Hepatic activities of thiamine-dependent enzymes, glucose-6 phosphate dehydro-genase and cytochrome P4501A in Baltic salmon (Salmo salar) yolk-sac fry after t....

Article *in* Aquatic toxicology (Amsterdam, Netherlands) · May 2000

DOI: 10.1016/S0166-445X(00)00089-8 · Source: PubMed

CITATIONS
24

READS
37

6 authors, including:

Gun Åkerman
Stockholm University
43 PUBLICATIONS **545** CITATIONS

SEE PROFILE

Leif Norrgren
Swedish University of Agricultural Sciences
138 PUBLICATIONS **3,635** CITATIONS

SEE PROFILE

Lennart Balk
Stockholm University
134 PUBLICATIONS **2,842** CITATIONS

SEE PROFILE

ELSEVIER

Aquatic Toxicology 48 (2000) 391–402

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Hepatic activities of thiamine-dependent enzymes, glucose-6-phosphate dehydrogenase and cytochrome P4501A in Baltic salmon (*Salmo salar*) yolk-sac fry after thiamine treatment

Patric Amcoff [a,*], Gun Åkerman [b], Hans Börjeson [c], Ulla Tjärnlund [b], Leif Norrgren [a], Lennart Balk [b]

[a] *Department of Pathology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, PO Box 7028, S-750 07 Uppsala, Sweden*

[b] *The Institute of Applied Environmental Research (ITM), Stockholm University, S-106 91 Stockholm, Sweden*

[c] *Swedish Salmon Research Institute, S-814 94 Älvkarleby, Sweden*

Received 29 July 1999; received in revised form 19 January 2000; accepted 20 January 2000

Abstract

Sea-run Baltic salmon (*Salmo salar*) populations have been affected by the M74 syndrome since 1974 causing high yolk-sac fry losses in Swedish compensatory rearing plants. M74 has been shown to be a maternally transmitted thiamine (vitamin B_1) deficiency. The aim of this study was to investigate possible relationships between thiamine and hepatic activities of the thiamine-dependent enzymes transketolase (TK) and α-ketoglutarate dehydrogenase (α-KGDH) in addition to glucose-6-phosphate dehydrogenase (G6PDH) and cytochrome P4501A (CYP1A), measured as 7-ethoxyresorufin *O*-deethylase (EROD), in Baltic salmon yolk-sac fry after treatment with thiamine. Thiamine concentrations and activities of TK, α-KGDH and EROD were significantly lower ($P < 0.05$) in M74 groups compared to controls (not developing M74) and family groups of thiamine injected females. In M74-developing groups the thiamine immersions reduced the mortality from 86 to 13% and restored thiamine concentrations and activities of TK, α-KGDH and EROD to levels slightly lower than the immersed controls. An interesting fact was that the controls showed significantly elevated ($P < 0.05$) TK and α-KGDH-activities after immersions in thiamine, indicating that they also may have a stressed thiamine metabolism. The TK and α-KGDH-activities of unimmersed groups correlated significantly ($P < 0.05$) with the thiamine content. We suggest that the low activities of TK and α-KGDH in M74 groups may be an integrative part in the pathogenesis of M74 development.

Keywords: Baltic salmon; M74; Thiamine deficiency; Cytochrome P4501A; Transketolase, α-ketoglutarate dehydrogenase; Glucose-6-phosphate dehydrogenase

* Corresponding author. Tel.: +46-18-671178; fax: +46-18-673532.
E-mail address: patric.amcoff@pat.slu.se (P. Amcoff)

0166-445X/00/$ - see front matter
PII: S0166-445X(00)00089-8

1. Introduction

Sea-run Baltic salmon (*Salmo salar*) populations have, since 1974, been afflicted by a severe reproduction disturbance designated the M74 syndrome (Börjeson and Norrgren, 1997). The M74 syndrome has been shown to be a vertically transmitted thiamine (vitamin B_1) deficiency from females to their progeny (Amcoff et al., 1998a). Family groups developing M74 demonstrate typical clinical signs with neurological aberrations followed by high mortality rates usually reaching 100% (Börjeson and Norrgren, 1997; Lundström et al., 1998; Åkerman and Balk, 1998). The syndrome frequently occurs in Swedish compensatory rearing plants, where sea-run broodfish are caught and stripped of milt and roe. The offspring are raised until smoltification when released into their native river. There are also strong indications that naturally reproducing Baltic salmon populations are affected by M74 (Karlström, 1999). The M74 syndrome has been described as a hierarchical thiamine deficiency since in severe cases also broodfish may be affected by neurological disturbances as wiggling and incoordinative behavior which occasionally results in death prior to spawning (Amcoff et al., 1998a). Wiggling female broodfish have low ovarian and hepatic thiamine content and their progeny is predestined to develop M74 (Amcoff et al., 1998a, 1999a). Development of M74 is effectively counteracted by injections of thiamine in broodfish or newly fertilized eggs or by immersions in a solution of thiamine at the egg or yolk-sac fry stage (Amcoff et al., 1998b; Åkerman and Balk, 1998; Börjeson et al., 1999).

The cause of the low thiamine concentrations in adult Baltic salmon is unknown, even though, oxidative stress due to biotransformation of xenobiotics, changed nutritional status and extensive feeding on prey items containing the thiamine decomposing enzyme thiaminase are factors that have been considered as potentially involved in the etiology (Börjeson and Norrgren, 1997; Amcoff et al., 1998a). Recent studies have suggested increased NADPH use due to biotransformation of xenobiotics as the driving force for the thiamine depletion (Åkerman et al., 1998a).

The diphosphate ester of thiamine (TDP) is the coenzyme of transketolase (TK; EC 2.2.1.1), the pyruvate dehydrogenase complex (PDH; EC 1.2.4.1, EC 2.3.1.12, EC 1.8.1.4) and the α-ketoglutarate dehydrogenase complex (α-KGDH; EC 1.2.4.2, EC 2.3.1.61, EC 1.8.1.4) which are situated in the pentose–phosphate shunt (PPS), the glycolytic pathway and the citric acid cycle, respectively (Stryer, 1988). The PPS produces NADPH and ribose 5-phosphate (R5P) and the rate limiting steps of the PPS are the glucose-6-phosphate dehydrogenase (EC 1.1.1.49; G6PDH) and the TK (Sabate et al., 1995).

Thiamine deficient rainbow trout (*Oncorhynchus mykiss*) have reduced TK-activities (Lehmitz and Spannhof, 1977; Morito et al., 1986; Masumoto et al., 1987), while rainbow trout yolk-sac fry exposed to PCB # 77 have shown increased TK-activities, possibly indicating an enhanced need of products derived from the PPS during induced biotransformation (Åkerman et al., 1998b). M74-developing Baltic salmon yolk-sac fry show thiamine correlated reductions of cytochrome P4501A-activities (CYP1A), measured as 7-ethoxyresorufin *O*-deethylase (EROD), indicating a possible connection between thiamine status, thiamine-dependent enzymes and CYP1A-activities (Börjeson et al., 1999; Lundström et al., 1999a). This relationship is also supported by the low activities of CYP1A observed in Baltic salmon yolk-sac fry exposed to the thiamine antagonist oxythiamine (Amcoff et al., 1999b).

This study was performed to study whether Baltic salmon yolk-sac fry affected by M74 have altered hepatic activities of TK, α-KGDH, G6PDH and CYP1A compared to healthy offspring, and also, to study the potential effects of different thiamine status and treatments on the activities of these enzymes.

2. Materials and methods

2.1. Fish material and thiamine treatment

The fish material used originated from sea-run ascending Baltic salmon of the River Dalälven population that were caught in July to August in

1996 by means of a fish trap. The broodfish were kept at the Broodfish Station of the National Board of Fisheries in Älvkarleby in large indoor pools with flowing river water until maturation. Approximately 3 weeks before maturation four randomly selected females, including one wiggling female predestined to produce M74- developing offspring (Amcoff et al., 1998a), were anaesthetized in MS222® (Sandoz Ltd., Basel, Switzerland; 5 min in 170 mg/l) and injected i.p. with a sterile solution of 100 mg thiamine hydrochloride (buffered to pH 6.9 with NaOH) per kg bodyweight. All fish were stripped of their eggs on 5th of November and females were measured of weight and length and their fecundity was evaluated. The thiamine injected females were dissected and their abdominal cavity was inspected for lesions caused by the injections. Milt from one sea-run male of River Daläven origin was added to each egg batch together with 0.5 l river water and left for 3 min for fertilization. Eggs were then rinsed in river water and thereafter water-hardened for 3 h in 5–6°C river water. After water-hardening, all egg batches were disinfected in a iodophore solution of Buffodine® (1%, v/v; Evans Vanodine International Ltd., Preston, UK) for 10 min after which the eggs were incubated in separate hatching trays with flowing river water. The water temperature varied between 0.1 and 6°C in November to hatching in late April and between 6 and 12°C in May during the yolk-sac fry development.

By means of prognostic hatching (i.e. hatching of 200 eggs at an elevated temperature, 7°C, to screen in advance for groups developing M74) four M74-prognosticated family groups (M74) and four healthy groups (controls) were selected. In addition offspring from four thiamine injected females (TI) were included in the study. Five days after hatching, at 32 posthatch degree-days (32 d°C), 50% of each group was immersed for a period of 2 h in an aerated recirculating thiamine solution (2.000 mg thiamine hydrochloride/l per 1.000 individuals) with the pH adjusted to 6.9 with NaOH. The treatment was repeated after an additional 10 days at 124 d°C. The immersion concentration was in agreement with previously performed studies and was estimated to counteract development of M74 (Amcoff et al., 1998b). The hatching trays were monitored daily for dead individuals that were removed. Two days after the onset of the first clinical signs of M74, at 182 d°C, 10 whole alive yolk-sac fry from each group were put in airtight zip-lock® bags, killed in liquid nitrogen and stored at −80°C for whole body thiamine analysis. For analysis of hepatic TK, α-KGDH, G6PDH and CYP1A-activities, a total of 15 yolk-sac fry from each group were measured of their length and placed in ice cold saline during dissection of their liver. The livers were pooled in threes in 300 μl sucrose 0.25 M and immediately homogenized (glass-Teflon homogenizer, # 18, Kontes, Vineland, NJ) at 0°C using 5 up-and-down strokes at 400 rpm and thereafter directly divided in aliquots to cryotubes and plunged into liquid nitrogen. For the enzymatic analysis the samples were stored at −120°C and rapidly thawed just before activity measurements.

2.2. Thiamine analysis

Total thiamine was analyzed based on the method by Roser et al. (1978) and performed as described by Amcoff et al. (1999a). Samples were extracted using acid hydrolysis at 121°C after which the extract was cooled to 23°C and the pH adjusted to 4.0. For enzymatic degradation and dephosphorylation of the thiamine phosphate esters a suspension of Taka-Diastase (Pfaltz and Bauer, Chemicon, Stockholm, Sweden) in water was added to the extract at a ratio of 0.1 g/g of sample and incubated at 45°C for 4 h. The extracted thiamine was converted to the fluorescent compound thiochrome using an automated pre-column derivation technique and analyzed with High-Pressure Liquid Chromatography (HPLC). The conversion efficiency of the Taka-Diastase preparation was checked by analysis of known amounts of thiamine and TDP added to the yolk-sac fry homogenate. All samples were analyzed in duplicates and presented on a wet weight basis (ww).

2.3. Enzyme assays

TK-activity was measured with a coupled spectrophotometric assay as essentially described by Smeets et al. (1971) and Tate and Nixon (1987). The incubation media consisted of 100 mM Tris–HCl buffer at pH 7.6, $MgCl_2$ 1.2 mM, D-xylulose 5-phosphate 0.8 mM, NADH 0.2 mM, 8 U/ml triosephosphate isomerase (EC 5.3.1.1) and 0.8 U/ml α-glycerophosphate dehydrogenase (EC 1.1.1.8) at 30°C. To obtain a stable background we allowed the sample to incubate for 5–10 min before start by adding of D-ribose 5-phosphate 10 mM. The results are presented in nmol NADH oxidized/min per liver.

The α-KGDH-activity was analyzed according to Heddi et al. (1993) with some modifications. The incubation media contained 50 mM KH_2PO_4/Na_2HPO_4 at pH 8.0, sucrose 200 mM, rotenone 0.012 mM, $MgCl_2$ 1 mM, HCl cysteine 2.5 mM, NAD^+ 1.25 mM and coenzyme A 0.1 mM at 10°C. Before initiating the enzymatic activity with 2 mM α-ketoglutarate and measurement of increase in optical density at 340 nm with a 4 nm wide band the sample was treated with 2% Triton X-100®. Treatment with 0.1–2% Triton X-100® for 2 min was found to significantly increase the activity while further increase of Triton X-100® concentration did not improve the activity. Comparison with the detergents CHAPS® or Lubrol PX® indicated that Triton X-100® was the best solubilizer of mitochondrial membrane for α-KGDH measurements. Treatment with detergent was performed at 20°C while the enzymatic activity was run at 10°C since higher temperatures indicated lower activities. The results are presented in nmol NADH formed/min per liver.

The G6PDH-activity was measured according to the method of Taketa and Watanabe (1971) with slight modifications, 50 mM Tris–Cl buffer at pH 7.7, $MgCl_2$ 20 mM, $NADP^+$ 0.5 mM, glucose-6-phosphate 1 mM, and the results are expressed as nmol NADPH formed/min per liver at 30°C.

Ethoxyresorufin *O*-deethylase (EROD) was analyzed according to the methods of Prough et al. (1978) at 22°C and results expressed as pmol resorufin formed/min per liver.

All enzyme assays were demonstrated to be linear with time and protein concentration under conditions used, and appropriate background and control incubations were performed. Measurements of different enzymatic activities were done in duplicates for each five pools. Figures are presented as means ± SD of the five pools, each pool consisting of livers from three yolk-sac fry. The values for these different samples agreed within 15%. All chemicals used were of p.a. quality and obtained from Sigma, St. Louis, US, if not stated otherwise.

2.4. Statistics

Effects of thiamine bathings on enzyme activities were statistically evaluated by using Student's *t*-test, after a normal distribution of data was confirmed using *Z*-score histograms. For testing of statistical differences in female biodata, mortality rates, thiamine concentrations and enzyme activities the M74 and TI groups were tested against the controls by using Student's *t*-test. In cases where data were not normally distributed, the non-parametric Mann–Whitney *U*-test was used. To compare whether the different enzyme activities corresponded with the thiamine concentrations and to compare the activities of the thiamine-dependent enzymes with the EROD and G6PDH-activities, simple regression analysis was applied. Data were converted by $\log_{10}$ transformation $[X' = \log(X+1)]$ and *P*-values were calculated using analysis of variance (ANOVA). In all testing the work of Zar (1984) was consulted and the statistics were calculated using the StatView 4.5 data analysis system (Abacus Concepts, Inc., Berkeley, CA). Figures were considered statistically different at a significance level of 0.95 and are presented as a *P*-value with the attached symbol; * $P < 0.05$.

3. Results

The progeny of the thiamine injected females developed into viable offspring including the offspring from the wiggling individual. Females giving rise to M74-developing yolk-sac fry weighed

significantly less and gave eggs with smaller diameter than control females ($P < 0.05$) (Table 1). The mean prevalence of spinal deformities were between 0.5 and 2.6% with a tendency toward higher frequencies in M74 groups. All family groups hatched during a period of 2–3 days and groups developing M74 displayed typical signs of M74 including irregular and upward swimming, convulsions, lethargy, white liver, darkening of the skin and exophthalmus, as described by Lundström et al. (1998). The thiamine immersions did not affect the length of yolk-sac fry at 182 d°C within the same family group.

3.1. Mortality rates

Mean cumulative mortalities at swim-up in unbathed and thiamine bathed TI and control groups ranged between 3.4 and 5.5% (Table 1). Two of the untreated M74 groups showed partial M74-development with 66 and 77% mortality, respectively, while the other two groups died before 220 d°C. The thiamine treatments significantly reduced ($P < 0.05$) mean mortality in M74 groups from 86 to 13% even if M74 groups still had significantly higher ($P < 0.05$) mortality rates than controls and TI groups.

3.2. Thiamine concentrations

The recovery of added thiamine and thiamine diphosphate was found to be between 95 and 98% in yolk-sac fry homogenate. The thiamine concentrations in the M74 groups ranged between 0.20 and 0.33 nmol/g and mean thiamine content was 0.25 nmol/g, which was below the previously suggested threshold limit for development of M74 of 0.34 nmol/g (Amcoff et al. 1998b) and about half of that in the controls ($P < 0.05$) and 1/22 of that in the TI groups ($P < 0.05$; Table 2). The immersions in thiamine elevated the thiamine concentrations of the M74 and control groups by an average of 1.0 nmol/g and the TI groups with 0.7 nmol/g, respectively.

Table 1
Female Baltic salmon (*S. salar*) biodata, yolk-sac fry deformities and cumulative mortality in groups developing M74 ($n = 4$), groups from thiamine injected (TI) females ($n = 4$) and in healthy controls ($n = 4$)[a]

	Thiamine immersions[b]	M74	TI[c]	Controls
Female biodata				
Weight (kg)	—	4.8 ± 1.3*	7.6 ± 3.4	7.4 ± 0.42
Condition factor[d]	—	1.0 ± 0.11	1.0 ± 0.10	1.1 ± 0.081
Fecundity[e]	—	1600 ± 96	1600 ± 190	1500 ± 120
Egg diameter (cm)	—	0.56 ± 0.02*	0.61 ± 0.04	0.61 ± 0.01
Spinal deformities (%)				
At swim-up	No	2.2 ± 1.7	0.5 ± 0.5	1.5 ± 0.9
At swim-up	Yes	2.6 ± 2.0	0.9 ± 0.5	1.0 ± 0.4
Cumulative mortality (%)				
Fertilization to eyed egg	—	1.9 ± 0.9	3.1 ± 3.1	0.9 ± 0.5
Eyed egg to swim-up	No	86 ± 17*	5.5 ± 2.1	3.9 ± 2.3
Eyed egg to swim-up	Yes	13 ± 3.8*	4.5 ± 3.0	3.4 ± 1.8

[a] Figures are means ± S.D. To test for statistical differences M74 and TI groups were tested against controls by using Student's *t*-test.

[b] Immersed in 2.000 mg thiamine hydrochloride/1.000 yolk-sac fry/l for a period of 2 h at 32 and 124 d°C.

[c] Females injected i.p. approximately 3 weeks before maturation with 100 mg thiamine hydrochloride (buffered to pH 6.9 with NaOH) per kg body weight.

[d] (Body weight (g))/(length (cm^3))*100.

[e] Mean number of eggs per kg body weight.

* $P < 0.05$.

Table 2
Thiamine concentrations (nmol/g; ww) and hepatic activities of transketolase (TK), α-ketoglutarate dehydrogenase (α-KGDH), glucose-6-phosphate dehydrogenase (G6PDH), the ratio [TK/G6PDH] and cytochrome P4501A-activity (EROD) in Baltic salmon (*S. salar*) yolk-sac fry sampled 15 days after hatching at 182 d°C[a]

Analyzed parameter	Thiamine bath[b]	M74 ($n=4$)	TI[c] ($n=4$)	Controls ($n=4$)
Thiamine (nmol/g)	No	0.25 ± 0.043*	5.5 ± 2.0*	0.49 ± 0.15
Thiamine (nmol/g)	Yes	1.3 ± 0.11	6.2 ± 1.7*	1.5 ± 0.26
TK (nmol/min per liver)	No	0.42 ± 0.13*	3.4 ± 0.47*	1.8 ± 0.96
TK (nmol/min per liver)	Yes	2.4 ± 0.13	3.7 ± 0.81	3.6 ± 1.1
α-KGDH (nmol/min per liver)	No	0.0008 ± 0.002[d,*]	0.26 ± 0.05*	0.11 ± 0.04
α-KGDH (nmol/min per liver)	Yes	0.12 ± 0.03	0.21 ± 0.04	0.17 ± 0.04
G6PDH (nmol/min per liver)	No	4.8 ± 1.6	4.3 ± 0.75*	5.8 ± 0.79
G6PDH (nmol/min per liver)	Yes	3.6 ± 0.57*	5.0 ± 1.5	5.6 ± 0.77
[TK/G6PDH]	No	0.09 ± 0.02*	0.80 ± 0.04*	0.33 ± 0.18
[TK/G6PDH]	Yes	0.67 ± 0.12	0.74 ± 0.04	0.64 ± 0.16
EROD (pmol/min per liver)	No	17 ± 2.6*	42 ± 15*	80 ± 14
EROD (pmol/min per liver)	Yes	28 ± 12	54 ± 30	59 ± 26

[a] Figures are means ± S.D.; four family groups for each category. To test for statistical differences M74 and thiamine injected (TI) groups were tested against controls by using Student's *t*-test.
[b] Immersed in 2.000 mg thiamine hydrochloride/1.000 yolk-sac fry/l for a period of 2 h at 32 and 124 d°C.
[c] Females injected i.p. approx. 3 weeks before maturation with 100 mg thiamine hydrochloride (buffered to pH 6.9 with NaOH) per kg body weight.
[d] The obtained low mean value reflect a number of animals analyzed being below the detection limit, see Table 3.
* $P<0.05$.

3.3. Enzyme activities

All enzyme activities, except for G6PDH in the M74 groups, were statistically different when comparing M74 and TI groups with the controls (Fig. 1a). Untreated M74-developing family groups had significantly lower activities of TK and α-KGDH ($P<0.05$) compared to controls (Fig. 1a; Table 2), this while the unbathed TI groups were significantly elevated ($P<0.05$). The unbathed TI groups had significantly reduced ($P<0.05$) activities of G6PDH compared to controls. The ratio [TK/G6PDH] was significantly lower ($P<0.05$) in M74 groups than in TI and control groups mainly due to the low TK-activities. The TI group had a [TK/G6PDH] ratio value of 0.80, which was significantly higher ($P<0.05$) than in the controls (0.33). The EROD-activities in unbathed M74 and TI groups were both significantly lower ($P<0.05$) than in controls.

The thiamine immersions after hatching resulted in normalized enzyme activities that were non-significantly different from the controls with the exception of G6PDH in M74 groups ($P>0.05$) (Fig. 1b). All control and M74 family groups significantly elevated ($P>0.05$) their TK-activities after immersions in thiamine (Tables 3 and 4), as did the α-KGDH-activities of all M74 family groups and in two of the control groups. Three of the four M74 groups showed significantly lower ($P>0.05$) G6PDH-activities after immersions than controls indicating a lower activity of the pentose-phosphate shunt. The equilibrium ratio [TK/G6PDH] for all thiamine immersed M74 and control groups were significantly elevated ($P<0.05$) after immersions in thiamine indicating a restoration of the TK-activities. The study indicates a potential thiamine threshold level for saturation of the TK and α-KGDH enzymes of below 1.3 nmol/g, since groups with less thiamine content had reduced TK and α-KGDH-activities. In the TI groups only one group (TI # 4) showed significantly higher activities ($P>0.05$) of TK and G6PDH after thiamine bathings (Table 5).

In unimmersed family groups ($n=12$) the TK and α-KGDH-activities were found to be significantly ($P<0.05$) correlated with the thiamine

content when plotted and subjected to regression analysis (regression equation for TK: $Y = -0.22 + 0.54x$; $R^2 = 0.64$; and for α-KGDH: $Y = -0.01 + 0.11x$; $R^2 = 0.75$).

EROD-activities after thiamine immersions showed a variable pattern between the groups with significantly increased ($P < 0.05$) activities in three M74 and two TI groups. This while the controls demonstrated significant reductions ($P < 0.05$) of EROD-activities in three family groups. In thiamine immersed groups ($n = 12$) the EROD-activities correlated significantly ($P < 0.05$) with TK and G6PDH-activities when subjected to regression analysis (regression equations for TK: $Y = 0.59 + 1.7x$; $R^2 = 0.55$; and for G6PDH: $Y = 0.29 + 1.8x$; $R^2 = 0.62$). In addition, the TK-activities in the immersed groups showed a significant ($P < 0.05$) correlation with G6PDH-activities (regression equation: $Y = 0.041 + 0.76$; $R^2 = 0.60$).

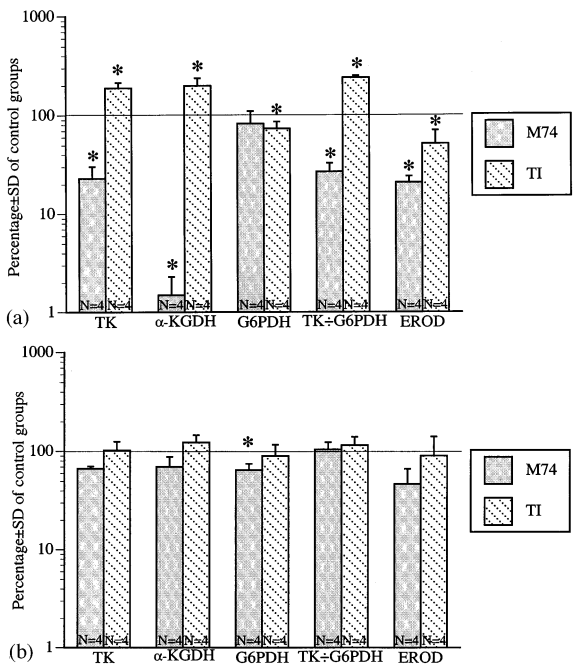

Fig. 1. Hepatic activities of transketolase (TK), α-ketoglutarate dehydrogenase (α-KGDH), glucose-6-phosphate dehydrogenase (G6PDH), the ratio [TK ÷ G6PDH] and EROD in unbathed (a) and thiamine bathed (b) Baltic salmon (*S. salar*) yolk-sac fry with M74 development ($n = 4$) or from thiamine injected (TI) females ($n = 4$) as percentage of healthy controls ($n = 4$). Figures are means ± S.D. Statistical differences between controls and the M74 and TI groups were tested using Student's *t*-test. The significance level is described as a *P*-value with the attached symbol; * $P < 0.05$.

4. Discussion

This study provides new data demonstrating that M74-affected Baltic salmon yolk-sac fry show reduced activities of the thiamine-dependent enzymes TK and α-KGDH. Furthermore, healthy developing offspring had significantly lower activities of these enzymes compared with the thiamine injected groups. The similarly low thiamine threshold limit interval in yolk-sac fry for development of M74, found in this (0.20–0.33 nmol/g) and in previous studies (0.31–0.47 nmol/g; Amcoff et al., 1998a,b) indicates a concentration range of thiamine where activities of the thiamine-dependent enzymes may be severely constrained. We suggest that the physiological disturbances observed in M74-affected yolk-sac fry may be implemented by the disturbed thiamine-dependent enzyme activities.

Thiamine deficiency can be monitored by assaying TK-activity in a subcellular fraction, with or without preincubation with TDP, in fish as well as in mammals (Smeets et al., 1971; Masumoto et al., 1987). The rise in TK-activity after adding TDP in vitro reflects the thiamine status of the animal, the so-called TPP-effect (thiamine pyrophosphate). The additional information obtained by preincubation with TDP may be of limited value with fish, since previous investigations have not shown increased sensitivity or relevance with such a procedure (Brin et al., 1960; Morito et al., 1986). One reason that the TPP-effect might not be convenient to monitor when assaying thiamine-dependent enzymes may be the relatively long preincubations necessary to reconstitute the active holoenzyme (Tate and Nixon, 1987). In addition, another uncertainty with this preincubation technique is that under certain in vitro conditions the apoenzyme may not bind TDP. Since we have obtained results suggesting a close connection between thiamine status and enzymatic activity of TK and α-KGDH in Baltic salmon yolk-sac fry without studying the TPP-effect, we suggest that this is satisfactory.

Table 3
Four family groups of M74-developing Baltic salmon (*S. salar*) yolk-sac fry after thiamine immersions[a]

Group	Thiamine immersions[b]	TK ($n=5$)	α-KGDH ($n=5$)	G6PDH ($n=5$)	EROD ($n=5$)	[TK/6PDH] ($n=5$)
M74 # 1	No	0.59 ± 0.092	0.003 ± 0.007	6.6 ± 0.76	20 ± 4.2	0.09 ± 0.014
M74 # 1	Yes	2.3 ± 0.26*	0.10 ± 0.076*	4.6 ± 0.57*	46 ± 6.4*	0.50 ± 0.071*
M74 # 2P	No	0.45 ± 0.33	0[c]	4.3 ± 0.40	16 ± 2.2	0.10 ± 0.08
M74 # 2P	Yes	2.3 ± 0.073*	0.15 ± 0.057*	3.3 ± 0.21*	24 ± 3.4*	0.70 ± 0.075*
M74 # 3	No	0.34 ± 0.10	0[c]	5.5 ± 0.74	14 ± 3.7	0.062 ± 0.019
M74 # 3	Yes	2.6 ± 0.43*	0.15 ± 0.018*	3.8 ± 0.40*	25 ± 7.0*	0.68 ± 0.12*
M74 # 4P	No	0.30 ± 1.2	0[c]	2.9 ± 0.42	16 ± 3.4	0.10 ± 0.064
M74 # 4P	Yes	2.2 ± 0.22*	0.097 ± 0.063*	2.8 ± 0.37	20 ± 2.2	0.79 ± 0.075*

[a] Two family groups showed partial (P) development of M74. Hepatic activities (nmol/min per liver) of transketolase (TK), α-ketoglutarate dehydrogenase (α-KGDH), glucose-6-phosphate dehydrogenase (G6PDH), cytochrome P4501A (EROD; pmol/min per liver) and the ratio [TK/G6PDH] 15 days after hatching at 182 d°C. Figures are means ± S.D.; $n=5$. Groups immersed in thiamine and unimmersed groups were statistically compared by using Student's t-test with the significance level 0.95 (* $P<0.05$).
[b] Immersed in 2.000 mg thiamine hydrochloride/1.000 yolk-sac fry/l for a period of 2 h at 32 and 124 d°C.
[c] Below detection limit.

The fact that M74 offspring had smaller eggs than controls in this study did not seem to affect the obtained results.

The stored glycogen depots in yolk-sac fry constitute one important source of glucose for the glycolytic pathway and the PPS. Low glycogen levels in M74-affected yolk-sac fry (Norrgren et al., 1993; Lundström et al., 1999b) and in yolk-sac fry with experimentally induced thiamine deficiency (Amcoff et al., 1999b) indicate a stressed carbohydrate metabolism. The PPS is divided into an oxidative and a nonoxidative part where the former mainly generates NADPH and the latter R5P. NADPH is necessary in de novo lipogenesis and as a reducing equivalent for several biotransformation systems e.g. CYP, while the R5P is required in synthesis of nucleotides (Segner and Böhm, 1994; Halliwell and Gutteridge, 1996). Disorders in lipid composition have been observed among Baltic M74 salmon (Pickova et al., 1998). TK is situated to the nonoxidative part, however, reduced TK-activities in experimentally induced thiamine deficiency may also reduce production of NADPH and R5P probably due to a blocked recycling of R5P from the nonoxidative part of the PPS into glucose-6-phosphate (Boros et al., 1997). The rate limiting, and irreversible step, of the oxidative part of the PPS is the G6PDH the rate of which is controlled by the ratio [NADPH/$NADP^+$], while the nonoxidative part of the PPS is mainly regulated by the availability of D-ribulose 5-phosphate (Sabate et al., 1995).

In this study, yolk-sac fry from thiamine injected females were considerably higher in thiamine than M74 and controls which was reflected in their higher TK-activities. The thiamine immersions caused six and two-fold mean elevations of the TK-activities in the M74 (2.4 nmol/min per liver) and control (3.6 nmol/min per liver) groups, respectively. This while only one TI group displayed increased TK-activities after immersions indicating an already sufficient content of thiamine with TK-activities probably being close to saturation.

The positive response to the thiamine immersions of the healthy developing controls, regard-

ing the TK and α-KGDH-activities, suggest that they are affected by a moderate thiamine deficiency (sublethal M74). Whether adult wild Baltic salmon also have unsaturated activities of thiamine-dependent enzymes remains to be seen. The results from this study give at hand a thiamine threshold limit interval in yolk-sac fry of 1.3–1.5 nmol thiamine/g for full activity of TK. This is considerably higher than the previously suggested thiamine threshold limit intervals (Amcoff et al., 1998a,b) for development of M74 in yolk-sac fry denoting a wide range from 0.3–1.3 nmol thiamine/g where activities of thiamine-dependent enzymes may be negatively affected. This is of great importance when therapeutic treatments of Baltic salmon with thiamine are used in Swedish compensatory rearing plants.

The α-KGDH-activities of M74 groups were around and below the detection limit of the enzymatic assay used and responded to the thiamine bathings with elevated activities being close to those of the untreated controls. As with the TK-activity, the α-KGDH-activities were also improved in the controls after thiamine immersions, however not to the same degree as for TK. Several studies have indicated that reductions in α-KGDH-activity caused by thiamine deficiency are more readily observed than decreases in activity of the thiamine-dependent PDH. The α-KGDH demonstrates decreased activity in presymptomatic animals, while decreases of the PDH-activity develop in parallel with, or after, manifestations of overt symptoms of thiamine deficiency (Gibson et al., 1984; Butterworth et al., 1985, 1986).

The ratio [TK/G6PDH], which gives an impression of the equilibrium level between the oxidative and the nonoxidative parts of the PPS, was considerably lower in M74 (0.09) and control groups (0.33) than in the TI groups (0.80). By immersions in thiamine the ratio was adjusted to levels comparable for all groups further indicating that both M74 and healthy Baltic salmon yolk-sac fry may suffer from a poorly regulated nonoxidative part of the PPS.

The lower EROD-activities for M74 yolk-sac fry obtained in this and in other studies performed indicate a possible link between thiamine deficiency and the CYP1A system (Amcoff et al., 1999b; Börjeson et al., 1999; Lundström et al., 1999a). Low activities of TK may constrain the intracellular availability of NADPH and R5P, possibly resulting in reduced CYP1A-activities in M74 groups. However, there are other sources e.g. the malate dehydrogenase that may provide intracellular NADPH for CYP1A (Baldwin and Reed, 1976). The thiamine antagonist oxythiamine has been used as an inhibitor of the nonox-

Table 4
Four family groups of controls (healthy) Baltic salmon (*S. salar*) yolk-sac fry after thiamine immersions[a]

Group	Thiamine immersions[b]	TK ($n=5$)	α-KGDH ($n=5$)	G6PDH ($n=5$)	EROD ($n=5$)	[TK/G6PDH] ($n=5$)
Control #1	No	1.3 ± 0.11	0.17 ± 0.024	5.3 ± 0.54	72 ± 7.5	0.25 ± 0.035
Control #1	Yes	2.1 ± 0.58*	0.21 ± 0.054*	5.1 ± 0.72	40 ± 10*	0.41 ± 0.086*
Control #2	No	0.84 ± 0.22	0.082 ± 0.049	6.9 ± 0.54	66 ± 6.0	0.12 ± 0.04
Control #2	Yes	4.0 ± 0.53*	0.18 ± 0.047*	6.4 ± 0.41	33 ± 3.8*	0.62 ± 0.074*
Control #3	No	3.0 ± 0.16	0.10 ± 0.042	5.6 ± 0.76	93 ± 3.5	0.54 ± 0.05
Control #3	Yes	3.6 ± 0.35*	0.12 ± 0.065	4.8 ± 0.36	76 ± 6.8*	0.75 ± 0.09*
Control #4	No	2.2 ± 0.39	0.17 ± 0.048	5.3 ± 0.63	90 ± 14	0.41 ± 0.13
Control #4	Yes	4.7 ± 0.33*	0.18 ± 0.022	6.1 ± 0.63	86 ± 12	0.77 ± 0.076*

[a] Hepatic activities (nmol/min per liver) of transketolase (TK), α-ketoglutarate dehydrogenase (α-KGDH), glucose-6-phosphate dehydrogenase (G6PDH), cytochrome P4501A (EROD; pmol/min per liver) and the ratio [TK/G6PDH] 15 days after hatching at 182 d°C. Figures are means ± S.D.; $n=5$. Groups immersed in thiamine and unimmersed groups were statistically compared by using Student's t-test with the significance level 0.95 (* $P<0.05$).

[b] Immersed in 2.000 mg thiamine hydrochloride/1.000 yolk-sac fry/l for a period of 2 h at 32 and 124 d°C.

Table 5
Four family groups of Baltic salmon (*S. salar*) yolk-sac fry from thiamine injected (TI) females (100 mg thiamine hydrochloride/kg) after thiamine immersions[a]

Group	Thiamine immersions[b]	TK ($n=5$)	α-KGDH ($n=5$)	G6PDH ($n=5$)	EROD ($n=5$)	[TK/G6PDH] ($n=5$)
TI # 1 (W)	No	2.9 ± 0.37	0.20 ± 0.071	3.4 ± 0.42	22 ± 2.3	0.85 ± 0.062
TI # 1 (W)	Yes	3.0 ± 0.53	0.15 ± 0.027	3.9 ± 0.17	33 ± 5.6*	0.77 ± 0.16
TI # 2	No	3.4 ± 0.22	0.24 ± 0.049	4.5 ± 0.40	50 ± 9.5	0.76 ± 0.12
TI # 2	Yes	3.4 ± 0.50	0.20 ± 0.091	4.5 ± 0.35	42 ± 12	0.76 ± 0.12
TI # 3	No	3.4 ± 0.32	0.33 ± 0.051	4.1 ± 0.55	41 ± 6.3	0.83 ± 0.11
TI # 3	Yes	3.5 ± 0.21	0.24 ± 0.069	4.6 ± 0.26	43 ± 2.8	0.76 ± 0.022
TI # 4	No	4.0 ± 0.32	0.26 ± 0.068	5.2 ± 0.53	56 ± 8.1	0.77 ± 0.065
TI # 4	Yes	4.8 ± 0.23*	0.25 ± 0.025	7.2 ± 0.26*	99 ± 3.4*	0.67 ± 0.045

[a] One female displayed wiggling behavior (W) prior to injection. Hepatic activities (nmol/min per liver) of transketolase (TK), α-ketoglutarate dehydrogenase (α-KGDH), glucose-6-phosphate dehydrogenase (G6PDH), cytochrome P4501A (EROD; pmol/min per liver) and the ratio [TK/G6PDH] 15 days after hatching at 182 d°C. Figures are means ± S.D.; $n=5$. Groups immersed in thiamine and unimmersed groups were statistically compared by using Student's t-test with the significance level 0.95. (* $P<0.05$). Females injected i.p. approx. 3 weeks before maturation with 100 mg thiamine hydrochloride (buffered to pH 6.9 with NaOH) per kg body weight..

[b] Immersed in 2.000 mg thiamine hydrochloride/1.000 yolk-sac fry/l for a period of 2 h at 32 and 124 d°C.

idative PPS-synthesis of R5P in cancer therapy showing that the thiamine status may play a crucial role in protein synthesis (Boros et al., 1997).

Thiamine deficiencies affecting young life stages of fish are not a local Baltic Sea problem since similar problems are also frequently occurring in the Laurentian Great Lakes region in North America where Atlantic salmon (*S. salar*) suffer from the Cayuga Syndrome (CS) and several other salmonid species are affected by the Early Mortality Syndrome (EMS) (Fisher et al., 1995; Marcquenski and Brown, 1997). Offspring developing these syndromes are treatable with thiamine to gain full survival (Fitzsimons, 1995; Fisher et al., 1996). Fisher et al. (1996) and Ji and Adelman (1998) have pointed at that monotonous feeding on thiaminase-rich alewife (*Alosa pseudoharengus*) may be involved in the etiology of CS and EMS.

The findings of this study emphasize the importance of knowing the nutritional status of fish material in experimental and monitoring research when analyzing for hepatic activities of the thiamine-dependent enzymes TK and α-KGDH and the activities of G6PDH and CYP1A. The positive response of thiamine treatments on activities of the thiamine-dependent enzymes in controls indicates that also seemingly healthy developing Baltic salmon yolk-sac fry that contain moderate thiamine levels may have restrained TK and α-KGDH-activities. The results indicate that administration of thiamine to sea-run ascending female Baltic salmon by injection may be a more appropriate method than bathing to counteract potential M74 development.

Acknowledgements

This investigation was in part supported by an EC-grant, ENV4-CT97-0468 and Tryggers foundation. The Oscar and Lili Lamm's Memorial Foundation, the Hierta–Retzius Foundation at the Royal Swedish Academy of Sciences and the Helge Ax:son Johnsons Foundation are also gratefully acknowledged for financial support. Also the staff at the Broodfish Station of the National Board of Fisheries in Älvkarleby are gratefully acknowledged for assistance in the selection of fish material.

References

Amcoff, P., Börjeson, H., Lindeberg, J., Norrgren, L., 1998a. Thiamine (vitamin B1) concentrations in feral Baltic salmon exhibiting the M74 syndrome. In: McDonald, G., Fitzsimons, J.D., Honeyfield, D.C. (Eds.), Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and Baltic Sea. Proceedings of the 126th Annual Meeting of the American Fisheries Society, August 25–29. Am. Fish. Soc. Symp. 21, Dearborn, Michigan, pp. 82–89.

Amcoff, P., Börjeson, H., Eriksson, R., Norrgren, L., 1998b. Effects of thiamine (vitamin B1) treatments on survival of M74-affected feral Baltic salmon. In: McDonald, G., Fitzsimons, J.D., Honeyfield, D.C. (Eds.), Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and Baltic Sea. Proceedings of the 126th Annual Meeting of the American Fisheries Society, August 25–29. Am. Fish. Soc. Symp. 21, Dearborn, Michigan, pp. 31–40.

Amcoff, P., Börjeson, H., Landergren, P., Vallin, L., Norrgren, L., 1999a. Thiamine (vitamin B1) concentrations in salmon (*Salmo salar*), brown trout (*Salmo trutta*) and cod (*Gadus morhua*) from the Baltic Sea. Ambio 28, 48–54.

Amcoff, P., Lundström, J., Teimert, L., Börjeson, H., Norrgren, L., 1999b. Physiological and morphological effect of microinjection of oxythiamine and PCBs in embryos of Baltic salmon (*Salmo salar*): a comparison with the M74 syndrome. Ambio 28, 55–66.

Åkerman, G., Balk, L., 1998. Descriptive studies of mortality and morphological disorders in early life stages of cod and salmon originating from the Baltic Sea. In: McDonald, G., Fitzsimons, J.D., Honeyfield, D.C. (Eds.), Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and Baltic Sea. Proceedings of the 126th Annual Meeting of the American Fisheries Society, August 25–29. Am. Fish. Soc. Symp. 21, Dearborn, Michigan, pp. 41–61.

Åkerman, G., Tjärnlund, U., Noaksson, E., Balk, L., 1998a. Evidence for anthropogenic substances causing thiamine deficiency in fish larvae when using model substances. Abstract from the 8th Annual Meeting of SETAC-Europe Congress, Bordeaux, France. April 14-18, 265.

Åkerman, G., Tjärnlund, U., Noaksson, E., Balk, L., 1998b. Studies with oxythiamine to mimic reproduction disorders among fish early life stages. Mar. Environ. Res. 46 (1–5), 493–497.

Baldwin, J., Reed, K.C., 1976. Cytoplasmic sources of NADPH for fat synthesis in rainbow trout liver: effect of thermal acclimation on enzyme activities. Comp. Biochem. Physiol. 54B, 527–529.

Boros, L.G., Puigjaner, J., Cascante, M., Lee, W.-N.P., Brandes, J.L., Bassilian, S., Yusuf, F.I., Williams, R.D., Muscarella, P., Melwin, W.S., Schirmer, W.J., 1997. Oxythiamine and dehydroepiandrosterone inhibit the nonoxidative synthesis of ribose and tumor cell proliferation. Cancer Res. 57, 4242–4248.

Brin, M., Tai, M., Ostashever, A.S., Kalinsky, H., 1960. The effect of thiamine deficiency on the activity of erythrocyte hemolysate transketolase. J. Nutr. 71, 273–281.

Butterworth, R.F., Giguère, J.-F., Besnard, A.-M., 1985. Activities of thiamine dependent enzymes in two experimental models of thiamine-deficiency encephalopathy. 1. The pyruvate dehydrogenase complex. Neurochem. Res. 10, 1417–1428.

Butterworth, R.F., Giguère, J.-F., Besnard, A.-M., 1986. Activities of thiamine dependent enzymes in two experimental models of thiamine-deficiency encephalopathy. 2. α-ketoglutarate dehydrogenase complex. Neurochem. Res. 11, 567–577.

Börjeson, H., Norrgren, L., 1997. M74 syndrome: a review of potential etiological factors. In: Rolland, R.M., Gilbertson, M., Peterson, R.E. (Eds.), Chemically Induced Alterations in Functional Development and Reproduction of Fishes. SETAC Press, Pensacola, FL, pp. 153–166.

Börjeson, H., Amcoff, P., Ragnarsson, B., Norrgren, L., 1999. Reconditioning of sea-run Baltic salmon (*Salmo salar*) that have produced progeny with the M74 syndrome. Ambio 28, 30–36.

Fisher, J.P., Spitsbergen, J.M., Getchell, R., Symula, J., Skea, J., Babenzein, M., Chiotti, T., 1995. Reproductive failure of landlocked Atlantic salmon from New York's Finger Lakes: investigations into the etiology and epidemiology of the 'Cayuga Syndrome'. J. Aquat. Anim. Health 7 (2), 81–94.

Fisher, J.P., Fitzsimons, J.D., Combs, G.F., Jr., Spitsbergen, J.M., 1996. Naturally occurring thiamine deficiency causing reproductive failure in Finger Lakes Atlantic salmon and Great Lakes lake trout. Trans. Am. Fish. Soc. 125 (2), 167–178.

Fitzsimons, J.D., 1995. The effect of B-vitamins on a swim-up syndrome in Lake Ontario lake trout. J. Great Lakes Res. 21 (1), 286–289.

Gibson, G.E., Ksiezak-Reding, H., Sheu, K.-F.R., Mykytyn, V., Blass, J.P., 1984. Correlation of enzymatic, metabolic, and behavioral deficits in thiamin deficiency and its reversal. Neurochem. Res. 9 (6), 803–814.

Halliwell, B., Gutteridge, J.M.C., 1996. Free Radicals in Biology and Medicine. Oxford University Press, Oxford.

Heddi, A., Lefebvre, F., Nardon, P., 1993. Effect of endocytobiotic bacteria on mitochondrial enzymatic activities in the Weevil *Sitophilus oryzae* (*Coleoptera*: *Curculionidae*). Insect Biochem. Mol. Biol. 23 (3), 403–411.

Ji, Y.Q., Adelman, I.R., 1998. Thiaminase activity in alewifes and smelt in Lakes Huron, Michigan and Superior. In: McDonald, G., Fitzsimons, J.D., Honeyfield, D.C. (Eds.), Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and Baltic Sea. Proceedings of the 126th Annual Meeting of the American Fisheries Society, August 25–29. Am. Fish. Soc. Symp. 21, Dearborn, Michigan, pp. 154–159.

Karlström, Ö., 1999. Development of the M74 syndrome in wild populations of Baltic salmon (*Salmo salar*) in Swedish rivers. Ambio 28, 82–86.

Lehmitz, R., Spannhof, L., 1977. Transketolase activity and thiamine deficiency in the kidney of rainbow trout (*Salmo gairdneri*) fed crude herring (in German with English abstract). Arch. Tierernae. 27 (4), 287–295.

Lundström, J., Börjeson, H., Norrgren, L., 1998. Clinical and pathological studies of Baltic salmon suffering from yolk sac fry mortality. In: McDonald, G., Fitzsimons, J.D., Honeyfield, D.C. (Eds.), Early Life Stage Mortality Syndrome in Fishes of the Great Lakes and Baltic Sea. Proceedings of the 126th Annual Meeting of the American Fisheries Society, August 25–29. Am. Fish. Soc. Symp. 21, Dearborn, Michigan, pp. 62–72.

Lundström, J., Carney, B., Amcoff, P., Pettersson, A., Börjeson, H., Förlin, L., Norrgren, L., 1999a. Antioxidative systems, detoxifying enzymes and thiamine levels in Baltic salmon (*Salmo salar*) that develop M74. Ambio 28, 24–29.

Lundström, J., Börjeson, H., Norrgren, L., 1999b. Histopathological studies of yolk-sac fry of Baltic salmon (*Salmo salar*) with the M74 syndrome. Ambio 28, 16–23.

Marcquenski, S.V., Brown, S.B., 1997. Early mortality syndrome in salmonid fishes from the Great Lakes. In: Rolland, R.M., Gilbertson, M., Peterson, R.E. (Eds.), Chemically Induced Alterations in Functional Development and Reproduction of Fishes. SETAC Press, Pensacola, FL, pp. 135–152.

Masumoto, T., Hardy, R.W., Casillas, E., 1987. Comparison of transketolase activity and thiamin pyrophosphate levels in erythrocytes and liver of rainbow trout (*Salmo gairdneri*) as indicators of thiamin status. J. Nutr. 117, 1422–1426.

Morito, C.L.H., Conrad, D.H., Hilton, J.W., 1986. The thiamin deficiency signs and requirement of rainbow trout (*Salmo gairdneri*, Richardson). Fish Physiol. Biochem. 1 (2), 93–104.

Norrgren, L., Andersson, T., Bergqvist, P.-A., Björklund, I., 1993. Chemical, physiological and morphological studies of feral Baltic salmon (*Salmo salar*) suffering from abnormal fry mortality. Environ. Toxicol. Chem. 12, 2065–2075.

Pickova, J., Kiessling, A., Pettersson, A., Dutta, P.C., 1998. Comparison of fatty acid composition and astaxanthin content in healthy and by M74 affected salmon eggs from three Swedish river stocks. Comp. Biochem. Physiol. B120, 265–271.

Prough, R.A., Burke, M.D., Mayer, R.T., 1978. Direct fluorometric methods for measuring mixed-function oxidase activity. In: Fleischer, S., Packer, L. (Eds.), Methods in Enzymology, LII: C. Academic Press, New York, pp. 372–377.

Roser, L., Andrist, A.H., Harrington, W.H., Naito, H.K., Lonsdale, D., 1978. Determination of urinary thiamine by high-pressure liquid chromatography utilizing the thiochrome fluorescent method. J. Chromat. 146, 43–53.

Sabate, L., Franco, R., Canela, E.I., Centelles, J.J., Cascante, M., 1995. A model of the pentose phosphate pathway in rat liver cells. Mol. Cell Biochem. 142, 9–17.

Segner, H., Böhm, R., 1994. Enzymes of lipogenesis. In: Hochachka, P.W., Mommsen, T.P. (Eds.), Biochemistry and Molecular Biology of Fishes. Exeter, Devon, pp. 313–325.

Smeets, E.H.J., Muller, H., de Wael, J., 1971. A NADH-dependent transketolase assay in erythrocyte hemolysates. Clin. Chim. Acta 33, 379–386.

Stryer, L., 1988. Biochemistry. Freeman, New York.

Taketa, K., Watanabe, A., 1971. Interconvertible microheterogeneity of glucose-6-phosphate dehydrogenase in rat liver. Biochem. Biophys. Acta 235, 19–26.

Tate, J.R., Nixon, P.F., 1987. Measurement of Michaelis constant for human erythrocyte transketolase and thiamin diphosphate. Anal. Biochem. 160, 78–87.

Zar, J.H., 1984. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, New Jersey.