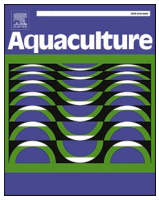
Aquaculture 576 (2023) 739836

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Effect of spawning induced starvation on nutrient loss in males of the annual spawner Atlantic halibut (*Hippoglossus hippoglossus*)

G.M. Berge [*], T. Åsgård [1], J. Kottmann [2], I. Lein

Nofima, Sjølsengvegen 22, NO 6600 Sunndalsøra, Norway

ARTICLE INFO

Keywords:
Atlantic halibut
Broodstock nutrition
Starvation
Spawning period

ABSTRACT

Atlantic halibut go through a period with reduced or no feed intake during their spawning period. This study with male Atlantic halibut aimed to assess the effect of a two-month starvation period corresponding to the naturally occurring spawning induced starvation. The actual loss of nutrients compared to initial body burden, was estimated to 25% for gross energy, 32% for total lipid, 14% for crude protein, and 40% and 44% respectively, for the essential fatty acids EPA (C20:5 n-3) and DHA (C22:6 n-3). This indicates that lipid is the preferred energy source during starvation related to spawning, and the n-3 fatty acids are used to a larger extent than other fatty acids, although mono-unsaturated fatty acids (MUFAs) comprised the largest energy reserve. The difference in relative amount of energy and nutrients used should be considered when formulating diets for re-feeding broodstock of annual spawners like the Atlantic halibut.

1. Introduction

Due to several positive characteristics such as high flesh quality, mild taste, long shelf life and a high fillet yield the Atlantic halibut (*Hippoglossus hippoglossus*) is highly appreciated in the market (Brown, 2009) and an excellent candidate for cold water aquaculture. Once Atlantic halibut reaches sexual maturation, the potential for egg production is high and spawning happens annually over a 2–3 month period, in nature probably over several decades (Haug and Gulliksen, 1988). Rapid growth and high fecundity are considered important traits for aquaculture species. However, high variability in gamete quality and low survival during early offspring development are major bottlenecks in aquaculture and optimal broodstock management, including broodstock nutrition, needs to be adapted for each species (Izquierdo et al., 2001; Migaud et al., 2013). Every year during the spawning season, halibut broodstock cease feeding for a period of at least 1–1.5 months. These seasonal fluctuations in feed intake in Atlantic halibut has been well documented over a four-year period (Brown, 2009). The energy invested in developing gonads and the energy loss during the starvation period must be compensated by feed uptake after spawning, before the fish can continue both somatic and reproductive growth for the next spawning season. This is particularly important for farmed fish which are intended to grow and spawn for several years.

Being exposed to periods of food deprivation is common in the life cycle of fish (Navarro and Gutierrez, 1995). This can be related to e.g. seasonal changes in food availability, migration, or reproduction, the latter being the case for halibut. The physiological strategies and mechanisms behind individual resilience of fish to food deprivation are largely unknown (Bar, 2014). Nonetheless, starvation periods that last for several weeks will have nutritional implications and the nutrient loss needs to be restored, often in the form of subsequent compensatory growth (Ali et al., 2003). In juvenile halibut, starvation/re-feeding experiments showed size-specific compensatory response, where higher feed-conversion efficiency and full growth recovery was achieved for halibut >2 kg, while fish <0.5 kg achieved only partial growth compensation (Foss et al., 2009). Moreover, starvation/re-feeding resulted in delayed sexual maturation (Foss et al., 2009), which has also been found in sea bass, *Dicentrarchus labrax* (Chatzifotis et al., 2011) Nile tilapia, *Oreochromis niloticus* (Sales et al., 2020) and tilapia, *Oreochromis mossambicus* (Pikle et al., 2017).

The metabolic response to starvation will likely vary among species, life stage, season, and individuals. However, fish metabolism depends largely on lipids and proteins stored in liver, viscera and muscle (Ali et al., 2003); (Einen et al., 1998). In flatfish the main energy reserves are

* Corresponding author.
E-mail addresses: gerd.berge@nofima.no (G.M. Berge), johanna.kottmann@aquagen.no (J. Kottmann), ingrid.lein@nofima.no (I. Lein).
[1] Present address: 6612 Grøa, Norway.
[2] Present address: AquaGen AS, Havnegata 9, 7010 Trondheim, Norway.

https://doi.org/10.1016/j.aquaculture.2023.739836
Received 30 March 2023; Received in revised form 23 June 2023; Accepted 24 June 2023
Available online 27 June 2023

recognized to be stored in the muscle and liver (Haug and Gulliksen, 1988). Lipids are known to be broken down early and constitute the major energy source during starvation periods (Bar, 2014). The resulting decrease in lipid content following food deprivation has been shown in many fish species, e.g. Antarctic fish *Notothenia coriiceps* and *Notothenia rossii* (Stepanowska and Nędzarek, 2020) sea bass (Chatzifotis et al., 2011), Nile tilapia (Sales et al., 2020), meagre, *Argyrosomus regius* (Chatzifotis et al., 2018), Japanese flounder, *Paralichthys olivaceus* (Yang et al., 2019) and Atlantic salmon (Einen et al., 1998).

However, the dietary composition can affect the metabolic response towards nutritional challenges related to starvation and re-feeding as observed in sea bass (Pérez-Jiménez et al., 2007), and dentex, *Dentex dentex* (Pérez-Jiménez et al., 2009). Moreover, feeding Siberian sturgeon a high protein diet during starvation/re-feeding cycles promoted higher growth (Babaei et al., 2020). Knowledge on the metabolic response and nutritional requirements related to the seasonal spawning induced starvation period in halibut is needed to optimize nutritional broodstock management. Thus far, nutritional studies on halibut have mainly focused on larval (Hamre et al., 2005; Sæle et al., 2003) and juvenile stages (Foss et al., 2009; Hamre et al., 2003). However, three diets commonly used in halibut production were fed to female broodstock resulting in biochemical and histological alterations of the liver compared to wild-caught halibut (Bolla et al., 2011). Moreover, the effect of halibut broodstock diets on the reproductive performance was tested (Mazorra et al., 2003) and showed that feeding with a diet enhanced in arachidonic acid (C20:4 n-6)) resulted in improved fertilisation success, blastomere morphology as well as hatching success.

The aim of the present study was to gain information about nutrient losses in sexually mature Atlantic halibut during a two-month feed deprivation period, which could influence requirements during maturation and re-feeding after spawning. Only males were used in the experiment because mature females were considered too valuable to sacrifice. Under farming conditions Atlantic halibut females reach sexual maturity after >6 years while males mature after 2–3 years.

2. Material and methods

2.1. Fish and facilities

A total of 50 captive Atlantic halibut males with a mean weight of 4126 g were obtained from a commercial halibut farm (Nordic Seafarms, Averøy, Norway). All fish were first-time sexually mature males. The fish were collected from commercial sea cages, and transported for 20 min by boat, with constant water flow, to the former AKVFORSK research facility at Ekkilsøy, Averøy, at the western coast of Norway.

The fish were anesthetised with tricaine mesylate (Finquel vet®, MS222) before they were individually tagged (PIT: Passive Integrated Transponder, Sokymat Unique 12 mm), and individual weights were recorded. All fish were transferred to one common flat-bottom 7x7x7 m net pen. A fine-meshed net was used to avoid “trespassing” of edible small fish. During the two-month experimental period, the fish were totally deprived from food. The experiment was started mid-December and terminated in February, corresponding to the peak spawning season of Atlantic halibut at this latitude. Temperature was recorded daily at 3 m water depth during the starvation period.

2.2. Collection of data

After one and two months all fish were individually weighed. The first nine fish randomly netted out of the pen were sampled for chemical analyses at start and at each sampling time. Sampled fish were euthanized using an overdose of Finquel. Round weight, gutted weight, liver weight, gonad weight and intestinal weight including stomach were recorded for each of the sampled fish. Due to loss of blood and slime during the slaughter process, there was a discrepancy between recorded body weight and the sum weight of the analysed compartments that was used in calculations of whole-body composition. Samples of gutted fish, liver, gonads, and intestines were frozen separately for each individual. Remaining fish were slaughtered after termination of the experiment.

2.3. Analyses and calculations

The nine fish from each sampling were randomly divided in groups of three fish each. Each compartment from these three fish were pooled when homogenized. Pooled samples of liver, gonad, stomach/intestines, and dress-out carcass were analysed for dry matter (105 °C, 16–18 h), lipid (Folch et al., 1957), fatty acids (methylation, (Mason and Waller, 1964); separation by gas chromatography, (Røsjø et al., 1994)) nitrogen (Semi Micro Kjeldahl, Kjeltech-Auto system) and crude protein calculated (nitrogen * 6.25), ash (16–18 h at 550 °C, until stable weight) and energy (Parr 1271 automatic bomb calorimeter).

Whole body weights of fish sampled at each time point, and the initial weight of the same individuals, were used to calculate % reduction of body weight. Body burden of the nutrients (total content in a fish of average weight from the actual sample) were calculated for fish sampled at start, and after 1 and 2 months. At sampling, the body was divided in different compartments (liver, gonad, stomach/intestines, and dress-out carcass). The compartments were weighed and analysed, and whole-body composition estimated based on sum of each analysed substance in each compartment and the sum weight of all compartments. Loss of nutrients were calculated as % reduction in body burden during the 2-month starvation period.

The following parameters were also calculated:

$$\text{Hepatosomatic index (HSI)} : \text{liver weight/body weight}^{*}100;$$

$$\text{Gonad index (GSI)} : \text{gonad weight/body weight}^{*}100;$$

$$\text{Intestinal index (ISI)} : \text{intestine weight/body weight}^{*}100;$$

$$\text{Dress} - \text{out percentage (DP)} : \text{gutted weight/body weight}^{*}100;$$

All data were subjected to statistical analysis by one-way ANOVA and ranked by Tukeys HSD test. The model assessed the effect of time. Weight measurements of individual fish were used as replicates ($n = 9$), as all fish were kept in one common cage. For chemical analyses, three pooled samples per timepoint were used as replicates ($n = 3$). Significant effects were indicated at a 5% level. Statistical analyses were conducted using the software package SAS System for Windows Release 9.4 (SAS Institute Inc., Cary, NC, USA).

2.4. Fish welfare

The experiment described in this publication was evaluated prior to start according to the Norwegian legislation which was valid at the time of the experiment (2004), that is “Lov om dyrevern” (LOV-1974-12-20-73) and “Forskrift om forsøk med dyr” (FOR-1996-01-15-23). The application was approved by the local authority on behalf of the Norwegian Animal Research Authority (NARA) (in operation until 2015).

3. Results

Temperature in the seawater (Fig. 1) was 7.5 °C at start and decreased gradually to 5 °C during the experimental period (mean 5.7 °C), a total of 325 day-degrees (d°). Six fish died during the experimental period. In addition, two sampled fishes had wounds that were difficult to interpret but may resemble bite marks indicating attacks from predators like otter or mink, which had been observed in the area.

Mean weights of whole fish and body compartments, as well as calculated organ indices, are shown in Table 1. Initial average weight of all fish (50) in the experiment was 4126 g. The random sampling gave a trend towards different initial weight in the groups sampled at different

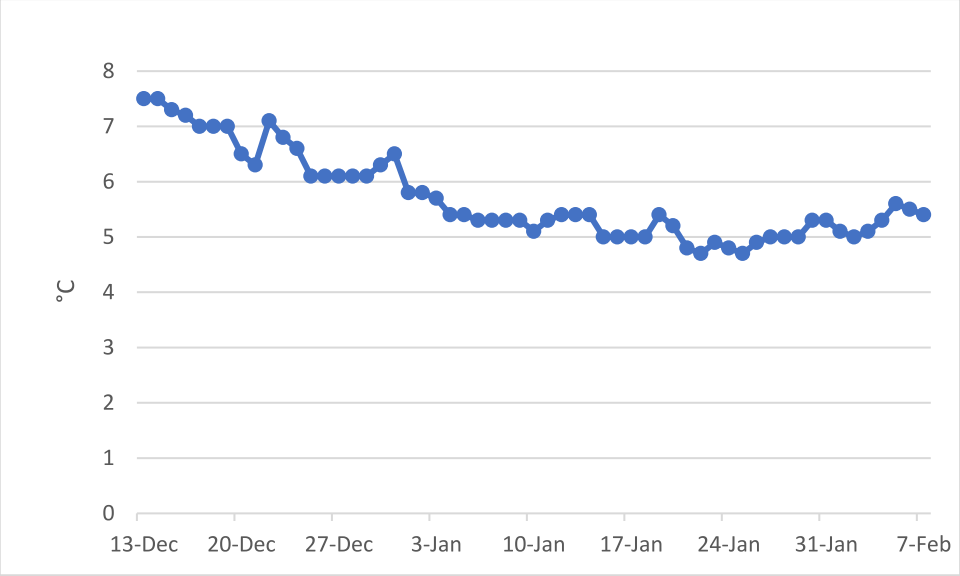


Fig. 1. Water temperature measured at 3 m depth during the starvation experiment.

Table 1
Weight data of whole body and body compartments in food deprived Atlantic halibut males, after 0, 1 and 2 months, data given as mean ± SEM (n = 9). Different letters within a row indicate significant differences.

	0	1 month	2 months	*p*-value
Initial weight, g	4126 ± 118	4317 ± 142	3913 ± 97	0.09
Sampling weight, g	4126 ± 75 [a]	4025 ± 93 [ab]	3642 ± 104 [b]	**0.03**
Control sum weight, g	4038 ± 119 [a]	3970 ± 133 [ab]	3597 ± 106 [b]	**0.03**
Gutted weight	3683 ± 100	3692 ± 128	3379 ± 98	0.11
Liver weight, g	58.8 ± 5.3	73.3 ± 4.7	58,1 ± 6.6	0.11
Gonad weight, g	232.2 ± 30.4 [a]	141.1 ± 22.2 [b]	81.9 ± 8.8 [c]	**0.0005**
Intestinal weight, g	64.2 ± 2.6	63.9 ± 2.7	68.8 ± 3.5	0.45
HSI, %	1.42 ± 0.11	1.81 ± 0.07	1.58 ± 0.16	0.06
GSI, %	5.57 ± 0.67 [a]	3.54 ± 0.57 [b]	2.25 ± 0.23 [b]	**0.001**
ISI, %	1.56 ± 0.05 [b]	1.59 ± 0.05 [b]	1.90 ± 0.10 [a]	**0.004**
DP, %	89.3 ± 0.5 [b]	91.7 ± 0.5 [a]	92.8 ± 0.2 [a]	**<0.0001**

HSI: hepatosomatic index.
GSI: gonadosomatic index.
ISI: intestine-somatic index.
DP: dress-out persentage.

times. Compared to actual initial weight, the fish sampled after one month had lost 6.7% body weight, and fish sampled after two months had lost 7.8% of the body weight recorded at the 1-month sampling. A drop in gonad weight was also observed during the two months of starvation. This was reflected in the gonadosomatic index (GSI) which significantly decreased from 5.6% to 2.3%. Hepatosomatic index (HSI) did not change significantly, while the intestine and stomach index (ISI) and dress-out percentage (DP) increased. Running milt was observed in nearly all sampled fish at the 1-month sampling. The weight of stomach and intestines, which was constant over time seen as absolute values, increased as relative weight (ISI).

Table 2 shows the proximate composition of the body compartments as well as calculated values for whole body composition. Content of the essential fatty acids C20:5 n-3 (EPA) and C22:6 n-3 (DHA) and groups of fatty acids in the body compartments and whole body, are presented in Table 3. There was a decrease in energy, total fat, DHA, EPA, and groups of fatty acids both in liver, gonads, and dressed-out carcass. As the

Table 2
Proximate composition (g/100 g) and energy (MJ/kg) in different body compartments, and in whole body of Atlantic halibut deprived of food for up to 2 months, data given as mean ± SEM(n = 3). Different letters within a row indicate significant differences.

	0	1 month	2 months	p-value
Liver				
Dry matter	52.8 ± 1.7 [a]	43.9 ± 0.9 [b]	37.5 ± 1.4 [c]	**0.0006**
Protein	9.0 ± 0.2 [b]	10.2 ± 0.2 [b]	12.6 ± 0.4 [a]	**0.0003**
Fat	44.2 ± 3.6 [a]	34.8 ± 2.0 [b]	21.6 ± 2.3 [c]	**0.003**
Ash	0.9 ± 0.04 [c]	1.2 ± 0.03 [b]	1.4 ± 0.03 [a]	**0.0001**
Energy	19.0 ± 0.8 [a]	15.0 ± 0.5 [b]	12.0 ± 0.6 [c]	**0.0007**
Gonad				
Dry matter	14.1 ± 0.1 [a]	13.0 ± 0.1 [b]	12.1 ± 0.2 [c]	**0.0004**
Protein	12.4 ± 0.2 [a]	11.6 ± 0.5 [ab]	10.2 ± 0.3 [b]	**0.02**
Fat	2.3 ± 0.1 [a]	2.1 ± 0.05 [b]	1.8 ± 0.04 [c]	**0.002**
Ash	1.7 ± 0.06 [a]	1.5 ± 0.02 [b]	1.4 ± 0.01 [c]	**0.003**
Energy	3.1 ± 0.1 [a]	2.9 ± 0.1 [ab]	2.6 ± 0.1 [b]	**0.01**
Stomach/ intestines				
Dry matter	16.7 ± 0.1 [a]	16.3 ± 0.3 [ab]	15.0 ± 0.6 [b]	0.04
Protein	13.6 ± 0.2	13.5 ± 0.3	11.8 ± 1.0	0.13
Fat	2.2 ± 0.1	2.0 ± 0.04	1.9 ± 0.1	0.14
Ash	1.3 ± 0.01	1.7 ± 0.2	1.3 ± 0.04	0.06
Energy	3.4 ± 0.1	3.3 ± 0.1	3.0 ± 0.1	0.06
Dress-out				
Dry matter	29.6 ± 0.6 [a]	28.6 ± 0.2 [a]	26.2 ± 0.6 [b]	**0.009**
Protein	16.5 ± 0.6	16.2 ± 0.4	15.8 ± 0.2	0.51
Fat	12.8 ± 0.4 [a]	13.2 ± 0.2 [a]	10.2 ± 0.6 [b]	**0.006**
Ash	2.3 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	0.47
Energy	8.0 ± 0.2 [a]	7.8 ± 0.9 [a]	6.7 ± 0.1 [b]	**0.001**
Whole body*				
Dry matter	28.8 ± 0.6 [a]	28.1 ± 0.2 [a]	25.9 ± 0.5 [b]	**0.01**
Protein	16.2 ± 0.5	15.9 ± 0.4	15.5 ± 0.2	0.56
Fat	10.5 ± 0.5 [a]	10.0 ± 0.4 [a]	8.0 ± 0.7 [b]	**0.03**
Ash	2.2 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	0.38
Energy	7.9 ± 0.2 [a]	7.7 ± 0.1 [a]	6.6 ± 0.1 [b]	**0.002**

* Content in whole body was estimated based on analyses and weight of the different compartments.

Table 3
Fatty acids content (g/100 g) in body compartments, and in whole body of Atlantic halibut deprived of food for up to 2 months, data given as mean ± SEM (n = 3). Different letters within a row indicate significant differences.

	0	1 month	2 months	p-value
Liver				
EPA	2.21 ± 0.24 [a]	1.85 ± 0.07 [a]	0.88 ± 0.08 [b]	**0.002**
DHA	3.20 ± 0.44 [a]	3.07 ± 0.27 [a]	1.61 ± 0.07 [b]	**0.02**
Sum n-3	7.37 ± 0.68 [a]	6.62 ± 0.36 [a]	3.63 ± 0.27 [b]	**0.003**
Sum n-6	3.58 ± 0.21[a]	2.73 ± 0.12 [b]	1.79 ± 0.20 [c]	**0.001**
SFA	7.54 ± 0.61[a]	5.88 ± 0.33 [ab]	3.93 ± 0.38 [b]	**0.004**
MUFA	18.78 ± 1.36 [a]	14.45 ± 1.06 [a]	9.00 ± 1.05 [b]	**0.003**
Gonad				
EPA	0.22 ± 0.01 [a]	0.19 ± 0.01 [ab]	0.17 ± 0.01 [b]	**0.004**
DHA	0.45 ± 0.01 [a]	0.43 ± 0.02 [a]	0.35 ± 0.01 [b]	**0.0008**
Sum n-3	0.75 ± 0.01 [a]	0.70 ± 0.02 [a]	0.59 ± 0.01 [b]	**0.002**
Sum n-6	0.11 ± 0.002	0.11 ± 0.003	0.10 ± 0.003	0.37
SFA	0.55 ± 0.02 [a]	0.47 ± 0.01 [b]	0.42 ± 0.01 [b]	**0.002**
MUFA	0.57 ± 0.02 [a]	0.51 ± 0.01 [ab]	0.47 ± 0.01 [b]	**0.01**
Stomach/ intestines				
EPA	0.21 ± 0.01	0.18 ± 0.002	0.16 ± 0.01	0.06
DHA	0.44 ± 0.02	0.41 ± 0.01	0.37 ± 0.02	0.10
Sum n-3	0.70 ± 0.04	0.65 ± 0.01	0.59 ± 0.03	0.10
Sum n-6	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.66
SFA	0.49 ± 0.02	0.43 ± 0.01	0.40 ± 0.03	0.07
MUFA	0.52 ± 0.03	0.47 ± 0.01	0.45 ± 0.02	0.24
Dress-out				
EPA	0.48 ± 0.03 [a]	0.48 ± 0.01 [a]	0.32 ± 0.02 [b]	**0.004**
DHA	0.53 ± 0.04 [a]	0.49 ± 0.01 [a]	0.34 ± 0.01 [b]	**0.002**
Sum n-3	1.28 ± 0.09 [a]	1.30 ± 0.02 [a]	0.94 ± 0.04 [b]	**0.007**
Sum n-6	0.79 ± 0.03 [a]	0.84 ± 0.02 [a]	0.64 ± 0.03 [b]	**0.005**
SFA	2.33 ± 0.07 [a]	2.44 ± 0.05 [a]	1.90 ± 0.13 [b]	**0.01**
MUFA	6.61 ± 0.22 [a]	6.85 ± 0.10 [a]	5.39 ± 0.34 [b]	**0.01**
Whole body*				
EPA	0.48 ± 0.03 [a]	0.49 ± 0.01 [a]	0.33 ± 0.02 [b]	**0.002**
DHA	0.56 ± 0.03 [a]	0.54 ± 0.01 [a]	0.36 ± 0.01 [b]	**0.0005**
Sum n-3	1.33 ± 0.07 [a]	1.36 ± 0.02 [a]	0.96 ± 0.04 [b]	**0.002**
Sum n-6	0.79 ± 0.03 [a]	0.84 ± 0.02 [a]	0.64 ± 0.03 [b]	**0.005**
SFA	2.28 ± 0.07 [a]	2.40 ± 0.04 [a]	1.87 ± 0.13 [b]	**0.01**
MUFA	6.34 ± 0.23 [a]	6.67 ± 0.10 [a]	5.25 ± 0.33 [b]	**0.01**

* Content in whole body was estimated based on analyses and weight of the different compartments.

carcass constitutes the largest compartment, the largest energy reserves were drawn from this compartment. Monounsaturated fatty acids (MUFA) comprised the largest part of the lipids in both carcass and liver.

Calculations showed that 93.5% of total body fat was found in the dressed-out carcass (Table 4), while only 5% was found in the liver at start of the trial. Looking at total amount of n-3 fatty acids at start of the trial, 88% was found in the dressed-out carcass, while 8% was found in liver. There was a slight but significant change in the proportions during the two months of starvation as at termination of the experiment 96% of total fat was found in dressed-out carcass and 3.5% in liver. The amount of fat and fatty acids found in gonads were significantly reduced during the experiment, as the total weight of gonads was reduced.

Loss of nutrients during the 2-month starvation period is presented as % reduction in body burden for each nutrient, and Table 5 shows the average reduction in protein, lipid, energy and fatty acids. The loss of DHA was highest with a 44% reduction of initial body burden, while the lipid loss was 32%, and protein loss was 14%. The estimated losses are also given per 100 day-degrees. Estimated values for reduced body burden are given without variance because the estimates were based on overall average numbers, since only initial body weight and not the initial chemical content of the actual sampled fish after 1 and 2 months was known.

4. Discussion

In aquaculture, knowledge of nutritional requirements for gonad development is needed to optimize broodstock management and thereby improve gamete and offspring quality. The present study

Table 4
Proportion of fat and fatty acids in each body compartment, % of total amount, in Atlantic halibut deprived of food for up to 2 months, data given as mean ± SEM (n = 3). Different letters within a row indicate significant differences.

	0	1 month	2 months	p-value
Liver				
Total fat	5.16 ± 0.55 [a]	4.93 ± 0.28 [a]	3.46 ± 0.33 [b]	0.05
EPA	6.79 ± 1.19	7.00 ± 0.27	4.34 ± 0.40	0.08
DHA	8.43 ± 1.70	10.57 ± 0.57	7.27 ± 0.58	0.17
n-3	8.23 ± 1.31	8.96 ± 0.41	6.07 ± 0.58	0.12
n-6	6.66 ± 0.65 [a]	6.03 ± 0.30 [ab]	4.45 ± 0.42 [b]	0.04
SFA	4.83 ± 0.51	4.53 ± 0.25	3.38 ± 0.27	0.07
MUFA	4.31 ± 0.38 [a]	4.01 ± 0.30 [ab]	2.75 ± 0.29 [b]	0.03
Gonad				
Total fat	1.05 ± 0.02 [a]	0.56 ± 0.01 [b]	0.42 ± 0.01 [c]	<0.0001
EPA	2.63 ± 0.11 [a]	1.42 ± 0.05 [b]	1.16 ± 0.03 [b]	<0.0001
DHA	4.69 ± 0.19 [a]	2.85 ± 0.14 [b]	2.24 ± 0.14 [b]	<0.0001
n-3	3.27 ± 0.14 [a]	1.83 ± 0.08 [b]	1.41 ± 0.07 [b]	<0.0001
n-6	0.81 ± 0.01 [a]	0.45 ± 0.01 [b]	0.37 ± 0.01 [c]	<0.0001
SFA	1.39 ± 0.02 [a]	0.70 ± 0.03 [b]	0.51 ± 0.02 [c]	<0.0001
MUFA	0.52 ± 0.01 [a]	0.27 ± 0.00 [b]	0.20 ± 0.01 [c]	<0.0001
Stomach and intestines				
Total fat	0.28 ± 0.01 [b]	0.25 ± 0.01 [b]	0.36 ± 0.03 [a]	0.02
EPA	0.67 ± 0.02 [b]	0.59 ± 0.03 [b]	0.94 ± 0.06 [a]	0.001
DHA	1.24 ± 0.04 [b]	1.24 ± 0.02 [b]	1.98 ± 0.09 [a]	0.0001
n-3	0.84 ± 0.03 [b]	0.76 ± 0.03 [b]	1.16 ± 0.06 [a]	0.001
n-6	0.37 ± 0.01 [b]	0.33 ± 0.02 [b]	0.51 ± 0.04 [a]	0.006
SFA	0.34 ± 0.01 [ab]	0.29 ± 0.01 [b]	0.41 ± 0.03 [a]	0.02
MUFA	0.13 ± 0.002 [ab]	0.11 ± 0.01 [b]	0.16 ± 0.01 [a]	0.01
Dressed out carcass				
Total fat	93.51 ± 0.53 [b]	94.26 ± 0.28 [ab]	95.77 ± 0.36 [a]	0.02
EPA	89.90 ± 1.31 [b]	90.99 ± 0.23 [ab]	93.55 ± 0.47 [a]	0.05
DHA	85.65 ± 1.85	85.34 ± 0.46	88.51 ± 0.63	0.18
n-3	87.66 ± 1.43	88.45 ± 0.43	91.36 ± 0.65	0.07
n-6	92.17 ± 0.63 [b]	93.18 ± 0.31 [ab]	94.67 ± 0.44 [a]	0.03
SFA	93.44 ± 0.48 [b]	94.48 ± 0.25 [ab]	95.69 ± 0.30 [a]	0.01
MUFA	95.04 ± 0.38 [b]	95.61 ± 0.31 [ab]	96.88 ± 0.30 [a]	0.022

Table 5
Estimated loss of energy and nutrients during two months of starvation, expressed as % of initial body burden, total and per 100 day-degrees.

	% of initial body burden	% of initial body burden per 100 d°
Energy	25	8
Protein	14	4
Lipid	32	10
DHA	44	13
EPA	40	12
n-3	35	11
n-6	27	8
SFA	27	8
MUFA	26	8

demonstrated the nutrient loss during a spawning related starvation period in sexually mature Atlantic halibut males and gave an indication of the amount of energy and nutrients that, at least, need to be restored in an annual spawner like the halibut.

The two-months starvation period resulted in an overall body weight reduction, which was expected as reserves were used during this period. This is in agreement with findings in other species, such as Atlantic salmon (Einen et al., 1998) and meagre (Chatzifotis et al., 2018). Moreover, a decrease in GSI was observed, while HSI stayed consistent, and ISI and DP even increased. This indicates that a large part of the weight loss was due to loss from gonads, which may partly be due to excretion of sperm, as supported by the observations of running milt, and partly to reabsorption of the tissue. Lower GSI in starvation/refeeding fish has also been found in juvenile halibut (Foss et al., 2009), as well as in Nile tilapia (Sales et al., 2020) and sea bass (Chatzifotis et al., 2011). In addition, a decrease in HSI has been found during starvation periods for meagre (Chatzifotis et al., 2018) and burbot (Binner et al., 2008). For halibut it has been shown that the HSI can be reduced during the spawning season because the liver represents an important energy source (Haug and Gulliksen, 1988). Even though the present study did not show a significant reduction in HSI, we observed a steady decrease in the liver fat confirming the importance of liver fat as metabolic energy during a starvation period in halibut, which is in line with results from Atlantic salmon (Einen et al., 1998). Also, a constant relative weight of an organ indicates an absolute reduction in weight of the organ at the same rate as the reduction in total body weight.

Overall, a significant decrease in lipid concentration was found in the present study, which is a common and expected response to starvation and has been documented in several other fish species like sea bass, Nile tilapia, Antarctic fish *Notothenia coriiceps* and *Notothenia rossii,* and Japanese flounder (Chatzifotis et al., 2011; Sales et al., 2020; Stepanowska and Nędzarek, 2020; Yang et al., 2019). The protein concentration was slightly reduced, although not statistically significant, but taking the weight reduction into account, the amount of protein was reduced by around 14%. This is in accordance with results from Atlantic salmon (Einen et al., 1998), sea bass (Chatzifotis et al., 2018), black rockcod (Stepanowska and Nędzarek, 2020) and burbot (Binner et al., 2008), and indicates that halibut favors the utilization of lipid as the major energy source during starvation. However, this appears to be specific to species and/or season, as there are studies showing a decline in protein in species like sea bass (Pérez-Jiménez et al., 2007) and marbled rock cod (Stepanowska and Nędzarek, 2020). This underlines the importance of case specific starvation experiments to gain important knowledge for future formulation of broodstock diets in annual spawning species.

For lipids, the largest decrease in body burden was found for DHA and EPA, in particular for DHA, and sum of n-3 fatty acids. As marine species have limited or no ability to synthesize EPA and DHA (Sargent et al., 2003) the dietary intake of these essential fatty acids (EFA) is crucial, in particular for broodstock prior to the spawning season. MUFAs represent the largest energy reserve in the body. However, the EFAs are extensively used as an energy source, with a higher reduction in body burden than SFA and MUFA. The large decrease in EPA and DHA indicates that there is no particular effort to conserve these essential fatty acids in the body, probably due to an evolutionary adaption to the abundance in the natural diet for marine fish. The high capability to utilize DHA as an energy source, may be due to high capacities for peroxisomal β-oxidation in agreement with what is shown in other marine species (Crockett and Sidell, 1993a, 1993b). We have previously compared peroxisomal β-oxidation capacities of livers from Atlantic Halibut with capacities in livers of Rainbow trout and Atlantic salmon and found approximately 2-fold higher activities in halibut than in salmonids (~46 compared to ~20 nmol*min^{-1}mg liver protein^{-1}, respectively (unpublished data)., A recent study (Wang et al., 2022), showed that the expression of peroxisomal fatty acid β-oxidation genes were linearly up-regulated in muscle and subcutaneous adipose tissue during a 1-month starvation period in the marine flatfish turbot (*Scophtalamus maximus*), which may indicate that the peroxisomal fatty acid β-oxidation capacity could be even higher in starved halibut than previously measured in fed fish.

The majority of lipid losses, including relative losses of EPA and DHA, occurred during the second month of the starvation experiment, which indicates different phases of starvation between month one and two. Three different phases of starvation can be defined (Bar, 2014), including (I) a short transitional phase of lipid and protein mobilization followed by (II) a longer phase with utilization of lipid reserves as the main energy source and finally (III) a shift to the mobilization of protein sources. According to the author, the beginning of phase III was difficult to determine but occurs likely between day 28 and 56 (Bar, 2014). Temperature- and species-specific differences in the timeline of starvation phases can be expected. It appears that the current starvation period only included phase I and II and it remains unclear whether halibut would reach a third phase of starvation. Since the halibut was kept at a lower temperature than turbot, the lipid reserves were not depleted and thus the third phase of starvation was not reached during the 2 months in the present trial. Biologically, it makes sense to use lipid reserves throughout a normal spawning season, while proteins should be spared. Nonetheless, broodstock nutrition needs to ensure sufficient nutrient availability throughout the whole spawning season. A significant decrease in the DHA levels in halibut eggs with time was observed in a feeding trial, indicating that DHA levels in the broodstock required for incorporation into the eggs were insufficient as the spawning season progressed (Mazorra et al., 2003). This indicates that formulation of future broodstock diets needs to consider requirements throughout the whole spawning season, and for repeated spawning seasons. The losses observed after 2 months of starvation in the present study can be interpreted as an indication of the requirement for maintenance and activity. This amount of nutrients and energy must be replaced, in addition to nutrients needed for new somatic and reproductive growth, and for further maintenance, digestive processes and activity. However, the fact that only males were studied in the present experiment needs to be considered, because the requirements for production of eggs may differ from that of milt. In the absence of species-specific knowledge, general knowledge of fish energetics and nutrient retention efficiencies could be used, in addition to our data on nutrient losses, as input in calculations and provide a basis for development of a broodstock diet.

5. Conclusions

The current study evaluated the nutrient loss in male halibut during a food deprivation period, which mimics starvation during the natural spawning season. Important knowledge on nutrient utilization was gained, showing favored metabolization of lipids including the essential fatty acids DHA and EPA as well as MUFAs which represented the largest reserves. A major part of the nutrient loss took place during the second month of the starvation period, emphasizing the importance of sufficiently stored nutrients in the broodstock throughout the spawning period. The collected data can be used as a basis for further work on

formulation of broodstock diets for the annual spawner Atlantic halibut, to ensure high quality offspring. Focus should be set on sufficient lipid and EFA levels in future halibut broodstock nutrition.

Author contribution statement

Gerd Marit Berge: conceptualization, investigation, statistical analysis, writing and editing.

Torbjørn Åsgård: conceptualizations, review, and editing.

Johanna Kottmann: writing and editing.

Ingrid Lein: conceptualization, investigation, writing and editing.

Funding

This work was supported by The Innovation Norway (Grant nr 2004/009991) and The SkatteFUNN R&D.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Thanks to Nordic Seafarms (Nordic Halibut AS) and Edvard Henden, for providing fish for the experimental work, and to Dr. Bente Ruyter for sharing unpublished data on peroxisomal β-oxidation in halibut and salmonids.

References

Ali, M., Nicieza, A., Wootton, R.J., 2003. Compensatory growth in fishes: a response to growth depression. Fish Fish. 4 (2), 147–190.

Babaei, S., Abedian-Kenari, A., Naseri, M., Yazdani-Sadati, M.A., Metón, I., 2020. Impact of starvation on digestive enzymes activities and plasma metabolites in Siberian sturgeon (Acipenser baerii, Brandt, 1869). Aquac. Res. 51 (4), 1689–1699.

Bar, N., 2014. Physiological and hormonal changes during prolonged starvation in fish. Can. J. Fish. Aquat. Sci. 71 (10), 1447–1458.

Binner, M., Kloas, W., Hardewig, I., 2008. Energy allocation in juvenile roach and burbot under different temperature and feeding regimes. Fish Physiol. Biochem. 34, 103–116.

Bolla, S., Nicolaisen, O., Amin, A., 2011. Liver alterations induced by long term feeding on commercial diets in Atlantic halibut (Hippoglossus hippoglossus L.) females. Histological and biochemical aspects. Aquaculture 312 (1–4), 117–125.

Brown, N., 2009. Development of Broodstock Diets for the Atlantic Halibut *Hippoglossus hippoglossus*. Center for Cooperative Aquaculture Research, University of Maine, Franklin, Maine, p. 51.

Chatzifotis, S., Papadaki, M., Despoti, S., Roufidou, C., Antonopoulou, E., 2011. Effect of starvation and re-feeding on reproductive indices, body weight, plasma metabolites and oxidative enzymes of sea bass (Dicentrarchus labrax). Aquaculture 316 (1–4), 53–59.

Chatzifotis, S., Clavero, S., Kounna, C., Soumalevris, A., Feidantsis, K., Antonopoulou, E., 2018. Effects of long-term feed deprivation on body weight loss, muscle composition, plasma metabolites, and intermediate metabolism of meagre (Argyrosomus regius) under different water temperatures. Fish Physiol. Biochem. 44, 527–542.

Crockett, E.L., Sidell, B.D., 1993a. Peroxisomal beta-oxidation is a significant pathway for catabolism of fatty acids in a marine teleost. Am. J. Phys. Regul. Integr. Comp. Phys. 264 (5), R1004–R1009.

Crockett, E.L., Sidell, B.D., 1993b. Substrate selectivities differ for hepatic mitochondrial and peroxisomal β-oxidation in an Antarctic fish, Notothenia gibberifrons. Biochem. J. 289 (2), 427–433.

Einen, O., Waagan, B., Thomassen, M.S., 1998. Starvation prior to slaughter in Atlantic salmon (Salmo salar): I. Effects on weight loss, body shape, slaughter-and fillet-yield, proximate and fatty acid composition. Aquaculture 166 (1–2), 85–104.

Folch, J., Lees, M., Stanley, G.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226 (1), 497–509. https://www.ncbi.nlm.nih.gov/pubmed/13428781.

Foss, A., Imsland, A.K., Vikingstad, E., Stefansson, S.O., Norberg, B., Pedersen, S., Sandvik, T., Roth, B., 2009. Compensatory growth in Atlantic halibut: effect of starvation and subsequent feeding on growth, maturation, feed utilization and flesh quality. Aquaculture 290 (3–4), 304–310.

Hamre, K., Øfsti, A., Næss, T., Nortvedt, R., Holm, J.C., 2003. Macronutrient composition of formulated diets for Atlantic halibut (Hippoglossus hippoglossus, L.) juveniles. Aquaculture 227 (1–4), 233–244.

Hamre, K., Moren, M., Solbakken, J., Opstad, I., Pittman, K., 2005. The impact of nutrition on metamorphosis in Atlantic halibut (Hippoglossus hippoglossus L.). Aquaculture 250 (3–4), 555–565.

Haug, T., Gulliksen, B., 1988. Fecundity and oocyte sizes in ovaries of female Atlantic halibut, Hippoglossus hippoglossus (L.). Sarsia 73 (4), 259–261.

Izquierdo, M., Fernandez-Palacios, H., Tacon, A., 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197 (1–4), 25–42.

Mason, M.E., Waller, G.R., 1964. Dimethoxypropane induced transesterification of fats and oils in preparation of methyl esters for gas chromatographic analysis. Anal. Chem. 36 (3), 583–586. https://doi.org/10.1021/ac60209a008.

Mazorra, C., Bruce, M., Bell, J., Davie, A., Alorend, E., Jordan, N., Rees, J., Papanikos, N., Porter, M., Bromage, N., 2003. Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (Hippoglossus hippoglossus). Aquaculture 227 (1–4), 21–33.

Migaud, H., Bell, G., Cabrita, E., McAndrew, B., Davie, A., Bobe, J., Herraez, M.P., Carrillo, M., 2013. Gamete quality and broodstock management in temperate fish. Rev. Aquac. 5, S194–S223.

Navarro, I., Gutierrez, J., 1995. Fasting and starvation. In: Biochemistry and Molecular Biology of Fishes, vol. 4. Elsevier, pp. 393–434.

Pérez-Jiménez, A., Guedes, M.J., Morales, A.E., Oliva-Teles, A., 2007. Metabolic responses to short starvation and refeeding in Dicentrarchus labrax. Effect of dietary composition. Aquaculture 265 (1–4), 325–335.

Pérez-Jiménez, A., Cardenete, G., Morales, A.E., García-Alcázar, A., Abellan, E., Hidalgo, M.C., 2009. Digestive enzymatic profile of Dentex dentex and response to different dietary formulations. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 154 (1), 157–164.

Pikle, R., Jatiger, R., Ganesh, C., 2017. Food-deprivation-induced suppression of pituitary–testicular-axis in the tilapia Oreochromis mossambicus. Int. Aquatic Res. 9 (3), 203–213.

Røsjø, C., Berg, T., Manum, K., Gjøen, T., Magnusson, S., Thomassen, M.S., 1994. Effects of temperature and dietary n-3 and n-6 fatty acids on endocytic processes in isolated rainbow trout (Oncorhynchus mykiss, Walbaum) hepatocytes. Fish Physiol. Biochem. 13, 119–132.

Sæle, Ø., Solbakken, J., Watanabe, K., Hamre, K., Pittman, K., 2003. The effect of diet on ossification and eye migration in Atlantic halibut larvae (Hippoglossus hippoglossus L.). Aquaculture 220 (1–4), 683–696.

Sales, C.F., Pinheiro, A.P.B., Ribeiro, Y.M., Weber, A.A., de Oliveira Paes-Leme, F., Luz, R.K., Bazzoli, N., Rizzo, E., Melo, R.M.C., 2020. Effects of starvation and refeeding cycles on spermatogenesis and sex steroids in the Nile tilapia Oreochromis niloticus. Mol. Cell. Endocrinol. 500, 110643.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2003. The lipids. Fish Nutr. 181–257.

Stepanowska, K., Nędzarek, A., 2020. Changes in the body chemical composition and the excretion of nitrogen and phosphorus during long-term starvation of Antarctic fish Notothenia coriiceps and Notothenia rossii. Eur. Zool. J. 87 (1), 571–579.

Wang, D., Ma, Q., Liao, Z., Bi, Q., Wei, Y., Liang, M., Xu, H., 2022. Tissue distribution and nutritional regulation of peroxisomal fatty acid β-oxidation genes: a preliminary study in two marine teleosts, turbot (Scophthalmus maximus) and tiger puffer (Takifugu rubripes). Aquac. Res. 53 (15), 5434–5443.

Yang, M., Deng, K., Pan, M., Gu, Z., Liu, D., Zhang, Y., Zhang, W., Mai, K., 2019. Glucose and lipid metabolic adaptations during postprandial starvation of Japanese flounder Paralichthys olivaceus previously fed different levels of dietary carbohydrates. Aquaculture 501, 416–429.