Reflex impairment as a measure of vitality and survival potential of Atlantic cod (*Gadus morhua*)

Odd-Børre Humborstad (contact author)[1]

Michael W. Davis[2]

Svein Løkkeborg[1]

E-mail address for contact author: oddb@imr.no

[1] Institute of Marine Research
Nordnesgaten 50
P.O. Box 1870
Nordnes, N-5817 Bergen, Norway

[2] NOAA Fisheries, Alaska Fisheries Science Center
Fisheries Behavioral Ecology Program
Hatfield Marine Science Center
Newport, Oregon 97365

Abstract—Assuring the vitality and survival potential of live-caught Atlantic cod (*Gadus morhua*) is important for improving the sorting of fish before net penning operations designed to hold fish for growth and later market. When Atlantic cod are captured by Danish seine, the most commonly used fishing gear for live-caught fish, they undergo stressors such as forced swimming, net abrasion, and air exposure. Laboratory experiments (at an air temperature of 9°C and water temperature of 8°C) were conducted with the aim of constructing a RAMP (reflex action mortality predictor) curve for prediction of vitality and survival potential in Atlantic cod captured in Danish seines, by varying the levels of these stressors. Atlantic cod exposed to increased duration in air (5–20 min) showed increased reflex impairment and mortality, with 75% mortality at 10 minutes of air exposure. Forced swimming in combination with net abrasion and air exposure did not increase reflex impairment or mortality above that associated with air exposure alone. The Atlantic cod RAMP curves indicated that fish with reflex impairment less than 50% would not show mortality and would likely recover from capture stress.

Manuscript submitted 29 October 2008.
Manuscript accepted 6 May 2009.
Fish. Bull. 107:395–402 (2009).

In capture-based aquaculture (CBA; Ottolenghi et al., 2004), fish are caught live and held in net pens to supply high-quality fresh fish throughout the year and thereby increase the value of the catch, given a fixed boat quota. Animal welfare issues in CBA arise when handling stress and adaptation costs to new environments are added to the capture stress. Also the duration of stress will increase dramatically compared to that of traditional commercial fishing where the human impacts on fish end with slaughtering immediately after capture. Improving fish welfare in CBA should be achieved by keeping and storing only vital specimens after a sorting process. Operational indicators of vitality and survival potential are therefore needed.

Current sorting criteria include removing specimens showing any visual sign of damage such as injuries from net abrasion and mechanical handling. Also specimens having clear signs of barotrauma such as gas filled eyes (exophthalmia) or overinflated abdomens due to punctured gasbladders are poor candidates for live storage and should be removed. This practice combined with the use of specialized transport tanks and net pens, and the implementation of careful handling routines and monitoring of water quality have greatly decreased instantaneous and postsorting mortality. Still some delayed mortality occurs in seemingly unharmed specimens because the internal status of the animal is not readily evaluated by visual inspection. Thus there is the potential for improving the sorting process before the transfer of fish to tanks or net pens by developing operational indicators of vitality and survival potential.

To date there are several physiological and behavioral indicators of fish condition available; however, they share the unfavorable attributes of being expensive and labor intensive and are therefore not suited for routine use in commercial fisheries. More importantly, although applicable for determining sublethal stress levels, they show a lack of concordance with mortality outcomes (Davis et al., 2001; Davis and Schreck, 2005). Recently, reflex impairment has been tested as an indicator of vitality and survival potential (Davis and Ottmar, 2006; Davis, 2007). Correlations between stressor intensity, reflex impairment, and increased mortality were found for several species of round fish and flat fish exposed to simulated capture stressors.

The main objective of this study was to test whether reflex impairment could be used as a rapid real-time operational indicator to predict vitality and survival outcomes in fishing operations designed to hold Atlantic cod (*Gadus morhua,* hereafter referred to as "cod") in net pens

Table 1

Reflex actions that were consistently present in 20 control (unstressed) Atlantic cod (*Gadus morhua*) and used to measure reflex impairment in fish exposed to stressors (forced swimming, net abrasion, and air exposure) associated with capture by Danish seines.

Reflex	Description
1. Body flexion 1	Fish flexed body when placed on a flat surface (sorting table).
2. Body flexion 2	Fish flexed when restrained.
3. Head complex	Restrained fish presented alternating mouth gaping and operculum flaring, as if gasping for air.
4. Operculum	Restrained fish either flared operculum rigidly open or strongly clamped operculum shut when operculum was lifted with a probe.
5. Gag	Restrained fish gaged when back of throat was stimulated with a probe.
6. Vestibular-ocular response (VOR)	Restrained fish tracked observer when it was rotated around long axis.
7. Tail flexion	Fish held under belly flexed when tail flanks were stroked by hand along long axis.

for additional growth and later marketing (CBA purposes). The Danish seine is the most commonly used fishing gear for CBA, and the stressors tested in this study, forced swimming, net abrasion, and air exposure, are typical in and inherent to this type of fishing gear. A series of experiments were conducted to construct a RAMP (Reflex Action Mortality Predictor) curve for cod exposed to these stressors and we broadly followed the three step procedures in Davis (in press) to validate reflex impairment as an indicator of vitality.

First we established appropriate procedures for testing fish reflexes by determining consistent reflexes for testing; we established the effect of repeated reflex testing in control fish to test whether cod were distressed by the testing procedure; we determined the recovery time after exposure to a stressor; and we determined the effect of disturbance from repeated netting of fish from a holding tank.

Second we established experimental methods for exposing fish to key stressors and conducted stress experiments to determine the effect of air exposure and the combined effect of forced swimming, net abrasion, and air exposure on reflexes and survival.

Third after observing immediate and delayed mortality, we correlated reflex impairment and mortality by producing RAMP curves for the effect of air exposure and the combined effect of forced swimming, net abrasion, and air exposure.

Materials and methods

Experimental fish

Cod were hatched and later reared in net pens at Austevoll Research Station (60°05′17″N 5°15′50″E), Austevoll, Norway. Five months prior to the experiments, fish were moved (after six months in net pens) to indoor ~1600-L green fiberglass tanks with rounded corners (145 cm × 145 cm × 100 cm depth, water depth 80 cm), supplied with aerated sea water (8.0 ±1.0°C), and fed standard commercial diets (Skretting Ltd, Stavanger, Norway) to satiation by using 24-hr disc feeders (1% of total biomass per day) under a continuous daylight regime. A total of 169 fish (31–49 cm total length [TL]) were tested for reflex impairment and mortality experiments. In order to maintain independence of observation no fish was used more than once.

Step 1: Consistent reflexes and appropriate procedures for reflex testing

A number of reflex actions in cod for potential inclusion in this study were identified based on previous work (e.g., Davis, 2007) and preliminary trial and error screening on fishing vessels and in the laboratory. In order to identify the reflexes for testing and to standardize methods and stimuli to elicit these reflex actions, twenty cod were initially tested. Seven reflexes were found to be consistently present in control fish (Table 1). For subsequent reflex impairment tests, the reflexes were scored as 1 (present) if a vigorous response was evident, or 0 if the response was not present or of weak or questionable strength. Reflex scoring insured consistent, high quality response data (Davis, in press).

To test reflex actions, fish were initially placed on a flat surface, corresponding to a sorting table on a fishing vessel and observed for spontaneous body flexing. Then fish were placed and held in a restraining device (Fig. 1) consisting of two plastic pieces (60 cm length × 10 cm width), each lined with a sponge to contact and hold fish, and connected at one end to form a hinge, while the other end was secured with a Velcro strap. Fish were then observed for body flexion in response to restraint. The restrained fish were then tested for reflexive head movements (a spontaneous alternating head and operculum movement), operculum movement (either tight closure or flaring after having been lifted with a probe), gag in response to throat stimulation, and vestibular-ocular response (eye rotation while the fish was rotated around its long axis). Finally fish were released from restraint and tested for body flexion in

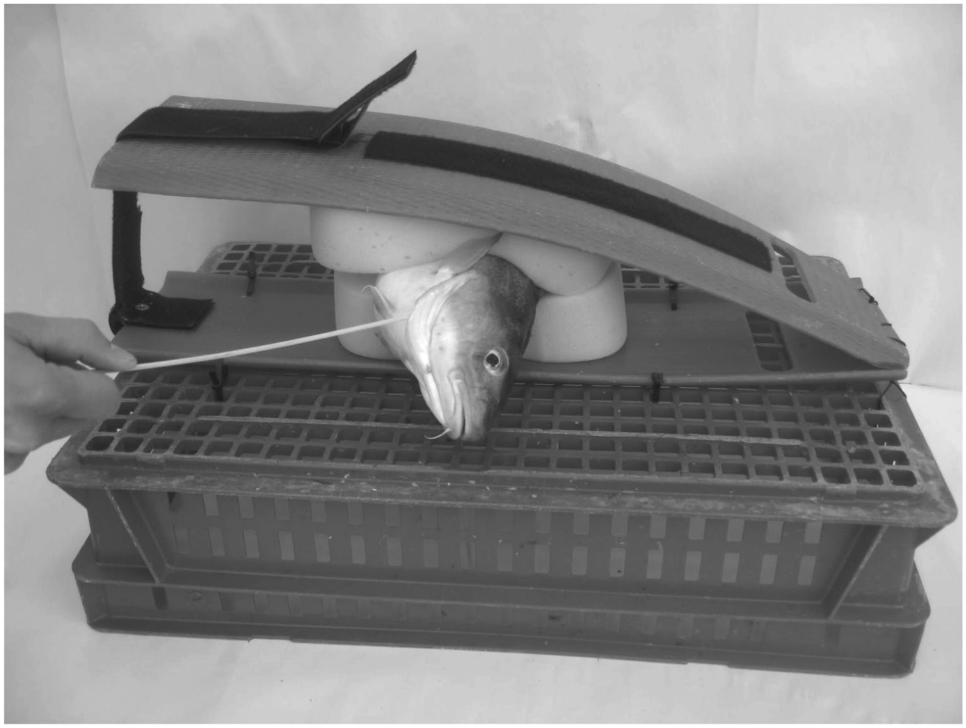

Figure 1
Fish restraining device used to test reflex actions in Atlantic cod (*Gadus morhua*). In this photograph, the fish is being tested for operculum reflex action.

response to hand stroking of the tail flanks along the long axis while being held under the abdomen. The entire sequence for reflex testing in a replicate fish was conducted within 60 seconds.

Repeated reflex testing was conducted with a group of ten fish to determine if cod were stressed by the reflex testing procedure itself. These fish were netted, restrained, and their reflexes were tested at 0, 60, and 180 minutes after the initial netting and restraint. Between each sampling time, fish were placed back into a tank with sea water and recaptured for the next reflex test.

The effect of recovery time (0–15 min) on reflexes after exposure of fish to stressors was tested to determine an appropriate time to sample cod reflexes. Replicate fish (n=35) were exposed to standard stressors (see detailed description of stressor administrations below in step 2) of 5 minutes of swimming, 1 minute of net abrasion, and 5 minutes in air (all at 9.0°C). Then fish were returned to the stock tank and reflex testing was begun at 0, 5, 7, 10, or 15 minutes after exposure to stressors and completed within 8 minutes from starting time of testing for each exposure group (n=7 per group).

It was possible that disturbance from repeated netting of fish from a stock tank before netting replicate fish for stressor treatment could have affected the level of reflex impairment resulting from exposure to stressors, because fish could have been repeatedly stressed by being chased before capture. To test this possibility, fish were exposed to 5 minutes of swimming, 1 minute of net abrasion, and 10 minutes of air exposure (all at 9.0°C) after being either captured from a tank (n=8) in which four fish had been previously captured or from a tank (n=8) in which 16 fish had been previously captured. Then fish were returned to the tanks and reflex testing began 5 minutes after exposure to stressors and was completed within 9 minutes from the start of testing for each exposure group. Fish were then held to determine mortality. Immediate and delayed mortality resulting from stressors was observed by holding fish in tanks for 60 days and removing and counting dead fish daily (mortality observed as common death signs, e.g., motionlessness, static flaring of operculum, nonresponsiveness to touch stimuli, *rigor mortis*). The rearing regime for the stressed fish was similar to stock tank fish.

Step 2: Stressors, exposure experiments, and stress induction

Stressors were selected to mimic the stressors cod experience during capture during Danish seine operations and to create a gradient of stress from low to moderate to lethal. Exposure to air was chosen for its known importance as a stressor for fish sorted on deck. Forced swimming is a relevant capture stressor. Net abrasion is important during haulback operations and transfer of fish from the seine to the boat in CBA. Moderate intensity of abrasion was administered in the experiment because these injuries are likely to occur during sorting and may increase the risk for infection, disease, and delayed mortality. Fish exposed to these stressors (air exposure, forced swimming, moderate net abrasion) may not have obvious external injury and the effects of such stressors may be difficult to assess by visual inspection during sorting. Moreover fish experiencing different levels of such stressors may differ in internal status and survival potential.

Exposure to air was caused by placing fish in a tank without water to simulate conditions that would occur during sorting on deck (on the sorting table) after capture, and the intensity of the stressor was controlled by altering exposure duration. The swimming stressor was created by forcing fish to swim against a current created by lowering the water level in a tank (145 cm × 145 cm × 8 cm water depth) in which water was introduced along the tank wall to cause a circular current of approximately 20 cm/s. This stressor intensity was varied by controlling the amount of time that the fish was forced to swim against the current. Net abrasion was caused by capturing the fish in a net and sloshing them up and down at the water surface in a tank for a fixed time of one minute. After administration of stressors the fish were placed into a stock tank, and one individual at a time was taken out for reflex testing.

The effect of exposure to air alone was tested in cod (n=32) exposed to either 5, 7, 10, or 20 minutes of air (all at 9.0°C). After air exposure, fish were placed into a stock tank and then individuals were taken out for reflex testing. Testing began 5 minutes after the exposure to air ended and was completed within 9 minutes from the start of testing for each exposure group (n=8 per group). Fish were then held to determine mortality.

The effect of exposure to seven combinations of swimming, net abrasion, and air was determined to simulate stressors associated with capture by Danish seine. Replicate fish (n=56) were exposed to swimming for 5, 10, or 15 minutes, net abrasion for 1 minute, and air for 5, 10, or 15 minutes (all at 9.0°C). Then fish were returned to tanks and reflex testing began 5 minutes after exposure to stressors and was completed within 9 minutes from the start of testing for each exposure group (n=8 per group). Fish were then held to observe mortality.

Step 3: RAMP curves and statistics

The proportion of reflex impairment in each fish was calculated as follows: 1 – (the sum of individual reflex scores/the total possible score of 7), i.e,. the proportion of reflex impairment was 2/7 if five out of the seven reflex actions (see Table 1) were present. Mortality for individual fish was scored as 1 (present) or 0 (absent) and the mortality date was noted. Statistical significance of correlation between reflex impairment and mortality was tested by using Spearman rank correlation. Sigmoid curves (RAMP curves) showing relationships between reflex impairment and mortality were fitted by using SigmaPlot vers. 10.0 (Systat Software, Inc., Chicago, IL). When control fish reflexes were sampled repeatedly, the effects on reflex impairment were tested with Friedman analysis of variance (ANOVA), whereas the effects of sampling time, air exposure time, forced swimming, net abrasion, and air exposure were tested with Kruskal-Wallis ANOVA. The effect of disturbance on reflexes was tested with a two sample t-test. Statistical significance was accepted at $P \leq 0.05$.

Results

Step 1: Appropriate procedures for reflex testing

Reflexes in control (unstressed) cod were not impaired by repeated testing at 0, 60, and 180 minutes after initial capture from a stock tank (ANOVA, $F_{2,9}$=0.64, P=0.728), indicating that cod were not stressed by the reflex-testing procedure itself. Mean reflex impairment (proportion) for each testing period ranged from 0.03 to 0.06 and no mortality occurred.

Reflex impairment occurred immediately after exposure to 5 minutes of swimming, 1 minute of net abrasion, and 5 minutes of air exposure and was not significantly changed (mean ±1 standard error) by the time of sampling when sampled at 0 (0.71 ±0.08), 5 (0.65 ±0.03), 7 (0.61 ±0.05), 10 (0.50 ±0.07), or 15 minutes (0.51 ±0.07) after exposure to stressors (ANOVA, F_{34}=7.92, P=0.095). These results indicate that reflex actions are real-time indicators of stress, and recovery does not happen within the first 15 minutes after exposure to stressors.

Repeatedly disturbing fish (16 times) in a stock tank by chasing and netting fish out of the tank before netting fish for stressor treatment did not result in increased mean (±1 standard error) reflex impairment (0.86 ±0.08) or mortality (0.75 ±0.16) after the fish were subjected to 5 minutes of swimming, 1 minute of net abrasion, and 10 minutes of air exposure when compared to repeatedly disturbing fish (4 times) (reflex impairment=0.82 ±0.08; mortality=0.50 ±0.19), after the fish were exposed to the same stressors (reflex impairment t-test, t_{14}=0.32, P=0.751; mortality t-test, t_{14}=1.00, P=0.334).

Step 2: Exposure experiments

Cod exposed to increased duration in air (5, 7, 10, and 20 minutes) showed increased reflex impairment (ANOVA, F_{31}=17.11, P < 0.001; Fig. 2). Mortality also increased with increased air exposure (ANOVA, F_{31}=17.87, P<0.001; Fig. 2). Air exposure was an important stressor and mortality increased rapidly as the duration of time that fish were exposed to air increased, and 75% mortality was observed at 10 minutes of air exposure. At 20 minutes of air exposure 100% mortality was observed. No immediate mortality was observed from any of the air exposure experiments, whereas 94% mortality (delayed mortality) occurred within 1 day (16 fish) and one fish died after 6 days.

Forcing cod to swim for 5, 10, or 15 minutes at 20 cm/s in combination with net abrasion and air exposure did not increase reflex impairment or mortality above that associated with air exposure alone. Reflex impairment increased with increased exposure to a combination of swimming, net abrasion, and air, and this effect appeared to be associated primarily with increased duration in air (ANOVA, F_{55}=28.82, P<0.001; Fig. 3). Likewise, mortality increased with increased exposure to these stressors, and mortality appeared to be primarily associated with air exposure (ANOVA, F_{55}=25.15, P<0.001; Fig. 3). Cod injury from net abrasion was not obvious, other than the presence of sloughed scales on the net. No immediate mortality was observed from any of the combined stressors, whereas 94% of observed delayed mortality occurred within 1 day (15 fish) and one fish died after 14 days.

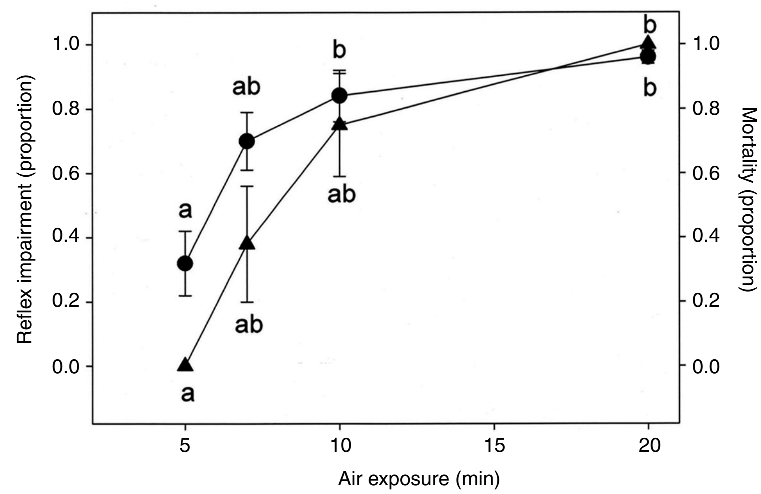

Figure 2

Atlantic cod (*Gadus morhua*) mean proportion of reflex impairment (●) and mortality (▲) for treatment groups (n=8 fish per treatment group) increased with increased duration (minutes) of exposure to air. There were two groups of mean (±1 standard error) values that were significantly different (indicated by a, b).

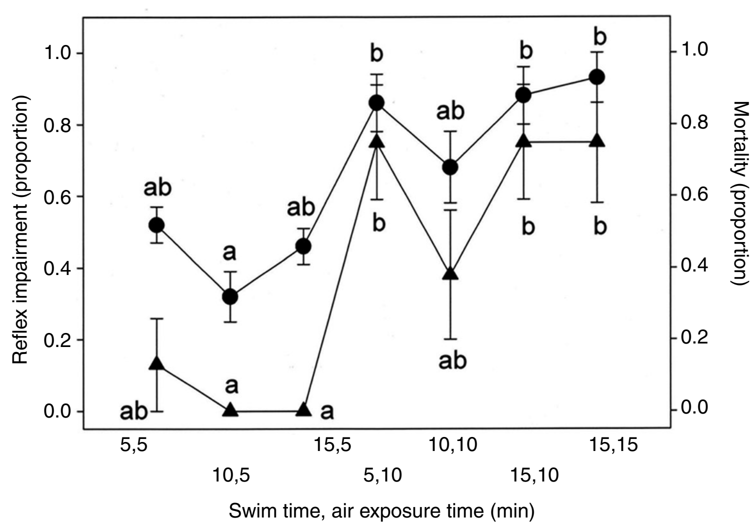

Figure 3

Atlantic cod (*Gadus morhua*) mean proportion of reflex impairment (●) and mortality (▲) for seven treatment groups (n=8 fish per treatment group) increased with increased stressor intensity. Fish were exposed to combinations of swimming (5, 10, and 15 minutes), net abrasion (1 minute), and air (5, 10, and 15 minutes). There were two groups of mean (±1 standard error) values that were significantly different (indicated by a, b).

Step 3: RAMP curves

Reflex impairment and mortality were correlated for individual cod exposed to air (Spearman rank correlation=0.85, P<0.001, n=32) and when exposed to swimming, net abrasion, and air (Spearman rank correlation =0.85, P<0.001, n=64). The relationship between reflex impairment and mortality was sigmoid (RAMP curve), initially showing increasing reflex impairment with no mortality, followed by a sharp increase in mortality at reflex impairment values > 0.6 (Fig. 4A). The relationship between reflex impairment and mortality calculated for cod in groups defined by experimental treatments that combined swimming, net abrasion, and air exposure (Fig. 4B) showed a similar pattern to that shown by the RAMP curve for individual fish, indicating that reflex impairment could predict mortality in populations of cod, as well as in individual fish.

Discussion

Capture-based aquaculture (CBA) is a combination of capture by commercial fisheries and rearing by aquaculture. In this article we address key stressors associated with the capture portion of CBA. Stress and mortality in fish from commercial fisheries often result from several classes of interacting acute stressors. These classes include capture stressors (net entrainment, mesh passage, crushing, wounding, sustained swimming until exhaustion, and pressure changes), fishing conditions (towing time, light conditions, water and air temperatures, hypoxia, sea conditions, time on deck, and handling procedures), and biological attributes (behavior, size, and species) (Davis, 2002). This study demonstrated that reflex impairment can be used to assess vitality and survival potential of cod exposed to capture related stressors such as forced swimming, net abrasion, and air exposure. Cod showed reflex impairment immediately after exposure to stressors, suggesting that reflex actions are sensitive real time indicators that integrate neurological, hormonal, and behavioral states that can be related to changes in fish vitality and welfare (Davis, in press). Importantly, the testing of reflex actions did not cause reflex impairment, indicating that cod were not stressed by the procedure. Also, disturbances caused by repeated netting of fish from the holding tank did not affect the level of reflex impairment resulting from exposure to stressors.

The RAMP curves for individuals and groups indicated that cod with less than 50% reflex impairment would not die and would likely recover from capture stress to resume normal feeding and growth. Similar correlations between stressor intensity, reflex impairment, and increased mortality were found for several species of roundfish and flatfish exposed to simulated capture stressors (Davis and Ottmar, 2006; Davis, 2007). Future measurements of reflex impairment and mortality in cod caught by Danish seine and held in net pens can be used to validate the laboratory RAMP curves for use in CBA operations. Experiments could be designed to expose cod to gradients of capture-related stressors in the field, to test for reflex impairment, and then to hold fish in the field in order to determine delayed mortality. Reflex impairment and mortality results from field stressor experiments could be compared with mortality rates that were predicted with the laboratory RAMP curve for cod. Possible differences in prediction of mortality rates between laboratory- and field-derived RAMP curves may be evident as a shift in the relationship between reflex impairment and mortality. These difference would be likely caused by the addition of stressor types in Danish seine operations that were not included in the laboratory experiments. In general the types of stressors and not the intensity of stressors control the shape of the RAMP curve (Davis, in press). The RAMP curves could also be applied to estimate survival of escapees and discarded fish (e.g., Davis and Ottmar, 2006; Ingolfsson et al., 2007; Enever et al., 2009) and to improve selection of cod with high survival potential in tagging studies (Fowler and Stobo, 1999; Brattey and Cadigan, 2004). Also our result for time of delayed mortality is of interest in relation to tagging studies; because 94% of delayed mortality occurred within 24 hours, holding fish for one day before release should reduce tagging mortality to negligible levels.

Exposure to air should be avoided when possible for cod, but if inevitable, it should be kept below safe levels. Air exposure was the most important stressor associated with mortality in cod with 40% mortality observed at 7 minutes, 75% at 10 minutes, and 100% at 20 minutes (Fig. 2). However, during the reflex testing procedures, air exposure of one minute or less did not induce reflex impairment or mortality and thus represents a conservative, safe level of ex-

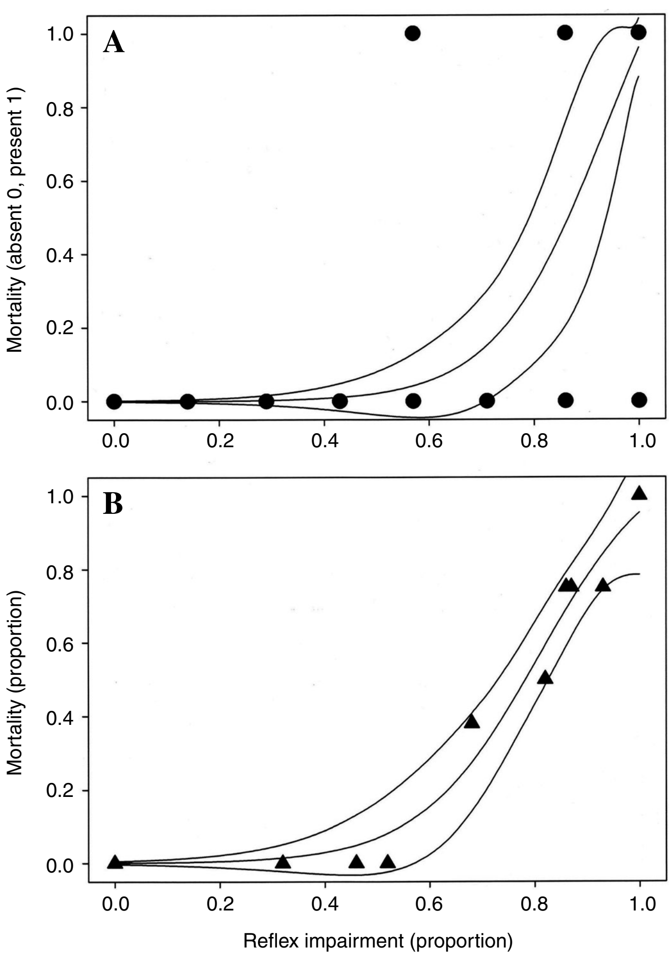

Figure 4

Reflex impairment (proportion) could predict (**A**) mortality (absent 0, present 1) in individual Atlantic cod (*Gadus morhua*), or (**B**) mean mortality (proportion) in populations of cod exposed to combinations of forced swimming, net abrasion, and air (see *Methods* section). For individual (●) cod in (A), the sigmoid curve (y=1.471/(1 + $e^{-(x-0.935/0.104)}$) ±95 % confidence intervals was significant (r^2=0.84, P < 0.001, n=64). Note that many points overlap. For populations (▲) of cod (B), defined by calculating mean proportions for reflex impairment and mortality in ten combination stressor treatment groups (including control and 100% mortality groups), the sigmoid curve (y=1.130/(1 + $e^{-(x-0.809/0.113)}$) ±95 % confidence intervals were significant (r^2=0.97, P<0.001, n=10). Note that two points overlap.

posure. Sensitivity to air exposure has been shown to vary among species, with mortality occurring at 7 to 45 minutes of exposure (Davis, 2002). Air exposure is often inevitable during sorting in live-capture operations, and duration in air can be reduced to safe levels, e.g., by the introduction of water-filled sorting tables.

Increased temperature is another stressor that has been associated with mortality in captured fish and may become important if cod are captured or sorted at temperatures above 12°C (Davis, 2002; Sartoris et al., 2003; Brattey and Cadigan, 2004; Suuronen et al., 2005). Increased temperature may also raise sensitivity to air exposure (Davis and Parker, 2004; Davis and Schreck, 2005). Temperatures in deep (200 m) and shallow (surface) water peak in July and August (at ~3 and ~8°C, respectively) in coastal areas suitable for CBA in northern Norway (Loeng, 1991). During spring months when capture for CBA purposes occurs, mixing in these areas is high, and temperatures remain around ~3°C throughout the water column and air temperature only rarely raises above 12°C in May (data from The Norwegian Meteorological Institute, Blindern, Oslo). Temperature should thus not be a critical factor at the temporal and spatial scales of current CBA practices in Norway, and accordingly we did not include temperature as a variable in our study. However attempts to develop CBA outside this temporal and spatial window (e.g., North Sea during summer months), warrant a high vigilance of temperature effects.

A likely effect of adding a stressor is that it may cause an increase in the overall stress response. However, forcing cod to swim for 5, 10, or 15 minutes at 20 cm/s in combination with net abrasion and air exposure did not increase reflex impairment or mortality above that associated with exposure to air alone. This result even indicated that swimming for 10 minutes may have enhanced resistance to air exposure, having a palliative effect on the induction of further stress. In a study of free swimming cod, oxygen consumption did not increase until fish were swimming at 30 cm/s or higher, indicating that at less than 30 cm/s cod would not be stressed (Claireaux et al., 1995). Although not directly relevant to this study, sustained slow swimming in rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*) has reduced the duration of recovery from exhaustive exercise (Farrell et al., 2001; Lee-Jenkins et al., 2007). Little is known about the palliative effects of low-intensity exercise in fish, either before or after exposure to stressors. Studies of the swimming performance of cod have been focused on measurements of endurance and burst swimming (40–130 cm/s) and have included an evaluation of changes in the scope for metabolic activity (Reidy et al., 2000). Further study of the interactive effects of low-intensity exercise, the perception of stressors by fish, and their management of stress may contribute to methods for the significant reduction of stress in captured, released, transported, and net-penned fish.

Injury to cod from net abrasion was not obvious, other than the presence of sloughed scales on the net, and did not appear to contribute to delayed mortality or reflex impairment. Fish with obvious damage to the skin, fin erosion (split fins), cataracts or opaque eyes, resulting from severe abrasion from net material will not pass the sorting procedure in CBA. Therefore a moderate intensity of abrasion was administered in this experiment because these injuries are not likely to be detected during sorting and may increase the risk for infection, disease, and delayed mortality. Such effects of moderate net abrasion were, however, not demonstrated in our experiment. Mortality rarely occurred in cod that were injured by net abrasion when escaping from a demersal trawl (Soldal et al., 1993). Injury to cod from fishing gear can occur, however; Baltic cod have been observed with a high incidence (48%) of skin infection probably associated with escape from fishing gear, but associated mortality was not determined (Mellergaard and Bagge, 1998). In other studies of net abrasion and consequent mortality, some species (herring, [*Clupea harengus*], and walleye Pollock [*Theragra chalcogramma*] were sensitive to net abrasion and showed delayed mortality associated with skin infection (Suuronen et al., 1996; Davis and Ottmar, 2006), whereas other species (sablefish [*Anoplopoma fimbria*] and Pacific halibut [*Hippoglossus stenolepis*] were more resistant and mortality was not correlated with skin abrasion (Davis and Ottmar, 2006).

Capture, transport, and holding of fish are often associated with induction of stress and reduction of vitality. Reflex impairment could be used to evaluate the role of different stressors at each stage of the live-fish fishery and to identify fish with the highest probability for survival. Successful live-fish capture, transport, and rearing operations should aim to minimize stress, optimize water quality, and minimize the increase of metabolic wastes (Huntingford et al., 2006; Ashley, 2007). Water quality and temperature must be controlled through inputs, tank and pen configurations, and flow rates. Food (e.g., Olsen et al., 2008) and stocking density (Staurnes et al., 1994) may be important factors because they control physiological and behavioral states of fish. The adjustments of wild fish to confinement and unnatural densities and how these short-term adjustments affect future performance and welfare are also important considerations. Reflex testing may be performed also in free-swimming fish (Davis and Ottmar, 2006; Stien et al., 2007), and monitoring reflex impairment in captive fish can be a rapid real-time method for identifying optimal transport and rearing conditions and for tracking recovery in cod after live capture.

Acknowledgments

The authors are grateful to Anders Mangor-Jensen for organizing and providing facilities and fish at the Austevoll Research Station in Norway, and for assistance during experiments. Shale Rosen is thanked for assistance carrying out experiments. Anne-Britt Skaar Tysseland is thanked for calculations of swimming speed and Ørjan Eknes is thanked for rearing our fish. The work was funded by the Norwegian Research Council (173193, Fish welfare in capture-based aquaculture and 173538, Physiological and behavioral indicators for fish welfare assessment in aquaculture and fish capture).

Literature cited

Ashley, P. J.
2007. Fish welfare: current issues in aquaculture. Appl. Anim. Behav. 104:199–235.

Brattey, J., and N. Cadigan.
2004. Estimation of short-term tagging mortality of adult Atlantic cod (*Gadus morhua*). Fish. Res. 66:223–233.

Claireaux, G., D. M. Webber, S. R. Kerr, and R. G. Boutilier.
1995. Physiology and behaviour of free-swimming Atlantic cod (*Gadus morhua*) facing fluctuating temperature conditions. J. Exp. Biol. 198:49–60.

Davis, M. W.
2002. Key principles for understanding fish bycatch discard mortality. Can. J. Fish. Aquat. Sci. 59:1834–1843.
2007. Simulated fishing experiments for predicting delayed mortality rates using reflex impairment in restrained fish. ICES J. Mar. Sci. 64:1535–1542.
In press. Fish stress and mortality can be predicted using reflex impairment. Fish and Fisheries.

Davis, M. W., B. L. Olla, and C. B. Schreck.
2001. Stress induced by hooking, net towing, elevated sea water temperature and air in sablefish: lack of concordance between mortality and physiological measures of stress. J. Fish Biol. 58:1–15.

Davis, M. W., and M. L. Ottmar.
2006. Wounding and reflex impairment may be predictors for mortality in discarded or escaped fish. Fish. Res. 82:1–6.

Davis, M. W., and S. J. Parker.
2004. Fish size and exposure to air: potential effects on behavioral impairment and mortality rates in discarded sablefish. N. Amer. J. Fish. Manag. 24:518–524.

Davis, M. W., and C. B. Schreck.
2005. Responses by Pacific halibut to air exposure: lack of correspondence among plasma constituents and mortality. Trans. Am. Fish. Soc. 134:991–998.

Enever, R., A. S. Revill, and A. Grant.
2009. Discarding in the North Sea and on the historical efficacy of gear-based technical measures in reducing discards. Fish. Res. 95:40–46.

Farrell, A. P., P. E. Gallaugher, and R. Routledge.
2001. Rapid recovery of exhausted adult coho salmon after commercial capture by troll fishing. Can. J. Fish. Aquat. Sci. 58:2319–2324.

Fowler, G. M., and W. T. Stobo.
1999. Effects of release parameters on recovery rates of tagged groundfish species. Can. J. Fish. Aquat. Sci. 56:1732–1751.

Huntingford, F. A., C. Adams, V. A. Braithwaite, S. Kadri, T. G. Pottinger, P. Sandøe, and J. F. Turnbull.
2006. Current issues in fish welfare. J. Fish Biol. 68:332–372.

Ingolfsson, O. A., A. Soldal, I. Huse, and M. B. Breen.
2007. Escape mortality of cod, saithe, and haddock in a Barents Sea trawl fishery. ICES J. Mar. Sci. 64:836–1844.

Lee-Jenkins, S. S. Y., T. R. Binder, A. P. Karch, and D. G. McDonald.
2007. The recovery of locomotory activity following exhaustive exercise in juvenile rainbow trout (*Oncorhynchus mykiss*). Physiol. Biochem. Zool. 80:88–98.

Loeng, H.
1991. Features of the physical oceanographic conditions of the Barents Sea. Pol. Res. 10 (1):5–18.

Mellergaard, S., and O. Bagge.
1998. Fishing gear-induced skin ulcerations in Baltic cod, *Gadus morhua* L. J. Fish Dis. 21:205–213.

Olsen, R.E., K. Sundell, E. Ringø, R. Myklebust, G. I. Hemre, T. Hansen, and Ø. Karlsen.
2008. The acute stress response in fed and food deprived Atlantic cod, *Gadus morhua* L. Aquacult. 280:232–241.

Ottolenghi, F., C. Silvestri, P. Giordano, A. Lovatelli, and M. B. New.
2004. Capture-based aquaculture. The fattening of eels, groupers, tunas and yellowtails, 308 p. FAO, Rome.

Reidy, S. P., S. R. Kerr, and J. A. Nelson.
2000. Aerobic and anaerobic swimming performance of individual Atlantic cod. J. Exp. Biol. 203:347–357.

Sartoris, F. J., C. Bock, I. Serendero, G. Lannig, and H. O. Pörtner.
2003. Temperature-dependent changes in energy metabolism, intracellular pH and blood oxygen tension in the Atlantic cod. J. Fish Biol. 62:1239–1253.

Soldal, A. V., B. Isaksen, and A. Engås.
1993. Survival of gadoids that escape from a demersal trawl. ICES Mar. Sci, Symp.
196:122–127.

Staurnes, M., T. Sigholt, H. P. Pedersen, and T. Rustad.
1994. Physiological effects of simulated high-density transport of Atlantic cod (*Gadus morhua*). Aquacult. 119:381–391.

Stien, L. H., S. Bratland, I. Austevoll, F. Oppedal, and T. S. Kristiansen.
2007. A video analysis procedure for assessing vertical fish distribution in aquaculture tanks. Aquacult. Eng. 37:115–124.

Suuronen, P., D. L. Erickson, and A. Orrensalo.
1996. Mortality of herring escaping from pelagic trawl codends. Fish. Res. 25:305–321.

Suuronen, P., E. Lehtonen, and P. Jounela.
2005. Escape mortality of trawl caught Baltic cod (*Gadus morhua*)—the effect of water temperature, fish size and codend catch. Fish. Res. 71:151–163.