EFFECT OF DIET ON GROWTH, SURVIVAL AND REPRODUCTIVE PERFORMANCE OF FIRST GENERATION (F1) ATLANTIC COD, *GADUS MORHUA* L. BROODSTOCK

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by

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ABSTRACT

Hamoutene, D., Lush, L., Pérez-Casanova, J.C., Hobbs, K., Burt, K., Walsh, A., and Moir, J. 2013. Effect of diet on growth, survival and reproductive performance of first generation (F1) Atlantic cod, *Gadus morhua* L. broodstock. Can. Tech. Rep. Fish. Aquat. Sci. 3026: v + 21 p.

This study evaluated the influence of three diets (a commercial on-growing pellet, a commercial pellet formulated for marine finfish broodstock, and a diet of baitfish with marine broodstock vitamin supplement) on spawning and growth of first generation photomanipulated cod broodstock. All fish belonged to the same families and hatched in 2006. Feeding trials commenced in August 2008 after fish had spawned for the first time (May 2008). Results showed that broodstock cod fed baitfish had better growth and condition factor than those fed pelleted diets. The first spawning season monitored (February 2009) revealed no differences in egg quality between diets but higher sperm quality in males fed baitfish. Results from the following spawning season (October 2009) showed higher fertilization and hatch rates in the baitfish diet group. Evaluation of sperm performance showed higher quality in both broodstock diet and baitfish fed males. An improved synchronicity of spawning between males and females was observed in the broodstock diet group. Our results confirm the importance of diet in overall broodstock performance and suggest that the baitfish diet results in superior outcomes. The broodstock diet may also contribute to increased reproductive performance when compared to the regularly used on-growing diet.

RÉSUMÉ

Hamoutene, D., Lush, L., Pérez-Casanova, J.C., Hobbs, K., Burt, K., Walsh, A., and Moir, J. 2013. Effect of diet on growth, survival and reproductive performance of first generation (F1) Atlantic cod, *Gadus morhua* L. broodstock. Can. Tech. Rep. Fish. Aquat. Sci. 3026: v + 21 p.

Cette étude évalue l'influence de 3 régimes alimentaires (un régime de croissance classique, des granulés spécialement formulés pour des géniteurs, ainsi qu'un régime « naturel » à base de chair de poisson et enrichi avec des vitamines conçues pour les géniteurs) sur la reproduction et la croissance d'une génération de géniteurs de morues d'élevage. Tous les poissons nés en 2006 sont issus des mêmes familles (13 familles au total). Les essais ont débuté en Août 2008 et ce après la première saison de reproduction des poissons qui a eu lieu en Mai 2008. Nos résultats démontrent que les géniteurs nourris à la chair de poisson (et poulpes) ont une croissance supérieure et sont en meilleure condition que les poissons nourris aux granulés. La seconde saison de reproduction (Février 2009, après le commencement des essais) ne révèle aucune différence dans la qualité des œufs de poisson mais une qualité supérieure du sperme des mâles nourris au régime « naturel ». Les résultats de la saison qui a suivi (Octobre 2009) montrent des taux de fertilisation et d'éclosion supérieurs dans le groupe nourri au régime naturel. La qualité du sperme est supérieure chez les mâles nourris au régime « naturel » et aux granulés conçus pour les géniteurs. Une meilleure synchronicité entre la maturité des mâles et femelles est observée dans le groupe de poissons nourris aux granulés conçus pour les géniteurs. Notre étude confirme l'importance du régime alimentaire dans l'obtention de géniteurs performants et suggèrent que le régime « naturel » donne de meilleurs résultats. Les granulés conçus pour les géniteurs semblent également contribuer à une meilleure reproduction chez les géniteurs recevant ces granulés comparativement à ceux nourris aux granulés classiques.

INTRODUCTION

Broodstock nutrition affects the viability and health of offspring as well as that of the broodfish, and therefore feeding broodstock a diet to fulfill the optimal reproductive potential is of vital importance (Pavlov et al. 2004). For example, Sink & Lochmann (2008) have shown in a study on channel catfish that spawning success in broodstock improved as dietary lipid concentration increased. Similarly, percentages of morphologically normal eggs have been found to increase with an increase in the *n-3* highly unsaturated fatty acid level in broodstock diets (Fernandez-Palacios et al. 1995). Vitamin E is important for the control of reproduction, testes function, macrophage function and intracellular oxidation in mammals and fish (Pavlov et al. 2004). A deficiency of dietary vitamin E will affect the number of spawning fish as well as hatching success and juvenile survival (Watanabe 1985). In addition, the synchronicity of spawning (fish from a specific group spawning simultaneously), which is quite important for a commercial hatchery, is closely linked to diet. Growth during the months prior to spawning has been found to affect the time of spawning in cod, with well-fed fish commencing spawning earlier than poorer fed fish (Kjesbu et al. 1996). Despite the fact that a number of studies have demonstrated that reproductive performance and egg quality are influenced by nutrients, broodstock nutrition still remains one of the most poorly understood areas of finfish nutrition (Zakeri et al. 2009).

Numerous parameters can be used as endpoints for monitoring reproductive performance and egg quality in fish. While fertilization success has been proven to be a good predictor of egg quality in salmon, its reliability in marine finfish remains questionable (Kjorsvik et al. 1990; Bromage 1994; Sheilds et al. 1997). Low fertilization success is usually related to poor hatching success; however, high fertilization success does not necessarily lead to high egg and larval viability for all species (Pavlov et al. 2004). Regardless, this parameter allows a quick determination of whether a batch is highly fertilized and will potentially lead to a high yield of larvae. Moreover, Kjorsvik et al. (1990) suggested that an assessment of cell symmetry at the early stages of cleavage (normal blastomeres) may be a possible general indicator of egg quality for marine fish. In a study on blastomere morphological criteria, Avery et al. (2009) found that even if hatching success was not significantly different between normal and abnormal eggs, overall abnormal eggs had significantly greater egg mortality than normal eggs.

Atlantic cod (*Gadus morhua* L.) has become a candidate for cold ocean aquaculture in many countries. The majority of cod commercial hatcheries maintain broodstock consisting almost exclusively of captured wild fish (Pavlov *et al*. 2004). Selective breeding has led to improvements in growth for many fish species (e.g. Bondari 1983; Symonds et al. 2005), and has been investigated in Atlantic cod, aiming to improve production through enhanced growth, survival, disease resistance, environmental tolerance, and final marketable quality (Symonds et al. 2005). As it is likely that many hatcheries will develop captive-bred broodstock, increased knowledge on hatchery-reared spawners and their nutritional requirements is of importance to ensure appropriate use of any farmed first (F1) and subsequent generation broodstock. In Atlantic Canada, the use of wild cod broodstock in experimental hatcheries has led to the development of a standard diet consisting of wild baitfish [herring, *Clupea harengus*

L., mackerel, *Scomber scombrus* L. and squid, *Ilex illecebrosus* (Lesueur)] with vitamin supplementation (used by other authors, e.g. Penney et al. 2006a). However, a wild baitfish diet has numerous drawbacks including inconsistent supply, unpredictable quality, prohibitive costs, high risks for disease transfer and may not always provide adequate levels of nutrients (Pavlov et al. 2004). This project aimed to determine the influence of diet on the spawning and growth of first generation photomanipulated Atlantic cod broodstock. The diets studied included 1) a commercial on-growing pellet, 2) a commercial pellet manufactured specifically for marine finfish broodstock, and 3) the current standard diet of wild baitfish, supplemented with vitamins. Cod broodstock were monitored for growth, reproduction, feed intake, and general status for three years. We also assessed fertilization success, blastomere morphology, egg and sperm quality, hatch success, and subsequent larval lengths. In addition to information on broodstock diet, this study focused on first generation spawners born in captivity as many questions remain as to whether poor spawning is influenced by captive bred broodstock, improper diet, or both. Our results will contribute to enhance the understanding of the nutritional requirements of cod broodstock, in order to improve reproduction of hatchery-reared spawners in support of the long-term sustainability of cod culture.

MATERIALS AND METHODS

EXPERIMENTAL SET-UP AND DIET DESCRIPTION

All fish used for this study were held in seawater tanks at the Joe Brown Aquatic Research Building (Memorial University of Newfoundland, St. John's, NL, Canada) as part of a broodstock development project in collaboration with the Atlantic Cod Genome Project (CGP) selective breeding program (www.codgene.ca). First generation broodstock hatched in 2006 from 13 first generation families were used for the experiment. Before the initiation of these trials, all fish were fed a commercial pelleted on-growing cod diet (EWOS Marine Grower, EWOS Canada, Moncton, NB, Canada). Every fish was tagged with a passive integrated transponder (PIT) and information on sex (obtained after their first spawning in May 2008) and origin (e.g. parents) recorded. In June 2008, sexually mature fish (~500 g) were divided into 6 groups of 20 individuals (40 fish per diet). Each tank (15 m^3 flow-through) of 20 fish was stocked with 10 males and 10 females. Fish were distributed in the tanks in a manner to ensure that families were almost equally represented in all treatments; a design with sex ratio and family representation was used to achieve this result. After 2 months of acclimatization, feeding trials started in August 2008. Fish were held under a photo-advanced light regime using a six month phase shifted photoperiod (Penney et al. 2006b). Light/dark cycles were adjusted every five days using a computerized timer connected to the tank light source. Temperatures were controlled at an average of 6.3 °C throughout spawning with a total annual temperature range of 5-11 °C due to natural fluctuations in ambient seawater temperatures and chilling capacity of the facility.

Three diets were tested: 1) the on-growing diet (hereafter "On-growing diet"), 2) a commercial pelleted marine broodstock diet (hereafter "Broodstock diet"), (Vitalis CAL, Skretting España, Burgos, Spain), and 3) the current standard broodstock diet of wild

baitfish (herring, mackerel and squid not given simultaneously)(hereafter “Baitfish diet”) cut into ~2-3 cm pieces, with each piece supplemented with a vitamin and mineral premix pellet (Marine Finfish Vitamin/Mineral Supplement manufactured at the St. Andrews Biological Station, NB, Canada). The general composition of pelleted diets (as provided by manufacturers) is summarized in Table 1. Ash, dry mass and moisture content of herring, mackerel, and squid were completed by processing 5 samples of each product. After weighing 10-15 g of thawed product, homogenization was completed using a Polytron pt 3000 tissue homogenizer (Brinkmann Instruments, Mississauga, ON, Canada) and ash, dry mass and moisture content was obtained after drying the samples in a drying oven, a dessicator and a muffle furnace for ash (e.g. Gaylean 1980). To calculate lipid percentages, baitfish samples (n=3 samples per baitfish type) were homogenized and lipids extracted in a mixture of ice cold chloroform:methanol (2:1) to a final dilution of 20-fold the volume of the sample. Percentages were determined gravimetrically as described in Folch et al. (1957). Dry mass of the baitfish samples was used to calculate the lipid content as percent dry mass as follows:

$$\text{Lipid content (\% dry mass)} = (\text{lipid content} * \text{dry mass}^{-1}) * 100$$

These calculations were necessary in order to compare the composition of the 3 diets and the food conversion ratio (FCR) between diets. Ash, moisture, as well as lipid percentage values of baitfish (values represent means of the three baitfish types analyzed) are listed in Table 1. The amount of food consumed during each meal was calculated as the difference between the mass of the food offered and that of the uneaten. FCR for each tank and for all diets was calculated according to Tacon (1990) as:

$$\text{FCR} = (\text{g feed consumed} * \text{gain in fish mass}^{-1})$$

RATION DETERMINATION

Experimental set-up occurred in a semi-commercial setting where broodstock were traditionally fed twice a week to satiation. Fish were manually fed the selected diet until apparent satiation was reached. Satiation was determined as the point when fish stopped actively feeding and the feed remained at the bottom of the tanks for more than 5 min. Considering that the broodstock diet had not been tested for cod, a preliminary trial was set-up to ensure that feeding the fish twice per week was appropriate. Four additional tanks were stocked with F1 cod randomly selected (n=8 fish per tank) with two tanks fed daily and two fed twice per week. Both groups were fed to satiation and fish were monitored for growth during a 3 month period.

GROWTH AND FISH GENERAL CONDITION

Fish wet body mass (BM) and total length (TL) were measured on July 30, 2008 (just prior to the initiation of the feeding trials), February 3, 2009 (during second spawning),

April 22, 2009 and March 17, 2010 and used to calculate specific growth rate (SGR) using the formula outlined by Ricker (1979):

$$SGR\ (\%\ BM\ day^{-1}) = 100 * (\ln final\ mass - \ln initial\ mass) * days^{-1}$$

and Fulton's condition factor (CF) as:

$$CF = (BM * TL^{-3}) * 100$$

Fulton's condition factor was chosen as it has previously been used in other studies on Atlantic cod reproduction (Lambert and Dutil 2000).

SYNCHRONICITY OF SPAWNING

All fish in every tank were monitored twice a week during two spawning periods (February 2009 and October-November 2009) to record the number of fish spawning for every day of monitoring (expressed as a percentage of spawning males/females per sampling). Fish were removed by net from the tanks (one tank at a time) and placed in 100 L plastic holding containers supplied with sea water. Individual fish were then manually stripped and eggs/sperm collected in plastic jars or glass vials respectively. The handling process did not last more than 2-3 min per fish. An observation frequency of two days per week was chosen to avoid overstressing the fish by daily handling. A total of seven observations per spawning were gathered, mainly targeting the middle and end of the reproductive period. The percentage of fish spawning at these bi-weekly samplings (percentage of spawners per sampling), as demonstrated by the ability to collect gametes, was recorded. These percentages were also calculated for males and females separately, across the diets. As all fish were PIT-tagged, any fish that did not spawn during the observation period was identified allowing us to determine a total number of spawners for every diet regardless of the number of times they produced gametes.

EGG AND SPERM QUALITY PARAMETERS

Crosses were completed by strip spawning according to Lush et al. (2005) while taking into account levels of relatedness, to avoid crossing siblings. As stated earlier, two spawning events were monitored, February 2009 (second spawning for this population) and October-November 2009 (third spawning). Egg quality for each batch was assessed based on the following parameters: fertilization success (# eggs with visible cell divisions $* 100^{-1}$), egg diameters of ten eggs, total volume of eggs, volume of sunken eggs, and volume of floating eggs. A series of blastomere normality criteria including cell symmetry, uniformity, adhesion, margins, clarity and number was also investigated following an adaptation of Shields et al. (1997) and Penney *et al*. (2006a). Blastomere normality parameters were measured on 100 eggs per batch and expressed as percentages of normal eggs. All successful egg batches (fertilization > 20 %) from the study groups were disinfected with ozone and incubated in 50 L conical incubators,

supplied with seawater at 1 L min^{-1} and aeration, and maintained at 5-6 °C. Estimated hatch success was calculated by stopping water flow to tank with continued aeration to distribute larvae through water column for 5 min, after which triplicate counts of larvae were performed by taking a 100 mL water sample from the incubator. Counts were averaged and extrapolated to determine larvae number in volume of incubator. Hatch success was then calculated based on the total batch volume using Kjesbu's (1989) calculation for number of eggs per batch based on batch volume and average egg diameter. Sperm quality was monitored (single observer) by evaluating motility under the microscope and establishing a ranking of samples according to the following scale: no motility detected (0); little motility detected and slow movement (1); over half motile and movement not vigorous (1.5); vast majority motile and swimming actively (2); all cells vigorously motile (2.5).

LARVAL REARING AND MEASUREMENTS

When 100 % of the eggs had hatched (0 day post hatch; dph), larvae were transferred to 500 L rearing tanks greened with algal paste (Reed Mariculture, Campbell, CA, USA) twice per day (12.5 mL of algae paste per tank). Tanks were supplied with 20 µm filtered UV treated seawater. Temperature was maintained at approximately 10.5 °C. Larvae were fed Ori-Green (Skretting, Bayside, NB, Canada) enriched rotifers from 1 until 30 dph three times daily. At 25 dph, 10 larvae from each tank (7 families for on-growing diet, 9 families for the broodstock diet and 10 for the baitfish diet group) were randomly chosen for length measurements.

Larvae were euthanized with an overdose of tricaine methanesulfonate (TMS) (Syndel Laboratories, Vancouver, BC, Canada) and individually measured to the nearest 0.1 mm for standard length using a stereoscope equipped with an eyepiece micrometer.

STATISTICAL ANALYSIS

One way ANOVA and/or two-way ANOVA were employed to compare data using SIGMA STAT software (Systat Software Inc., Chicago, IL, USA); multiple comparisons were tested with the Holm-Sidak method. Two-way ANOVA was used to explore tank effect on all data. It was also used to check potential sex effect on growth data. As all parameters of blastomere normality, as well as fertilization success and hatch success are expressed as percentages, data were arcsine square root transformed prior to statistical analyses but this transformation showed no effect on results. Arcsine square root transformation is necessary when a sizeable number of the observed proportions are either relatively small ($P < 0.2$) or large ($0.8 < P < 1$); if most of the computed proportions lie between 0.2 and 0.7, it should have little impact on the results (Snedecor & Cochran 1980). Cumulative mortality data was used to calculate regression equations for each group by plotting mortality data and time. To test for statistical differences, an ANCOVA was used for multiple comparisons of the regression coefficients (slopes) using the software Statistica 8 (StatSoft, Inc., Tulsa, OK, USA). Significance level was set at 0.05.

RESULTS

DIET DESCRIPTION

Moisture, ash, and lipid contents were determined on herring, mackerel and squid (baitfish diet) and presented in Table 1. All the other information found in Table 1 is provided by the manufacturers of the pelleted diets (on-growing and broodstock). No statistical analysis was performed on this data as no access to the raw data for either of the pelleted diets was available. We can note the fact that the broodstock diet is enriched with vitamins A and E (as compared to the on-growing diet). The baitfish diet has the highest lipid content of all the diets. Baitfish lipid content obtained gravimetrically was: 13.67 % for squid, 30.63 % for herring, and 69.11 % for mackerel; the number presented in Table 1 represents the mean of these values.

RATION DETERMINATION

After a 3 month preliminary trial, fish fed twice a week and fish fed daily (broodstock diet) showed no significant differences ($P > 0.05$) in BM and SGR (0.48 % BM day^{-1} for fish fed daily, 0.60 % BM day^{-1} for fish fed twice per week) by two-way ANOVA (rations, no tank effect). Fish initially had a mean BM of 455.38 ± 24.66 g (n=16), and 465.56 ± 21.33 g (n=16), and had a final BM of 728.15 ± 35.45 g and 839.04 ± 54.64 g when fed daily or twice per week respectively. This information alleviated concerns regarding adequate feeding frequency of the broodstock diet considering this was the only diet that had not been previously used for cod aquaculture. The previous standard of feeding twice per week was therefore maintained for all three diets in this study.

GROWTH AND FISH GENERAL CONDITION

No tank effect was found when considering growth of all groups of fish allowing us to group data by diet. At initial assessment, no significant differences were detected in body mass or condition factors of fish ($P < 0.05$, Table 2). At the following assessment, fish fed the baitfish diet had BM values higher than fish fed the two pelleted diets ($P < 0.05$, Table 2). Similarly, SGR of cod fed the baitfish diet was higher than in both remaining groups ($P < 0.05$). Though, it is important to note that after the last BM measurement the fish fed the broodstock diet exhibited higher SGR than the group fed the on-growing diet ($P < 0.05$). Data also showed that fish fed the baitfish diet had a higher condition factor than the on-growing and broodstock diet groups ($P < 0.05$). At the last weighing, these differences were significant ($P < 0.05$) only when baitfish diet fish were compared to the on-growing diet group as no statistical differences in CF was found between broodstock and baitfish diet fish at that sampling.

Table 1. Diet composition for pelleted diets as provided by manufacturer and for baitfish diet with moisture and lipid contents analyzed as described herein.

Diet	Pro %	Lip %	Fib %	Ash %	Moi %	Vit A IU kg^{-1}	Vit D3 IU kg^{-1}	Vit E IU kg^{-1}	Ca %	P %	Na %
On growing	53	12	1	11	7	3000	3000	200	2.2	1.8	0.9
Broodstock	54	18	1.1	11	7-9	7500	1125	600		1.6	
Baitfish (supplemented with vitamins and minerals		37.8		7.4	72.2	500*	400*		1.2*		

1-Pro: protein, Fib: Fibre, Ca: Calcium, Na: Sodium, Lip: Lipid, Moi: Moisture, P: Phosphorus
2-The percentages for the baitfish diet represent the average of percentages obtained for herring, mackerel and squid (n=5 subsamples for each)
*-depnotes amount available from vitamin/mineral supplement only.

When calculating growth data per sex (Table 3), the baitfish diet males and females had higher BM than the ones fed the on-growing diet ($P < 0.05$). SGR values were also higher for the baitfish diet; however, differences between the broodstock diet group and the baitfish diet group can be seen only in females, with no differences in SGR between males of the two diets. No statistical differences ($P > 0.05$) in SGR were found between on-growing and broodstock diet groups. Condition factor values show that the trend observed for all fish (Table 2) is due to a male effect and that females show no statistical differences ($P > 0.05$) in final condition factors between diets. No differences in initial values of CF were found in males and/or females.

Feed conversion ratios (FCR) were calculated for the three diets and for individual tanks (two per diet). As consumption by single fish was not monitored, statistical analyses were not completed on these values (n=2). For the on-growing diet the FCR was 2.50 (2.35 and 2.65 for each tank) and the FCR for the broodstock diet was lower: 1.87 (1.79 and 1.96 for each tank). The lowest FCR was obtained by the fish fed the baitfish diet: 0.86 (0.93 and 0.80 for each tank).

Fish cumulative mortality (% of initial number of fish) was evaluated for all diets and significant differences were detected among treatments ($p<0.0001$; $F=54.7147$) (Fig. 1). Results of the ANCOVAs with Newman-Keuls post hoc testing showed that the fish fed the baitfish diet had lower cumulative mortality than the other two groups ($p<0.001$). Differences were also found in cumulative mortality between the group of fish fed the on-growing diet and the one fed the broodstock diet, with mortality rate being higher in the broodstock diet group ($p < 0.0001$).

Some mortality during and post-spawning was observed, mostly as a result of females retaining, and never releasing the ovulated oocytes, resulting in an ‘eggbound’ state. This accounted for a higher percentage of the total mortality in the on-growing diet group

with 24% eggbound mortalities than in the broodstock diet and baitfish diet groups, where 16% and 11% eggbound females were observed respectively. Other spawning related mortality was low, with only a further 3% of total mortality occurring in the on growing diet group at or post spawning, and 7% in the baitfish diet group. The broodstock diet group has no further spawning related mortality. A few noticeable and sudden increases in mortality were observed, particularly September/October 2008 with the broodstock diet fed group and August/September 2009 with both the on-growing and baitfish fed groups. Some difficulties in broodstock accepting the broodstock diet were noted at the commencement of the feeding trials and this may have contributed to the mortality seen at this earlier phases of the study. In addition, warmest annual seawater temperatures are observed in August/September which may have contributed to higher mortality as a result of environmental stress. It is also important to note that an outbreak of the microsporidian, *Loma morhua* was responsible for some of the mortality observed in the three groups.

Table 2. Means (±SE) for wet body mass (BM), specific growth rate (SGR) and condition factor for cod fed three different diets.. Different superscripted letters denote significant differences ($P < 0.05$) after application of two-way ANOVA (tank effect, diet effect) and Holm-Sidak method as ad-hoc test.

Parameter	Diet	Sampling Period			
		Jul 2008*	Feb 2009	Apr 2009	Mar 2010
Body Mass (g)	On Growing	523.81 ± 16.95 [a]	928.15 ± 44.95[a]	1051.30 ± 47.61[a]	1609.25 ± 92.95[a]
	Broodstock	492.53 ± 12.46 [a]	874.82 ± 42.58[a]	1002.62 ± 49.69[a]	1705.24 ± 79.32[a]
	Baitfish	509.23 ± 13.84 [a]	1190.89 ± 72.16[b]	1404.27 ± 71.56[b]	2223.70 ± 126.01[b]
Specific Growth Rate (% BM day^{-1})	On Growing	NA	0.30 ± 0.10[a]	0.29 ± 0.01[a]	0.18 ± 0.01[a]
	Broodstock	NA	0.35 ± 0.04[a]	0.29 ± 0.02[a]	0.21 ± 0.01[b]
	Baitfish	NA	0.47 ± 0.03[b]	0.40 ± 0.02[b]	0.24 ± 0.01[c]
Condtion Factor	On Growing	0.82± 0.02 [a]	1.14 ± 0.12[a]	1.01 ± 0.02[a]	0.99 ± 0.03[a]
	Broodstock	0.79 ± 0.02 [a]	1.02 ± 0.03[a]	1.02 ± 0.03[a]	1.14 ± 0.05[a,b]
	Baitfish	0.78 ± 0.01 [a]	1.20 ± 0.05[b]	1.20 ± 0.05[b]	1.28 ± 0.07[b]

*Initial measurement

Table 3. Means (± one SD) for wet body mass (BM), specific growth rate (SGR), and condition factor (CF) across diets by sex. Means within sexes not sharing the same superscripted letter are significantly different (P<0.05) after application of two-way ANOVA (diet effect, and tank or sex effect) and Holm-Sidak method as ad-hoc test.

Diet	BM (g) (Initial)		BM (g) (Mar 2010)		SGR (%BM day^{-1})		Condition Factor (Mar 2010)	
	Males	Females	Males	Females	Males	Females	Males	Females
On-growing	525.89 ± 1.71^{a}	529.73 ± 21.71^{a}	1637.92 ± 148.08^{a}	1573.41 ± 165.56^{a}	0.18 ± 0.01^{a}	0.16 ± 0.01^{a}	0.95 ± 0.07^{a}	1.03 ± 0.08
Broodstock	481.64 ± 21.18^{a}	509.36 ± 21.18^{a}	1763.35 ± 165.56^{a,b}	1647.13 ± 165.56^{a,b}	0.22 ± 0.01^{a,b}	0.20 ± 0.02^{a}	1.14 ± 0.09^{a,b}	1.11 ± 0.01
Baitfish	505.10 ± 21.18^{a}	509.90 ± 22.27^{a}	2235.25 ± 125.15^{b}	2200.59 ± 176.99^{b}	0.25 ± 0.01^{b}	0.25 ± 0.01^{b}	1.33 ± 0.08^{b}	1.24 ± 0.09

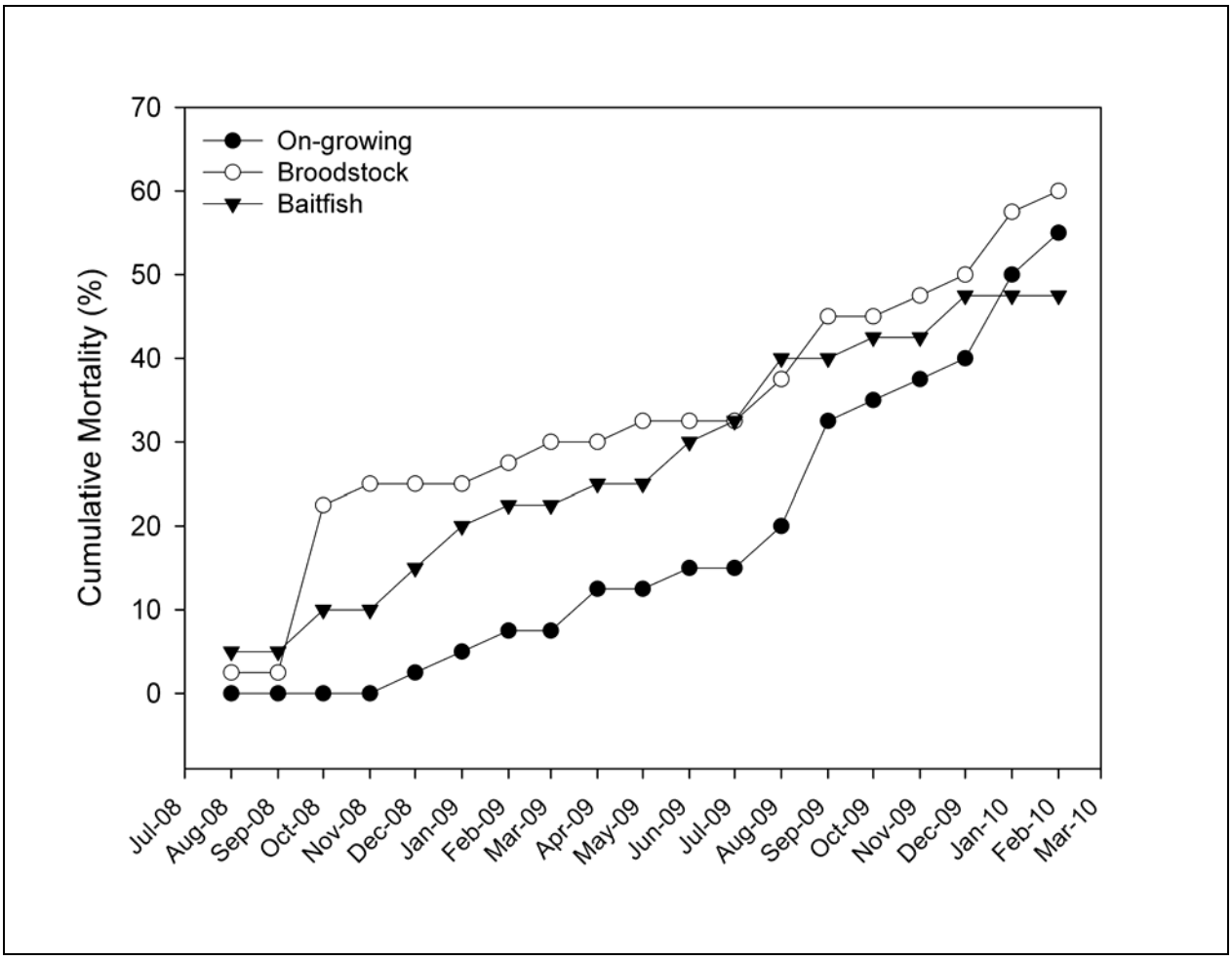

Figure1. Cumulative mortality recorded from July 2008 to March 2010 across diets.
●: On-growing diet, ○: Broodstock diet, ▼: Baitfish diet.

SYNCHRONICITY OF SPAWNING

No tank effect was found when considering the number of fish spawning per tank for each diet, thus, data was grouped according to diet. The total number of spawning fish checked at the bi-weekly samplings (n=7) are summarized in Table 4. Data show that the number of spawners increased from the second spawning to the third spawning (no statistical analysis was done on these numbers as they represent a unique value for each diet). The bi-weekly observations gathered throughout the spawning periods showed that the lowest number of spawners per sampling was found in the baitfish diet fish ($P < 0.05$). Data also demonstrate a poor synchronicity of spawning in the baitfish diet group as compared to the two other diets with a significantly lower percentage of females spawning when males were spawning. Interestingly, the broodstock diet group showed the highest number of females spawning simultaneously at the third spawning.

No significant differences were found in male spawners and their spawning readiness in relation to diet.

Table 4. Means (± one SD) for percentages of spawners after strip spawning across diets (n=7observations). Means not sharing the same superscripted letter are significantly different (P<0.05) after application of two-way ANOVA (diet effect, and tank or sex effect) and Holm-Sidak method as ad-hoc test.

	Second spawning			Third spawning		
	OG	BR	BA	OG	BR	BA
% spawning of all females	40.0	43.6	23.8	92.9	87.5	75.0
% spawning of all males	60.7	47.9	37.5	100.0	90.0	100.0
% spawners per sampling (n=7)	27.5 ± 2.6	24.4 ± 4.3	17.4 ± 2.2	55.4 ± 3.2	66.9 ± 5.4	51.4 ± 3.9
% females spawning per sampling (n=7)	25.0 ± 2.6^{a}	21.4 ± 4.9^{a}	7.7 ± 3.4^{b}	37.5 ± 3.1^{a}	58.7 ± 7.7^{b}	15.2 ± 4.0^{c}
% males spawning per sampling (n=7)	31.1 ± 4.8	27.0 ± 9.4	24.4 ± 2.8	87.3 ± 7.0	74.3 ± 5.3	87.6 ± 4.5

1- OG: on-growing diet, BR: broodstock diet, BA: baitfish.

GAMETE QUALITY AND LARVAL LENGTH

Sperm data presented in Table 5 summarizes the evaluation of motility for one sample for every male which spawned. Data showed that both the broodstock diet and baitfish diet fed males had significantly ($P < 0.05$) more actively swimming sperm than the ones fed the on-growing diet at both spawning times.

Table 5. Mean (± SE) ranking of sperm motility across diets (only one sample for every male was considered for this evaluation). Means within a column not sharing the same superscripted letter are significantly different ($P < 0.05$) after application of two-way ANOVA (diet effect, and sex effect) and Holm-Sidak method as ad-hoc test.

Diet	Second spawning	Third spawning
On-growing (n=10)	1.19 ± 0.18^{a} (n=10)	0.74 ± 0.11^{a} (n=12)
Broodstock (n=9)	1.86 ± 0.18^{b} (n=9)	2.32 ± 0.11^{b} (n=11)
Baitfish (n=8)	2.29 ± 0.22^{b} (n=8)	2.41 ± 0.09^{b} (n=18)

No motility detected (0); little motility detected and slow movement (1); over half motile and movement not vigorous (1.5); vast majority motile and swimming actively (2); all cells are vigorously motile (2.5).

Egg quality was evaluated by measuring fertilization success, hatch success (Table 6) and blastomere normality percentages (results not shown). Blastomere normality percentages showed no differences between diets at both of the monitored spawning seasons. Moreover, no differences ($P > 0.05$) between diets were found in fertilization and hatch success assessed at the second spawning (results not presented). At the third spawning (Table 6), statistically significant differences between diets were found in fertilization, and hatch success with the baitfish diet group having the highest values ($P < 0.05$).

Values for larval standard lengths were higher ($P < 0.05$) in crosses originating from the baitfish diet parents when compared to the larvae originated from parents fed the other two diets (Table 6). The standard length of larvae obtained from fish fed the broodstock diet was not significantly different from the ones obtained from the on-growing diet group ($P > 0.05$). Not all the crosses completed for these trials were used to stock the hatchery tanks due to space restrictions. Therefore (as can be seen in Table 6), the number of samples completed and evaluated for fertilization, hatch, and blastomere normality percentages are higher than the number of families processed for larval length measurements.

Table 6. Mean (±SE) for fertilization rates, hatch rates and larval lengths of crosses completed per diet for third spawning season. Means within a column not sharing the same superscripted letter are significantly different ($P < 0.05$) after application of two-way ANOVA (diet effect, and tank effect) and Holm Sidak method as ad-hoc test.

Diet	Fertilization rates (%)	Hatch rates (%)	Standard Length (mm)
On-growing	14.72 ± 3.03^{a} (n=15)	$10.37 \pm 3.56^{a,b}$ (n=15)	8.909 ± 0.174^{a} (n=7)
Broodstock	$24.40 \pm 4.08^{a,b}$ (n=21)	9.09 ± 2.10^{a} (n=21)	9.209 ± 0.12^{a} (n=9)
Baitfish	36.22 ± 5.84^{b} (n=12)	22.87 ± 5.20^{b} (n=12)	9.728 ± 0.112^{b} (n=10)

Standard length values represent the mean of 10 larvae per family (70 larvae for on-growing, 90 larvae for broodstock, 100 larvae for baitfish).

DISCUSSION

Our results show that the baitfish diet is the superior choice in terms of fish growth, general condition and reproduction and that the broodstock diet resulted in overall better fish performance than the on-growing diet currently used for F1 feeding. In particular, the main differences between the broodstock and the on-growing diet groups were a better synchronicity of females, improved sperm quality and a higher SGR for the spawners fed the broodstock diet. Since the broodstock used in this study originated from the same families, we can affirm that differences between groups are likely due to a dietary effect and not a genetic difference between fish. As it is difficult to effectively discuss differences between diets without a detailed diet composition, we will be limiting our discussion to information provided by the manufacturers and the differences in lipid amounts. The gravimetric analyses completed by us, in addition to the pelleted diets manufacturer's information suggest that the baitfish diet has the highest lipid percentage (37.8 %). Sink & Lochmann (2008) showed in a study on channel catfish that spawning success in broodstock improved as dietary lipid concentration increased. This is in accordance with our results showing that the highest spawning success (as evaluated by the criteria used in this study) was obtained in the fish fed the baitfish diet. However, detailed analyses of lipid composition (in particular fatty acid content) are necessary for a proper understanding of this aspect. Vitamin E is important for the control of reproduction, testes function, macrophage function and intracellular oxidation in mammals and fish (Pavlov et al. 2004). A deficiency of dietary vitamin E will affect the number of spawning fish as well as hatching success and juvenile survival (Watanabe 1985). The broodstock diet did not result in neither an overall higher number of spawners at both monitored seasons (for on-growing diet: 40 % to 92.9 % of the females spawned, 60 % to 100 % for males; for broodstock diet: 43.6 % to 87.5 % for females and 47.9 % to 90.0 % for males) nor an improved hatching success. However, the

number of females spawning simultaneously and repeatedly was higher, suggesting a better female “readiness” in the broodstock diet groups. A major difference between the broodstock diet used in this study and the on-growing diet is the squid meal inclusion (www.skretting.com/internet/SkrettingGlobal). Among different feed ingredients, cuttlefish, squid and krill meals are recognized as valuable components of broodstock diets. The protein component of cuttlefish and squid meals together with their optimal concentration of highly unsaturated fatty acids appears to be responsible for their positive effect on reproductive performance (Izquierdo et al. 2001). Squid’s efficacy has been ascribed to its superior protein quality, higher phospholipid and cholesterol content (Watanabe et al. 1991) and/or its better apparent protein digestibility coefficients (Fernandez-Palacios et al. 1997).

The baitfish diet gave better results in terms of growth, as well as a lower mortality than both pelleted diets. Moreover, our results show that the baitfish diet had the lowest FCR of all three diets, however, it is important to point out that no statistical analysis was conducted on the FCR data since calculations were completed per tank (n=2 for each diet). The inherent high moisture content of the baitfish diet would attribute to the lower FCR described in the broodstock fed the baitfish diet, as a higher mass of feed (compared to a dry pelleted diet) is required to result in growth. It is surprising to note that in terms of condition factor, the main differences between diets are related to males. Males generally utilize less energy for gonad maturation for a given BM than do females (Jonsson et al. 1991). Moreover, experiments on some marine fish have indicated that food manipulation may not affect the proportion of mature males and the mass of the testes, but can affect ovarian investment (Karlsen et al. 1995; Bromley et al. 2000). In this study, no significant differences between the proportions of mature males were found between diets but the influence of feed on the male’s condition factor had a larger contribution than for females. As males use less energy for gonad maturation than females (Jonsson et al. 1991), it is plausible that the amount of surplus energy available (from a different diet) to improve their condition factor is higher. Furthermore, male gonadal tissue does not occupy the body cavity to the same extent as that of a female during later stages of annual reproductive development. Thus, there is likely to be more space available for food within a male compared with a female’s body (Fordham AND Trippel 1999).

In our study, the broodstock diet group showed that during the third spawning period, 58.7 % of the females were spawning when monitored twice weekly versus 37.5 % for the on-growing diet group and 15.2 % for the baitfish fed fish. It is known that in cod mass spawning tanks not all females are perfectly synchronized (Kjorsvik et al. 2004). These results suggest that the broodstock diet might improve female readiness and ability to spawn repeatedly. We can hypothesize a potential effect of the broodstock diet on the inter-ovulatory period and/or gonad atresia, though without a precise account of batch production for every female this remains speculative. The impact of atretic processes on the potential fecundity of cod was examined by Kjesbu et al. (1991). Erosion of potential fecundity during the spawning season was linked to maternal nutritional status, with females spawning between 20 % to 80 % of their potential fecundity depending on their somatic condition (Kjesbu et al. 1991). In addition, Kjesbu et al. (1990) reported that acclimated cod are regular serial batch spawners with

60 hour-intervals between spawning events. No specific effect of the nutritional status, or stress (Morgan et al. 1999) other than temperature related (McEvoy and McEvoy 1992) has been reported to affect the length of the inter-ovulatory period in cod. The fact that both pelleted diet groups had higher female spawning synchronicity than the baitfish group also suggests a possible inconsistency inherent with feeding fish a “mixed diet” versus pellets of invariable composition. The baitfish diet consisted of a mix of mackerel, herring and squid supplemented with vitamins; fish would be fed either of these preys per meal (mackerel, herring and then squid sequentially). Nonetheless, this pattern did vary in some instances with supply. Knowing the hierarchical feeding behaviour of cod in tanks (e.g. Hoglund et al. 2005) and the fact that fish were fed different baits, females and males may have consumed different baitfish (in different amounts) during the gonad build-up phase consequently influencing their readiness to spawn at the same time.

We have used fertilization, hatch success, and blastomere normality criteria to evaluate the reproductive output of spawners. The criteria used in this study are reflective of effects of diets on reproductive products of both males and females post fertilization; though we have also evaluated by microscopic observation the general motility of sperm in males. Since the fatty acid composition of sperm is determined by the essential fatty acid composition of broodstock diet in species such as rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Watanabe et al. 1984) and European sea bass, *Dicentrarchus labrax* L. (Asturiano 1999), it is possible that sperm motility and in turn fertilization would be affected by the dietary fatty acid makeup (Izquierdo et al. 2001). Our spawning results show that the baitfish diet gave better results in terms of reproductive output than both pelleted diets. It is often reported that female broodstock fed on “natural diets” produce eggs of better quality than those on formulated commercial diets (Brooks et al. 1997), though, we cannot comment on this point as we have not assessed egg quality in females prior to fertilization. The use of unprocessed products does not always provide adequate levels of nutrients and nutrition of broodstock can be improved by feeding marine fish on fresh marine organisms in combination with commercial diets (Pavlov et al. 2004). It is important to note that the baitfish used in this study was enriched with vitamin pellets that would have contributed to complete the nutritional requirements of the broodstock, made apparent by their growth and improved reproductive output.

The fact that no differences in egg quality were found in the first reproductive period monitored (second spawning) might be explained by the fact that at that point fish did not have enough time to take advantage of the diet change. The diet trials were initiated in August 2008 and fish spawned in February 2009 representing at this point seven months of feeding. In cod, ovarian development starts eight to nine months prior to the onset of spawning, and nutrients are incorporated into the oocytes up to the final maturation of the different egg batches (Kjesbu et al. 1991). To date, no experiments have been performed to determine the required feeding period for good nutritional status at the start of gonad growth in cod. A dietary influence on both fertilization and hatch success was seen at the following spawning period after 15 months of feeding trials. Moreover, in a study on first-time spawning male and female cod, Yoneda and Wright (2005a, 2005b) suggest that first-time spawning cod have a minimal level of growth that must be maintained if spawners are to invest in reproduction. This suggests that once committed, cod do not reduce gamete production in response to low food or unfavorable

temperatures during the first reproductive season (Yoneda and Wright 2005b). Nonetheless, this statement might be valid only for first-time spawners as food manipulation experiments on repeat spawning cod demonstrated that a lowering of body condition led them to reabsorb oocytes and to reduce fecundity (Kjesbu et al. 1991). An individual's growth trajectory can be roughly divided into three phases: first, only somatic growth takes place up to the age at sexual maturation; second, growth is balanced with reproduction during some years following maturation, and third, reproduction receives the primary focus of energy allocation (Jorgensen et al. 2006). This suggests a stronger link between body condition and reproductive outcomes after the first spawning.

The cod used in this study are part of the first generation of farmed broodstock obtained through selective breeding (www.codgene.ca). While most cod broodstock are still caught from the wild (Pavlov et al. 2004); our study brings novel information on hatchery-reared broodstock. The fertilization and hatch success data obtained across diets proved to be quite low with values below 10 % in some instances. For example, in a study on wild-caught captive cod broodstock, Trippel (1998) found fertilization rates of 20 % and 84 % for first-time and second-time spawners respectively, as well as hatching success lower for first time than for second-time spawners (55 % versus 75 %). Considering the fact that the fertilization and hatch success presented in Table 6 are descriptive of the cod's third spawning, the values observed in this study remain surprisingly low. This might be attributed to the fact that the cod broodstock considered here are hatchery reared versus wild fish held captive, and that inherent differences due to life history might have influenced their reproductive performance. A study on sperm of wild and farmed cod published by Skjaeraasen et al. (2009) showed impaired sperm quality in farmed cod. One of the explanations given for the better performance of wild broodstock is the contrast in social dynamics and competition in the farmed and wild environments (Locatello et al. 2007; Skjaeraasen et al. 2009). As found in wild cod stocks (e.g. Murawski et al. 2001), an increase in the proportion of mature spawners can be observed with age. The total number of spawners across the diets increased from 23.8-60.7 % of the total population in the first year of the study (second spawning season) to 75-100 % in the subsequent season.

Our results suggest that the baitfish diet gives the best results in terms of growth and reproductive output. The broodstock diet is more efficient than the regularly used on-growing diet, and provides a better synchronicity in female reproduction than the two other diets. The on-growing diet used in this study is not fulfilling the nutritional requirements of cod broodstock and should not be used for that purpose. The debate remains whether to use unprocessed fish products considering their benefits and known shortcomings versus improving the nutritional quality of formulated feeds. These improvements would increase feed production costs, which would be even higher if diets are developed for each species (Izquierdo et al. 2001). Nonetheless, the harvesting of squid, cuttlefish, mussels, krill and other fish species to feed cultured broodstock is cost prohibitive and defeats the purpose of the promotion of a sustainable development of the aquaculture industry. In addition, lower FCRs for the baitfish diet may potentially be prohibitive as increased feed volume and costs will occur if this diet is chosen to feed cod broodstock and it will have to be determined if the trade off of improved broodstock performance for higher FCR is preferential. From a research perspective, more

information is necessary to understand the diet effect on female cod egg production as well as the completion of detailed diet lipid analyses to better explain the contribution of these lipids to cod broodstock growth and reproduction.

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