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Structure of and energy reserves in the liver of wild and cultured yellowtail flounder, *Limanda ferruginea*

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Abstract Knowledge of the structure and energy reserves, in the liver of commercially important fish species, is important in understanding metabolic processes and in assessing the impact of potential environmental physical and chemical stressors in both wild and cultured stocks. The present study investigated the microscopic morphology and histochemistry (total and neutral lipids, glycogen) of liver tissue of wild (3⁺) and cultured (1⁺) sexually immature female and male yellowtail flounder (*Limanda ferruginea* Storer), sampled in late April 2001. Hepatosomatic indices [HSI: (liver weight/body weight–liver weight)×100] of cultured fish were significantly higher than those of wild fish. Females in the cultured group had significantly lower HSIs than males. The liver of both wild and cultured *L. ferruginea* was interspersed with pancreatic tissue. The main components of the liver tissue were irregular cords of hepatocytes arranged in tubules which surrounded vascular sinusoids. The hepatocytes contained an abundance of lipid, much of which appeared to be neutral lipids, in both sexes of the cultured fish. Total and neutral lipid droplets were larger, and the area occupied by these droplets was significantly greater in both cultured females and males compared to the wild fish, suggesting lipidosis in the cultured fish. In the cultured fish these differences were sex-dependent, the females having significantly more total and neutral lipids in hepatocytes than the males. This suggests a potentially greater storage capacity in females and/or a higher lipid metabolism in males. There were no statistically significant differences in glycogen content between the cultured and wild fish, or between the sexes in both sampling groups.

Introduction

Yellowtail flounder (*Limanda ferruginea*, formerly *Pleuronectes ferrugineus*) is a benthic species of the family Pleuronectidae, found along the North American Atlantic coast, mainly at depths around 60 m at temperatures of 3–5°C (Langton 1979; Pitt 1983). Before the fishing moratorium established in 1992, this groundfish species was important commercially on the Grand Banks of Newfoundland, and its decline, along with the decline of other fish stocks, is a concern. It is well known that the marine ecosystem is increasingly exposed to a wide variety of physical and chemical stressors, such as those produced by oil and gas exploration and exploitation, which may harm fisheries through direct and indirect destruction of fish stocks. The level of contaminants, such as polycyclic aromatic compounds, in sediments correlates with increased prevalence of hepatic lesions in fish, particularly in species of Pleuronectidae (Myers et al. 1998, and references therein). *L. ferruginea* could be an excellent candidate in monitoring programs to protect the quality of the stocks (Collier et al. 1998) and an important species for the aquaculture industry (Brown et al. 1995). Hatchery conditions (i.e. diet, temperature, population density, interspecific competition) may also affect several physiological processes such as growth and reproduction in fish (Blaxter 1975; Herlinger and Friars 1992; Whalen 1999).

Age and size of wild yellowtail flounder, at sexual maturity, vary depending on the geographic region, year class, and sex. Males (<26 cm) and females (32–40 cm) from southern areas (off New England) mature at ages 2–4 years, while males and females from northern areas (Grand Banks of Newfoundland) mature at ages 4.4–5.5 years (25–30 cm) and 6.1–6.8 years (34 cm), respectively (Royce et al. 1959; Morgan and Walsh 1997). Sexually mature females and males develop a stock of vitellogenic oocytes and spermatozoa, respectively, during the winter months. The peak spawning period is in mid-May for fish off New Eng-

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land (4.9–12.3°C) and during the latter half of June for fish on the Grand Banks of Newfoundland (2–4°C) (Royce et al. 1959; Pitt 1970; Howell 1983; Clearwater 1996; Manning and Crim 1998). Cultured yellowtail flounder sexually mature earlier and grow faster than wild fish, with males maturing at age 22 months (mean total length of 17.3–20.1 cm) and females at age 22–34 months (mean total length of 18.3–24.3 cm). Both sexes start to spawn in June (A.J. Manning, unpublished data).

The liver of fish plays an important role in intermediary metabolism, storage of reserve compounds such as lipids (triacylglycerol) and glycogen, the biotransformation and detoxification of lipophilic organic compounds (e.g. xenobiotics), and reproduction (vitellogenesis). Therefore, monitoring the effects of chemical and physical stressors in fish has been focussed on the liver. Many histological, histochemical, and enzyme cytochemical and biochemical parameters have been used to diagnose stress in the liver of marine teleosts, flatfish species in particular (e.g. Köhler 1990; Carr et al. 1991; Köhler and Pluta 1995; Köhler et al. 1992, 1998; Husøy et al. 1996; Vethaak and Wester 1996; Collier et al. 1998; Myers et al. 1998), notably, differences in the quantities of stored lipids and glycogen in the hepatocytes. Metabolism of these energy-rich storage products is variable, especially depending on water temperature, reproductive, and nutritional status, as well as age and state of health of the fish (Welsch and Storch 1973, and references therein). Early signs of liver injury may be accompanied by lipid accumulation and/or excessive storage or depletion of glycogen in the hepatocytes (Köhler et al. 1992; Hinton 1993).

The histomorphology of liver of flatfish such as winter flounder (*Pseudopleuronectes americanus*), European flounder (*Platichthys flesus*), English sole (*Pleuronectes vetulus*), and dab (*Limanda limanda*) resembles that of other teleosts (Hampton et al. 1985, 1988; Morrison 1987; Stehr et al. 1988; Köhler 1989; Vethaak and Wester 1996, and references therein). The basic morphology and physiology of the liver of yellowtail flounder have not been studied to date, and sex differences in the studied species, and the potential differences between wild and cultured fish have also received little attention.

The main objectives of the present study were to: (1) examine the liver morphology of yellowtail flounder and (2) investigate potential differences in lipid and glycogen storage in female and male hepatocytes in wild and cultured sexually immature yellowtail flounder.

Materials and methods

Fish sampling

Wild and cultured *Limanda ferruginea* Storer were selected on the basis of sex, size, age, and gonadal maturity stage for comparison of the microscopic liver structure and its content of energy reserve material. Wild gonadally immature (juvenile) yellowtail flounder

[12–27 cm body length; 2–4 years old, according to the growth curves published in Pitt (1974)] were collected by otter trawl from Witless Bay, Newfoundland and Labrador, during April 2001 (Methven and Schneider 1998). Fish were transported to the Ocean Sciences Centre, and males and females were kept in separate tanks with flowing seawater at ambient temperature (1–2°C) for 2 days, during which time they were not fed. Otoliths were collected from the cranial cavity of each fish for age determination in years (Chilton and Beamish 1982). Ten specimens (5 females and 5 males; 13.5–21.5 cm body length) of the same age and in the same gonadal maturity stage were selected for liver investigation. Seventeen specimens (9 females and 8 males; 15.5–21 cm body length) of cultured sexually immature yellowtail flounder (21 months, 1⁺ year class) were obtained from the Ocean Sciences Centre in late April 2001. Fish were reared from eggs collected from a captive adult broodstock and raised using standard hatchery protocols (Rabe and Brown 2000; Dwyer et al. 2002). From the age of 6 months until the day of sampling, the fish were kept at an 18 h light:6 h dark photoperiod at temperatures of 8–13°C. They were fed, to satiation, twice a day with a dry pellet salmonid diet containing 24% fat and 48% protein (Moore-Clarke NutraFry Extruded Fish Feed, St. Andrew's, NB) until 2 days before sampling.

Tissue preparation

Wild and cultured *L. ferruginea* were euthanized with 2-phenoxyethanol (3 ml in 4 l of seawater), weighed, and their standard length measured. The liver was removed quickly, without rupturing the gall bladder, and weighed. A piece of tissue from the largest lobe of the liver was fixed in 10% neutral buffered formalin. This tissue was dehydrated through ascending grades of ethanol, cleared in Hemo-De (Fisher Diagnostics), then impregnated and embedded in paraffin wax (Paraplast Plus, Fisher Diagnostics) for histological and histochemical study. A second piece of liver from the same lobe was submerged in Histo Prep and supercooled in petroleum ether (–40°C to –60°C) which had been precooled in a chilling bath of acetone (Chayen and Bitensky 1991) for histochemical study. The frozen tissue was stored at –70°C until sectioning and histochemical procedures could be performed. In addition, a piece of the gonad was fixed in 10% neutral buffered formalin and processed, as described for the liver, for histological study to assess the sex and the gonadal maturity stage of each fish. The hepatosomatic indices (HSI) were calculated according to the formula: (liver weight/body weight–liver weight)×100.

Histology and histochemistry

Liver tissue embedded in paraffin wax was sectioned at 6 µm, and frozen tissue, at 8 µm. To investigate the general morphology of the liver tissue, paraffin sections were stained in Mayer's hematoxylin and eosin (H&E), Gomori's one-step trichrome, and Masson's trichrome (Carson 1990; Kiernan 1990). The following methods were used to demonstrate lipid and glycogen storage. Sudan black B and oil red O were used as lysochromes for the determination of all available lipids (other than steroids) and of unsaturated neutral lipids, respectively, in frozen sections (Chayen and Bitensky 1991). Paraffin sections were used in the Periodic Acid-Schiff (PAS) reaction to assess the amount of glycogen present (Sheehan and Hrapchak 1980). Pretreatment of the sections with 0.1% malt diastase (Sigma) in sodium phosphate buffer was used to determine whether or not the PAS-positive material in the hepatocytes was indeed glycogen.

Gonad (ovary and testis) tissue embedded in paraffin wax was sectioned at 6 µm and stained with Mayer's H&E. Gonad development was defined according to the relative abundance of each germinal type found within the gonad. Oogenetic stages were characterized according to the classification of Howell (1983), and spermatogenic stages were characterized according to the classification used for winter flounder (Harmin et al. 1995).

Evaluation

Relative amounts of PAS, malt diastase/PAS, Sudan black B, and oil red O were measured using computerized image analysis. Images were attained using a Sony video camera mounted to a Nikon Eclipse E 600 microscope. The images were subsequently captured using Adobe Premiere version 6 software and analyzed using Mocha video analysis software. This program measures the amount of PAS-positive material and lysochromes in arbitrary units, by counting the number of pixels of each image that meet a set threshold. The amount of glycogen and lipid was calculated per tissue area. Eight readings under medium power (total magnification, $\times 400$) per liver were carried out on duplicate sections.

Statistical analysis

Data were analyzed using the statistical package Minitab for Windows release 13 (Minitab). Significant differences between the sampling groups (wild and cultured) and between males and females were tested using general linear ANOVA, followed by Tukey's pairwise comparisons with a significance level of $\alpha = 0.05$.

Results

General characteristics of wild and cultured fish

Age, standard length, body and liver weight, and hepatosomatic index of the sampled wild and cultured *Limanda ferruginea* are summarized in Table 1. Otolith reading determined that the wild fish used were of the year class 3⁺. In general, cultured females and males were heavier than wild females and males, cultured females being significantly heavier than wild females and males. Liver weight of cultured females was also significantly greater than that of wild females and males. Macroscopic examination of the livers revealed that the livers of laboratory-reared fish were pale pink, while the livers of wild fish were red colored. Hepatosomatic indices of cultured fish were significantly higher than those of wild fish, and were different between males and females. The HSI of cultured males was significantly higher than that for females.

The ovaries of all female fish (wild and cultured) had oögonia and numerous follicles with previtellogenic oocytes (stages II through IV, Howell 1983) arranged in ovigerous lamellae (Fig. 1A). In contrast to the females, the males (wild and cultured) were in more advanced stages of sexual maturity (prespawning, Harmin et al.

1995). Their seminiferous tubules were filled with numerous spermatids and spermatozoa, and sparse clusters of spermatogonia (Fig. 1B).

Liver morphology

Microscopic examination of the liver sections stained with Mayer's H&E or Masson's trichrome demonstrated circular and ellipsoidal profiles of hepatic tubules (Fig. 1C). Liver tissue was interspersed with pancreatic tissue, which was more or less concentrated in one area (Fig. 1C). Liver tissue was composed of irregular, two-cell-thick cords of hepatocytes arranged in tubules surrounding vascular sinusoids. The sinusoids were lined by endothelial cells. Hepatocytes were large, irregularly shaped, with eosinophilic cytoplasm around clear spaces. The peripherally located nuclei were large with prominent nucleoli. The nuclei of the hepatocytes appeared to be mostly located at the base of the hepatocytes in close proximity to the sinusoids. Hepatocytes in cultured fish were larger than those in wild fish, and in both groups females had larger cells than males. Clear spaces within the cytoplasm were large and numerous in cultured fish, and minute and fewer in number in wild fish (Fig. 1H–K).

We did not observe the typical portal triads found in higher vertebrates, consisting of connective tissue containing terminal branches of the portal vein and hepatic artery, and a small tributary of the bile duct. It was difficult to distinguish between portal (afferent) venules and hepatic (efferent) venules. Occasionally diads were seen, consisting of a putative afferent venule and an arteriole within connective tissue (Fig. 1D). Randomly distributed venules were close to arterioles or to biliary ductules, suggesting afferent (portal) venules (Fig. 1E). Venules in which branching sinusoidal channels converge were scattered throughout the liver tissue, suggesting efferent (terminal hepatic) venules (Fig. 1E). In addition, randomly distributed small arteries were located near biliary ductules which have lumens lined with low cuboidal epithelium (Fig. 1F). Occasionally a medium-sized bile duct, its lumen lined with single cuboidal epithelium, an arteriole and a small vein were grouped together within a connective tissue sheath (Fig. 1G). The lumina of large bile ducts were lined with single columnar epithelium.

Table 1 *Limanda ferruginea*. Group, sex, number of individuals, age, standard length, body and liver weight, and hepatosomatic indices (HSI) of juvenile wild and cultured yellowtail flounder (mean $\pm$ SE)

Group	Sex	N	Age	Standard length (cm)	Body weight (g)	Liver weight (g)	HSI
Wild	Female	5	3 ⁺	14.5 $\pm$ 1.0	39.9 $\pm$ 7.3	0.95 $\pm$ 0.07	0.97 $\pm$ 0.08
	Male	5	3 ⁺	15.9 $\pm$ 1.8	63.5 $\pm$ 20.3	0.65 $\pm$ 0.22	1.07 $\pm$ 0.10
Cultured	Female	9	1 ⁺	18.9 $\pm$ 0.3	106.8 $\pm$ 5.4 ^{a1}	1.91 $\pm$ 0.19 ^{a2}	1.85 $\pm$ 0.22 ^b
	Male	8	1 ⁺	17.2 $\pm$ 0.7	69.3 $\pm$ 3.6	1.61 $\pm$ 0.11	2.40 $\pm$ 0.19 ^{a3}

^{a1}Significantly different from female (^{a1} $P = 0.002$; ^{a2} $P = 0.000$; ^{a3} $P = 0.001$) and male (^{a1} $P = 0.048$; ^{a2} $P = 0.000$; ^{a3} $P = 0.003$) wild fish

^bSignificantly different from female ($P = 0.002$) and male ($P = 0.007$) wild fish, and cultured males ($P = 0.029$)

Liver histochemistry

PAS-positive material appeared as fine magenta-stained granular material in the cytoplasm of the hepatocytes, with greater intensity surrounding the sinusoids (Fig. 2A, B). Pretreatment of the sections with malt diastase resulted in disappearance of the fine magenta-stained granular material within the cytoplasm (Fig. 2C), showing that the granular material is glycogen. The amount of glycogen per unit area was not significantly different between the wild and cultured fish or between females and males (Fig. 3). However, the values obtained for the glycogen storage in cultured fish were higher in comparison to wild yellowtail flounder.

Total lipids stained with Sudan black B and neutral lipids stained with oil red O were located within the cytoplasm of the hepatocytes (Fig. 2D and E, respectively). The hepatocytes contained an abundance of lipids, particularly in the cultured fish, much of which appeared to be neutral lipid (Fig. 2F–I and J–K, respectively). In the cultured females the lipid droplets stained with Sudan black B were often large, frequently irregular, and had bridges with smaller droplets (Fig. 2F). These droplets were dramatic in comparison to those in wild females (Fig. 2G). The lipid droplets in cultured males were smaller, and they were regular and had no bridges (Fig. 2H), but still were much larger than those in wild males (Fig. 2I). Figure 2J–M shows similar

Fig. 1A–K *Limanda ferruginea* **A** Ovary of cultured sexually immature yellowtail flounder showing previtellogenic oocytes, Mayer's hematoxylin and eosin ($\times 100$); **B** maturing testis of cultured yellowtail flounder with seminiferous tubules full of spermatids and spermatozoa, Mayer's hematoxylin and eosin ($\times 100$); **C** liver of cultured sexually immature female yellowtail flounder with interspersed pancreatic tissue (*thick arrow*) and circular (*arrowhead*) and ellipsoidal profiles (*thin arrow*) of hepatic tubules, Gomori ($\times 250$); **D** liver of cultured sexually immature female yellowtail flounder with small vein (*v*) and arteriole (*arrowhead*) within connective tissue sheath, Masson's trichrome ($\times 500$); **E** liver of cultured sexually immature female yellowtail flounder with venule (*v*) and bile ductule (*arrowhead*) and sinusoids (*arrow*), converging in a putative efferent venule, Mayer's hematoxylin and eosin ($\times 250$); **F** liver of cultured sexually immature female yellowtail flounder with small artery (*arrow*) and bile ductule (*arrowhead*) and sinusoids (*thin arrow*), Mayer's hematoxylin and eosin ($\times 250$); **G** liver of cultured sexually immature female yellowtail flounder with medium-sized bile duct (*thick arrow*), its lumen lined with single cuboidal epithelium, an arteriole (*arrowhead*) and a branching vein (*thin arrow*), grouped within a connective tissue sheath, Mayer's hematoxylin and eosin ($\times 250$); **H** liver of cultured sexually immature female yellowtail flounder, showing large hepatocytes with numerous large clear spaces in cytoplasm, Mayer's hematoxylin and eosin ($\times 500$); **I** liver of wild sexually immature female yellowtail flounder, showing small hepatocytes with small clear spaces in cytoplasm, Mayer's hematoxylin and eosin ($\times 500$); **J** liver of cultured sexually immature male yellowtail flounder, showing large hepatocytes with numerous large clear spaces in cytoplasm, Mayer's hematoxylin and eosin ($\times 500$); **K** liver of wild sexually immature male yellowtail flounder, showing small hepatocytes with small clear spaces in cytoplasm, Mayer's hematoxylin and eosin ($\times 500$)

results for neutral lipids stained with oil red O in cultured (Fig. 2J, L) and wild (Fig. 2K, M) fish of both sexes. The amounts of total and neutral lipids per unit area in the liver of cultured female and male fish were significantly higher than those in wild female and male fish (Fig. 4). In addition, there was a sex-dependent difference in cultured fish: females contained significantly higher amounts of total and neutral lipids compared to males.

Discussion

In the present study we have shown that sexually immature wild and cultured female and male *Limanda ferruginea* have similar liver structures, but differ in hepatic lipid accumulation between sampling groups and between sexes.

Although wild and cultured fish were of different year classes, 3⁺ and 1⁺, respectively, the gonadal development was at the same level. The majority of males in both sampling groups was at a much more advanced gonadal stage than the females. The wild and cultured fish used in the present study were all sexually immature fish sampled in early spring, when reproductively mature yellowtail flounder at this latitude tend to be in a spawning stage of development (Howell 1983; Clearwater 1996; Manning and Crim 1998).

Hepatosomatic indices (HSIs) of cultured *L. ferruginea* were significantly higher than those of wild fish and

different between females and males, which may be due to greater storage. The HSIs of the cultured male fish were significantly higher than those of females, mainly due to the lower body weight of males. The HSIs of gonadally immature wild winter flounder (*Pseudopleuronectes americanus*) and scup (*Stenotomus chrysops*) were not sexually differentiated, while those of mature fish were. The HSIs of mature female winter flounder and scup were significantly greater than those in males, likely due to the liver enlargement in females associated with vitellogenesis (Snowberger Gray et al. 1991). Ando et al. (1993) found a significant relationship between HSI and lipid content in five species of fish, including cultured flounder (*Paralichthys olivaceus*); the hepatic lipid content was positively correlated with the HSI. In our study cultured fish had both higher HSIs and higher liver lipid content than wild fish.

The liver of sexually immature *L. ferruginea* is invested with pancreatic tissue, which is more or less concentrated in one area of the liver tissue. In sexually mature flatfish species, such as European flounder (*Platichthys flesus*), winter flounder (*Pseudopleuronectes americanus*), and plaice (*Pleuronectes platessa*), the pancreatic tissue is more scattered throughout the liver (Timashova 1982; Köhler 1990; Murchelano and Wolke 1991; March and Reisman 1995; Vethaak and Wester 1996). As in other fish, the histomorphology of the liver in sexually immature *L. ferruginea* causes difficulties in determining the functional liver units and in explaining the appearance of histopathological changes (Hampton et al. 1985, 1988; Morrison 1987; Stehr et al. 1988; Köhler 1989; Krause and Cutts 1994; Vethaak and Wester 1996). In vivo microscopy of liver microvasculature in rainbow trout revealed a repeating pattern of alternation between afferent and efferent regions (Hinton and Lauren 1989). In a number of teleost species, diads were found consisting of bile ducts and arterioles ensheathed with connective tissue (Hampton et al. 1988). The diads found in liver tissue of juvenile yellowtail flounder consisted of arterioles and venules within a connective tissue sheath. To distinguish between an arteriole and a bile ductule may be difficult sometimes, due to the contraction of the arteriole during the dissecting process, resulting in a cuboidal appearance of the endothelium. The structure of the liver in cultured and wild juvenile yellowtail flounder looked alike, except that the hepatocytes in cultured fish were larger and contained larger cytoplasmic clear spaces, than those in wild fish. The clear spaces as seen in H&E and Masson's trichrome-stained, formalin-fixed tissues are caused mainly by the removal of lipids during the dehydration (alcohol treatment) steps of routine histological processing.

In the present study, the levels of cytoplasmic glycogen and lipids within the hepatocytes were greater in cultured fish than in wild fish, most likely resulting from hatchery conditions such as diet and temperature. These differences in lipid content between cultured and wild fish, and between cultured females and males were

statistically significant, while there were no significant differences among the groups or sexes for glycogen content. Hatchery-reared rainbow trout (*Salmo gairdneri*) and channel catfish (*Ictalurus punctatus*) showed an abundance of glycogen within the hepatocytes (Hinton and Pool 1976; Hampton et al. 1985), likely caused by diet. Exposure to chemical contaminants may cause depletion of hepatocellular glycogen, as has been suggested in several species such as winter flounder (*P. americanus*) and male bullheads (*Cottus gobio* L.) (Carr et al. 1991; Bucher et al. 1992).

As shown by the Sudan black B and oil red O methods, the liver of cultured sexually immature yellowtail flounder contained an abundance of lipids,

particularly neutral lipids. Apparently the cultured fish store lipids, and not all the energy is used for growth, or the magnitude of dietary uptake of lipid into the liver overwhelms the organism's capacity to utilize this lipid for growth.

The cultured females stored more lipids than males, suggesting that the capacity for lipid storage in males is less than in females and/or the males are metabolizing lipids at a higher rate. The same results were obtained in cultured 0⁺ year class yellowtail flounder (Scott et al. 2002). Biochemical studies demonstrated that the amount of lipid stored by cultured sexually immature yellowtail flounder (0⁺) was significantly higher than the amount stored by wild fish, but the differences were not

Fig. 2A–M *Limanda ferruginea*. **A** Liver of cultured sexually immature female yellowtail flounder, with PAS-positive material, fine droplets within the hepatocytes (arrowhead) and around sinusoids (arrow), Periodic Acid Schiff (×250); **B** liver of wild sexually immature male yellowtail flounder, with PAS-positive material, fine droplets within the hepatocytes (arrowhead) and around sinusoids (arrow), Periodic Acid Schiff (×500); **C** liver of the same wild sexually immature male yellowtail flounder, shown in panel B, with PAS-positive material around sinusoids (arrow), malt diastase and Periodic Acid Schiff (×500); **D** liver of cultured sexually immature female yellowtail flounder, with medium to large lipid droplets stained with Sudan black B within hepatocytes located around venules (v) (×250); **E** liver of cultured sexually immature female yellowtail flounder, with numerous medium to large unsaturated neutral lipid droplets stained with oil red O within hepatocytes located around venules (v), oil red O with hematoxylin counterstain (×250); **F** liver of cultured sexually immature female yellowtail flounder, with variably sized, irregularly shaped lipid droplets (arrowhead) and bridges between droplets (arrow) stained with Sudan black B within hepatocytes (×400); **G** liver of wild sexually immature female yellowtail flounder, with small lipid droplets (arrowhead) stained with Sudan black B within hepatocytes (×400); **H** liver of cultured sexually immature male yellowtail flounder, with medium to large lipid droplets (arrowhead) stained with Sudan black B within hepatocytes (×400); **I** liver of wild sexually immature male yellowtail flounder, with small lipid droplets (arrowhead) stained with Sudan black B within hepatocytes (×400); **J** liver of cultured sexually immature female yellowtail flounder, with numerous medium to large unsaturated neutral lipid droplets (arrowhead) stained with oil red O within hepatocytes, oil red O with hematoxylin counterstain (×400); **K** liver of wild sexually immature female yellowtail flounder, with small unsaturated neutral lipid droplets (arrowhead) stained with oil red O within hepatocytes, oil red O with hematoxylin counterstain (×400); **L** liver of cultured sexually immature male yellowtail flounder, showing medium to large unsaturated neutral lipid droplets (arrowhead) stained with oil red O within the hepatocytes, oil red O with hematoxylin counterstain (×400); **M** liver of wild sexually immature male yellowtail flounder, with small unsaturated neutral lipid droplets (arrowhead) stained with oil red O within the hepatocytes, oil red O with hematoxylin counterstain (×400)

Fig. 3 *Limanda ferruginea*. Glycogen in livers of wild and cultured female and male yellowtail flounder (mean ± SE)

sex dependent (Whalen 1999; Scott et al. 2002). In contrast, sex differences in hepatocytes have been reported in sexually mature winter flounder, with males having a lower and less variable lipid content during the reproductive cycle than females, reflecting the process of vitellogenesis in females (March and Reisman 1995). Köhler et al. (1992, and references therein) describe lipid

Fig. 4 *Limanda ferruginea*. Total lipids (Sudan black B) and neutral lipids (oil red O) in livers of wild and cultured female and male yellowtail flounder (mean ± SE) [* , significantly different from wild females ($P=0.000$), wild ($P=0.000$) and cultured males ($P=0.000$); **, significantly different from wild females ($P=0.000$), wild ($P=0.000$) and cultured males ($P=0.000$)]

accumulation as one of the first results of exposure of organisms to pollutants (such as organochlorines and heavy metals), as seen in livers from flatfish from polluted parts of the North Sea. Induced lipidosis caused by pollution or other factors like hatchery or aquaculture nutrition programs will affect the health of the fish. Wild juvenile yellowtail flounder are a so-called “lean” fish (high protein, low fat), reflecting their natural diet of amphipods and polychaete worms, which are low in lipids with high proportions of $n-3$ polyunsaturated fatty acids (Libey and Cole 1979; Parrish et al. 1996). The lipids in liver and muscle of wild sexually immature yellowtail flounder (1.5–2 years) were mainly triacylglycerols (TAG) and phospholipids (PL), and there was more TAG and less PL in muscle than in liver, suggesting low lipid storage in the liver (Whalen 1999).

Cultured sexually immature yellowtail flounder store glycogen and lipids, particularly lipids. Based on the amount of glycogen and lipids found in the liver of several other pleuronectids, fish may be divided into the lipid- and glycogen-storing type (winter flounder, *Pseudopleuronectes americanus*), the lipid-storing type (dab, *Limanda limanda*), or the glycogen-storing type (European flounder, *Pleuronectes flesus*) (Köhler 1990; Köhler et al. 1992; March and Reisman 1995). It is difficult to classify species absolutely since many factors affect the amount of storage material within the hepatocytes. Differences in types of storage material may even exist among specimens of the same species, as has been demonstrated in English sole (*Pleuronectes vetulus*) (Stehr et al. 1988).

We conclude that sexually immature yellowtail flounder demonstrate sex-specific lipid storage within the hepatocytes in cultured fish, fed on a relatively high lipid/low protein diet.

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