www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 15: 39-48 (2015) DOI: 10.4194/1303-2712-v15_1_05

PROOF

Metabolic Potential, Respiration Rate and Their Relationship in Offspring of Different Sizes of Marble Trout (*Salmo marmoratus* Cuvier)

Tatjana Simčič[1],*, Dušan Jesenšek[2], Anton Brancelj[1]

[1] National Institute of Biology, Ljubljana, Slovenia.
[2] Tolmin Angling Association, Tolmin, Slovenia.

* Corresponding Author: Tel.: +386 59 232 738 ; Fax: +386 1 24 129 80 ;
E-mail: tatjana.simcic@nib.si

Received 07 April 2014
Accepted 26 February 2015

Abstract

The size and composition of fish eggs are related to female's characteristics, such as age, size and individual conditions, and they have an impact on the properties of offspring that are important for their fitness. Electron transport system (ETS) activity and respiration rate (R) of early life history stages (i.e. non-fertilized eggs, eggs at eyed stage and larvae with yolk sac) of 13 females of marble trout (*Salmo marmoratus*) were measured separately in order to determine their metabolic properties in relation to size. The results showed that larger females produced larger eggs in higher numbers. Growth experiments on the survival of offspring of a single female revealed that the survival rate of early embryos was higher for smaller eggs during the earliest stages, but ultimately the percentage of surviving larvae did not correlate with egg size. The ETS activities and respiration rates of non-fertilized eggs, eyed eggs and larvae differed significantly between 13 females. Both parameters increased with increasing dry mass of the early life history stages, but the increase of respiration rate was greater than that of ETS activity. The lower ETS/R ratios in larger individuals therefore indicate that their energy metabolism was less adaptable to environmental changes than that of smaller ones. Larger egg size could be an advantage under favourable conditions, whereas smaller size could be optimal under stressing circumstances in which the higher metabolic potential enables production of the energy required for metabolism. This is first report on the relationship between ETS activity and respiration rate of the early life history stages in salmonids.

Keywords: Fish, ETS activity, ETS/R ratio, fitness, metabolism

Introduction

Fish eggs have wide fluctuations in size and composition that can be of great importance for the fish production in hatchery and the success of fish populations in their natural environments. Several energy-related mechanisms can influence the amount of energy allocated to gonads during a reproductive cycle and thus the number and size or quality of eggs that are spawned (Van Winkle *et al.*, 1997). Most studies addressing maternal provisioning and egg quality in fish consider various measures of egg and alevin size, such as diameter, wet mass, dry mass (e.g., Hutchings, 1991; Lobon-Cervia *et al.*, 1997; Olofsson and Mosegaard, 1999; Berg *et al.*, 2001; Hendry *et al.*, 2001; Heinimaa and Heinimaa, 2004), the yolk mass and the yolk sphere diameter and volume (Heming and Buddington, 1988) and biochemical composition (Lahnsteiner *et al.*, 1999; Keckeis *et al.*, 2000; Berg *et al.*, 2001; Yanes-Roca *et al.*, 2009). Enzyme activity has been used less frequently as an indicator of egg quality (Lahnsteiner *et al.*, 1999; Lahnsteiner *et al.*, 2001). Since eggs and developing embryos rely on aerobic metabolism (Rombough, 1988), the latter is important for egg viability (Lahnsteiner *et al.*, 1999). However, it is well established that large females produce larger eggs and stronger offspring, but information on metabolic potential and its exploitation for metabolic requirements that could explain differences in smaller and larger eggs are still lacking.

Electron transport system (ETS) activity is often used to estimate metabolic activity for various groups of organisms (Kenner and Ahmed, 1975a; King and Packard, 1975; Packard and Williams, 1981; del Giorgio, 1992; Simčič *et al.*, 2012). Previous studies show that ETS-assay is an appropriate method for estimating metabolic activity in early life history stages of elopomorph fish (Pfeiler and Govoni, 1993) and the subtropical teleost fish medaka (*Oryzias latipes* Temminck and Schlegel) (G.-Tóth *et al.*, 1995). ETS activity is considered as a biochemical

measure of the metabolic potential of organisms that would occur if the multienzyme complex of respiratory chain functioned at maximum (Muskó *et al.*, 1995). Relation between ETS activity and actual respiration rate (ETS/R) is a measure of the exploitation of the metabolic potential and can provide useful information about the fitness of an organism. The ETS/R ratio reflects the different degree of adaptation of an organism to its environment in related invertebrate species (Muskó *et al.*, 1995; Simčič and Brancelj, 1997; Fanslow *et al.*, 2001; Simčič, 2005; Simčič *et al.*, 2005). In those exhibiting high ratios, the capacity for elevated metabolism is already present (Fanslow *et al.*, 2001). It is therefore reasonable to assume that the ETS/R ratio can be used as an indicator of fitness of different sized early life history stages of fish.

Marble trout (*Salmo marmoratus* Cuvier) is considered to be one of the highly endangered freshwater fish of the Adriatic basin due to hybridization with brown trout (*Salmo trutta* L.) (Povž, 1995) which is important for conservation, but also for aquaculture and sport fishing.

Early salmonid development can be divided into two intervals – from fertilization to hatching, and from hatching to emergence from the gravel (Berg *et al.*, 2001). Since the benefit of the greater egg size could be followed for 2-3 months (Springate *et al.*, 1985), we have determined the metabolic activity of marble trout in three stages of development – non-fertilized eggs, eggs at the eyed stage, and the pre-feeding larval stage in which yolk was resorbed.

Size of eggs has already been proved to be one of the indicators of offspring quality (Berg *et al*., 2001; Hendry *et al.*, 2001), but information about the metabolic processes in eggs and offspring of different size is scarce. Thus, the aim of this research was to determine ETS activity, respiration rate and an exploitation of metabolic potential for actual metabolic demands (i.e. the ETS/R ratio) in order to explain the differences in studied metabolic parameters that could be responsible for the different viability in small and large eggs and early developmental stages of marble trout. We expected that smaller females will produce a lower number of smaller eggs than larger ones. We hypothesised that the small and large eggs will have different ETS activity, respiration rate, and the ETS/R ratio that will change during development. Moreover, we expected that these parameters can be used as indicators of fitness of the different sized early life history stages of marble trout.

Materials and Methods

Fertilization

In November and December we stripped 13 female marble trout from the River Zadlaščica (females Nos. 1, 2, 3, 4, 5, 6, 7) and the River Trebuščica (females Nos. 8, 9, 10, 11, 12, 13) and fertilized their eggs with milt from males from the same locations (Table 1). For each stripped female one male was stripped. The females were collected at two locations due to low number of genetically pure individuals in order to include a wide range of the sizes of females. Previous genetic studies (Snoj *et al*., 2000; Fumagalli *et al*., 2002) assured that only genetically pure individuals were selected for experiments. Unfortunately, it was impossible to get small and large females from both populations. Since egg size varies among and within populations of salmonids (Fleming and Gross, 1990), in the present study both effects cannot be distinguished. Eggs and sperm were transported to a trout hatchery (Vališče Modrej), where the eggs were fertilized using extender solution: 2.42 g TRIS, 3.76 g glycine and 5.50 g NaCl per liter of water. Sperm of each male was used only once. Basic data (length, body mass, age) were recorded individually for the studied females (Table. 1). Age was determined by counting the annuli in fish scales.

Rearing of Experimental Organisms

Embryos and larvae were reared in a trout hatchery supplied with river water at a constant temperature of 10 ± 0.5 ºC, pH = 7.4 – 7.6 and oxygen concentration 10.2 – 10.6 mg L^{-1}, that is similar to the native conditions. After hardening in river water (2 h), eggs were placed in hatching tubes made of plastic tube (70 mm diameter). Tubes were 150 mm long with four holes of 25 mm in diameter drilled in the middle. The bottom of the tube was covered with plastic net with mesh size 2 x 2 mm, attached with a metal ring. The four holes were covered with the same plastic netting, attached to the tube with gluing tape. Tubes were placed in hatching boxes in which an upwelling water flow was created. The tops of the tubes were 30 mm above water surface so the holes in the middle served as an outflow.

Hatching success and time required to reach different embryonic phases were followed without taking into account the different sizes of eggs. The embryogenesis was monitored by naked eye on a white background. During the first period of hatching egg mortality was checked daily and dead eggs were removed. The time interval for hatching of eggs was 4 days (40 day degrees). Eggs reached the eyed stage after 260 - 280 day degrees. Eyed eggs were tested after 30 days of hatching (300 day degrees), since determination of the appearance of the eye pigment depends on the egg capsule properties, which can vary within batches. Eggs hatched at 460 - 480 day degrees.

On the day of the analyses for respiration and ETS measurements experimental organisms were placed in 500 mL plastic bottles filled with river water from the hatchery and transported in a thermally-

Table 1. Characteristics of females and their hardened eggs, eyed stage eggs and larvae of *Salmo marmoratus*. Data for wet mass: mean (± SD); N = 10

	Female 1	Female 2	Female 3	Female 4	Female 5	Female 6	Female 7	Female 8	Female 9	Female 10	Female 11	Female 12	Female 13
Female													
Age (years)	3	3	3	4	4	4	4	5	6	6	7	7	8
Length (cm)	23	27	28	24.5	24	28	31	55	58	66	76	89	65.5
Mass (g)	88	158	162	118	120	168	220	1710	2130	3296	4282	8225	2648
No of eggs	228	351	416	226	180	252	667	2351	2443	4181	-	8229	2906
Egg													
Wet mass (g)	0.061 (0.001)	0.075 (0.002)	0.067 (0.001)	0.077 (0.002)	0.078 (0.005)	0.092 (0.002)	0.065 (0.002)	0.100 (0.002)	0.136 (0.007)	0.106 (0.003)	0.139 (0.005)	0.101 (0.004)	0.093 (0.006)
% DW of WW	33.1	34.6	33.2	34.2	34.1	33.3	34.1	34.5	35.9	35.1	36.7	36.0	34.8
Eyed stage egg													
Wet mass (g)	0.059 (0.002)	0.075 (0.001)	0.068 (0.002)	0.075 (0.004)	0.08 (0.004)	0.087 (0.002)	0.064 (0.002)	0.093 (0.007)	0.129 (0.004)	0.102 (0.004)	0.134 (0.011)	0.095 (0.004)	0.080 (0.003)
% DW of WW	34.1	35.2	34.7	35.1	34.6	33.6	34.8	35.9	35.9	35.5	37.2	35.3	34.8
Larva													
Wet mass (g)	0.076 (0.003)	0.074 (0.005)	0.065 (0.003)	0.077 (0.001)	0.096 (0.004)	0.094 (0.005)	0.075 (0.003)	0.091 (0.008)	0.140 (0.003)	0.100 (0.006)	0.124 (0.015)	0.094 (0.007)	0.090 (0.012)
% DW of WW	20.0	26.6	25.0	26.6	21.9	24.9	22.2	30.4	29.1	29.2	25.6	30.0	27.5
Survival (%)													
Pre-eyed stage	94	98	91	98	61	98	83	77	76	93	75	81	96
Eyed to hutch	95	99	80	100	100	90	83	93	91	92	84	93	87

insulated box to the laboratory. Before measurements in the laboratory (for 2 - 3 hours), organisms were kept in aired river water at 10 ºC. ETS activity of all experimental organisms was measured the same day. Equipment for measuring respiration rate was set up the same day and measurements were conducted the next day (after 17 - 19 h). The ETS activity of non-fertilized eggs, and ETS activity and respiration rate of eggs at the eyed stage (304 day degrees) and of larvae at 267 day degrees after hatching were measured. Newly stripped eggs were allowed minimum 3 hours to harden in river water, and they were weighted before measurements.

ETS Activity Measurement

ETS activity was measured using the method introduced by Packard (1971) and modified by Kenner and Ahmed (1975b) and G.-Tóth *et al.* (1995). A single egg or larva was used for each analysis. Before experiments, animals were rinsed with filtered water to minimize adherence of bacteria to the surface. Animals were placed between two filter papers for a few seconds to remove water, then placed on a pre-weighed piece of aluminium foil and weighed on an electrobalance to the nearest 0.1 mg. Each freshly weighed animal was homogenized in 4 mL of homogenization buffer for 2 min at 700 rpm with a tissue homogeniser (IKA, Eurostar; Staufen, Germany) in ice bath. Homogenization buffer consisted of: 0.1 M sodium phosphate buffer, pH = 8.4; 75 µM $MgSO_4$; 0.15% (w/v) polyvinyl pyrrolidone; 0.2% (v/v) Triton-X-100. The homogenate was centrifuged for 4 min at 0 ºC at 8500 g (centrifuge Sigma 2K15; St. Louis, MO, USA).

Three 0.5 mL samples from each homogenate were incubated in 1.5 mL substrate solution (0.1 M sodium phosphate buffer, pH = 8.4; 1.7 mM NADH; 0.25 mM NADPH; 0.2% (v/v) Triton-X-100) with 0.5 mL 2.5 mM 2-p-iodo-phenyl 3-p-nitrophenyl 5-phenyl tetrazolium chloride (INT) solution for 40 min at a temperature of 10 ºC. The reaction was stopped by adding 0.5 mL of stopping solution (formalin (conc.): H_3PO_4 (conc.) in volume ratio 1:1). Blanks (substrate and INT solution) were incubated and stopped as for the samples; 0.5 mL of homogenate was added after stopping. Formazan production was determined spectrophotometrically (Perkin-Elmer, Lambda 25; Waltham, MA, USA) from the absorbance of the sample at 490 nm against the blank. ETS activity was measured as the rate of tetrazolium dye reduction which was converted to equivalent oxygen utilised per egg or larva in a given time interval, as described by Kenner and Ahmed (1975b).

Respiration Measurement

Respiration rate was estimated by the closed bottle method (Lampert, 1984). Ground glass stoppered bottles (300 mL) were filled with aerated water (oxygen concentration 10.5 - 11.0 mg L^{-1}). Three bottles with no animals served as controls,

while test bottles received five eyed eggs and two or three larvae each. Bottles were stoppered and kept at 10 °C. After 17 to 19 h the concentration of dissolved oxygen in the experimental and control bottles was measured by a polarographic sensor (membrane electrode WTW Oxi 320/Set; Munich, Germany). In control bottles oxygen concentration was 8 - 9 mg L^{-1}, and in experimental bottles concentration did not fall below 5 mg L^{-1}. The difference between the oxygen concentration in each experimental bottle and the concentration in control bottles was assumed to equal the oxygen consumed by the animals.

Weight Determination

The dry mass (DW) of eggs and animals was determined by drying samples at 105 ºC for 24 h. They were weighed on an electrobalance to the nearest 0.1 mg.

Statistical Analyses

Analysis of variance (ANOVA) was carried out to test differences between females. Prior to ANOVA, data were log-transformed to achieve homogeneity of variance and normality of distribution. Linear regressions of log-log transformed data were used to describe relationships between fecundity and female length, between female length and mean dry mass of single egg, between dry mass of eggs and larvae and ETS activity, respiration rate and ETS/R ratio, and between ETS activities and respiration rates. Slopes of regressions between ETS activity and dry mass and respiration rate and dry mass were compared in order to determine variation of increasing ETS activity and respiration rate with increasing size. Pearson's correlation coefficients were calculated between mass and survival, and between masses of eggs, eyed stage eggs and larvae. A *t*-test was used to test the differences between the offspring produced by smaller (i.e. younger, ≤ 4 years old) and larger (i.e. older, > 4 years old) females. All statistical analyses were performed using Microsoft Excel and SPSS.13.0 (SPSS Inc. Chicago, Illinois, USA).

Results

Size and Survival

The age of females from River Zadlaščica was 3 and 4 years, but from River Trebuščica it ranged from 5 to 8 years (Table 1). The length of younger females ranged from 22.8 to 31.0 cm and of older ones from 55.0 to 89.0 cm. Their weight ranged from 88 to 220 g and from 1710 to 8225 g, respectively (Table 1). There was a significant positive correlation between female length and number of eggs per female (Pearson's coefficient r = 0.989; P < 0.001), and between female length and egg mass (r = 0.811; P < 0.001). Significant correlations were observed between egg mass and eyed stage egg mass (r = 0.956; P < 0.001), between eyed stage egg mass and larva mass (r = 0.878, P < 0.001) and between egg mass and larva mass (r = 0.919, P < 0.001). A negative correlation was observed between egg mass and survival of eggs before hatching (r = -0.381; P < 0.05), but no correlation between egg size and survival of larvae after hatching (r = -0.173; P > 0.05).

ETS Activity

ETS activities ranged from 0.92 to 3.70 µL O_2 ind^{-1} h^{-1} for eggs, from 2.53 to 4.76 µL O_2 ind^{-1} h^{-1} for eyed stage eggs, and from 9.06 to 12.57 µL O_2 ind^{-1} h^{-1} for larvae (Figure 1). ANOVA revealed that ETS activities of eggs (F = 55.69; d.f. = 51; P < 0.001), eyed stage eggs (F = 7.77; d.f. = 51; P < 0.001), and larvae (F = 3.49; d.f. = 51; P < 0.01) increased significantly with increasing mass of eggs, eyed stage eggs and larvae, and differed significantly between females (Figure. 2). Mean ETS activity was lower in smaller eggs (≤ 0.092 g wet mass) produced by younger females (1.18 ± 0.41 µL O_2 ind^{-1} h^{-1}; n = 24) than in larger ones (≥ 0.093 g wet mass) produced by larger females (2.69 ± 0.62 µL O_2 ind^{-1} h^{-1}; n = 28) (t-test, t = 10.44, d.f. = 50, P < 0.001). The mean ETS activity was also lower in the eyed stage eggs of smaller females (2.92 ± 0.34 µL O_2 ind^{-1} h^{-1}; n = 28) than in eyed stage eggs of larger ones (3.69 ± 0.87 µL O_2 ind^{-1} h^{-1}; n = 24) (t-test, t = 4.32, d.f. =50, P < 0.05). The ETS activity of larvae did not differ significantly between smaller (10.42 ± 1.49 µL O_2 ind^{-1} h^{-1}; n = 28) and larger (10.15 ± 1.58 µL O_2 ind^{-1} h^{-1}; n = 24) females (t-test, t = 0.63, d.f. = 50, P > 0.05).

Respiration Rate

The respiration rates of eyed stage eggs ranged from 0.83 to 1.74 µL O_2 ind^{-1} h^{-1} and those of larvae from 8.37 to 14.89 µL O_2 ind^{-1} h^{-1} (Figure 1). Respiration rates differed significantly between females for both eyed stage eggs (F = 2.37; d.f. = 32; P < 0.05), and larvae (F = 3.36; d.f. = 38; P < 0.01). The regression between respiration rate and dry mass showed greater oxygen demands in larger individuals than in smaller ones (Figure 3), but the mean respiration rate of eyed stage eggs produced by smaller females (1.08 ± 0.34 µL O_2 ind^{-1} h^{-1}; n = 19) did not differ significantly from those measured in eyed stage eggs produced by larger females (1.20 ± 0.30 µL O_2 ind^{-1} h^{-1}; n = 14) (t-test, t = 1.08, d.f. = 31, P > 0.05). The larvae of smaller females (9.03 ± 1.41 µL O_2 ind^{-1} h^{-1}; n = 21) had lower respiration rates than those of larger ones (12.36 ± 2.30 µL O_2 ind^{-1} h^{-1}; n = 18) (t-test, t = 5.54, d.f. = 37, P < 0.001).

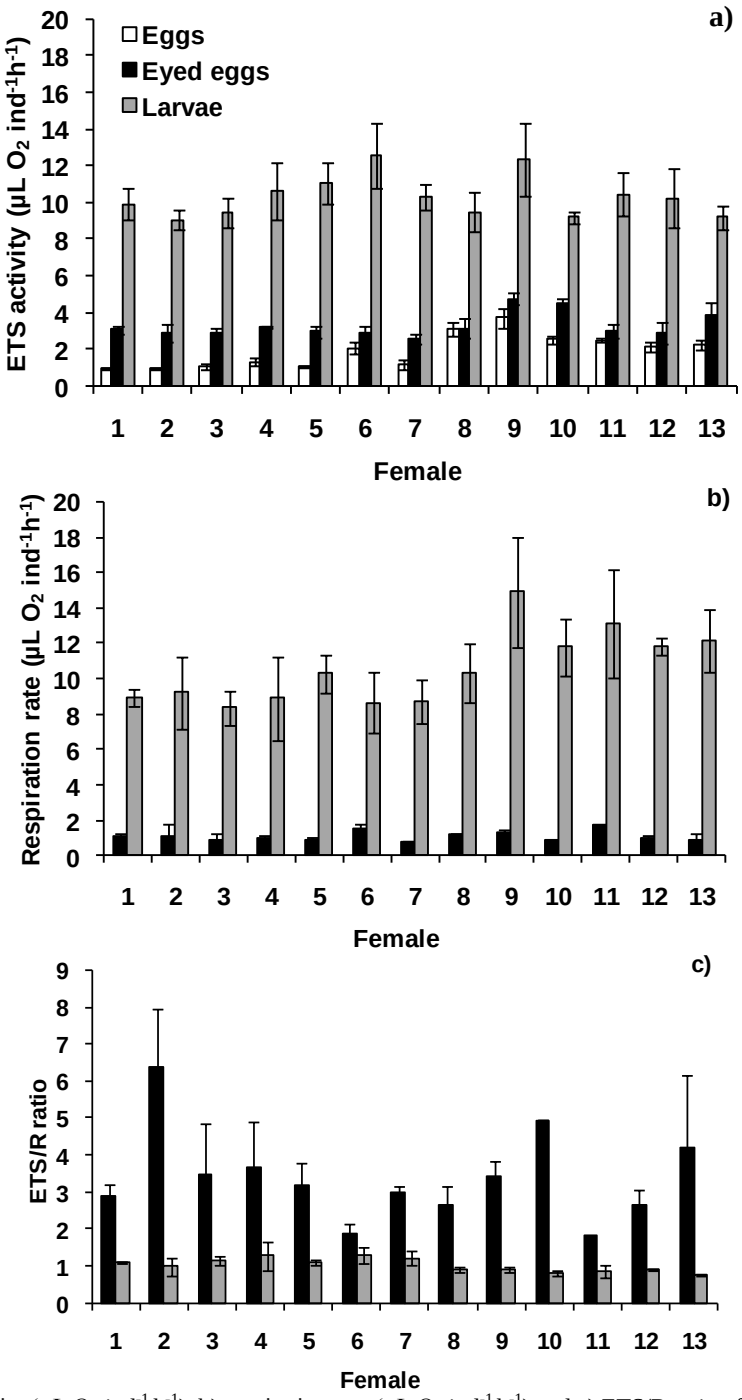

Figure 1. a) ETS activity (µL O_2 ind^{-1} h^{-1}), b) respiration rate (µL O_2 ind^{-1} h^{-1}) and c) ETS/R ratio of eggs, eyed stage eggs and larvae of *Salmo marmoratus*. Bars represent ± SD (N=3 - 4).

Relationship Between ETS Activity and Respiration Rate

ETS activities for the larvae correlated significantly with their respiration rates according to the function: log R = 0.150 + 0.854 * log ETS (r = 0.440, n = 39, P < 0.01) but, in the case of eyed stage eggs, the correlation was not significant (r = 0.158; n = 33; P > 0.05). The ETS/R ratios of eyed stage eggs (F = 2.37; d.f. = 32; P < 0.05) and of larvae (F = 4.63; d.f. = 38; P < 0.01) differed significantly between the females (Figure 1). No correlation was observed between ETS/R ratios and dry mass in eyed stage eggs (P > 0.05), but those of larvae decreased significantly with increasing dry mass (Figure 4). Since the ratios for eyed stage eggs of smaller and larger females were similar, a mean ETS/R ratio (3.06 ± 1.06; n = 33) was calculated for the eyed stage eggs of all females. Significantly higher ratios were observed for the larvae developed from the eggs of smaller females (1.15 ± 0.20; n = 21) than those developed from the larger eggs (0.85 ± 0.10; n = 18) (t-test, t = 5.86, d.f. = 37, P < 0.001). Comparison of the slopes of the regressions between ETS activity

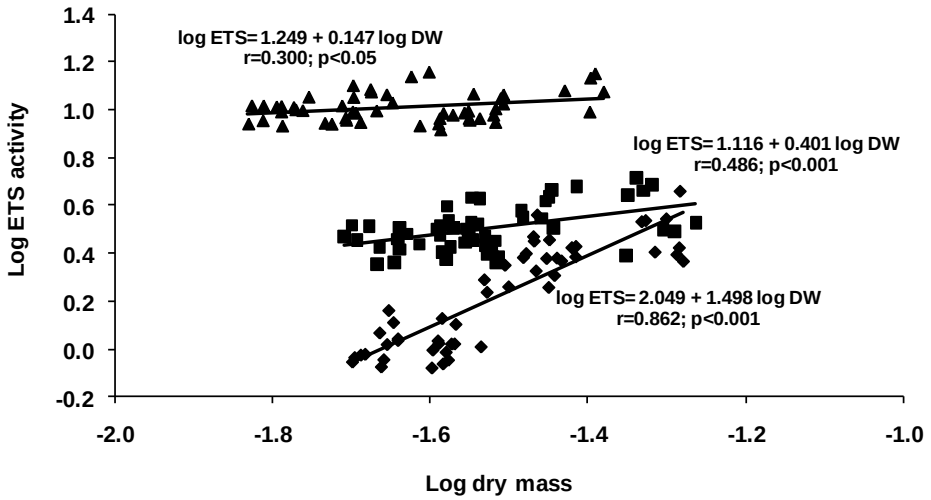

Figure 2. Relationship between ETS activity (µL O_2 ind^{-1} h^{-1}) and egg dry mass (DW; g) for eggs (♦), eyed stage eggs (■), and larvae (▲) of *Salmo marmoratus;* N = 52.

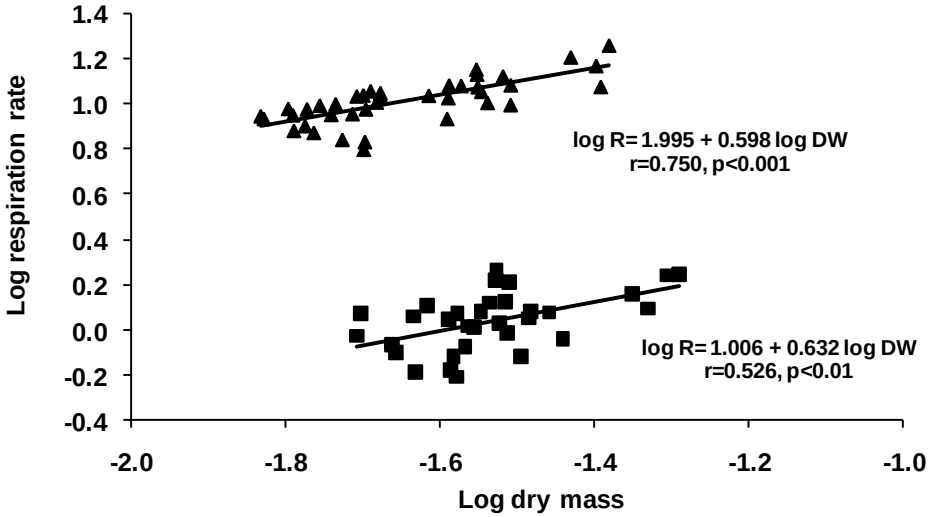

Figure 3. Relationship between respiration rate R (µL O_2 ind^{-1} h^{-1}) and egg dry mass (DW; g) for eyed stage eggs (■), and larvae (▲) of *Salmo marmoratus;* N = 39

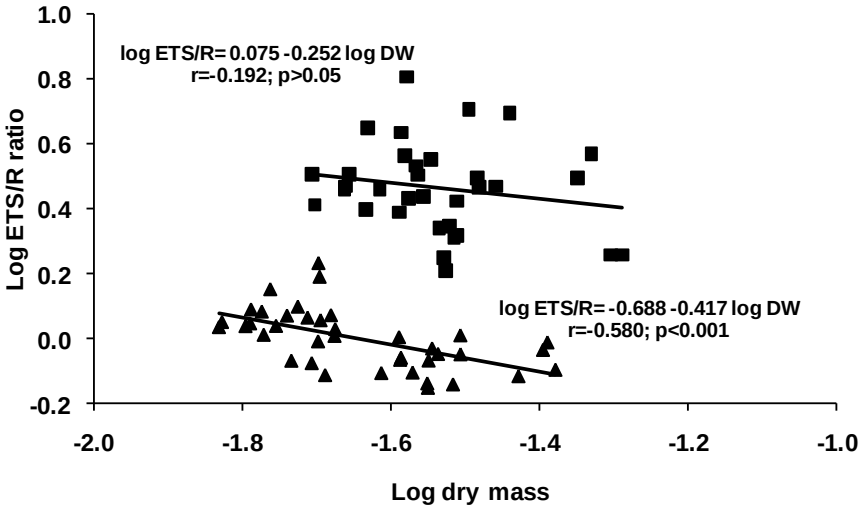

Figure 4. Relationship between ETS/R ratio and egg dry mass (DW; g) for eyed stage eggs (■), and larvae (▲) of *Salmo marmoratus;* N = 39.

and dry mass (Figure 2) and between respiration rate and dry mass (Figure 3) showed a greater increase in respiration rate than in ETS activity with increasing size of larvae ($F = 16.97$; $P < 0.001$), but it was insignificantly different for the eyed stage eggs ($F = 1.40$; $p > 0.05$).

Discussion

Eggs and early developmental stages produced by different sized females of the marble trout vary in size, viability and energy metabolism. As expected, size and number of produced eggs increased with maternal size. Moreover, small and large eggs possessed different metabolic properties, namely ETS activity, respiration rate and the ratio between metabolic potential and actual respiration rate that could explain various fitness of smaller and larger eggs in particular environmental conditions.

Size and Survival

The results of the present study are consistent with the previous findings that, in salmonids, both egg size and fecundity correlate positively with maternal size (Fleming and Gross, 1990; Lobon-Cervia *et al.*, 1997; Rollinson and Hutchings, 2010). Studies have shown that larger eggs give rise to larger juveniles, and that larger juveniles exhibit increased survival, competitive ability, swimming performance, growth and overall fitness (Blaxter, 1988). However, under some circumstances such as poor gravel quality, smaller eggs with less energy may actually be optimal (Hendry *et al.*, 2001). Larger eggs have a low surface to volume ratio, making oxygen transfer more difficult, and have greater metabolic oxygen demands (Berg *et al.*, 2001). To test this assumption, Einum *et al.* (2002) exposed small and large eggs of brown trout (*Salmo trutta*) to high (14 mg L^{-1}) and low (2.3 mg L^{-1}) levels of dissolved oxygen. They found that at high oxygen levels eggs survival was higher and independent of eggs size, while at low oxygen levels survival decreased overall, and was higher for large-egged than small-egged siblings. Our results show that egg size influences larval size, with larger larvae being developed from larger eggs, but that the percentage of surviving embryos during the earliest life history period (i.e. egg to eyed stage egg) declines with larger egg size. Later, however, survival of the eyed stage eggs that have developed to the larval stage no longer correlates with egg size. Similarly, Hendry *et al.* (2001) reported that, in those aquatic environments in which the survival of eggs is influenced by oxygen availability, offspring survival before hatching decreases with increasing egg size, whereas offspring survival after hatching increases with increasing egg size.

ETS Activity and Respiration Rate

The activity of enzymes and respiration rate can be expressed in activity units per whole embryo, per protein content or dry weight of the whole egg, or only embryo tissue without adjacent yolk (Neyfakh and Abramova, 2014). Since in developing embryo the mitochondria are closely bound to the yolk and the increase in respiration during the embryogenesis occurs mainly at the expense of mitochondrial fraction bound to the yolk (Abramova *et al.*, 1966; cited in Neyfakh and Abramova, 2014), in the present study ETS activity and respiration rate expressed per whole embryo, the yolk included, i.e. per whole egg or larva. ETS activity and respiration rate increased during early development from the egg stage to the larva stage (Figure 1). Similarly, ETS activity has been found to increase exponentially during embryonic development in the fish medaka (G.-Tóth *et al.*, 1995) and respiration rate has also been reported to increase exponentially from fertilization to the first feeding of the common nase (*Chondrostoma nasus* L.) (Kamler *et al.*, 1998). Increasing ETS activity probably can be attributed to the mitochondrial biogenesis, increase in density and biochemical differentiation of mitochondria (Stackley *et al.*, 2011), since ETS activity is an enzymatic process, depending on the concentration (Båmstedt, 1980) and characteristics (Packard, 1971) of the multienzyme complex in respiratory chain. Respiration is a complex physiological process that is further influenced by an intact intracellular environment, concentrations of substrates, coenzymes, phosphates, ADP (Neyfakh and Abramova, 2014) and structure and properties of intact lipid membranes (Withers, 1992), Thus, increasing of the respiration intensity is attained through intensification of mitochondrial functions that is regulated by genetic mechanisms (Neyfakh and Abramova, 2014).

Lahnsteiner *et al.* (1999), who measured the respiration rate in lake trout (*Salmo trutta lacustris* L.) *via* ETS rate, observed positive correlations between the percentage of eyed stage eggs and the respiration rate, the malate dehydrogenase activity, and the NADH to NAD ratio, all of which indicate the importance of the aerobic metabolism for egg viability. The different ETS activities and respiration rates may therefore reflect differences in offspring quality. The ETS activities and respiration rates for eggs, eyed stage eggs and larvae differed between females. The increase of respiratory potential with size was evident for the earliest embryonic stages, but later on, in larvae, the correlation between ETS activity and dry mass was low (Figure 2). The metabolic potential of small eggs increased by 8.8-fold during the development to larvae, while the increase of metabolic potential in large eggs was considerably lower (by 3.8-fold). Consequently, the larvae produced by the smaller females exhibit

metabolic potentials similar to those of the larvae that developed from the larger eggs. Thus, the differences in metabolic potential between the different sized eggs are minimized during the earliest embryonic development before the larval stage is reached. Similarly to ETS activity, respiration rate increased with increasing mass in eyed stage eggs and larvae. Einum *et al.* (2002) found that oxygen consumption increased relatively slowly with increasing egg mass in Atlantic salmon (*Salmo salar* L.) (allometric constant 0.443). Higher scaling factor was found for the eyed eggs (0.632) and larvae (0.598) of the marble trout, indicating higher increase of oxygen demands with increasing egg mass. In these studies, intensity of metabolism was calculated per dry mass of whole egg or larva, without considering the metabolically inert part of the total mass. Since the proportion of metabolically inactive tissues varies between different species and between different sized animals or developmental stages of the same species, it would be useful to determine and include other egg parameters, such as the yolk mass, the yolk sphere diameter and volume, the total and dechorionated embryonic mass, the perivitelline space and the egg surface-volume ratio, in order get more information on the viability of the different sized early life history stages of fish. For example, determination of the rate of yolk absorption or efficiency of yolk utilization could provide valuable information on energy content of different sized early life history stages (Heming and Buddington, 1988). Thus, these parameters should be examined in further studies of the marble trout.

Relationship Between ETS Activity and Respiration Rate

The ETS/R ratios of marble trout reported here are similar to those found in the early development stages of the fish medaka (G.-Tóth *et al.*, 1995), but there are no published data for salmonids to be compared with. The ETS/R ratio was three-fold lower for the larvae than for the eyed stage eggs. The latter thus exploit a low proportion of the estimated metabolic potential, while larvae use the total metabolic potential to cover energy demands. Total respiration increased with the age of the zebrafish embryo mostly due to the increase in mitochondrial content (Stackley *et al*., 2011) that is reflected in ETS activity and respiration rate measurements. Moreover, increasing of the respiration rate is attained through intensification of mitochondrial functions via several mechanisms (Neyfakh and Abramova, 2014). Thus, an intensive growth of body tissues that develop gradually during the embryonic and larval period (Rombough, 1988) resulted in lower ETS/R value in larvae than in eyed stage eggs. This is first report on the relationship between ETS activity and respiration rate of the early life history stages in salmonids showing the increase in exploitation of metabolic capacity for energy production.

Decrease in the ETS/R ratio with increasing size is also observed within both egg eyed and larval developmental stages. Comparison of the regression slopes showed a greater increase in respiration relative to ETS activity with increasing dry mass of larvae. Thus, larger individuals exhibited lower ETS/R ratios than smaller ones. The ETS/R ratio is an important index of the organisms' metabolism (Muskó *et al.*, 1995) – in organisms with high ratios, the capacity for increased metabolism is maintained (Fanslow *et al.*, 2001). It seems that the lower ETS activity and respiration rate of small individuals is accompanied by relatively higher metabolic potential that can be exploited in the case of increased respiration. On the other hand, the high ETS activity and respiration rate of large sized individuals enables their rapid development, but only within the optimal ecological limits. In poor habitats, the high metabolic demands of large eggs require more ATP than can be generated by existing ETS machinery. Therefore, it is reasonable to assume that the smaller size would be an advantage in unfavourable conditions that cause increased respiration. These findings are in agreement with those of Hendry *et al.* (2001) and Berg *et al.* (2001) who reported that larger eggs are favoured in richer habitats. Moreover, the ratio between ETS activity and respiration rate is one of the factors that could explain the disadvantage of larger size in the early life history stages under unfavourable conditions, as evidenced by Rollinson and Hutchings (2010) and Marshall *et al.* (2010).

Thus, the findings of the present study support the statements of Marshall *et al.* (2010) that larger offspring fare better than smaller offspring size when reared in the same, optimal conditions, but in different, unfavourable conditions smaller offspring size would be optimal. These findings could be taken into account in the process of marble trout restoration which has been practiced for more than 20 years in Slovenia. There are eight genetically pure populations of marble trout that serve as a basis for stocking the Soča river system in order to replace brown trout non-native genes with the marble trout ones and slowly regain the situation characteristic of the Soča river system before brown trout introduction (Snoj *et al.* 2000; Fumagalli *et al.* 2002).

Conclusions

In conclusion, it is assumed that the smaller females of marble trout produce fewer, smaller eggs which have a relatively high metabolic potential and are thus at an advantage relative to the larger ones under unfavourable conditions. During embryogenesis this advantageous high potential in small eggs is lost. The low ETS/R ratio in larger larvae leads to the expectation that they have lower fitness than smaller ones. This could be true for their survival under poor environmental conditions but under optimal conditions, the larger larvae give rise to larger

juveniles having greater competitive ability, swimming performance and growth. However, further studies on eggs and offspring of different sized marble trout exposed to various environmental conditions and concurrent appropriate biochemical and physiological measurements are needed to test these assumptions. This is first report on the relationship between metabolic potential and respiration rate of the early life history stages in salmonids showing the increase of exploitation of metabolic capacity for energy production with development and size.

Acknowledgement

We thank Roger Pain for English revision of the manuscript, and two anonymous reviewers for their constructive and helpful comments on the manuscript. We acknowledge financial support by the Slovenian Research Agency (Research Programme P1-0255).

References

Båmstedt, U. 1980. ETS activity as an estimator of respiratory rate of zooplankton populations. The significance of variations in environmental factors. J. Exp. Mar. Biol. Ecol., 42: 267-283.

Berg, O.K., Hendry, A.P., Svendsen, B., Bech, C., Arnekleiv, J.V. and Lohrmann, A. 2001. Maternal provisioning of offspring and the use of those resources during ontogeny: variation within and between Atlantic Salmon families. Funct. Ecol., 15: 13-23. DOI: 10.1046/j.1365-2435.2001.00473.x

Blaxter, J.H.S. 1988. Pattern and variety in development, p. 1-57. In: W.S. Hoar and D.J. Randall (Eds.), Fish Physiology, The Physiology of Developing Fish, Eggs and Larvae. Vol. 11, part A. Academic Press, London.

del Giorgio, P.A. 1992. The relationship between ETS (electron transport system) activity and oxygen consumption in lake plankton: a cross-system calibration. J. Plankton Res., 14: 1723-1741. doi: 10.1093/plankt/14.12.1723

Einum, S., Hendry, A.P. and Fleming, I.A. 2002. Egg-size evolution in aquatic environments: does oxygen availability constrain size? Proc. R. Soc. Lond. B 269, 2325–2330 The Royal Society DOI 10.1098/rspb.2002.2150

Fanslow, D.L., Nalepa, T.F. and Johengen, T.H. 2001. Seasonal changes in the respiratory electron transport system (ETS) and respiration rate of the zebra mussel, *Dreissena polymorpha* in Saginaw Bay, Lake Huron. Hydrobiologia, 448: 61-70. doi: 10.1023/A:1017582119098

Fleming, I.A. and Gross, M.R. 1990. Latitudinal clines: A trade-off between egg number and size in Pacific salmon. Ecology, 71: 1-11. doi: 10.2307/1940241

Fumagalli, L., Snoj, A., Jesenšek, D., Balloux, F., Jug ,T., Duron, O., Brossier, F., Crivelli, A.J. and Berrebi, P. 2002. Extreme genetic differentiation among the remnant populations of marble trout (*Salmo marmoratus*) in Slovenia. Mol. Ecol., 11: 2711-2716. doi: 10.1046/j.1365-294X.2002.01648.x

G.-Tóth, L., Szábo, M. and Webb, D. 1995. Adaptation of the tetrazolium reduction test for the measurement of the electron transport system (ETS) activity during embryonic development of medaka. J. Fish Biol., 46: 835-844. doi: 10.1111/j.1095-8649.1995.tb01606.x

Heinimaa, S. and Heinimaa, P. 2004. Effect of female size on egg quality and fecundity of the wild Atlantic salmon in the sub-arctic River Teno. Boreal Environ. Res., 9:55-62.

Heming, T.A. and Buddington, R.K. 1988. Yolk absorbtion in embryonic and larval fishes, p. 408-438. In: W.S. Hoar and D.J. Randall (Eds.), Fish Physiology, The Physiology of Developing Fish, Eggs and Larvae. Vol. 11, part A. Academic Press, London.

Hendry, A.P., Day, T., Cooper, A.B. and McPeek, M.A. 2001. Optimal size and number of propagules: Allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. Am. Nat., 157: 387-408. doi: 10.1086/319316

Hutchings, J.A. 1991. Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. Evolution, 45: 1162-1168. doi: 10.2307/2409723

Kamler, E., Keckeis, H. and Bauer-Nemeschkal, E. 1998. Temperature-induced changes of survival, development and yolk partitioning in *Chondrostoma nasus*. J. Fish Biol., 53: 658-682. doi: 10.1111/j.1095-8649.1998.tb01009.x

Keckeis, H., Bauer-Nemeschkal, E., Menshutkin, V.V., Nemeschkal, H.L. and Kamler, E. 2000. Effects of female attributes and egg properties on offspring viability in a rheophilic cyprinid, *Chondrostoma nasus*. Can. J. Fish. Aquat. Sci., 57: 789-796. doi: 10.1139/f00-006

Kenner, R.A. and Ahmed, S.I. 1975a. Correlation between oxygen utilization and electron transport activity in marine phytoplankton. Mar. Biol., 33: 129-133. doi: 10.1007/BF00390717

Kenner, R.A. and Ahmed, S.I. 1975b. Measurements of electron transport activities in marine phytoplankton. Mar. Biol., 33: 119-127. doi: 10.1007/BF00390716

King, F.D. and Packard, T.T. 1975. Respiration and respiratory electron transport system in marine zooplankton. Limnol. Oceanogr., 20: 846-854. doi: 10.1007/BF00393750

Lahnsteiner, F., Urbanyi, B., Horvath, A. and Weismann, T. 2001. Bio-markers for egg quality determination in cyprinid fish. Aquaculture, 195: 331-352. doi: 10.1016/S0044-8486(00)00550-0

Lahnsteiner, F., Weismann ,T. and Patzner, R.A. 1999. Changes in eggs of brown trout, rainbow trout, and grayling during short-term storage. N. Am. J. Aquaculture, 61: 213-219. doi: 10.1577/1548-8454(1999)

Lampert, W. 1984. The measurement of respiration. In: J.A. Downing and F.H. Rigler (Eds.), A Manual on Methods for the Assessment of Secondary Productivity in Fresh Water, p. 413-468. IPB Handbook 17, second edition. Blackwell Scientific Publications, Oxford.

Lobon-Cervia, J., Utrilla, C.G., Rincon, P.A. and Amezcua, F. 1997. Environmentally induced spatio-temporal variations in the fecundity of brown trout *Salmo trutta* L.: trade-offs between egg size and number. Freshw. Biol., 38: 277-288. doi: 10.1046/j.1365-2427.1997.00217.x

Marshall, D.J., Heppell, S.S., Munch, S. and Warner, R.R. 2010. The relationship between maternal phenotype

and offspring quality: Do older mothers really produce the best offspring. Ecology, 210: 862-2873. doi: 10.1890/09-0156.1

Muskó, I.B., G.-Tóth, L. and Szábo, E. 1995. Respiration and respiratory electron transport system (ETS) activity of two amphipods: *Corophium curvispinum* G. O. Sars and *Gammarus fossarum* Koch. Pol. Arch. Hydrobiol., 42: 547-558.

Neyfakh, A.A. and Abramova, N.B. 2014. Biochemical embryology of fishes. In: M. Florkin and B.T. Sheer (Eds.), Chemical Zoology, Deuterostomians, Cyclostemes and Fishes. 275-283. books.google.hu/books?isbn=0323163343.

Olofsson, H. and Mosegaard, H. 1999. Larger eggs in resident brown trout living in sympatry with anadromous brown trout. Ecol. Freshw. Fish, 8:59-64. doi: 10.1111/j.1600-0633.1999.tb00054.x

Packard, T.T. 1971. The measurement of respiratory electron- transport activity in marine phytoplankton. J. Mar. Res., 29: 235-244.

Packard, T.T. and le B Williams, P.J. 1981. Rates of respiratory oxygen consumption and electron transport in surface seawater from the northwest Atlantic. Oceanol. Acta, 4: 351-358.

Pfeiler, E. and Govoni, J.J. 1993. Metabolic rates in early life history stages of elopomorph fishes. Biol. Bull., 185: 277-283. doi: 10.2307/1542007

Povž, M. 1995. Status of freshwater fishes in the Adriatic catchment of Slovenia. Biol. Conserv., 72: 171-177. doi: 10.1016/0006-3207(94)00079-6

Rollinson, N. and Hutchings, J.A. 2010. Why does egg size increase with maternal size? Effects of egg size and egg density on offspring phenotypes in Atlantic salmon (*Salmo salar*). Evol. Ecol. Res., 12: 949-960.

Rombough, P.J. 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life, p. 59-161. In: W.S. Hoar and D.J. Randall (Eds.), Fish Physiology, The Physiology of Developing Fish, Eggs and Larvae. Vol. 11, part A. Academic Press, London.

Simčič, T. and Brancelj, A. 1997. Electron transport system (ETS) activity and respiration rate in five *Daphnia* species at different temperatures. Hydrobiologia, 360: 117-125. doi: 10.1023/A:1003117221455

Simčič, T., Lukančič, S. and Brancelj, A. 2005. Comparative study of electron transport system activity and oxygen consumption of amphipods from caves and surface habitats. Freshw. Biol., 50: 494-501. doi: 10.1111/j.1365-2427.2005.01339.x

Simčič, T. 2005. Respiratory electron transport system (ETS) activity and respiration rate in cold-stenothermal and eurythermal chironomid larvae from high-mountain lakes. Arch. Hydrobiol., 162: 399-415. doi: 10.1127/0003-9136/2005/0162-0399

Simčič, T., Pajk, F., Vrezec, A. and Brancelj, A. 2012. Size scaling of whole-body metabolic activity in the noble crayfish (*Astacus astacus*) estimated from measurements on a single leg. Freshw. Biol., 57: 39-48. doi: 10.1111/j.1365-2427.2011.02692.x

Snoj, A., Jug, T., Melkič, E., Sušnik, S., Pohar, J., Dovč, P., Jesenšek, D. and Budihna, N. 2000. Mitochondrial and microsatellite DNA analysis of marble trout in Slovenia. Quaderni ETP, 29: 5-11.

Springate, J.R.C., Bromage, N.R. and Cumaranatunga, P.R.T. 1985. The effects of different ration on fecundity and egg quality in the rainbow trout (*Salmo gairdneri*), p. 371-393. In: C.B. Cowey, A.M. Mackie and J.G. Bell (Eds.), Nutrition and feeding in fish. Academic Press, London.

Stackley, K.D, Beeson, C.C., Rahn J.J, and Chan, S.S.L. 2011. Bioenergetic profiling of zebrafish embryonic development. PLoS ONE 6(9): e25652. doi:10.1371/journal.pone.0025652

Van Winkle, W., Shuter, B.J., Holcomb, B.D., Jager, H.I., Tyler, J.A. and Whitaker, S.Y. 1997. Regulation of energy aqusition and allocation to respiratory, growth and reproduction: simulation model and example using rainbow trout, p. 103-137. In: R.C. Chambers and E.A. Trippele (Eds.), Early life history and recruitment in fish populations. Chapman & Hall, London.

Withers P.C. 1992. Comparative Animal Physiology, Saunders Collage Publishing, Fort Worth, pp. 82-187.

Yanes-Roca, C., Rhody, N., Nystrom, M. and Main, K.L. 2009. Effects of fatty acid composition and spawning season patterns on egg quality and larval survival in common snook (*Centropomus undecimalis*). Aquaculture, 287: 335–340. doi: 10.1016/j.aquaculture.2008.10.043