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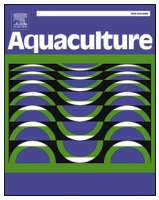

Turbidity and temperature effects on growth and gene transcription of threatened juvenile Longfin Smelt (*Spirinchus thaleichthys*)

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ABSTRACT

The Longfin Smelt (LFS, *Spirinchus thaleichthys*) population within the San Francisco Estuary, California, has experienced a substantial reduction, diminishing to <1% of their historical abundance. This decline has culminated in their classification as a threatened species under the purview of the California Endangered Species Act. Understanding their physiology and stress response in relation to varying environmental conditions, such as temperature and turbidity, is crucial for LFS culturing, management, and conservation. In this study, we assessed juvenile LFS (age range during exposure: 181 to 228 days post hatch, dph) performance as measured by growth and gene expression following four weeks at two temperatures (11 °C and 14 °C) and three turbidity levels (1, 4, and 11 nephelometric turbidity units (NTU)). At the end of the 4-week exposure period, we conducted assessments encompassing fork length, wet weight, condition factor, and examined alterations in the transcription of 12 genes. The selection of these genes aimed at determining responses associated with osmoregulation, growth, metabolism, and general stress, as all of which are potentially influenced by temperature and/or turbidity. Weight and condition factor was significantly higher at lower temperature, whereas turbidity had no effect on growth, condition factor, and transcriptomic stress-response. Instead, the lower expression levels of *Catalase*, *Citrate Synthase* and *Growth Factor Receptor Bound Protein 10* at 14 °C were indicative of metabolic and growth-related changes governed by temperature. This suggests that rearing of LFS at 11 °C and low turbidity (<11 NTU) is suitable for the juvenile stage, whereas growth as well as metabolic capacity is limited at slightly warmer temperatures.

1. Introduction

Across the globe, human influences have led to the depletion of >90% of species that were once ecologically significant from previously diverse and productive estuaries and coastal seas (Lotze et al., 2006). Such impacts in the San Francisco Estuary, California, have resulted in numerous fish species being listed as threatened or endangered (Moyle et al., 2012; Brown et al., 2016; Brennan et al., 2022). Of particular interest for fish conservation in the estuary are Longfin Smelt (LFS, *Spirinchus thaleichthys*) and Delta Smelt (DSM, *Hypomesus transpacificus*), for which the roles of multiple environmental stressors in population declines have been studied (Nally Mac et al., 2010; Glibert et al., 2011; Brooks et al., 2012; Moyle et al., 2016; Nobriga and Rosenfield, 2016; Hobbs et al., 2017). Both species face similar threats and have overlapping habitats, making insights from DSM studies invaluable for LFS conservation efforts. DSM was listed as threatened in 1993 under the U. S. Endangered Species Act (USFWS, 1993) and as endangered in 2010 by the State of California (CDFG, 2010). The southernmost populations of LFS are listed as threatened by the State of California in 2009 (CDFG, 2009). Due to the occurrence of both species in the estuarine mixing zone, which is where pronounced changes in turbidity, salinity, and temperature co-occur (Moyle et al., 2016), an understanding of the

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impact of such changes on the physiological performance of smelt is of crucial importance. Turbidity is suspected to be a key factor influencing the biotic resources for fishes, such as the abundance and diversity of prey items as well as predator-prey relationships (Baskerville and Lindberg, 2004; Nelson et al., 2015; Kurobe et al., 2022).

Longfin Smelt is an anadromous fish species that inhabits waters from the Aleutian Islands to the San Francisco Estuary (Moyle, 2002; Sommer et al., 2007). Recent efforts to rear LFS in captivity have provided novel opportunities for hypothesis-driven research regarding the ecological niche of this species. In particular, life-stage specific information is required for culturing of LFS in aquaculture facilities, which aim at providing adequate water quality conditions to promote fish health and optimal growth and performance of captive-bred specimens that can be used for population augmentation (Jeffries et al., 2016; Hobbs et al., 2017; Lewis et al., 2019, 2020; Tempel et al., 2021; Yanagitsuru et al., 2021, 2022; Mulvaney et al., 2022; Hung et al., 2023). Currently, the capacity to culture LFS is limited to early life stages; however, if LFS can successfully undergo its entire life cycle in a controlled culture environment, this development will not only help with population augmentation, but it would also facilitate LFS utilization in fundamental and applied research, offering valuable insights into the species that can inform conservation, management decisions, and practical research efforts aimed at refining their breeding methods.

While substantial research has been conducted on the impact of altered environments on DSM, facilitated by the availability of captive culture populations (e.g., Baskerville and Lindberg, 2004; Lindberg et al., 2013), relatively little empirical research has been conducted to determine the habitat requirements of LFS. In this study, we focus on understanding temperature and turbidity requirements for juvenile LFS, both of which have already demonstrated their importance in the cultivation of DSM (Hasenbein et al., 2016; Tigan et al., 2020; Pasparakis et al., 2022, 2023) and the embryonic/larval stage of LFS (Yanagitsuru et al., 2021, 2022). Elevated temperatures and the growing frequency of heatwaves pose a substantial threat to the future survival of smelt species (Moyle et al., 2016), especially because of impacts to pivotal life history events such as spawning and migration (Basevkin et al., 2023). Temperature also directly impacts the solubility of oxygen, which has implications for all gill-breathing species with high oxygen demands (Chapra et al., 2021). Understanding life-stage specific physiological responses to elevated water temperature is crucial to predict species declines and inform management.

Turbidity results from the scattering and absorption of light by suspended particles and is frequently described as the 'cloudiness' of water (Kirk, 1985; Henley et al., 2000). The level of turbidity depends on suspended organic and/or inorganic materials, while factors such as light intensity and water depth further contribute to effects on organisms (Lee and Rast, 1997). Light reflected off these particles is used as a means of measuring turbidity, which is expressed as nephelometric turbidity units (NTU). Turbidity is an understudied, yet significant driver of ecosystem function that influences trophic interactions and species compositions (Lunt and Smee, 2014). It is also a crucial factor in determining optimal rearing conditions for fish in aquaculture production systems (Becke et al., 2018, 2020). Turbidity requirements can vary significantly across fish species (Utne-Palm, 2002), affecting for example larval and juvenile DSM feeding ability and resulting growth rates, as well as impacting predator prey interactions, and levels of stress (Hasenbein et al., 2013; Tigan et al., 2020; Pasparakis et al., 2023).

It has been postulated that visual feeders benefit from certain levels of turbidity that facilitate contrast to see their prey. Turbidity levels that are too high, however, may result in clogged and damaged gills, diminished visibility and thus reduced feeding (Gardner, 1981; Grimaldo et al., 2009). For DSM late-larvae (60 dph), Hasenbein et al. (2016) identified turbidity levels ranging from 25 to 80 NTU as beneficial in the context of feeding rates; diminished cortisol concentrations were observed within the range of 35 to 80 NTU, while heightened levels were noted at 5, 12, and 25 NTU. Additionally, turbidity was shown to influence the feeding conditions of DSM larvae, which are visual predators, by limiting light penetration (Baskerville and Lindberg, 2004; Tigan et al., 2020). This also accounts for initial studies on the larval stage of LFS, which have indicated the necessity for low turbidity levels in captivity (Yanagitsuru et al., 2021, 2022). Turbidity requirements for DSM have been described as life-stage dependent, and play an essential role in feeding behavior and potential predator evasion (Feyrer et al., 2007; Nobriga et al., 2008; Grimaldo et al., 2009; Sommer et al., 2011; Brown et al., 2013; Hasenbein et al., 2013; Sommer and Mejia, 2013; Ferrari et al., 2014; Bennett and Burau, 2015; Pasparakis et al., 2023).

Considering the heightened sensitivity and the current conservation status of LFS in the natural estuary habitat, it is imperative to gain a comprehensive understanding of the impacts of changes in water quality parameters, notably temperature and turbidity, on the physiological performance of LFS. Moreover, rearing the juvenile stage of LFS presents specific challenges, and establishing suitable conditions for the juvenile life stage necessitates a deep comprehension of physiological thresholds in performance. While there is established information regarding the importance of turbidity for culturing DSM, there is a dearth of data on the turbidity needs for LFS. When considering aquaculture, one might anticipate that LFS and DSM would share similar requirements due to their comparable distribution patterns in the estuary during certain life stages. This suggests that their juvenile stages are likely to thrive under similar environmental conditions characterized by specific temperature ranges and levels of water turbidity. However, the tendency of LFS to concentrate in San Francisco Bay during the late juvenile and adult stages, as part of their anadromous life cycle, likely accounts for the shift in the population center from landward to seaward (Moyle, 2002). It is important to note that their life cycle differs from DSM in that the juvenile to adult stages of LFS, depending on water temperature and if it is a low or high flow year, are typically found in oceanic environments, suggesting that clear waters may be more suitable for LFS at this life stage (Grimaldo et al., 2020).

This study had two main objectives: 1) to contribute to the fundamental knowledge of LFS early life stage physiology and 2) to better understand water quality requirements of juvenile LFS to be practically applied to enhance LFS culture. Therefore, we assessed growth and stress-related gene expression under holding conditions of different turbidity (1–11 NTU) and temperature levels (11 °C and 14 °C). Many of the selected genes are connected to the hypothalamus–pituitary–interrenal (HPI) axis and its end product cortisol. Cortisol is a bioindicator of stress, which combines glucocorticoid and mineralocorticoid actions and is therefore essential in the context of hydromineral homeostasis (Wendelaar Bonga, 1997) as well as osmoregulation, growth, and reproduction (Mommsen et al., 1999). Specifically, we hypothesized that:

(i) For the larval stage preferences, a temperature of 11 °C would yield accelerated growth rates in comparison to those at 14 °C.
(ii) Given that the rearing and acclimation occurred closer to 11 °C, less pronounced transcription levels in stress- and growth-related genes would be expected at 11 °C when compared to treatments conducted at 14 °C.
(iii) Since juvenile LFS exhibit a migratory behavior and occur in clearer ocean waters, turbidity conditions within the range of 1–11 NTU would result in limited impacts on growth and the expression of examined genes for this life stage. This would suggest that tested turbidities fall within a shared tolerance range. Nonetheless, a probable threshold response to turbidity is anticipated, with an optimal range expected between insufficient and excessive levels.

Consequently, lower temperature and low turbidity may be beneficial while also implementable for the rearing of LFS.

2. Methods

2.1. Fish source, experimental setup, and maintenance

Longfin Smelt were spawned from wild-caught broodstock and reared at the UC Davis Fish Conservation and Culture Lab (FCCL). The experimental setup was similar to that described in Pasparakis et al. (2022). At 164–183 dph, fish were transferred from the FCCL to the UC Davis Putah Creek Facility (PCF) research lab. Upon arrival, groups of 30 fish (720 total) were distributed into 24, 57-L black polyethylene tubs (referred to herein as sub tanks) fitted with standpipes, allowing for ca. 57 L. Sub tanks were distributed in groups of three within four 400-L tanks (referred to hereafter as holding tanks, eight in total) which were contained across two separate recirculating aquaculture systems (Fig. S1). The holding tanks served as experimental water baths with each system having separate temperature control units. Water was continuously pumped from external reservoirs that maintained experimental algal concentrations used to attain the selected turbidity levels (Table S2). Fish were fed twice daily and directly via filtering a standardized volume of live brine shrimp (*Artemia franciscana*) culture with a brine shrimp net. External reservoirs were maintained to 7.0–7.4 ppt salinity with Instant Ocean (Aquarium Systems, Mentor, OH) to guarantee optimal conditions in all treatments for brine shrimp. Each sub tank was fitted with independent, external biofiltration units through which water was treated with seeded k1 biomedia, returning to the tanks using an airlift mechanism via airstones. These external biofiltration units also served to maintain the oxygen levels in the sub tanks as well as help maintain the turbid mixture in suspension. Sub tanks were covered with circular lids made from shade cloth, and styrofoam lids were used to cover each holding tank.

Longfin Smelt were subjected to a daily 12-h light/12-h dark cycle, which was maintained using a timer connected to the lighting system. Lights reached 7 to 120 lx as measured above the water surface by a portable digital light meter (LX1330B, Dr. Meter) and were placed within each holding tank lid to investigate turbidity-light interactions and their effects on feeding efficiency and stress responses. Water quality parameters were measured during the acclimation and exposure period (Table S2). Temperature (°C), dissolved oxygen (mg/L), and salinity (ppt) were measured daily with a hand-held YSI 556 MPS meter (YSI Inc., Yellow Springs, OH). Ammonia, nitrite, and nitrate concentrations, as well as pH, were measured biweekly. Ammonia, nitrite, and nitrate measurements were conducted using a marine care multi test kit (Red Sea, Houston, TX), with additional ammonia measurements taken using a Hach pocket colorimeter (Hach Company, Loveland, CO), whereas pH was measured using a pinpoint pH monitor (American Marine Inc., Ridgefield, CT). Mortality was checked daily, and any dead fish were removed. Fish care and use protocols were reviewed and approved by the UC Davis Institutional Animal Care and Use Committee (IACUC Protocol #16591).

Since temperature is directly related to performance traits such as growth and development (Claireaux et al., 2006; Eliason et al., 2011), we tested two sublethal thermal regimes (11 °C and 14 °C) based on recommendations for rearing early life stages (9–12 °C) and one degree lower than the temperature threshold (15 °C) above which has been shown to be harmful to LFS performance, hatch success, growth, and mortality (Grimaldo et al., 2017; Yanagitsuru et al., 2021). Following a two-week laboratory acclimation period and reaching an age of 181–200 dph, acclimation at two different temperatures (11 °C and 14 °C) and turbidities (1, 4, and 11 NTU) was conducted over a period of four weeks. Sampling was conducted following this 4-week acclimation, at an age ranging from 209 to 228 dph. This was the first study to use juvenile LFS in a prolonged experimental setup. Decreased turbidity levels relative to environmental conditions were chosen based on our limited understanding of tolerances and to facilitate a thorough mortality assessment. This approach was chosen to minimize mortality risks and enhance the feasibility of accurately monitoring survival rates over four weeks. The study was conducted using four replicates of 30 fish for each treatment. During the two-week laboratory acclimation, fish were initially acclimated to the system for 11 days, at 11 °C in clear water, and after which they were transitioned to treatment conditions over a period of three days. The temperature for the higher temperature treatment was gradually increased from 11 °C to 14 °C at a rate of 1.3 °C per day, while the 11 °C treatment was kept constant over the same three-day period. Turbidity was adjusted using predetermined volumes of *Nannochloropsis* spp. algae (Nanno 3600 – High yield grow out feed; Reed Mariculture Inc., USA). No algae was added in the 1 NTU treatments and turbidity levels were increased from 1 to 4 and 11 NTU at a rate of 3 NTU per day. Algae were added daily to reservoir tanks connected to each sub tank based on the FCCL protocol for DSM. Temperature was measured using eight HOBO data loggers, set at 15 min intervals, randomly distributed in one sub tank per holding tank set to 11 °C or 14 °C. Turbidity was measured daily using a Hach pocket colorimeter (Hach 2100q portable turbidity meter, Hach Company, Loveland, CO).

Longfin Smelt were not fed during the 24 h prior to the sampling. At the end of the exposure, juvenile LFS were sampled under low luminosity after switching off the lights to minimize handling stress. All fish were euthanized using an overdose of buffered tricaine methanesulfonate (MS-222; 50 mg/L; buffered to a neutral pH with sodium bicarbonate; Western Chemical, Ferndale, WA), weighed (to the nearest 0.001 g) and measured (fork length; to the nearest 0.1 cm) to calculate Fulton's fish condition factor (K). Fulton's condition factor was calculated using the following eq. $K = 100\ W/\ L^3$, where W is the weight (g), and L is the fork length (cm) (Fulton, 1904). After body measurements, the whole fish was immediately snap-frozen in liquid nitrogen, with 12 fish per treatment archived at −80 °C for gene expression analysis.

2.2. Water physicochemistry

Pretreated clean water from outdoor reservoir tanks and biofiltration in each sub-tank maintained stable water quality throughout the system. In conjunction with daily temperature assessments via YSI, each tank was equipped with a designated HOBO temperature logger (Onset). The logger was systematically rotated among treatment sub-tanks every two days, capturing temperature readings (°C) at 30-min intervals. The average water temperatures during the exposure period were 11.2 ± 0.0 (Low) and 13.8 ± 0.0 (High) °C, and average turbidity levels were 1.0 ± 0.1, 3.6 ± 0.1, and 10.9 ± 0.5 NTU, respectively, closely matching nominal values. More detailed water parameters are shown in Table S2.

2.3. Gene transcription analysis

To aid RNA extraction, whole fish were cryogenically ground using a mortar and pestle. Mortars were placed on dry ice, into which the samples were placed, and grinding was aided using liquid nitrogen to break down the tissue into fine, homogeneous powder. Immediately after grinding, the powder was transferred into cryogenic tubes, set on dry ice, and stored at −80 °C until further processing.

Total RNA extraction was performed using the RNeasy Plus Mini Kit (Qiagen, Valencia, CA, USA) in an automated QIAcube (Qiagen). Extraction efficiency and RNA quantity were verified using a NanoDrop ND1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). Total RNA integrity was visually verified by non-denaturing gel electrophoresis using a 1% (*w/v*) agarose gel. QuantiTect Reverse Transcription Kit (Qiagen) was used to synthesize cDNA from 1 μg of total RNA, following the manufacturer's protocol, to a final volume of 40 μl. Quantitative PCR (qPCR) was conducted using a 2× PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Foster City, CA, USA). Cycling conditions were 2 min at 50 °C; 2 min at 95 °C; 40 cycles: of 15 s at 95 °C, 15 s at 55 °C, 1 min at 72 °C; and 15 s at 95 °C. Mean cycle threshold (Ct) values of triplicate technical replicates were used relative to reference genes. Gene expression data were normalized to the geometric mean of 3 reference genes (*GAPDH, RPL7,* and *ACTB*),

which showed the highest consistency across treatments (Table S1). Expression of target genes were then calibrated to fish maintained at a turbidity of 1 NTU and at 11 °C (i.e., data is presented relative to this condition, which corresponds to current rearing practices). The genes of interest for this study and reference genes are listed in Table 1, their predicted expression in response to experimental parameters in Table 2.

Primers used in this study were designed in house using whole transcriptomic data available from a prior LFS study (Jeffries et al., 2016). Primers were designed using PrimerQuest™ (Integrated DNA Technologies) or Primer-BLAST (NCBI), and their efficiencies were determined using a five-point standard curve in three replicates established from a 10-fold serial dilution of cDNA. The amplification efficiency (E) of primers was calculated as $E = 10^{-1/slope}$ on a calibration curve.

2.4. Statistical analyses

Analyses were conducted and graphs generated with GraphPad Prism (Version 10.1.2, © 1992–2021 Graphpad Software, LLC). Statistical assumptions were tested using jamovi (Version 2.3.16). Normal distribution was verified via Shapiro-Wilk tests and homogeneity of variances via Levene's tests. Weight, length, and Fulton's condition factor data did not meet assumptions for normal distribution and homogeneity of variances; therefore, a non-parametric test (Kruskal-Wallis) was used followed by Dunn's multiple comparison test.

Relative expression of target genes was determined using the method described by Pfaffl (2004) and presented as log2-fold change. A two-way ANOVA, followed by Tukey multiple comparison tests with a single pooled variance, was used to test the main effects of temperature and turbidity as well as their interaction on gene expression of 12 genes of interest. *POMCB* was not normally distributed ($p = 0.003$), and *HSD11b2* did not show homogenous variances ($F = 2.79$; $p = 0.024$) so non-parametric tests (Kruskal Wallis test followed by Dunn's multiple comparison test) were applied. Because there were no significant differences across turbidity treatments, we also were able to consider them graphically as ad hoc replicates within each temperature. Quantitative PCR data are presented as average ± standard error (SEM); significance was set at $p < 0.05$.

3. Results

3.1. Survival, length, weight, and condition factor

Average survival per treatment was high and ranged between 79.2% - 90.6%, with no significant differences between treatments (Table S3). Fork length, weight, and K were not significantly different between turbidity treatments ($p = 0.07$, $p = 0.08$, and $p = 0.36$). However, weight and K were affected by temperature: At 11 °C, weight ($p = 0.01$) and K ($p < 0.001$) were significantly higher than at 14 °C (Fig. 1). Over the 4-week exposure, this resulted in a difference of the final weight by 77.4 mg (771.3 ± 23.7 mg at 11 °C versus 693.9 ± 24.2 mg at 14 °C) and of K by 0.054 (0.539 ± 0.011 at 11 °C versus 0.486 ± 0.006 at 14 °C). Examined via ANOVA, no statistically significant interactions were identified between temperature and turbidity concerning survival, length, weight, or Fulton's condition factor.

3.2. Gene transcription

The impact of turbidity and temperature-turbidity interactions on gene transcription was non-significant for all 12 genes of interest (Fig. S2). This suggests that, at the time of sampling, the metabolic, growth, oxidative, and physiological stress associated with these

Table 1
Selected longfin smelt (*Spirinchus thaleichthys*) genes. Primer sequences and efficiencies for quantitative PCR analysis.

Gene name	Gene code	General function	Primer forward	Primer reverse	Efficiency (%)
Catalase	*CAT*	Defense against oxidative stress	GGTTCGCTGTCTGGTGCTCTA	TCGAGGTGGTCGTCATAGCG	96.3
Citrate Synthase	*CS*	Synthesis of citrate from oxaloacetate and acetyl coenzyme A, capable of oxidative metabolism	GCATTCCGGAGTGTCAGCA	CCTTCAGGGAGAGGCTCTTGA	106.8
Growth Factor Receptor Bound Protein 10	*GRB10*	Encodes growth factor receptor-binding protein that interacts with insulin receptors and insulin-like growth-factor receptors	TGCATCAAGCCTAGCAAGGTG	CATCTTCCGCGCACATCAT	98.4
Potassium sodium hyperpolarization-activated cyclic nucleotide-gated channel 2-like	*HCN2*	Voltage-gated potassium channel activity	ACCTTGTGGAGGAGGAGGGA	TGATGAGGCGAGCCTTCGAG	101.3
Heat Shock Protein Family A	*HSP70*	Thermal Stress/General Stress – protein chaperone	GACCGTGCGATTGGTCGTCA	ATCAGGGCGACGATGCAGTT	107.1
Heat Shock Protein 30	*HSP30*	Thermal Stress/General Stress – protein chaperone	TCGCTTCTCCAGAACGACTTC	GCCTTGCTGGAGTTCCTTCAT	97.0
Cholesterol 7-alpha-monooxygenase-like; full = cytochrome *p*450 7a1	*CYP7a1*	Metabolism and synthesis of cholesterol, steroids, and other lipids. This endoplasmic reticulum membrane protein converts cholesterol	GGTGTCGGACGTGAGCATGA	GGCCACACCACAGAGAACCT	96.5
Proopiomelanocortin b precursor	*POMCb*	Precursor of adrenocorticotrophic hormone; HPI axis	GTGTGTCGTGGCTGTTGACC	TATCTTTGCAGTAGGGGCGGT	99.2
Corticosteroid 11-beta-dehydrogenase isozyme 2-like	*HSD11b2*	Cortisol conversion; HPI axis	CACACATCCACTGCTCCCAG	CTCTGGGGCGTGGTGAACAAT	104.4
Transferrin	*TFR* (HIF1A mediated)	Transport of iron to cells, response to hypoxia	TGCTCTCCTCAAAGGCCTCG	GCTGATACAGCACCAACGCA	109.2
Insulin-Like Growth Factor 1 Receptor	*IGF1r*	Tyrosine kinase activity, transformation events.	AGCTGGAGACCTTCATGGGC	CCAGCGTGTGGGAGTGTCTT	106.9
Glucose Transporter 5	*GLUT5* (= SLC2A5)	Glucose transportation	TCATCGCCCAGCCTGATCTC	TGGCTGGTGCTACCAGAGAC	101.3
Glyceraldehyde-3-Phosphate Dehydrogenase	*GAPDH*	Reference	GACCTGACCTGCCGTTTGAC	TCCGTGAGCAGCTTTCTTGA	94.9
Ribosomal Protein L7	*RPL7*	Reference	GCATCGCCCTCACTGACAA	CTCATGGATCAGGTCCTCAACA	94.5
Actin Beta	*ACTB*	Reference	CCATCGGCAACGAGAGGTT	GCAGGACTCCATACCGAGGAA	101.5
Ribosomal Protein S9	*RPS9*	Reference	TGGAACTGGAGGAGCTGATGA	CAGCCAATACTTCCAGGACGAC	92.2

Table 2
Predictions and observations related to the expression of genes of interest (A) and the condition factor (B) in Longfin Smelt (*Spirinchus thaleichthys*, 209–228 dph) in response to experimental parameters turbidity of 1–11 NTU, and temperature of 11–14 °C (+, increased expression; −, decreased expression; nee, no expected effect; ns, non-significant).

Gene code	Described function/pathway	Related literature	Prediction in response to increasing turbidity (NTU)			Prediction in response to increasing temperature (°C)		Observation in response to increasing turbidity (NTU)			Observation in response to increasing temperature (°C)	
			1	4	11	11	14	1	4	11	11	14
A)												
CAT	maintenance of pro- and antioxidant balance	Di Giulio et al. (1989); Ahmad et al. (2000); Bagnyukova et al. (2005a, 2005b); Lesser (2006); Shin et al., 2006; Vinagre et al. (2012)	+			+		ns			–	
CS	indicator for the metabolic and aerobic capacity and growth rates of fish	Pelletier et al. (1993, 1994); Majed et al. (2002)	nee			–		ns			–	
GRB10	growth suppressor, overexpression can inhibit tyrosine kinase activity	Charalambous et al. (2003); Plasschaert and Bartolomei (2015)	nee			+		ns			–	
HCN2	cardiac activity, acute stress events	Jackson et al., 2007; Hassinen et al. (2017); Kolesnikova (2021)	nee			+		ns			ns	
HSP70	influences the activin/nodal/transforming growth factor-β and bone morphogenetic protein receptors	Yamashita et al. (2010); Hasenbein et al. (2016)	+			+		ns			ns	
HSP30	can regulate the H + -ATPase induced by various kinds of stress, ubiquitinated proteins degraded via proteasomes or lysosomes can cause upregulation	Piper et al., 1997; Young and Heikkila (2010)	nee			+		ns			ns	
CYP7a1	involved in the cholesterol catabolic process and growth, as well as the bile acid synthesis; bile acid was described to be beneficial for energy metabolism and health condition of farmed fish	Yun et al. (2012); Chiang (2017); Kong et al. (2021); Wang et al. (2023)	nee			–		ns			ns	
POMCb	precursor of adrenocorticotropic hormone (ACTH) which is involved in cortisol production	Alsop and Aluru (2011); Hasenbein et al. (2013); Pasparakis et al., 2023	–			+		ns			ns	
HSD11b2	regulation of glucocorticoids converting cortisol into inactive cortisone	Krozowski et al. (1999); Tomlinson and Stewart (2001); Hasenbein et al. (2016); Pasparakis et al. (2023)	+			–		ns			ns	
TFR	immune response of macrophages to, for example, bacterial infections	Pxytycz and Józkowicz (1994); Stafford and Belosevic (2003); Neves et al. (2009)	nee			+		ns			ns	
IGF1r	stimulates cartilage sulfation of growth hormones, can result in reduced growth but increased longevity and stress resistance	Jones and Clemmons (1995); Gabillard et al. (2003); Holzenberger et al. (2003); Muñoz (2003); Cypser et al. (2006); Zhang et al. (2018)	nee			–		ns			ns	
GLUT5	indicator of nutritional status and transports fructose, in fish liver and muscle, glucose transporters react to fasting and hypoxia	Polakof et al. (2012), Hasenbein et al. (2013), Wang et al. (2019); Koepsell (2020); Pasparakis et al. (2023)	+			–		ns			ns	
B)												
	Condition Factor	Yanagitsuru et al. (2021); Pasparakis et al. (2023)	+			–		ns			–	

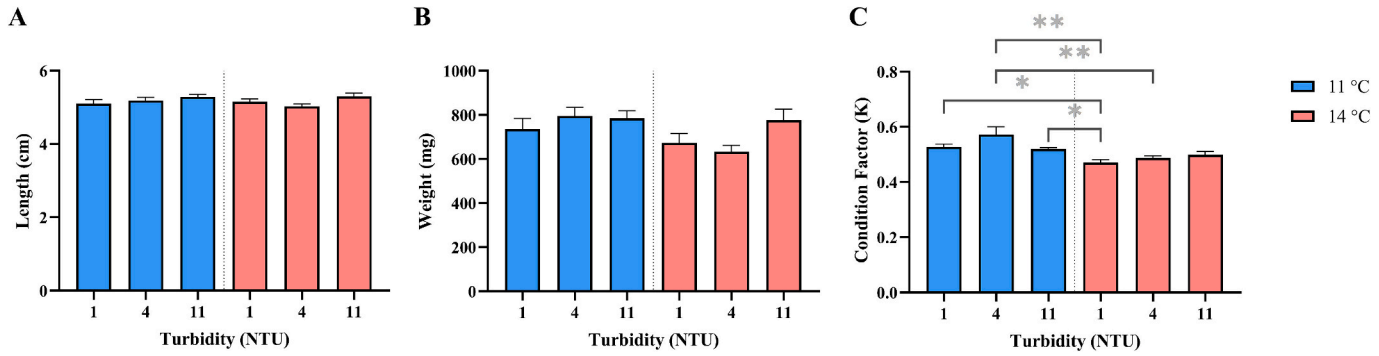

Fig. 1. Longfin Smelt (*Spirinchus thaleichthys*) growth: Length (A), weight (B) and condition factor K (C) of Longfin Smelt (209–228 dph) after 4 weeks at rearing conditions of turbidity (1 NTU, 4 NTU, and 11 NTU) and temperature (11 °C in blue, 14 °C in red) treatments. Data ($n = 4$) are presented as mean ± SEM (* $p < 0.05$, ** $p < 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pathways were not affected or that these biological processes might be more strongly regulated by other genes not analyzed. In contrast, temperature had a significant effect on transcription of *Catalase* (*CAT*; temperature F = 4.73, $p = 0.033$; turbidity F = 0.46, $p = 0.63$; interaction F = 1.60, $p = 0.21$), *Citrate synthase* (*CS*; temperature F = 6.19, $p = 0.015$; turbidity F = 0.82, $p = 0.44$; interaction F = 0.029, $p = 0.97$), and *Growth Factor Receptor Bound Protein 10* (*GRB10*; temperature F = 4.45, $p = 0.039$; turbidity F = 0.55, $p = 0.58$; interaction F = 0.76, $p = 0.47$) [Fig. 2].

Non-significant linear trends of gene expression with increasing

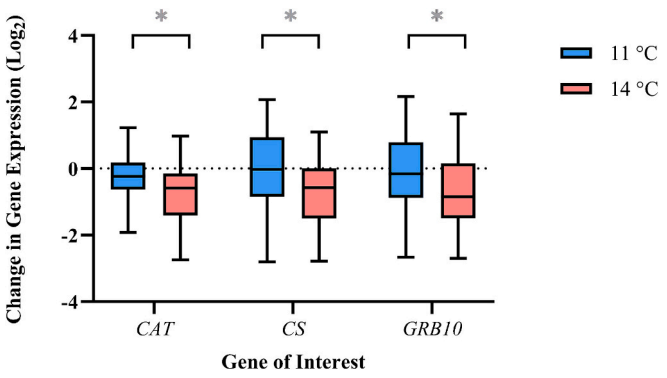

Fig. 2. Longfin Smelt (*Spirinchus thaleichthys,* 209–228 dph) gene expression levels: *Catalase*, *Citrate Synthase*, and *Growth Factor Receptor Bound Protein 10* at 11 °C (blue) vs. 14 °C (red) treatments at the end of the 4-week exposure period. Data ($n = 12$) are presented as mean (Box and whiskers, min to max), (* $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

turbidity were observed: Increasing expression means of *HSP30* (general stress) and *GLUT5* (nutritional status) at 11 °C, and *Cyp7a1* (cholesterol catabolic process and growth) at 14 °C; decreasing expression means for *POMCb* (HPI axis, precursor of adrenocorticotropic hormone, cortisol production) at 11 °C, and *IGF1r* (influencing growth hormones and transformation events) at 14 °C.

4. Discussion

Survival rates of juvenile LFS within the evaluated temperature range of 11 °C to 14 °C and turbidity levels ranging from 1 NTU to 11 NTU over a 4-week duration imply that these environmental conditions are suitable for their successful rearing at this life stage. At lower temperature, both weight and condition factor exhibited significant increases, while turbidity did not exert any discernible effect on growth, condition factor, or transcriptomic stress-response. The significant outcomes over the 4-week period represent crucial findings for aquaculture, indicating that 11 °C is likely more favorable than 14 °C, aligning with our initial hypothesis (i). Our findings are supported by those reported by Baxter et al. (2010) and Yanagitsuru et al. (2021), where temperatures of 9 °C and 12 °C were found to be more suitable than 15 °C for rearing larval stages of LFS. At 11 NTU, juveniles exhibited greater length compared to lower turbidity conditions; however, this was non-significant. This was also observed in similar studies for juvenile DSM weights and condition factors at 14 °C, where increased growth was determined at 10–11 NTU compared to 1–2 NTU (Pasparakis et al., 2023), and at up to 9 NTU for late-stage DSM larvae (Tigan et al., 2020); this could suggest the presence of a visual element in the feeding process, with turbidity potentially affecting prey contrast and, consequently, influencing feeding ability. Consequently, increased weight and condition factor, an indicator of overall health and well-being at 11 °C, suggest that this condition is beneficial, particularly since growth-related endpoints have been highly connected to survival on the basis that "bigger is better" (Sogard, 1997; Brander, 2003).

The transcription of genes associated with stress and growth does not conclusively establish that a lower temperature of 11 °C is closer to optimum for the cultivation of juvenile LFS. Three genes were significantly influenced by temperature: *Citrate Synthase* (*CS*), *Catalase* (*CAT*), and *Growth Factor Receptor Bound Protein 10* (*GRB10*). *CS* catalyzes the synthesis of citrate from oxaloacetate and acetyl coenzyme A and plays an important role in oxidative metabolism. It can be an indicator for the metabolic and aerobic capacity of fish (Pelletier et al., 1993; Majed et al., 2002) as well as growth rates (Pelletier et al., 1994). The higher expression level at 11 °C compared to 14 °C, supports our hypothesis that 11 °C can be beneficial for aerobic metabolism and growth. *CAT* and *GRB10*, however, showed lower expression at 14 °C than at 11 °C. *CAT* is a key enzyme in the bodies defense against oxidative stress and is a main driver in the maintenance of pro/antioxidant balance (Di Giulio et al., 1989; Ahmad et al., 2000; Bagnyukova et al., 2005a, 2005b; Lesser, 2006). Differences in *CAT* expression suggests potential modulation of cellular defense mechanisms and responses to oxidative stress. The lower expression levels of *CAT* at 14 °C compared to 11 °C could potentially indicate less oxidative stress at 14 °C. *GRB10*, a suppressor of growth and cellular proliferation interacts with insulin and insulin-like growth-factor receptors. It is known that overexpression can inhibit tyrosine kinase activity and result in growth suppression (Charalambous et al., 2003; Plasschaert and Bartolomei, 2015). At a temperature of 14 °C, the expression levels of *GRB10* were lower compared to those at 11 °C, making it improbable, based on the temperatures examined, that its expression was sufficiently high to impact growth but potentially affecting tyrosine kinase activity. We hypothesized (ii), that stress- and growth-related gene expression (e.g., *Proopiomelanocortin b precursor* (*POMCb*), *Heat shock protein 30* (*HSP30*), *Glucose Transporter 5* (*GLUT5*)) would indicate a preference for treatments at 11 °C. However, given that there were no notable expression alterations in any other genes of interest, hypothesis (ii) could not be confirmed. It remains plausible that temperatures tested may not impact the pathways associated with investigated genes. Based on our findings and those of other researchers (e.g. Yanagitsuru et al., 2021), temperatures below 14 °C can be considered to be within the thermal tolerance of LFS which explains the absence of cellular thermal stress responses (e.g., HSP expression changes; Jeffries et al., 2016).

Turbidity can elicit both favorable and unfavorable outcomes in fish, contingent on the species (Utne-Palm, 2002). Prior research has utilized *CAT* (e.g., Shin et al., 2006), *Heat shock protein 70* (e.g., Hasenbein et al., 2016), *Proopiomelanocortin* (e.g., Hasenbein et al., 2013), and *11-beta-hydroxyteroid-dehydrogenase* (e.g., Hasenbein et al., 2016) as candidate genes to evaluate responses to turbidity. Nevertheless, there were no significant differences in gene expression in LFS at tested turbidities up to 11 NTU, supporting hypothesis (iii), indicating that the migratory behavior of juvenile LFS to clearer ocean waters would lead to diminished dependence on turbid conditions. However, particularly at turbidity levels higher than those examined in this study, it is possible that turbidity could influence the HPI axis and consequently cortisol levels. This turbidity effect on stress is suggested by e.g., the observed reduction in the means of *POMCb* expression with increasing turbidity at 11 °C. The outcomes of this investigation indicate that, for culturing of the juvenile stage, turbidity might not be a crucial water criterion to uphold, as long as it remains at low levels. This result might not be surprising given that this developmental stage migrates toward ocean waters characterized by low turbidity and lower temperatures (Rosenfield and Baxter, 2007). In contrast to analogous investigations on DSM with different experimental designs (such as those conducted by Hasenbein et al. (2013, 2016)), we examined the impact of turbidity at relatively lower NTU levels and over a 4-week exposure duration, allowing adequate time for potential acclimation of LFS. This design employed herein may explain the observed lack of significance in gene expression outcomes due to e.g., significant plasticity and acclimation capacity to various turbidity levels over time, which was shown for guppy, *Poecilia reticulata* (Ehlman et al., 2015) and zebrafish, *Danio rerio* (Suriyampola et al., 2018). Overall, the absence of significant gene expression changes could be due to the environmental conditions being within the optimal range, the four-week acclimation period allowing animals to normalize any acute genomic alterations, or the influence of different pathways not detected by the candidate gene-targeted examination.

The relationship between growth and gene expression is intricate and dynamic. Under conditions of stress, organisms divert energy toward the restoration of homeostasis, consequently diminishing the energy allocation available for developmental and growth processes (Schreck and Tort, 2016). During development and differentiation,

however, there is a tendency for oxidative metabolism to be elevated, and concurrently, for body size to impact gene expression (Kocmarek et al., 2014). Findings by Kocmarek et al. (2014) indicate heightened expression of general stress and immune response genes in larger juvenile fish, relative to earlier larval life stages, indicative of enhanced adaptability to environmental changes. Despite the comparable size and age of LFS within the study, the modulated expression of *CAT*, *CS*, and *GRB10* might have also been influenced by size. Under hypothesis (ii), our prediction was that growth-related gene expression (e.g., *GRB10*, *GLUT5*) would indicate a preference for 11 °C. We could not support this hypothesis. Despite the growth data favoring 11 °C, gene expression data was inconclusive, not discarding 14 °C as a suitable rearing temperature. Multiple physiological endpoints need to be investigated in future to determine optimal conditions for LFS. Particularly, further research is warranted for various life stages, given that physiology can be influenced by the size of the fish and the distinct developmental stages occurring in diverse environments.

5. Conclusion

In an ecosystem such as the San Francisco Estuary, which has experienced substantial alterations in its physical, chemical, and biological aspects (Kimmerer, 2002; Cloern and Jassby, 2012), rapidly changing conditions challenge aquatic organisms. Rearing LFS in captivity is becoming increasingly important for population conservation and potential augmentation in the wild, as well as for the provision of research organisms toward better understanding specific requirements of this species. Taken together, temperature ranges of 11–14 °C and turbidity levels <11 NTU were found to be generally suitable for rearing juvenile LFS at an age range of 181 to 228 dph. Our results indicate that aquaculture practices could potentially forego adding algae into rearing tanks, toward increasing turbidity, leading to reduced costs and efforts (Hung et al., 2023). Reporting our non-significant gene expression results in response to turbidity is crucial as they often go unreported, leading to biased meta-analyses, despite the ethical obligation to document all findings from well-designed studies, which may help minimize publication bias and improve scientific dissemination (Visentin et al., 2020). Knowledge of the life-stage-specific impacts of culturing temperature and turbidity levels on LFS, as documented in this study, is an essential prerequisite for minimizing stress, optimizing growth, and supporting animal health. Future research and conservation efforts should integrate additional environmental factors (e.g., salinity), husbandry parameters (e.g., stocking density and life history), or life-related aspects (e.g., food type and habitat) to elucidate their interactions comprehensively. Understanding the tolerance thresholds and ecological needs for each developmental stage of LFS is crucial for advancing both basic science and its application in derived conservation initiatives.

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CRediT authorship contribution statement

Felix Biefel: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Christina Pasparakis:** Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dennis E. Cocherell:** Resources, Methodology. **Tien-Chieh Hung:** Writing – review & editing, Resources, Methodology, Funding acquisition. **Evan W. Carson:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Nann A. Fangue:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Juergen P. Geist:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Anne E. Todgham:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Richard E. Connon:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.741296.

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