Possible Factors Responsible for the High Variation in the Cholecalciferol Contents of Fish

Pirjo Mattila,* Vieno Piironen, and Riitta Haapala

Department of Applied Chemistry and Microbiology, University of Helsinki, P.O. Box 27, FIN-00014 University of Helsinki, Finland

Timo Hirvi and Esko Uusi-Rauva

National Veterinary and Food Research Institute, P.O. Box 368, 00231 Helsinki, Finland

Previously a high variation was found in the cholecalciferol (vitamin D_3) contents of fish samples representing the same species but caught in different habitats in Finland. The present study was designed to provide reasons for this variation. The influences of various factors, e.g. weight, age, and sex of the fish as well as the cholecalciferol content of zooplankton, on the cholecalciferol contents of fish flesh were studied. Perch, vendace, and pike samples were caught in three different types of lakes, and perch, Baltic herring, and pike samples were caught in the Baltic Sea. Zooplankton samples were caught in all four habitats. The cholecalciferol determinations were performed with HPLC after purification with preparative HPLC. No consistent relationship between the weight, sex, or age of the fish and cholecalciferol content was found. According to this study, it is possible that diet is a likely factor which causes the variation in the cholecalciferol contents of the fish.

Keywords: *Vitamin D; cholecalciferol; fish; food; HPLC*

INTRODUCTION

Studies aiming at clarifying the origin of vitamin D in fish have led to contradictory conclusions. It has been assumed that there are three possible mechanisms: photochemical biosynthesis, nonphotochemical biosynthesis, and accumulation of vitamin D from the food supply. Bills (1927) concluded that photochemical biosynthesis is improbable. He suggested that fish might be able to synthesize vitamin D by a nonphotochemical process. Blondin et al. (1964) supported the idea of nonphotochemical synthesis of vitamin D in fish. On the other hand, based on more recent studies both photochemical and nonphotochemical formations are unlikely at least in the case of several fish species (Sugisaki et al., 1974; Takeuchi et al., 1991).

Evidence for the assumption that a food supply derived from plankton can be the origin of vitamin D in fish has been presented by Copping (1934), Takeuchi et al. (1991), and Sunita Rao and Raghuramulu (1994). According to these papers, vitamin D is probably synthesized in plankton by solar radiation. High contents of cholecalciferol (vitamin D_3) in fish liver may therefore be a consequence of accumulation of this vitamin in food chains starting from plankton (Takeuchi et al., 1991; Sunita Rao and Raghuramulu, 1994). Furthermore, the findings that e.g. cultivated rainbow trout (*Oncorhynchus mykiss*; Barnett et al., 1979) and channel catfish (*Ictalurus punctatus*; Lovell and Yin-Pen, 1978; Brown and Robinson, 1992) need vitamin D in their diet for maximum growth suggest that the role of food supply as a source of vitamin D for fish is important.

* To whom correspondence should be addressed (telephone +358 9 7085240; fax +358 9 7085475; e-mail Pirjo.Mattila@Helsinki.Fi).

Previously, a high variation has been found in the cholecalciferol contents of fish flesh samples representing the same fish species when the samples were caught in three different locations in Finland (Mattila et al., 1995a). This variation was not caused by the varying fat contents of the fish. In addition, only a minor variation was found between seasons. Because fish and fish products are one of the most important dietary sources of vitamin D for humans it was decided to study the reasons for the high variation. In order to study relationships between different factors and cholecalciferol contents, samples of fish and zooplankton were taken from four habitats whose quality of water differed in their chemical and physical parameters. The fish samples were pooled according to weight and habitat before cholecalciferol analysis. Furthermore, the cholecalciferol contents of pike individuals whose sex, weight, and age were known were studied.

MATERIALS AND METHODS

Choice of the Habitats. Fish and zooplankton samples were taken from the Baltic Sea and different types of lakes: Saimaa/Puruvesi, Vesijärvi, and Haarajärvi. The lakes are located in southern Finland. Lake Saimaa/Puruvesi is an oligotrophic lake with clear water, Lake Haarajärvi is an oligotrophic lake with brown water and a high concentration of humic substances and Lake Vesijärvi is an eutrophic lake with turbid water. The physical and chemical parameters of the water of the habitats were obtained from the Finnish Game and Fisheries Research Institute and from the Water Quality Data Base coordinated by the Finnish Environment Institute.

Fish Samples. Samples of perch and vendace were caught during the autumn of 1995 in the three lakes. Samples of two marine fish species (Baltic herring and perch) caught in Baltic Sea were also obtained in autumn 1995. With the exception of vendace caught in Lake Vesijärvi (one weight class), two weight classes of each fish species were purchased from each habitats, and there were at least 10 individuals belonging to

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Table 1. Cholecalciferol (D_3), Fat and Dry Matter Contents in Fish Samples Representing Two Weight Classes Caught in the Baltic Sea, Lake Saimaa/Puruvesi, Lake Haarajärvi, and Lake Vesijärvi, and Ergocalciferol (D_2) and Cholecalciferol (D_3) Contents in the Corresponding Plankton Samples

habitat	av wt/variation range (g)	D_2 (μg/100 g)[a]	D_3 (μg/100 g)[a]	fat (%)	dry matter (%)
Baltic Sea					
plankton		2.7	10.5		
perch[b]	230/168−338	nd	24.7	1.1	20.1
perch	53/39−62	nd	16.3	0.8	18.6
Baltic[c] herring	150/112−189	nd	31.9	15.4	31.8
Baltic herring	29/−	nd	22.5	5.7	23.0
Lake Saimaa/Puruvesi					
plankton		2.5	6.9		
perch	126/98−180	nd	4.4	0.8	21.1
perch	61/32−81	nd	2.2	0.7	21.5
vendace[d]	31/26−36	nd	7.3	6.9	26.3
vendace	9/−	nd	11.4	2.9	22.1
Lake Haarajärvi					
plankton		3.8	3.8		
perch	131/85−165	nd	5.9	0.9	20.2
perch	42/23−75	nd	7.5	1.0	19.7
vendace	86/78−105	nd	2.6	5.0	25.1
vendace	48/36−64	nd	2.4	5.6	24.6
Lake Vesijärvi					
plankton		1.3	1.5		
perch	188/127−263	nd	1.6	0.7	19.6
perch	43/−	nd	3.8	0.7	18.3
vendace	49/−	nd	16.8	4.9	25.0

[a] Contents are μg/100 g of dry matter for plankton samples and μg/100 g of fresh weight for fish samples. [b] *Perca fluviatilis*. [c] *Clupea harengus membras*. [d] *Coregonus albula*.

each weight classes (Table 1). In the laboratory the fishes were skinned (except for Baltic herring and vendace), filleted, and pooled according to their species, habitats, and weight classes so that one fillet of each fish was added to the pool. The pooled samples were homogenized using a blender (Moulinex), vacuum packed in small portions (50−100 g), and stored at −20 °C until analyzed for cholecalciferol, total fat, and dry matter.

Variation in the cholecalciferol contents of three perch individuals (caught in Lake Vesijärvi) and three vendace individuals (caught in lake Haarajärvi) of similar weights was studied in autumn 1995. Both fillets of the fish individuals were homogenized, vacuum-packed, and stored as described above until analyzed for cholecalciferol. In summer 1996, a more comprehensive study was carried out to study the variation in the cholecalciferol contents of pike individuals. Pike samples (15 individuals/habitat) were caught in Lake Vesijärvi, Lake Saimaa/Puruvesi, Lake Haarajärvi, and brackish waters of the Baltic Sea. One fillet of each individual fish was homogenized and handled as described above. In addition to determining cholecalciferol, pike samples were examined for weight, age, and sex.

Zooplankton Samples. The samples of zooplankton were caught by a plankton net in Lake Vesijärvi, Lake Saimaa/Puruvesi, Lake Haarajärvi, and Baltic Sea in autumn 1995. The genera of the species was determined by microscopic examination. The zooplankton from Lake Vesijärvi (dry matter 1.3%) and Lake Haarajärvi (dry matter 0.3%) included species belonging to the genera *Rotatoria*, *Cladocera*, *Copepoda*, *Calanoida*, and *Cyclopoida*. The same genera except the *Rotatoria* were represented in zooplankton from Lake Saimaa/Puruvesi (dry matter 0.8%). The zooplankton from Baltic Sea (dry matter 1.9%) included the genera *Ciliata*, *Rotatoria*, *Cladocera*, *Copepoda*, and *Calanoida*. The samples were stored at −20 °C until analyzed for cholecalciferol, ergocalciferol (vitamin D_2), and dry matter.

Cholecalciferol Determinations. A method which has been previously described (Mattila et al., 1995a) was used for the fish samples. The method involves saponification, extraction, purification using semipreparative normal-phase HPLC, and quantification with reversed-phase HPLC using an internal standard (ergocalciferol) method. A modification of the method (Mattila et al., 1995a) was made in that the semipreparative step was carried out according to Mattila et al. (1992). The quality of the analytical data was monitored with recovery tests (recoveries varied from 91 to 110%) and repeatability tests by running an in-house reference sample (perch from Lake Saimaa/Puruvesi in autumn 1995 and pike from Baltic Sea in summer 1996). The coefficient of variation was <4% for both in-house reference materials.

The cholecalciferol and ergocalciferol contents of the zooplankton samples were determined using the method of Mattila et al. (1995b) which involves saponification, extraction, normal-phase HPLC purification, and reversed-phase HPLC purification. Quantification was performed with two reversed-phase columns connected in series. Quantification was based on the external standard method with recovery corrections (recoveries varied from 86.5 to 90.4%). The amount taken for analysis varied from 10 to 30 g. The results were calculated as μg/100 g of plankton (dry matter).

Fat and Moisture Determinations. The moisture of fish and zooplankton samples was determined by drying at 100 ± 2 °C to a constant weight (AOAC 952.08, modified; AOAC, 1990). An AOAC method was also used to determine the fat content of fish (AOAC 948.15; AOAC, 1990).

RESULTS AND DISCUSSION

The methods developed and validated earlier (Mattila et al., 1995a,b) were used for cholecalciferol (fish samples) and cholecalciferol and ergocalciferol (plankton samples) determinations. The cholecalciferol and ergocalciferol peaks separated well from each other and from the matrix, and the compounds could be quantified reliably (Figures 1 and 2). The recoveries and repeatabilities of the method for the fish were good. Because plankton samples contained both cholecalciferol and ergocalciferol (Figure 2), the external standard method with recovery corrections was used. The recoveries were good also for plankton samples but repeatabilities were somewhat poorer due to the small quantities of the samples of low dry matter content.

This study confirmed the conclusion reached in the previous report (Mattila et al., 1995a) that there is no correlation between the fat and cholecalciferol contents in fish. Thus, the assumption that only fat fish is good

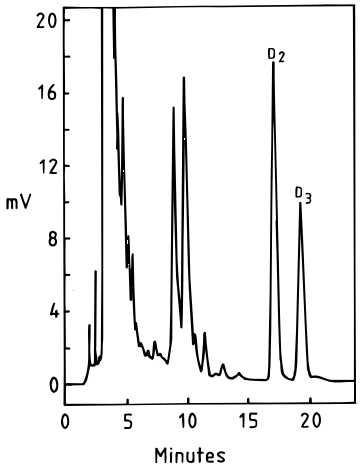

Figure 1. Analytical HPLC chromatogram of a vendace sample caught in Lake Haarajärvi (D_2, ergocalciferol, int. std; D_3, cholecalciferol).

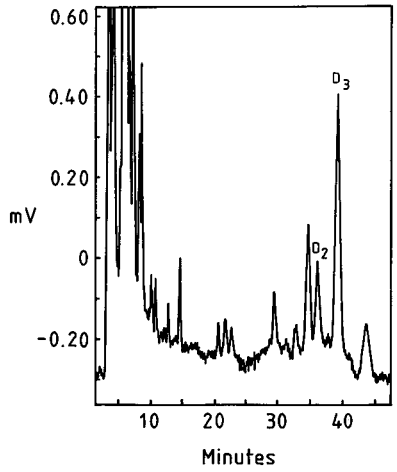

Figure 2. Analytical HPLC chromatogram of zooplankton sample caught in the Baltic Sea (D_2, ergocalciferol; D_3, cholecalciferol).

source of vitamin D was proved to be untenable. In the first place, however, this study was designed to highlight some other factors that might affect the cholecalciferol contents in fish. These factors were the individual properties of the fish (sex, age, weight), cholecalciferol contents in plankton, and chemical and physical properties of the water of the habitats.

Table 2. Cholecalciferol (D_3) Contents in Vendace and Perch Individuals of Similar Weights

	wt (g)	D_3 (μg/100 g)
Lake Haarajärvi vendace	99	1.0
	98	2.1
	98	1.1
Lake Vesijärvi perch	237	1.1
	263	0.2
	262	1.6

Significance of Individual Properties of the Fish. The body weight of the fish has been considered as one factor which might affect the vitamin D contents in fish liver (Takeuchi et al., 1987), although contradictory results have also been presented (Pugsley, 1939a,b). In the present study, no clear relationship between body weight and cholecalciferol contents in the flesh of fish was found (Table 1, Figures 3−6). In addition, fish individuals having similar weights differed in their cholecalciferol contents (Table 2).

There was a high variation in cholecalciferol contents between pike individuals. The vitamin levels in pikes from Lake Vesijärvi, Lake Haarajärvi, Lake Saimaa/Puruvesi, and Baltic Sea varied 0.4−2.0, 0.6−1.6, 0.9−11.9, and 1.3−5.9 μg/100 g, respectively. No relationships between weight, age, or sex and cholecalciferol contents were found (Figures 3−6). As seen in Figures 3−6, there were differences in the cholecalciferol levels in pikes caught in the four habitats. The finding that habitat strongly affects the cholecalciferol levels in fish has also been reported earlier (Mattila et al., 1995a). In addition to the variation between habitats, there was high variation in the cholecalciferol contents between pike individuals.

Influence of Cholecalciferol Contents in Zooplankton. Cholecalciferol contents in zooplankton caught in the Baltic Sea, Lake Saimaa/Puruvesi, Lake Haarajärvi, and Lake Vesijärvi were 10.5, 6.9, 3.8, and 1.5 μg/100 g, respectively. The contents and variation were lower in the case of ergocalciferol (1.3−3.8 μg/100 g, Table 1). The magnitude of these vitamin contents was similar to the results of Takeuchi et al. (1991). On

Weight (g)	Age (yr.)	Sex	Vitamin D3 content (μg/100 g)
270	2	m*	3.9
325	4	f*	2.5
331	3	f	2.9
396	3	f	6.9
528	5	m	9.4
552	3	m	9.9
569	5	m	7.3
727	5	f	9.5
796	5	m	11.9
810	5	m	11.5
1007	8	m	3.0
1112	5	f	6.8
1116	5	f	3.7
1164	5	m	0.9
1465	4	f	1.4

* f = female
m = male

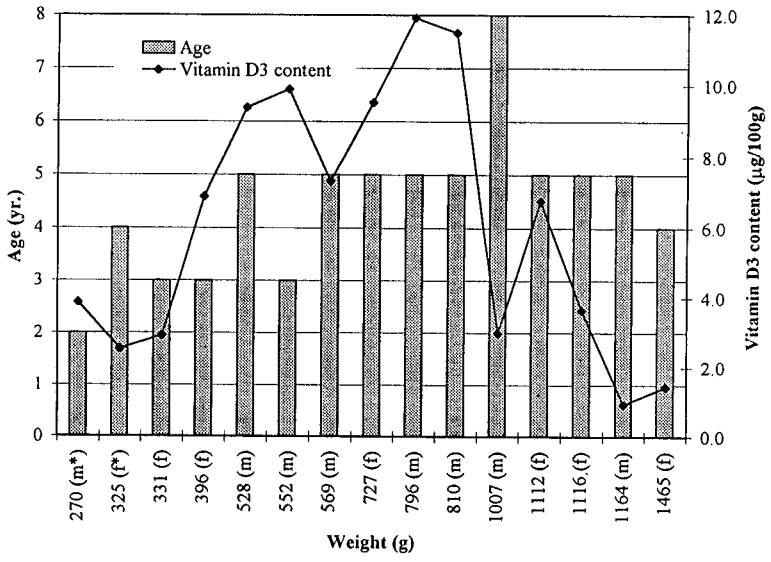

Figure 3. Influence of weight, age, and sex on the cholecalciferol contents in pike (*Esox lucius*) caught in Lake Saimaa/Puruvesi.

Weight (g)	Age (yr.)	Sex	Vitamin D3 content (μg/100 g)
270	5	m*	1.6
369	5	m	1.1
537	6	f*	1.0
560	7	m	0.8
570	7	m	0.9
626	5	f	0.7
1000	7	m	0.8
1033	7	m	0.7
1109	8	m	1.0
1111	9	m	1.4
1224	7	f	0.7
1519	8	m	1.2
1584	7	f	0.7
1661	10	m	0.9
1668	8	f	0.6

* f = female
m = male

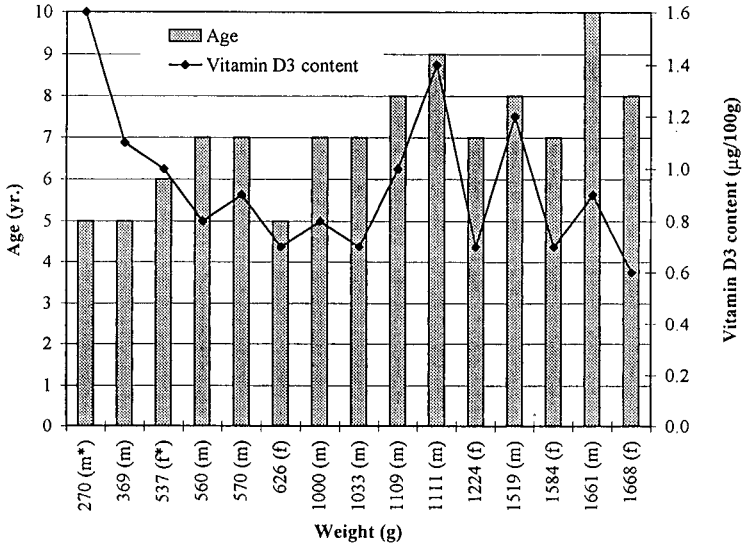

Figure 4. Influence of weight, age, and sex on the cholecalciferol contents in pike (*Esox lucius*) caught in Lake Haarajärvi.

Weight (g)	Age (yr.)	Sex	Vitamin D3 content (μg/100 g)
441	5	m*	0.7
468	5	f*	0.6
469	5	f	0.9
471	5	m	1.6
962	7	m	1.7
1000	7	m	0.4
1001	5	f	0.7
1263	8	m	1.2
1313	7	f	1.7
1394	7	f	1.4
1455	8	f	1.5
1457	7	f	1.6
1640	8	f	1.6
1700	11	f	1.0
2052	8	f	2.0

* f = female
m = male

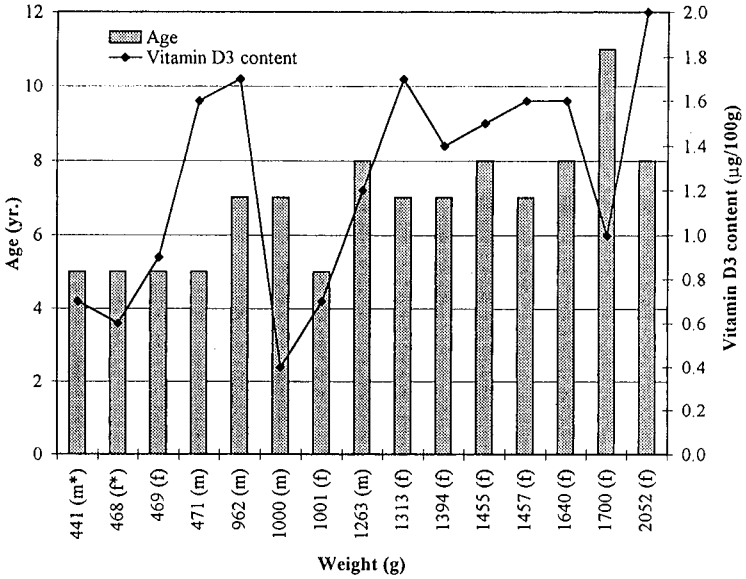

Figure 5. Influence of weight, age, and sex on the cholecalciferol contents in pike (*Esox lucius*) caught in Lake Vesijärvi.

Weight (g)	Age (yr.)	Sex	Vitamin D3 content (μg/100 g)
446	5	m*	2.3
529	3	f*	1.8
664	5	m	5.9
690	4	f	1.5
692	5	f	3.4
707	4	m	3.8
744	5	f	2.6
813	7	m	1.3
823	6	m	2.5
864	5	m	1.7
1279	5	f	1.5
1302	5	f	1.7
1325	11	m	2.4
1355	7	f	2.3
1371	6	f	1.8
2006	7	f	1.9

* f= female
m = male

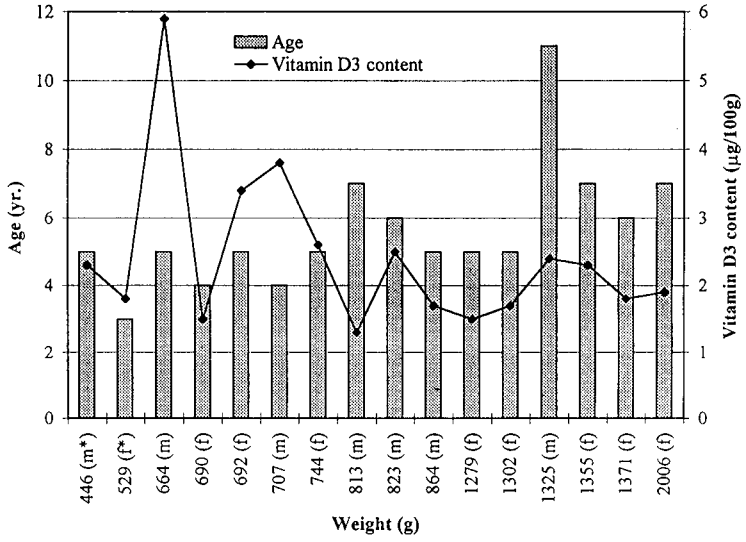

Figure 6. Influence of weight, age, and sex on the cholecalciferol contents in pike (*Esox lucius*) caught in the Baltic Sea.

the basis of the results of this study, no clear correlation was found between the cholecalciferol contents of the zooplankton and the fishes. A slight relationship was, however, seen. The fishes caught in Baltic Sea and Lake Saimaa/Puruvesi, where zooplankton contained the highest amount of cholecalciferol, contained quite

Table 3. Some Physical and Chemical Parameters of the Water of Lake Vesijärvi, the Baltic Sea, Lake Haarajärvi, and Lake Saimaa/Vesijärvi (Year 1995) As Derived from the Finnish Game and Fisheries Institute and from the Water Quality Data Base Coordinated by the Finnish Environment Institute

	color unit (mg Pt/L)	turbidity (FTU)	total N (μg/L)	secchi disk transparency (m)
Lake Vesijärvi	10−30	0.8−5	380−1000	1.6−3.8
Baltic Sea	5	0.33−1.3	200−360	1.5−3.8
Lake Haarajärvi	65−70	*a*	382−487	*a*
Lake Saimaa/ Puruvesi	5	0.3	230−390	7.8

[a] Information missing.

high amounts of this vitamin (Table 1; Figures 3−6; see also Mattila et al., 1995a). However, the diet of fish is very diverse and varies in response to many factors (e.g. season and the size of the fish; Linko et al., 1985). Using this kind of comparison, it is difficult to clearly indicate whether the high cholecalciferol contents in the flesh of fish are due to nutritional conditions. In addition, besides quality, the magnitude of the food supply may also affect the vitamin D status of the fish. On the other hand, the moderately high contents of cholecalciferol in plankton support the possibility that it might be the origin of vitamin D in fish.

Zooplankton samples caught in all the habitats (Lake Vesijärvi, Lake Haarajärvi, Lake Saimaa/Puruvesi, and Baltic Sea) contained in addition to cholecalciferol also ergocalciferol. Ergocalciferol was not, however, found in the fish samples which gives reason to believe that the bioavailability of ergocalciferol in fish is lower than that of cholecalciferol. The studies of Andrews et al. (1980), Takeuchi et al. (1991), and Barnett et al. (1982) support this assumption. Barnett et al. (1982) and Andrews et al. (1980) found that ergocalciferol is less potent than cholecalciferol in supporting the weight gain of channel catfish and rainbow trout. According to Takeuchi et al. (1991), ergocalciferol appears to be stored less efficiently than cholecalciferol in bastard halibut (*Paralichthys olivaceus*) and carp (*Cyprinus caprio*). In the rainbow trout, dietary cholecalciferol was estimated to be 3.27 times as potent as ergocalciferol (Barnett et al., 1982).

Influence of Chemical and Physical Properties of the Water of the Habitats. Whether the clearness and transparency of the water of the habitat affect the cholecalciferol contents of zooplankton and fishes was also examined. It was assumed that vitamin D found in zooplankton originates from the solar irradiation of provitamins D and that the more clear and transparent the water is, the more provitamins D present in plankton can be irradiated to form vitamin D. There was some confirmation for this assumption. The cholecalciferol contents of fresh water plankton caught in the habitat whose water was most transparent (Lake Saimaa/Puruvesi, Table 3) were highest (Table 1). Also the cholecalciferol contents of fish caught in this habitat were high (Table 1, Figures 3−6; Mattila et al., 1995a).

Conclusions. There was no consistent relationship between the weight of the fish (perch, vendace, pike, and Baltic herring) and their cholecalciferol content. There was also no relationship between the age and sex of the fish (pike) and the cholecalciferol content. According to this study, it is possible that diet is a likely factor which causes variation in cholecalciferol contents of fish.

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