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Are there lingering effects of egg thiamine deficiency (M74) in thiamine-treated Baltic Sea salmon (*Salmo salar*) juveniles?

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ABSTRACT

Temporal fluctuation of thiamine deficiency is a well-documented phenomenon in Atlantic salmon (*Salmo salar)* from the Baltic Sea. The deficiency causes increased levels of fry mortality (M74-syndrome). Although the cause for the deficiency in salmon remains somewhat unclear, the mortality rate can be significantly reduced by bathing alevins in thiamine solution. However, it is not known whether thiamine supplementation at the alevin stage successfully treats all other potential adverse effects associated to thiamine deficiency during the embryonic stages of development. To investigate how thiamine deficiency affects growth and survival in thiamine treated and untreated salmon during the first summer, fertilized eggs with known thiamine content were sorted into four groups: *Very low*, *Low*, *Medium* and *High* thiamine content (all having thiamine levels above critical levels where mortality is inevitable if left untreated). Half of each group was treated with thiamine according to standardized hatchery procedures and half was left untreated as control. Mortality was monitored continuously, and body length and -mass were recorded after the initial growing season (five months after hatching). The results showed that fish with very low initial levels of thiamine grew smaller even if treated with supplemental thiamine. This indicates a potential disturbance of individuals suffering from early thiamine deficiency.

1. Introduction

Periodic incidences of thiamine (vitamin B_1) deficiency, causing major impact on egg hatching rates and yolk-sac fry development, is a common management concern and a regularly occurring phenomenon in Baltic Sea salmon (*Salmo salar* L.) (Hylander et al., 2020). To limit the effects in aquaculture using wild or sea-ranched brood stocks, standardized thiamine treatments of alevins (from hatching until first-feeding) are carried out in Swedish salmon hatcheries rearing fish for compensatory smolt stocking (i.e. stocking compensating for lost salmon production due to hydropower development) (Norrgård, 2020). This treatment has proven to be very successful to counteract fry mortality. However, whether or not there are lingering effects of thiamine deficiency in treated fish is still an open question. In this study, we investigate effects of thiamine deficiency on growth and survival of Atlantic salmon fry over their first summer.

M74 is the term for a reproductive disorder in Atlantic salmon foraging in the Baltic Sea ('Baltic salmon'). Signals of the disorder can be found in fish foraging in either of the two main basins of the Baltic Sea (Jones et al., 2022). The disorder, generally characterized by yolk-sac fry (alevin) abnormalities and mortality, received the designation "M74" (Swedish abbreviation for *Miljöfaktor-74*; English translation: *Environmental factor-74*) after the initially unknown causation and the year of recognition (1974) (Norrgren et al., 1994). Since 1995, it is known that M74 is caused by thiamine deficiency in the eggs, just like the "Cayuga"- and "early mortality" syndromes observed in salmonids in the North American Finger- and Great Lakes systems (Bylund and Lerche, 1995; Fisher et al., 1995; ICES WGPDMO, 1995; Fitzsimons et al., 1999). The offspring of a thiamine deficient female do not develop normally, and the hatched fry tend to die early. Typical directly observable symptoms in M74-affected fry include uncoordinated movements (wobbling), inability to maintain an upright swimming position, inactivity, pallor, and body deformities such as a curved spines and swollen eyes and head, followed by death at the alevin stage (Keinänen et al., 2000). In addition to low levels of thiamine, M74 is also associated with necrotic brain cells, oxidative stress, imbalance in fatty

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acids, and low levels of carotenoids (e.g., astaxanthin) (Amcoff et al., 1999; Pickova et al., 1999, 2003; Lundström et al., 1999a; Pettersson and Lignell, 1999; Vouri and Nikinmaa, 2007).

In marine systems, thiamine is primarily produced by bacteria and phytoplankton while animals have their thiamine requirements met via their food (Sañudo-Wilhelmy et al., 2014). Thiamine pyrophosphate (TDP) is a cofactor for enzymes that control, among other things, the metabolism of carbohydrates, fatty acids, and amino acids, as well as the production of energy carrying molecules (ATP; Whitfield et al., 2018). In addition to its role as a cofactor, thiamine is also an antioxidant that protects against oxidative stress (Lukienko et al., 2000). Thiamine is essential and deficiency thereby means that vital processes in an organism cannot be maintained. The clinical signs of thiamine deficiency are multifaceted, as described for the M74 syndrome above. In a deficient state, toxic metabolites (e.g., glyoxales, lactate, and phytanic acid) that damage the central nervous system accumulates (Whitfield et al., 2018). Thus, thiamine deficiency can lead to neurological impact and associated memory- and learning disorders, behavioural anomalies and reduced food intake (see Balk et al., 2016, with associated references). In salmon hatcheries, the development of M74 can be counteracted by thiamine treatment of eggs (thiamine injections in the females) and alevins (bath in thiamine solution) (Norrgård, 2020). Today, there is much knowledge about the uptake and distribution of thiamine to the fry and what concentrations of thiamine are adequate for good fry survival. However, possible lingering effects as a consequence of low levels of thiamine deficiency during the embryonic development such as permanent cognitive dysfunction due to brain damage (Amcoff et al., 1999; Whitfield et al., 2018), are not yet sufficiently investigated in Baltic salmon. In other salmonids, the influence of thiamine deficiency has demonstrated affecting fry and larval growth (Fitzsimons et al., 2009; Reed et al., 2023) as well as behaviour (Fitzsimons et al., 2009).

We hypothesize that individuals hatching from eggs with low thiamine concentrations will not develop as well as individuals hatching from eggs with higher thiamine concentrations and that there are lingering effects of thiamine deficiency, despite thiamine treatment. In this study, we examined growth and survival of groups of salmon fry originating from eggs with variable thiamine concentrations, but all above the critical lethal threshold levels (Vuorinen et al., 2021), where half of the groups were treated with thiamine and half of the groups left untreated as control. Survival was monitored from the egg stage and growth evaluated at the end of the first growing season (5 months after hatching).

2. Material and methods

The study was conducted at the Swedish University of Agricultural Sciences' research- and hatchery facility in Älvkarleby, Sweden (WGS84 dec: 60.561798, 17.440463). Here, adult wild and sea-ranched (hatchery-reared, released as smolts) salmon are caught in the river Dalälven during their spawning migration and used as brood stock in the hatchery, to produce salmon smolts for compensatory stocking.

2.1. Egg thiamine measurements

At stripping (Oct 24 and Nov 2, 2022), ca. 15 mL of unfertilized eggs from each female was collected for determination of thiamine content. Thiamine was extracted from a 1 g aliquot from each female. The eggs were homogenization in acetone (2 mL) followed by addition of water (4 mL) and dichloromethane (4 mL), the sample was mixed further and after centrifugation the aqueous phase was isolated. The water extract was washed with an additional portion of dichloromethane and analysed using liquid chromatography mass spectrometry (LC-MS), with an Acquity UPLC system, coupled to a Xevo TQ-S micro, mass spectrometer (Waters Corporation, Millford, MA), with an electrospray source working in in positive mode (ESI+), and a X-bridge phenyl column [2.1 × 100 mm, 3.5 μm (Waters Corp.)] (Larsson et al., 2024). Additional calibration and quality parameters can be found in the supplement, Fig. S1.

2.2. Experimental groups

Based on the results of the thiamine analyses, 25 females were selected to form four thiamine level groups: *Very low*, *Low*, *Medium*, and *High* (Table 1). Thiamine level groups were formed based on information from the literature on lethal thiamine concentration inducing 50 or 100 % mortality of embryos, with incipient, LC 50 at 0.47 nmol g^{-1} and LC 100 at 0.22 nmol g^{-1} or salmonids (Balk et al., 2016; Vuorinen et al., 2021;) and the distribution of the thiamine analysis data, aiming to create discrete groups containing eggs from as many females as possible, without too much intra-group variation in egg thiamine concentration (Table 1). On March 30, 2023, fertilized eggs from each group of females were placed in two hatching trays, 900 eggs in each. Number of eggs per female varied due to variable number of females per thiamine level group (Table 1). After hatching in April, alevins in one of the hatching trays from each thiamine level group were bathed in a thiamine solution (details below). The alevins in the other hatching tray for each thiamine level group were left untreated as control. Mortality was monitored from March 30, 2023, until the end of the experiment.

2.3. Thiamine treatment

Thiamine treatment is used across Swedish hatcheries producing Baltic salmon smolts. The procedures can vary slightly among the hatcheries (e.g., concentrations) but the aim is to counteract mortality caused by M74. Half of the experimental alevins (yolk sac stage)(one of the two hatching trays in each thiamine level group) was treated with a thiamine solution three times: April 25, May 2, and May 10, 2023. During treatment, the regular inflow of water to the hatching trays was closed and a thiamine solution consisting of dissolved thiamine mononitrate granule (>96 %; Jiangsu Brother Vitamins Co., Ltd., Jiangsu) and water, was added to reach a concentration of 2 g · L^{-1} (normal concentration used in this hatchery, J. Rask pers. comm.). A pump was used to circulate the water and oxygen was continuously added during the treatment. After 1 h of treatment the circulation of water was started again, and the thiamine solution flushed out of the system.

2.4. Post-treatment rearing and data collection

On May 22, 2023, the experimental individuals were moved to rearing tanks where they started external feeding (fry stage ~1 month post hatch). Fry from each hatching tray (treatment or control), were split into three tanks (in total 24 tanks). Due to some egg and fry mortality, the number of fry that was put in each tank varied between 263 and 295 (Table 2). Rearing tanks had a bottom area of 1 m^2, 23–25 cm water depth, and a continuous water flow (ca. 0.5 L s^{-1}) creating a

Table 1
Data on females used to produce experimental groups of Atlantic salmon fry based on thiamine content of the eggs (nmol · g^{-1}). Mass, length and egg thiamine concentration are given as mean ± standard deviation. 'Eggs per female' indicate number of eggs collected per female.

Group	*N* females	Mass (kg)	Length (cm)	Eggs per female	Egg size (cm)	Thiamine (nmol · g^{-1})
Very low	7	5.7 ± 0.8	82.7 ± 4.6	258	0.60	1.14 ± 0.20
Low	5	7.4 ± 2.5	88.8 ± 8.4	360	0.60	2.03 ± 0.20
Medium	9	6.4 ± 1.9	86.9 ± 9.2	200	0.61	3.50 ± 0.23
High	4	10.1 ± 1.9	101.5 ± 7.9	450	0.64	6.57 ± 0.60

Table 2

Information about hatching and mortality during the last part of the egg stage and alevin stage for fish from different thiamine level groups (TH). Thiamine treatment (TR) done three times, once weekly, during the alevin stage). Start = number of eggs at the start; D4 = dead during hatching; D5 = dead alevins; D Tot = total number of dead individuals. Dates (YYY-MM-DD) are given for start date of hatching and date when half of the eggs had hatched; hatching in all groups had finished by 2023-04-23. *P*-values are given for differences in frequency (Control vs. Treated) based on 4 × 2 contingency tables [or 4 × 2 Fisher's Exact Test (Freeman and Halton, 1951), marked by '*'].

TH	TR	Start	D4	D5	D Tot	Survivors	Start hatch	Half hatched
Very low	Control	900	3	111	114	786	2023-04-04	2023-04-14
Low	Control	900	38	64	102	798	2023-04-13	2023-04-20
Medium	Control	900	4	14	18	882	2023-04-06	2023-04-17
High	Control	900	2	19	21	879	2023-03-30	2023-04-15
Very low	Treated	900	2	43	45	855	2023-04-04	2023-04-18
Low	Treated	900	54	57	111	789	2023-04-12	2023-04-21
Medium	Treated	900	3	12	15	885	2023-04-04	2023-04-17
High	Treated	900	0	24	24	876	2023-04-06	2023-04-17
P-value			0.284*	< 0.001	< 0.001			

single-direction current in the tanks. The majority of the water was provided from the River Dalälven, with a small addition of ground water to secure constant water flow to the tanks. The water temperature was measured once a week and varied from about 14 °C in May, to between 19 and 21.5 °C at the end of June and throughout July (see Fig. S2 in the supplement). At the end of August, the temperature was 17 °C, and at the end of the experiment, on September 18 (~ 5 months post hatch), 2023, the temperature was about 15 °C. Fish were fed ad libitum through automatic feeders (Arvo-tec feeding system; Aller Aqua feed pellets: 0.5–1.0 mm until July 25, thereafter 0.9–1.6 mm). Tanks were cleaned twice a week and dead fish were removed every day. On Sep 18, 50 fish from each tank were randomly netted, anaesthetised with tricaine methanesulfonate ('MS222', 175 g L^{-1}; Pharmaq Ltd., Hampshire) and measured for wet mass (precision: 0.1 g) and total length (precision: 1 mm).

2.5. Data analyses

Body size after summer (Sept 18, 2023) was analysed in two maximum likelihood linear mixed models, one on total length L_T (Eq. (1)) and one on $\log_{10}$ wet body mass M (Eq. (2)), in R using the lme4 package (Bates et al., 2014):

$$L_T \sim TH + TR + TH \times TR + (1|Tank) \tag{1}$$

$$log_{10}(M) \sim TH + TR + TH \times TR + (1|Tank) \tag{2}$$

where *TH* is thiamine level group (fixed factor; four levels, Table 1), *TR* is treatment (fixed factor; two levels: thiamine treated and untreated controls), and *Tank* is the rearing tray of the fish (random intercepts factor; 24 levels). Factor effects were determined by analysis of deviance [ANODEV; type III χ^2-test through the car package (Fox et al., 2013)]. The inclusion of *Tank* as a random factor was evaluated using a likelihood ratio test (LRT) between the full model and a reduced model excluding *Tank*, with a significant effect ($p < 0.05$) signaling that the inclusion of *Tank* has relevant explanatory value in the model; the inclusion of *Tank* was supported for both models ($p < 10^{-15}$). Pairwise comparisons were done based on estimated marginal means through the emmeans package (Lenth, 2023), with degrees of freedom determined by Kenward-Rogers estimation and p-value adjustment by the Tukey method. Threshold for statistical significance was $p < 0.05$, with p-values derived through t-tests based on the Satterthwaite's method (lmerTest package; Kuznetsova et al., 2017).

Body condition was analysed as the body mass relative to body length in a linear model (Eq. (3)):

$$log_{10}(M) \sim log_{10}(L_T) + TH + TR + TH \times TR + (1|Tank) \tag{3}$$

with the same term designation as above. Inclusion of *Tank* as a random factor was supported by LRT-test, as per above ($p \langle 10^{-10}$). To further assess body condition graphically, we calculated a cube law condition factor (K; Eq. (4)) for each individual:

$$K = M/{L_T}^3 \times 10^{-5} \tag{4}$$

where a value of 1 was considered a normal condition for a healthy fish.

Survival was analysed using a logistic model (binomial generalized linear model, logit link function; Eq. (5)). The response variable was the proportion surviving fish *S*, i.e. the number of remaining fish in rearing trays on Sept 18, 2023, relative to the initial number of fish at the transfer to the trays on May 22, 2023:

$$S \sim TH + TR + TH \times TR \tag{5}$$

with the same term designation as above.

All models were run and evaluated in R (R Core Team, 2023) through RStudio (version 2023.03.0; Posit Software, PBC, Boston, MA). Data handling was conducted using the tidyverse suite of R packages (Wickham et al., 2019) and figures were produced using ggplot2 (Wickham et al., 2019) and cowplot (Wilke, 2020).

3. Results

3.1. Survival during the egg- and alevin (thiamine treatment) stages

From March 30 to hatching there was no mortality. During hatching, there was no differences between the groups destined to become either controls or thiamine treated, but the *Low* thiamine level group showed a substantially higher mortality (Table 2). From hatching to the initiation of rearing in experimental trays, i.e. during the alevin stage when the *Treated* experimental groups were treated with thiamine, the mortality was higher in the *Control* group for the *Very low* thiamine level group, as compared to the *Treated* group (Table 2); the *Low* thiamine level group still had a higher mortality than the *Medium*- and *High* thiamine level groups in general, but there were no clear difference between *Treated* and *Control* in the *Low* group (Table 2).

3.2. Post-treatment survival

The logistic model of survival revealed a significant treatment × thiamine level group interaction effect (Table 3). Significant pairwise contrasts among groups were detected between the *Very low* control group and all other groups (all $p < 0.001$; Fig. 1). Furthermore, a very small but significant contrast was detected between the *High* and *Very*

Table 3

ANODEV table for the logistic model of survival.

Independent variables	χ^2	d.f.	p
Treatment (TR)	0.525	1	0.469
Thiamine group (TH)	9.462	3	**0.024**
TR × TH	179.3	3	**< 0.001**

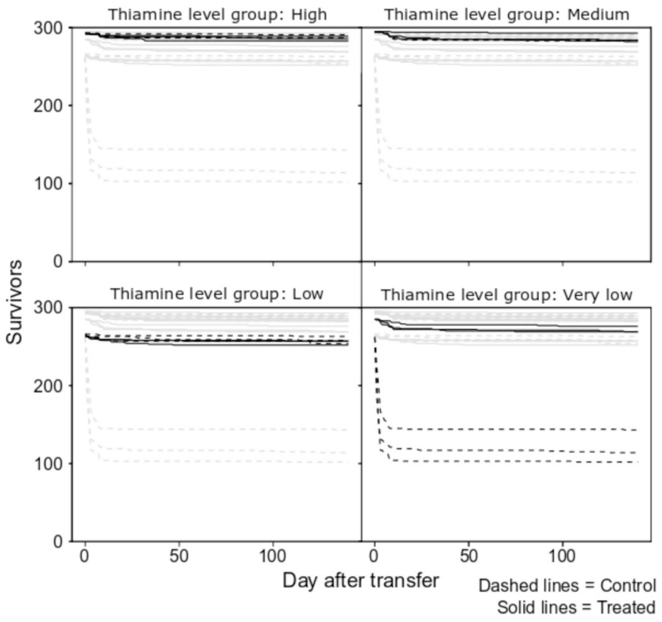

Fig. 1. Survival from transfer to rearing trays (May 22, 2023) to the date of measurements (September 18, 2023), for each rearing group (note differences in initial number, due to alevin mortality prior to transfer to rearing tanks). Each thiamine level group is highlighted in black lines in a panel each, with all other groups being presented in grey for comparison.

low thiamine treated groups ($p = 0.023$) (effect barely visible; Fig. 1). No other contrasts were significant ($p > 0.173$).

3.3. Body size

For body length, a significant effect of thiamine level group was detected (Table 4). Pairwise contrasts among the thiamine level groups (irrespective of treatment) revealed a single significant contrast between the groups *High* and *Very low* (estimated difference ± SE: 12.6 ± 3.5 mm; $t = 3.631$, $p = 0.005$). The *Very low* group was also shorter on average than the *Medium* (estimated difference: 9.1 ± 3.5 mm) and *Low* (estimated difference: 8.1 ± 3.5 mm) groups, but without statistically clear results ($p = 0.058$ and $p = 0.107$, respectively) (Fig. 2A,C).

For body mass, no statistically clear effects were detected (Table 4), although the patterns were qualitatively similar to that of body length (Fig. 2B,D).

3.4. Body condition

The body condition differed among some groups, as indicated by a significant treatment × thiamine level group interaction effect (Table 5A). Significant pairwise effects among control groups were detected between the *High* and *Very low* groups, and between the *Medium* and *Very low* groups, with the *Very low* group having higher body condition in both cases (Table 5B; length-mass relationship shown in Fig. 3A). For the thiamine treated groups, significant contrasts were detected between the *High* and *Medium* groups, and between the *High* and *Low* groups, with the *High* group having lower condition in both cases (Table 5B, Fig. 3A).

Table 4
ANODEV tables for the models of body length and body mass.

Independent variables	χ^2	d.f.	*p*
Body length			
intercept	1607	1	**< 0.001**
Treatment (TR)	0.095	1	0.757
Thiamine group (TH)	9.129	3	**0.028**
TR × TH	2.615	3	0.455
Body mass			
intercept	862.1	1	< 0.001
Treatment (TR)	0.212	1	0.645
Thiamine group (TH)	6.294	3	0.098
TR × TH	1.006	3	0.800

For all groups in the study, the majority of the fish had a relatively high condition factor ($K > 1.0$; Fig. 3B), and there were no indications of poorer condition in fish with lower initial thiamine levels. The graphical illustration of K presents the same results as the statistical analysis of the length-mass relationships in the experimental groups.

4. Discussion

Thiamine is a life essential coenzyme, important to maintain cell function and normal growth for all animals. Severe thiamine deficiency is ultimately lethal, but also has a number of sublethal health effects (Balk et al., 2016) that may cause permanent damages. In this study, thiamine deficiency in the egg of Baltic salmon caused some mortality at the very early stages after hatching, after which survival rates were essentially constant among groups regardless of treatment with supplemental thiamine. However, associated with thiamine deficiency a difference in length was observed, both in survivors in the control group and in fish from the thiamine treatment group.

The fish from the thiamine group with lowest initial concentrations (*Very Low* thiamine group) were on average smaller (in both length and mass) than fish from the high initial concentrations (*High* thiamine group), with no statistically significant differences detected in any other pairwise comparisons among the thiamine level groups. According to previous research, poor growth performance has been observed in various fish species fed thiamine-deficient diets including rainbow trout *Oncorhynchus mykiss* (Morito et al., 1986) and Japanese eel *Anguilla japonica* (Hashimoto et al., 1970). Poor growth in itself may well be an early sign of low thiamine supply (Sun et al., 2022) and has been documented in relation to thiamine deficiency in lake trout *Salvelinus namaycush* (Fitzsimons et al., 2009) and steelhead trout *O. mykiss* (Reed et al., 2023). Here, however, the fish were supplied with food that within hatchery regimes previously has demonstrated contain sufficient thiamine content during rearing from the termination of the alevin stage. Given rearing in a food rich environment and that the condition factor was relatively high in the *Very Low* groups, it is reasonable to assume that compensatory growth would have been possible over summer (Ali et al., 2003). However, restricted or no compensation in structural growth (body length) has been observed in closely related brown trout *Salmo trutta* fry (Näslund et al., 2017a, 2017b). Hence, it is possible that the shorter body lengths are lingering effects from reduced growth performance from the thiamine deficient egg and alevin period. Another potential explanation for shorter body length in the *Very Low* group could be size biased mortality during the pre-treatment period, if larger individuals died to a higher extent within this group. Lastly, the apparent difference in egg size could potentially have contributed to the size difference but since fish were fed *ab libitum* and reared separately from each other, it is conceivably unlikely that egg size effects would remain for such a long period (see Einum and Fleming, 1999). While there are a few alternative hypotheses for the smaller average body size of the *Very Low* group fish, the explanation that these fish are performing poorly due to lingering effects of the early thiamine deficiency still remains as a feasible hypothesis.

In their work on estimating thiamine deficiency-related mortality of Atlantic salmon offspring and variation in the Baltic salmon M74 syndrome, Vuorinen et al. (2021) demonstrated incipient M74 mortality and 100 % offspring mortality (LC100) at concentrations of thiamine of 0.71 and 0.22 nmol g^{-1} in ovulated unfertilized eggs, respectively. In the present study, thiamine concentrations in all groups exceeded 0.83 nmol g^{-1} and were thus not at lethal levels. While the effects of slightly

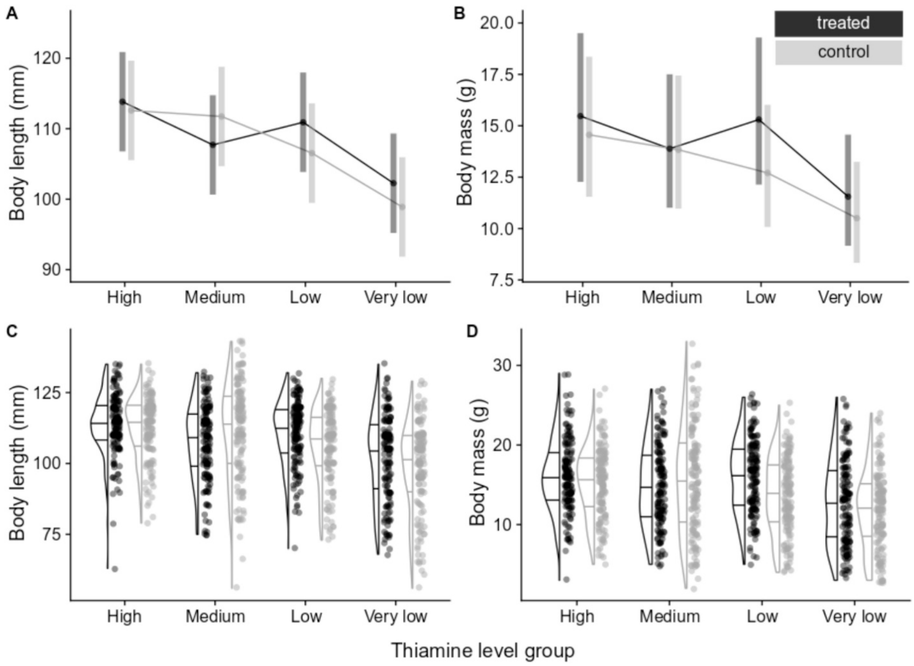

Fig. 2. Body size of juvenile Baltic salmon from different experimental groups. A-B) Estimated marginal means with 95 % confidence intervals for A) body length (total length), and B) body mass. C-D) Raw data plots showing the distribution of data for each group in combined half-violin/dot plots (half-violin plots include lines demarking data quartiles.

Table 5
Results from statistical analyses. A) ANODEV table for main- and interaction effects. B) Significance of pairwise contrasts between thiamine level groups within the treatment groups.

A) Analysis of deviance (type III Wald χ^2 test)

Independent variables	χ^2	d.f.	*p*
intercept	19,084	1	**< 0.001**
$\log_{10}$(length)	30,213	1	**< 0.001**
Treatment (TR)	2.154	1	0.142
Thiamine group (TH)	16.809	3	**< 0.001**
TR × TH	18.771	3	**< 0.001**

B) Pairwise contrasts

	TR = Control		TR = Treated	
Pair: TH groups	*t*-ratio	*p*	*t*-ratio	*p*
High – Medium	0.280	0.992	−2.828	**0.036**
High – Low	−1.574	0.405	−2.931	**0.028**
High – Very low	−3.664	**0.004**	−2.000	0.206
Medium – Low	−1.854	0.265	−0.101	0.999
Medium – Very low	−3.947	**0.002**	0.823	0.843
Low – Very low	−2.104	0.171	0.922	0.793

smaller size of initially thiamine deficient individuals, in itself, may not have any major significance for the predicted performance of the salmon, given that hatchery produced fish in general are larger than wild fish, the effect may still signal a disturbance in the individuals that suffers from early thiamine deficiency. Thus, a potentially poorer performance of these fish may be evident in the longer perspective (especially in more life-skills demanding natural environments; Johnsson et al., 2014), which is an issue worth looking further into in future studies. In our study, we observed a relatively high apparent LC50 mortality rate, as compared to a previous estimate for Baltic salmon. Vuorinen et al. (2021) estimated an LC50 level of 0.47 nmol g-1 for fish, but our results show 50 % survival at >0.83 nmol g-1 (Fig. 1). This suggests that our fish may a relatively high thiamine requirement, or that different environmental/genetic factors affect their survival. However, it should be noted that Vourinen et al.'s estimated LC50 value is based on a large sample where 50 % mortality is observed within the span of approximately 0.3 to 0.8 nmol g-1. Early rearing survival rates varied among groups with similar egg thiamine levels, highlighting the complexity of M74 syndrome and the need for tailored management strategies to maintain healthy fish populations. Another potentially important line of investigation is to look further into brain cell damage caused by thiamine deficiency during embryonal development (i.e. prior to thiamine treatment) (Lundström et al., 1999b). Neural damage may lead to permanent performance issues through the life of a salmon, and the effects may be more evident in the more cognitive demanding natural environments experienced after stocking. Investigations of such effects are difficult, but still potentially achievable through e.g., standardized behavioural- and cognitive tests (Näslund, 2021; Salena et al., 2021), followed up by large scale mark-recapture studies (e.g., Siira et al., 2006), or telemetry studies (Thorstad et al., 2013).

In accordance with previously documented linear relationship of thiamine deficiency induced mortality in salmon (Vuorinen et al., 2021), higher levels of mortality were recorded in the *Very low* and *Low* group as part of this study. However, a survivorship bias was observed in the *Very low* group, where number of individuals in the thiamine treated group had higher survival than those in the *Low* group. This is potentially a consequence of embryos in the *Very low* group dying even before the study was initiated with thiamine treatments, or an unintentional selection on large individuals that later died to a higher extent (with larger individuals containing less thiamine in relation to body mass). This relationship needs to be examined and investigated more in depth.

Although the juveniles in the *Low* group were shorter, a coherent condition factor above 1.0 in the majority of the fish across all groups and treatments indicated that no meaningful difficulties involving feeding were present during this study. However, the situation in more natural settings and harsher conditions is unknown, and investigations recommended for future studies.

5. Conclusions

Based on the observed results we can conclude that thiamine deficiency in Baltic salmon not only cause fry mortality, but also that there is

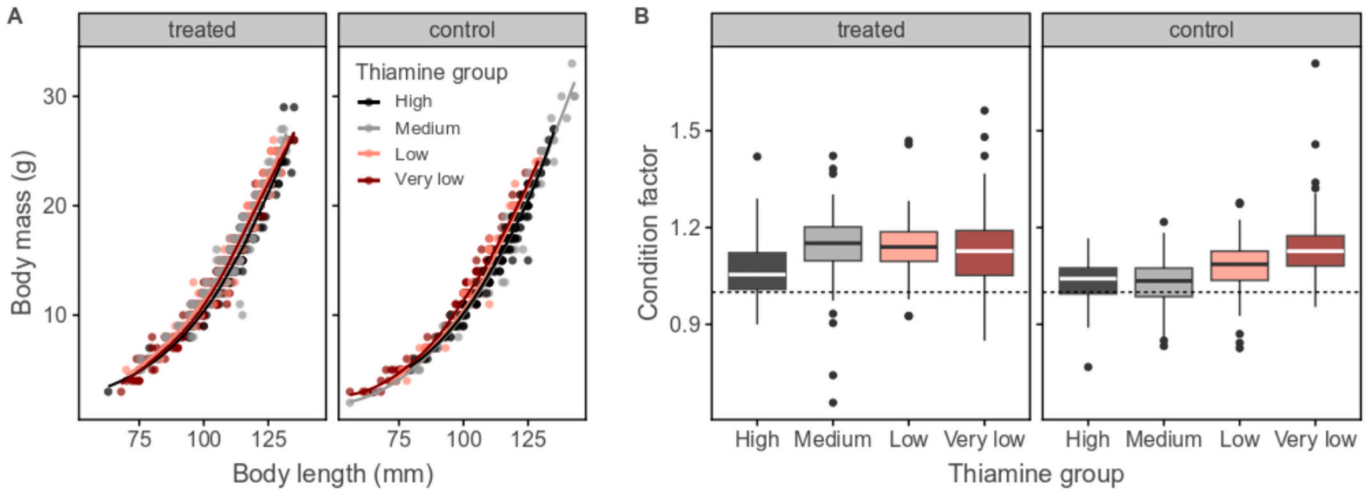

Fig. 3. Body condition of juvenile Baltic salmon from different thiamine level- and treatment groups. A) Length-mass plots, with average condition represented by non-linear loess regression lines. B) Total variation in condition factor (*K*) for measured individuals, represented by Tukey box-plots.

a risk that it leads to sublethal effects on growth despite being treated by thiamine baths. As there is good documentation on effects that may affect the brain and, hence, e.g., cognitive ability, knowledge on the relationships between thiamine status and salmon lifetime fitness are needed. Even if the effects on size are due to size bias (from reduced growth, size selective mortality or initial egg size depending on thiamine deficiency in the brood fish) before the thiamine treatment period, it may still have implications for stocking performance.

Ethical statement

The experiment was conducted under an ethical permit for animal research issued by the Swedish Board of Agriculture 2023-03-24 - 2025-12-31 (Dnr 5.8.18-03489/2023; reviewed by the Uppsala ethical committee).

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CRediT authorship contribution statement

Elin Dahlgren: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Lo Persson:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Dennis Lindqvist:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Joacim Näslund:** Conceptualization, Methodology, Formal analysis, Validation, Writing – original draft, Writing – review & editing, Visualization.

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 sets and R code used for analyses are deposited in the figshare database: https://doi.org/10.6084/m9.figshare.24981606.

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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.2024.741581.

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