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Fish stress and mortality can be predicted using reflex impairment

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Abstract

Fish stress and mortality can be significant problems in both capture and culture operations. In addition to inexpensive and real-time metrics, ones that are simple to use are also desirable for measuring fish stress and predicting mortality. Current methods to define stress rely on expensive, laboratory-based measurements of changes in fish pathology such as disease, necropsy and histology, in physiology such as plasma cortisol, lactate, glucose and ions and in complex behaviour determined from swimming, feeding and predator evasion. All of these methods are often not rigorously linked to fitness outcomes. An alternative is to observe reflex impairment as a direct sign of stress which can be easily and rapidly measured in free swimming or restrained fish responding to peripheral stimuli such as gravity, light, sound and touch. Reflex impairment is correlated with stress and mortality outcomes, eliminating the need for prolonged holding or monitoring of fish. A few examples of reflexes that may be impaired include orientation, startle responses, fin erection, body flex upon restraint, operculum and mouth clamping or gaping, gag response and vestibular–ocular response. Reflex impairment combines the effects of stressors and their interactions and is not dependent on fish size, motivation states and acclimation which make it a consistent sign of stress across a wide range of stressor types and fish ages. Use of reflex impairment to measure stress and predict mortality would significantly improve monitoring of fish health and welfare in many types of field operations such as commercial and recreational fishing, aquaculture, live transport, stock enhancement and tagging.

Keywords Aquaculture, discards, disease, stock enhancement, vitality, welfare

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Introduction

Fish stress and mortality can cause significant losses of resources and productivity in both capture and culture systems. Reduction of these losses requires knowledge of stressors, stress levels and fitness outcomes (Power 1997; Anderson 2000). Fish welfare is compromised by stress and has become an increasing concern in the operation of capture, rearing and research operations (Huntingford *et al.* 2006; Browman and Skiftesvik 2007). Measurement of fish welfare and stress has been hampered by a lack of real-time field methods that are easy and inexpensive to use (Morgan and Iwama 1997; Dawkins 2004; Huntingford *et al.* 2006). Determination of stress outcomes requires either tagging and recapture or prolonged holding of fish in controlled conditions (Broadhurst *et al.* 2006; Portz *et al.* 2006; Pollock and Pine 2007). The results of outcome experiments can then be used to identify important stressors in the management of welfare and stress.

A direct approach to the problem of insuring fish welfare and reducing stress is to measure characteristics of whole fish, such as reflex impairment. A reflex action is an involuntary or stereotyped movement induced by a peripheral stimulus (Landau 1986). Reflex responses can be quantified as present or absent after stimulation by gravity, light, sound or touch in free swimming or restrained fish. Responses of several reflexes can be combined into an index for reflex impairment in individuals or populations. Reflex impairment is a whole animal sign of stress that can be correlated with outcomes, including reduced growth, impaired predator evasion, and delayed mortality (Olla and Davis 1989; Davis 2007; Fisher *et al.* 2007).

The goal of this paper is to show how reflex impairment can be used for rapid real-time assessment of fish stress and for predicting delayed and total mortality in a wide range of operational conditions and research. Integrated observations of stressors, reflex impairment and stress outcomes will enhance knowledge of specific stressor effects on fish vitality and contribute to improved fitness modelling, informed changes in management and operational conditions, and enhanced fish welfare, health and production.

Current methods for determining stressors, stress and outcomes

Many types of factors and their interactions can act as fish stressors (Wedemeyer 1997; Barton 2002; Ashley 2007). Stressor types may be grouped as physical having an influence through exercise, pressure, temperature and silt, ecological which derive from social stress, predation and food availability, and chemical sources resulting from changes in pH, O_2, CO_2 and xenobiotics. Stressors may be acute (short term) or chronic (long term) and their strength can range from mild to severe which can be gauged by the induced stress response and its outcomes (Barton 1997; Huntingford *et al.* 2006). Exposure to multiple stressors produces cumulative stress that may be more than the sum of individual stressor effects (Power 1997).

Analysis and prediction of stress and mortality outcomes requires knowledge of stressors and their interactions, stress states and outcomes (Fig. 1). When a healthy fish is exposed to short-term acute stress brought on by handling or predator avoidance, this will result in adaptive physiological and behavioural responses enabling the fish to recover to a healthy state. However, mortality can occur if the stressor is extreme. Of more concern is when a healthy fish is exposed to long-term chronic stress deriving from inappropriate temperatures, hypoxia or xenobiotics. Adaptive changes in physiology and behaviour may occur in response to chronic stress as the organism attempts to establish new states of equilibrium (allostasis). Increased metabolic costs associated with chronic stress (allostatic load) may lead to compromised welfare indicated by a suppression in growth, reproduction and most seriously immunocompetence, resulting in the increased

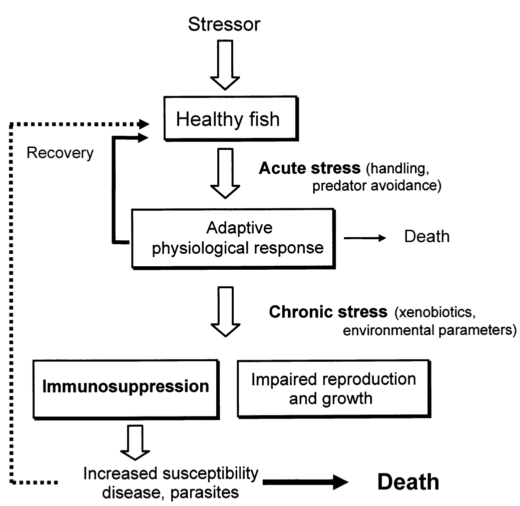

Figure 1 Diagram of stress and mortality outcomes for healthy fish exposed to stressors.

likelihood for mortality through disease (McEwen 2000; Sterling 2004).

Fish mortality can result immediately after exposure to acute stressors and is easily observed in the field. A problem in many field operations is the occurrence of delayed mortality after the onset of stress (Cato and Brown 2003; Coggins *et al.* 2007; Cooke and Schramm 2007). Mortality can occur from hours to weeks after exposure to stressors (Fig. 2). To identify factors associated with mortality and to measure true mortality rates, fish must be held for prolonged periods under controlled conditions such that they do not contribute to the original stressors causing delayed mortality. This holding requirement is often difficult and expensive to satisfy in field operations and does not include predation (Suuronen 2005; Portz *et al.* 2006; Pollock and Pine 2007). Tag and recapture, and electronic tagging and tracking have been used to measure delayed mortality and may include predation; but these methods often cannot identify causes or rates for mortality. Statistical models have been used to predict delayed mortality based on correlations with stressors or with signs of stress (Adams *et al.* 1993; Turnbull *et al.* 2005; Broadhurst *et al.* 2006). These mortality models require initial holding or tagging of fish for model validation.

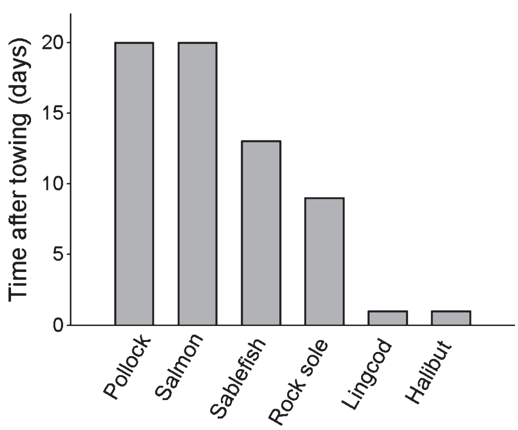

Figure 2 Time (days) to reach maximum delayed mortality after towing in a net for walleye pollock (*Theragra chalcogramma*, Gadidae), coho salmon (*Oncorhynchus kisutch*, Salmonidae), sablefish (*Anoplopoma fimbria*, Anoplopomatidae), northern rock sole (*Lepidopsetta polyxystra*, Pleuronectidae), lingcod (*Ophiodon elongates*, Hexagrammidae) and Pacific halibut (*Hippoglossus stenolepis*, Pleuronectidae). Methods and data are described in Davis and Olla (2002), Davis and Ottmar (2006) and Davis (2007).

Changes in fish pathology normally associated with lesions and infection are clear signs of stress and have often been linked to mortality outcomes, but some stressors deriving from physical and ecological factors may not produce pathological signs (Noga 2000; Barton *et al.* 2002; Davis and Ottmar 2006). Stress has also been measured as changes in physiological and behavioural parameters including, but not limited to, plasma hormones and ions, blood gases, heat shock proteins, gene expression, respiration, heart rate, swimming, searching for food, feeding, social interactions and predator evasion after stressor challenges (Beitinger 1990; Iwama *et al.* 1997; Huntingford *et al.* 2006). Impaired fish performance in response to stressors may be linked to mortality outcomes (Beitinger and McCauley 1990; Little 2002; Le Vay *et al.* 2007). Performance impairment has been evaluated using biotelemetry and bio-logging devices to monitor changes in vital physiological parameters and behaviour (Wikelski and Cooke 2006; Young *et al.* 2006; Donaldson *et al.* 2008). However, the design, expense and operational complexity of performance tests and field tracking makes these whole fish measures poor candidates for rapid field assessment of stress and prediction of mortality on a routine basis.

Relatively few stress studies have attempted to link stress signs with welfare, vitality or mortality outcomes (Anderson 2000; Wikelski and Cooke 2006; Young *et al.* 2006). Interpretation of performance measures as signs of stress linked to mortality has been difficult as they can be altered by factors like acclimation, size, species and motivation which are not measures of health status (Kieffer 2000; Barton 2002; Little 2002; Peake and Farrell 2006). For example, normal adaptive stress responses may result in quick recovery to homeostasis (Barton 1997; Huntingford *et al.* 2006; Ashley 2007). Negative outcomes can occur when fish show chronic stress or switch to new stable states through adaptation and compensation and then experience additional allostatic load (McEwen 2000; Sterling 2004). It appears that departures of measures from normality which define stress enable us to say that fish are stressed and have impaired welfare and health, but not by how much, or when they might return to normality or show mortality.

Reflex impairment measures stress

Fish vitality is a visual impression of survival potential that is familiar to anyone who has handled fish. The elements of this impression include spontaneous activity, reflex actions, wounding and swimming away after release. Technical personnel in field operations routinely observe spontaneous activity, responsiveness and gross wounding or infection as pre-clinical signs of fish vitality, and subsequently make intuitive decisions about welfare, health care and culture conditions based on previous experience of mortality outcomes (Noga 2000; Dawkins 2004; Turnbull *et al.* 2005). Reflex impairment appears to be a useful sign of fish stress and potential mortality as it is easily and rapidly measured in the field and can be consistently correlated with fitness outcomes (Davis 2005, 2007; Davis and Ottmar 2006). Anyone who has been examined by a medical doctor is probably familiar with reflex testing to assess neurological condition and as part of an evaluation of health outcomes. Veterinarians routinely test animal reflexes as part of a neurological assessment and evaluation of outcomes. Exposure of fish to anaesthetic results in characteristic stages for loss of responsiveness, which appear to parallel the effects of stress on reflex responses. Anaesthesia stages are described by loss of reactivity to external stimuli, reduction in respiration rate, loss of equilibrium and loss of reflex reactivity (Summerfelt and Smith 1990; Keene *et al.* 1998). In light of the anaesthesia model, reflex impairment appears to be a good measure for loss of fish responsiveness. Assessment of fish welfare has been linked with measures of responsiveness and possible pain perception (Chandroo *et al.* 2004; Huntingford *et al.* 2006). The relationships between reflex responses, neurological condition and responsiveness suggest that reflex impairment may be a useful measure for fish welfare in capture and rearing operations.

Reflex impairment can be quantified as a measure of stress in a standardized manner in both field and laboratory operations using a minimum of equipment, expense and time. Well-defined external stimuli including light, gravity, sound and touch, trigger reflexes which can then be measured in free swimming or restrained animals. Reflex responses can be scored as present or absent, without observer bias and then scores are summed to give a measure of impairment relative to healthy control fish responses (see Davis and Ottmar 2006; Davis 2007 for methods). Impairment can then be correlated with stressors and delayed mortality for statistical prediction and modelling of stressor effects and fitness outcomes. Reflex impairment occurs quickly and may remain constant for several hours after stress induction (Fig. 3). The strength of a reflex response can be measured with respect to force, acceleration, range of movement, latency and repeatability of response amongst other criteria, but may not be useful for stress assessment in populations over a wide range of operational conditions as response strength can be a function of factors such as size, sex, hunger and fear that are separate from the fish's health status (Takeda and Takii 1992; McKenzie *et al.* 1997; Shingles *et al.* 2005). However, reflex response strength is useful for assessing stress in fish with similar size, sex and motivation state and this approach has been used in studies of the neurological effects of stressors (Carvalho *et al.* 2002; Herbert *et al.* 2002; Beck *et al.* 2004; Weber 2006).

Several types of reflexes have shown impairment after stress induction. In free swimming fish, studied reflexes included orientation where the fish should normally be upright, righting reflex where the fish returns to an upright position and the startle response in which the fish shows rapid forward motion in response to stimuli (Lutnesky and Szyper 1990; Artigas *et al.* 2005; Davis and Ottmar 2006). In restrained fish, studied reflexes included body flex upon restraint where the fish attempts to escape when restrained, dorsal fin erection in which the fins become erect when the fish is restrained, operculum and mouth closure where the operculum or mouth clamps shut when lifted or opened, the gag response where the fish opens its mouth and

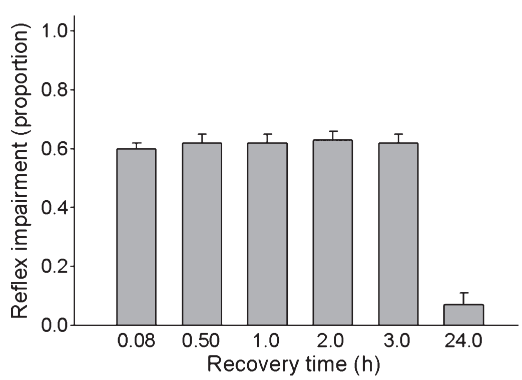

Figure 3 Impairment (proportion) of a suite of three reflexes (orientation and startle responses to light and sound) in free swimming sablefish was induced quickly and remained constant for 3 h after towing in net. Values are mean ± one SE. Methods and data are described in Davis (2005) and Davis and Ottmar (2006).

flexes the body when the throat is stimulated and the vestibular–ocular response (VOR) shown by eye rolls when the body is rotated around the long axis (Trumble *et al.* 2000; Davis 2007). Other studies of reflexes in free swimming fish have included atonic immobility, dorsal light reaction, and optomotor and optikinetic responses (Douglas and Hawryshyn 1990; McCormack and McDonnell 1994; Wells *et al.* 2005; Hasegawa 2006).

Patterns of impairment for five individual reflexes varied between species in a study where restrained fish were tested after towing in a net (Fig. 4; Davis 2007). Impairment of operculum closure occurred first and dominated reflex impairment in walleye pollock. The VOR was impaired first in Pacific halibut. Impairment of gag response occurred first and dominated impairment in coho salmon. The gag response and body flex were impaired first in northern rock sole. The VOR was not present in both control and stressed coho salmon and northern rock sole.

Reflex responses are involuntary components of complex behaviours that have direct ecological relevance for fitness (Mesa *et al.* 1994; Shingles *et al.* 2005). Normal orientation is necessary for swimming, while startle responses are an integral part of threat evasion (Wakeling 2001; Webb

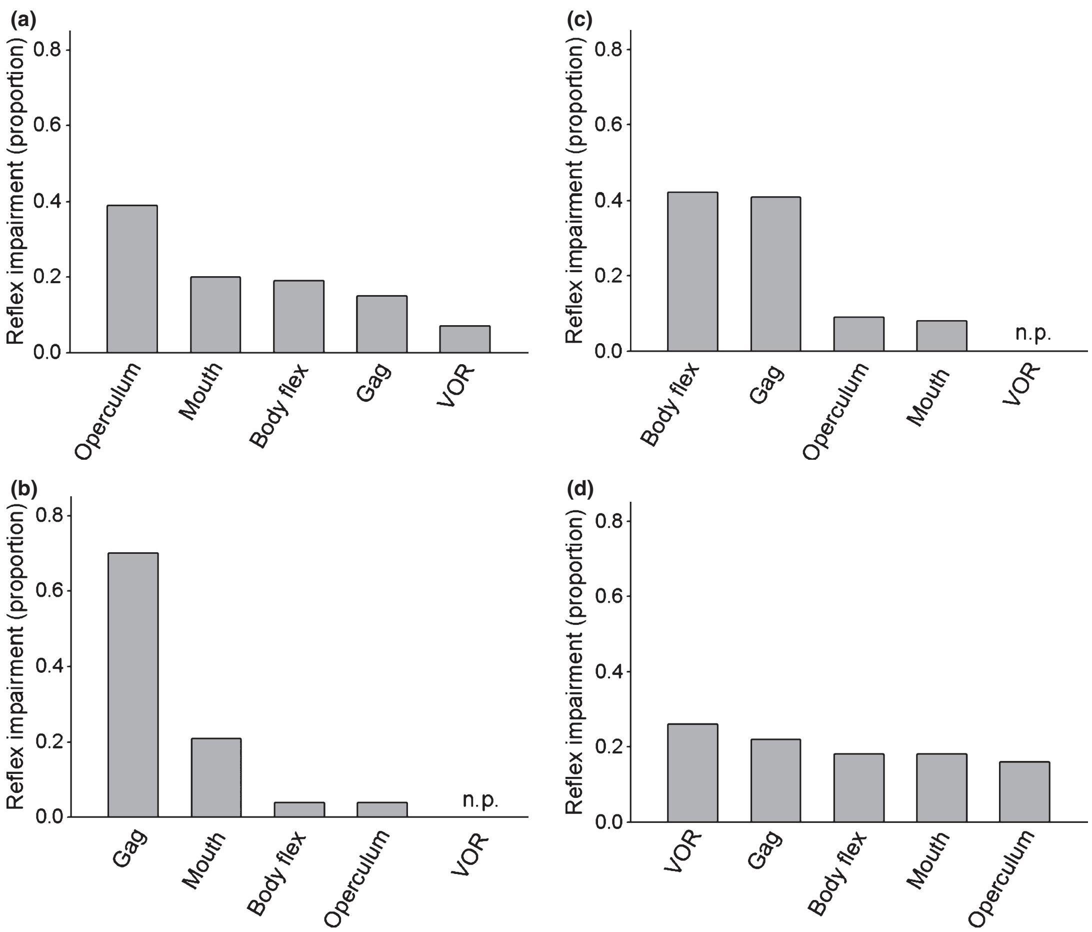

Figure 4 The proportional contribution of impairment from each tested reflex (body flex, operculum closure, mouth closure, gag and VOR) to total reflex impairment in restrained (a) walleye pollock (n = 21), (b) coho salmon (n = 14), (c) northern rock sole (n = 15) and (d) Pacific halibut (n = 18). Contribution proportions were calculated by summing values for mean reflex impairment in each reflex (reported in Davis 2007) and then dividing each individual reflex sum by the grand sum of all reflex impairment means for each species. Reflexes for each species are listed in order of impairment and contribution to total impairment. VOR was not present (n.p.) for control and stressed coho salmon and northern rock sole. Fish were towed in a net prior to reflex testing. Methods and data are described in Davis (2007).

2004). Operculum and mouth closure are important in coughing and fish respiration (Ballintijn and Hughes 1965; Ballintijn 1969). Gag response is necessary for expelling objects from the mouth or throat (Sims *et al.* 2000). Visual responses are important in orientation, food searching, social interactions and threat evasion (Douglas and Hawryshyn 1990).

How to validate reflex impairment as a research tool

Fish stress and mortality can be predicted by using a reflex action mortality predictor (RAMP) curve. Separate curves should be derived and used for individual species through assay validation and fish stressor experiments (Figs 5 and 6; Davis and Ottmar 2006; Davis 2007). The RAMP curve includes information about the combined effects of stressors and their interactions, reflex impairment resulting from stress induction, and the relationship between impairment and mortality outcomes. Reflex tests for fish larvae may be different than for juveniles and adults as larvae show rapid ontogenetic development of reflexes compared to juveniles and adults, which have fully developed reflexes.

The process of validating a reflex impairment assay for stress and prediction of mortality (RAMP) includes several components (Fig. 5). Step 1 is to set up a control by holding fish with minimum stress and test reflex responses in free swimming and restrained fish to identify the reflexes that consistently respond to stimulation. Examples of candidate reflex responses were given above and others are probably present in other species. The key point is to answer the question, what stimuli including gravity, light, sound and touch cause fish to consistently respond with involuntary or stereotypical movements that are not dependent on size, sex or motivation. Identify at least five to eight reflex responses so that a composite measure of impairment can be calculated. As different stressor types belonging to the physical, ecological and chemical groupings may affect reflex responses in different ways, testing combinations of reflexes insures that the effects of multiple stressor types are included in the calculated impairment index. To minimize observer bias, a reflex response should be scored as present only when it is clearly evident. If there is doubt about reflex presence or the response is weak, then it should be scored as not present. These simple criteria for reflex response scoring make the training of fishers and other non-technical personnel relatively easy and insure consistent, high quality response data.

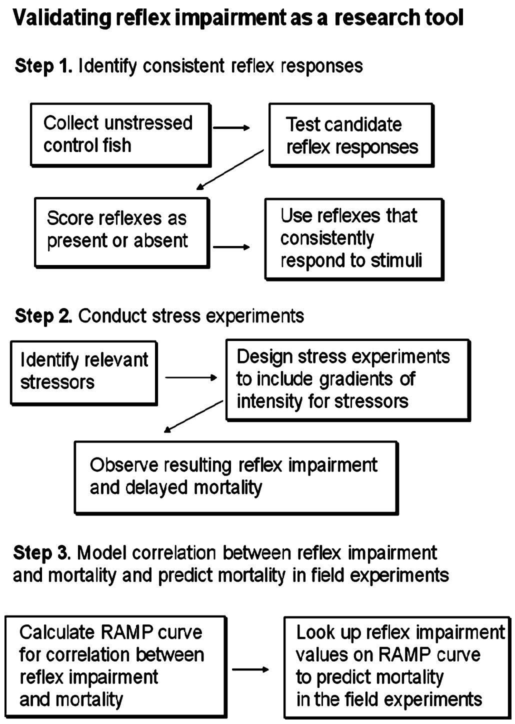

Figure 5 Diagram of a template for validating reflex impairment as a research tool to measure stress and predict delayed and total mortality.

Step 2 is to identify stressor types that may be present in the operational system which may be commercial or recreational fishing, aquaculture, transport or tagging for which stress and mortality is to be modelled. Then replicate fish are exposed to gradients of relevant stressor types and intensities which might include temperature, capture injury, crowding, hypoxia or rapid decompression. A fishing experiment could be conducted in a sport fishing tournament or in a commercial trawling operation that included systematic variation of important stressors, or fish could be transported under varying conditions to a release site, or fish could be exposed to a gradient of culture conditions (Davis 2002; Iversen *et al.* 2005; Killen *et al.* 2006; Portz *et al.* 2006). To insure that the RAMP curve is robust, the experiments should include operational conditions which result in a wide range of reflex impairment values and subsequent delayed mortality, observed by holding fish for an extended period of

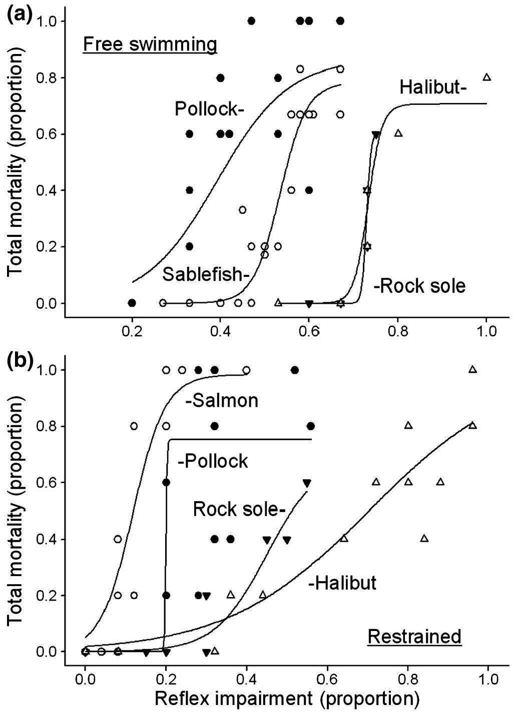

Figure 6 Reflex action mortality predictor (RAMP) curves (reflex action mortality predictor) that describe relationships between reflex impairment and total mortality (immediate + delayed mortality). Curves are for (a) free swimming walleye pollock (●), sablefish (○), and Pacific halibut (▲) and for (b) restrained coho salmon (▼), northern rock sole (□), and Pacific halibut (▲). Fish were exposed to towing in a net and exposure to air and elevated temperature prior to reflex testing. Methods and data are described in Davis and Ottmar (2006) and Davis (2007).

time of up to 30 days. Several reflex responses should be measured as present or absent for each fish, summed for replicate individuals or groups of fish, and impairment calculated as one – (total reflex responses observed/total reflex responses possible).

Step 3 is to model correlation between reflex impairment and mortality using non-linear or logistic regression. RAMP curves are typically sigmoid, characterized by an initial increase in reflex impairment without concomitant mortality, followed by further increases in reflex impairment and associated increasing mortality (Fig. 6). Future measurements of reflex impairment can then be used as inputs to RAMP curve models to predict sub-lethal stress and mortality in operational conditions, which include the stressor types used for assay validation. Correlations can also be modelled between reflex impairment, operational conditions, and other measures for welfare and effects of stress, which may be more difficult or expensive to measure than reflex impairment. Finally, relationships between stressors, reflex impairment and mortality can be determined and operational conditions can be adjusted and managed to decrease stressors and reflex impairment and improve welfare, health and survival.

Several potential limitations for the use of reflex impairment to measure stress and predict mortality should be evaluated in future research. All fish have reflexes and these differ among species. When some species are handled, for example flatfish and other cryptic species, they may not be very responsive for a period of time, which makes observation of reflex responses in restrained control fish difficult. In such cases, observation of free swimming fish may be more appropriate. Choice of reflexes to be tested may be guided by the ontogeny and ecology of the species in question which may be cryptic, benthic or pelagic. For the species that have been tested so far, relationships between reflex impairment, stress and mortality were consistent within assumed operational conditions. However, when other types of stressors are present, relationships between reflex impairment and outcomes may be altered and new RAMP curves should be derived for those stressor types. The synergistic effects of stressor types on reflex impairment are not well tested and characterized and there should also be more research on the links between reflex impairment, welfare, physiology, injury and mortality across a broader range of stressor types and fish species.

Mortality prediction: stressors and fish performance vs. reflex impairment

Mortality can be predicted using data on stressors or signs of stress, for example through pathology, fish performance by measuring cortisol, feeding and swimming, or reflex impairment as predictors in validated logistic, or non-linear regression models. Reflex impairment is a sign of stress that can be correlated with mortality outcomes, making it a useful predictor in operational conditions for which it has been validated, regardless of whether the stressor intensities or interactions change beyond the original range of the validation model. In contrast, stressors, pathology and fish performance each have limitations as predictors that are not present for reflex impairment.

When stress outcomes are well known in stable operational conditions, then stressors, pathology or fish performance may be useful mortality predictors based on the operator's past experience. However, there are operational conditions under which predictor model assumptions are not satisfied. Stressor conditions may change to include values which range outside of the original model or new stressor type interactions and synergisms can occur which were not accounted for in the model. Stressor factor interactions are common and often include the effects of varying combinations and intensities of stressors present at the time of exposure (Power 1997; Springman *et al.* 2005). Pathological signs of stress may not be present in fish that are exposed to physical and ecological stressors and could not be assigned to mortality outcomes. Impaired performance may result from factors not related to health such as size, motivation and acclimation, and may not be correlated with mortality.

The natural units for mortality are whole fish or populations of fish. Reflex impairment is an immediate sign of stress that can be correlated with mortality in whole fish and populations. When wounding is present, it may be correlated with mortality. Similarly, pathology can predict mortality when overt signs of disease are present, but which occur after a period of time when stress has become chronic. Physiological performance measures are poor predictors of mortality because they are signs of stress in separate body systems including the neural, hormonal, circulatory, digestive and excretory systems, and therefore are linked to whole fish outcomes only through interactions with other body systems, which can be uncoupled from fitness outcomes. Complex behaviours may be signs of stress in whole fish but are poor predictors of mortality because they can be altered by factors not related to health.

Conclusions

Monitoring and protection of the fish themselves and of the health and welfare of the ecosystem in which they live has become a priority as increased pressure is exerted by human activities (Wikelski and Cooke 2006; Browman and Skiftesvik 2007). Efficient field operations require frequent monitoring of fish health and modification of operational conditions where needed to improve welfare, health, fitness and production (Adams *et al.* 1993; Huntingford *et al.* 2006; Ashley 2007). Developing the capability for rapid real-time field assessment of fish stress and the prediction of delayed mortality using reflex impairment would facilitate data collection and support an expanded scope for studies on stress, fitness outcomes and welfare. In addition to mortality prediction, reflex impairment may be linked with impairment of feeding, predator evasion, habitat use and social interactions for prediction of fish production, recruitment and management outcomes. New technologies for computerized monitoring of fish reflex responses in tanks and in the field makes this application appropriate for aquaculture, stock enhancement and the ornamental fish trade (Kristiansen *et al.* 2004; Stien *et al.* 2007; Donaldson *et al.* 2008).

A wide range of fish systems that would benefit from real-time stress and mortality analysis are evident. Recreational and commercial capture fishery operations and the gear used to catch the fish such as rod and reel, trap, pot, seine, trawl, longline and trolling, may be modified to reduce discard and escapee mortality (Broadhurst *et al.* 2006; Coggins *et al.* 2007). Aquacultural operations may be modified to mitigate deteriorating environmental and social conditions and optimize fish welfare, health and growth before outbreaks of disease associated with chronic stress can occur (Portz *et al.* 2006; Ashley 2007). Collection of fish from the wild, shipping and the rearing of aquarium specimens may be optimized to reduce mortality and shipping costs and increase specimen quality (Cato and Brown 2003; Lim *et al.* 2003). The effects of environmental stressors on fishery ecosystems may be monitored and mitigated in the field (Adams *et al.* 1993; Schleiger 2004). Fish released for stock enhancement or that are used in laboratory and field research may be screened for health and survival probability (Huntingford *et al.* 2006; Le Vay *et al.* 2007).

Reliable prediction of stress and mortality requires development of consistent measures for whole fish impairment that combine the interacting effects of stressors and can be correlated with outcomes. Moreover, to be widely useful, these fitness predictors should be easy and inexpensive to use in real-time field operations. Reflex impairment may appear to be a simplistic measure that is technologically naive and lacks power to explain mechanisms for induction of fish stress and mortality. However, shifting focus from mechanistic studies of fish stress to finding correlations between stressors, reflex impairment and mortality can offer a powerful method for predicting stress and delayed mortality, as well as for identifying important stressors and their synergistic effects on individuals, populations and ecosystems. Better estimates for fish stress and mortality will reduce uncertainty in field operations and management and improve design of innovative solutions to production problems in both fish capture and culture.

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