Repeatability of fin length measurements using digital image analysis, and studies of fin morphology and erosion as indicator of social interactions of cod

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Master Thesis 30 credits 2012

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Gjentaksgrad av målinger av finnelengde gjennomført ved hjelp av digitale bildeanalyser,
og studier av finnemorfologi og -erosjon som indikatorer for sosiale interaksjoner mellom
torsk i oppdrett

Master Thesis in Aquaculture (30 credits)

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PREFACE

This master thesis is part of the BreedWell project, aiming to developing methods to improve fish welfare in aquaculture breeding programs. The project is run by the Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima) and funded by the Research Council of Norway. This study was carried out at Nofima Marin, over the period from December 2011 to August 2012.

Animal welfare is a subject of increasing interest for ethical and legal reasons. Various traits can be used as indicators of animal welfare. For selection and breeding purposes, it has been shown that the trait frequency of injury (e.g. fin damage) can be taken into account in fish selective breeding and have generally been used in cannibalistic fish species such as rainbow trout. However, evidence related to the fin condition of the Atlantic cod is lacking. I believe this study relating to fin morphology and changes in cod will provide useful knowledge for making logical and meaningful inferences of the cause of fin damage due to possible social interactions (or cannibalism) among conspecifics.

Changes in fin length, is one of the methods commonly used to assess the degree of fin damage. Differing from normal methods of measuring fish length, we use digital image analysis to measure fin length of fish in this study which showed the advantage of being convenient and time-saving. Assessor reliability test indicated that it was feasible to record fin length using digital image analysis which further proved its accuracy in measurements. I suggest that more advanced and sophisticated technologies ought to be applied in research studies with various purposes to speed up the development of scientific industry in the future.

Yajing He
Ås, Aug 2012

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I would extremely like to pay homage to my three supervisors, **Prof. Ingrid Olesen, Dr. Hanne Marie Nielsen** and **Dr. Jørgen Ødegård**. I am very much thankful to Prof. Ingrid Olesen for answering my questions extremely patiently, despite her busy and intense research works in Malaysia. I also sincerely acknowledge Dr. Hanne Marie Nielsen for her encouragement, and taking time to discuss various issues in relation to SAS program which help me successfully overcame many difficulties and learned a lot. Last but not least, I warmly thank Dr. Jørgen Ødegård, for his valuable advice in statistical analysis and his extensive discussions around my work.

I would feel pride to dedicate this thesis to my philanthropic mother **Xiuyun Lu** and father **Yuantong He**, who have been a source of encouragement and inspiration to me throughout my life.

Thank you all and may God bless you!

ABSTRACT

Fin damage, commonly termed as fin erosion, has been paid considerable attention as a worldwide welfare issue especially in cannibalistic fish species. As cannibalistic fish, Atlantic cod (*Gadus morhua*) has been showed to have fin damage. The present study was conducted with 2100 juvenile cod with a mean initial body weight of 34.6g. The lengths of four fins (three dorsal and the caudal) for each fish were measured, and the measurements were made by three different assessors at three different points of time (recording 1-3) within six weeks of the experiment. This paper demonstrates the application of digital image analysis for analyzing fin length of cod. In order to provide an indication of the repeatability of digital image analysis, a reliability test was performed. The image of 42 randomly chosen juvenile cod taken at recording 3 was analyzed repeatedly by three different assessors. Significant differences in fin length measurements were found both between and within assessors. However the Pearson's correlation in fin length measurements between each of the two assessor replicates was equal to or higher than 0.45. There were moderate correlations of fin length measurements between different assessors (r=0.45-0.84), and the correlations between replicates within same assessor were strong (r=0.57-0.94). In addition, majority of the variance was found to be attributed to the fish effect rather than assessor effect. Generally there were moderate repeatability of the fin lengths analyzed using digital image (*R*=0.46-0.61). With the support of all the statistical results from the reliability test, it is justified to say that this digital image based approach to measure fin length is accurate and feasible for genetic analyses.

All the data set obtained from 2100 juvenile cod was used to assess the changes for all four fins during the experiment due to growth or possible erosion. "Relative fin length" expressed as the percentage of fin length to the total body length, was applied in this study to assess the fin erosion. All four fins of fish suffered damages within the first two weeks (recording 1-2), and the caudal fin showed the most injury. In the following four weeks until the end of the experiment (recording 2-3), the cannibalism diminished with anterior dorsal fin still suffering degeneration. Later the incidence of fin erosion also reduced compared to that described for the first two weeks of the experiment. In general, the damage was concentrated on anterior dorsal and caudal parts of the fish. A possible hypothesis is that the fin erosion was probably due to attacks among the cohorts in the same rearing unit for the establishment of dominance hierarchy (i.e., social interactions). More relevant research is however needed to understand the underlying reasons that may explain the observed fin damage.

Key words: Atlantic cod *Gadus morhua*, digital image analysis, fin erosion, fin length measurement, reliability test

LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization of the United Nations
MATLAB	MATrix LABoratory
PIT	Passive Integrated Transponder
MS-222	Tricaine methanesulfonate
JPEG	Joint Photographic Experts Group
FIN1	Anterior dorsal fin (1st dorsal fin)
FIN2	Mid dorsal fin (2nd dorsal fin)
FIN3	Posterior dorsal fin (3rd dorsal fin)
FIN4	Caudal fin
M	Assessor M
B	Assessor B
K	Assessor K
SS	Sum of Squares
MS	Mean of Square
DF	Degree of Freedom

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1 INTRODUCTION

Aquaculture industry is one of the fastest growing sectors of animal production in the world (FAO 2012). In recent years, the scale of gadoid fish culture like cod has expanded (Rosenlund & Skretting 2006). Norway is one of the most famous cod producing nations in the world. In the 1980s, Norway started to develop commercial cod farming, and in 2002, the first breeding program Cod Culture Norway (Bergen) was introduced (Moksness et al. 2004). The boom in the cod fishery industry can be attributed to the tasty meat and extraordinary nutritional value of the cod fish. Since cod is cannibalistic, studying relevant welfare indicators (e.g. fin damages) affecting cod growth with the purpose of improving its domestication shows substantial meanings. However, published literature about fin damage in cod is lacking. One thing that has been confirmed is that the cannibalistic phenomenon seems to be most prevalent in larval and juvenile cod (Puvanendran et al. 2008). Moreover, fin damage can be considered as an indication of the level of aggressive activity for a cod population (Hatlen et al. 2006).

Fin condition can be described by fin status or quantified by fin length and profile (Latremouille 2003). The inevitable problem in using descriptive and subjective scoring methods to assess fin losses is the inherent subjectivity (Branson 2008). Kindschi (1987) proposed the term named “relative fin length” to assess the fin damage of steelhead trout through comparing the changes of the percentage of the specific fin length to total fish length. Later this method was developed and proved feasible in many fish species such as rainbow trout (Bosakowski & Wagner 1994). In order to ensure the accuracy of the assessment using relative fin length, precise body and fin lengths of the fish must be obtained. Traditionally, the length of fish is measured using measuring tools such as measuring tapes, but this method has many drawbacks such as being time-consuming. In recent years, digital image analysis technology has developed and has been used in the fishery researches (Blonk et al. 2010). Using digital image based approaches to analyze morphological trait of the fish has been proved feasible. For instance, it is well documented that the length of tuna fish can be well measured through using digital imaging approach (Hsieh et al. 2011). Unfortunately, to our knowledge, relevant research on the cod fish is lacking.

The main objective of this thesis was to investigate the possibilities to use digital image analyses to measure fin length, in order to use these fin length measurements to assess morphological change of the fins due to growth and possible social interactions during the experiment. From this deduce the following:

- To determine the correlations between assessors and between replicates within assessor in fin length measurements obtained from 42 fish for the reliability test and the repeatability of fin length measurements.
- To evaluate the advantages and the disadvantages in recording fin length using digital image analysis.
- To determine morphological change of four different fins (three dorsal and the caudal) by studying changes of the fin lengths and the relative fin lengths [(fin length×100)/total body length] obtained from 2100 fish at three different points of time during the experiment (lasting for 6 weeks).
- Discussion of the possible methods for the assessment of fin erosion

2 BACKGROUND

2.1 Species traits and culture attributes of Atlantic cod

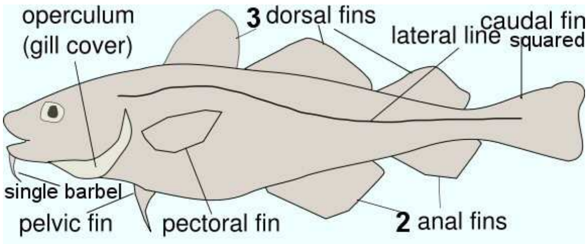


Figure 1. Fins of Atlantic cod (*Gadus morhua*) http://www.allfishingbuy.com/Fish-Species/Atlantic-Cod.htm accessed 30.07.2012

Atlantic cod (*Gadus morhua*) is a well-known groundfish, also known as codfish or codling (Torsk in Norwegian). It has a heavy and tapered body, a large mouth and many small teeth in it. Cod has three dorsal fins, none of which contain spines. The tail fin is almost squared. A characterized white lateral line runs from the gill slit to the base of the tail fin. Cod is a highly fecund species with pelagic eggs and larvae (Kjesbu 1989). The larvae also show extraordinarily high growth potential (>20% per day) (Rosenlund & Halldórsson 2007). Not only that, even at low temperatures, cod are still capable to have similar growth rate to other farmed fish species (Finn et al. 2002).

In traditional cod farming, the cod fry depends on wild stocks. However, the annual landings from wild stocks have been declining and some stocks have showed a significant decrease compared to historical levels (Moksness et al. 2004). Considering the limitation of wild fishery of Atlantic cod and the considerable profit, an applicable commercial cod farming method was needed. Fortunately, the potential for the development of cod commercial farming is large, and the main limitation is probably in the juvenile cod production (Moksness et al. 2004). Attempts have been conducted continuously. For example, in Norway, comparing the successful commercial production of Atlantic salmon, a passionate interest has been created to develop commercial cod farming (Rosenlund & Halldórsson 2007). Great efforts were made around 1990s for cod farming, however, the commercialization process stopped later due to various reasons (Rosenlund & Skretting 2006). The production of farmed cod (*Gadus morhua*) has increased rapidly from 2000, but the production volumes of cod are still low compared with salmon (*Salmo salar*) (Fig. 2 and 3).

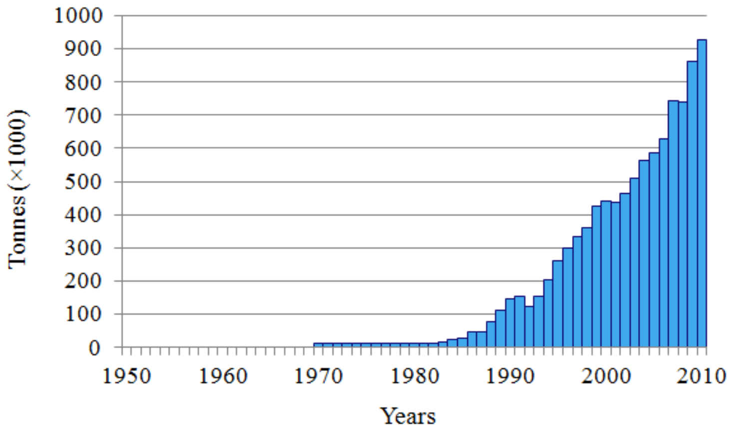


Figure 2. Production of Atlantic salmon (*Salmo salar*) in Norway from 1950 to 2010 (FAO Fishery Statistic).

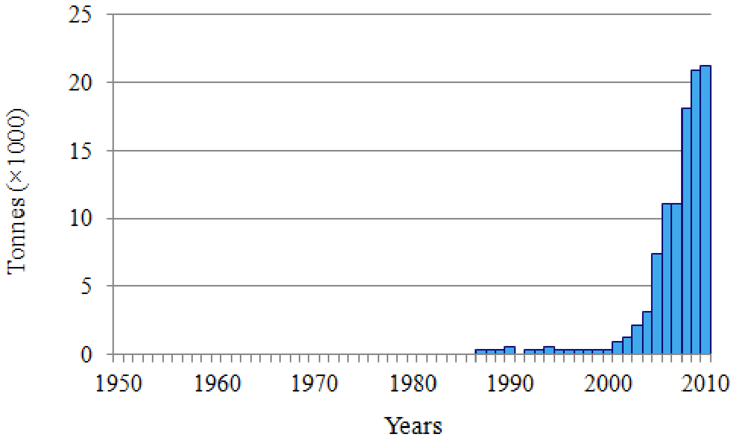


Figure 3. Production of Atlantic cod (*Gadus morhua*) in Norway from 1950 to 2010 (FAO Fishery Statistic).

2.2 Cannibalism

The research history of Atlantic cod is long, and the majority of the basic culture details have been established. However, problems in some areas still exist, including larval and juvenile mortalities due to cannibalism (Brown et al. 2003). Cannibalism is an extreme form of predation within group members (Puvanendran et al. 2008), and it can be attributed to the social interactions among cohorts. The causes of social interaction are various. In aquaculture species, large amount of individuals are reared together in one unit. The density of individuals is much higher than that in the wild, and considerable phenotypic variation in the fish population can be found. As a result, aggression happens more frequently among interacting individuals (Muir & Bijma 2006). These aggressive (or cannibalistic) behaviors may cause harms to the fish and may lead to devastating losses if it is not managed properly (Folkvord 1991). For example, Turnbull et al. (1998) mentioned that aggression in fish is frequently shown in the form of fin damage. In contrast, the incidence and severity of fin injury can be also used as the criteria to evaluate the aggression of fish, which has been used in salmonids (MacLean et al. 2000). Cod are well-known aggressive fish species and can be cannibalistic if food availability is suboptimal (Rosenlund & Halldórsson 2007), and the cannibalism phenomenon found to be most prevalent in larval and juvenile cod (Puvanendran et al. 2008). Therefore, reducing cannibalism should be treated as an essential and critical issue for further cod farming, since juvenile cod production is the key point of the cod commercial farming.

2.3 Fin erosion phenomenon

The commercial fish should look healthy and aesthetically pleasing, at least without visible signs of suffering or deformities. Person-Le Ruyet et al. (2007) thought that the commercial value of hatchery-reared juveniles is related to the fin condition. Damaged fins will affect the appearance of fish anyhow. Further, fin condition can be used as a potential indicator of fish quality such as dorsal fin length (Winfree et al. 1998). For example, the Norwegian industry standard for fish incorporates the absence of fin loss (or damage) into the judgment of superior fish (*Norwegian Industry Standard for Fish* 1999).

Fin erosion can be defined as degradation of the fin skin or fin rays of teleost fish and cause various morphological changes such as splitting and histological reduction in fin size (Sharples & Evans 1996). Latremouille (2003) reviewed methods used for the assessment of fin erosion, which can be mainly divided into methods describing the fin status and methods quantifying the area or length of fins. A relatively objective method called “relative fin length” (fin length×100/total body length) for quantifying the extent of fin erosion, proposed by Kindschi (1987), has been widely used for the assessment of fin erosion with the measurement of fin lengths. Fin erosion is best documented in farmed salmonids such as rainbow trout (*Oncorhynchus mykiss*) (Bosakowski & Wagner 1994). For instance, Moutou et al. (1998) found that the severity of erosion on the dorsal fins seemed greater than that on the caudal fins, and the cause of dorsal fin damage is of a behavioral origin. Moreover, aggressive nipping in juvenile steelhead trout was proved due to the establishment of dominance hierarchies, and the erosion was also observed primarily at the dorsal fin (Abbott & Dill 1985). For cod, relevant research appears to be less. Fin nipping has been found in juvenile cod, and higher incidence of fin erosion was found on small cod (55g) than large cod (250g and 450g) (Hatlen et al. 2006). However, according to the results shown in the report commissioned by the Scottish Aquaculture Research Forum (SARF), minimal level of fin erosion was seen in farmed adult cod (4 to 8kg), except for the first dorsal fin (Smith et al. 2009).

2.4 Digital Image Analysis

From the design of digital image analysis system (Fig. 4), a digital image analysis system can be systematically divided into the following parts: digital camera, illumination, digitizer, computer hardware and software (Wang & Sun 2002). Good illumination can significantly reduce inappropriate external effects, such as shadow. A brief explanation of the workflow is that the digitizer will change the pictorial images into numerical form for subsequent image processing.

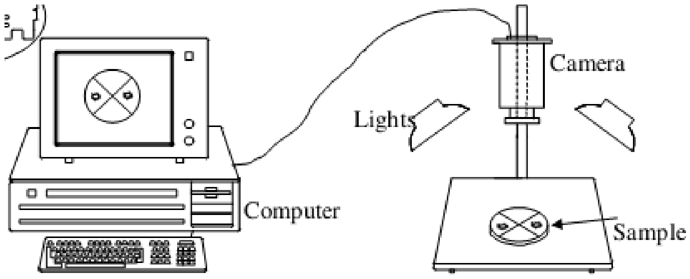


Figure 4. Main components of the digital image analysis system (Wang & Sun 2002)

The traditional measuring method is awkward and time consuming. Each time before measuring the fins, fish should be narcotized and fixed to obtain an optimal measuring condition. Fish may experience potential damage or stress, if the evaluation work is done regularly. It seems like this method is unacceptable in some cases. With the development of advanced technology, digital image analysis can easily solve this problem. The images can be used repeatedly without interrupting the fish. Sometimes the experiment and the data analysis are conducted in different places. Thus, experimenters can take pictures of fishes then send these pictures through the internet to researchers in different places for further analysis. To some extent, using digital image measuring system can not only reduce the unnecessary repetitive manual operations but make reanalysis easy. In reality, digital image analysis has been used in descriptive work in aquaculture (Blonk et al. 2010), and it is supposed to do outstanding contributions in aquaculture researches.

2.5 Feasibility analysis in statistics

In research studies, the term reliability refers to “repeatability” or “consistency”. Simply put, a measurement is deemed reliable if the same result can be obtained again and again assuming the subject under measuring is invariable. Two measures of reliability are mainly of interest: change in the mean and the correlation between test and retest (Hopkins 2000). Change in the mean simply means the difference between the means of two tests. The change can be derived from both random change and systematic change. Pearson correlation coefficient (r) is a typical parameter to assess the correlation between test and retest:

$$\mathrm{Corr}=\rho=\frac{\mathrm{Cov}(\mathrm{X},\mathrm{Y})}{\sqrt{Var(\mathrm{X})}\sqrt{Var(\mathrm{Y})}}$$

Where X, Y are normal distributed and independent of each other.

A preferable measure of reliability is intraclass correlation coefficients (ICCs). When more than two tests are taken, it can be calculated as a single correlation. Intraclass correlation was typically used for measuring homogeneity (Shrout & Fleiss 1979).

In the classical test theory, the actual measurement consists of two parts:

$$X=T+e$$

X = the measurement in the study;
T = the true score;
e = the measurement error.

Assuming the measurement error is uncorrelated with true score:

$$Var(\mathrm{X})=Var(\mathrm{T})+Var(\mathrm{e})$$

The reliability of a measuring task is defined as the true score variance to the total variance.

$$R=\frac{Var(T)}{Var(\mathrm{X})}=\frac{Var(T)}{Var(T)+Var(e)}$$

The variance of true score cannot be calculated, due to the true value are never known for a measurement. The best way is to estimate it. X_1 and X_2 are two measurements of the same subject, either from same assessor or two different assessors.

$$X_1=T+e_1 \text{ and } X_2=T+e_2$$

With $Var(X_1)=Var(X_2)=Var(X)$ and $Var(\mathrm{e}_1)=Var(\mathrm{e}_2)=Var(\mathrm{e})$

$$\mathrm{Cov}(X_1,X_2)=\mathrm{Cov}(T+e_1,T+e_2)=\mathrm{Cov}(T)$$

$$\text{Corr}=\frac{\text{Cov}(\text{X}_1,\text{X}_2)}{\sqrt{Var(\text{X}_1)}\sqrt{Var(\text{X}_2)}}=\frac{Var(T)}{Var(T)+Var(e)}=R$$

It is clearly showed that the reliability coefficient is an intraclass correlation coefficient. When the scores assigned by assessors are numerical data such as length and weight, measurement reliability can be assessed by analysis of variance model (Landis & Koch 1975).

In the most elementary reliability testing study with repeated measurement, each of j (j=1, 2…n) assessors independently measuring one characteristic once on each of the same i (i=1, 2…k) subjects.

$$Y_{ij}=\mu+\tau_i+e_{ij}$$

Y_{ij}= the measurement of the i th subject made by the j th assessor
μ= overall population mean of the measurements
τ_i= the i th subject effect
e_{ij}= residual error

τ_i and e_{ij} are assumed to vary normally with means of 0 and variances of σ_s^2 and σ_e^2.

The estimated intraclass correlation coefficient of reliability can be used as an index to assess the reliability of the measuring procedure (Shrout & Fleiss 1979), which is denoted as $\hat{\rho}$:

$$\hat{\rho}=\frac{\sigma_s^2}{\sigma_s^2+\sigma_e^2} \qquad (1)$$

3 MATERIALS AND METHODS

A 6 weeks experiment was carried out on Atlantic cod, and the experiment was performed at the experimental base, the Cod Breeding Station, of Nofima Marin in Tromsø in November 2009. Pictures were taken of the cod in the experiment at three different points of time during the experiment, and fin lengths of the fish were measured using these digital images. Two different analyses on the digital image were done, and the image materials were all obtained from the fish in this experiment. The first analysis was a reliability test with the purpose of testing the feasibility to record fin length using digital image analysis. The analyzed images were obtained from the fish derived from two randomly chosen tanks in this experiment. In addition, the second analysis was made based on all the data set obtained from the whole fish in the experiment aiming at assessing the morphological changes of the fins on the fish.

3.1 Experimental system

3.1.1 Fish materials

The number of tanks, the number of fish and families per tank in the experiment was decided after initial power calculations and simulations (Ødegård & Olesen 2011). As a result, a total of 2100 tagged fingerlings (with a mean initial body weight of 34.6g hatched in March to April 2009) from 100 full sib families originating from the National Cod Breeding program were used. Each full-sib group were split into three sub-groups (300 sub-groups in total and 7 individuals in each sub-group), each sub-group was further represented in one of 100 tanks (190L) randomly. Meanwhile, three sub-groups (21 fish) were stocked in each of the 100 tanks.

3.1.2 Recordings at the station

During the experiment, the weight and length of each fish were measured at three different points of time. Body weight was measured to the nearest 0.1g, and body length to the nearest 0.1cm. First recording was at stocking (2nd and 3rd November, 2009), second recording was two weeks after the stocking (16th and 17th November, 2009), and third recording was four weeks later at the end of the experiment (14th to16th December, 2009). Dead fish were registered throughout the experiment.

Pictures were taken of all 2100 fish at all three recordings during the experiment, and they were identified by their individual PIT tags. Before taking pictures, fish received a temporary anesthesia with MS-222 to avoid stress and make them lay still during the photography. Later as shown in Figure 5, fish were placed on a uniform and white background with the true left side of fish body up. A calibration ruler was placed adjacent to the fish. Moreover, two papers were attached by the side of fish, on which the tank number and the fish number were written, respectively. The fish number represented the photographing sequence of each fish within one tank, since pictures of the 21 fish were taken one by one. All digital images were saved in JPEG-format for further image processing.

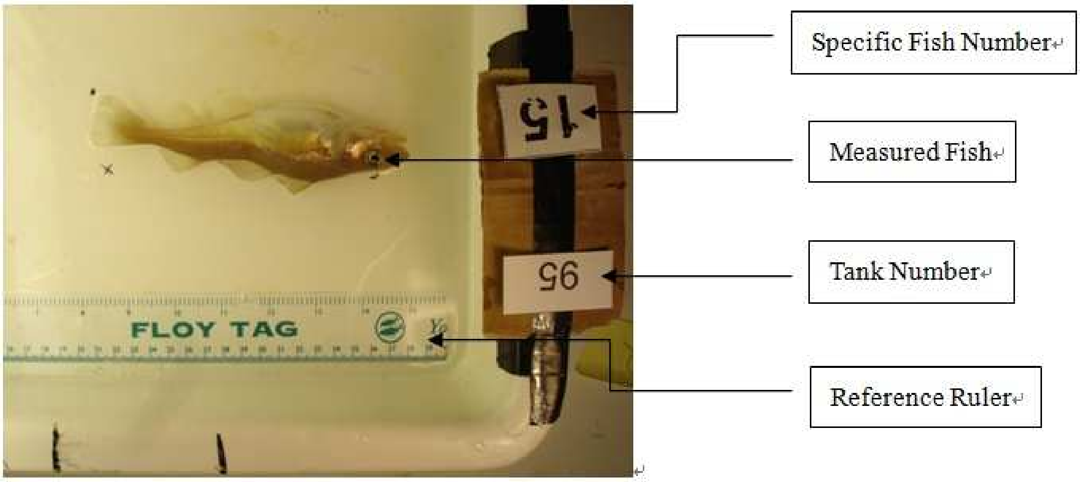


Figure 5. Sample digital image (photograph) of a juvenile cod with a calibration rule for length estimation and two labels for identification, photographed by technicians in the cod breeding station (Nofima Marin, Tromsø) in November 2009 (recording 1).

3.2 **Fin length measuring method** (digital image analysis)

The digital image (2048×1536 pixels) was performed using MATLAB software (version 7.12, r2011a). Each image was changed to grayscale after reading into the workspace. Ten centimeters was firstly measured from the ruler as a calibration vector.

Measurements were conducted by taking the maximum length reading (i.e., parallel to the fin rays; fig. 6). Three dorsal fins and the caudal fin with the abbreviated name of Fin1, 2, 3 and 4 were measured by three different assessors (named M, B and K), who did not have the experience of digital image analysis before. Assessors were taught to use ordinary cursor positioning and mouse clicks to measure the fin length through locating the starting points of fins on the base side and the terminal point of fins on the outer side along with the fin ray. For the caudal fin, the length of fin ray on the dorsal side of the fin was measured (Zimmerman et al. 2006). Fin length was estimated based on the proportional relationship between the fin vector and the 10cm vector from the calibration ruler. Finally, the numeric length of each fin was automatically recorded for further analysis.

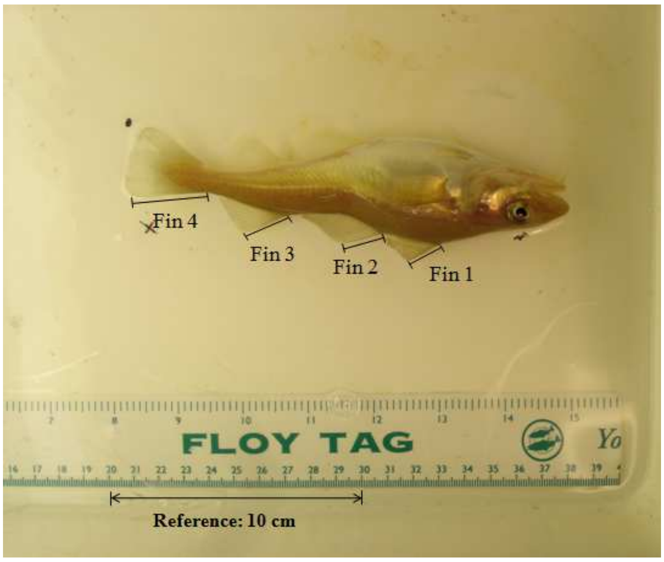


Figure 6. Location of the measurements taken for maximum fin lengths of three dorsal fins and the caudal fin.

3.3 **Analysis 1** (verification of digital image analysis to measure fin length)

3.3.1 The reliability test design of the digital image analysis

The investigation for verifying the feasibility of implementing the digital image based approach to analyze fin length was essential. It included whether the image analyses carried out by different persons on the same fins are coherent, and whether the image analyses done by the same person is reliable (i.e., not too discrepant resulting fin lengths when done twice or more times by the same person or different persons). The pictures of 42 fish taken at recording 3 were analyzed repeatedly by the three assessors, in order to investigate the correlation of the measurements carried out by same and different assessors. In detail, M, K and B made measurements three times, twice and once, respectively. Therefore, assessors with replicates were divided into six groups: M1, M2, M3, B1, K1 and K2. The capital letters refer to the assessors, and the digits refer to the number of replicates (Table 1). There were 252 (42 fish×6 measures) observations obtained from all the assessors with replicates for each of the four fins.

Table 1. Descriptions of the abbreviations for assessor with replicated measurements.

Abbreviations	Description
M1	First measurement of assessor M (11:00 on19th Dec, 2011)
M2	Second measurement of assessor M (13:00 on19th Dec, 2011)
M3	Third measurement of assessor M (4th Jan, 2012)
B1	One measurement of assessor B
K1	First measurement of assessor K (Autumn 2010)
K2	Second measurement of assessor K (Jan 2012)

3.3.2 Statistical analysis - Analysis 1

The statistical analyses were conducted using various statements of the Statistical Analysis Software (SAS®) release 9.2 for Windows (SAS Institute Inc., Cary, USA). Means of fin length measured by same assessor and different assessors were compared separately by using the dependent group TTEST statement (paired comparisons *t*-test). The degree of association between the measurements made by the assessors was analyzed by running PROC CORR statement to estimate Pearson’s correlations. Data set was also statistically analyzed by analysis of variance (ANOVA) using the general linear model (GLM) statement and the MIXED statement. When running the statistical model, fin length measurement was used as the dependent variable. The effect of assessor, fish and repetition were used as the class variables. Different combinations of these variables were tested, and the GLM statement was used to test the significance of these class variables. The level of significance was indicated at $P \leq 0.05$. Four different mixed linear models were later used to estimate the variance component of those effects which had been tested significant by GLM statement before. In the Mixed Model ANOVA, the variance of the random effect parameters was referred to as variance component. Variance components were estimated using restricted maximum likelihood approach (REML). Reliability (or repeatability) is expressed as the ratio of between-subject variance to the total variance (Eq. 2). It ranges from “0” to “1” (more close to “1” indicates more excellent reliability). Furthermore, the LSMEANS statement (least-square means) was used to detect differences of the fin length measurements between the assessors in the reliability test (used in model 4).

$$R = \frac{\operatorname{cov}(P_1 P_2)}{\sigma_{P_1}\sigma_{P_2}} = \frac{\operatorname{cov}(fish + e_1,\, fish + e_2)}{\sigma_P^2} = \frac{\sigma_f^2}{\sigma_T^2} \quad (2)$$

With $\sigma_{P_1} = \sigma_{P_2} = \sigma_P$; $\operatorname{cov}(fish, e) = 0$; $\operatorname{cov}(e_1, e_2) = 0$

$\sigma^2{}_f$ =variance component due to fish;
$\sigma^2{}_T$ = total variance.

***Model 1**: Assess the variance due to fish effect to see the repeatability of the measurements.*

First, we assumed a linear mixed model with the fish effect as a random effect.

$$Y_{ij} = \mu + f_i + e_{ij} \quad (3)$$

Y_{ij}= *ij*th measurement of the fin length;
μ= overall mean;
f_i= random effect of fish *i*;
e_{ij}= residual errors.

From Eq. (2) we easily derived the formula for the reliability (repeatability) for this model. Such a coefficient was referred to as intraclass correlation coefficient between any two measurements assigned to the same subject (fish) was calculated as:

$$R = \frac{\sigma^2_{fish}}{\sigma^2_{fish} + \sigma^2_{error}} \quad (4)$$

Model 2*: Assess the variance due to assessor effect.*

Both assessor effect and fish effect were included as random effects (in order to take into account the effect due to assessor).

$$\mathrm{Y}_{ijk} = \mu + f_i + a_j + e_{ijk} \quad (5)$$

Y_{ijk} = ijkth measurement of the fin length;
μ = overall mean;
f_i = random effect of fish i;
a_j= random effect of assessor j;
e_{ijk} = residual errors.

Model 3*: Assess the variance due to the interaction between assessor and repetition.*

Fish effect and the interaction between assessor and replication were presented as random effects in this model. Assessor*rep variable (the interaction between assessor and repetition) was introduced to take into account the differences between all the replicates made by the three assessors, due to the replications made by each assessor was uneven and performed at different times.

$$\mathrm{Y}_{ijkl} = \mu + f_i + (a \times r)_{jk} + e_{ijkl} \quad (6)$$

Y_{ijkl} = $ijkl$th measurement of the fin length;
μ = overall mean;
f_i = random effect of fish i;
$(a \times r)_{jk}$= interaction between assessor j and replication k;
e_{ijkl} = residual errors.

Model 4: *Three assessors were the only assessors involved in this study.*

Assessor effect was included as fixed effect. In such case, assessor effect should be parameterized as: $\Sigma A_j = 0$. Fish effect and the effect due to interaction between assessor and replication were designated as random effects.

$$Y_{ijkl} = \mu + f_i + A_j + (a \times r)_{jk} + e_{ijkl} \qquad (7)$$

Y_{ijkl} = ijkth measurement of the fin length;
μ = overall mean;
f_i = random effect of fish i;
A_j= fixed effect of assessor j;
$(a \times r)_{jk}$ = random effect of interaction between assessor j and repetition k;
e_{ijkl} = residual errors.

The intraclass correlation coefficient for this model was calculated as:

$$R' = \frac{\sigma^2_{fish}}{\sigma^2_{fish} + \sigma^2_{assessor \times rep} + \sigma^2_{error}} \qquad (8)$$

3.4 **Analysis 2** (assessment of morphological changes of the fins)

3.4.1 Assessment methods

The whole data set of the 2100 fish was used to assess the fin change for each of the four fins during the experiment. The measurements together were done by either assessor K, B or M (i.e., each fish was analyzed one time by one of the three assessors at each recording) (Table 2).

Table 2. The number of measurements obtained by each assessor at each recording, and the total number of measurements obtained across all assessors at each recording.

	Assessor M	Assessor B	Assessor K	Total Number [1]
Recording 1	—	1163	785	1948
Recording 2	685	1199	126	2010
Recording 3	565	—	1473	2038

[1] With 152, 90 and 62 missing values at recording 1, 2 and 3, respectively.

The mean of the raw fin lengths for each of the four fins at the three recordings was calculated in order to describe the changes in the different fins during the experiment. Relative fin length, also called “fin index”, was used to assess the fin erosion. It was calculated as:

$$\text{Relative fin length} = \frac{\text{Fin length}}{\text{Total body length}} \times 100 \qquad (9)$$

The total body length refers to the length of the fish from the snout to the end of the tail fin (i.e., the maximum length of the fish) (Fig. 7).

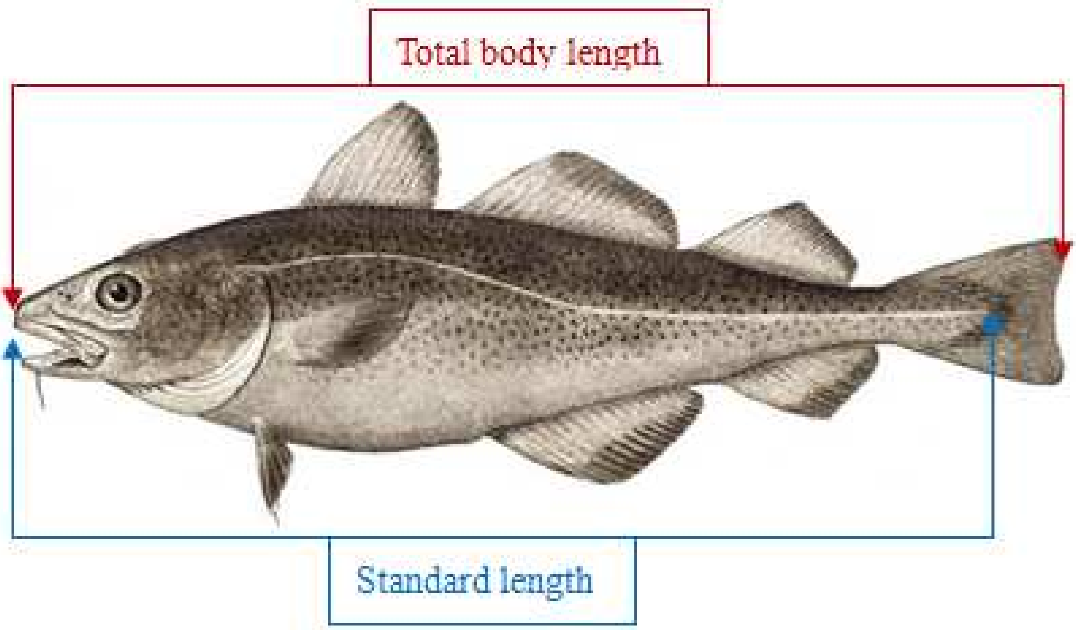


Figure 7. Total body length is the maximum length of the fish from the snout to the end of the tail fin, and the standard length is the length of the fish from the snout to the end of the vertebral column.

3.4.2 Statistical analysis – Analysis 2

Data from the whole experiment were statistical analyzed by analysis of variance (ANOVA) using the GLM statement of the SAS® release 9.2 for Windows (SAS Institute Inc., Cary, USA). In the model, fin length measurement was used as dependent variable. Assessor and recording time were used as class variables.

Furthermore, the LSMEANS statement was used to detect differences of the measurements of fin length and the relative fin length between assessors and between recordings during the experiment.

The main GLM model:

$$\mathrm{Y}_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk} \qquad (10)$$

Y_{ijk}= *ijk*th measurement of the fin length;
μ = general mean;
α_i = effect of assessor *i*;
β_j= effect of recording *j*;
ε_{ijk} = residual errors.

4 RESULTS

This section consists of the results from the reliability test (42 fish in two tanks at recording 3 only) and the results from the assessment of the changes in different fins during the whole experiment (2100 fish).

4.1 The results of the reliability test (42 fish)

4.1.1 Measurement differences

4.1.1.1 Measurement differences between fins

The coefficient of variation (CV) was highest for Fin1 (CV of 20 and 19 for tank 35 and tank39, respectively; table 3) followed by Fin2 (CV of 18 for both of the two tanks, respectively; table 3) then Fin3 (CV of 14 and 16 for tank35 and tank39, respectively; table 3) and lowest for Fin4 (CV of 8 and 10 for tank35 and tank39, respectively; table 3). In other words, the CV across all means within each fin progressively decreased from Fin1 to Fin4 in both tanks.

4.1.1.2 Measurement differences between assessors

Measurement differences due to assessor bias were observed on the three dorsal fins. Assessor K appeared to make lower measurements, especially K1 which maintained the lowest measurements (K1 line in Fin1 to 3; table 3). Assessor M by contrast had three consistently high measurements (M1, M2 and M3 lines in Fin1 to 3; table 3). In general, significant differences in fin length measurements were observed not only between different assessors but also between the replicates within same assessor (Table 4). The noted difference between measurements in all fins was less than 5mm.

Table 3. Descriptive statistic parameters of fin lengths (cm) analyzed using digital images with respective assessor replicates, including three replicates of assessor M (M1, M2, M3), two replicates of assessor K (K1, K2) and one measurement of assessor B (B1). Fin 1 to 4 refers to the 1st dorsal fin, the 2nd dorsal fin, the 3rd dorsal fin and the caudal fin, respectively. CV represents the coefficient of variation. CV (%) = (SD / Mean) ×100.

		Tank 35			Tank 39		
	N	Mean	SD	CV (%)	Mean	SD	CV (%)
				Fin1			
M1	21	1.72	0.42	24	1.83	0.33	18
M2	21	1.64	0.37	23	1.68	0.26	15
M3	21	1.51	0.18	12	1.66	0.24	14
B1	21	1.68	0.32	19	1.62	0.30	19
K1	21	1.36	0.21	15	1.32	0.21	16
K2	21	1.47	0.24	16	1.48	0.16	11
				Fin2			
M1	21	1.87	0.29	16	1.56	0.25	16
M2	21	1.74	0.25	14	1.53	0.23	15
M3	21	1.59	0.21	13	1.56	0.22	14
B1	21	1.59	0.22	14	1.35	0.26	19
K1	21	1.39	0.28	20	1.26	0.22	18
K2	21	1.56	0.23	15	1.32	0.17	13
				Fin3			
M1	21	2.04	0.16	8	1.87	0.22	12
M2	21	2.03	0.18	9	1.82	0.24	13
M3	21	1.91	0.23	12	1.70	0.26	16
B1	21	1.83	0.19	10	1.67	0.19	12
K1	21	1.60	0.27	17	1.48	0.25	17
K2	21	1.72	0.20	12	1.50	0.22	15
				Fin4			
M1	21	2.74	0.17	6	2.66	0.23	9
M2	21	2.73	0.21	8	2.63	0.23	9
M3	21	2.76	0.21	7	2.67	0.22	8
B1	21	2.48	0.20	8	2.37	0.24	10
K1	21	2.80	0.21	8	2.59	0.25	10
K2	21	2.64	0.21	8	2.52	0.25	10

Table 4. Paired *t*-test results between the measurements obtained from three different assessors (replicates are included) across two tanks (DF = 41). Fin 1 to 4 refers to the 1st dorsal fin, the 2nd dorsal fin, the 3rd dorsal fin and the caudal fin, respectively. The abbreviations of the assessors with replicates are listed in table 1. The comparison of the fin length measurements within assessor is indicated by a *. Insignificant differences in the fin length measurements between assessors with replicates are bold marked.

	Fin 1			Fin 2			Fin 3			Fin 4		
Replicates	Mean±SE	t	P-value	Mean±SE	t	P-value	Mean±SE	t	P-value	Mean±SE	t	P-value
*M1 vs. M2	0.11±0.02	5.45	<.0001	0.08±0.02	3.85	0.0004	0.03±0.02	1.65	**0.1074**	0.02±0.02	1.51	**0.1384**
*M1 vs. M3	0.19±0.05	4.11	0.0002	0.14±0.04	3.51	0.0011	0.15±0.03	5.31	<.0001	-0.01±0.02	-0.64	**0.5279**
*M2 vs. M3	0.07±0.04	1.95	**0.0584**	0.06±0.03	1.86	**0.0695**	0.12±0.02	5.05	<.0001	-0.04±0.02	-2.19	0.0345
*K1 vs. K2	-0.13±0.03	-4.53	<.0001	-0.12±0.03	-3.92	0.0003	-0.07±0.03	-2.51	0.0162	0.12±0.03	4.26	0.0001
M1 vs. B1	-0.12±0.04	-3.34	0.0018	-0.24±0.04	-6.67	<.0001	-0.21±0.02	-9.38	<.0001	-0.28±0.02	-11.94	<.0001
M1 vs. K1	0.43±0.05	8.31	<.0001	0.39±0.04	8.68	<.0001	0.41±0.03	12.75	<.0001	0.01±0.03	0.27	**0.7852**
M1 vs. K2	0.30±0.04	7.12	<.0001	0.27±0.03	8.05	<.0001	0.34±0.02	15.60	<.0001	0.13±0.02	5.81	<.0001
M2 vs. B1	-0.01±0.03	-0.30	**0.7656**	-0.16±0.03	-4.94	<.0001	-0.18±0.02	-7.24	<.0001	-0.26±0.03	-10.03	<.0001
M2 vs. K1	0.32±0.04	7.35	<.0001	0.31±0.04	7.51	<.0001	0.38±0.03	12.98	<.0001	-0.02±0.02	-0.69	**0.4953**
M2 vs. K2	0.19±0.03	5.54	<.0001	0.19±0.03	5.89	<.0001	0.32±0.02	15.18	<.0001	0.10±0.02	4.55	<.0001
M3 vs. B1	0.07±0.04	1.74	**0.0896**	-0.11±0.04	-2.96	0.0051	-0.06±0.03	-1.92	**0.0624**	-0.29±0.03	-10.16	<.0001
M3 vs. K1	0.24±0.03	7.30	<.0001	0.25±0.03	8.09	<.0001	0.26±0.03	9.16	<.0001	0.02±0.03	0.76	**0.4509**
M3 vs. K2	0.11±0.03	3.97	0.0003	0.13±0.03	3.98	0.0003	0.20±0.03	7.58	<.0001	0.14±0.02	5.86	<.0001
B1 vs. K1	0.31±0.04	7.62	<.0001	0.15±0.03	4.97	<.0001	0.21±0.03	6.45	<.0001	-0.27±0.03	-9.01	<.0001
B1 vs. K2	0.18±0.04	4.91	<.0001	0.03±0.03	1.10	**0.2768**	0.14±0.02	5.86	<.0001	-0.15±0.02	-7.13	<.0001

4.1.2 Pearson's correlation

4.1.2.1 Correlation between assessors, replicates for the four fins

Almost all the correlation coefficients shown in table 5 were numerically greater than 0.6, except for the few correlations between K1 and M, which were relatively low with the values below 0.5 (bold markers in table 5). The first two replicates made by assessor M (M1 and M2) were made within two hours on the same day (19th Dec, 2011) showed "almost perfect" correlations for all four fins (r=0.94, 0.91, 0.88 and 0.89, respectively). However, the correlations between M3, which was done approximately 2 weeks later (4th Jan, 2012), and the previous two replicates (M1 and M2) were numerically reduced (r=0.60-0.89).

4.1.2.2 Mean correlation coefficients for all four fins

The mean correlation coefficient refers to the overall mean of the correlation coefficients across all the 15 correlation coefficients within each fin (table 5).

For example:

For Fin1, $\bar{r}$ = (0.94+0.61+0.63+0.77+0.81+0.62+0.45+0.50+0.50+0.54+0.71+0.73+ 0.63+0.65+0.57)/15 = 0.68

The mean correlation coefficients tended to increase from Fin1 to Fin4 ($\bar{r}$ = 0.68, 0.71, 0.79 and 0.81, respectively; fig. 8).

Table 5. Pearson's correlation in fin length measurements between assessors and between replicates within same assessor across two tanks (N = 42) as well as across all measurements of four fins (N = 168). Fin 1 to 4 refers to the 1st dorsal fin, the 2nd dorsal fin, the 3rd dorsal fin and the caudal fin, respectively. The abbreviations of the assessors with replicates are listed in table 1. The correlation coefficients equal or lower than 0.5 were bold marked.

Assessor × rep	M2	M3	B1	K1	K2
			Fin1		
M1	0.94	0.61	0.77	**0.45**	0.71
M2		0.63	0.81	**0.50**	0.73
M3			0.62	**0.50**	0.63
B1				0.54	0.65
K1					0.57
			Fin2		
M1	0.91	0.60	0.68	**0.50**	0.72
M2		0.65	0.67	**0.46**	0.64
M3			0.55	0.65	0.52
B1				0.73	0.78
K1					0.69
			Fin3		
M1	0.88	0.74	0.76	0.63	0.80
M2		0.82	0.75	0.71	0.84
M3			0.70	0.75	0.78
B1				0.63	0.76
K1					0.76
			Fin4		
M1	0.89	0.83	0.76	0.75	0.80
M2		0.89	0.73	0.81	0.79
M3			0.65	0.76	0.77
B1				0.67	0.82
K1					0.72

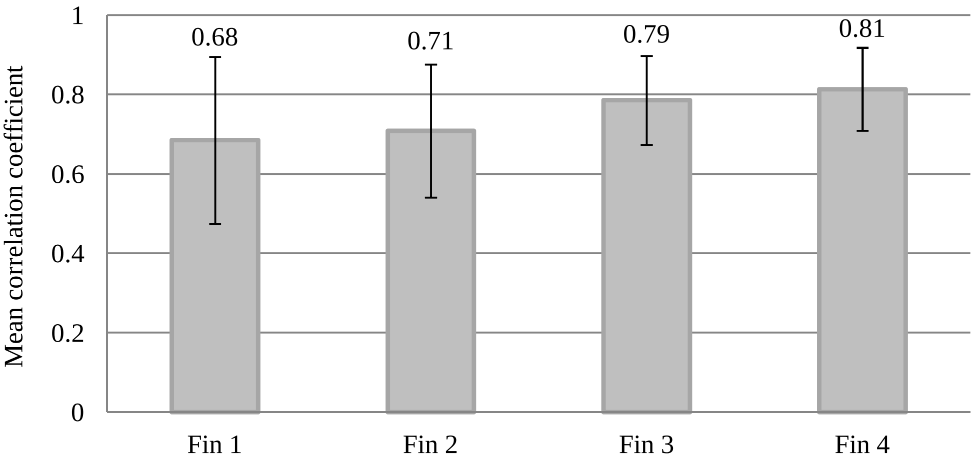


Figure 8. The mean correlation coefficient for each of the four fins. Bar height represents the mean, with error bars indicating the standard deviation (s.d.). Fin 1 to 4 refers to the 1st dorsal fin, the 2nd dorsal fin, the 3rd dorsal fin and the caudal fin, respectively.

4.1.3 ANOVA-Variance Components

4.1.3.1 Significance tests

The GLM statement was used to look at the fixed effects to see which factors were significant. Firstly, assessor effect, fish effects and the interaction between them were estimated. Significant differences in fish and assessor effects were observed (P<0.0001; table 6), but no significant difference was found in the interaction between fish and assessor (*P*=0.3002; table 6). Therefore, the interaction between assessor and fish was not included into the mixed models for calculating variance components.

Table 6. Significance tests for fixed effects, including assessor effect, fish effect and the interaction between fish and assessor. Significance level was set at 5%.

Source	DF	MS	F-value	*P*
Assessor	2	1.9015	58.18	<.0001
Fish	41	0.3234	9.90	<.0001
Assessor*fish	82	0.3620	1.11	0.3002

The interaction between assessor and repetition was included replacing the interaction between assessor and fish in the second significance test. All three factors were significant (P<0.0001; table 7), which would be taken into account in the mixed models for variance estimation.

Table 7. Significance tests for fixed effects, including assessor effect, fish effect and the interaction between assessor and replicate. Significance level was set at 5%.

Source	DF	MS	F-value	P
Assessor	2	1.9015	65.26	<.0001
Fish	41	0.3234	11.10	<.0001
Assessor*rep	3	0.3710	12.73	<.0001

4.1.3.2 Analysis of variance

When the mean squares were equated to their expectations, the estimated variance components were as follows (Fin1 in model 1 as example; table 8):

From the residual mean square, $\sigma_{e_1}^2 = 0.0519$ (residual error);

$6\sigma_{f_1}^2 = 0.3235 - 0.0519$, $\sigma_{f_1}^2 = 0.045$ (variance due to fish effect);

$\sigma_{T_1}^2 = \sigma_{f_1}^2 + \sigma_{e_1}^2 = 0.097$ (total variance)

Table 8. Analysis of variance and the mean square expectations for Fin1in Model 1.

Source of variation	DF	SS	MS	Expected MS	F-value	P
Total	251	24.1510				
Fish	41	13.2616	0.3235	Var(Error) + 6Var(fish)	6.24	<.0001
Residual	210	10.8893	0.0519	Var(Error)		

Model 1: *fish effect was included as random effect*

As shown in table 9, the intraclass correlations of Fin1 and Fin3 were close (0.464 and 0.468). Compared to Fin1 and 3, Fin2 showed higher intraclass correlation coefficient (0.482). The intraclass correlation of Fin4 was the highest among the four fins (0.607).

Table 9. Variance components of all the assessor measurements across two tanks for all four fins and the ICCs of the fin length measurements estimated using Model 1(N = 252). Fin 1 to 4 refers to the 1st dorsal fin, the 2nd dorsal fin, the 3rd dorsal fin and the caudal fin, respectively.

Fin	Variance due to [a]			Percentage of total variance due to (%)		R [b]
	Fish ($\sigma^2_{f_1}$)	Error ($\sigma^2_{e_1}$)	Total($\sigma^2_{T_1}$)	Fish	Error	
Fin1	0.045	0.052	0.097	46.4	53.6	0.464
Fin2	0.040	0.043	0.083	48.2	51.8	0.482
Fin3	0.037	0.042	0.079	46.8	53.2	0.468
Fin4	0.037	0.024	0.061	60.7	39.3	0.607

[a] $\sigma^2_{f_1}$ = variance due to fish; $\sigma^2_{e_1}$ = residual variance; $\sigma^2_{T_1}$ = total variance

[b] R refers to the intraclass correlation of reliability for each fin in Model 1.

R = fish variance ($\sigma^2_{f_1}$) ÷ total variance ($\sigma^2_{T_1}$).

***Model 2:** Both fish and assessor effect were included as random effects*

As shown in table 10, the variance due to assessor effect (0.021, 0.017, 0.026 and 0.020 for the four fins, respectively) was much lower than the variance due to fish effect (0.048, 0.043, 0.041 and 0.039 for the four fins, respectively). However, the assessor effect was indicated significant for all four fins through the likelihood ratio testing between model 2 and model 1 ($P<0.0001$).

Table 10. Variance components of all the assessor measurements across two tanks for all four fins estimated using Model 2(N = 252). Fin 1 to 4 refers to the 1st dorsal fin, the 2nd dorsal fin, the 3rd dorsal fin and the caudal fin, respectively.

Fin	Variance due to				Percentage of total variance due to (%)		
	Assessor (σ^2_a) [a]	Fish ($\sigma^2_{f_2}$)	Error ($\sigma^2_{e_2}$)	Total ($\sigma^2_{T_2}$)	Assessor	Fish	Error
Fin1	0.021	0.048	0.034	0.103	20.4	46.6	33.0
Fin2	0.017	0.043	0.027	0.087	19.5	49.5	31.0
Fin3	0.026	0.041	0.017	0.084	31.0	48.8	20.2
Fin4	0.020	0.039	0.013	0.072	27.8	54.2	18.0

[a] σ^2_a = variance due to assessors

***Model 3:** Fish effect, interaction between assessor and repetition were included as random effects*

As shown in table 11, the percentage of the total variance due to the effect of assessor with replications (22.8, 23.0, 32.5 and 20.3% for the four fins, respectively) was much smaller than the proportion of the total variance due to fish effects (48.5, 49.4, 50.6 and 60.9% for the four fins, respectively). However, the assessor*rep effect was proved significant for all four fins through the likelihood ratio testing between model 3 and model 1(P<0.0001).

Table 11. Variance components of all assessor measurements across two tanks for Fin1, 2, 3 and 4 (N = 252) estimated using Model 3. Fin 1 to 4 refers to the 1st dorsal fin, the 2nd dorsal fin, the 3rd dorsal fin and the caudal fin, respectively.

Fin	Variance due to				Percentage of total variance due to (%)		
	Assessor*rep ($\sigma^2_{a\times r}$)[a]	Fish ($\sigma^2_{f_3}$)	Error ($\sigma^2_{e_3}$)	Total[2] ($\sigma^2_{T_3}$)	Assessor *rep	Fish	Error
Fin1	0.023	0.049	0.029	0.101	22.8	48.5	28.7
Fin2	0.020	0.043	0.024	0.087	23.0	49.4	27.6
Fin3	0.027	0.042	0.014	0.083	32.5	50.6	16.9
Fin4	0.013	0.039	0.012	0.064	20.3	60.9	18.8

[a] $\sigma^2_{a\times r}$ = variance due to the interaction between assessor and repetition

***Model 4:** Assessor effect was designated as fixed effect, and the fish effect and the interaction between assessor and repetition were included as random effects*

The variance components due to assessor*rep (0.0023-0.0081; table 12) in this model markedly decreased compared to the corresponding variance components in model 3 (0.013-0.027; table 11). Tendencies towards an increase in the intraclass correlation coefficients of reliability from Fin1 to 4 were found in both model 1 and 4, whereas the intraclass correlation coefficient of reliability for each of the four fins in model 4 (0.569, 0.597, 0.688 and 0.732 for the four fins, respectively; table 12) was detected higher than that in model 3 (0.464, 0.482, 0.468 and 0.607 for the four fins, respectively; table 9). Moreover, systematic error due to assessor bias was observed on both Fin3 and Fin4 (*P* = 0.0347 and 0.0419 for Fin3 and 4, respectively; table 13).

Table 12. Variance components of all assessor measurements across two tanks for Fin1, 2, 3 and 4 (N = 252) estimated using Model 4. Fin 1 to 4 refers to the 1st dorsal fin, the 2nd dorsal fin, the 3rd dorsal fin and the caudal fin, respectively.

Fin	Variance due to				Percentage of total variance due to (%)			R'[a]
	Assessor*rep ($\sigma^2_{(a\times r)'}$)	Fish ($\sigma^2_{f_4}$)	Error ($\sigma^2_{e_4}$)	Total^2 ($\sigma^2_{T_4}$)	Assessors *rep	Fish	Error	
Fin1	0.0081	0.049	0.029	0.086	9.4	56.9	33.8	0.569
Fin2	0.0049	0.043	0.024	0.072	6.8	59.7	33.2	0.597
Fin3	0.0045	0.042	0.014	0.061	7.5	68.8	23.7	0.688
Fin4	0.0023	0.039	0.012	0.053	4.3	73.2	22.0	0.732

[a] R' refers to the intraclass correlation of reliability for each fin in Model 4.

R' = fish variance ($\sigma^2_{f_4}$) ÷ total variance ($\sigma^2_{T_4}$)

Table 13. The least square means of the fin lengths analyzed by the three assessors for all four fins across two tanks (N=42). Significance level was set at 5%. *P*-value equals to or lower than 0.5 is indicated by a *. Fin 1 to 4 refers to the 1st dorsal fin, the 2nd dorsal fin, the 3rd dorsal fin and the caudal fin, respectively.

	Least square means			P-value
	Assessor M	Assessor B	Assessor K	
Fin1	1.67	1.65	1.41	0.1077
Fin2	1.64	1.47	1.38	0.0662
Fin3	1.89	1.75	1.57	0.0347*
Fin4	2.70	2.43	2.64	0.0419*

4.2 Morphological changes of the four fins during the experiment (2100 fish)

The status of the fin situation on each fin prior to the experiment was not considered. As shown in table 14, the body length increased during the experiment (15.4 to 18.4cm), and the increase of fin lengths was proportional to the body length with the caudal fin having the highest increase (2.34 to 2.80cm). In addition, the length of Fin4 seemed to be longer than the other three dorsal fins, which was observed on all three recordings.

Table 14. Mean body length and weight, and the mean fin lengths for the four fins analyzed at the three recordings (2nd and 3rd November, 16th and 17th November and 14th and 16th December, respectively) during the experiment. All values were means (s.d.). Fin 1 to 4 refers to the 1st dorsal fin, the 2nd dorsal fin, the 3rd dorsal fin and the caudal fin, respectively.

	Body weight (g)	Body length (cm)	Fin1 (cm)	Fin 2 (cm)	Fin3 (cm)	Fin4 (cm)
Recording 1	34.6 (11.8)	15.4 (1.50)	1.38 (0.32)	1.33 (0.21)	1.49 (0.22)	2.34 (0.29)
Recording 2	42.2 (13.8)	16.2 (1.57)	1.53 (0.35)	1.44 (0.22)	1.61 (0.23)	2.42 (0.29)
Recording 3	63.5 (20.7)	18.4 (1.69)	1.58 (0.41)	1.56 (0.31)	1.77 (0.32)	2.80 (0.34)

In accordance with the data shown in table 3 obtained from the reliability test, some systematic errors from different assessors did occur during the measuring process. As shown in the “fin length” part of table 15, assessor M had a tendency to make higher measurements than the other two assessors for all four fins, and the measurements of B (1.55, 1.46 and 1.70, respectively) in the three dorsal fins were found to be higher than K (1.34, 1.35 and 1.48, respectively). Same tendencies were also found in the “relative fin length” part. However, the assessor bias did not significantly affect the accuracy of the mean length of the fin in the experiment, since the assessor arrangement for the overall measuring works did compensate well in terms of the combination of assessors that one had higher or lower measurement and the other(s) had the opposite one, see table 2.

The least square mean of the fin lengths increased during the experiment for all four fins (table 15). These were estimated using the GLM model for analyses 2 (eq. 10), which was consistent with the situation observed in table 14. When using relative fin length, the least square means of the percentage of fin length over total body length decreased for all four fins over the course of period 1 (2nd and 3rd November to 16th and 17th November) in the experiment (-0.12%, -0.19%, -0.39% and -0.53% for Fin1 to 4, respectively). In the following four weeks (16th and 17th November to 14th and

16th December), the least square means of relative fin lengths of the second and the third dorsal fins as well as the caudal fin increased (0.01%, 0.54% and 0.31% for Fin2 to 4, respectively), except for the first dorsal fin (-0.09%).

Table 15. The least square means of fin lengths and relative fin lengths [relative fin length (%) = (fin length×100)/total body length] obtained from the three assessors at three points of time (2nd and 3rd November, 16th and 17th November and 14th and 16th December, respectively) during the experiment for all four fins. Fin 1 to 4 refers to the 1st dorsal fin, the 2nd dorsal fin, the 3rd dorsal fin and the caudal fin, respectively.

	Least square means			
	Fin1	**Fin2**	**Fin3**	**Fin4**
	Fin length (cm)			
Assessor M	1.67	1.58	1.77	2.58
Assessor B	1.55	1.46	1.70	2.50
Assessor K	1.34	1.35	1.48	2.50
Recording 1	1.43	1.37	1.53	2.37
Recording 2	1.47	1.41	1.55	2.42
Recording 3	1.67	1.61	1.86	2.80
	Relative fin length (%)			
Assessor M	10.02	9.51	10.62	15.66
Assessor B	9.31	8.75	10.15	14.97
Assessor K	8.22	8.19	8.93	15.06
Recording 1	9.29	8.94	9.94	15.48
Recording 2	9.17	8.75	9.61	14.95
Recording 3	9.08	8.76	10.15	15.26

5 DISCUSSION

The discussion section is mainly divided into the following two sections. Firstly is a discussion of the feasibility to analyze fin length using digital image. Secondly, the study of the morphological changes of different fins during the experiment in farmed cod is discussed.

5.1 The feasibility to analyze fin length using digital image

In this study, fin length was measured with digital image analysis. It is an elaborate method and has been developed and used in fisheries research (Blonk et al. 2010; Hsieh et al. 2011) due to its advantages such as it is time efficient. In the present study, the assessors measured the fin length by marking two points of each fin on the image from base side of the fish to the outer end of the fin using cursor locating with mouse clicks. This method is the same as that used in the software *AnalyzingDigitalImages* for measuring the size of leaf (Pickle 2008). To provide an indication of reliability of digital image analysis and of effects of assessor, the images of 42 fish from two tanks were repeatedly analyzed by three different assessors. Various indices and coefficients were used in the study, since there is no general agreement about the rigorous statistical approach for the reliability test (Rosati et al. 2004).

5.1.1 Statistical parameters

As mentioned in the background, the repeatability of measurements in repeated trials on the same subjects can be assessed by the reliability test (Hopkins 2000). In present study, there were positive correlations between assessors (r=0.45-0.84) and between replicates within assessor (r=0.57-0.94) across all four fins. This indicates that there are strong correlations between replicates within assessor, but the correlations between different assessors are moderate. In addition, the correlations between the replicates obtained from assessor (M) made on the same day (with two hours interval) were observed to be significantly higher than that made on different two days (with two weeks interval). It is possible that the effects of memory are more pronounced if two measures are made within a short interval compared to measurements done far apart. Further, it is notable that there was an interval of almost two years between K1 (autumn 2010) and K2 (Jan 2012), but the correlations between this two replicates obtained from assessor K were found at moderate level. This indicates that the fin lengths analyzed by different assessors using digital image are consistent and repeatable.

Further we ran mixed model ANOVA to estimate variance components of the effects in the models to calculate the intraclass correlation coefficient (ICC). Intraclass correlation coefficient is another powerful and preferable parameter used to estimate the repeatability, especially when more than two tests are compared (Wong & McGraw 1996). Generally, the intraclass correlation coefficient was classified as follows: 0-0.20, “Slight”; 0.21-0.40, “Fair”; 0.41-0.60, “Moderate”; 0.61-0.80, “Substantial”; and >0.80, “Almost perfect”(Kho et al. 2008).

Model 1 only included fish effect as the random effect, and the variance due to assessor effect was then included into the error. Thus, the intraclass correlation coefficient of the first model does not yield information about differences among assessors but the accuracy of the measurement process. As shown in table 9, there was moderate level of repeatability (R=0.46-0.61) between the measurements. From a genetic analysis point of view, it also indicates that the maximum heritability of the trait fin length is moderate. As presented by Whitlock (1996) “the maximum heritability estimate possible from a single measure is the repeatability”. In addition, the repeatability obtained in our study is high, when compared with the results presented in the clinical trials. For example, the repeatability for pathological diagnosis between trained pathologists ranged from 0.38 (fair) to a high of 0.43 (moderate) (Nicholson 2004). Therefore, it is justified to say that this digital image based method to record fin length is reproducible.

In model 2 to 4, the variance due to assessor effect was estimated in different ways. Because the assessors make uneven number of measurements (M: three times; K: twice; B: once), which makes it incorrect to assess the variance due to assessor replicates directly from the residual error (Steiner et al. 2003). For this reason the variable called assessor*rep was used in model 3. In model 4, the assessor effect was included as the fixed effect to see the differences of measurements between assessors. The variance due to assessor*rep decreased sharply in model 4 after getting rid of the systematic error from different assessors, compared with that in Model 3. This implies that most of the variation due to assessor effect derives from the inter-assessor variances, and using different assessors may increase the variation. Yet the assessor variances were much lower than the variance due to fish effect in the three models (the former was roughly equal to or lower half of the latter). The dominant source of variation therefore was shown to be attributed to the fish, even though all the effects included in the models were reported to be significant ($P<0.0001$). This indicates that means of a large number of analyses made by many different assessors will not differ a lot (Kazmierczak et al. 2006).

In this study, correlations of fin length measurements across assessors and replicates were generally high and the repeatability for digital image analysis was estimated on 0.46-0.61 providing consistency of the measurement between and within assessors. Majority of the variation was attributed to the fish effect. However, results showed a significant effect of assessor ($P<0.0001$). This coincides with another study performed on common sole, *Solea solea* (Blonk et al. 2010).

5.1.2 Factors affecting the accuracy of the measurements using digital image

One possibility of causing assessor bias is different understandings of fin length definition by different assessors, especially when judging the fins which are in a poor condition. In this study, fin length was measured from the base to the outer edge of each fin along with the fin ray. The assessor use cursor positioning and mouse clicks to locate the starting and the terminal point of each fin. Unfortunately, when the photo was taken, some dorsal fins (especially the first dorsal fin) were not fully open or had been seriously damaged when the photos were taken (Fig. 8). Hence, the click points of those fins could not be identified clearly and consistently by different assessors. In our study, all three assessors tended to have higher CV on the three dorsal fins than the caudal fin (table 3), and the mean correlation coefficient also found to be increasing from Fin1 to Fin4 (fig. 9). This indicates that the disagreement between assessors may increase when the complexity of the measurement increases.

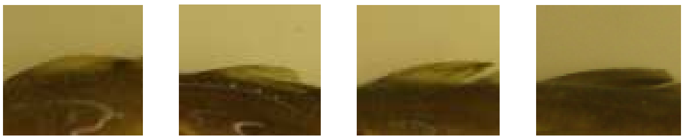
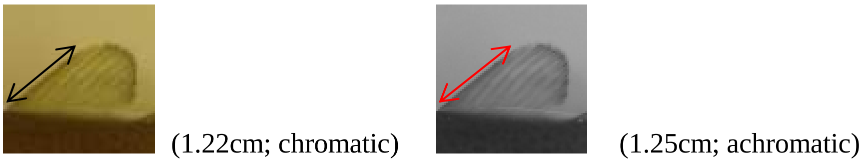


Figure 9. Images of first dorsal fin which are seriously damaged or not completely open.

In addition, a few factors may adversely affect the accuracy of measuring the length of fins. One undesirable factor is derived from the lack of ability of using chromatic diagrams analysis. Those original chromatic images automatically switched to grayscale after being read into the MATLAB workspace. Based on the feedback received from two of the assessors (B and M), they would often backtrack the original image to verify the position of fin ray especially for the first dorsal fin.



(1.22cm; chromatic) (1.25cm; achromatic)

Figure 10. Length of the same intact first dorsal fin analyzed using chromatic or achromatic image.

5.1.3 Benefits of digital image analysis

This digital image analysis is far more time efficient than traditional manual method. The assessors in our study could complete the analyses of the fin length of all four fins on an individual fish using digital image within one minute. In addition, the images can be easily stored in computer for later check or possible reassessment as we used in the current study during the fin length analyses. The procedure does not include manual records, therefore avoiding possible transcribing errors. What’s more the digital images can provide more information such as the deformity situation of each fish than just a single fin length message. From the economic point of view, an ordinary digital camera with high pixel resolution is inexpensive (price of US$399 for the digital camera used in this study) compared with the total fee for a long distance sampling trip.

5.1.4 Considerations for future improvements

There could be some improvements in accuracy of using digital image analysis to do fishery researches. In accordance with the suggestion by Chang et al. (2010), the assessors with more common trainings could provide more fin length estimations with high accuracy. The training should be based on more careful and precise definition of the analysis. Moreover as presented in Kazmierczak et al. (2006), the assessors should be more patience to backtrack the original pictures to make reassessment in case of uncertainty. In addition, we consider the advent of more sophisticated digital camera technology with a finer resolution (clear detection of the measuring points), developed user-friendly digital image analysis interface (chromatic image analysis ability) may also contribute to improve the accuracy of using digital image analysis.

5.2 Assessment of the morphological changes of fins

“Relative fin length” expressed as the ratio of fin length over total body length was used in our study to assess the degree of fin erosion. This ratio assumes that fin length grows in proportion to total body length in the absence of fin erosion, and Bosakowski and Wagner (1994) provided fin length analyses for wild rainbow trout in support of this assumption that the fin growth is isometric. Whereas, other studies were found to use standard body length replacing total body length to calculate relative fin length

(Ellis et al. 2009).

5.2.1 *Change of the fin lengths during the experiment*

Atlantic cod (*Gadus morhua*) are cannibalistic, and cannibalism seems to be most prevalent in larval and juvenile cod (Puvanendran et al. 2008). As mentioned before, high frequency of fin damage has been found on 55g juvenile cod (Hatlen et al. 2006). Cod with a mean weight of 34.6g introduced in the current study therefore may also be aggressive. The obtained results from our study showed that the increase of total length is accompanied by the increses of length of all four fins. Further, from the changes in relative fin length, we can see that all four fins were eroded during the first two weeks (recording 1-2) of the experiment with caudal fin exposing the most serious damaged condition (decreased 5.3%). It indicates that the susceptibility and mode of action for fin erosion is likely to vary between different fins, as previously reported in North et al. (2006). At the final recording on 14th to 16th December however, siginificant erosion was still found on the first dorsal fin compared with the other three fins (table 16). As significant fin erosion was confined to limited areas of the body (mainly the anterior dorsal part of fins), it is likely that the observed fin erosion in our study may partly result from the cannibalism of cod.

5.2.2 *What causes the fin damage – possible hypotheses*

Fin erosion resulting from aggressive conspecifics is well documented in fish species such as Atlantic salmon parr (Turnbull et al. 1996). Based on an earlier study, groups of fish tend to form dominance hierarchies after moving in a new stable environment, and then aggression tends to diminish when the hierarchy is established (Sloman et al. 2001). A group of cod were reported to be organized in dominance hierarchies (Brawn 1961). As mentioned before, all four fins were damaged in the first two weeks of the experiment (recording 1-2), and it is possible that individuals act aggressively fighting each other to establish their own hierarchies in the group at the beginning. At recording on 14th and 16th December (recording 3) the damages diminished and were found mainly on the anaterior part of the dorsal fins. I hypothesize that the fin damage during this period may still be caused by the cannibalism among the cod in the same tank, since the dorsal fin has shown to be a preferred site of attack by conspecifics in the same rearing unit (Turnbull et al. 1998). The third dorsal fin showed a remarkable high relative fin length (10.15%) which was higher than the value at stocking (9.94%), suggesting that there may be fin erosion existing prior to the experiment. Moreover, caudal fin length change leads to total body length change (Fig. 6), which then affects the relative fin length. This might be another reason to explain the strange increase of the relative fin length in the third dorsal fin. Therefore, I suggest that standard length (the length of the fish from the snout to the end of the fleshy part of the body; see fig 6) is probably more suitable than total body length in calculating relative fin length. Because the standard length has the characteristic of not being affected by the damages to the caudal fin. However I must emphasize that these are only hypotheses, and there may be many other reasons for the observed fin damage. Several physical and physiological factors are known to cause fin erosion, to understand more about the underlying triggers that may influence the fin erosion, more research is needed.

5.2.3 Discussion of methods for the assessment of fin erosion

Various methods can be used to describe or quantify fin erosion, as shown in Latremouille (2003). Monsen et al. (2010) assessed fin erosion of the fish in this experiment by subjective scoring fin loss on a scale from 0% fin erosion to 100% fin erosion in 5% interval on the first dorsal fin and the caudal fin. At recording 3, two fins were reported suffered 23.1% and 13.3% erosion, respectively. This is in line with the observations of more erosion in the first dorsal fin from our study. More advanced descriptive scales like photographic key has been used for visual assessment of fin erosion in adult cod (4 to 8kg) (Smith et al. 2009), see fig. 11. Photographs are taken of the interested fins from fish, representing the entire range of erosion (i.e., intact to absent fins). The images are then subjectively divided into several levels, and these levels are defined according to a certain standard. For example, as shown in fig. 11, these four point scales classify fins erosion as insignificant (score 0), moderate (score 1), significant (score 3) and severe (score 4), based on the area of the fin lost. Later, assessors assess the photographic key score for the fins subjectively. Hoyle et al. (2007) considered that this photographic key is visual and more informative for assessment of fish erosion, because photographic key shows extents of fin tissue loss instead of degrees of fin shortening. However, Person-Le Ruyet et al. (2007) thought that it is easier to determine the fin length that may be expressed as relative fin length of all fins than erosion levels. In my opinion, fin length (or relative fin length) is easier to determine, and it is less affected by assessors. As described in our study, by recording the maximum length of the fin rays, assessor bias is limited. However, photographic keys for assessing fin damage may be more informatics and convictive to be used as the indicator to assess fish welfare. Moreover, it is possible to do rapid descriptions of fin condition in the field using photographic key. Further experiment are required to analyze the fin condition using photographic key, which might be useful to get a more accurate estimation of fin damages of the fish in the experiment.

Atlantic Cod (*Gadus morhua*) Fin Erosion Key

1st Dorsal Fin	Caudal Fin	Pectoral Fins	Score	Description
			0	Insignificant 0-5% Loss
			1	Moderate 6-20% Loss
			2	Significant 21-50% Loss
			3	Severe 50+% Loss

Figure 11. Photographic key for assessing fin erosion in Atlantic cod (4 to 8kg) (Smith et al. 2009).

6 CONCLUSIONS

A computer based software program (MATLAB) to measure fin length of fish using digital image analysis technology was used in this study to assess the morphological changes of four different fins in Atlantic cod. To our knowledge, this is the first study to investigate the feasibility of implementing digital image based approach to analyze the length of fins. In our study, image analyses carried out by different persons on the same fins were coherent and the image analyses done by the same person was reliable. Repeatability for the measuring process varied from 0.46 to 0.61 for the four fins, proving satisfying consistency of the method within each assessor. Bias in estimation due to different assessors was observed in our study. However, to our knowledge, recent developments in digital image analysis program are unable to fully automate the analysis of the length of fish without introducing assessor effect. Therefore, we consider digital image analysis can be used as the measuring tool to accurately analyze the fin length of cod in this experiment. Digital image analysis technique has the potential to be included in the fishery researches, because it is time efficient and makes reassessment possible.

The method used in this study for quantifying fin damage is “relative fin length”, which reflects the degrees of fin shortening. The results from our study showed that the fin damage was observed on all four fins at the first two weeks (recording 1 to 2). Later until the end of the experiment, siginificant erosion was only found on the first dorsal fin compared with the other three fins. We further observed that the fin erosion mainly focused on specific part of the fish, which is the anterior dorsal fin. Therefore, I hypothesize that the obtained fin erosion might be close linked to the cannibalistic behavior of the juvenile cod. Fish fought each other to establish their own hierarchies at the beginning causing fin damages, and the aggressive behaviors tended to diminish after the establishment of the dominance hierarchies. In addition, because of the limitation of using fin length (or relative fin length) to assess fin erosion, I suggest that further research is required to analyze fin erosion using photographic key.

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