceosome or transcription termination factors are depleted from cells. Therefore, the change in the expression of *snu114, sf3a, and u1a* for spliceosome assembly may likely result in changing the levels of many-core spliceosomal components, which in turn will profoundly impact the expression of circRNA splice variants in Nile tilapia muscle.

Recent studies have demonstrated that both mRNA and circRNAs act in the ceRNA network by competing for shared miRNAs. It is well known that circRNAs regulate miRNA activity through binding with miRNA-responsive elements [16]. miRNAs have similar regulatory functions and serve a decisive role in growth by inhibiting targeted mRNA. For this reason, several studies have evaluated mRNA, miRNA, and circRNA co-regulatory relationships during muscle development. For instance, circLMO7 in bovine muscle can competitively absorb miR-378a-3p, which targets the *hdac4* gene, thus promoting muscle cell proliferation and inhibiting muscle cell differentiation [40]. Wang et al. [41] reported the overexpression of circTTN in bovine skeletal muscle induces an inhibitory effect on miR-432 by complementary binding. Inhibition of miR-432 activates the IGF-II/phosphatidylinositol 3-kinase (PI3K)/AKT signal pathway, which promotes the proliferation and differentiation of bovine myoblasts.

We have identified in silico miRNAs that show complementary binding to circRNAs and mRNAs that are possibly involved in the ceRNA network. circMef2c exhibited target correlation with oni-miR-34, oni-miR-130b-5p, and oni-miR-202 expression that, in turn, correlate with their targeted genes under the same growth-associated trajectory. Oni-miR-202, oni-miR-130b-5p, and oni-miR-34 were significantly expressed in slow-growing group comparing with fast-growing. Interestingly, many miRNAs, such as miR-202 and miR-34, have been previously identified during skeletal muscle development of Nile tilapia [42,43]. Moreover, muscle-specific miRNA can target muscle-specific PCMs to mediate skeletal muscle cell proliferation and differentiation. Oni-miR-34 and miR-202 are highly expressed in slow-growing fish muscle and predicted to target down-regulated *igfbp2* and *myod1,* respectively. It is assumed that *igfbp-2* in many fish species positively regulates the *igf-i* activity through binding to their receptors [44]. *igf-i* can activate PI3-kinase and MAPK signalling pathways, which regulate myoblast proliferation and differentiation [45]. In salmonid, *igfbp-2* follows a directional regulation of plasma *igf-i* abundance, which suggests their co-regulation to achieve a specific free-to-bound *igf* ratio that promotes an appropriate physiological response [46,47]. Similarly, *fgf14* has been reported in Nile tilapia muscle which plays an essential gene for somatic growth. A recent study on *fgf14* showed that the overexpression of this gene influences cell proliferation and induces cell apoptosis via mediating PI3K/AKT/mTOR pathway [48]. Currently, it is unknown whether oni-miR-130b-5p is involved in proliferation, differentiation or autophagy in skeletal muscle. However, oni-miR-130b-5p was expressed at a higher level in slow-growing fish compared to the fast-growing group. It has been shown that overexpression of miR-130b dramatically suppressed both in vitro and in vivo proliferation of cells, which could be attributed to influence apoptosis and cell cycle arrest [49]. In addition, the bioinformatic analysis showed that oni-miR-130b-5p (homologous to mmu-miR-130b-5p) target downregulated *myod1,* and the inhibition of *myod1* (*myod1*, usually referred to as *myod*) in cells negatively regulate the proliferation of self-renewing myogenic stem cell population thus, reducing post-natal growth [50].

In summary, this is the first study to provide an overview of circRNA, miRNA, and mRNA co-expression in Nile tilapia muscle tissues. Hundreds of circRNAs, miRNAs, and mRNA were identified; several showed significant differences in expression when comparing fast and slow-growing fish. We established a multiple ceRNA network, including circMef2c-oni-miR-34-igfbp2, circMef2c-oni-miR-130b-5p-myod1, and circMef2c-oni-miR-202-fgf14, which could serve an important role in

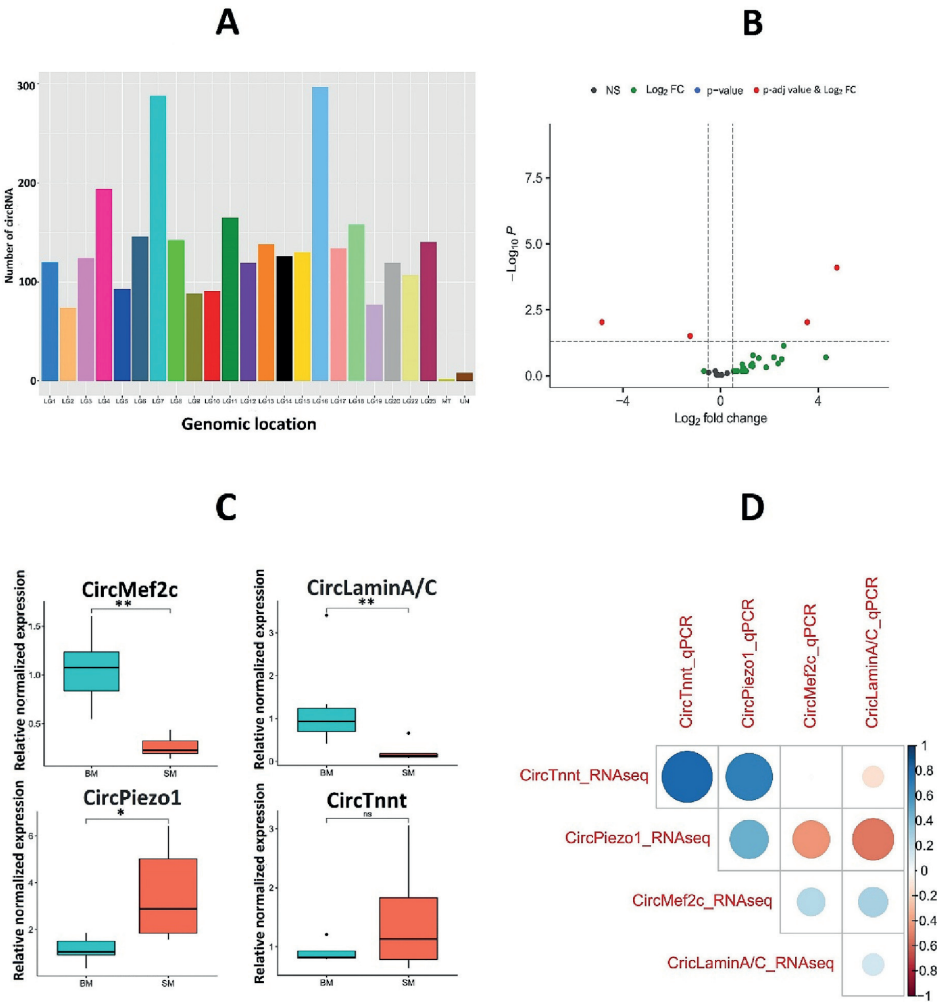

Fig. 9. Characterization and expression profile of circRNAs. A) Distribution of circRNAs in different linkage groups (LG). MT – mitochondrial genome, UN – Unknown. Combined data for BM and SM Nile tilapia groups. B) Volcano plot showing differential expression of circular RNAs between BM and SM groups. Red points represent up- and down-expressed circRNAs with a padj-value below 0.05 and $|Log_2 fold\ change| \geq 1$. The black dots represent non-significant results, green indicates genes having $|Log_2 fold\ change| \geq 1$, and blue represents genes with a padj-value ≤ 0.05. C) qPCR analysis of differentially expressed circRNAs. circRNA expression levels were normalized using the ΔΔCT method, considering the geometric mean of two reference genes (*β-actin* and *elongation factor 1-alpha*). $*p < 0.05$;$**p < 0.01$ D) Correlation between the normalized counts from the circRNA sequencing data and relative gene expression from qPCR data. Differential expression results from next-generation sequencing data were normalized with DESeq2, which performs an internal normalization where geometric means are calculated for each gene across all samples. The colour scale indicates Pearson's correlation coefficient. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Differentially expressed circRNAs between slow- (SM) and fast-growing (BM) Nile tilapia fast muscle, their location, and host genes.

CircRNA ID	CircRNA name	Genomic location	Expression Log_2FC	Structure	Host gene
NC_031978.2:30702661–30,704,116	CircPiezo1	LG13	3.55	Exonic	*piezo type mechanosensitive ion channel component 1*
NC_031976.2:36016595–36,018,688	CircLamin A/C	LG11	−4.86	Exonic	*lamin A/C*
NC_031972.2:9033630–9,035,959	CircMef2c	LG7	−1.24	Exonic	*myocyte-specific enhancer factor 2C-like (mef2c)*
NC_031972.2:48086230–48,086,927	CircTnnt	LG7	4.76	Antisense	*troponin T, cardiac muscle isoforms*

Table 3
CircRNAs and primer sets used for PCR validation.

CircRNA	Primer sequence (5′–3′)	Annealing temperature (°C)	Amplicon size (bp)	Efficiency (%)
circMef2c	Forward: GCGACTGTGAGATTGCCCT Reverse: ACTGGAAGCACACTGTGAAA	58	364	89
CircLaminA/C	Forward: CTATGAGACGGAGCTGGCG Reverse: CTTCTGACTGCAGCCTGCT	58	270	86
CircPiezo1	Forward: AACAGTCGTCCTCTGCAGC Reverse: ATCAGCCCATCAGCGACAG	58	302	105
CircTnnt	Forward: GATCCAGTGGGGGAGCTGT Reverse: GCTTGACAGCATACCCCCA	58	351	94

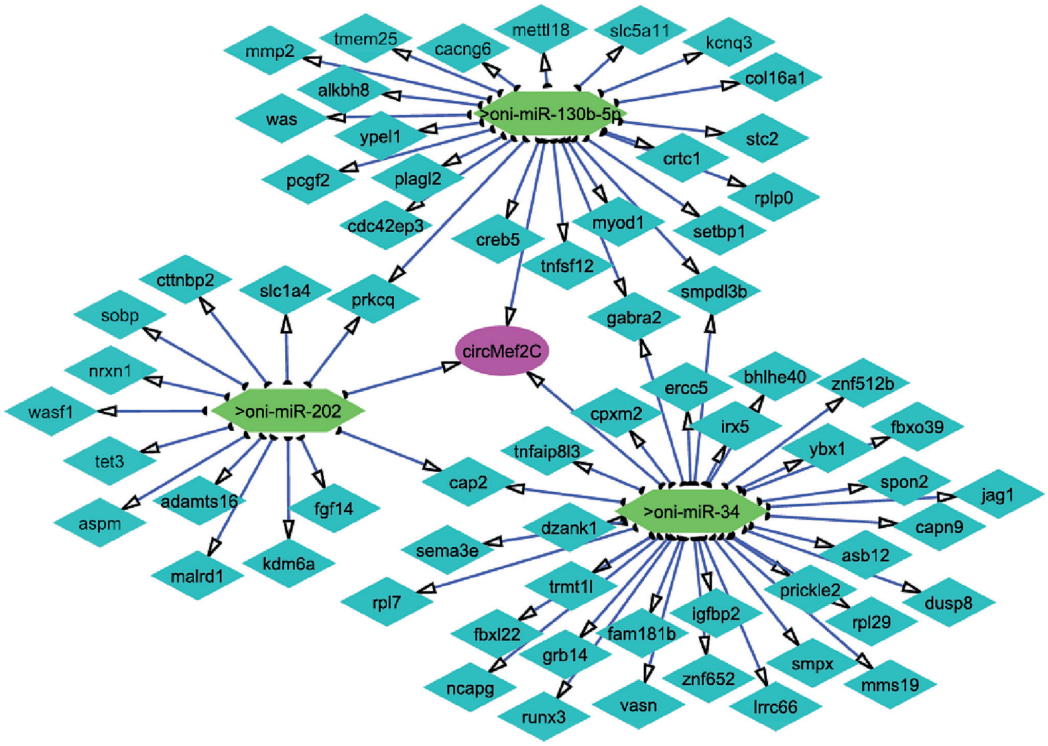

Fig. 10. CircRNA–miRNA–mRNA regulatory network. The network consists of one circRNA(circMef2c), three miRNAs (on-miR-34, oni-miR-130b-5p, oni-miR-202) and 65 protein-coding genes.

muscle gain throughout ontogeny. These results point to a new direction for understanding muscle development and exploring growth biomarkers in teleost species.

4. Materials and methods

4.1. Ethical approval

Research involving live fish was approved by the ethical committee of Nord University (Bodø, Norway) and performed following the regulation and instructions of the Norwegian Animal Research Authority (FOTS ID 1042). All procedures were conducted according to the EU Directive 2010/63 on the use of animals for scientific purposes.

4.2. Sample collection

Fertilized eggs were captured using traditional fishing nets and traps at the river Nile in Luxor (Egypt) and transferred to Nord University as the base population (F0) for our Nile tilapia breeding program. The fish were kept in a freshwater recirculating aquaculture system maintained at the temperature = 28 °C, pH = 7.6 [9,10]. The photoperiod was adjusted at 11:13 h dark:light. All fish were fed ad libitum with 0.15–0.8 mm Amber Neptun pellets (Skretting, Norway). They were maintained for 3 generations and selected for improved growth [8]. The samples for this study were collected from 9-month-old F3 fish reared in common garden to minimize environmental effects. Males were selected because they grow faster than females and are preferred for fish farming. Six slow- (SM) and six fast-growing (BM) full-sib male Nile tilapia were randomly selected. Prior to sampling, fish were euthanized with clove

oil (Sigma Aldrich, USA) before sample collection using a 1:10 mix of 15 mL clove oil with 95% ethanol diluted in 10 L of system water. Fish gender was confirmed by checking the genital papilla. Fast (white) muscle was collected by careful dissection from the left dorsal quadrant and snap-frozen in liquid nitrogen. Muscle samples were stored at −80 °C until total RNA extraction.

4.3. Total RNA extraction and quality control

The frozen fast muscle samples were briefly homogenized using beads (Qiagen GmbH, Germany) and TRI reagent (Zymo Research, USA) at 6500 rpm for 3 × 20s in a Precellys 24 homogenizer (Bertin Instruments, France). Total RNA was extracted from the homogenized tissue using the Direct-zol RNA kit (Zymo Research, USA) according to the manufacturer's instructions. RNA purity was assessed on a NanoDrop ND-1000 spectrophotometer (Thermofisher Scientific, USA) with the criteria of A260/280 $\geq$ 1.8 and A260/A230 $\geq$ 2.0. Afterwards, the concentration of RNA was determined using Qubit RNA Assay Kit in Qubit 3.0 Fluorometer (Thermofisher Scientific, USA), and the RNA integrity was evaluated using TapeStation 2200 (Agilent Technologies, USA). Only RNA samples with an RNA integrity number (RIN) $\geq$ 7 were used for library preparation and sequencing.

4.4. mRNA, miRNA and circRNA library preparation

All samples were processed for mRNA, miRNA and circRNAs library construction. An amount of 200 ng total RNA per sample was used for mRNA sequencing library construction. The libraries were constructed using the NEBNext Ultra II Directional RNA Library Prep (NEB, USA) with the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB, USA). After poly(A) enrichment, mRNA was fragmented to 100–200 nucleotide (nt) length for first and second-strand cDNA synthesis. The cDNA was purified, end-repaired and used for adaptor ligation followed by multiplexing using NEBNext Multiplex Oligos (NEB, USA). Nine PCR cycles were used to amplify the libraries; cDNA library purification was carried out with AMPure XP beads (Beckman Coulter Inc., USA).

Small RNA multiplexed libraries were prepared from 200 ng RNA and using NEXTFLEX® Small RNA-Seq Kit v2 (PerkinElmer, USA). Adaptor ligation, PCR, clean up and size selection were performed according to the manufacturer's instructions with minor modifications, as reported [51].

For circRNA library preparation, 1 μg of total RNA for each sample was used as input. Total RNA was treated with RNase R (Lucigen, USA) for one hour at 37 °C to digest all linear RNA and then purified with Agencourt RNAClean XP beads (Beckman Coulter Inc., USA). Subsequently, the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads (NEB, USA) was used to ensure the complete removal of ribosomal RNA (rRNA). At the end of this procedure, RNA samples contain mainly circular RNA fragments. CircRNA multiplexed libraries were prepared using the NEBNext Ultra II RNA Library Prep Kit (NEB, USA). In brief, the purified RNA samples were used for the first-strand and second-strand cDNA synthesis and adaptor ligation. A unique barcode was tagged in each 12 samples and amplified with 16 PCR cycles.

The quality and quantity of individual mRNA, miRNA, and circRNA libraries were assessed using Agilent High Sensitivity D1000 ScreenTape assay in Agilent 2200 TapeStation (Agilent Technologies, USA). The multiplexed circRNAs, miRNA and mRNA libraries, were then pooled separately at equimolar concentrations and loaded on the flow cells at 1.5 pM. Sequencing was performed on Illumina NextSeq 500 platform (Illumina, USA) with the NextSeq 500 High Output Kit for paired-end (75 bp for circRNA and 150 bp for mRNA) and single-end (75 bp for miRNA) reads at the Nord University genomics facility (Bodø, Norway).

4.5. Bioinformatic analyses

4.5.1. Quality control and mapping

The quality of the raw DNA reads (both Q20 and Q30) and GC percentage was assessed with fastqc (v0.11.5). From circRNA and mRNA data, the reads containing adapter, poly-N and poor quality reads (<Q20) were removed using fastp v0.19.10 [52], and further downstream analyses were performed only with high-quality clean data. The reference genome (NCBI accession: GCF_001858045.2_O_niloticus_UMD_NMBU) [53] and respective annotation file were downloaded from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/). To identify circRNAs, clean reads were mapped to the Nile tilapia reference genome using BWA (v0.7.17) with the parameters: -T 19 -t 8 [54]. The resulting mapped reads from each sample were used as candidates for back-spliced junction detection in CIRI2 v2.0.6 with the -T 4 parameter [55].

In the case of mRNA, cleaned data were mapped to the same reference genome with HISAT2 (v2.2.1) using the default parameters [56]. Next, mapped reads were annotated to reference transcriptome with featureCounts [57] to obtain read count for each gene; subsequently, all samples were concatenated into one count matrix.

For miRNA analysis, adapter sequences and low-quality (Phred quality score 〈20) sequences were removed using Cutadapt v1.12 [58]. Clean reads outside the range of 15–35 nt were excluded. All sequences were aggregated based on the group, and representative reads were then mapped to the same Nile tilapia reference genome through a custom perl script (https://github.com/Qirui4172/Salmon_RNA-seq_miRNA-seq_integrative_analysis). With mirDeep2 v0.1.3 [59], the mapped small RNA tags were used to identify known mature Nile tilapia miRNAs with a single mismatch using miRBase version 22 (http://www.mirbase.org). Customized perl scripts were used to obtain the miRNA counts and concatenate them into a combined count matrix (https://github.com/Qirui4172/Salmon_adherent_cells_RNAseq_miRNAseq_integrative_analysis).

4.5.2. Splice variant analysis

Quantification of gene expression levels from RNA-seq reads also depends on accurately identifying isoforms (splice variants) of a given gene produced in each read. To identify splice variants, all the clean mRNA reads obtained in this study were mapped to the Nile tilapia reference genome using Tophat v1.3.2 [60], guided by its corresponding annotation (GTF) file. Mapped reads were then assembled with the Cufflinks program v1.3.0 [61] using the default parameters. Next, differentially expressed isoforms (adjusted *p-value* $\leq$ 0.05) were identified by comparing BM and SM groups using Cuffdiff (part of the Cufflinks package). The Cuffdiff program also reports genes showing differential levels of alternatively spliced transcripts.

4.5.3. Differential expression of mRNAs, miRNAs and circRNAs

The expression analysis of circRNAs was conducted with circMeta, which uses junction reads without considering the host gene [62]. It performs the deviance goodness of fit test for each circRNA. circRNAs with $|\text{Log}_2\text{fold change}| \geq 1$ and p adjusted value $\leq$0.05 were considered significantly differentially expressed. Finally, host genes of differentially expressed circRNAs were predicted using CircParser [23].

Differential expression of mRNA and mature miRNAs between the BM and SM groups were assessed with the DESeq2 algorithm [63], which uses normalized counts. To be considered differentially expressed, mRNA and miRNA transcripts displayed a minimum $|\text{Log}_2\text{-fold change}| \geq 1$, as well as an adjusted *p-value* $\leq$ 0.05 (Benjamini–Hochberg multiple test correction method).

4.5.4. Functional analysis

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed with significantly differentially expressed mRNA genes (adjusted *p-value* $\leq$ 0.05). Gene IDs were

extracted and annotated in GO and KEGG databases using clusterProfiler [64]. A corrected *p-value* (q-value) ≤ 0.05 (Benjamini–Hochberg multiple test correction) was used as a threshold level of significance for the functional enrichment. Visualization of GO terms was generated using the GOplot R package [65].

4.5.5. Construction of ceRNA network

To explore the co-expression of circRNA, miRNA and mRNA, circRNA–miRNA–mRNA (ceRNA) networks were constructed based on possible functional relationships between DE-circRNAs, DE-miRNAs, and DE-mRNAs. Interaction between DE-circRNA and its targeted DE-miRNA was predicted using Miranda v3.3a software [66] with a threshold of energy score $\Delta G = -12$ kcal/mol and a paring score $S = 120$. The targets of the DE miRNAs were predicted with the parameters $\Delta G = -20$ kcal/mol and $S = 140$. Finally, circRNA–miRNA–mRNA networks were constructed and visualized using Cytoscape 3.8.2 [67].

4.5.6. RT-PCR, Sanger sequencing, and quantitative PCR

Primers and PCR conditions for circRNA validation (Table 3) were developed using a custom docker-based workflow, which includes the Primer3 tool [68]. The same total RNA samples used for the RNA library preparation were used to validate the presence of differentially expressed circRNAs by PCR. We selected circMef2c produced from the growth-associated gene *mef2c* for back-splice junction validation. Complementary DNA (cDNA) was synthesized from 200 ng total RNA using the QuantiTect Reverse Transcription Kit (Qiagen, Germany). The obtained cDNA was further diluted five times with nuclease-free water and used as the PCR template. Divergent primers were expected to amplify the circRNAs fragment near the junction point. PCR amplification was performed with 40 cycles using AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific, USA), and the PCR products were visualized by 2% (*w/v*) agarose gel electrophoresis. Subsequently, the amplified PCR product was used to confirm the back-splice junction sequences by Sanger sequencing. Quantitative qPCR analysis of differentially expressed circRNAs was performed using divergent primers and SYBR green in LightCycler® 96 Real-Time PCR System (Roche Holding AG, Switzerland). The relative expression levels of the target circRNAs were calculated using the ΔΔCT method considering *actin beta* and *elongation factor 1-alpha* as reference genes.

Similarly, the reliability of mRNA and miRNA sequencing data was confirmed by qPCR. Using the NCBI Primer-BLAST tool, we designed the primers for the chosen mRNAs. The primers were then examined by NetPrimer for secondary structures such as hairpins, repetitions, self-dimers, and cross-dimers (Premier Biosoft, Palo Alto, USA). Supplementary table S5 contains a list of selected mRNA primers and their annealing temperature. A Bio-Rad CFX96 real-time PCR machine (Bio-Rad Laboratories, Hercules, CA, USA) was used for qPCR amplification under the following conditions: an initial enzyme activation/cDNA denaturation step at 95 °C for 1 min, followed by 45 cycles at 95 °C for 15 s, 58–61 °C (specific to each primer; Supplementary Table S5) for 15 s and 72 °C for 15 s, with a final standard dissociation protocol to obtain the melting profiles. Data were acquired and analyzed using the CFX Manager software (Bio-Rad). The relative expression levels of the target circRNA were calculated using the delta-CT method considering *actin beta* and *elongation factor 1-alpha* as reference genes.

For validation of the small RNA-seq data, cDNA was synthesized from 200 ng total RNA using the miRCURY LNA RT Kit (Qiagen, Germany), according to the manufacturer's instructions. The obtained cDNA was further diluted 20 times with nuclease-free water and used as qPCR template. Relative expression of 8 DEmiRNAs (Table S6 in Supplementary material) was analyzed using the miRCURY LNA SYBR Green PCR kit and appropriate miRCURY LNA miRNA PCR assay primers (both QIAGEN, Hilden, Germany), following the manufacturer's instructions. Thermocycling was conducted on a CFX96 Touch Real-Time PCR detection system (BioRad, Munich, Germany). The qPCR reactions were set under the following conditions: initial heat activation at 95 °C for 2 min, 40 cycles at 95 °C for 10 s, and 56 °C for 60 s followed by a melt curve analysis. qRT-PCR reactions were performed in triplicates of 6 biological replicates in each group. Data were acquired and analyzed using the CFX Manager software. The relative expression levels of the target miRNAs were calculated using the ΔΔCT method considering oni-miR-10c and oni-miR-26b as reference genes.

Author contributions

J.R., J.G-V. and J.F. designed this study, conceptualized the methodology and experiments; G.R., A.N. and P.S. performed sampling; G.R. performed library preparation and sequencing; G.R., A.N. and F.S. analyzed the data; G.R wrote the draft manuscript; A.N., P.S., F.S., J.R., J.G-V. and J.F. revised the manuscript; J.F. supervised the study. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Data availability

The Nile tilapia mRNA, microRNA, and circRNA sequencing data are available at NCBI Bioproject with the accession number PRJNA825740.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2023.110598.

References

[1] FAO, The state of world fisheries and aquaculture 2020, in: Sustainability in Action. Rome 63, 2020, pp. 8–17.
[2] J. Yáñez, R. Joshi, G. Yoshida, Genomics to accelerate genetic improvement in tilapia, Anim. Genet. 51 (5) (2020) 658–674.
[3] L.M. Valente, K.A. Moutou, L.E. Conceicao, S. Engrola, J.M. Fernandes, I. A. Johnston, What determines growth potential and juvenile quality of farmed fish species? Rev. Aquac. 5 (2013) S93–S168.
[4] J.M. Fernandes, M.G. Mackenzie, G. Elgar, Y. Suzuki, S. Watabe, J.R. Kinghorn, et al., A genomic approach to reveal novel genes associated with myotube formation in the model teleost *Takifugu rubripes*, Physiol. Genomics 22 (3) (2005) 327–338.
[5] G. Rbbani, A. Nedoluzhko, J. Galindo-Villegas, J.M. Fernandes, Function of circular RNAs in fish and their potential application as biomarkers, Int. J. Mol. Sci. 22 (13) (2021) 7119.
[6] C. Campos, A.Y. Sundaram, L.M. Valente, L.E. Conceição, S. Engrola, J. M. Fernandes, Thermal plasticity of the miRNA transcriptome during *Senegalese sole* development, BMC Genomics 15 (1) (2014) 1–13.
[7] M. Horak, J. Novak, J. Bienertova-Vasku, Muscle-specific microRNAs in skeletal muscle development, Dev. Biol. 410 (1) (2016) 1–13.
[8] I. Konstantinidis, D. Anastasiadi, P. Sætrom, A.V. Nedoluzhko, R. Mjelle, T. Podgorniak, et al., Epigenetic mapping of the somatotropic axis in Nile tilapia reveals differential DNA hydroxymethylation marks associated with growth, Genomics. 113 (5) (2021) 2953–2964.
[9] I. Konstantinidis, P. Sætrom, R. Mjelle, A.V. Nedoluzhko, D. Robledo, J. M. Fernandes, Major gene expression changes and epigenetic remodelling in Nile tilapia muscle after just one generation of domestication, Epigenetics. 15 (10) (2020) 1052–1067.
[10] T. Podgorniak, S. Brockmann, I. Konstantinidis, J.M. Fernandes, Differences in the fast muscle methylome provide insight into sex-specific epigenetic regulation of growth in Nile tilapia during early stages of domestication, Epigenetics. 14 (8) (2019) 818–836.
[11] V.S. Patil, R. Zhou, T.M. Rana, Gene regulation by non-coding RNAs, Crit. Rev. Biochem. Mol. Biol. 49 (1) (2014) 16–32.

[12] B. Yan, C.-D. Zhu, J.-T. Guo, L.-H. Zhao, J.-L. Zhao, miR-206 regulates the growth of the teleost tilapia (*Oreochromis niloticus*) through the modulation of IGF-1 gene expression, J. Exp. Biol. 216 (7) (2013) 1265–1269.

[13] X. Zhu, D. Chen, Y. Hu, P. Wu, K. Wang, J. Zhang, et al., The microRNA signature in response to nutrient restriction and refeeding in skeletal muscle of Chinese perch (*Siniperca chuatsi*), Mar. Biotechnol. 17 (2) (2015) 180–189.

[14] E. Lasda, R. Parker, Circular RNAs: diversity of form and function, RNA. 20 (12) (2014) 1829–1842.

[15] S. Memczak, M. Jens, A. Elefsinioti, F. Torti, J. Krueger, A. Rybak, et al., Circular RNAs are a large class of animal RNAs with regulatory potency, Nature. 495 (7441) (2013) 333–338.

[16] Q. Zheng, C. Bao, W. Guo, S. Li, J. Chen, B. Chen, et al., Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs, Nat. Commun. 7 (1) (2016) 1–13.

[17] I.M. Dykes, C. Emanueli, Transcriptional and post-transcriptional gene regulation by long non-coding RNA, Gen. Proteom. Bioinformat. 15 (3) (2017) 177–186.

[18] Z. Li, C. Huang, C. Bao, L. Chen, M. Lin, X. Wang, et al., Exon-intron circular RNAs regulate transcription in the nucleus, Nat. Struct. Mol. Biol. 22 (3) (2015) 256–264.

[19] C.-Y. Yu, H.-C. Kuo, The emerging roles and functions of circular RNAs and their generation, J. Biomed. Sci. 26 (1) (2019) 1–12.

[20] S. Peng, C. Song, H. Li, X. Cao, Y. Ma, X. Wang, et al., Circular RNA SNX29 sponges miR-744 to regulate proliferation and differentiation of myoblasts by activating the Wnt5a/Ca2+ signaling pathway, Mol. Ther. Nucl. Acids. 16 (2019) 481–493.

[21] L. Liu, Y. Chen, J. Diao, L. Luo, Z. Gao, Identification and characterization of novel circRNAs involved in muscle growth of blunt snout bream (*Megalobrama amblycephala*), Int. J. Mol. Sci. 22 (18) (2021) 10056.

[22] R. Joshi, A. Skaaurd, Alvarez A. Tola, Experimental validation of genetic selection for resistance against *Streptococcus agalactiae* via different routes of infection in the commercial Nile tilapia breeding programme, J. Anim. Breed. Genet. 138 (3) (2021) 338–348.

[23] A. Nedoluzhko, F. Sharko, M.G. Rbbani, A. Teslyuk, I. Konstantinidis, J. M. Fernandes, CircParser: a novel streamlined pipeline for circular RNA structure and host gene prediction in non-model organisms, PeerJ. 8 (2020), e8757.

[24] R. Ashwal-Fluss, M. Meyer, N.R. Pamudurti, A. Ivanov, O. Bartok, M. Hanan, et al., circRNA biogenesis competes with pre-mRNA splicing, Mol. Cell 56 (1) (2014) 55–66.

[25] C.M. Anderson, J. Hu, R.M. Barnes, A.B. Heidt, I. Cornelissen, B.L. Black, Myocyte enhancer factor 2C function in skeletal muscle is required for normal growth and glucose metabolism in mice, Skelet. Muscle 5 (1) (2015) 1–10.

[26] B. Yue, J. Wang, W. Ru, J. Wu, X. Cao, H. Yang, et al., The circular RNA circHUWE1 sponges the miR-29b-AKT3 axis to regulate myoblast development, Mol. Ther. Nucl. Acids. 19 (2020) 1086–1097.

[27] M. Chen, X. Wei, M. Song, R. Jiang, K. Huang, Y. Deng, et al., Circular RNA circMYBPC1 promotes skeletal muscle differentiation by targeting MyHC, Mol. Ther. Nucl. Acids. 24 (2021) 352–368.

[28] X. Liu, S. Zeng, S. Liu, G. Wang, H. Lai, X. Zhao, et al., Identifying the related genes of muscle growth and exploring the functions by compensatory growth in mandarin fish (*Siniperca chuatsi*), Front. Physiol. 11 (2020) 1215.

[29] L. Zhao, K. He, Q. Xiao, Q. Liu, W. Luo, J. Luo, et al., Comparative transcriptome profiles of large and small bodied large-scale loaches cultivated in paddy fields, Sci. Rep. 11 (1) (2021) 1–10.

[30] N.I. Bower, X. Li, R. Taylor, I.A. Johnston, Switching to fast growth: the insulin-like growth factor (IGF) system in skeletal muscle of Atlantic salmon, J. Exp. Biol. 211 (24) (2008) 3859–3870.

[31] L.-L. Shi, K.-C. Zhu, H.-L. Wang, Characterization of myogenic regulatory factors, myod and myf5 from *Megalobrama amblycephala* and the effect of lipopolysaccharide on satellite cells in skeletal muscle, Gene. 146608 (2022).

[32] L.J. Oost, M. Kustermann, A. Armani, B. Blaauw, V. Romanello, Fibroblast growth factor 21 controls mitophagy and muscle mass, J. Cachexia. Sarcopenia Muscle 10 (3) (2019) 630–642.

[33] V. Romanello, M. Sandri, The connection between the dynamic remodeling of the mitochondrial network and the regulation of muscle mass, Cell. Mol. Life Sci. 78 (4) (2021) 1305–1328.

[34] D.-H. Lee, D. Kim, S.T. Kim, S. Jeong, J.L. Kim, S.M. Shim, et al., PARK7 modulates autophagic proteolysis through binding to the N-terminally arginylated form of the molecular chaperone HSPA5, Autophagy. 14 (11) (2018) 1870–1885.

[35] A. Gao, J. Jiang, F. Xie, L. Chen, Bnip3 in mitophagy: novel insights and potential therapeutic target for diseases of secondary mitochondrial dysfunction, Clin. Chim. Acta 506 (2020) 72–83.

[36] A.N. Ladd, T.A. Cooper, Finding signals that regulate alternative splicing in the post-genomic era, Genome Biol. 3 (11) (2002) 1–16.

[37] K.K. Ebbesen, T.B. Hansen, J. Kjems, Insights into circular RNA biology, RNA Biol. 14 (8) (2017) 1035–1045.

[38] S. Starke, I. Jost, O. Rossbach, T. Schneider, S. Schreiner, L.-H. Hung, et al., Exon circularization requires canonical splice signals, Cell Rep. 10 (1) (2015) 103–111.

[39] D. Liang, D.C. Tatomer, Z. Luo, H. Wu, L. Yang, L.-L. Chen, et al., The output of protein-coding genes shifts to circular RNAs when the pre-mRNA processing machinery is limiting, Mol. Cell 68 (5) (2017) 940–954 (e3).

[40] X. Wei, H. Li, J. Yang, D. Hao, D. Dong, Y. Huang, et al., Circular RNA profiling reveals an abundant circLMO7 that regulates myoblasts differentiation and survival by sponging miR-378a-3p, Cell Death Dis. 8(10):e3153-e (2017).

[41] X. Wang, X. Cao, D. Dong, X. Shen, J. Cheng, R. Jiang, et al., Circular RNA TTN acts as a miR-432 sponge to facilitate proliferation and differentiation of myoblasts via the IGF2/PI3K/AKT signaling pathway, Mol. Ther. Nucl. Acids. 18 (2019) 966–980.

[42] C. Huang, Y. Li, S. Hu, J. Chi, G. Lin, C. Lin, et al., Differential expression patterns of growth-related microRNAs in the skeletal muscle of Nile tilapia (*Oreochromis niloticus*), J. Anim. Sci. 90 (12) (2012) 4266–4279.

[43] Y. Huang, X.Y. Ma, Y.B. Yang, H.T. Ren, X.H. Sun, L.R. Wang, Identification and characterization of microRNAs and their target genes from Nile tilapia (*Oreochromis niloticus*), Z. Naturforsch. C 71 (7–8) (2016) 215–223.

[44] J. Zhou, W. Li, H. Kamei, C. Duan, Duplication of the IGFBP-2 gene in teleost fish: protein structure and functionality conservation and gene expression divergence, PLoS One 3 (12) (2008), e3926.

[45] F. Mourkioti, N. Rosenthal, IGF-1, inflammation and stem cells: interactions during muscle regeneration, Trends Immunol. 26 (10) (2005) 535–542.

[46] A. Pierce, M. Shimizu, B.R. Beckman, D. Baker, W.W. Dickhoff, Time course of the GH/IGF axis response to fasting and increased ration in Chinook salmon (*Oncorhynchus tshawytscha*), Gen. Comp. Endocrinol. 140 (3) (2005) 192–202.

[47] M. Shimizu, K.A. Cooper, W.W. Dickhoff, B.R. Beckman, Postprandial changes in plasma growth hormone, insulin, insulin-like growth factor (IGF)-I, and IGF-binding proteins in coho salmon fasted for varying periods, Am. J. Phys. Regul. Integr. Comp. Phys. 297 (2) (2009) R61–R352.

[48] T. Su, L. Huang, N. Zhang, S. Peng, X. Li, G. Wei, et al., FGF14 functions as a tumor suppressor through inhibiting PI3K/AKT/mTOR pathway in colorectal cancer, J. Cancer 11 (4) (2020) 819.

[49] G. Zhao, J.-g. Zhang, Y. Shi, Q. Qin, Y. Liu, B. Wang, et al., MiR-130b is a prognostic marker and inhibits cell proliferation and invasion in pancreatic cancer through targeting STAT3, PLoS One 8 (9) (2013), e73803.

[50] I.A. Johnston, N.I. Bower, D.J. Macqueen, Growth and the regulation of myotomal muscle mass in teleost fish, J. Exp. Biol. 214 (10) (2011) 1617–1628.

[51] C.C. Oliveira, E. Fatsini, I. Fernández, C. Anjos, F. Chauvigné, J. Cerdà, et al., Kisspeptin influences the reproductive axis and circulating levels of microRNAs in Senegalese sole, Int. J. Mol. Sci. 21 (23) (2020) 9051.

[52] S. Chen, Y. Zhou, Y. Chen, J. Gu, Fastp: an ultra-fast all-in-one FASTQ preprocessor, Bioinformatics. 34 (17) (2018) i90–i884.

[53] M.A. Conte, R. Joshi, E.C. Moore, S.P. Nandamuri, W.J. Gammerdinger, R. B. Roberts, et al., Chromosome-scale assemblies reveal the structural evolution of African cichlid genomes, Gigascience. 8 (4) (2019) giz030.

[54] H. Li, R. Durbin, Fast and accurate short read alignment with Burrows–Wheeler transform, Bioinformatics. 25 (14) (2009) 1754–1760.

[55] Y. Gao, J. Zhang, F. Zhao, Circular RNA identification based on multiple seed matching, Brief. Bioinform. 19 (5) (2018) 803–810.

[56] D. Kim, B. Langmead, S.L. Salzberg, HISAT: a fast spliced aligner with low memory requirements, Nat. Methods 12 (4) (2015) 357–360.

[57] Y. Liao, G.K. Smyth, W. Shi, featureCounts: an efficient general purpose program for assigning sequence reads to genomic features, Bioinformatics. 30 (7) (2014) 923–930.

[58] M. Martin, Cutadapt removes adapter sequences from high-throughput sequencing reads, EMBnet J. 17 (1) (2011) 10–12.

[59] M.R. Friedländer, S.D. Mackowiak, N. Li, W. Chen, N. Rajewsky, miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades, Nucleic Acids Res. 40 (1) (2012) 37–52.

[60] C. Trapnell, L. Pachter, S.L. Salzberg, TopHat: discovering splice junctions with RNA-Seq, Bioinformatics. 25 (9) (2009) 1105–1111.

[61] C. Trapnell, B.A. Williams, G. Pertea, A. Mortazavi, G. Kwan, M.J. Van Baren, et al., Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation, Nat. Biotechnol. 28 (5) (2010) 511–515.

[62] L. Chen, F. Wang, E.C. Bruggeman, C. Li, B. Yao, circMeta: a unified computational framework for genomic feature annotation and differential expression analysis of circular RNAs, Bioinformatics. 36 (2) (2020) 539–545.

[63] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, Genome Biol. 15 (12) (2014) 1–21.

[64] G. Yu, L.-G. Wang, Y. Han, Q.-Y. He, clusterProfiler: an R package for comparing biological themes among gene clusters, OMICS: J Integrat. Biol. 16 (5) (2012) 284–287.

[65] W. Walter, F. Sánchez-Cabo, M. Ricote, GOplot: an R package for visually combining expression data with functional analysis, Bioinformatics. 31 (17) (2015) 2912–2914.

[66] A. Enright, B. John, U. Gaul, T. Tuschl, C. Sander, D. Marks, MicroRNA targets in Drosophila, Genome Biol. 4 (11) (2003) 1–27.

[67] P. Shannon, A. Markiel, O. Ozier, N.S. Baliga, J.T. Wang, D. Ramage, et al., Cytoscape: a software environment for integrated models of biomolecular interaction networks, Genome Res. 13 (11) (2003) 2498–2504.

[68] F. Sharko, G. Rbbani, P. Siriyappagouder, J.A. Raeymaekers, J. Galindo-Villegas, A. Nedoluzhko, et al., CircPrime: a web-based platform for design of specific circular RNA primers, bioRxiv (2022), https://doi.org/10.1101/2022.12.20.521155.

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Fish muscle is a focal point in the aquaculture industry due to its commercial importance as the edible portion of fish. The regulation of muscle development and growth is coordinated by a multitude of genetic and environmental factors, including temperature. To date, the molecular mechanisms associated with these factors and their intricate relationships are not fully understood. Therefore, this PhD thesis aims to understand the complex regulatory network of circular RNAs (circRNAs) in the context of muscle growth regulation in fish and explore the relationship between the thermal developmental plasticity of circRNA expression and muscle growth. To achieve this, we employed an integrative omics approach to quantify and compare the expression profiles of circRNAs, miRNAs, and mRNAs in fast muscle from Nile tilapia. We further manipulated embryonic temperatures to assess circRNA expression and its correlation with long-term growth. Our results indicate that circRNA-miRNA-mRNA networks may play a significant role in enhancing muscle growth. Furthermore, circRNA expression changes with embryonic temperature, which correlates with growth at the juvenile stage.

This thesis significantly advances our understanding of the molecular mechanisms underlying muscle growth in Nile tilapia, with a specific focus on circRNAs, laying a robust foundation for new research lines in muscle development. Additionally, it highlights the potential of circRNAs as molecular growth markers, opening a promising avenue for evaluating and monitoring how fish respond to temperature fluctuations, which is particularly important in the context of global climate change.

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