

Timing and determination of potential fecundity in Atlantic cod (*Gadus morhua*)

Jon Egil Skjæraasen, Trygve Nilsen, and Olav S. Kjesbu

Abstract: Studies using annual averages of lipid storage or estimated quality of the feeding season have shown that energy reserves influence egg production in Atlantic cod (*Gadus morhua*). However, vitellogenesis starts months before spawning. Therefore, energy reserves near the start of vitellogenesis might provide better proxies of fecundity and hence egg production than yearly averages. If so, proxies with large temporal variations (e.g., weight and lipid energy) should vary similarly in their predictive power, and females with different spawning periods should have their fecundity determined at different times. We exposed cod to two photoperiods to induce different spawning seasons. Growth before spawning was monitored, and potential fecundity was measured at the onset of spawning. The date yielding the greatest explanatory power differed between photoperiods. As proxies, length varied less and had lower explanatory power than weight. Lipid energy at the onset of spawning was a poor proxy. The greatest explanatory power was found ~3–4 months before spawning around the start of vitellogenesis, indicating that potential fecundity was highly influenced by female energy reserves at this time. Determination of potential fecundity early in vitellogenesis may be a common feature for determinate teleost spawners.

Résumé : Les études qui tiennent compte des moyennes annuelles de stockage des lipides ou d'une autre estimation de la qualité de la saison d'alimentation montrent que les réserves énergétiques influencent la production d'oeufs chez la morue franche (*Gadus morhua*). Cependant, la vitellogenèse commence plusieurs mois avant la fraye. Les réserves énergétiques vers le début de la vitellogenèse sont donc peut-être de meilleurs variables de remplacement de la fécondité et ainsi de la production d'oeufs que les moyennes annuelles. Si cela est vrai, les variables de remplacement qui changent considérablement dans le temps (par exemple, la masse et l'énergie lipidique) devraient aussi varier dans leur pouvoir de prédiction et les femelles qui ont des périodes de fraye différentes devraient avoir leur fécondité évaluée à des moments différents. Nous avons exposé des morues à deux photopériodes distinctes afin de générer deux saisons de fraye différentes. Nous avons suivi la croissance avant la fraye et mesuré la fécondité potentielle au début de la fraye. La date qui permet l'explication potentielle la meilleure n'est pas la même sous les deux régimes de photopériode. Comme valeur de remplacement, la longueur varie moins mais a un pouvoir explicatif moindre que la masse. L'énergie lipidique au début de la fraye n'est pas une bonne valeur de remplacement. Le meilleur pouvoir d'explication s'obtient ~3–4 mois avant la fraye à peu près au moment du début de la vitellogenèse, ce qui indique que la fécondité potentielle est fortement influencée par les réserves énergétiques des femelles à ce moment. La détermination de la fécondité potentielle tôt durant la vitellogenèse peut s'avérer généralement possible chez les reproducteurs téléostéens à fécondité prédéterminée.

[Traduit par la Rédaction]

Introduction

Owing to its fundamental importance for fisheries management, predictable relationships between the sizes of fish stocks and recruitment to them have long been sought. These models have evolved in complexity. Traditionally models have used correlations between spawning stock biomass and recruitment (e.g., Hilborn and Walters 1992). However, life-history theory predicts that the energy available for reproduction and growth in iteroparous spawners is surplus energy after energy requirements for basic metabolic needs

have been fulfilled. The energy state of each fish therefore influences its egg production, and recent models of egg production and recruitment have also included various indices of individual condition (Marshall et al. 1999; Marteinsdottir and Begg 2002; Blanchard et al. 2003).

In lean fish such as cod (*Gadus morhua*), the main energy stores are found in the liver, which contains high levels of lipids, and in the white muscle, which is dominated by protein (Kjesbu et al. 1991; Lambert and Dutil 1997). Lipids are partly included in the synthesis of vitellogenin, the major precursor protein of all yolk proteins (Tyler and Sumpter

Received 28 June 2005. Accepted 30 July 2005. Published on the NRC Research Press Web site at <http://cjfas.nrc.ca> on 21 December 2005.
J18763

J.E. Skjæraasen.¹ University of Bergen, Department of Biology, N-5020 Bergen, Norway.

T. Nilsen. University of Bergen, Department of Mathematics, N-5020 Bergen, Norway.

O.S. Kjesbu. Institute of Marine Research, P. O. Box 1870 Nordnes, N-5817 Bergen, Norway.

¹Corresponding author (e-mail: jon.skjaeraasen@bio.uib.no).

1996). Marshall et al. (1999, 2000) showed that total lipid energy of the spawning stock was a better proxy of egg production and recruitment than spawning stock biomass for northeast Arctic cod. Body weight in relation to total length (condition) or food availability during the main feeding season, both of which are indicators of energy reserves, have also improved predictions of fecundity and egg production for Icelandic (Marteinsdottir and Begg 2002) and Baltic Sea cod (Kraus et al. 2002). For another gadoid (haddock (*Melanogrammus aeglefinus*)), incorporating condition has improved predictions of both inter- and intra-annual variation in fecundity and egg production (Marshall and Frank 1999; Blanchard et al. 2003). The merit of including individual energy reserves in a model has therefore been shown using both direct measurements (lipid energy) and indicators.

These studies, however, used yearly averages of lipid energy (Marshall et al. 1999), the quality of the feeding season as whole (Kraus et al. 2002), or data from fish caught just prior to the start of the spawning season (Kjesbu et al. 1998; Marteinsdottir and Begg 2002; Blanchard et al. 2003). Empirical evidence suggests, however, that potential fecundity level may largely be determined by energy reserves a considerable time before the onset of spawning. For Atlantic cod, low individual energy reserves might cause fish to skip spawning altogether (Burton et al. 1997; Marshall et al. 1998; Rideout et al. 2000) or severely reduce fecundity (Kjesbu et al. 1991; Lambert and Dutil 2000). It has therefore been suggested that there might exist a critical time window where investment into sexual maturation (Metcalf 1998) or gamete production either starts or is skipped, depending on an individual energy threshold value (Burton et al. 1997; Rideout et al. 2000).

During vitellogenesis, vitellogenin is sequestered into the developing oocytes, which thereby are recruited into the maturing pool to become a year's potential egg production (Tyler and Sumpter 1996). A study on rainbow trout (*Oncorhynchus mykiss*) found that fecundity was unaffected in fish that were fed on a low diet during the latter parts of vitellogenesis, while it was substantially reduced for fish fed on a small ration during early vitellogenesis (Bromage et al. 1991). Similarly, cod fed on a low ration prior to the start of vitellogenesis and then on a high ration during early stages of vitellogenesis had similar fecundities to fish fed on a high ration during the entire time (Kjesbu and Holm 1994). Woodhead and Woodhead (1965) and Kjesbu et al. (1991) found that although oocytes in cod are recruited to the maturing pool during early vitellogenesis, subsequent down-regulation through atresia at more advanced maturity stages alters potential fecundity. Emerging evidence suggests that down-regulation during the course of the maturation cycle is a normal process but that this fall in oocyte number is accelerated at poor conditions (e.g., Atlantic herring, *Clupea harengus*; see Kurita et al. 2003). Taken together, energy reserves early in vitellogenesis should be highly influential for potential fecundity at the start of the spawning season. It is therefore possible that estimates of potential fecundity and hence egg production may benefit from including data on energy reserves of fish during early vitellogenesis along with yearly averages or data obtained at the start of the spawning period to further increase accuracy and precision in fecundity determination. The inclusion of these early fecundity

proxies could also have a clear value in predictions, since cod enter vitellogenesis about 6 months before spawning (Kjesbu 1994).

In the present study, we exposed female cod to two different photoperiods, since photoperiod is known to alter the timing of spawning and hence vitellogenesis (Dahle et al. 2003; Norberg et al. 2004). If energy levels during early vitellogenesis are highly influential for final egg production, the individual status at this time might provide better proxies of potential fecundity and hence egg production than yearly averages do. If so, predictors of fecundity that show large temporal variations at the individual level (i.e., weight and liver energy) should show similar variation in their power as proxies for fecundity during the course of the year. Also, females with different spawning periods should have their annual fecundity determined by energy reserves at different times of the year. To test this hypothesis, we exposed cod to two photoperiods, which caused their spawning period to differ, and monitored development of individual females for a prolonged period prior to spawning. Finally, their potential egg production was measured at the onset of the spawning season. In light of the results of Marshall et al. (1999, 2000), we also examined the usefulness of lipid energy as a proxy for potential fecundity at the onset of spawning.

Materials and methods

History of the experimental fish

Coastal cod were caught using traps off the coast of Bergen, Norway, in late February and early March 2000. The fish were then kept in sea cages until they were transported to the Department of Fisheries and Marine Biology (University of Bergen, Norway) at the end of March and housed in a 7000 L (10 °C, flow rate 5000 L·h⁻¹) tank under a natural light regime for Bergen (60°25'N, 5°20'E). We fed the cod thawed, sliced herring twice a week for a 3 month conditioning period. Individuals were anaesthetized using Metakain (0.5 g·10 L⁻¹ of water), externally tagged with Hallmark Flow Tags, weighed (g), and measured (cm) before the start of the experiment. In addition, a blood sample was obtained for measurement of sex steroid levels.

The experiment

On 20 June 2000 the fish were divided into two groups consisting of 31 (17 males and 14 females) and 32 (12 males and 20 females) individuals, which were housed in two 7000 L tanks (8–10.5 °C through the experiment, 34.5 psu, water renewal rate 3000 L·h⁻¹). Note that the fish could not be sexed until later in the maturation cycle, so the sex ratio was only known in hindsight and each individual fish were therefore randomly allocated to each group at start of the experiment. One group (hereafter denoted as Short day) was exposed to a simulated photoperiod (8 h light:16 h dark), representative of January in Bergen. The other group (hereafter denoted as Normal day) was exposed to a simulated natural photoperiod for Bergen. The photoperiod for Short day was chosen because it resembles that associated with time of spawning of coastal cod in their natural habitat. A change in photoperiod will alter the timing of maturation and onset of spawning in cod (Hansen et al. 2001; Norberg

Table 1. Dates of capture, measurements, start of spawning, and sacrifice for both groups.

| Date | Short day | Normal day |
|---------------------|--|-----------------------------|
| February–March 2000 | Wild cod caught off the coast off Bergen and transported to lab facility | |
| 20 June 2000 | Measurements | Measurements |
| 20 June 2000 | Measurements | Measurements |
| 25 August 2000 | Measurements | Measurements |
| 23 October 2000 | Start of spawning | — |
| 2 November 2000 | Short day group sacrificed | — |
| 13 November 2000 | — | Measurements |
| 29 January 2001 | — | Start of spawning |
| 7 February 2001 | — | Normal day group sacrificed |

et al. 2004). Thus, the two groups should enter vitellogenesis at different times of the year.

Cod were fed herring corresponding to ~10% of their biomass (calculated at the last measurement date; see Table 1) in the tank per week (i.e., a fixed diet close to satiation). Food were cut into similar-sized pieces and weighed before and, if the entire ration was not finished and food remained, after feeding. We terminated food deliveries whenever the whole ration had been eaten or if four pieces in a row had sunk to the bottom of the tank without being consumed.

All individuals were weighed and measured and individual blood samples taken every other month. All measurements were done after the cod had been sedated using Metakain.

The experimental groups were terminated when eggs were detected in a net filtering the outlet of each tank (Table 1). When sacrificed, total length (cm), total weight (g), liver weight (g), and gonad weight (g) were measured for all individuals. For Short day fish, one female was allowed to complete spawning (to study behavioural aspects; not reported here), while one died. For Normal day fish, similar data were two and one, respectively. Thus, five females were excluded for these reasons. Consequently, at the time of sacrifice, we have data from 12 and 17 females from the Short and Normal day groups, respectively. Otolith examination showed that almost all females were 4 years old (range 3–5 years), and all fish were deemed to be first-time (recruit) spawners based on the otolith patterns (S. Lemvig, Institute of Marine Research, P.O. Box 1870 Nordnes, N-5817 Bergen, Norway, personal communication). Further details can be found in Skjæraasen et al. (2004).

Plasma steroid levels

Blood was collected from all individuals into a heparinized, 2 mL syringe by heart puncture. After collection, blood was centrifuged at 4 °C and 3000 r·min⁻¹ (1 r = 2 πrad; 2000g) for 3 min to determine initial plasma sex steroid levels. Plasma aliquots were frozen on dry ice and stored at -80 °C until extraction. Steroids were extracted from 100–200 μL plasma with ether:heptane (4:1) (Hyllner et al. 1994). Plasma levels of testosterone (T) (Rodriguez et al. 2000) and estradiol-17β (E2) (Weltzien et al. 2002) in both Short day females (*n* = 11) and Normal day females (*n* = 14) were analysed by enzyme-linked immunosorbent assay (ELISA). The inter-assay variations were 15.8% for E2 (*n* = 10) and 13.5% for T (*n* = 10). Intra-assay variations were 11.1% for E2 (*n* = 6) and 5.9% for T (*n* = 10). Analy-

sis of sex steroid levels was performed because these data could be used in conjunction with published data on oocyte development in cod (e.g., Dahle et al. 2003; Norberg et al. 2004) to evaluate the maturation stage of females through the course of the experiment.

Lipid energy

In view of the results of Marshall et al. (1999, 2000), we also estimated liver energy of females at the time of sacrifice to evaluate its usefulness as a proxy for fecundity at the onset of spawning. This was done by using the formula in Marshall et al. (1999) derived from Lambert and Dutil (1997):

$$(1) \quad \text{LEC} = 24.77 \{1 - \exp[-0.52(\text{LCI} - 0.48)]\}$$

where LEC is specific liver energy content (kJ·g⁻¹), and LCI is percent liver weight of total weight. Although this equation was developed for northern Gulf of St. Lawrence cod with LCI between 1% and 8%, the asymptotic increase in LEC shows that specific energy content increases rapidly up to 5%, but levels off above this value.

Lipid energy (LE) was calculated as follows:

$$(2) \quad \text{LE} = \text{LEC} \times \text{liver weight}$$

Potential fecundity

Potential fecundity was estimated as follows:

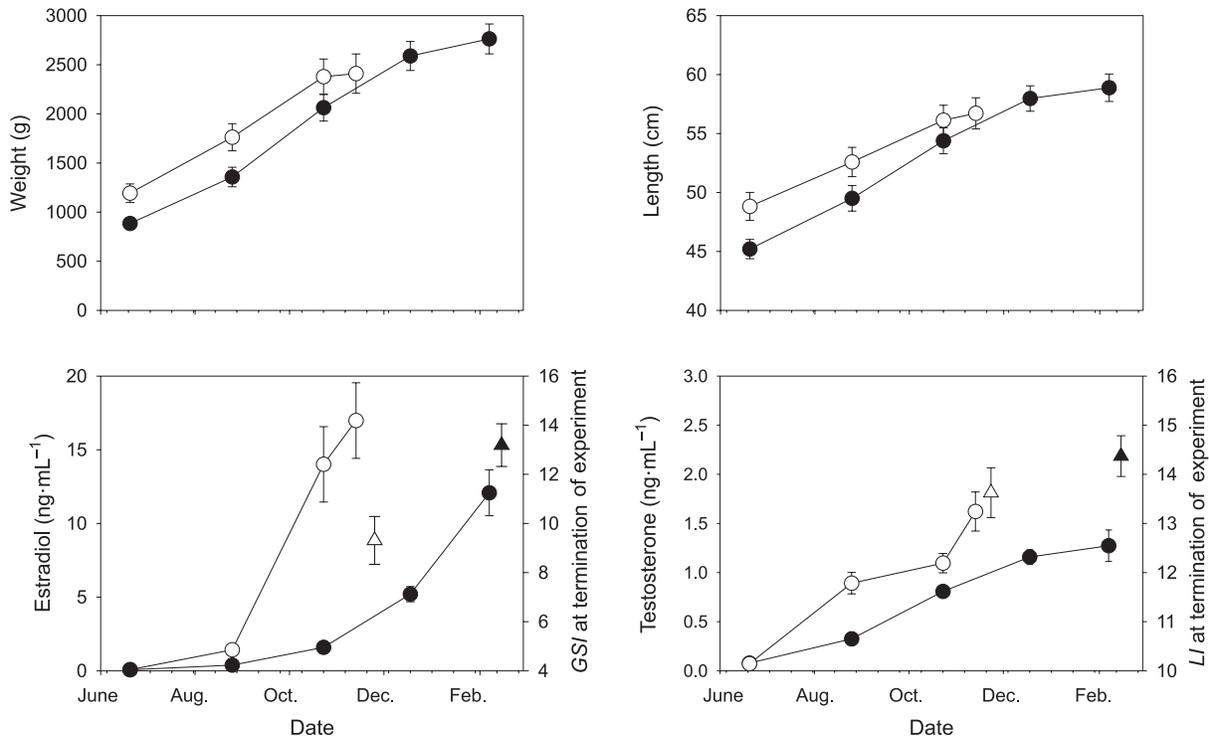
$$(3) \quad F_p = 2.139 \times 10^{11} \times \text{OD}^{-2.7} \times \text{OW}$$

based on Thorsen and Kjesbu (2001) and protocols therein, where F_p is potential fecundity, OD is average vitellogenic oocyte diameter estimated by advanced digital image analysis, and OW is ovary weight (g) at the time of sacrifice. Between 160 and 180 oocytes were measured from each ovary for calculation of the average diameter.

Data analyses

For each group and date, we used a linear regression between total weight or length (ln-transformed) as the independent variables and potential fecundity (ln-transformed) as the dependent variable. These analyses were also performed with inclusion of both weight and length simultaneously as independent variables to see if this increased the amount of variation explained. At the time of sacrifice, we also performed a regression between lipid energy and potential fecundity for each group. If there was no difference in investment patterns between photoperiods (i.e., investment per unit weight or length) at the time of investment, we

Fig. 1. Variation in length, weight, and levels of sex steroids in both Short and Normal day groups through the course of the experiment. Single points (triangles) are the values for gonadosomatic index (GSI) and liver index (LI) at the time of sacrifice. Numbers of females contributing to the weight and length figures are 12 for Short day and 17 Normal day. For the sex steroid figures, there are 11 and 14 females in the Short and Normal day groups, respectively. Error bars denote standard errors. Short day, open symbols; Normal day, solid symbols.



would expect similar relationships between proxy and potential fecundity for females from the two photoperiods. To test this, we performed analyses of covariance (ANCOVA) between the groups at these times using weight or length (ln-transformed) as the independent variable and potential fecundity (ln-transformed) as the dependent variable. We checked for homogeneity of slopes before performing these analyses.

Upon sacrifice, some fish in each light regime had begun spawning (based on macroscopic examination of gonads and hydrated-ovulated oocytes reported from the image analysis). Since these fish most likely had released eggs, which could not be accounted for, these fish were omitted from the analysis (three individuals from Short day and seven from Normal day). Potential fecundity could thus be reported from a total of 9 and 10 females in Short and Normal day, respectively.

Results

At the start of the experiment, there was a small but significant size difference between the females in the different photoperiods (two tailed *t* test, $p < 0.05$; weight and length, Fig. 1). No difference, however, was found in levels of testosterone ($T = 0.07 \text{ ng}\cdot\text{mL}^{-1}$ (Short day) and $T = 0.08 \text{ ng}\cdot\text{mL}^{-1}$ (Normal day); *t* test, $p = 0.66$; Fig. 1) or estradiol ($E2 = 0.07 \text{ ng}\cdot\text{mL}^{-1}$ for both Short and Normal day; $p = 0.77$; Fig. 1) at the start of the experiment. Patterns of weight and length development were similar in both groups.

First, there was a period of almost linear increase, and then in the final month before spawning, the increase halted and stopped (Fig. 1). These decreases in growth happened concurrently with a general decrease in appetite (the groups did not finish their entire ration) 2–3 months before the onset of spawning. All females were in good condition throughout the experiment, with a Fulton's condition factor ($CF = \text{weight} \times \text{length}^{-3} \times 100$) ~ 1.0 at the start of the experiment and ~ 1.3 at spawning (data not shown). Plasma sex steroid levels of Short day females increased much more rapidly than Normal day females. In August, average levels of T and E2 for Short day females were 0.9 and $1.4 \text{ ng}\cdot\text{mL}^{-1}$, respectively, while the corresponding values were 0.4 and $0.3 \text{ ng}\cdot\text{mL}^{-1}$ for Normal day females (Fig. 1). Hormonal levels in the Short day group remained higher until sacrifice (Fig. 1). Peak values were around 17 and $11 \text{ ng}\cdot\text{mL}^{-1}$ for E2 and 1.6 and $1.2 \text{ ng}\cdot\text{mL}^{-1}$ for T between the Short and Normal day females, respectively, (Fig. 1). The short photoperiod advanced spawning by approximately 3 months to early November (Table 1) for the Short day group compared with the Normal day group.

Individual status and potential fecundity

Group differences

All predictors of fecundity were positively and significantly ($p < 0.05$) correlated to potential fecundity at several of the measurement dates. The power of the relationship between the predictors and potential fecundity varied between

Fig. 2. Plots of weight and length (ln-transformed) versus potential fecundity (ln-transformed) at all measurement dates for the Short day group. Lines are the predicted values for potential fecundity based on the result of the regression between the predictor at the respective measurement date and potential fecundity. The R^2 -adjusted value (R^2 -adj.) and the equation resulting from the regression are given for each date and predictor.

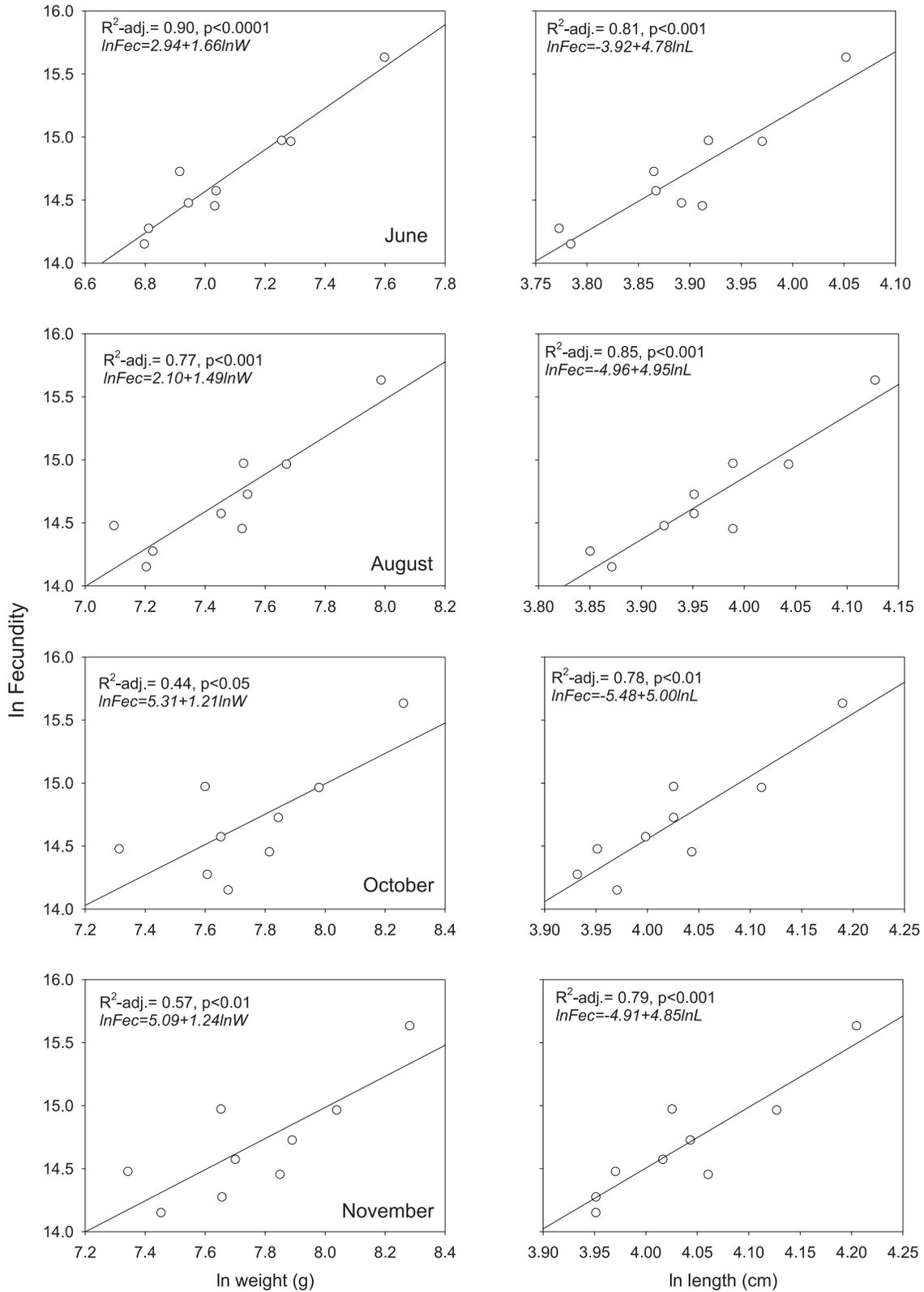
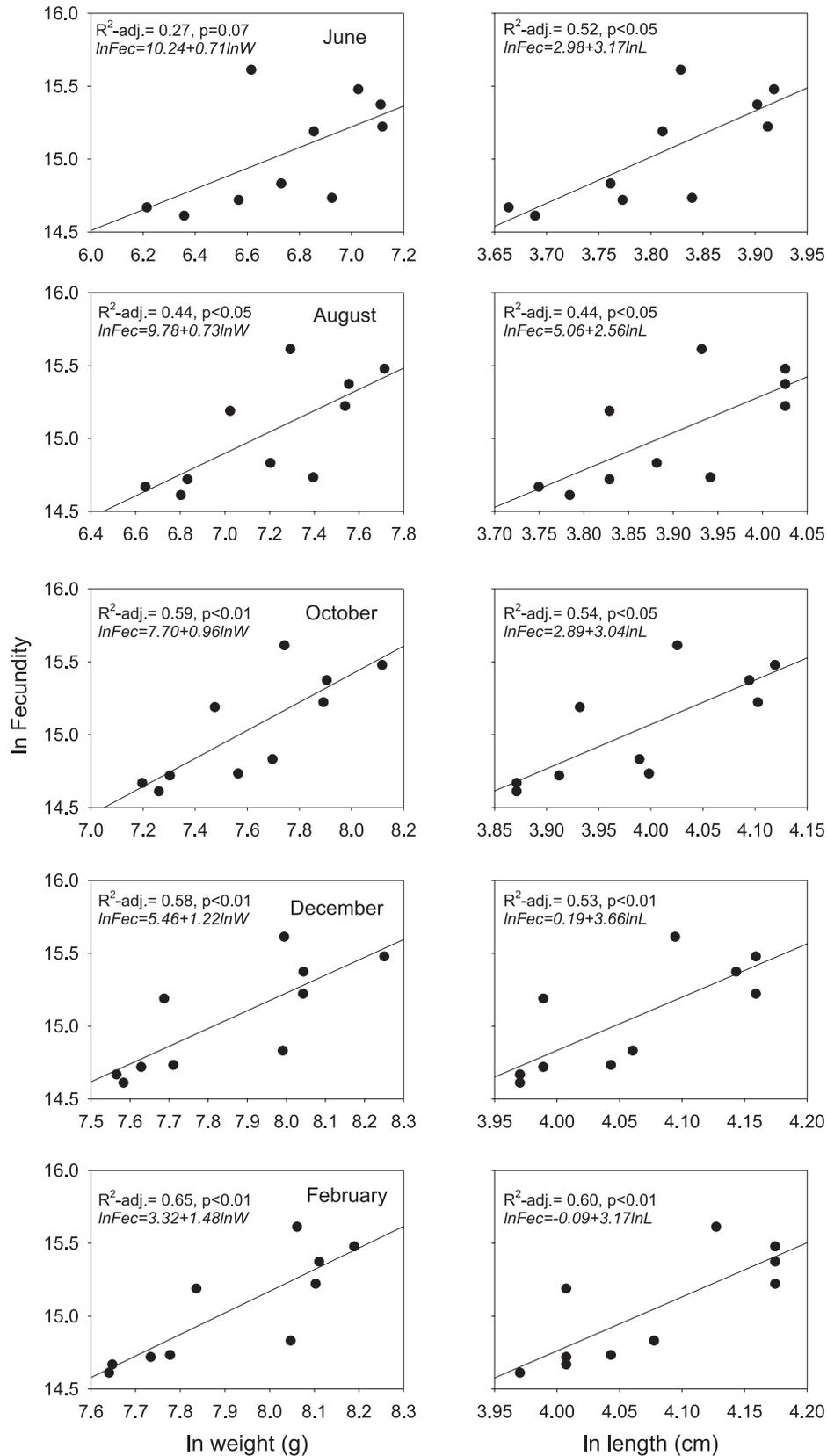


Fig. 3. Plots of weight and length (ln-transformed) versus potential fecundity (ln-transformed) at all measurement dates for the Normal day group. Lines are the predicted values for potential fecundity based on the result of the regression between the predictor at the respective measurement date and potential fecundity. The R^2 -adjusted value (R^2 -adj.) and the equation resulting from the regression are given for each date and predictor.



measurements though, and there was also a clear and consistent difference between groups for measurement date(s) that explained the most of the variation in potential fecundity (Figs. 2, 3). While the status of the fish in June (weight) and August (length) explained most of the observed variation in potential fecundity for Short day females (Fig. 2), these dates generally provided the least explanatory power for the Normal day group (Fig. 3). Instead, February (time of sacrifice) gave the best overall fit for Normal day females, but while a large increase in explanatory power occurred for weight from June to October (Fig. 3), measurements from October to February differed less (Fig. 3). Overall, the June measurements provided the greatest explanatory power when considering both weight and length in the Short day group (Fig. 2).

When comparing the June measurements from the Short day group with the October through February measurements for the Normal day group, the ANCOVA tests showed that overall the October measurements provided the greatest consistency between predictors in the two groups. Even so, Short day females tended to produce more eggs per unit weight than Normal day females when using the weight of Normal day females in February ($F_{[1,16]} = 29.79$, $p < 0.0001$), December ($F_{[1,16]} = 18.39$, $p < 0.001$), and also October ($F_{[1,16]} = 4.98$, $p = 0.04$; Fig. 4) as the independent variable for the Normal day group. For female length, however, the October measurements for the Normal day group produced a similar relationship between length and potential fecundity ($F_{[1,16]} = 0.029$, $p = 0.87$; Fig. 4) as that of the measurements in June for the Short day females, while female length in December and February did not ($p < 0.05$).

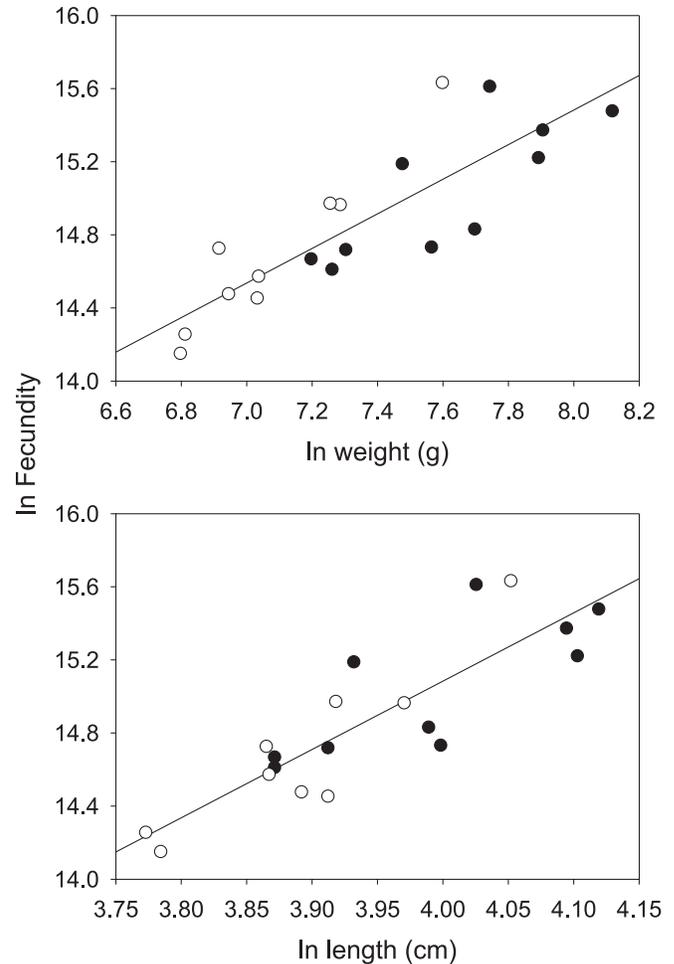
Predictor differences

There were clear differences between weight and length in the amount of variation they explained and also on their resilience (i.e., how much of the amount of variation explained changed with measurement date). Length was the most resilient predictor. The power of the regressions varied only by 7 percentage points from the best to the poorest fit in the Short day group (Fig. 2) and by 16 percentage points between the best and worst fit in the Normal day group (Fig. 3). In comparison, there was a 46 and 38 percentage points difference between the best and worst fit when using weight as the predictor for Short day and Normal day, respectively. Even though length was the most resilient predictor, it explained 5% less of the variation than weight did at its peak. Using weight and length simultaneously as independent variables did not increase the amount of variation explained in potential fecundity in this study (Table 2). Lipid energy at the time of sacrifice explained between 30 (Normal day) and 45 (Short day) percentage points less than did weight and length of females at earlier measurements (Figs. 2, 3, 5).

Individual energy levels at different time points

There was a gradual change in relative energy levels of individual fish through the course of the experiment (Table 3). As expected, the higher resilience of length as a predictor was explained by the relatively small changes among individuals through the experiment. Accordingly, relative changes in weight were larger, explaining the greater varia-

Fig. 4. Relationship between potential fecundity (ln-transformed) and weight and length (ln-transformed) using the June measurements of weight and length for the Short day group (open circles) and the October measurements for the Normal day group (solid circles). Lines are the predicted values of a regression between proxy and potential fecundity including all data points.



tion in the amount of potential fecundity explained from different measurement dates. From October to February, the period that yielded very similar results for the Normal day group, almost no relative change in weight or length among individual females occurred (Table 3).

Discussion

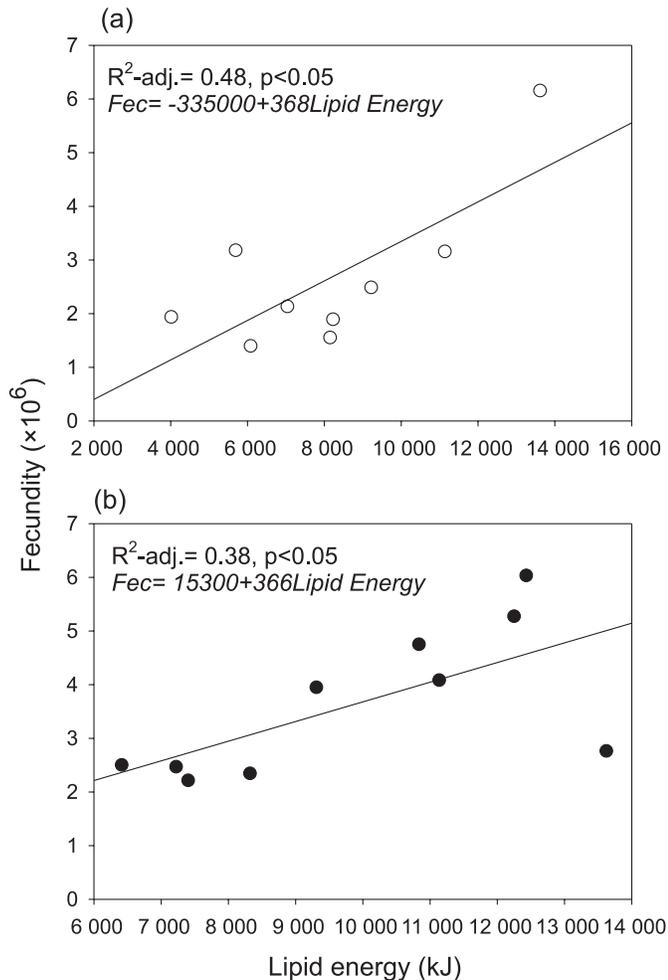
Timing and determination of potential fecundity

The different photoperiod experienced by the females in the two groups changed the maturation–hormonal cycle and thus the onset of spawning. The light regimes applied caused the females in the Short day group to advance the onset spawning by approximately 3 months compared with the Normal day group. These results are in agreement with previous studies on the effect of photoperiod on the timing of spawning in cultured cod (Hansen et al. 2001; Norberg et al. 2004). If there exists a certain time window prior to spawning when the final level of potential fecundity is determined, then the egg production of females in the different groups in

Table 2. Results of multiple regression using both weight (*W*) and length (*L*) (ln-transformed) as independent variables and potential fecundity (*fec*) (ln-transformed) as the dependent variable.

| Group | Month | Regression | Length | Weight | <i>R</i> ² -adjusted |
|------------|----------|---|--------|--------|---------------------------------|
| Short day | June | $\ln \text{ fec} = 2.13 + 0.48 \ln L + 1.51 \ln W$ | 0.81 | 0.053 | 0.89 |
| | August | $\ln \text{ fec} = -3.22 + 3.74 \ln L + 0.48 \ln W$ | 0.11 | 0.54 | 0.83 |
| | October | $\ln \text{ fec} = -9.14 + 7.57 \ln L - 0.85 \ln W$ | 0.01 | 0.21 | 0.80 |
| | November | $\ln \text{ fec} = -7.82 + 6.65 \ln L - 0.56 \ln W$ | 0.037 | 0.47 | 0.77 |
| Normal day | June | $\ln \text{ fec} = -5.12 + 7.74 \ln L - 1.38 \ln W$ | 0.02 | 0.10 | 0.64 |
| | August | $\ln \text{ fec} = 7.54 + 1.18 \ln L + 0.40 \ln W$ | 0.81 | 0.77 | 0.37 |
| | October | $\ln \text{ fec} = 9.76 - 1.19 \ln L + 1.32 \ln W$ | 0.79 | 0.34 | 0.54 |
| | December | $\ln \text{ fec} = 4.31 + 0.68 \ln L + 1.01 \ln W$ | 0.52 | 0.85 | 0.52 |
| | February | $\ln \text{ fec} = 2.43 + 0.73 \ln L + 1.22 \ln W$ | 0.81 | 0.31 | 0.61 |

Note: The value under length and weight is the *p* value for the respective independent variable. (i.e., Was it correlated to potential fecundity or not?) *R*²-adjusted is the overall explanatory power of the regression equation.

Fig. 5. Relationship between lipid energy and potential fecundity at the time of sacrifice in the (a) Short day and (b) Normal day groups. The *R*²-adjusted value (*R*²-adj.) and the equation resulting from the regressions are given.

this study should be influenced by energy reserves at different dates because of the difference in their spawning periods. This appears very much to be the case. While potential egg production of the Short day group is closely linked to individual status of females in June, this date only poorly

explains egg production for the Normal day females. Instead, status of females later in the study from October to February provides the best results for the Normal day group. Is February or October likely to be closer to the true time of determination of egg production for the Normal day females? Firstly, very little change in the predictive power occurred from October to February (6 percentage points) compared with June to October (32 percentage points). Our results show that very little relative change in female weight or length occurred during these final months in the Normal day group. We believe that this explains the lack of a clear peak in explanatory power and that the small rise in February is an artefact caused by the small sample size. Secondly, the overall results of the ANCOVA indicate that October produces the greatest consistency when comparing results from the two groups. Consequently, we conclude that potential egg production of females in the Normal day group was largely determined by individual energy reserves close to the October measurement.

How does present knowledge of the maturation cycle of cod agree with our results? Our results indicate that the potential fecundity is best described statistically by the June predictors of Short day females (135 days prior to spawning, mean temperature 9.5 °C) and by October predictors in the Normal day group (~100 days prior to spawning, mean temperature 9.9 °C). These dates correspond reasonably well with the assumed start of vitellogenesis in relation to the start of spawning among these females; time to start of spawning from early developing oocytes (diameter 300 µm) to spawning is documented to vary from 105 days (9 °C) to 120 days (7 °C) (Kjesbu 1994). Considering the fact that both groups were kept in the same tank under an identical normal photoperiod until June, we do not expect vitellogenesis to have started in the Short day group before this date.

Hormonal studies on reared cod have shown that E2 increases steadily in maturing females, while E2 decreases sharply after spawning (Dahle et al. 2003; Norberg et al. 2004). Although T presently shows a relatively regular increase, it has been found to only increase during the later maturation stages in other studies, making it unuseful for separating previtellogenic females from females possessing early maturing oocytes (Dahle et al. 2003; Norberg et al. 2004). Dahle et al. (2003) measured E2 levels in conjunction

Table 3. Results of linear regressions (Pearson) of predictors between measurement dates (given as R^2 -adjusted).

| Predictor | | June | | August | | October | | December |
|-----------|----------|-------|-------|--------|-------|---------|-------|----------|
| | | Sd | Nd | Sd | Nd | Sd | Nd | Nd |
| Length | August | 0.98* | 0.95* | — | — | — | — | — |
| | October | 0.91* | 0.94* | 0.97* | 0.99* | — | — | — |
| | November | 0.93* | — | 0.97* | — | 0.99* | — | — |
| | December | — | 0.89* | — | 0.96* | — | 0.99* | — |
| | February | — | 0.85* | — | 0.93* | — | 0.97* | 0.99* |
| Weight | August | 0.88* | 0.90* | — | — | — | — | — |
| | October | 0.70 | 0.83* | 0.91* | 0.96* | — | — | — |
| | November | 0.76* | — | 0.95* | — | 0.95* | — | — |
| | December | — | 0.72* | — | 0.89* | — | 0.98* | — |
| | February | — | 0.76* | — | 0.88* | — | 0.97* | 0.98* |

Note: An asterisk (*) indicates significant result ($p < 0.05$). Sd, Short day; Nd, Normal day.

with microscopic examination of oocytes and found that previtellogenic oocytes (oocyte size $< 240 \mu\text{m}$) were associated with E2 levels of $0.6 \text{ ng}\cdot\text{mL}^{-1}$, while early maturing ($260\text{--}370 \mu\text{m}$) and maturing oocytes ($360\text{--}440 \mu\text{m}$) were associated with E2 levels of 1.3 and $2.6 \text{ ng}\cdot\text{mL}^{-1}$, respectively. Based on this information, both Short and Normal day females possessed only previtellogenic oocytes in June (T and E2 $0.07\text{--}0.08 \text{ ng}\cdot\text{mL}^{-1}$ in both groups), while Short day females were at the early maturing stage in August (i.e., vitellogenesis had started (E2 = $1.4 \text{ ng}\cdot\text{mL}^{-1}$)). Normal day females, however, still only possessed previtellogenic oocytes based on the observed E2 levels ($0.3 \text{ ng}\cdot\text{mL}^{-1}$). In October, E2 levels of Normal day females indicated that vitellogenesis had started ($1.6 \text{ ng}\cdot\text{mL}^{-1}$).

These results support our hypothesis that energy reserves early in vitellogenesis is highly influential for potential egg production. The altered photoperiod experienced by Short day females from June likely caused vitellogenesis to start soon thereafter, as indicated by the higher hormonal levels of Short day females in August. Individual energy status in June therefore seemingly influenced the level of egg production of Short day females. Changes in individual weight and length (energy status) after this date did not boost potential egg production, as indicated by the weakened strength of our proxies for potential fecundity at the other measurement dates for this group. In contrast, vitellogenesis in the Normal day group started later (close to October), and energy acquired after June therefore boosted potential egg production for the Normal day females. Vitellogenesis likely started close to the end of October based on our proxies for fecundity and levels of E2.

Although energy levels in both groups were positively correlated to fecundity, the amount of variation explained was somewhat higher in the Short day group. In addition, the fish in the two groups differed substantially in size at experimental start. A random splitting of samples, such as the one undertaken in June, does not guarantee that the groups become identical (Kjesbu et al. 1991). We believe, however, that these noticed differences in weight and length did not influence our conclusions. Firstly, spawning time should not be affected (Kjesbu 1994), nor should steroid levels within maturity stages (Dahle et al. 2003). Secondly, our current emphasis is on size-specific effects (length- or weight-specific fecundity) rather than group effects. The females

were all recruit spawners and of similar age, so potential age effects (Kjesbu et al. 1996; Marteinsdottir and Begg 2002) could not be a probable explanation. However, a source of potential error in estimates of fecundity is atresia. It is known that the level of atresia accelerates at poor condition (see Rideout et al. 2000). Day length might also be influential (Hansen et al. 2001). As all present females were in good condition, atresia should in principle be low (Kjesbu et al. 1991) and not differ importantly between groups. On the other hand, a switch from natural light to 24 h continuous light in December (i.e., at advanced vitellogenesis, the largest oocytes being around $500 \mu\text{m}$), apparently negatively affects fecundity in cod, measured as number of spawned eggs (Hansen et al. 2001). The significant ($p < 0.05$) difference found in the ANCOVA when using weight as the independent variable may indicate that the altered photoperiod caused Short day females to invest more per unit weight into egg production than Normal day females. We are unaware of any previous study showing this. An alternative explanation is that the true time of determination of egg production is somewhere between 25 August and 23 October for the Normal day females (albeit clearly closer to 23 October). A more frequent sampling regime might have pushed the date of determination back further than 23 October in the Normal day group. This could potentially have increased the amount of variation explained in the Normal day group.

Differences between proxies

We found that total weight provided the greatest explanatory power, while total length was the most time-resilient proxy for fecundity. The same difference between weight and length as proxies for fecundity and egg production was noted by Blanchard et al. (2003) in their study on haddock. Previous studies on gadoids have used yearly averages (Marshall et al. 1999), the quality of the feeding season as whole (Kraus et al. 2002), or data from fish caught just prior to the start of the spawning season (Kjesbu et al. 1998; Marteinsdottir and Begg 2002; Blanchard et al. 2003) to estimate egg production. We found that the time of measurement may greatly influence the prediction of reproductive potential and the amount of variation explained. While it has been argued that weight is a potentially spurious predictor of fecundity because of its high degree of seasonal variation (e.g., Blanchard et al. 2003), we argue that the seasonal vari-

ation of weight (Marshall et al. 1999; Hansen et al. 2001) instead can be coupled with knowledge of the onset and duration of vitellogenesis to examine if this can improve estimates of total egg production. The importance of lipid energy for egg production in cod has been shown conclusively by Marshall et al. (1999, 2000). The large seasonal variation in liver size (similar to weight) (Eliassen and Vahl 1982; Yaragina and Marshall 2000), however, leads us to believe that bioenergetic indexes of reproductive potential depending on lipid energy (e.g., Marshall et al. 2000) also could be tuned by only including data from time periods sensitive for egg production. The poor explanatory power of lipid energy at the time of sacrifice compared with proxies using female weight and length at earlier measurements in this study also indicates this. In this respect, however, it is important to be aware that cod is classified among the determinate spawners (i.e., there is no or very rarely de novo recruitment of oocytes to the vitellogenic mode during spawning; Kjesbu et al. 1998; Thorsen and Kjesbu 2001) in contrast with indeterminate spawners, such as the common sole (*Solea solea*), in which oocyte recruitment is a much more continuous process (Witthames 2003). In other words, the timing of fecundity determination and thereby the optimal reference points for proxies vary among species depending on the reproductive style.

Using length and weight as independent variables in the same regression did not improve estimates, contrary to what has been found in numerous other studies (e.g., Kjesbu et al. 1998; Marteinsdottir and Begg 2002; Blanchard et al. 2003). This may have been caused by the relatively small differences in condition in our material, which will reduce the importance of scaling weight to length. In a recent review, however, Koops et al. (2004) argued that using length and maternal condition to predict fecundity could substantially overestimate the effect of maternal condition from the effect of body size alone.

In conclusion, since condition and energy reserves observed in the field may lead to markedly different relationships between female weight–length and egg production than presently found in our laboratory work, the presented results should not be applied uncritically on wild fish. Cod held in captivity generally have a higher size-specific fecundity than its wild counterparts (Kjesbu et al. 1991). Our results do, however, show that energy reserves during early vitellogenesis are highly influential for final potential fecundity in cod, a determinate spawner. This is a result that we believe may be possible to extrapolate to the field situation. We therefore suggest that there may be merit in using field data from early vitellogenesis to tune forecast models and hence ultimately increase knowledge and power of stock–recruitment relationships. Although the spawning season of cod varies little between years (Brander 1994), oocyte diameter could also be used to verify that females are at the early vitellogenic stage. In our experiment, the fish had high energy reserves and all females produced eggs. In the wild, adult cod with limited energy reserves may skip spawning (Burton et al. 1997; Rideout et al. 2000). Using data from time periods close to the predicted onset of gonad development would likely also improve estimates of the proportions of skipped spawners if energy thresholds required for reproduction could be identified.

Acknowledgements

We thank J. Titelman, A.G.V. Salvanes, Ø. Fiksen, and A. Folkvord for comments and thoughts on the manuscript. M. Hordnes and coworkers at the Laboratory of the High Technology Centre in Bergen kindly assisted with the experiment. Ø. Karlsen and Merete Fonn at the Institute of Marine Research gave advice on experimental protocol and carried out the fecundity analysis, respectively. This project was funded by the Norwegian Research Council (Project No. 130192/140, Environmental Influence on Fish Stocks). The write-up of this paper was supported by EU RASER Q5RS-2002-01825 (O.S. Kjesbu).

References

- Blanchard, J.L., Frank, K.T., and Simon, J.E. 2003. Effects of condition on fecundity and total egg production of eastern Scotian Shelf haddock (*Melanogrammus aeglefinus*). *Can. J. Fish. Aquat. Sci.* **60**: 321–332.
- Brander, K. 1994. Spawning and life history information for North Atlantic cod stocks. ICES Coop. Res. Rep. 205.
- Bromage, N.R., Jones, J., Randall, C., Thrush, M., Springate, J., Duston, J., and Barker, G. 1991. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **100**: 141–166.
- Burton, M.P.M., Penney, R.M., and Biddiscombe, S. 1997. Time course of gametogenesis in Northwest Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* **54**(Suppl. 1): 122–131.
- Dahle, R., Taranger, G.L., Karlsen, Ø., Kjesbu, O.S., and Norberg, B. 2003. Gonadal development and associated changes in liver size and sexual steroids during the reproductive cycle of captive male and female Atlantic cod (*Gadus morhua* L.). *Comp. Biochem. Physiol.* **136**: 641–653.
- Eliassen, J.E., and Vahl, O. 1982. Seasonal variation in biochemical composition and energy content of liver, gonad and muscle of immature and mature cod, *Gadus morhua* (L.) from Balsfjorden, northern Norway. *J. Fish Biol.* **20**: 707–716.
- Hansen, T., Karlsen, Ø., Hemre, G.I., Holm, J.C., and Kjesbu, O.S. 2001. Growth, gonadal development and spawning time of Atlantic cod (*Gadus morhua*) reared under different photoperiods. *Aquaculture*, **203**: 51–67.
- Hilborn, R., and Walters, C.J. 1992. Quantitative fisheries stock assessment. Chapman and Hall, New York.
- Hyllner, S.J., Norberg, B., and Haux, C. 1994. Isolation, partial characterization, induction, and the occurrence in plasma of the major vitelline envelope proteins in the Atlantic halibut (*Hippoglossus hippoglossus*) during sexual maturation. *Can. J. Fish. Aquat. Sci.* **51**: 1700–1707.
- Kjesbu, O.S. 1994. Time of start of spawning in Atlantic cod (*Gadus morhua*) females in relation to vitellogenic oocyte diameter, temperature, fish length and condition. *J. Fish Biol.* **45**: 719–735.
- Kjesbu, O.S., and Holm, J.C. 1994. Oocyte recruitment in first-time spawning Atlantic cod (*Gadus morhua*) in relation to feeding regime. *Can. J. Fish. Aquat. Sci.* **51**: 1893–1898.
- Kjesbu, O.S., Klungsøyr, J., and Kryvi, H. 1991. Fecundity, atresia and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body composition. *Can. J. Fish. Aquat. Sci.* **48**: 2333–2343.

- Kjesbu, O.S., Solemdal, P., Bratland, P., and Fonn, M. 1996. Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* **53**: 610–620.
- Kjesbu, O.S., Witthames, P.R., Solemdal, P., and Greer Walker, M. 1998. Temporal variations in the fecundity of Arcto Norwegian cod (*Gadus morhua*) in response to natural changes in food and temperature. *J. Sea Res.* **40**: 303–321.
- Koops, M.A., Hutchings, J.A., and McIntyre, T.M. 2004. Testing hypotheses about fecundity, body size and maternal condition in fishes. *Fish Fish.* **5**: 120–130.
- Kraus, G., Tomkiewicz, J., and Köster, F.W. 2002. Egg production of Baltic cod (*Gadus morhua*) in relation to variable sex ratio, maturity, and fecundity. *Can. J. Fish. Aquat. Sci.* **59**: 1908–1920.
- Kurita, Y., Meier, B., and Kjesbu, O.S. 2003. Oocyte growth and fecundity regulation by atresia of Atlantic herring (*Clupea harengus*) in relation to body condition throughout the maturation cycle. *J. Sea Res.* **49**: 203–209.
- Lambert, Y., and Dutil, J.D. 1997. Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of Atlantic cod (*Gadus morhua*)? *Can. J. Fish. Aquat. Sci.* **54**(Suppl. 1): 104–112.
- Lambert, Y., and Dutil, J.D. 2000. Energetic consequences of reproduction in Atlantic cod (*Gadus morhua*) in relation to spawning level of somatic energy reserves. *Can. J. Fish. Aquat. Sci.* **57**: 815–825.
- Marshall, C.T., and Frank, K.T. 1999. The effect of interannual variation in growth and condition on haddock recruitment. *Can. J. Fish. Aquat. Sci.* **56**: 347–355.
- Marshall, C.T., Kjesbu, O.S., Yaragina, N.A., Solemdal, P.S., and Ulltang, Ø. 1998. Is spawner biomass a sensitive measure of the reproductive and recruitment potential of Northeast Arctic cod? *Can. J. Fish. Aquat. Sci.* **55**: 1766–1783.
- Marshall, C.T., Yaragina, N.A., Lambert, Y., and Kjesbu, O.S. 1999. Total lipid energy as a proxy for total egg production by fish stocks. *Nature (London)*, **402**: 288–290.
- Marshall, C.T., Yaragina, N.A., Adlandsvik, B., and Dolgov, A.V. 2000. Reconstructing the stock–recruitment relationship using a bioenergetic index of reproductive potential. *Can. J. Fish. Aquat. Sci.* **57**: 2433–2442.
- Marteinsdottir, G., and Begg, G.A. 2002. Essential relationships incorporating the influence of age, size and condition on variables required for estimation of reproductive potential in Atlantic cod, *Gadus morhua*. *Mar. Ecol. Prog. Ser.* **235**: 235–256.
- Metcalf, N.B. 1998. Interaction between behavior and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **55**(Suppl. 1): 93–103.
- Norberg, B., Brown, C.L., Halldorsson, O., Stensland, K., and Björnsson, B.T. 2004. Photoperiod regulates the timing of sexual maturation, spawning, sex steroid and thyroid hormone profiles in the Atlantic cod (*Gadus morhua*). *Aquaculture*, **229**: 451–467.
- Rideout, R.M., Burton, M.P.M., and Rose, G.A. 2000. Observations on mass atresia and skipped spawning in northern Atlantic cod, *Gadus morhua* L., from Smith Sound, Newfoundland. *J. Fish Biol.* **57**: 1429–1440.
- Rodriguez, L., Begtashi, I., Zanuy, S., and Carillo, M. 2000. Development and validation of an enzyme immunoassay for testosterone: effects of photoperiod on plasma testosterone levels and gonadal development in male sea bass (*Dicentrarchus labrax*, L.) at puberty. *Fish Physiol. Biochem.* **23**: 141–150.
- Skjæraasen, J.E., Salvanes, A.G.V., Karlsen, Ø., Dahle, R., Nilsen, T., and Norberg, B. 2004. The effect of photoperiod on sexual maturation, appetite and growth in wild Atlantic cod (*Gadus morhua* L.). *Fish Physiol. Biochem.* **30**(2): 163–174.
- Thorsen, A., and Kjesbu, O.S. 2001. A rapid method for estimation of oocyte size and potential fecundity in Atlantic cod using a computer-aided particle analysis system. *J. Sea Res.* **46**: 295–308.
- Tyler, C.R., and Sumpter, J.C. 1996. Oocyte growth and development in teleosts. *Rev. Fish Biol. Fish.* **6**: 287–318.
- Weltzien, F.A., Taranger, G.L., Karlsen, Ø., and Norberg, B. 2002. Testis development and plasma androgen levels in Atlantic halibut (*Hippoglossus hippoglossus* L.). *Comp. Biochem. Physiol. A*, **132**: 567–575.
- Witthames, P.R. 2003. Methods to assess maturity and realized fecundity illustrated by studies on Dover sole *Solea solea*. In Report of the Working Group of Modern Approaches to Assess Maturity and Fecundity of Warm- and Cold-Water Fish and Squids, Bergen, Norway, 4–7 September 2001. Edited by O.S. Kjesbu, J.R. Hunter, and P.R. Witthames. *Fisken og havet*. No. 12-2003. Institute of Marine Research, Bergen. pp. 125–138.
- Woodhead, A.D., and Woodhead, P.M.J. 1965. Seasonal changes in the physiology of Barents Sea cod, *Gadus morhua* L., in relation to its environment. 1. Endocrine changes particularly affecting migration and maturation. *ICNAF Spec. Publ.* **6**: 691–715.
- Yaragina, N.A., and Marshall, C.T. 2000. Trophic influences on interannual and seasonal variation in the liver condition index of Northeast Arctic cod (*Gadus morhua*). *ICES J. Mar. Sci.* **57**: 42–55.