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ORIGINAL ARTICLE

The expression of myogenic gene markers during the embryo-larval-transition in Pikeperch (*Sander lucioperca*)

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Abstract

Pikeperch (*Sander lucioperca*) has become a species of interest in aquaculture. It is a popular and economically valuable food fish and can produce high numbers of offspring. However, during early development, there are transition phases when high mortality rates concur with growth changes, vital organ transformations and a limited energy budget. Up to now, no study focused on the developmental adaption of muscle tissue in pikeperch, regardless of muscle tissue influencing essential traits such as locomotion and thus the competence to hunt prey and avoid predators. In the present study, therefore, the developmental myogenesis of pikeperch was analysed using specimens from early embryonic to larval development. Myogenic and developmental genes were utilized to gain insights into transcriptomic regulation during these stages by applying a nanofluidic qPCR approach. Result, three phases of myogenic gene expression, during somitogenesis, during the late embryonic development and during the larval development were detected. Increased myostatin expression showed an interim arrest of muscle formation between embryonic and larval myogenesis. Expression patterns of satellite cell gene markers indicated an accumulation of stem cells before myogenesis interruption. The here gained data will help to broaden the knowledge on percid myogenesis and can support pikeperch rearing in aquaculture.

KEYWORDS
Fluidigm array, gene expression, larval fish, ontogeny, Percidae, regulatory networks

1 | INTRODUCTION

Pikeperch is a new focus species in freshwater aquaculture. It has large economical potential as it already has a broad consumer base as well as the biological capability to produce large numbers of offspring (Lappalainen et al., 2003; Steenfeldt et al., 2015). Still, it is rare in aquaculture, as high mortality rates occur during its rearing and husbandry (Policar et al., 2019). This is especially severe during the early development between embryonic and larval ontogeny. During this period, the loss of around half or more of the reared specimens can occur, which highly affects the economic success of pikeperch aquaculture (Ostaszewska et al., 2005; Szkudlarek & Zakęś, 2007).

Several developmental changes occur during this time including late embryonic organ formation, hatching, the transition from endogenous to exogenous feeding and several organ changes connected to these (Bastl, 1978; Demska-Zakęś et al., 2003; Kimmel et al., 1995;

Ostaszewska, 2005). At the same time, the energy budget of fish larvae is generally restricted, which requires an adjustment of energy distribution (Rombough, 2011; Wieser, 1995). In pikeperch larvae, the present energy resource is especially sparse, leading to the death of larvae after overstepping a point of no return 1–2 days after the depletion of endogenous resources (Xu et al., 2017).

During the early development of fish and other vertebrates three phases of myogenic activity are present (Rowlerson & Veggetti, 2001; Steinbacher et al., 2007). In these early stages after hatching, muscle development is crucial for survival. In zebrafish (*Danio rerio*), variable swimming modes are already present in larval stages where they are applied as well for general locomotion as for capturing prey items (Budick & O'Malley, 2000). However, since the water resistance is different for smaller than for larger organisms, concurrent with smaller Reynolds numbers, the water becomes more viscous for smaller fish or developmental stages, and the energy expenditure is thus higher (Muller et al., 2000). This viscosity can lead to additional difficulties during development also for the young and small pikeperch larvae, causing locomotion to become crucial for survival. Thus, problems of, for example, hydrodynamic starvation during initial feeding events (China & Holzman, 2014) as well as difficulties with breaking through the water surface during swim bladder inflation (Blecha et al., 2019; Summerfelt, 2013) can occur occasionally during early development of pikeperch larvae. Up to now, no study was undertaken to analyse muscle development and its regulation during that time in pikeperch. To fill this gap, we conducted a gene expression analysis of near-wild reared pikeperch specimens from embryonic to larval development. We chose specimens from a wild population, which were reared under natural as possible conditions. While this involves unsteady environmental conditions (like water temperature), it also allows observing specimens free or otherwise present cultivation or breeding effects (Berg et al., 1990; Mas-Muñoz et al., 2013; Molnár et al., 2020), which possibly are present in aquaculture specimens.

Generally, muscle development relies on a cascade from myogenic progenitor cells derived from the dermomyotomal somite tissues, through myoblasts and myotubes, to the final myofibres (Chal & Pourquié, 2017; Cossu et al., 1995; Kato & Gurdon, 1993; Watabe, 1999). For vertebrates, different master genes are commonly associated with these steps. After the initial formation, further growth of muscle occurs via an increase in the number of cells (hyperplasia) or an enlargement of the cells (hypertrophia) in myofibres (Churova et al., 2017). Due to hyperplasia even in the adult stages, fish exhibit lifelong muscle growth, which differs from the other vertebrate groups (Rowe & Goldspink, 1969; Watabe, 1999).

In this study, the expression of selected master genes was analysed for the early developmental stages of pikeperch. Genes with a contribution to myogenesis in other teleosts were chosen (Watabe, 1999; Watabe, 2001). As this study focuses primarily on early ontogeny, we included genes that influence general development as well as genes that regulate local muscle formation (Degenhardt & Sassoon, 2001; Kimmel et al., 2001). As muscle localization during development is controlled by the musculoskeletal system (Sefton & Kardon, 2019), additional gene markers for collagenous (Gelse et al., 2003) and skeletal development (Gersch & Hadjiargyrou, 2009; Hadjiargyrou, 2018) were included. The goal of this study was to provide a basic expression profile of developmental genes to gain insight into early ontogeny, and in particular, the myogenesis of pikeperch.

2 | MATERIALS AND METHODS

2.1 | Specimen rearing and collection

Pikeperch specimens were gained from a wild population of parental animals from the Hohen Sprenz Lake in Mecklenburg Western-Pomerania, Germany. During spawning season, a group of eight females and three males were kept in a net cage of 4 m × 4 m × 2 m and fed with fish from the lake. Eggs were spawned on coco-mats and fertilized naturally. Afterwards, fertilized eggs were placed in Zuger-jars. Shortly before hatch, the specimens were transferred to a net cage of 1 m × 0.8 m × 0.8 m in size situated in a larger tank with 2500 L volume. A flow-through system provided natural water conditions including temperatures conforming to the lake during the whole period. The temperature was measured in the lake as well as in the flow-through system using temperature loggers (iButton MF1921G, Maxim Integrated) to control for similar temperature levels between the measuring points and to determine the developmental age in degree-days (dd). A spotlight lamp provided illumination with a 12:12 h day/night setting. Altogether, eight age stages were taken with embryonic stages at 1 dpf (26 dd), 3 dpf (53 dd), 5 dpf (80 dd), 8 dpf (127 dd) and 10 dpf (154 dd), and larval stages at 17 dpf (248 dd), 19 dpf (280 dd) and 22 dpf (325 dd). Embryonic and larval development were separated based on the start of external food ingestion (Peňáz, 2001). For each stage, three pools of 15 specimens were euthanized using MS222 (Serva) in a 0.25 g/L concentration. They were transferred to RNA-later (Thermo Fisher) and stored at −80°C until RNA extraction. To record developmental changes between the sampled stages, specimens were examined under the stereomicroscope (Leica SD9) and photographed using the Moticam 5.0 with associated Software (Motic, Motic Images Plus Version 3.0.). This study followed international, national and institutional guidelines for animal treatment and sacrifice and complied with Directive 2010/63/EU and the German Animal Welfare Act [§ 4(3) TierSchG].

2.2 | Selection of genes and primer design

Altogether, 19 genes were selected for analysis (Table 1). The focus was placed on genes regulating myogenesis with the transformation of myogenic progenitor cells to myoblasts (myogenic factor 5, myogenic differentiation 1, paired box 3, paired box 7a and msh homeobox 1), the formation of myotubes (myogenin, myocyte enhance factor 2a, insulin-like growth factor 1, insulin-like growth factor 2 and transforming growth factor beta 1) and muscle fibre

TABLE 1 Overview of selected genes and primers for Fluidigm qPCR analysis

Gene symbol	For/Rev sequence (5′- 3′)	Accession no./source	Product length [bp]
Target genes			
BMP4	CCGTAAACGCAACCGCAACTG AGACCGCCTAGTAGACTTGAGT	Schäfer et al. (2021)	151
COL1A1	TCCGCTGGCAACCTCAAGAAG TGATGGGCAGGCGAGATGTTTT	JN112557.1	181
COL1A2	AAGAGGCCAACCCGGAAACATT AGACCAGTGGGACCAGTGGAT	XM_028596714.1	99
EN2	CTCAAACCCAGCCCAGTCTCA CGTCCGTGGTCGCTTGTCCT	XM_028568870.1	142
IGF1	TGTGTGGAGAGAGAGGCTTTTAT AGCGAGCAGCCTTGCTAGTCT	XM_031313442.1	158
IGF2	GAGGCTTCTATTTCAGGTAGGC ACGGGTATGACCTGCAGAGAG	XM_031282902.1	162
MSX1	CGAGAGCCCCGACAGTAATGA GGGGTCCGGGGCTTTCTGTT	XM_028576570.1	132
MUSTN1	TATTGCCCCAAAGCGCTACAAG TTGATGGGCGACATCTTGGTAG	Swirplies et al. (2019)	153
MEF2A	AGAGTGCGGGAAGAAATCGTGT ACGCTCAGCTCATAGGCCTTTT	XM_028584207.1	182
MYOD1	CTCCGACGGCATGACGGATTT GAGATCCGCTCCACAATGCTG	XM_028584397.1	165
MYF5	GTGGAAAACTACTACGGCCTAC TCGTTCCTCGCATATGAATAACC	HM190249.1	152
MRF4	TGATGGGCTTATGTGGGAGTCT CTCTCCGACGGTCCATGGTAA	HM190251.1	177
MYOG	TTCTACGAGGGAGGGGACAG GAACTGGGCTCACTTGACGA	XM_031323467	512
MYH6	CTTGCTGGAGAAGTCACGGT CGAGAGCGCCAAGAGAGTAG	HM050076	485
MSTN	ACTGGGGCATCGAGATCAACG TTGGGGCCCTCTGAGATCTTAA	XM_028591409.1	121
PAX3	ACCCACGCTGGCTCAGAACTA CTCCACGATTTTATGTCGGATGT	XM_031283761.1	145
PAX7A	ACTGCGAGTTTCTCACGGTT ATGGAGCTCACAGATGAAGCC	XM_031302059.1	252
PGC1A	CCGTTTGAGAACAAAACCATTGAA TGACCGATGCTTTGAAAGGATTC	XM_028564706.1	124
TGFB1	GCGTGAGGAGCTTGTACATCG TGATGCTGGTACAGGGCCAAAA	XM_031285154	157
Reference genes			
EEF1A1	ATGGACAGACCCGTGAGCATG TTCTTGATGTAGGTGCTCACTTC	Swirplies et al. (2019)	151
RPL32	GGCGTAAACCCAGAGGTATTGA ACCTCGAGCTCCTTGACATTGT	Swirplies et al. (2019)	157
RPS5	GCAGGATTACATTGCTGTGAAAG TCATCAGCTTCTTGCCATTGTTG	Swirplies et al. (2019)	161

development (myogenic regulatory factor 4 and myostatin). Further, genes regulating regional specific myogenesis (engrailed 2 and myosin heavy chain 6), structural tissues formation (collagen type 1 alpha 1, collagen type 1 alpha 2, bone morphogenetic protein 4 and musculoskeletal embryonic nuclear protein 1) and energy metabolism (peroxisome proliferative activated receptor gamma coactivator 1 alpha) were chosen. Following the MIQE Guidelines (Bustin et al., 2009), three reference genes (elongation factor 1 alpha, ribosomal protein L32 and ribosomal protein S5) were chosen and validated to allow normalizing the expression data (Pfaffl, 2001; Swirplies et al., 2019).

For primer design, sequences were obtained from the NCBI GenBank database. If no pikeperch gene sequences (RefSeq NCBI: GCA_008315115) were available, sequences from other perciform species (*Perca flavaescens* and *Epinephelus coioides*) were chosen and their homology was checked using BLAST algorithms against the genome of *Sander lucioperca* (RefSeq NCBI: GCA_008315115; PMID: 31540274/DOI: 10.3390/genes10090708). The oligonucleotide primer sequences (Table 1) were designed using the software PSQ-Assay Design (Version 1.0.6, Biotage) and synthesized by SIGMA–Aldrich (Merck). Primers were evaluated by performing a standard PCR, gel electrophoresis, as well as qPCR on a LightCycler96 instrument (Roche) to ensure the formation of single products and to prevent primer dimer-formation.

2.3 | Extraction of RNA and RT-qPCR

For RNA extraction, pools of 15 specimens each were mechanically homogenized using Precellys Evolution (VWR) followed by Trizol-Chloroform precipitation. Total RNA was further purified using the RNeasy Micro Kit (Qiagen) combined with an RNase-free DNase Set (Qiagen) according to the manufacturer's instructions. The concentration of RNA was determined using the 260/280 nm absorbance ratio obtained with the NanoDrop ND-1000 spectrophotometer (Peqlab). Until further measurements, the RNA was stored at −80°C.

Transcriptional analysis was conducted using a quantitative real-time PCR based on a microfluid circuit system (Biomark, FLUIDIGM) using EvaGreen fluorescence dyes (Bio-Rad). The protocol was described in detail by Rebl et al. (2019). The design of Fluidigm 48.48 Dynamic Array IFC allows the parallel quantification of 48 transcripts in 48 samples each. Three biological replicates were assessed in a form of two technical replicates each. The raw cycle thresholds (CT-values) were retrieved using the real-time PCR analysis software (Fluidigm, Version 3.0.2).

2.4 | Data analysis and graph design

The resulting CT-values were used as a base for a relative quantification established by Pfaffl (2001) assuming a constant efficiency (E = 2.0) for all sequences. The values obtained from all examined stages were converted to fold changes (FC) to the expression of the first age stage. Consequently, the gene-expression data during the first stage was set at one. Based on the obtained relative ratios of the age stages, three biological replicates and their respective geometric mean data were analysed using R (Version 4.0.3). Statistically significant differences between the expression of all examined stages were determined by conducting an ANOVA followed by a Tukey-test ($p = 0.05$) with the library stats (Version 4.0.3). Graphs were designed by applying the ggplot2 library (Version 3.3.2). Correlation between gene expressions of embryonic and larval stages was checked using corrplot library (Version 0.84; provided as supplemental only, Tables S1 and S2). The depiction of the expression networks in comparison to the first age stage (26 dd) was generated using Ingenuity Pathway Analysis (IPA) Software (Qiagen, Release March 2021).

3 | RESULTS

3.1 | Development of the analysed stages

The earliest obtained embryonic stage, taken at 1 day post fertilization (dpf) with an age of 26 dd, progressed during the latest stages of gastrulation up to early segmentation. The following embryonic stages at 3 dpf (53 dd) had an increasing number of somites (Figure 1a), the eye anlagen became visible, the heart started to beat and the tail detached from the yolk (Figure 1b). At 8 and 10 dpf (127 and 154 dd) some first prehatchlings occurred. At this phase, the pectoral fin bud formed and subsequently started to shift laterventrally. The head, which was previously bent to the yolk, began to straighten up. The eyes were fully pigmented and the mandibular was positioned ventral to the eyes. The first larval stage at the 17 dpf stage (248 dd) had an open mouth and ingested the first food particles (Figure 1c). Additionally, the pectoral fins were well developed and arrived in their final body position (Figure 1c). In the 19 dpf stage (280 dd), the yolk was depleted in most specimens, but in a few specimens oil droplet remnants remained present. Simultaneously, the stomach was now distinguishable from the lower intestines. At 22 dpf (325 dd), all specimens had switched to solely exogenous nutrition (Figure 1d). The formation of skeletal elements progressed further (Figure 1d,e).

3.2 | Activation of myogenic progenitor cells and stem cell regulation

The expression of the genes associated with muscle stem cells and satellite cells was not uniform across the different age groups (Figure 2). However, myogenic differentiation 1 (MYOD1, Figure 2a) and myogenic factor 5 (MYF5, Figure 2b) followed similar expression patterns. A maximum expression was present at 53 dd. The following lower expression levels were interrupted by a slightly increased expression from 127 to 154 dd and again at 280 dd. The paired box

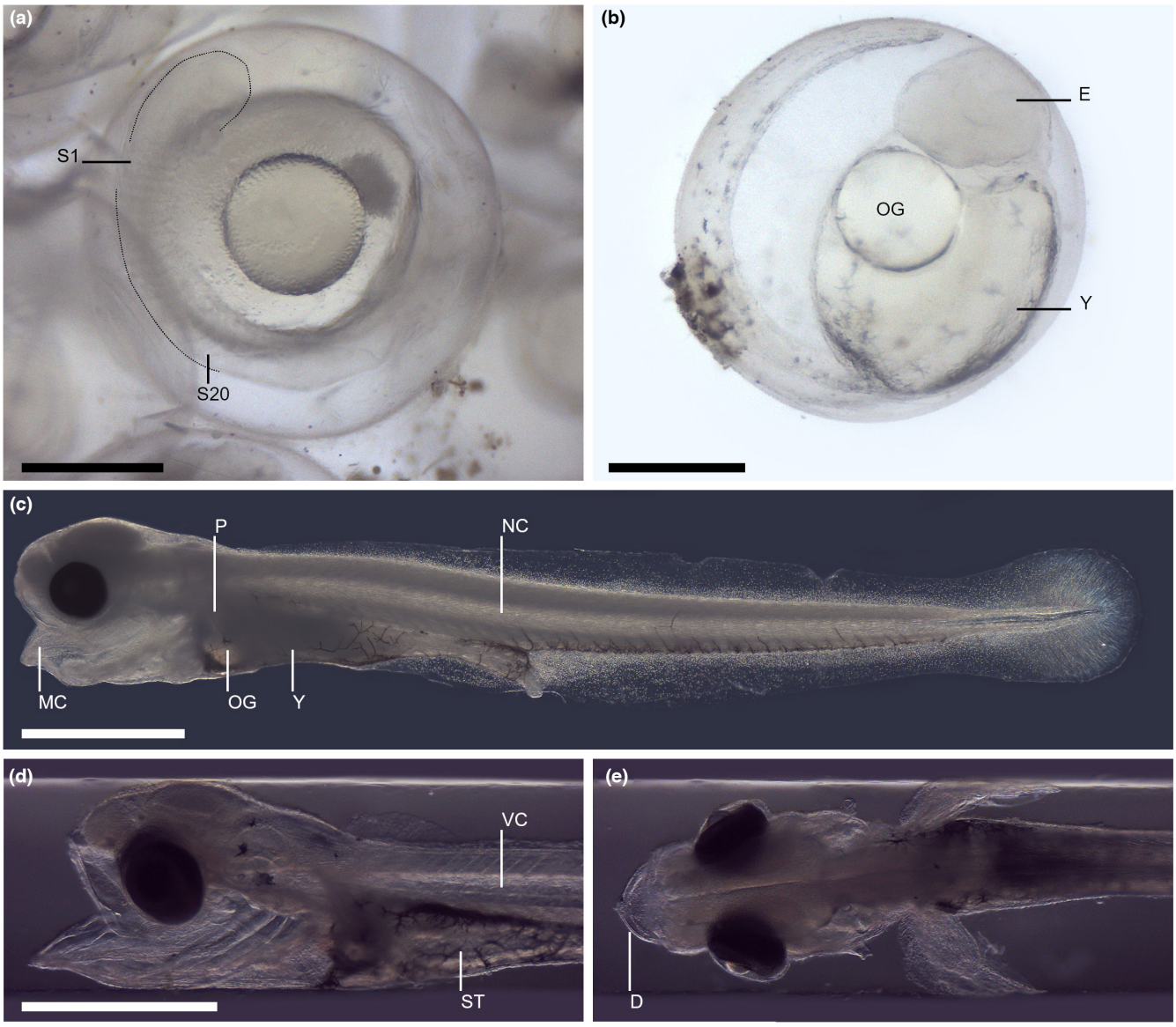

FIGURE 1 Light microscopy images of selected developmental stages of pikeperch. (a) 3 dpf/53 dd stage, the embryonic stage during somitogenesis with 20 visible somites, scale = 0.5 mm. (b) 5 dpf/80 dd stage, embryonic specimen showing early eye anlagen and a tail separated from the yolk, scale = 0.5 mm. (c) 17 dpf/248 dd stage, larval specimen during the onset of exogenous feeding. Scale = 1 mm. (d, e) 22 dpf/325 dd stage, lateral (d) and dorsal (e) anterior body of a larval specimen after the completed transition to exogenous feeding, oblique light applied to emphasize internal morphology. Scale = 1 mm. D, dental ossification; E, eye anlage; MC, meckels cartilage; NC, notochord; OG, oil globule; P, pectoral fin; S, somite; ST, stomach; VC, vertebral centrum ossification; Y, yolk.

genes (PAX3, PAX7A, Figure 2c,d) had both lowered expression at 248 dd and their maximum at the latest stage at 325 dd. The expression of msh homeobox 1 (MSX1, Figure 2e) was at its highest level during the first examined age at 28 dd. It decreased significantly afterwards, reaching a steady low expression level from stage 80 dd on, which was solely interrupted by a significantly increased expression at 280 dd. Peroxisome proliferative activated receptor gamma coactivator 1 alpha (PGC1A, Figure 2f) had a low-level expression during the earlier stages until 127 dd and as well at 248 dd. At 154 dd, and from 280 dd onward, the expression was significantly higher with around 2.3–2.6 FC.

3.3 | Proliferation of myoblasts, myotube and muscle fibre formation

The expression of myogenin (MYOG, Figure 3a) fluctuated modestly between an FC of 1.0 and 3.5 but had no significant expression changes overall. The myocyte enhancer factor 2A (MEF2A, Figure 3b) expression remained steady until 80 dd. Two significant increases between 80 dd and 154 dd lead to an FC of 5.0, which remained steady afterwards, except for a single intermediate drop at 248 dd. The expression of the insulin-like growth factor genes (IGF1, IGF2, Figure 3c,d) increased during the progressive ontogeny,

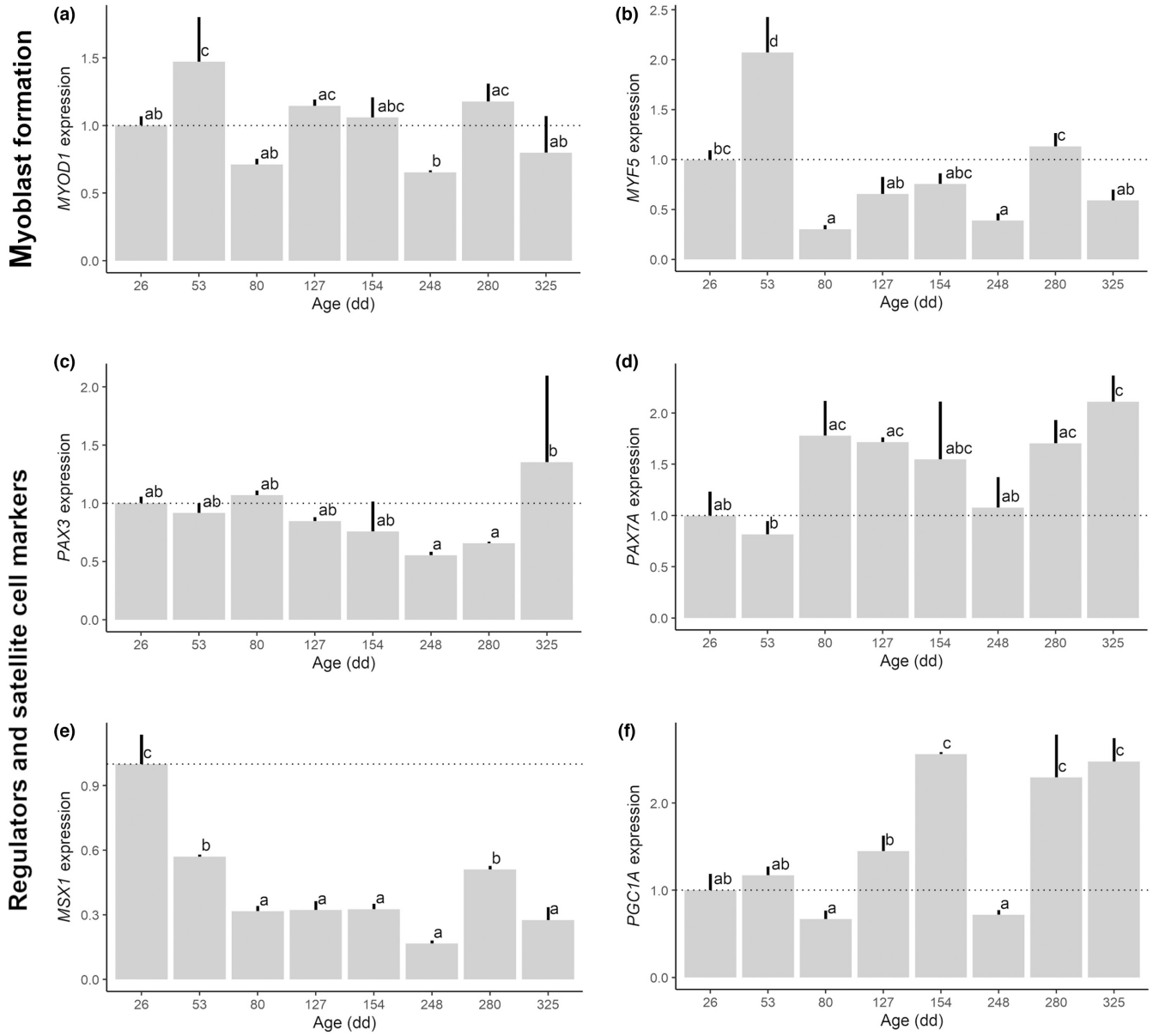

FIGURE 2 Relative expression patterns of genes regulating muscle stem cells and the formation of myogenic progenitor cells in pikeperch. (a) MYOD1. (b) MYF5. (c) PAX3. (d) PAX7A. (e) MSX1. (f) PGC1A. The dotted line indicates an FC of 1, significant differences are shown by groupwise letters given for $P \leq 0.05$.

leading to a maximum at the last examined stage. Compared with IGF2, IGF1 were more uniformly expressed during the observed stages. Transforming growth factor-beta 1 (TGFB1, Figure 3e) had an increased expression over time leading to a maximum of 4.5 FC in the latest studied stage. In between, a slight decrease occurred at 248 dd.

The expression pattern of myogenic regulatory factor 4 (MRF4, Figure 3f) was raised significantly three times during ontogeny. The first time at 53 dd had the overall highest FC of 2.4. Further, similar increases were present from 127 dd to 154 dd, and at 280 dd. Myostatin (MSTN, Figure 3g) expression did not change significantly during the first 4 examined age stages. Afterwards, expression increased to more than 10-fold expression at 248 dd, followed by a significant increase at 280 dd to a maximum of 32.4 FC. The latest stage decreased significantly again, reaching expression levels comparable to the ones measured at 248 dd.

3.4 | Regional muscle markers and structural tissue regulating genes

The expression of the cardiac-specific myosin heavy chain 6 (MYH6, Figure 4a) was elevated mainly during the earlier stages. Hereby, a maximum occurred at 80 dd. Following this maximum, the levels decreased significantly only showing an intermediately higher expression again at 248 dd. The expression of the

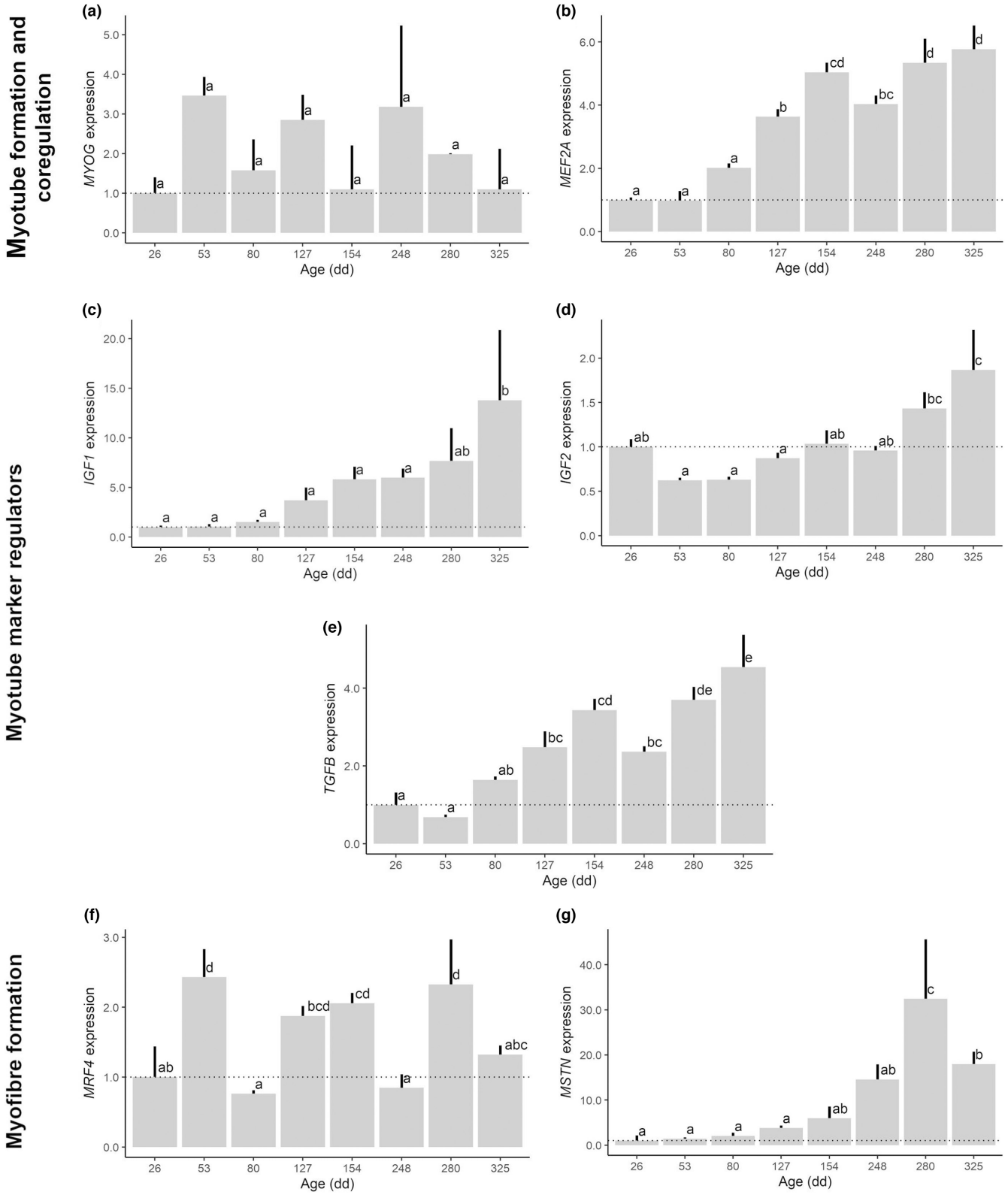

FIGURE 3 Relative expression patterns of genes associated with the proliferation of myoblasts and the formation of myotubes and myofibres in pikeperch. (a) MYOG. (b) MEF2A. (c) IGF1. (d) IGF2. (e) TGFB1. (f) MRF4. (g) MSTN. The dotted line indicates an FC of 1, significant differences are shown by groupwise letters given for $p \leq 0.05$.

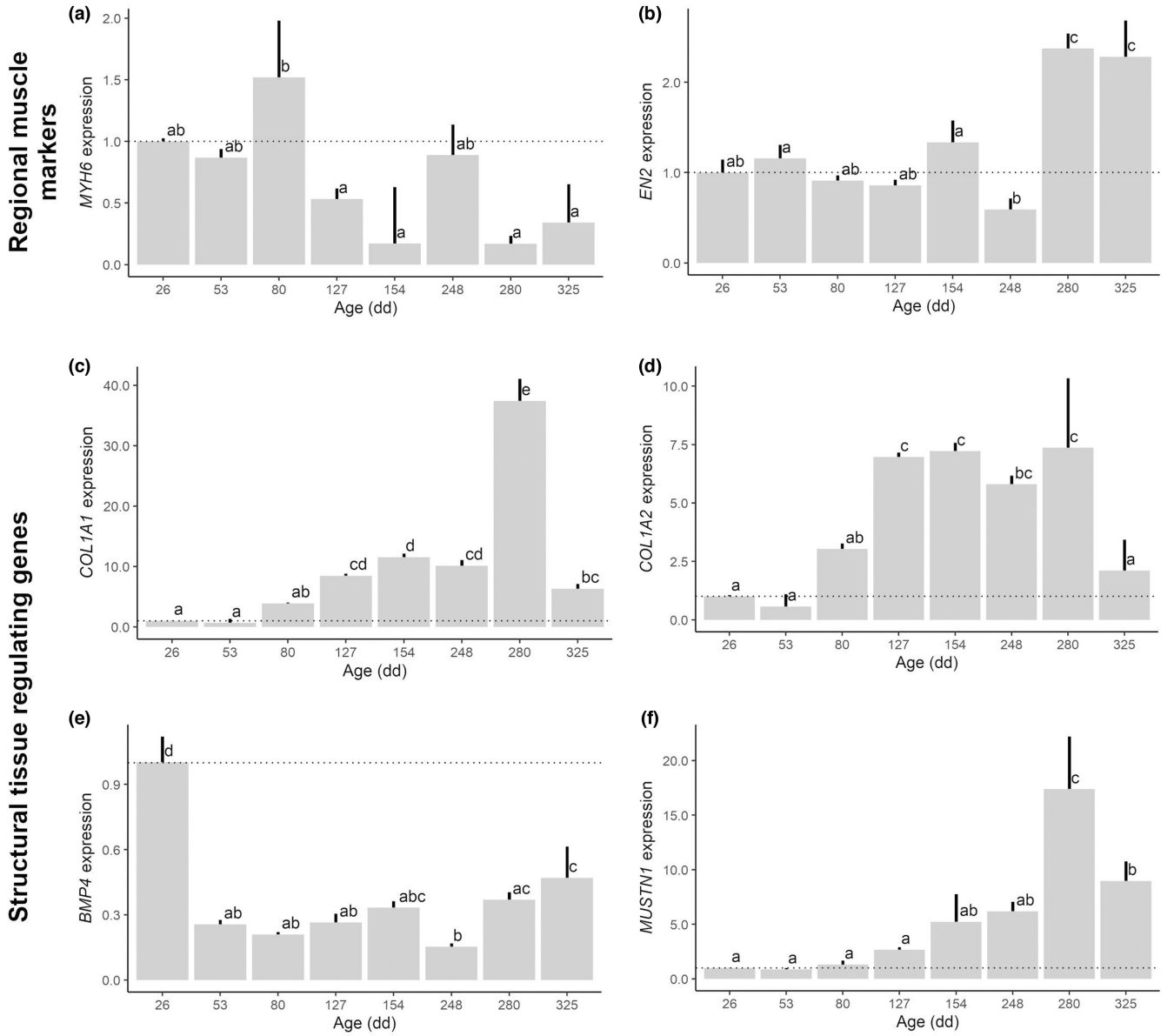

FIGURE 4 Relative expression patterns of genes controlling regional muscle development and structural tissue growth in pikeperch. (a) MYH6. (b) EN2. (c, d) COL1A1 and COL1A2. (e) BMP4. (f) MUSTN1. The dotted line indicates an FC of 1, significant differences are shown by groupwise letters given for $p \leq 0.05$.

mandibular arch-muscle specific engrailed 2 (EN2, Figure 4b) remained constant until 154 dd. A subsequent significant decrease in expression at 248 dd was followed by a significant increase at 280 dd to a steady level during the last two stages observed.

The two analysed collagen 1 alpha variants (COL1A1, COL1A2; Figure 4c,d) had overall similar expression patterns. Apart from the expression levels at 280 dd, which represent the maximum for both gene variants, the expressions correspond to a bell-shaped pattern, with a steady increase until stage 154 dd and a steadily decreasing expression in the following stages. For COL1A1, the change in expression level was more pronounced reaching 11.5 FC at the vertex and 37.4 FC at 280 dd.

The expression pattern of the gene coding for bone morphogenetic protein 4 (BMP4, Figure 4e) had its peak in the first stage. Afterwards, lower expression occurred with a mean FC of 0.3, a minimum of 0.2 FC at 248 dd, and a subsequent renewed increase until the latest stage. The musculoskeletal embryonic nuclear protein 1 (MUSTN1, Figure 4f) was expressed sparsely until 127 dd. From 154 dd, increasing expression levels occurred with a prominent maximum of 17.4 FC at 280 dd.

3.5 | Gene network

We used the IPA tool to illustrate linkages of analysed myogenic genes (Figure 5). Additional genes or regulators, next to the here

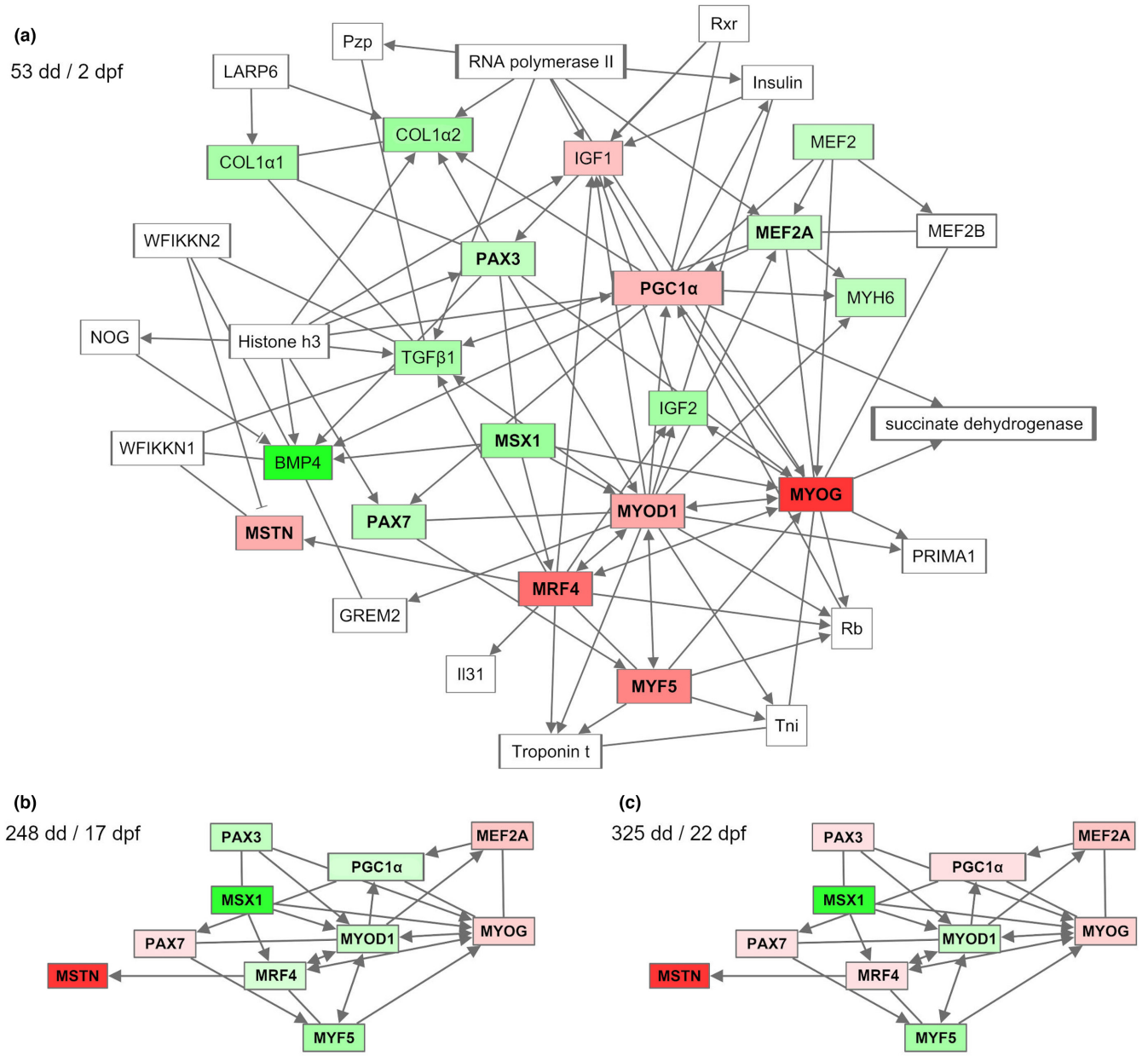

FIGURE 5 Network of myogenesis-associated genes displayed with the IPA-tool. Expression levels for pikeperch are included and given in relation to the first stage at 26 dd/1 dpf. Age is given in degree-days (dd) and days post fertilization (dpf). Interactions are depicted as arrows. Green labels show downregulation and red labels upregulations. (a) Myogenesis associated network for the 53 dd stage. (b, c) Section of the myogenesis associated network for 248 dd (b) and 325 dd (c). Full networks for 248 dd and 325 dd are added as supplemental (Figures S1–S3).

analysed ones, were given as output based on connections from the underlying IPA database (Release March 2021). Hereby, differences between the software-included genes were present in between the stages, especially for genes whose products have a plasma membrane localization (Figures S1–S3). At the same time, similarly connected genes were found between all stages, mainly in relation to gene expression and, in addition, to membrane transport and proteases (Figures S1–S3). This provides further genes that may be relevant for the here investigated regulatory and developmental patterns (Figure 5a; Figures S1–S3).

4 | DISCUSSION

4.1 | Activation of progenitor cells and myoblast formation

The main actors of the muscle progenitor activation and the resulting formation of myoblasts are MYOD and MYF5 (Berkes & Tapscott, 2005; Rudnicki et al., 1993; Rudnicki & Jaenisch, 1995; Tapscott, 2005; Ustanina et al., 2007; Watabe, 1999; Zhu et al., 2016). These genes are commonly regulated positively by PAX3 and PAX7

and negatively by MSX1 (Bendall et al., 1999; Buckingham, 2007; Buckingham & Relaix, 2007; Koganti et al., 2020; Song et al., 1992). In pikeperch, MYOD1 and MYF5 are positively correlated in embryonic and larval stages. Their elevated expressions coincide with somitogenesis and the phases of muscle growth before and after hatching. This supports the common coregulation and joint control of myogenic progenitor cells by MYOD1 and MYF5 in pikeperch. However, the IPA network shows that they are differentially regulated, as also inferred from the correlation of MSX1 and PAX7A expression with MYF5 but not with MYOD1. One reason for these differences could be an earlier onset in MYF5 expression compared with MYOD expression, as shown for common carp (*Cyprinus carpio*) (Kobiyama et al., 1998).

Here, MSX1 was found to negatively regulate myogenesis in pikeperch. Phases of elevated MSX1 expression coincided with times of low myogenic activity. The expression of the PAX genes differed in pikeperch, indicating varying roles during development. PAX3 had a constant expression level throughout embryonic development, whereas PAX7A expression showed significant differences between the stages. Since we analysed the whole specimen in this study, a clear statement on the influence on muscle development is not possible here.

4.2 | Satellite cell regulation

We found possible indications for satellite cell formation before hatch and during the later larval stages in pikeperch. As a marker for these quiescent muscle stem cells (Devoto et al., 2006; Jiao et al., 2015; Koganti et al., 2020; Nord et al., 2021; Relaix, Montarras, et al., 2005; Relaix, Rocancourt, et al., 2005), PAX7A showed elevated expression levels during these times. The additionally increased expression during the late embryonic development is of interest, as it may indicate the initial formation of satellite cells before the energy-demanding early larval stages and the subsequent proliferation of muscle tissue. Spatial expression data are required to fully clarify this finding. Control of energy metabolism that includes PGC1A represents a further mechanism regulating muscle growth and regeneration (Williamson et al., 2009). However, the functions of PGC1A gene variants were found to differ, at least partially, in fish (LeMoine et al., 2008). Thus, its connection to satellite cell formation remains ambiguous. Consequently, PGC1A itself remains useful as the regulator of the energy metabolism (Liang & Ward, 2006; Peng et al., 2017), by highlighting phases of elevated energy expenditure. For this reason, the significantly reduced expression at 248 dd is particularly interesting.

4.3 | Myotube and muscle fibre formation and regulation

MYOG is the key regulator in the formation of myotubes (Devoto et al., 2006; Hasty et al., 1993; Nabeshima et al., 1993). In pikeperch, it was expressed in a fluctuating wave-like pattern, which we attribute to three phases of myogenesis during pikeperch ontogeny.

The formation of muscle fibres out of single myotubes is promoted by MEF2A and MRF4 (Hinits et al., 2009; Hinits & Hughes, 2007; Moretti et al., 2016). The common reciprocal coregulation of MEF2A with MYOG (Watabe, 1999; Watabe, 2001) was not clearly supported by the obtained data. Still, the overall expression of MEF2A followed the increase in muscle tissues, confirming its role in muscle fibre formation. MRF4 expression resembled that of MYOG with three elevated phases, during somite formation, before hatch and during larval stages. The elevated expression at 53 dd implies some influence on somite formation, which would contradict previous work on *D. rerio* (Hinits et al., 2009). However, an initiating effect of MRF4 on somite myogenesis is supported by studies on mice (Kassar-Duchossoy et al., 2004).

While both IGFs seem necessary for overall muscle growth in pikeperch, their incorporation into myogenesis differs. IGF1 and IGF2 control the increase in muscle fibre mass (Barton-Davis et al., 1999; Fuentes et al., 2013; Fuentes et al., 2014; Johnston, 2006; Wood et al., 2005). For pikeperch, IGF1 and IGF2 expression correlated with one another during the embryonic and even more during the larval phase. Still, during the larval stages, only IGF1 had a positive correlation with MYOD1 and a negative correlation with MYOG. This pattern could be attributed to different developmental tasks (Fuentes et al., 2013; Wood et al., 2005), and differential association with overall growth (Fuentes et al., 2013; Mingarro et al., 2002), which becomes more dominant during larval stages.

The role of TGFB1 in pikeperch myogenesis remains ambiguous, but a connection to fibre type differentiation might is justifiable. In previous studies, TGFB1 inhibited myogenesis in chickens (Li & Velleman, 2009), but in rodents, a connection to the differentiation of fast muscle fibres was found (McLennan, 1993; McLennan et al., 2000; Noirez et al., 2006). Linked to fibre type differentiation, TGFB1 supports the formation of extracellular matrix proteins like collagen (Herpin et al., 2004; Schmidt et al., 2016; Vial et al., 2008). In pikeperch, TGFB1 expression coincided with the late embryonic and larval phase of myogenesis, possibly negating an inhibiting effect on myogenic activity. The strong correlations of TGFB1 with both analysed COL1A variants during embryonic stages support a connection to collagen formation. Nevertheless, as TGFB1 influences various developmental processes (Grafe et al., 2018; Massagué, 2012; Massagué & Xi, 2012; Zinski et al., 2018) no clear statement can be given here and further research remains necessary. In contrast to TGFB1, MSTN is clearly linked to myogenesis as a negative key regulator (Gabillard et al., 2013; Østbye et al., 2007; Rodgers et al., 2001; Rodgers & Garikipati, 2008). This can be concluded for pikeperch as well, as the MSTN expression maximum was situated in a growth gap, between the late embryonic and larval myogenesis phase. Hereby, a general increase in expression with age could be attributed to growth regulation and homeostasis, as also shown in tilapia and white bass (Rodgers & Garikipati, 2008).

4.4 | Regional muscle markers

In this study, two regional muscle markers MYH6 and EN2 were analysed, both of which can be attributed to local muscle formation

in pikeperch. MYH6 is specifically expressed in the heart muscle cells (Schiaffino, 2018; Singleman & Holtzman, 2012). In the gill arches, EN2 regulates the formation of muscles associated with the first visceral arch that forms the jaw elements (Degenhardt & Sassoon, 2001; Yokoyama et al., 2021). In our study, the expression maxima of MYH6 coincides with the observed formation of the heart and takes place shortly before the first heartbeats. The further increased expression occurs after hatching and coincides with the switch to gill respiration, which is accompanied by another increase in myocardial tissue and a combined increase in the circulatory system (Grimes & Kirby, 2009). EN2s expression maxima coincided with the point of full exogenous feeding, which was coupled with increased jaw development. Additionally, a first minor elevated expression was detected in the specimens at 154 dd shortly before hatching. This increase could additionally be influenced by the formation of slow muscle tissue, which is known to also express EN2 at higher levels (Du et al., 1997; Hammond et al., 2009; Hatta et al., 1991). Consistent with studies in zebrafish, this slow muscle tissue is known to form during embryogenesis, where they are required for initial post-hatching movements (te Kronnié, 2000).

4.5 | Markers for skeletal development

In our specimens, skeletal growth generally matched with the pikeperch skeletal development described by Löffler et al. (2008) and Ott et al. (2012). In the embryonic stages before hatching, fundamental cartilages with single ossifications were present. During larval stages, further skeletal elements formed and ossification considerably increased. The expression of the studied skeletal markers coincided with times of skeletal development and demonstrated differences between embryonic and larval skeletogenesis. Since muscles and bones develop partially codependently together as elements of the musculoskeletal system (Sefton & Kardon, 2019), these two skeletal growth phases could be linked to muscle development.

For the COL1A variants, the bell-shaped expression pattern in pikeperch could be linked to embryonic cartilage formation (Gelse et al., 2003; Min et al., 2021; Watson et al., 2017). An additional peak at 280 dd matched with increased skeletal development during this time. The transformation of intestinal tissues during the change to exogenous feeding shortly after hatching could further influence this peak value (Ostaszewska, 2005), as it also involves the formation of collagenous matrices (Nakase et al., 2006). Together with COL1A, the skeletal marker MUSTN1 (Hadjiargyrou, 2018; Lombardo et al., 2004) showed significant expression maxima during the increasing skeletal growth and ossification at 280 dd.

In contrast, BMP4 cannot be associated only with skeletal development. While it has been found to influence intermuscular ossification (Su & Dong, 2018) and bone repair (Rosen & Thies, 1992), it was also linked to other developmental processes (Hwang et al., 1997), including tooth development (Bei & Maas, 1998), inhibition of muscle pioneer cells (Du et al., 1997) and dorsoventral patterning (Stickney et al., 2007). The highest expression of BMP4 occurred during the earliest studied stage, while the expression in pikeperch otherwise correlated with age. This maximum could highlight a foremost attribution to general developmental processes in pikeperch.

5 | CONCLUSIONS

In this study on pikeperch myogenesis during early development, the three phases of myogenic activity were found to be chronologically adapted to the change from endogenous to exogenous feeding shortly after hatch. After a first myogenic phase during somitogenesis, a second occurs in embryos shortly before hatch (127–154 dd) and a third starts at around 280 dd during larval development. Additionally, skeletal gene marker expression was found to coincide with the second and third phases of myogenic gene expression.

The increased expression of some muscle gene markers in pikeperch indicates that the muscle tissue fundamental for initial locomotion is already formed before the embryos hatch. Between embryonic and larval development, an interruption in muscle development occurs, as evidenced by decreased expression of myogenesis marker genes. Additionally, two different phases of skeletal growth were supported by our expression analysis. A first phase is present before hatching, in which precursor cartilages are formed, and a second phase after hatching, in which ossification processes are enhanced.

In this fundamental study, gene expression was analysed using the whole specimen. To clarify the exact spatial expression and analyse the resulting phenotypic changes during myogenesis, follow-up studies are needed. Nevertheless, this study provides the first insight into the regulation of myogenic genes in near-natural raised pikeperch, which is important for comparisons with aquaculture-reared pikeperch.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are contained in the article or are available as supplementary material.

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