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Oxygen consumption and lactate accumulation in the intra-ovarian embryos and young of the viviparous fish, Zoarces viviparus (L.) in relation to decreasing water O_2 concentration

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The oxygen consumption of the young of *Zoarces viviparus* (L.) was measured at different O_2 concentrations in brackish water with a closed system respirometer. Changes in routine metabolic rate and hypoxia resistance related to age are presented. The routine metabolic rate remains at a steady level over a wide range of O_2 concentrations, the critical O_2-level being 3.5—2.0 mg O_2/l depending on the age and acclimatisation state of the young.

Lactate is produced under hypoxic conditions by both intra-ovarian embryos and by young. Both show high tolerance to hypoxia. Lactate is excreted at least partly in to the surrounding media (embryotrophe/brackish water). The lactate concentration in the young does not begin to increase in a brackish water environment until the water O_2 concentration is under 1 mg O_2/l. In the ovarian cavity, the lactate concentration in embryos and embryotrophe has increased already at 4 mg O_2/l, when it is still at a steady level in the blood and muscle tissue of parent fish.

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1. Introduction

Surprisingly little information is available about the physiology of embryos of viviparous fish during gestation (Wourms 1981). Webb & Brett (1972a) described the oxygen demands of the broods of two species of viviparous sea perch and the oxygen characteristics of the intra-ovarian environment and compared the brood — ovary system with the mammalian placenta because of the absence of such information for viviparous fish.

During the pregnancy of the eel-pout *Zoarces viviparus* (L.) which lasts for about 5 months in the northern Baltic, both the volume and consistency of ovarian fluid, embryotrophe, varies greatly (Kristoffersson & Pekkarinen 1975). During late gestation the amount of embryotrophe in the ovarian cavity is often so small that it is impossible to draw a fluid sample into a capillary tube. In any case, all the transfer of material between mother and embryos is via the embryotrophe. How the oxygen demands of embryos can be fulfilled under these conditions is an unanswered question. Both the resistance to hypoxia and the anaerobic pathways of viviparous fish have still to be studied. This paper describes the oxygen consumption of *Zoarces* young at different oxygen concentrations in brackish water and changes in the metabolic rate and resistance to hypoxia in relation to age. Progressive hypoxia has been studied by measuring the accumulation of lactate in the tissues of parent fish and embryos. Hypoxic conditions have been produced in both the intra-ovarian environment and in brackish water, which is the natural milieu for the young after birth.

2. Material and methods

Pregnant eel-pouts were caught with nets at depths of 25—40 m from the brackish water (salinity 6—7 ‰) areas of Tvärminne Zoological Station, Gulf of Finland.[1] Fish were kept for at least one week in an aerated aquarium (O_2 saturation at least 90 %) with free running (c. 2 l/min) natural brackish water before experiments. Water temperature was kept at 5±1 °C. Adult fish were not fed. All experiments with pregnant females were made between January 16 and February 9 (1974-1977) during the normal parturition period of the eel-pout in the Gulf of Finland (Kristoffersson et al. 1973).

[1]) This eel-pout population has been absent from these waters since 1980.

2.1. Experiment with young in brackish water

Pregnant females were lightly anaesthetized with neutralized 0.03 % MS-222® brackish water solution, which was at the same temperature at which the fish had been acclimatized. Some of the young were immediately transferred from opened ovaries (Fig. 1) to the respirometer. The rest were kept in 5 l aerated brackish water aquaria at 5±1 °C. The young were fed with commercial aquaria fish food every other day. Each brood was kept in a separate aquarium.

In the oxygen consumption measurements the young were grouped according to their age:

Group:	0	I	II	III
Age	0 hour	10-27 hours	3-5 days	14-19 days

The young in group 0 were transferred straight from the ovarian cavity to a respirometer.

In the lactate measurements the material was grouped according to the decreasing oxygen concentration of the water, which was developed in a closed system respirometer (Snow & Williams 1971). Lactate contents were analysed at the following O_2 concentrations: 6, 2, 1 and 0.5 mg O_2/l. The control group was taken from an aerated aquarium.

2.2. Experiments with pregnant females and intra-ovarian embryos

In these experiments too material was grouped according to the decreasing oxygen concentration of the water in Brett's (1964) closed system respirometer. Lactate concentrations were measured from intra-ovarian embryos, from embryotrophe and from blood and muscle tissue of the parent fish at the following O_2 concentrations: aerated, 4, 3, 2 and 1.5 mg O_2/l.

In order to minimize the effect of handling, pregnant females were rested in a continuously aerated open respirometer chamber for at least 10 hours before starting the respirometer run. For the same reason MS-222 solution was poured before sampling straight into the respirometer chamber, the final concentration again being 0.03 %.

2.3. Analytical methods

The oxygen consumption of the young was measured in a closed system respirometer (Snow & Williams 1971, Kangas & Lappalainen 1978). A Delta Scientific automatic dissolved oxygen analyser (series 2010) connected to a 10 mV recorder was used. The oxygen probe was fitted into a darkened beaker (volume 375 ml). Adequate stirring at constant speed was arranged with a magnetic stirrer. In order to avoid disturbing the young the mixing bas was placed under a net bottom. Filtered natural brackish water was used in the respirometer to avoid background oxygen consumption. The temperature was kept at 5±0.3 °C with a water bath. 8—14 young were used in each respirometer run. The results presented are means of different runs. The oxygen consumption is calculated at different oxygen concentrations at intervals of 0.5 ppm and expressed as μg O_2/ hour/g wet weight.

Brett's closed system respirometer (1964) was used in experiments with pregnant females. The temperature and circulation speed of water were controlled. The respirometer chamber was darkened during the runs.

Lactate was measured using Biochemica Boehringer's test combination for human blood (cat. No 15972) with some modifications. The young were weighed and immediately homogenized in cold 0.6 N perchloric acid (1:10 w/v). Samples were centrifuged for 10 min at 5 °C (5000 r.p.m) and filtered; the supernatant was used in the analyses. The same procedure was used for the adult muscle tissue prepared from the epacsonic part of the side muscle just caudal to the head. Blood and embryotrophe were analysed according to the manufacturer's instructions. Blood was drawn from the heart into heparinised syringes. The manufacturer's calculation factor was corrected by taking into account the difference in the water content of human blood and the eel-pout tissues used in the analyses and different dilution used (Boehringer Mannheim GmbH Biochemica's Test-Fibel).

In water content determinations, the young were lightly dried with filter paper and weighed immediately. They were then dried to constant weight at 105 °C. The difference between fresh weight and dry weight is considered the water content.

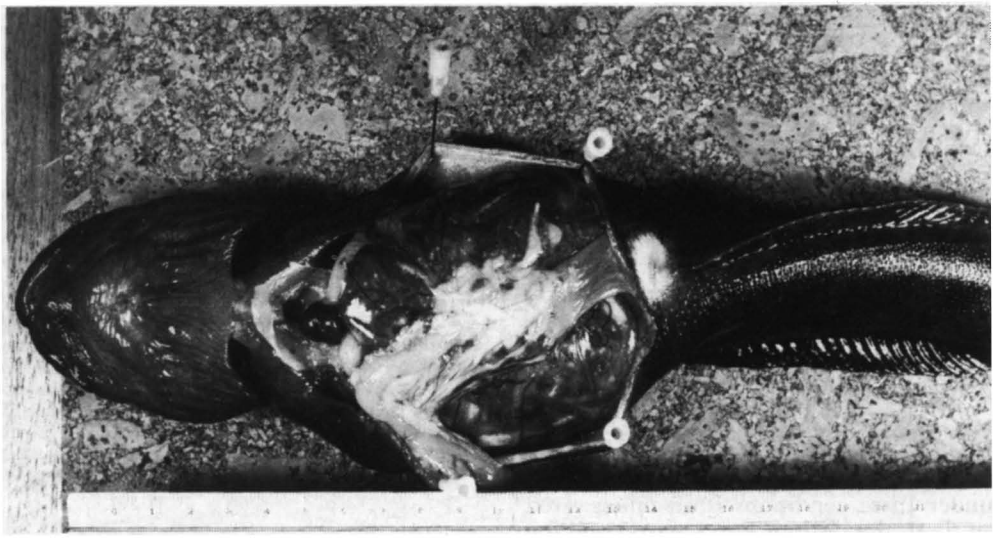

Fig. 1. Anaesthetized pregnant Zoarces after laparatomy showing the huge ovary packed with fully grown embryos (174 embryos). The empty intestine partly hides the ovary. The bladder is empty. The scale bar is in centimetres.

Table 1. Effect of different immobilization techniques on lactate concentrations in blood (mg/100 ml), muscle tissue (mg/100 g), embryotrophe (mg/100 ml) and intra-ovarian embryos (mg/100 g) of *Zoarces viviparus*. Means ± SDs are presented. Number of fish analysed in parentheses. Further explanation in text.

	Blow on head (A)		Anaesthetized		
		p[1]	after netting (B)	p[1]	after rest (C)
Blood	5.10± 2.13 (5)	NS	5.06±1.81 (7)	NS	5.02± 1.24 (7)
Muscle	189.91±23.91 (5)	<0.01	125.12±9.92 (4)	<0.02	95.33±17.31 (7)
E-trophe	2.39± 1.21 (3)	NS	1.47±0.83 (4)		
Embryos	27.16± 8.71 (15)	<0.01	21.04±3.77 (21)		

[1]) Student's *t* test, NS = not significant.

2.4. Methodological experiments

The effect of different immobilization techniques on the lactate levels of both parent fish and intra-ovarian embryos were tested in the following groups:

A. Eel-pouts netted and stunned by a blow on the head.

B. Eel-pouts netted straight into a neutralized 0.03 % MS-222 — brackish water anaesthesia bath which was well aerated and at the same temperature to which the fish had been acclimatized.

C. Eel-pouts netted into an aerated respirometer chamber and allowed to rest there for at least 10 hours before sampling. MS-222 was poured straight into the respirometer, the final concentration again being 0.03 %.

The results are presented in Table 1.

Blood lactate remained at a steady level in all groups analysed. Oikari & Soivio (1975) have compared the effects of immobilization techniques on some blood parameters including lactate in *Zoarces*. They used techniques similar to those in the first two groups with the present study. Lactate value of their stunned group was lower than in the present study; the reason is partly an arithmetical one. The corrected calculation factor used in this work (see 2.3) is higher than Biochemica Boehringer's calculation factor for human blood.

Oikari & Soivio (1975) preferred the stunning technique: "Because of the reduced muscular activity and shorter sampling time, blood from fish immobilized by stunning gave a better picture of the resting state than samples from anaesthetized fish". The discrepancy between those two results can partly be explained by the previous handling of the fish. In Oikari & Soivios (1975) experiments, fish were netted one after the other from a big aquarium into an anaesthesia bath during one day. Stress caused by repeated netting perhaps influenced their results. In the present study fish were kept in several smaller aquaria before sampling and only one fish was netted from same aquarium during one day. The effect of netting can be seen in the lactate levels in the muscle tissues in different groups (Table 1). It is at a significantly lower level in fish which have been rested for 10 hours in a respirometer chamber and then been anaesthetized without disturbance than in the group which was netted straight into the anaesthesia bath. This is in good agreement with the results of Soivio & Oikari (1976). They have shown that lactic acid level in pike rises steeply after handling and requires 12 h to return to normal. Stunning causes very strong muscular convulsions in this fish species, which is also reflected in the lactate content of embryos. This reaction is much stronger in *Zoarces* than in *Esox lucius* L. or in *Perca fluviatilis* L. The stunning technique was discarded because it has a greater effect on the embryos than anaesthesia of the parent fish.

The method of killing intra-ovarian embryos and young was chosen after comparing the lactate contents of the following groups:

A. Young weighed live and then immediately homogenized in cold perchloric acid.

B. Young killed by cutting their necks before weighing.

Results are given in Table 2.

Table 2. Lactate concentrations of *Zoarces* embryos and young at different ages and the effect of two immobilization techniques on their lactate levels. See Table 1 for explanations.

Killing method	Age	Lactate
Homogenizing (A)	0 hours	21.04±3.77 (21)
—"—	19 days	20.66±5.23 (11)
		$p < 0.05$
Cutting of neck (B)	14 days	27.04±5.85 (5)

The cutting technique was discarded in order to diminish the effect of handling. Age as such does not change the lactate level of youngs during the first 19 days. Embryos of the first group (age 0 hours) were taken straight from the ovarian cavity of anaesthetized parent fish for analysis.

3. Results and discussion

3.1. Oxygen consumption of young in brackish water

The oxygen consumption of the eel-pout young in group I (10—27 h old) was slightly, but not statistically, higher than in the youngest group 0 (0 hours old, see Fig. 2). An explanation for this difference can be found in the drastic change in the ambient medium: the young, which were living in a low oxygen concentration in the ovarian fluid were suddenly removed into Baltic water saturated with air. The acclimatisation state of a fish has an influence on its oxygen consumption (Beamish 1964). Both experimental groups showed, according to Fry's (1957) terminology, respiratory independence. In other words, they regulated their oxygen uptake over a wide range of environmental oxygen concentrations like adult *Zoarces* (unpublished). The critical oxygen level (Prosser 1973) was lower in group 0 (2 mg O_2/l) than in the older ones (group I: 3.5 mg O_2/l). Does the shift in critical oxygen level to the left depend on a change in the Hb oxygen binding capacity (foetal haemoglobin) was not studied.

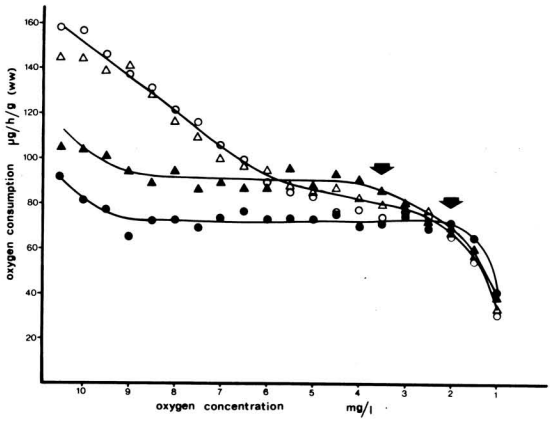

Fig. 2. The general course of oxygen consumption in *Zoarces viviparus* young at different ages in relation to the decreasing oxygen concentration of the water. Age is expressed as hours/days after sectioning. Group 0 = black dots (0 hour), Group I = black triangles (10—27 hours), Group II = open triangles (3—5 days) and Group III = open rings (14—19 days). The critical oxygen levels are indicated with arrows.

Brett (1972) discussed the problems of terminology for different levels of metabolic rate. He considers Fry's (1957) term 'routine metabolic rate' a useful one. According to Fry's definition, the routine metabolic rate of fish is independent of the oxygen pressure over a certain range of oxygen pressures, but can fluctuate widely under the influence of activity or excitement. This term is better suited to the measurements of oxygen consumption in the present study than 'standard metabolic rate' (Brett 1962), which must be measured at complete rest and in a post-absorptive state. The experimental arrangements of the present study meets the demands made for measurements of routine metabolic rate: temperature and stirring rate were controlled; darkness and quiet were maintained; young were acclimatised to the same temperature used in the respirometer runs. The oxygen consumption curves of the two oldest groups (3—5 and 14—19 days) show a relatively steeply decreasing slope at higher O_2 concentrations (10—6 mg O_2/l). The shape of the curve closely resembles the curves of active metabolic rate (cf. Fry 1957). Activity measurements were not made during respiratory runs, but no active swimming or excitement was observed. Perhaps the young become more easily disturbed as they become older. Disturbance caused by netting of the young into the respirometer chamber possibly caused increased activity and oxygen debt, which is repaid at the beginning of the respirometer run.

At the lower oxygen levels the consumption might be expected to go up as the work of ventilation increases (Beamish 1964, Shelton 1970). This transient elevation of oxygen uptake rate at lower ambient oxygen levels is not seen in our results. Webb & Brett (1972a) studied oxygen consumption in embryos of viviparous sea perch, *Rhacochilus vacca*. Their experimental arrangements differed from the present work. Both salinity and temperature were higher. At late gestation, the metabolic rate of embryos (222 mg O_2/kg/h) is about 3 times higher than that of *Zoarces* young, the temperature difference being 13 °C. The oxygen pressure in ovarian fluid changed when gestation proceeded from 36 mmHg to a minimum of 13.7 mmHg. At the same time the fluid volume increased (Webb & Brett 1972a and 1972b). The oxygen pressure in *Zoarces* embryotrophe varied at the end of gestation from 29.5 mmHg to 61.5 mmHg measured by Radiometer microtechnique (unpubl.). The volume was not measured, but it was noted that both the consistency and the volume varies greatly, and during late gestation the amount of embryotrophe is so small that it is often impossible to draw a sample into a capillary tube. This is an agreement with the considerations of several authors, that secretory activity of ovarian tissue is reduced or ceases during late gestation (Eigeman 1892, Turner 1938, Igrashii 1961, Wiebe 1968 cited by Webb & Brett 1972 b).

Because the oxygen consumption of a fish can be affected by its nutritional state (Fry 1957), the body weights of different experimental groups are given in Table 3. The water content of embryos and young was measured in order to estimate the osmotic effect of removal from the ovarian cavity to brackish water, which is hypotonic against the embryotrophe (Kristoffersson et al. 1973).

The young lost weight despite being fed every other day. They were observed to eat or at least to try to eat when fed, but perhaps suitable food was not found for them though several trials were done. It must be noticed that the ages given for each group are the ages at the beginning of the respiratory runs and the youngs were weighed at the end of experiments. In other words the young in group 0 had been in brackish water for 10—24 hours (duration of respiratory runs) when weighed. The greatest weight loss (13 %) happened during the first 2 days in brackish water, between groups 0 and I ($p < 0.01$). The water content of the young in group 0 was significantly ($p < 0.01$) higher than in intra-ovarian embryos, but began to decrease after the first day. This occurred at the same time as the weight loss, which was, however, greater than water loss.

Table 3. Body weight and percentage water content of *Zoarces viviparus* embryos and young at different ages. Means ± SDs are presented. In parentheses, the number of fish.

Group	Age	Weight (mg)	Water content (%)
In ovary		254.91±54.77 (21)	80.57±1.37 (21)
		$p < 0.01$	$p < 0.01$
0	0 hour	287.16±41.88 (67)	84.28±1.08 (67)
		$p < 0.01$	$p < 0.01$
I	10—27 hours	254.41±25.78 (40)	83.15±1.17 (40)
		NS	NS
II	3—5 days	241.57±40.37 (42)	82.20±2.21 (42)
		NS	$p < 0.05$
III	14—19 days	231.12±30.40 (57)	81.97±1.14 (57)

3.2. Lactate concentrations in intra-ovarian embryos and in different tissues of parent fish

Because the volume of embryotrophe decreases towards the end of the gestation period, it was assumed that the embryos of *Zoarces* must be able to tolerate hypoxia and make some use of the anaerobic pathways. In order to study the use of lactate pathway, hypoxic conditions for the embryos were arranged in the ovary in a closed system respirometer. The changes in the lactate concentrations of blood, muscle tissue and embryotrophe of parent fish were also measured for estimating the severity of hypoxia on the embryos.

No signs of hypoxia in parent fish can be seen before the oxygen concentration of the water has decreased to under 2 mg O_2/l, when the lactate concentration in blood and muscle tissue begins to increase (Table 4). The high lactate value in the muscle tissue of the control group (aerated) is the result of netting just before sampling (see Table 1).

In embryos and in embryotrophe, the lactate level increased ($p < 0.01$) much earlier, already at 4 mg O_2/l, than in the control group. The only possible source of excess lactate in embryotrophe is the embryos. This means that the embryos excrete at least part of their excess lactate via embryotrophe to the metabolic system of parent fish, where an adequate oxygen partial pressure still permits aerobic oxidation. It is known that at least some parasites excrete lactate into their hosts (Dejours 1975).

Lactate accumulates even more in embryos at lower water O_2 concentrations the upward leap being very pronounced ($p < 0.01$; 3—2 mg O_2/l) when the blood lactate concentration of the parent fish exceeds the lactate concentration of the embryotrophe and diffusion is blocked by a concentration gradient. The results show that embryos are able to tolerate severe hypoxia and make use of the lactate pathway. The experiments took 7—15 hours from 4 mg O_2/l to 1.5 mg O_2/l, depending on the size of the parent fish. In any case the embryos were very lively when removed to aerated water. Whether or not they need the lactate pathway in utero under natural conditions remains open because of the lack of lactate comparisons between the control group and 4 mg O_2/l.

Webb & Brett (1972a) described ovarian fluid as a food reserve as well as an oxygen reserve against temporary shortage. The scanty ovarian fluid of *Zoarces* can hardly be an oxygen reserve and therefore anaerobic metabolism is switched on.

Table 4. Lactate concentrations of blood, muscle tissue, embryotrophe and intra-ovarian embryos of *Zoarces viviparus* at decreasing ambient oxygen concentrations. See Table 1 for explanations.

O_2 mg/l	Blood	Muscle	E-trophe	Embryo
aerated	5.06±1.81 (7)	125.12± 9.92 (4)	1.47±0.83 (4)	21.04± 3.77 (21)
	NS	$p < 0.01$	$p < 0.02$	$p < 0.01$
4	3.95±1.00 (7)	94.37±31.69 (6)	6.70±2.93 (3)	74.10±28.55 (17)
	NS	NS		NS
3	4.61±0.33 (5)	84.55±21.12 (4)	5.78 (2)	85.90±25.61 (17)
	NS	NS		$p < 0.01$
2	6.48±2.63 (4)	108.98± 8.69 (3)	4.11±2.39 (3)	145.25±74.45 (10)
	$p < 0.05$	$p < 0.01$	NS	NS
1.5	14.85±6.43 (8)	164.51±16.83 (5)	7.11±2.44 (5)	183.48±37.11 (19)

3.3. Lactate concentrations in young in brackish water

On the basis of the previous oxygen consumption curves it was assumed that the lactate concentration in young ought to rise soon after the critical oxygen level (3.5 mg O_2/l). This assumption was wrong (Table 5). In all groups where the water O_2 content was above 1 mg/l and the oxygen uptake abowe 30 μg/h/g (w.w), lactate remained at a steady level. After this point it began to rise and reached a significantly ($p < 0.01$) higher level first after 1.5—2 hours, when the water O_2 content had reached 0.5 mg/l. An explanation for this unexpectedly high hypoxia tolerance might be that eel-pout young are still able to excrete lactate into the surrounding media after birth, as they do in the ovarian cavity (see 3.2). This assumption is also supported by the low lactate value of young in the group 0.5 mg O_2/l, which is less than 1/6 of the highest concentrations in the ovarian cavity (Table 4), where the small amount of embryotrophe restricts excretion. Excretion of lactate is an expensive way to repay oxygen debts, but after birth it is needed only in flight situations, because eel-pout young hardly meet low O_2 environments in these waters.

Table 5. Lactate concentrations in *Zoarces viviparus* young in relation to decreasing water oxygen concentration. See Table 1 for explanations.

O_2 mg/l	Age	Lactate
aerated	19 days	20.66±5.23 (11)
		NS
6	9 days	17.16±4.72 (7)
		NS
2	19 days	22.57±6.14 (9)
		NS
1	9 days	22.10±4.67 (6)
		$p < 0.01$
0.5	20 days	28.52±4.80 (6)

The oxygen consumption curve of the age groups used in the lactate analyses decreases steeply (Fig. 2) above 6 mg O_2/l. This was thought to be due to repayment of oxygen debt. Lactate analyses were unfortunately not made in this oxygen area. They would have thrown more light on the problem. Age as such does not change the lactate level during the first 20 days (see also Tables 2 and 4).

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