

**Theme session on the application of experimental laboratory studies to fisheries science**

**Estimating natural mortality of wild cod from controlled feeding and starvation experiments conducted in the laboratory**

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The contribution of individual factors to the rate of natural mortality is usually unknown because it can hardly be measured in the wild, particularly in large ecosystems. Atlantic cod experienced a decline of their nutritional condition in the early 1990s in the Gulf of St. Lawrence. Whether or not natural mortality increased as a result of that decline was determined from controlled experiments conducted in the laboratory. The maximum range of several variables reflecting the nutritional condition of fed and unfed cod >30 cm was determined. By comparing values observed in the wild and in the laboratory, we concluded that wild cod may die of inanition during the spring period. The decline in the nutritional condition of wild cod from January to April was slower however than in cod deprived of food during a similar period of time in laboratory experiments. This indicates that cod fed but did not meet their energy requirements in winter prior to spawning. In contrast, the decline in condition from April to May matched the rate observed in the laboratory experiments, indicating that wild cod probably fast at this time. Estimates of survival probability based on the frequency distribution of condition factor during the spawning period appear to be unbiased as the rate of energy mobilization observed in the field never exceeded the rate observed in the laboratory.

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## Introduction

When assessing stock status, fishery biologists account for natural mortality by adding a fixed value of natural mortality ( $M$ ) to a measured value of fishing mortality ( $F$ ).  $M$  has been given with few exceptions a constant value of 0.2 (18%) across species, ages and time, based on historic accounts and possibly also because  $M$  is not readily measurable. While natural mortality in unexploited stocks can be assessed readily, partitioning natural mortality into its major components is a difficult task. This is even more difficult in situations where stocks are exploited, particularly in large ecosystems. Several factors in the environment and within communities adversely affect the probability of any individual to survive. Some of these may act by having a negative impact on the physiological condition of the fish which in turn may for instance reduce its ability to secure preys and avoid predation.

Recently, one such impact has been assessed in Atlantic cod, *Gadus morhua*. Cod in the northern Gulf of St. Lawrence have experienced a period of changing environmental conditions. The cooling of the cold intermediate layer in the Gulf of St. Lawrence started in the 1980s (Gilbert and Pettigrew 1997). The cod stock moved to deeper waters and shifted its latitudinal distribution so as to avoid being exposed to colder waters (Castonguay et al. 1999). Nevertheless both size-at-age and energetic condition of individual fish declined through the 1980s and early 1990s (Chouinard and Fréchet 1994, Lambert and Dutil 1997). Surplus production also declined and was nil by the early 1990's (Dutil et al. 1999). Individual fish experienced marked seasonal changes in condition within a year with levels of energy reserves being minimal during the spring period when spawning takes place (Lambert and Dutil 1997, Schwalm and Chouinard 1999). Females in poor condition invested less energy in maturation, which occurs in a period when individual condition deteriorates, but energy expenditures were greater in relation to available energy reserves than in females in better condition (Lambert and Dutil 2000). As a result, energy invested in reproduction by poor-condition females increased their risk of mortality (Dutil and Lambert 2000).

The potential range of values for several variates indicative of cod condition has been determined experimentally (Lambert and Dutil 1997, Dutil and Lambert 2000). By comparing field observations to the frequency distribution for starved fish, the proportion of wild cod with energy reserves

as low as starved cod in the laboratory has been determined. The degree of overlap was used as an estimate of the natural mortality which can be ascribed directly to lack of food. There are two potential drawbacks to this method however. Fish in the wild may die before critical set points are reached, because life is more demanding in the wild than in the laboratory. Also, observed levels of energy reserves in wild fish may be biased if fish in poor condition die before being sampled. To investigate whether or not our estimates of natural mortality associated with poor condition had been underestimated, we have compared the change in physiological condition observed in the wild to the change in physiological condition measured in the laboratory in unfed fish kept at 3 temperatures over a period of 84 days.

## Materials and methods

Atlantic cod of 2 size group (38-48 cm and 48-58 cm) were deprived of food for a period of 84 days at 2, 6 or 10°C and changes in the condition factor and energy stores were monitored following the methods described previously (Dutil and Lambert 2000). Changes in condition factor were modelled for a standard 48 cm fish at each temperature and were compared to changes in condition observed in wild fish during the period January – June 1994. We used « condition factor » to refer to changes in total mass and « somatic condition factor » to refer to changes in somatic mass.

Field samples were obtained from the estuary and northern Gulf of St. Lawrence in 1994. Fish size varied among samples, but our analyses were restricted to a common size range (32 to 64 cm) : January, mean length = 41 cm (n=145); April, 37 cm (n=60); May, 47 cm (n=554); June, 40 cm (n=139). Within that size range, somatic condition factor and length were not correlated ( $P > 0.05$  and  $r^2 < 0.02$  in all four samples).

For the range of temperatures in the cod habitat during winter in the early 1990s (2-6°C) and for a higher level of temperature representative of fast-growing stocks (10°C), we tested whether the observed change in the somatic condition factor in 1994 differed from the expected change ( $\Delta K$ ) for unfed cod at these temperatures. The hypothesis for the January-April period for instance was :

$$\begin{aligned} K_J - K_A &= \Delta K \\ \text{or} \\ K_J - (K_A + \Delta K) &= 0 \\ \text{or} \end{aligned}$$

$$K_J = K_A + \Delta K$$

The alternate hypothesis would be :

$$K_J - K_A < \Delta K$$

or

$$K_J < K_A + \Delta K$$

where  $K_J$  and  $K_A$  are the observed somatic condition factor in January and April and  $\Delta K$  represents the expected change in the somatic condition factor for a 48 cm fish with a somatic condition factor at the start of the starvation period equal to  $K_J$ . The expected frequency distribution for the somatic condition factor in April was constructed by translation assuming a normal distribution with mean ( $K_{AP}$ ) :

$$K_{AP} = K_J - \Delta K$$

$K_J$  and  $K_{AP}$  were assumed to have equal coefficients of variability. The same procedure was repeated for the 3 levels of temperature.

The expected frequency distribution for the somatic condition factor in May was constructed by translation from the frequency distribution in April (28 days), assuming a normal distribution. The change in somatic condition factor from April to May ( $\Delta K_{28d}$ ) for a 47.3 cm fish (median length in the May sample) was calculated from the laboratory experiments. The variance (Var) was calculated as :

$$\text{Var}(K_A) + (28/84)^2 \cdot \text{Var}(\Delta K_{84d}) + 2 \cdot (28/84)^2 \cdot \text{Cov}(K, \Delta K_{84d})$$

where  $\text{Cov}(K, \Delta K_{84d})$  is the covariance between  $K$ , the condition factor at the start of the laboratory experiment, and  $\Delta K_{84d}$ .

## **Results**

### Laboratory observations

Fish length and temperature had a significant impact on the change in condition factor (Table 1) over the 84 days of the experiment ( $P < 0.001$ , 2 categories of fish length;  $P < 0.001$ , 3 levels of temperature), but there was no significant interaction between the two factors ( $P > 0.05$ ). Changes in condition factor followed normal distributions (Shapiro-Wilk's,  $P > 0.05$ ), and variances were homoscedastic (Bartlett's test,  $P > 0.05$ ).

Fish condition factor at the start of the experiment, used as a covariate for size and temperature separately, had a significant effect on the change in condition factor during the experiment, with fish in better condition initially

experiencing larger changes in condition ( $P < 0.01$ , whether size or temperature was used as the main factor). There were no differences in the average condition factor among tanks at the start of the experiment (Table 2) ( $P > 0.05$ ). Changes in the condition factor associated with size or temperature were accordingly very similar for the adjusted and unadjusted values (Table 1). When examined for each size and temperature combination, condition factor at the start of the experiment had a significant effect for both size categories at  $2^{\circ}\text{C}$  and for small fish at  $6^{\circ}\text{C}$ . No significant effect occurred for large fish at  $6^{\circ}\text{C}$  and for fish of both size categories at  $10^{\circ}\text{C}$ . Fish condition at the start of the experiment explained less than 30% of the observed variability (Table 2).

Fish length and condition factor at the start of the experiment were used as dependent variates to explain the variability in the change of the condition factor over the 84 day starvation period. This yielded similar results as using size category as a main factor and condition factor at the start of the experiment as a covariate in the analyses. The correlation matrix for the condition factor and fish length at the start of the experiment and the change in condition factor during starvation were calculated for each level of temperature (Table 3). This indicated a poor correlation between length and condition factor at the start of the experiment, particularly at  $2^{\circ}\text{C}$  (no collinearity). The correlation between the change in condition and fish length at the start of the experiment was stronger and significant at all 3 levels of temperature. The correlation between the change in condition and condition factor at the start of the experiment however was significant at  $2^{\circ}\text{C}$ , marginally significant at  $6^{\circ}\text{C}$  and not significant at  $10^{\circ}\text{C}$  (Table 3).

The change in condition factor ( $\Delta K_{84d}$ ) at each level of temperature over the 84 day starvation period could thus be described by the following equations :

$$2^{\circ}\text{C} : \quad \Delta K_{84d} = - 0.3071 - 0.1264 K + 0.000575 L$$

$$6^{\circ}\text{C} : \quad \Delta K_{84d} = - 0.3246 - 0.1017 K + 0.000471 L$$

$$10^{\circ}\text{C} : \quad \Delta K_{84d} = - 0.5356 + 0.000681 L$$

where K and L are the condition factor and fork length (mm) at the start of the experiment. The removal of K as a main factor in the equation at  $10^{\circ}\text{C}$  had no incidence on the statistics in Table 3. Based on weighings made after 0, 21, 42, 63 and 84 days, weight loss was linear at all levels of temperature ( $P < 0.001$ ,  $r^2 > 0.95$ ), but the fit was not as good for day 21 when predicted weight losses are less than actual weight losses. The equations were

recalculated with the somatic condition factor using an average gonadosomatic index value (n=20, 1.2%) for day 0 :

$$2^{\circ}\text{C} : \quad \Delta K_{84d} = - 0.1362 - 0.2423 K + 0.000376 L$$

$$6^{\circ}\text{C} : \quad \Delta K_{84d} = - 0.2371 - 0.2298 K + 0.000494 L$$

$$10^{\circ}\text{C} : \quad \Delta K_{84d} = - 0.5934 + 0.000771 L$$

### Field observations

The physiological condition of cod declined from January to May, but it improved from May to June. Somatic condition factor differed significantly among the four samples ( $P < 0.001$ ). January and May differed from all other samples (Tukey HSD,  $P < 0.05$ ), but April and June were not significantly different ( $P > 0.05$ ). Somatic condition factor was normally distributed for each monthly sample (Shapiro-Wilk,  $P > 0.05$ ) and the four variances were homoscedastic (Bartlett,  $P > 0.05$ ) (Table 4). This change in somatic condition factor is shown in Figure 1 for a theoretical population of 1 million fish assuming a normal distribution and using values in Table 4. The proportion of cod with somatic condition factor values below 0.75 for instance would be 36.2% in January, 70.1% in April, 90.1% in May and 80.8% in June

### Expected vs observed distributions

From January to April, the observed decline in somatic condition factor was less than predicted declines in unfed fish in the laboratory for the 3 levels of temperature ( $P < 0.001$ ). From April to May, observed and predicted declines did not differ at  $2^{\circ}\text{C}$  or  $6^{\circ}\text{C}$  ( $P = 0.79$  and  $P = 0.08$ , respectively), but they did at  $10^{\circ}\text{C}$  ( $P < 0.01$ ). From May to June, condition actually improved and thus observed and predicted values differed at all 3 levels of temperature. Had the wild cod not fed between January and April (76 days between sampling events) or had feeding only made for greater activity costs in the wild compared to the laboratory, a large proportion of fish would have had their survival threatened (Table 5).

Because the predicted and observed decline in the somatic condition factor looked similar over the April-May period ( $2$  and  $6^{\circ}\text{C}$ ), we may ask whether the May sample was biased in that it did not include fish that died from energy exhaustion during that critical period. Laboratory experiments do not support this possibility (Table 6). The percent proportion of fish below two critical thresholds described in Dutil and Lambert (2000) were

not markedly different for the observed distribution in May compared to the predicted distribution at 2 and 6°C. Furthermore, the observed distribution in May did not depart from normality (see above).

## **Discussion**

By comparing field data and the results of laboratory experiments, we were able to investigate a source of bias in our estimates of natural mortality associated with poor condition in cod (Dutil and Lambert 2000). Potential sources of bias included different lethal thresholds for wild and laboratory animals and a misrepresentation of fish in poor condition in the field samples. These would result in an underestimate of natural mortality when field and laboratory distributions are compared. The possibility that the death of fish in poor condition may result in a misrepresentation of these cod in the field samples and in a biased estimate of natural mortality was investigated in our study.

Higher mortality rates are expected among individuals in poor condition. Thus one would predict a markedly skewed distribution of the somatic condition factor in situations when the mean condition factor of the population approaches the level below which mortalities occur. The frequency distributions of the somatic condition factor in the field samples did not show any significant departure from normality. Furthermore, predicted and observed changes in condition during the January-April period suggest that cod had actually fed during that period. The decline of their energy reserves at all levels of temperature was much less than one would expect in unfed animals. While mid-winter in the northern Gulf of St. Lawrence appears to be a period of relative food shortage, as shown by the decline of condition from January to April in the field samples, laboratory experiments suggest that part of the energy costs are nevertheless met through occasional feeding during mid-winter. This is consistent with stomach contents data for that stock (Chabot et al., unpublished).

The laboratory experiments suggest that a total lack of food during the January-April period would have resulted in very high mortalities, not only at 6°C but also at 2°C, in the mid-1990s. The only period however when cod appear to have incurred similar energy costs in the wild as in the laboratory is the April-May period. This period would then appear to be a period when cod are most vulnerable to death from poor condition. Not only do they experience a period of food shortage, they must also prepare for spawning.

Our laboratory experiments do not support the hypothesis that mortalities from poor condition resulted in an underestimate of the incidence of mortality in May, but laboratory experiments were conducted in the fall period at the onset of sexual maturation in cod. Our results may have been different had the food deprivation experiment been conducted in the January-May period when the energy demands for maturation and mating increase.

Temperature had a marked effect on the change in condition factor with greater energy costs at 6°C than at 2°C. The occurrence of cod at higher temperatures in winter than in summer (Castonguay et al. 1999) may thus appear contradictory if food is not abundant. Indeed cod might be expected to seek lower temperatures in periods of food shortage in order to minimize energy expenditures and maximize survival. One potential explanation for this observation is that cod may require higher temperatures for successful maturation. Results on gonad maturation in food deprived fish however (Dutil et al., unpublished) tend to indicate that food deprivation is more detrimental to maturation at higher temperatures.

## References

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Table 1. Observed decline (mean and standard deviation) in the condition factor of Atlantic cod over a starvation period of 84 days. Fish of two size categories (38-48 cm and 48-58 cm) were kept at 3 temperatures (2, 6 and 10°C). Values for the 2 size categories represent average declines for the 3 temperatures combined and vice versa. Means adjusted for differences in the condition factor at the start of the experiment are also shown in parentheses.

	Change in condition factor	Number of observations
2°C	0.1477 ± 0.0378 (0.1483)	50
6°C	0.1926 ± 0.0393 (0.1932)	42
10°C	0.2077 ± 0.0462 (0.2063)	40
38-48 cm	0.2017 ± 0.0454 (0.2024)	65
48-58 cm	0.1592 ± 0.0416 (0.1585)	67

Table 2. Condition factor of Atlantic cod at the start of an experiment (mean and standard deviation) in which fish of two size categories (38-48 cm and 48-58 cm) were kept at 3 temperatures (2, 6 and 10°C). The statistics for the regression between the change in condition factor and the condition factor at the start of the experiment are shown for each size and temperature combination.

	Initial condition factor	Regression	
		P	r <sup>2</sup>
2°C, <48 cm	0.920 ± 0.135 (n=25)	<0.01	0.27
2°C, >48 cm	0.925 ± 0.125 (n=25)	<0.01	0.27
6°C, <48 cm	0.903 ± 0.127 (n=21)	<0.05	0.20
6°C, >48 cm	0.940 ± 0.102 (n=21)	>0.05	0.03
10°C, <48 cm	0.949 ± 0.101 (n=19)	>0.05	0.05
10°C, >48 cm	0.946 ± 0.115 (n=21)	>0.05	0.01

Table 3. Correlation coefficients for the relationship between the change in condition factor ( $\Delta K$ ) and the length (L) and condition factor (K) at the start of an experiment in which Atlantic cod were kept at 3 temperatures. The statistics for the multiple linear regression between the change in condition factor and the length and condition factor at the start of the experiment are shown for each level of temperature.

	Temperature		
	2°C	6°C	10°C
Correlation coefficient			
L - K	0.03	0.20	0.23
L - $\Delta K$	0.53	0.42	0.58
K - $\Delta K$	-0.42	-0.20	0.10
Model $\Delta K = f(L, K)$			
P	<0.001	<0.01	<0.001
$r^2$	0.47	0.26	0.34
Test for factor=0			
K	<0.001	0.04	0.78
L	<0.001	<0.01	<0.001

Table 4. Somatic condition factor (K, mean and standard deviation) of Atlantic cod ranging in size from 32 to 64 cm in January, April, May and June 1994 in the estuary and northern Gulf of St. Lawrence. P, probability for Shapiro-Wilk statistic.

	K	Range	P	Skewness	Kurtosis
January	0.780±0.058	0.62-0.98	0.942	0.17	0.45
April	0.733±0.052	0.59-0.85	0.939	-0.20	0.10
May	0.690±0.054	0.55-0.91	0.411	0.37	0.57
June	0.715±0.052	0.59-0.84	0.412	0.06	-0.34

Table 5. Predicted somatic condition factor (K) for Atlantic cod in April 1994. Predicted values were calculated from the January observations under the assumption of no feeding between January and April at 3 levels of temperature, 2, 6 and 10°C. The percent proportion of fish expected to reach two critical thresholds described in Dutil and Lambert (2000) is also shown assuming a similar coefficient of variability before and after starvation.

Temperature	K	K < 0.54	K < 0.66
2°C	0.649	1.2	58.7
6°C	0.618	4.4	81.8
10°C	0.578	18.7	97.1

Table 6. Predicted and observed somatic condition factor (K) for Atlantic cod in May 1994. Predicted values were calculated under the assumption of no feeding between April and May at 2 levels of temperature, 2 and 6°C. The percent proportion of fish expected to reach two critical thresholds described in Dutil and Lambert (2000) is also shown.

	K	K < 0.54	K < 0.66
2°C	0.687	<0.1	27.4
6°C	0.675	0.2	37.3
Observed	0.690	0.3	28.8

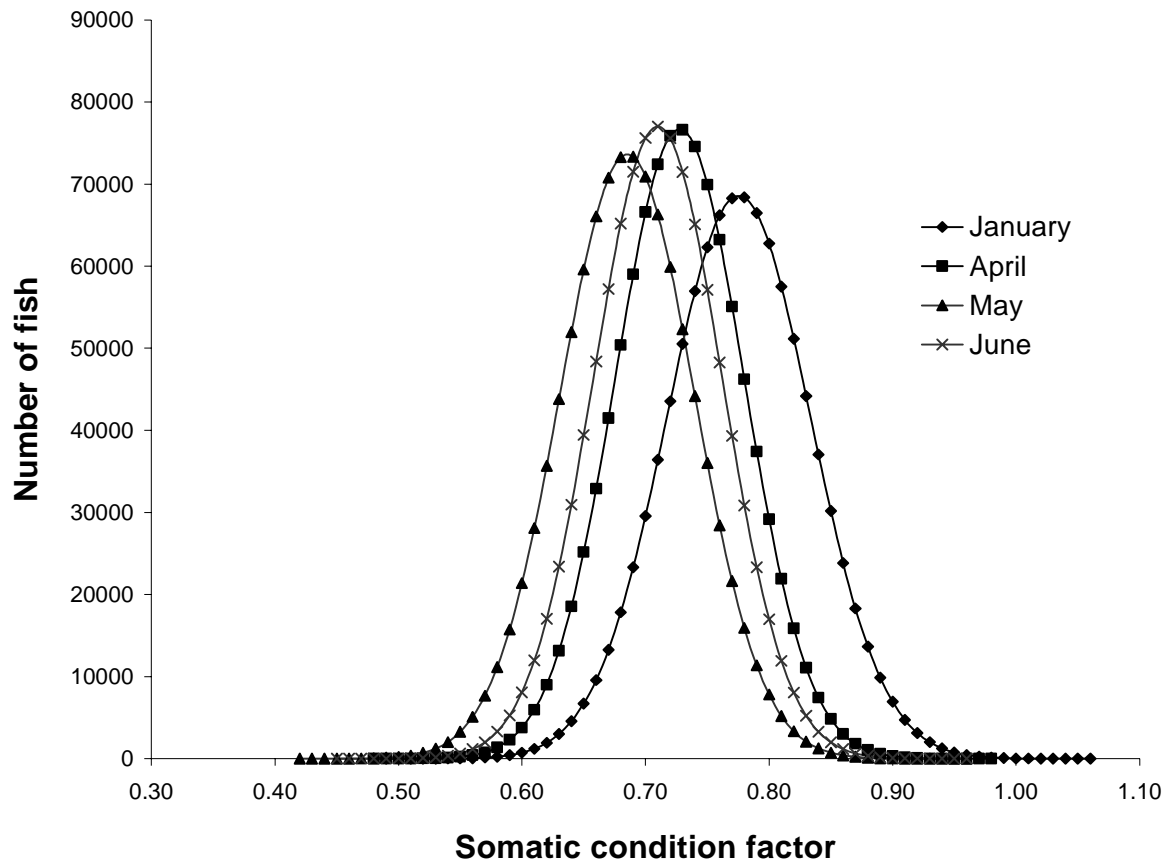


Figure 1. Frequency distribution of the somatic condition factor for Atlantic cod sampled in January, April, May and June 1994 assuming a normal distribution and a population of one million fish.