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Original article

Composition of the ovarian fluid in 4 salmonid species: *Oncorhynchus mykiss, Salmo trutta* f *lacustris, Salvelinus alpinus* and *Hucho hucho*

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Summary — The ovarian fluid of rainbow trout *(Oncorhynchus mykiss)*, charr *(Salvelinus alpinus)*, lake trout *(Salmo trutta* f *lacustris)* and Danube salmon *(Hucho hucho)* was analyzed for its inorganic and organic composition. The qualitative composition of the ovarian fluid of the investigated species was similar, but significant quantitative differences were found. The following components were determined: sodium 106.6–142.2 mmol/l, potassium 1.7–2.7 mmol/l, calcium 0.45–0.61 mmol/l, osmolality 256.4–291.6 mosmol/kg, pH 8.4–8.8, glucose 1 698–4 195 µmol/l, fructose 17–399 µmol/l, lactate 34–227 µmol/l, cholesterol 650–1 230 µmol/l, phosphatidylcholine 0.25–3.0 µmol/l, lysophosphatidylcholine 10–100 µmol/l, choline 0–1.1 µmol/l, protein 95.0–278.4 mg/100 ml. Arginine, cystine, glycine, histidine, lysine, proline, serine, tyrosine and valine were the free amino acids occurring in concentrations of more than 10 µmol/l. Activities of alkaline phosphatase (200–6 000 µmol substrate/l/h), lactate dehydrogenase (9–690 µmol substrate/l/h), β-D-glucuronidase (70–410 µmol substrate/l/h), proteases (140–215 µmol/l/h with collagen substrate, 25–90 µmol/h/l with gelatine substrate) and acid phosphatase (100–130 µmol substrate/l/h) were measured, but not the glucose-6-phosphate dehydrogenase and α-glucosidases activities.

ovarian fluid composition / *Oncorhynchus mykiss* / *Salmo tutta* f *lacustris* / *Salvelinus alpinus* / *Hucho hucho*

Résumé — **Composition du liquide cœlomique chez 4 espèces saumonées :** *Oncorhynchus mykiss, Salmo trutta* f *lacustris, Salvelinus alpinus, Hucho hucho. Les composants organiques et inorganiques du liquide cœlomique d'*Oncorhynchus mykiss, Salmo trutta* f lacustris, Salvelinus alpinus et Hucho hucho ont été caractérisés. Seules des différences quantitatives ont été mises en évidence entre espèces. Les composants suivants sont mesurés : sodium 106,6–142,2 mmol/l, potassium 1,7–2,7 mmol/l, calcium 0,45–0,61 mmol/l, osmolarité 256,4–291,6 mosmol/kg, pH 8,4–8,8, glucose 1698–4195 µmol/l, fructose 17–399 µmol/l, lactate 34–227 µmol/l, cholesterol 650–1230 µmol/l, phos-phatidylcholine 0,25–3,0 µmol/l, lysophosphatidylcholine 10–100 µmol/l, choline 0–1,1 µmol/l, pro-téines 95,0–278,4 mg/100 ml. Arginine, cystine, glycine, histidine, lysine, proline, sérine, tyrosine et valine étaient les acides aminés libres dont la concentration excédait 10 µmol/l. Des activités phosphatase alca-line (200–6000 µmol substrat/l/h), lactate déhydrogénase (9–690 µmol substrat/l/h), β-D-glucuroni-dase (70–410 µmol substrat/l/h), protéases (140–215 µmol/l/h avec le collagène, 25–90 µmol/l/h avec la gélatine) et phosphatase acide (100–130 µmol substrat/l/h) étaient détectables, mais la glucose-6-phosphate déhydrogénase et l'α-glucosidase étaient absentes.

compostion du liquide cœlomique / *Oncorhynchus mykiss* / *Salmo trutta* f lacustris / *Salvelinus alpinus* / *Hucho hucho*

INTRODUCTION

When gametes of externally fertilizing fishes are released into water, physiological alterations occur due to the changes in their surrounding milieu which result in the onset of sperm motility and in the activation of eggs (Jamieson, 1991). The short period of sperm motility and the closure of the micropyle limit the period during which fertilization is possible to only about 1 min (Billard *et al,* 1986). Therefore the fluids accompanying the gametes (seminal fluid, ovarian fluid) play an important role as storage medium of gametes to prevent activation *in vivo* in the gonads as well as under artificial conditions. While the seminal fluid is well investigated in many species (for review, Jamieson, 1991) data on the composition of the ovarian fluid are more limited. Holtz *et al* (1977) and Van Heerden *et al* (1993) analyzed the inorganic compounds of the ovarian fluid of *Oncorhynchus mykiss* for adaptation of fertilization solutions. Further, Matsubara *et al* (1985) investigated the protein composition of the ovarian fluid of *O mykiss* and *O keta* and Liley (1982) described pheromonal activities of the ovarian fluid. A fundamental analysis of the ovarian fluid composition and the possible interspecific differences is still lacking. Data on its composition could provide the basis for further work as the development of artificial extenders for storage and conservation of eggs or for quality control of ova.

Therefore, the present study gives a description of the composition of the ovarian fluid of Salmonidae. It investigates the main inorganic and organic (lipid, sugar, amino acid, enzyme activities) compounds in the rainbow trout *(O mykiss)* and compares it with 3 other salmonid species, the charr *(Salvelinus alpinus),* the lake trout (*Salmo trutta* f *lacustris*) and the Danube salmon *(Hucho hucho)* to see whether there exist species-specific differences.

MATERIALS AND METHODS

In the middle of their spawning time female rainbow trout (autumn strain) *(O mykiss)* and lake trouts (*S t f lacustris*) were obtained from fishery farms in Upper Austria and Salzburg, Danube salmon *(H hucho)* from a fishery farmer in Lower Austria and charr *(S alpinus)* were caught from wild populations in lake Grundlsee (Upper Austria). A characterization of the fish is given in table I. Fish used for the investigations were in a running stage; they were anaesthetized with MS 222 and the eggs stripped off by massage of their abdomen. Ovulation was evaluated by daily checking maturity of the fishes by trying them gently to strip off and sampling was done 24–36 h thereafter. Only egg batches were used which were well-rounded and unwrinkled in shape, transparent and did not stick together after having been stripped off. Special care was also taken to avoid contamination with urine, blood and broken eggs. The eggs were collected in a fine sieve and the ovarian fluid flowing through the sieve was collected *via* a funnel and filtered. Within 10 min after collection 1 ml portions of the filtrate were frozen in liquid nitrogen. Samples were thawed at room temperature and immediately analysed. For determination of metabolites, enzymatic activities were inactivated with 5% trichloroacetic acid immediately after thawing.

Determination of pH and osmolality

The pH value was determined with a mini-electrode using an external reference (3.0 M KCl). Osmolality was measured in an automatic micro-osmometer (Roebling).

Cation determination

Na^+, K^+ and Ca^{2+} were determined with electrodes supplied with ion sensitive membranes (Möller, Suisse) using external references. These references were 3 M KCl for Na^+ and Ca^{2+} and 3 M lithium actetate for K^+.

Thin layer chromatography (TLC)

For qualitative analysis of lipids, carbohydrates and free amino acids, sample pools and individ-

Table I. Characterization of the investigated species.

Species	Total length (cm)	Weight (g)	Spawning period	Volume eggs (ml)/ individual
Rainbow trout	35–55	520–1 510	October	300–400
Lake trout	40–65	580–3 100	December	400–600
Brown trout	30–40	420–730	December	120–150
Danube salmon	70–110	4 200–10 500	April	600–800

ual samples were used. Lipids were extracted from the ovarian fluid in a chloroform methanol solution (2:1) and silica gel plates were used for TLC. The solvent was chloroform, methanol and water (40:50:10) for polar lipids and petroleumether and diethylether (90:10) for non-polar lipids. TLC of carbohydrates was performed without extraction on silica-gel plates impregnated with 0.2 M sodium monophosphate. Chloroform, methanol and acetic acid (70:30:15) were used as solvent. For TLC of amino acids, untreated ovarian fluid was used. Analyses were performed on silica-gel plates with *n*-butanol, acetic acid and water (60:20:20) as solvent. Spots were determined with appropriate standard mixtures in various concentrations which were applied on the same plates as the samples.

Determination of enzyme activities

General methods for the determination of enzyme activities followed routine assays: acid phosphatase was determined according to Moss (Bergmeyer, 1985) and alkaline phosphatase according to Bretaudiere and Spillmann (Bergmeyer, 1985), both determinations were fixed-time methods. Measurements on the proteolytic activity of the ovarian fluid were performed according to assays of Weber, Tschesche and Macartney, and Tschesche *et al* (*in* Bergmeyer, 1985) with casein (Serva, cows' milk No 16 260), collagen (Sigma, acid soluble, calf skin C-3511) and gelatin (Sigma, porcine skin G-2500) as substrates and the liberated peptides were measured either by their absorbance at 280 nm or with Folin and Ciocalteu reagent. β-D-Glucuronidase activity was measured with the method of Stahl and Fishman (Bergmeyer, 1985) whereby phenolphthaleine glucuronide was the substrate. α-Glucosidases activities were investigated according to Dahlqvist (Bergmeyer, 1985)

using lactose and sucrose as substrates lactate dehydrogenase (LDH) activity determination followed the UV-spectrophotometric assay of Vassault (Bergmeyer, 1985) (substrate pyruvate). Glucose-6-phosphate dehydrogenase (G-6-PDH) activity was assayed using UV spectrophotometry (Deutsch, *in* Bergmeyer 1985) with glucose-6-phosphate as substrate; possibly occurring 6-phosphogluconate dehydrogenase activity was inhibited with 2,3-diphosphoglycerate.

The assays were standardized for fish tissue in aspects of substrate concentration, pH and reaction temperature whereby 3 samples of ovarian fluid of *O mykiss* and 3 samples of *H hucho* were used. The influence of temperature gradients (4°C, 20°C, 37°C) and of the following substrate concentrations and pH ranges were tested: acid phosphatase: 0.1–22 mmol/l *p*-nitrophenyl phosphate, (concentration in assay), pH 3-5; alkaline phosphatase: 0.1-22 mmol/l *p*-nitrophenyl phosphate, pH 9–11; β-D-glucuronidase: 0.2–20 mmol/l phenophthaleine glucuronide, pH 3.8–5; protease 0.01–0.75% collagen and gelatine, pH 6.5–8; lactate dehydrogenase activity: 0.1–20 mmol/l pyruvate, pH 6.8–8.0; glucose-6-phosphate dehydrogenase: 0.2–5 mmol/l glucose-6-phosphate, pH 7.0–8.0, α-glucosidases: lactose 5–50 mmol/l, sucrose 5–50 mmol/l, pH 5.5–8.0.

Finally, for analysis of the ovarian fluid all assays were performed at 20°C, further parameters are the following: acid phosphatase: 15.2 mmol/l *p*-nitrophenyl phosphate, pH 4.5, incubation 60 min, φ (ratio sample volume/total assay volume) = 0.2; alkaline phosphatase: 6.3 mmol/l *p*-nitrophenyl phosphate, pH 10, incubation 60 min, φ = 0.2; proteolytic activity: 0.25% collagen, 0.25% gelatine, pH 7.5, incubation 4 h, φ = 0.1; β-D-glucuronidase: 8 mmol/l phenolphthaleine glucuronide, pH 4.5, incubation 3 h, φ = 0.3; lactate dehydrogenase (LDH): 1.6 mmol/l pyruvate, pH 7.2, φ = 0.04. No α-glucosidase and glucose-6-

phosphate dehydrogenase (G-6-PDH) activities were found in samples used for standardization. Therefore the original assays of Dahlqvist and Deutsch (in Bergmeyer, 1985) (α-glucosidase: 28 mmol/l lactose and sucrose, pH 6.0, incubation 4 h, $\varphi = 0.1$; glucose-6-phosphate dehydrogenase: 3.3 mmol/l glucose-6-phosphate, pH 7.1, $\varphi = 0.05$) were used for investigation of the other samples.

Quantitative determinations of carbohydrates, lipids and proteins

Glucose, fructose, lactate, triglycerides, phosphatidylcholine and choline were determined using UV spectrophotometry at 339 nm, glucose with hexokinase and glucose-6-phosphate dehydrogenase according to Kunst et al (Bergmeyer, 1985), fructose with phosphoglucose isomerase, hexokinase and glucose-6-phosphate dehydrogenase according to Beutler (Bergmeyer, 1985), and lactate according to the assay of Noll (Bergmeyer, 1985). Phosphatidylcholine was determined with phospholipase C and choline kinase (Schiefer and Beutler, in Bergmeyer, 1985); for choline determination the same assay without hydrolysis of phosphatidylcholine was performed. Triglyceride determination followed the assay of Bucolo and David (1973) with lipase, glycerolkinase, pyruvate kinase and lactate dehydrogenase. Cholesterol was measured colorimetrically with cholesterol esterase and cholesterol oxidase as enzymes and 4-aminoantipyrine and p-hydroxybenzenesulfonate as colour reagents (Allain et al, 1974). To determine the occurrence of glycogen, ovarian fluid was incubated together with amyloglucosidase for 2 h at 37°C (Keppler and Decker, in Bergmeyer, 1985) and thereafter glucose levels were investigated as described. Protein was determined with the Lowry procedure. The amount of samples was adjusted to fall in the linear range of the assay ($\varphi = 0.05–0.25$).

Fertilization assays

To determine the fertilizability of egg batches used for analysis, fertilization assays were performed. After ovarian fluid was poured off, 25 ml egg batches were transferred into a 4°C cold fertilization solution modified after Scheerer and Thorgaard (1989) ($NaHCO_3$ 60 mM, glycine 20 mM, theophylline 5 mM, Tris 50 mM, pH 9) in a ratio of 1:2 (fertilization solution/eggs). Semen was stripped off from males, which were in a running stage and 4–5 samples were pooled in a ratio of 1:1. Semen (0.125 ml) was mixed with the eggs by gentle stirring. After 2–3 min about 50 ml well water was added and then the eggs were rinsed in well water. Fertilized eggs were incubated into flow incubators in the fishery farm of Kreuzstein (Salzburg). The eggs were hatched in spring water of 6.0–8.0°C, pH 7.8, conductivity 315 μS at 20°C, O_2 content 11.35 mg/l, Fe^{2+} 0 mg/l, NH_4^+ 0 mg/l, NO_2^- 0 mg/l, NO_3^- 1.42 mg/l, phosphate total 0.002 mg/l. Fertilization rate was determined by larvae reaching the eyed stage in relation to total number of eggs 28–30 d after fertilization in the rainbow trout, 35–38 d after fertilization in the lake trout and charr, and 25 d after fertilization in the Danube Salmon.

Statistics

All values represent mean ± standard deviation. Data were normalized by logarithmic transformation log ($a + 1$) and analysed with Student's t-test and Scheffe's multiple range test at a significance level of $p = 0.05$.

RESULTS

Inorganic components

Values for pH, osmolality, Na^+, K^+ and Ca^{2+} concentrations are listed in table II.

Organic components

Metabolites

As regards the carbohydrate composition, the ovarian fluid of the investigated species contained high glucose and low fructose levels (table II). No other carbohydrates could be detected by TLC. Glucose levels of the ovarian fluid were similar before and after incubation with amyloglucosidase. Lac-

tate occurred in the ovarian fluid of all species (table II). Among lipids, cholesterol, phosphatidylcholine and lysophosphatidylcholine were identified (table II). Triglycerides were absent, choline levels were fluctuating and could be determined in the ovarian fluid of some individuals only (table II).

The following free amino acids were detected by TLC: arginine ($\leq$ 10 µmol/l), cystine ($\leq$ 10 µmol/l), glycine ($\leq$ 10 µmol/l), histidine ($\leq$ 10 µmol/l), lysine ($\leq$ 10 µmol/l), proline ($\leq$ 20 µmol/l), serine ($\leq$ 20 µmol/l), tyrosine ($\leq$ 10 µmol/l) and valine ($\leq$ 20 µmol/l). There existed no intraspecific differences in the qualitative composi-

tion of free amino acids. The ovarian fluid of all species contained high amounts of protein.

Enzymatic activities

Standardization

Acid phosphatase, β-D-glucuronidase, LDH as well as proteases (collagenase, gelatinase) showed highest activity at 37°C, alkaline phosphatase at 20°C (fig 1). As regards the substrate concentrations, the maximal activity was reached at 6.3 mmol/l p-nitrophenyl phosphate for alkaline phosphatase

Table II. Composition of the ovarian fluid of rainbow trout *(O mykiss)* *(n* = 12), lake trout *(S t f lacustris)* *(n* = 11), charr *(S alpinus)* *(n* = 12) and Danube salmon *(H Hucho)* *(n* = 6).

Compound	Rainbow trout	Lake trout	Charr	Danube salmon
pH	8.4 ± 0.1[a]	8.6 ± 0.1[a]	8.6 ± 0.1[a]	8.8 ± 0.1[a]
Osmolality (mosmol/kg)	291.6 ± 12.9[b]	268.2 ± 7.7[c]	256.4 ± 16.2[c]	290.3 ± 4.4[b]
Na+ (mmol/l)	134.7 ± 7.4[d]	106.6 ± 10.7[e]	111.0 ± 13.6[e]	142.2 ± 11.7[d]
K+ (mmol/l)	2.7 ± 0.2[f]	1.7 ± 0.4[g]	1.9 ± 0.5[g]	2.2 ± 0.7[h]
Ca²⁺ (mmol/l)	0.45 ± 0.04[i]	0.58 ± 0.07[i]	0.61 ± 0.10[i]	0.6 ± 0.1[i]
Glucose (µmol/l)	1 798 ± 505[k]	4 195 ± 2 044[l]	2 365 ± 532[m]	1 700 ± 400[k]
Fructose (µmol/l)	53 ± 35[n]	32 ± 16[o]	17 ± 9[p]	399 ± 122[q]
Lactate (µmol/l)	34 ± 15[r]	67 ± 61[s]	26 ± 18[r]	227 ± 24[s]
Triglycerides (µmol/l)	0[t]	0[t]	0[t]	0[t]
Cholesterol (µmol/l)	970 ± 780[u]	650 ± 250[v]	890 ± 410[u]	1 230 ± 160[u]
Phosphatidylcholine (µmol/l)	0.25 ± 0.35[w]	1.30 ± 0.90[x]	3.00 ± 2.70[v]	0.28 ± 0.47[w]
Lysophosphatidylcholine (µmol/l) *	10–100	10–100	10–100	10–100
Choline (µmol/l)	0[z]	0.6 ± 0.7[z]	1.1 ± 2.1[z]	0.2 ± 0.3[z]
Alkaline phosphatase (µmol/l/h)	1 900 ± 900[A]	800 ± 200[B]	6 000 ± 1 200[C]	200 ± 20[D]
Acid phosphatase (µmol/l/h)	100 ± 40[E]	130 ± 10[E]	120 ± 8[E]	130 ± 30[E]
Collagenase (µmol tyrosin/l/h)	160 ± 105[FG]	140 ± 60[F]	215 ± 120[G]	160 ± 70[F]
Gelatinase (µmol tyrosin/l/h)	25 ± 15[H]	45 ± 41[H]	90 ± 77[I]	85 ± 45[I]
β-D-Glucuronidase (µmol/l/h)	154 ± 53[K]	410 ± 25[L]	70 ± 47[M]	183 ± 65[K]
LDH (µmol/l/h)	690 ± 190[N]	11 ± 5[O]	160 ± 65[P]	9 ± 3[Q]
α-Glucosidases (µmol/l/h)	0[R]	0[R]	0[R]	0[R]
G-6-PDH (µmol/l/h)	0[S]	0[S]	0[S]	0[S]
Protein (mg/100 ml)	117.3 ± 20.4[I]	146.8 ± 23.2[U]	95.0 ± 28.2[I]	278.4 ± 15.0[V]
Fertilization rate	74.4 ± 5.1[W]	88.7, ± 6.2[X]	65.0 ± 3.2[Y]	47.9 ± 5.4[Z]

Values represent mean ± SD; values superscripted by the same letter do not significantly differ ($p \leq 0.05$).
* Estimated on TLC plates.
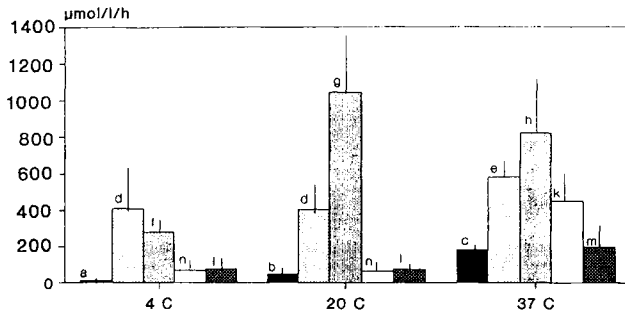
Fig 1. Influence of temperature of the ovarian fluid gelatinase, alkaline and acid phosphatase, β-D-glucuronidase and lactate dehydrogenase activity. Values represent mean ± SD ($n = 5$). Assays were performed at optimal substrate concentrations and pH (see *Materials and methods*). Values (= mean ± SD, $n = 6$) superscripted by the same letter do not significantly differ ($p \leq 0.05$). ■ gelatinase; ▨ β-D-glucuronidase; ■ alkaline phosphatase; □ acid phosphatase; ■ LDH.

(20°C) and at 15.2 mmol/l *p*-nitrophenyl phosphate for acid phosphatase (20°C) (fig 2). β-D-Glucuronidase activity was maximal at substrate concentrations of 8–14 mmol/l phenolphthaleine glucuronide (20°C) and decreased again at higher concentrations (fig 3). LDH activity was maximal at pyruvate concentrations of ≥ 0.8 mmol/l pyruvate (20°C) (fig 3) and also decreased at high concentrations of more than 20 mmol/l pyruvate. Proteolytic activity was found against collagen and gelatin, but not against casein. Optimal substrate concentration was at $\geq 0.2\%$ collagen and gelatine at 20°C (fig 4). The optimal pH ranges were the follow-

ing: alkaline phosphatase pH 9.5–10.5, acid phosphatase pH 4.5, β-D-glucuronidase pH 4.5, LDH pH 6.8–8.0, proteases pH 7.0–8.0. No differences in temperature-, substrate- and pH-dependence of enzymes were found between samples of rainbow trout and Danube Salmon.
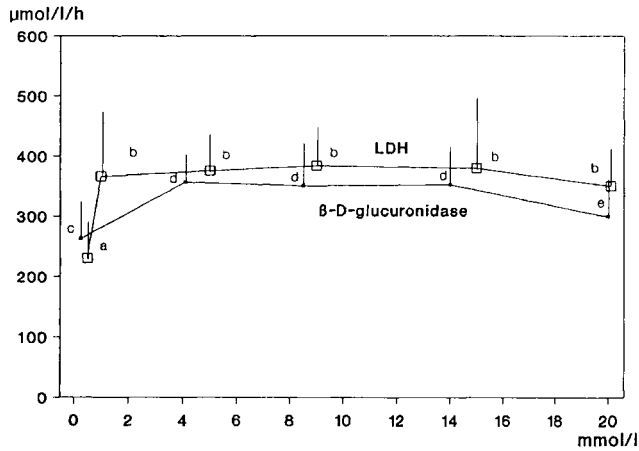
Enzyme activities of the ovarian fluid

Activities of alkaline and acid phosphatase, LDH, gelatinase, collagenase and β-D-glucuronidase were determined in the ovarian fluid of all investigated species (table II). No G-6-PDH and α-glucosidase activities could
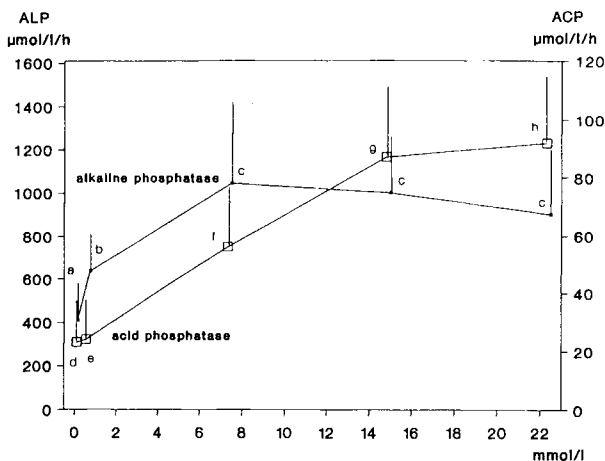

Fig 2. Influence of substrate concentrations of the ovarian fluid alkaline and acid phosphatase activity. Assays were performed at 20°C and at optimal pH (see *Materials and methods*). Values (= mean ± SD, $n = 6$) superscripted by the same letter do not significantly differ ($p \leq 0.05$).

Fig 3. Influence of substrate concentrations on the ovarian fluid lactate dehydrogenase and β-D-glucuronidase activity. Assays were performed at 20°C and at optimal pH (see *Materials and methods*). Values (= mean ± SD, $n = 6$) superscripted by the same letter do not significantly differ ($p \leq 0.05$).

Fig 4. Influence of substrate concentrations on gelatinase activity of the ovarian fluid. Assays were performed at 20°C and pH 7.5. Similar substrate optima are obtained for collagenase activity. Values (= mean ± SD, $n = 5$) superscripted by the same letter do not significantly differ ($p \leq 0.05$).
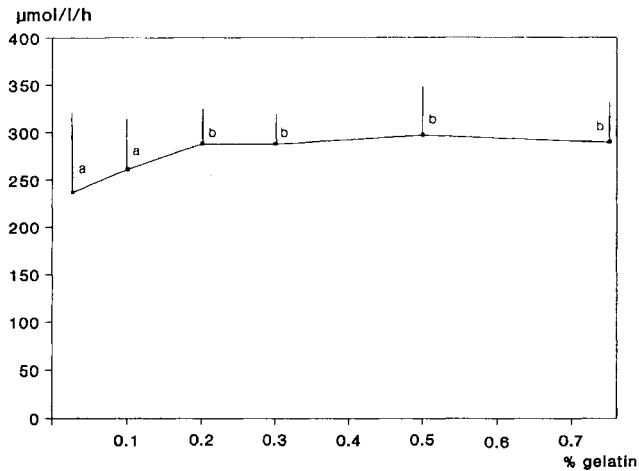
be measured. Activities of acid phosphatase were found only in the ovarian fluid of lake trout, Danube salmon and in some individuals of the charr (table II).

Fertilization assays

The fertilization results obtained with the egg batches used for analysis are listed in table II.

DISCUSSION

Origin of the ovarian fluid

In fishes mature oocytes that have been released out of the follicles discharge into the ovarian cavity (Nagahama, 1983). While in most species the ovarian cavity is in continuity with the oviduct, in the Salmonidae the ovaries have no oviduct and mature

oocytes must pass the body cavity to reach the genital porus *via* the genital papilla (Henderson, 1976; Nagahama, 1983; Guraya, 1986). Therefore the ovarian fluid could be of either ovarian or coelomic origin. We consider the ovarian fluid to be of ovarian origin for the following reasons. The ovarian cavity containing the mature oocytes consists of wide fluid-filled spaces (Van den Hurk and Peute, 1979) and the cells lining the ovarian cavity were found to be secretory active in the medaka *(Oryzias latipes)* (Yamamoto, 1963). This suggests that ovarian fluid is a specific secretion product of the ovaries released together with the eggs and for storage of eggs. In contrast to this, the cells of the coelomic epithelium are small and cuboidal, do not have secretive functions (Jew *et al*, 1971) and considerable amounts of ovarian fluid were never observed in the coelomic cavity (unpublished results).

Fertilization rates

The low intraspecific variations in fertilization results indicate that egg batches selected macroscopically by their appearance had a very uniform and high quality. Interspecific differences in the fertilization results therefore may also depend on semen quality which can show wide fluctuations (Jamieson, 1991).

Inorganic components

The analytical data on ionic composition and osmolality of the ovarian fluid here reported are similar to values obtained by Van Heerden *et al* (1993) and Holtz *et al* (1977). pH values are higher in our determinations than in measurements of Van Heerden *et al* (1993), but in accordance to Nomura (1964). The inorganic composition of the ovarian fluid of the Salmonidae is pre-

destined for storage of eggs and for prolonging the fertilization period during natural spawning as well as during artificial conditions. Osmolality is adequate to prevent activation, cortical reaction and swelling of eggs before fertilization (Billard *et al*, 1974; Billard, 1988). In fresh water activation of eggs starts immediately after immersion (Billard, 1988), the micropyle closes (Szöllösi and Billard, 1974), the egg swells and the egg shell hardens due to changes in the protein structure (Iuchi *et al*, 1991). Because of the low potassium levels and the alkaline pH (Morisawa, 1983; Billard, 1988), spermatozoa are activated when immersed in the ovarian fluid. As ovarian fluid is also isoosmolar to the sperm cells the motility is prolonged in comparison to fresh water similar to in artificial fertilization solutions (Billard *et al*, 1974). Fertilization is highest in alkaline media and strongly decreases by acidification (Billard *et al*, 1974). Therefore the alkalinity of the ovarian fluid may be important to stabilize fertilization under natural conditions and especially in acidic waters.

Organic components

Metabolites

The organic composition of the ovarian fluid of the investigated species qualitatively and quantitatively differs from that of the seminal fluid (Scott and Baynes, 1980; Piironen and Hyvärinen, 1983; Lahnsteiner *et al*, 1993). In comparison to the seminal fluid it is characterized by higher levels of protein, free amino acids, glucose, lactate, phospholipids and cholesterol and by the absence of triglycerides. During oogenesis glucose and amino acids are incorporated in developing oocytes *via* the follicular epithelium (Riehl, 1978; Nagahama, 1983; Guraya, 1986) and used for the synthesis of yolk components. However, it is uncertain whether mature oocytes can take up extraovarian compo-

nents and consequently if glucose and amino acids and also the anaerobic glycolytic end product lactate are associated with the metabolism of mature eggs. No glycogen is present in the ovarian fluid as glucose levels are similar before and after incubation with amyloglucosidase. Absence of glucose-6-phosphate dehydrogenase activity also indicates that gluconeogenetic processes (pentose phosphate route) do not occur in the ovarian fluid. Protein composition of the ovarian fluid was characterized in several studies (Nomura, 1964; Matsubara *et al*, 1985) and a positive influence on sperm motility was also discussed (Yoshida and Nomura, 1972).

Enzymes

The activities of LDH, protease, β-D-glucuronidase and acid and alkaline phosphatase increase with temperature as generally does the metabolism of fish. Temperature dependences and optima are not uniform for the investigated enzymes. While LDH, protease, β-D-glucuronidase and acid phosphatase have their maxima at 37°C, the maximal activity of alkaline phosphatase is at 20°C. Further no differences in activities of acid phosphatase, β-D-glucuronidase and LDH are observed at 4°C and 20°C. To be still in a physiological temperature range for salmonid fish tissue enzyme activities were determined at 20°C. Alkaline phosphatase is the most active of the investigated enzymes of the ovarian fluid, followed by lactate dehydrogenase, β-D-glucuronidase, protease and acid phosphatase while glucose-6-phosphate dehydrogenase activity and α-glucosidase activities are completely missing. Similar qualitative enzyme patterns are also found in the seminal fluid of these species, the quantities however are completely different. LDH is distributed in the cytoplasmic and mitochondrial fraction of cells and may be liberated by the breakdown of cells, *eg,* the rup-

ture of follicular epithelium when mature eggs are released or by the atresis of follicles. Alkaline phosphatase is derived in degenerating phospholipid metabolism, *ie* in the generation of free choline out of phosphatidylcholine. Proteolytic enzymes play an important role in the lysis of the follicular epithelium during evacuation of mature oocytes (Iwamatsu and Otha, 1977; Oshiro and Hibiya, 1982; Berndtson and Goetz, 1990) and may be liberated during this process. They also occur in the multivesicular bodies of the oocytes (Sire *et al*, 1994). β-D-Glucuronidase is generally a lytic enzyme involved in hydrolysis of β-glucuronides to glucuronic acid which represent a shuttle system for hydrophobic metabolites.

Interspecific differences

The ovarian fluid of the 4 investigated species reveals no qualitative differences in its composition. With exception of pH, quantitative species specific differences are found in inorganic as well as in organic components. There are also significant quantitative interspecific differences between these species cultured in fish farms (rainbow trout, lake trout and Danube salmon). Fluctuations in organic components of the ovarian fluid generally are higher than those of inorganic ones and may indicate dynamics in metabolism. Interspecific comparisons on egg physiology and metabolism in Salmonidae have not been performed until now. As regards egg morphology, Riehl (1980) found the micropylar structure to be a species-specific characteristic in salmonid eggs.

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