

Final Report

Selectivity and Survival of Atlantic Cod (*Gadus morhua*) [and Haddock (*Melanogrammus aeglefinus*)] in the Northwest Atlantic Longline Fishery.

By:

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NOAA/NMFS Saltonstall-Kennedy Program

A. Grant Number: NA86FD0108

B. Amount of Grant:

Federal \$163,244 Match \$122,634 Total \$285,878

C. Project Title: Increasing Survival of Juvenile Atlantic Cod (*Gadus morhua*) and Haddock (*Melanogrammus aeglefinus*) in the Northwest Atlantic Longline Fishery.

D. Grantee: New England Aquarium and Massachusetts Division of Marine Fisheries.

E. Award Period: 1 May 1998 to 30 September 2002.

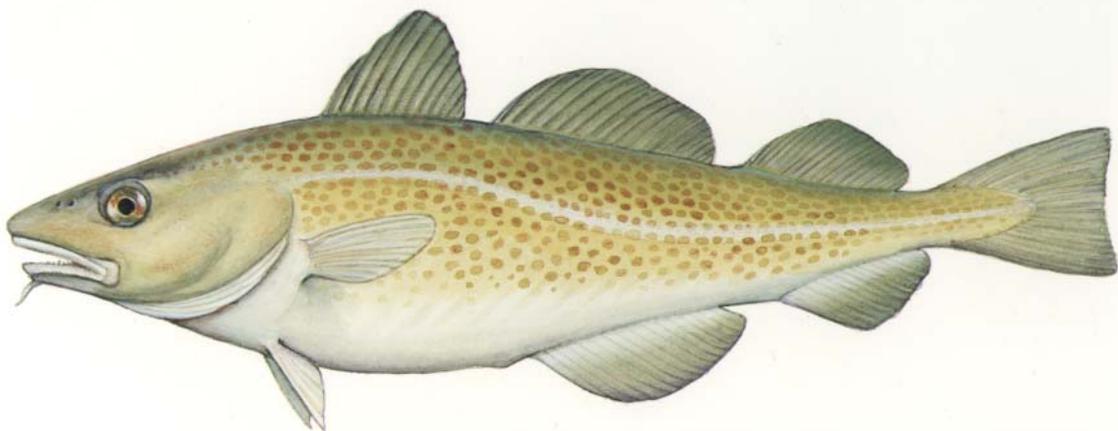


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Acknowledgements

The authors wish to thank the captain and crew of the F/V Isabel S for their help, input and professionalism. As well, Mark Leach, Captain of the F/V Sea Holly, provided tremendous assistance to our studies. In addition we would like to thank Debie Meck and Glenn Hovermale from the New England Aquarium and Vinny Manfredi from the MA Division of Marine Fisheries for their assistance with field collection and work. A special note of thanks goes to Henry Milliken from the National Oceanic and Atmospheric Administration for his help in the field up to and including statistical analysis advice.

The authors thank NOAA-NMFS for financial support for this project.



I. Abstract

Longline fishing practices use static components that minimally impact the substrate, especially compared to mobile fishing gear such as otter trawls. However, the catch is usually removed from the hook by force: the fish is held in place with a gaff braced against two parallel steel cylinders placed vertically on the gunwale, allowing the hydraulic hauler to pull the hook through the fish's flesh. This process can inflict severe injury to the fish. In order to minimize these injuries an alternate protocol was investigated. Using a two handed flip over the barb of circle hooks produced a single hole in the oral cavity of the fish. When this flip method was compared to the snub procedure, no difference in survival after 72 hours was observed in sublegal-sized cod (*Gadus morhua*) bycatch. Biochemical data that were gathered on a similar subset of these fish suggested that the protocols chosen to judge survival may have added a level of stress that could have confounded the results. Statistical significance could be obtained at the $\alpha = 0.1$ level when additional snub fish from a related study were added to these figures.

II. Executive Summary

Fleet effort, discard survival and gear selectivity of the longline fishery in New England remains largely unexamined. Previous work by New England Aquarium and Massachusetts Division of Marine Fisheries has documented selectivity and survival in Atlantic cod (*Gadus morhua*) sublegal-sized bycatch (SK report grant #NA66FD0028). Those findings concluded that although not all undersized fish died, survival was compromised when fish were mechanically removed from hooks by force. This report compared different strategies for removing fish automatically from longlines and critically examined one alternate method that resulted in fewer gross injuries, which should have augmented survival.

Several characteristics considered essential for a successful protocol were discussed with fishermen and engineers creating a unique opportunity to investigate whether several designs could improve survival by decreasing physical injury to the fish. After several false starts, a method already being employed by fishermen was modified. Briefly, a gaff is used to immobilize a circle hook and the tail of the fish is flipped over the barb. This process was converted into a one-man procedure by transferring hauling operations to a foot pedal.

Effectiveness of this method was tested in two ways: holding fish for 72 hours; and sampling blood chemistry. The first survival cruise took place in July 2000. High numbers of spiny dogfish (*Squalus acanthias*) were caught while cod catches remained low. The cod that were caught allowed for only two survival study cages to be deployed. In addition, the numbers of fish obtained for blood work were not enough for any meaningful analyses.

The second survival cruise took place in June 2001. After meetings with fishermen, fishing practices were designed to use two vessels: a 12-meter commercial longline vessel for fishing and a 27-meter trawler for handling all the equipment for the survival and biochemistry studies. Cod were removed from the longline gear mechanically (“snub”) or by the alternate method (“flip”). Additional cod were caught by jig and used for comparison (controls). Survival was ascertained by placing fish in cages that were retrieved after 72 hours. Overall 435 sublegal-sized cod were used for this study and an additional study that examined the role of potassium in snubbed survival and was described elsewhere (SK ID#NA06FD0177).

The 72-hour survival was 30% for snubbed fish while 41% of the flipped fish remained alive. However these data were not found statistically significant; flipped fish did not appear to survive at a higher rate than snubbed fish.

Does even minor injury cause high mortality in sublegal cod? These results contradict the distinct difference in the apparent severity of injuries using the two methods. Some inconsistencies in handling and stress due to caging (see below) may be obscuring differences in survival. Also, evidence is available that suggests that small sample size may have confounded our results.

The physiological responses to fishing were also measured to determine the relationship between fishing protocol and survivability (Robinson et al., 1993; Farrington et al., 1998). Normal blood profiles were inferred from cod that were caught by hand jigging and bled within one minute from the set of the hook. Control values were obtained from cod that were captured by jigging, not bled and then held in cages along with longlined fish for 72 hours to observe their survival.

Without exception, the serum cortisol levels measured in jigged cod hovered near the limit of detection. Since the secretion of cortisol is a primary response to stress in fish, this result was a reliable indication that other adjustments in the blood may not have occurred and would reflect the normal ranges for the concentration of the components found in cod blood.

Except for potassium ion and glucose, all physiological parameters that were measured from cod taken directly from the longline regardless of dehooking protocol were significantly elevated over normal values. These values were similar to previous results and indicate that longline-caught cod experienced a moderate level of stress. Lactate, sodium ions, cortisol and hematocrit values remained significantly elevated from normal values after 72 hours. In addition, lactate, sodium and chloride ions, osmolality, cortisol and hematocrit control values were elevated over normal values indicating some aspect relating to the survival process was stressful and may have contributed to the ambiguous observations between dehooking methods.

Biochemical analysis revealed that a subset of fish from a related study could be added to the snub totals and reevaluated. Although the survival percentages were not very different, the additional data did find significant differences in the survival for fish removed by the tail flip method.

III. Project Management

The two Principal Investigators, Dr. Farrington and Mr. Carr, jointly supervised all aspects of this project. They planned for the logistical support of the cruises, scheduled technical personnel, oversaw the collection and interpretation of the data, and submitted requisite technical reports. Mr. Carr arranged for the fishing boat charters. Dr. Farrington arranged for laboratory (shore-based) support, oversaw data entry, and supervised the statistical analysis and biochemical analyses. The Office of Sponsored Programs of the New England Aquarium prepared the semiannual financial reports.

In addition to the co-principal investigators, Mr. John Mandelman, a graduate student at Northeastern University and the Edgerton Research Laboratory, New England Aquarium assisted in the biochemical assays for the project.

The Division of Marine Fisheries received a subcontract from New England Aquarium to cover some personnel costs and miscellaneous supplies. Two additional subcontracts were administered with fishing vessels.

IV. Purpose

A. Description of the problem:

To assess the survival of juvenile cod (*Gadus morhua*) bycatch under mitigated haul-back procedures specifically designed to reduce juvenile bycatch mortality. In this report, if used, “juvenile cod” refers to the sublegal-sized cod bycatch (total length less than 50cm).

B. Objectives of the project:

(1) To modify the equipment used in longline hauling to reduce injury and increase survival of the bycatch and catch.

(2) To quantify the degree of stress induced by the modified methods of capture and relate the degree of stress of fish caught through the modified method to fish caught via current longline methods through the analysis of stress parameters in the blood.

(3) To continue to solicit advice from 4-5 longliners relative to increasing the survival of discarded groundfish and increasing selectivity of demersal longline gear.

V. Bycatch Survival

A. Background

Undersized individuals of commercially important species and noncommercial catch are often taken along with legal-sized adults during normal fishing practices. Current fishing regulations and the Fishery Management Plan for the Northeast Multi-Species Fishery dictate that this bycatch must be returned to the ocean. In hook fisheries, fish may sustain injuries to their mouth, gills, eyes and occasionally in the gut. These injuries, along with other factors encountered during the fish capture process, such as temperature and pressure changes, increase their vulnerability and mortality. Mouth hook injuries, while severe, seem to have a higher survival rate than gut hook injuries at least in the short term (Orsi et al., 1993). Consequently circle hooks were used exclusively during this investigation.

Although the commercial longline fishery has been touted as a clean, low impact fishing practice, some longline fishermen and biologists have expressed concern regarding the use of a mechanical hook removal component called the “crucifier”. This device usually consists of two parallel steel cylinders placed vertically on the gunwale. The longline passes through the freely rotating cylinders during the haul back of gear. Unwanted fish are removed from the gear by laying a gaff handle across the rollers, which “snub” them from the hook. That is, the fish are blocked and the hooks are pulled out of them by the action of the hydraulic hauler. These fish fall directly back into the ocean. Injuries can range from superficial to the entire jaw being ripped out from one side (Farrington et al., 1998). The injury, if significant, has been shown to diminish the 72 hour survival of the juvenile cod bycatch (Farrington et al., 1998). The magnitude of these injuries primarily depends on where the hook is imbedded in the oral cavity.

Relatively little research has been conducted on the survival of discards from commercial hook fisheries and even less specifically addresses the survival of juvenile, or sublegal-sized cod caught by demersal longlines (Milliken et al., 1999). Many factors can govern bycatch survival. For instance, fishing depth is a known factor because the over inflation of air bladders leads to the loss of buoyancy control. Additionally, time of year may influence survival due to changes in temperature upon ascent and on deck. The documentation and improved survivorship of juvenile cod discards taken from demersal longline gear has been the primary objective of this study.

Survival of the discarded cod may vary given the manner in which each fisherman removes discard from the gear. Devising an automated method to standardize hook removal and decrease the degree of injury should reduce the variations seen on different longline fishing vessels. Therefore, this would improve the overall survival statistics. Simply removing hooks carefully from individual fish to minimize injury would inordinately increase haul time and therefore decrease overall efficiency. In addition, this

procedure would endanger the fisherman in areas where there is a large current or when there are high seas.

B. Approaches

Several automated devices and methods of hook removal were investigated. In consultation with longline fishermen, a list of requirements were developed that seemed necessary for any method to be practical:

- Feeding the longline through the dehooking device would provide continuous operation promoting efficiency.
- Hooks with fish on them would have to be immobilized to facilitate the release of the fish. Fixing the hook in the device could exploit the weight of the fish by pulling the gangion down and away from the longline during retrieval.
- Once the hook is fixed, the least harmful release would be to back the fish off of the hook, leaving only a small entry wound.
- Hooks should be repeatedly stripped with as much finesse and as little time as by snubbing. After a fish is removed from the gear, the hook must then be freed from the device to allow uninterrupted retrieval of the longline. The increasing force generated by the continued operation of the hydraulic winches was assessed for this purpose.
- Any modifications aimed for a one-man operation of the device.

These insights were discussed with fishermen and engineers creating a unique opportunity to investigate whether these concepts could be compiled into a single device and whether the design improved survival by decreasing physical injury to the fish. All of the prototypes considered below assumed hooks imbedded within the external oral cavity, the most commonly hooked location when using circle hooks.

Method #1 Double roller by Jonathan Bennett. The devised apparatus was considered too cumbersome and impractical for daily commercial use. Completely automated designs were abandoned because it became immediately apparent that any such apparatus would require too complicated of a mechanism. See Appendix. Considerations then turned toward semi-automated designs.

Method #2 Dehooking scoop. This method used a gaff-like rod of a diameter that would fit inside the circle of the hook. Attached to this rod would be a split scoop fastened along the axis of the rod. The gangion would be placed in the split in the scoop and the rod at the bottom of the split would then be placed in the hook for immobilization. After careful consideration, it was decided that this design would not have enough force to immobilize the hook and scoop the fish back around the hook at the same time. Untried.

Method #3 Rotating slide. Sublegal sized fish were moved into another basket by rotating the feed slide to an alternate basket. These fish would then be released after hauling. This method was actually prototyped in the field. Unfortunately, this concept took more time

than the manual removal of individual sublegal sized fish as the catch was hauled and increased the time on deck further risking bycatch survival. Abandoned.

Method #4 Leach's "Tail Flip". Regular consultation with fishermen allowed the exploration of other measures for increasing the survival of juvenile bycatch. Firsthand knowledge of the intricacies of the haul led to a solution incorporating many elements of the required characteristics. In response to criticisms about the typical de-hooking process for sub-legal fish, one fisherman, Mark Leach, had already modified a technique that minimized the damage caused by the snubbing procedure. This fisherman employed a "tail-flip" maneuver on sub-legal fish during the hauling process. This technique involves several steps to remove a hooked fish. Slide the gaff down the gangion into the corner of the hook and hold the gaff outboard from the vessel so the gangion can be pulled taut. Cradle the fish with your other hand in preparation to rotate the fish around the hook. Once the fish is freed, release the hook by sliding the gaff out. This release method resulted in a hole in the oral cavity slightly larger than with carefully removed fish and no broken jaws (Figure 1).



Figure 1. Typical injury sustained by tail flip method of hook release.

Unfortunately the entire maneuver, including modifying the speed of the haul, required more than two hands. By adding a hydraulic foot pedal to govern the speed of incoming gear, the "tail-flip" turned into a one-man, two-handed operation (See Video, Szymanski et al., 2002). The purpose of this part of the study was to assess the post-capture survival of juvenile/sub-legal Atlantic cod using either the "snubbed" or "tail-flip" techniques.

C. Survival Methodology

Survival cruises were conducted over two field seasons to study the effects of the post-capture methods to release juvenile Atlantic cod. Cruise location included the waters east of Cape Cod, Massachusetts around the Great South Channel. The first cruise took place in July 2000 and the second was performed in June of 2001. Video footage was collected from survival cruises and on multiple fishing trips with the F/V Sea Holly to record the "flip" technique utilized by Captain Mark Leach. From the video footage collected, an edited movie was produced detailing the technique to instruct other fishermen on its use (See Video; Szymanski et al., 2002).

Survival Cruise 2000

Juvenile cod were collected from 19-24 July 2000 on a 27-meter trawler (F/V Isabel S) that was contracted to complete the study. Demersal longline gear using snap-on gangions was set and hauled. The F/V Isabel S was equipped with a set of stainless steel rollers, a winch to haul longline gear and with water chillers, air pumps and cages to serve as a laboratory platform to complete all the survival and physiology procedures. Also, a commercial longline fisherman was employed to assist with the longline fishing practices and locations. Fishing occurred during the two or three slack tide periods during daylight hours.



Figure 2. Stainless steel rollers installed on board the F/V Isabel S. One of the many dogfish caught in July 2000 being snubbed from the longline.

Atlantic cod used for survival experiments were captured using demersal longlines and removed using either the common industry practice of allowing the hook to be pulled out of the fish by the hauling gear (“snub”) or by backing the fish off the hook using the “flip” technique. Only fish that were sub-legal (<49 cm or 19 in) were included in the experiment. Jigged fish were also caught using hand-lines for use as controls for blood chemistry (Farrington et al., 1998).

The fish were measured, checked for damage, tagged, and placed in holding tanks. Two 1000-liter holding tanks were filled with seawater and chilled to the measured daily bottom temperatures, typically around 5-7°C. Oxygen was provided using an air pump and diffusers. When an adequate number of fish were captured, they were placed in cages, which were deployed around the fishing grounds. After approximately 72 hours, cages were recovered and fish were determined to be alive or dead. Number of fish per cage and volume of cages varied (1.82 to 4.48 cubic meters).



Figure 3. Transferring fish from the holding tank to the survival cage.

Survival Cruise 2001

Cod were collected from 10-14 June 2001. After meetings with fishermen and drawing on experiences from the June 2000 cruise, fishing practices were designed to utilize two vessels simultaneously. First, fish were caught on a 12-meter commercial vessel (F/V Sea Holly with Captain Mark Leach) using standard longline gear and wire cable gear with snap-on gangions. Jigged fish were also caught using hand-lines periodically during the day onboard the Isabel S and placed in seawater filled holding tanks on deck. These fish were tagged and randomly placed in cages with longlined fish for 72-hour survival trials. The Sea Holly made daily trips to the fishing location whereas the Isabel S remained on site for the duration of the cruise and served as the platform for conducting all the survival and physiology procedures. Fishing occurred only during the morning slack tide.

Onboard the Sea Holly standard fishing practices were followed with regard to the setting and hauling of the longline gear. During the hauling process juvenile fish were removed from the hooks using two techniques (“snub” or “flip”). Fish were either bled immediately during the hauling process on the Sea Holly or transferred to the Isabel S via bushel baskets for the survival study. Fish used for blood samples were not used in the survival studies.

Fish transferred to the Isabel S were measured, checked for damage, visually assessed, tagged, and placed in holding tanks with all information recorded on data sheets. As in the previous season, two 1000-liter holding tanks were filled with seawater on the deck of the commercial trawler. One holding tank contained untreated seawater and the second tank contained potassium-enriched seawater for a different but simultaneous study. These

results will be presented elsewhere. Water was chilled to bottom temperature readings, typically 5-7 °C. Oxygen was provided using an air pump and diffusers. After fishing practices were completed for the day, cages were prepared to contain fish from the holding tanks. Then the cages were deployed around the fishing grounds and re-checked for mortality after 72 hours. Cage dimensions varied and number of fish per cage varied as in the previous year.

D. Results

Survival Cruise 2000

High numbers of spiny dogfish (*Squalus acanthias*) were caught while cod catches remained low. The cod catch allowed for only two survival study cages to be deployed. The trip was terminated one day earlier than originally planned due to the large numbers of dogfish caught and the serious lack of juvenile cod present. In addition, the numbers of fish obtained for blood work were not high enough for any meaningful analysis.

Survival Cruise 2001

Survival after the two hook removal methods (snub and flip) were assessed by using the G-test for independence, which tests the goodness of fit of observed cell frequencies to their expected frequencies (Sokal and Rohlf 1995). The Williams correction was then used to ensure that the correct Type I error was determined. The corrected observed G was then compared with a chi-square distribution with one degree of freedom.

A total of 192 sublegal cod were used to compare the survival rates between the two dehooking techniques, and the potassium holding treatment. One hundred ninety four fish were placed in cages; two fish could not be accounted for. One hundred eighteen of these fish were removed using the snub technique while seventy-four were removed using the flip technique (Table 1). Although data for snubbed fish soaked in the potassium enriched seawater were co-analyzed, results and discussion of these data will be reported and discussed elsewhere (Farrington and Carr, 2003; SK#NA06FD0177). The following only consider snubbed fish untreated with potassium.

Table 1. Number of fish found alive or dead in holding cages following dehooking from a longline with two techniques.

Technique	Treatment	Alive	Dead	Total	% Alive
Snubbed	Seawater	13	31	44	30 %
Snubbed	Seawater + K ⁺	20	54	74	27%
Flip	Seawater	30	44	74	41%
Total		63	129	192	

Thirteen (30%) of the snubbed fish were alive upon cage retrieval; thirty (41%) of the flipped fish were alive. Using the G-test for independence ($\alpha=0.1$), the survival of cod in these data were not found to be dependent on the dehooking technique ($G_{\text{adjusted}} = 1.44$, $df = 1$, $p = 0.23$).

Lengths of fish used in survival treatments ranged from 38-52 cm total length (Figure 2). Mortalities had the same size range; surviving fish ranged from 39-50 cm. No quantitative analysis of differences in lengths between treatments was conducted.

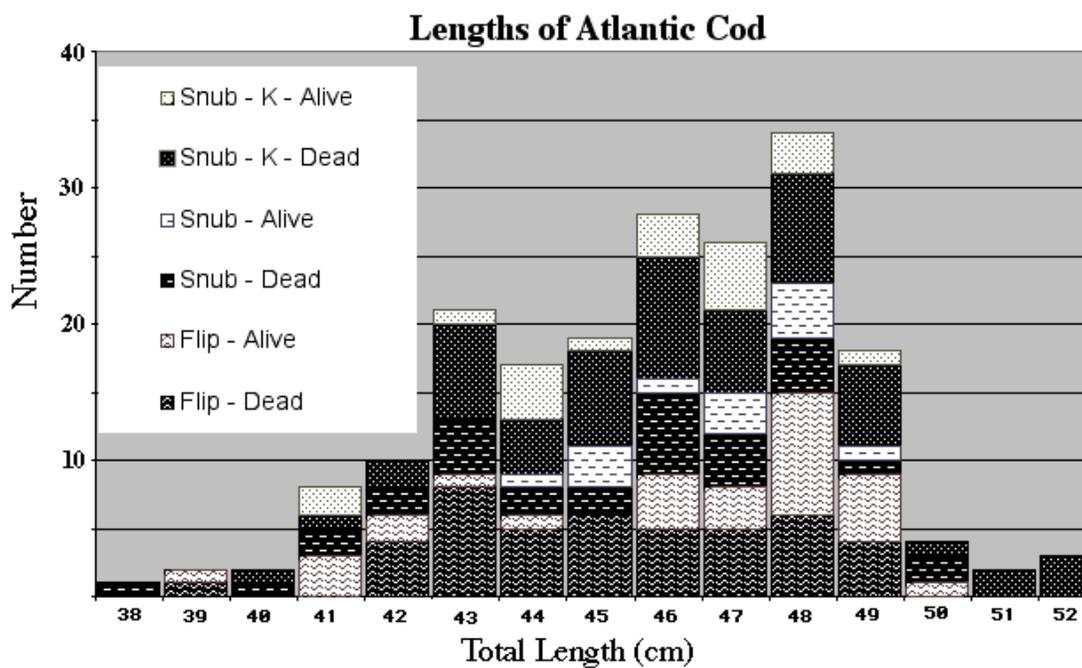


Figure 4. Lengths of Atlantic cod recovered during survival experiments.

Copies of the edited video were distributed to the Cape Cod Commercial Hook Fishermen’s Association, played at various trade show exhibitions and are made available upon request. The movie is catalogued as tape ID# 02MADMF765 in the Division of Marine Fisheries Conservation Engineering Program’s video database.

E. Discussion

The serious shortage of sublegal sized cod caught in July 2000 should have ended what could have been learned from this novel dehooking system because the subcontract budget only covered one field season. At that time however, the principal investigators were also completing another grant that used the same fishing platform for a related

bycatch survival study (Farrington and Carr, 2003; SK#NA06FD0177). Successful fishing in June 2001 collected fish for both studies. Although all data have been appended, this report will only discuss snub versus flip survival. Potassium enriched seawater treatment and its effect on survival will be discussed elsewhere (Farrington and Carr, 2003; SK#NA06FD0177).

Results from this study run counter to Milliken et al. (1999) who found that careful handling of cod resulted in a statistically supported increase in survival. In that study and this one, snubbed fish often exhibited visible, dramatic injury. The maximum observed injury in flipped fish was a small puncture wound (< 2 mm diameter) and a small flap of flesh. Usually injuries resulting from the flip technique did not show any apparent blood flow. Also, the fish were active and vigorous following removal. One important difference between these studies was the snubbing device itself. In the earlier work an eight-inch block guided the longline and acted as the crucifier. It is plausible that the block induced a perceptible inequality in the degree or type of injury induced in these fish.

Paradoxically, appropriate post-capture handling has been shown to increase survival in other fisheries (Farrell et al. 2001a, 2001b). Furthermore, techniques similar to the flip technique practiced in this study are advocated for the release of fish captured in other longline fisheries, most notably the Pacific halibut *Hippoglossus stenolepis* (Kaimmer and Trumble, 1998; Robb 2002).

We recognize that other factors may have contributed to the contradictory findings in this study. Foremost, the number of fish used in the flip protocol part of the study may have been inadequate to tease out statistical relevance in the spread of the data.

In addition, the confinement stresses experienced in the cages may have overwhelmed any advantage derived from handling and post-handling treatments (see below). It is conceivable that barometric and/or rapid temperature changes experienced while deploying and retrieving the cages complicated statistical analyses. Davis et al. (2001) found that temperature stress masked any differential impact of capture method in coho salmon, *Oncorhynchus kisutch*. Overcrowding may also have influenced survival however physiological responses to crowding typically have been difficult to quantify. Routine aquaculture methods put more emphasis on water quality that provides oxygen and removes wastes rather than the availability of physical space (Wedemeyer, 1997). Hatcheries routinely stock salmonids at densities of 60 to 120 kg/m³ for long periods of time (Wedemeyer, 1997; Westers, 1984). The largest densities in the test cages were 20 to 30 kg/m³ minimizing this concern in open ocean survival studies.

VI. Blood Biochemistry



Figure 5. Laboratory quarters inside Isabel S.

A. Background

Without exception, the capture of fish exerts physically stressful stimuli that include struggling, hypoxia, injury, fatigue and rapid changes in temperature and pressure. Identifying how sublegal-sized cod bycatch responds to these biological hardships has been evaluated by the biochemical analyses of blood components. An extensive characterization of the physiological status of these fish coupled to their known survival rates widens the means to investigate post-capture impact of fishing gear. Fisheries managers can use these data to improve bycatch regulations. In addition, once a large enough database has been collected and compared to actual survival rates, blood profiles may eventually provide an estimation of intrinsic survival. The hematological parameters that were chosen for biochemical analyses have been historically used to indicate stress in fish (Black 1958, Blaxhall and Daisley 1973, Wedemeyer and Yasutake 1977) and extend the data base collected for cod in previous work (Farrington et al., 1998),

B. Biochemistry Methodology

1. *Phlebotomy and assays.*

Blood was drawn from the caudal vein of Atlantic cod (*Gadus morhua*) using a non-heparinized 18-gauge stainless steel syringe needle fitted to a 5 ml plastic syringe. All fish were wrapped with sea water soaked towels that specifically covered their eyes to reduce escape activity. In addition, the towel provided a way to hold each animal securely.

Drawn whole blood was immediately prepared as four sub-samples for microhematocrit measurements, lactate measurements, plasma separation and serum separation. Blood was loaded into heparinized microhematocrit capillary tubes, kept at 4° C (Biron and Benfey, 1994) until spun at 6,400 x g for three minutes and then read to determine the percentage of red blood cells contained in whole blood. Samples for lactate analysis (Sigma Diagnostics □ Procedure No. 826-UV, St. Louis, MO) were deproteinated by adding 500 ul of whole blood to 1.0 ml of ice cold 8% perchloric acid. This solution was kept on ice for at least ten minutes to ensure complete protein denaturation before centrifuging at 300 x g for ten minutes to pellet cellular debris. The supernatant was transferred to cryovials and frozen immediately in liquid nitrogen. Plasma samples were obtained by spinning heparinized blood samples at 300 x g for five minutes to remove blood cells. Plasma samples were used to determine the soluble protein concentration in the non-cellular portion of the blood (Pierce BCA □ protein assay reagent kit 23225). Serum samples were obtained by allowing whole blood to clot (at least 30 minutes) and then centrifuging at 300 x g for five minutes. Serum samples were used to determine glucose (Sigma Trinder □ Procedure No. 315, St. Louis, MO), cortisol (outsourced to IDEXX Veterinary Services, Grafton, MA), chloride ion, sodium ion and potassium ion concentration (Baxter/AMDEV Lytning 5 Analyzer, Baxter Lytning Systems, Inc.) and osmolality (Fiske One-Ten freezing point depression osmometer) in the non-cellular portion of the blood. All supernatants were transferred to cryovials and immediately flash frozen in liquid nitrogen. All blood products were transferred to and kept in a -80 ° C freezer until analysis.

2. Longline and survival (72-hour)

Fish from each longline were randomly chosen for blood sampling as the gear was being hauled on board. Fish were removed by snubbing with the gaff on the “crucifier” (*snub*) or by flipping the fish over the immobilized hook (*flip*). It was then wrapped in a seawater soaked towel and blood was drawn. The time elapsed from when the fish broke the surface of the water to the completion of blood sample collection was routinely less than one minute. Although fish were sampled from the beginning, middle, and end of each haul-back operation, fewer fish were bled on the first two sea days so that more fish could be used to observe the post-capture survival at 72 hours. Once bled, fish were discarded and not used to assess 72-hour survival statistics.

Blood was also drawn from fish that survived being held in cages on the sea bottom for 72 hours. After the cages were retrieved, fish were immediately placed into on-deck holding tanks that were aerated and maintained at bottom sea temperatures to minimize aerial exposure and bottom to surface temperature discrepancies. Blood was collected from as many of these fish as was possible within 20 minutes of landing the cage on deck. In the majority of the hauls, all living fish were sampled. Twenty minutes was chosen based on the earliest time observed for serum cortisol response in fish (Roche and Bogé, 1996; Einarsdóttir and Nilssen, 1996).

3. *Normal and Control values*

Control animals for the survival portion of this study were jig-caught cod whose blood was sampled quickly. Cod were hauled from the water by hand, rapidly and carefully removed from the gear and bled within one minute of being hooked. The physiological parameters that were evaluated in this study, especially the primary response from cortisol, typically require longer than 10 minutes to detect a significant concentration change in the blood (Lowe and Wells, 1996; Ryan, 1995; Biron and Benfey, 1994). Within three minutes, catecholamines will only have just begun to affect the osmotic condition or concentration of ions in the blood (Mazeaud and Mazeaud, 1981). Since blood was obtained within one minute after just being in its natural habitat, cod captured and sampled in this manner were considered a good model to determine the molecular profile one would expect to find under “normal” conditions. This expectation assumed that the captured fish was not ill or had not been engaging in any activity that may have otherwise altered these parameters, such as active predation or predator avoidance. Damage to the jigged fish was restricted to the hole left by the hook, typically in the soft flesh of one jaw. Any animals that were gut hooked, snagged, or dropped on the deck were not used. Although gill condition was monitored as the hook was being removed from jigged cod, morphometric measurements were not taken until after the blood was sampled to minimize handling prior to taking blood. Accordingly, fish that were immediately bled from the hand line were assumed to represent the “normal” basal state of the animal. Jigged-cod that were placed in cages for 72 hours represented the “controls” for the survival study.

C. Biochemistry Statistics

Blood parameters were analyzed using the JMP statistical package (SAS Institute Inc. copyrighted 2001). Data from all cages were combined regardless of slight differences in recovery time. The influence of fish length on blood parameters was analyzed using a linear regression that revealed no significant impacts and that the animals were grouped randomly irrespective of length.

Tests of the impacts of hook removal protocol and subsequent recovery on blood parameters were conducted on samples that were divided by treatment and by injury level. This resulted in six treatment/injury groups (snub, flip, jig or normal/baseline, snub/cage, flip/cage, and jig/cage or control), that were used in the remainder of the analyses. “Flip” represents the grouping of cod that were removed using the Leach tail-flip protocol. “Snub” represents the grouping of cod that were mechanically removed as the gear was being hauled and typically sustained injuries such as a broken jaw. “Flip/cage” and “Snub/cage” represent subsets of cod from the groups mentioned above that were not previously bled but were retained in cages at fishing depths for 72 hours to assess short-term survival. “Jig” represents the grouping of cod that were individually jigged at fishing depths and immediately bled. Blood was obtained in vacutainer tubes within one minute of hooking by jig and results are considered to represent the *Normal* blood profiles of Atlantic cod (Farrington et al., 1998). Jig/cage or *Control* represents the grouping of cod that were individually jigged, removed carefully from gear and placed in

cages with Flip/cage and Snub/cage animals for 72 hours at fishing depths to assess survival.

Differences between these groups were tested by using one way analyses of variance (ANOVA) or the Kruskal-Wallis test as indicated by the distribution of the data. If significant differences were found, Tukey-Kramer tests ($\alpha=0.05$) were conducted to test all combination of pairs for significant differences among the means. These comparisons are used for reporting in the Results Section (D).

D. Results

Complete biochemistry spreadsheets for the June 2001 cruise can be found in the Appendix

Fish used to obtain blood samples ranged from 31 to 50 cm total length (Table 2). Although the groups of cod bled immediately from the longline were significantly ($\alpha=0.05$) different in length than the surviving jig or flip fish, there was no indication that the magnitude of any of the parameters measured was correlated to the range of sizes found in this study.

Table 2. Lengths of fish used to obtain blood samples within treatment groups.

Treatment	Range (cm)	Average \pm Standard Deviation (cm)
Jig	32 to 50	46.2 \pm 3.32
Flip	31 to 50	44.3 \pm 3.49
Snub	38 to 48	44.4 \pm 3.49
Jig/cage	39 to 50	46.6 \pm 2.77
Flip/cage	41 to 50	46.6 \pm 2.73
Snub/cage	44 to 50	46.7 \pm 1.72

For the combined studies (SK ID# NA86FD0108 and NA06FD0177), blood was collected from a total of 241 live fish. Each biochemical parameter was represented by at least 86% of the total catch of fish being measured across each treatment. The experimental data examined here excluded 20 snub cod that were treated with potassium ion for survival enhancement.

Jigged fish, without exception, had low serum cortisol levels ranging from, just above the detection limit (<0.2 ug/dL) to undetectable. Half the analyses returned a value of 0.2 or 0.3. These values were defined as normal data for analysis. The highest mean values of serum cortisol were obtained from fish directly off the longline regardless of dehooking procedure (Figure 6). No significant differences between snub or flip treated fish were found but these values were significantly elevated ($p<0.0001$) over the measurable levels of cortisol in jigged or normal cod. After 72 hours, these values had decreased enough to no longer significantly differ from the normal values. Control or jigged fish that were

caged presented values similar and indistinguishable from the longlined fish but were significantly different than the normal values represented by jigged cod prior to caging.

Plasma protein concentrations measured in snub and flip fish were significantly ($p < 0.0001$) elevated compared to jig fish (Figure 6). Although all caged fish exhibited mean values that were depressed from jig fish none were significantly different.

Hematocrits taken from cod immediately after being removed from the longline were significantly elevated ($p < 0.0001$) from the jigged fish (Figure 6). After 72 hours these values had recovered somewhat but only the snub fish were indistinguishable from the jigged fish. Notably the caged jigged fish were also significantly elevated over initial values after 72 hours.

Snub and flip fish bled immediately after being removed from longline gear showed significantly ($p < 0.0001$) higher serum lactate values over jig cod (Figure 7). Although the lactate values from snub and flip fish had decreased 72 hours later they were still significantly different ($p < 0.0001$) than the values obtained from jig cod. In addition lactate values obtained from snub fish were significantly ($p < 0.0001$) greater than all caged fish. Again, caged jigged fish were significantly ($p < 0.0001$) elevated over initial values.

Although the mean serum glucose values from both snub and flip fish were elevated over those obtained from jig fish, only the values from snub fish were significantly ($p < 0.0001$) greater than jig fish (Figure 7). After 72 hours, all cod survivors had glucose values indistinguishable to each other and to jig fish.

Serum osmolality measured in all cod taken directly from the longline showed considerable and significantly ($p < 0.0001$) greater values than jig cod (Figure 7). After 72 hours, values from both snub and flip cod (snub/cage and flip/cage) had returned to levels indistinguishable to jig cod. Although caged/jig cod exhibited serum osmolalities statistically similar to all other fish that survived in cages for 72 hours, they were significantly elevated from initial jig values.

Serum sodium ion levels measured in all cod taken directly from longlines showed considerable and significantly ($p < 0.0001$) greater values than jig cod (Figure 8). After 72 hours, snub/cage and flip/cage cod did recover but were still significantly ($p < 0.0001$) greater than initial jig cod values. Jig/cage values for serum sodium ion were similar to snub/cage and flip/cage.

Serum chloride ion levels exhibited similar patterns as the sodium ion except that flip/cage mean values, although somewhat elevated, became statistically indistinct from initial jig values (Figure 8)

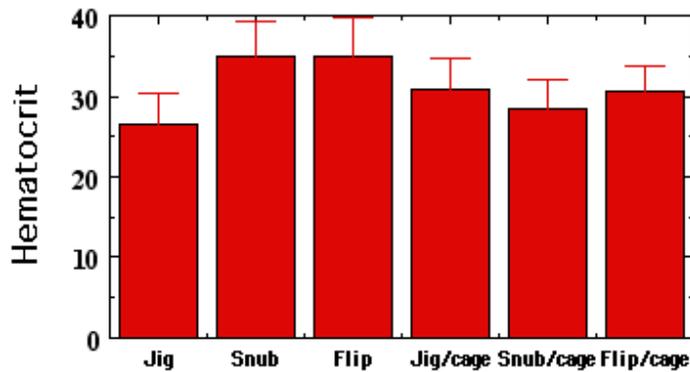
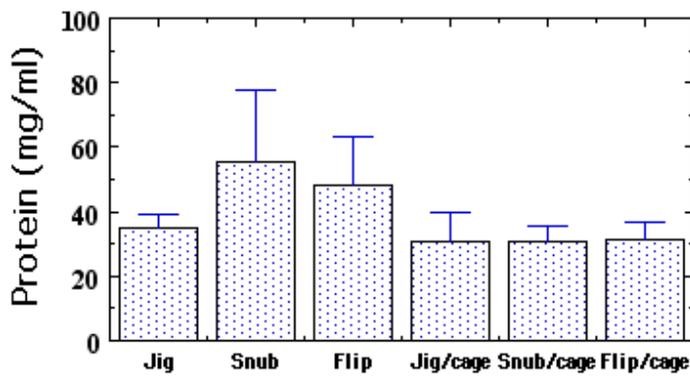
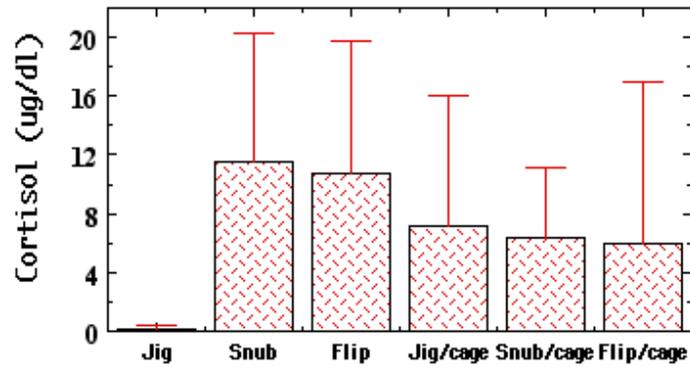


Figure 6. Comparison of cortisol concentration, protein concentration and hematocrit level in blood of sublegal-sized cod. Snub and flip samples are taken from cod immediately after removal from the longline as it is hauled. Flip/cage and Snub/cage represent samples taken from cod that have survived 72 hours after longline capture and have not been previously bled. Jig fish have been caught individually and bled within one minute of hooking. These most likely represent Normal values of blood components in cod. Jig/cage represent samples from fish that have survived 72 hours after capture and are used as the Control for these experiments. Sublegal-sized cod were obtained in the NW Atlantic in June 2001. Error bars represent one standard deviation of the means.

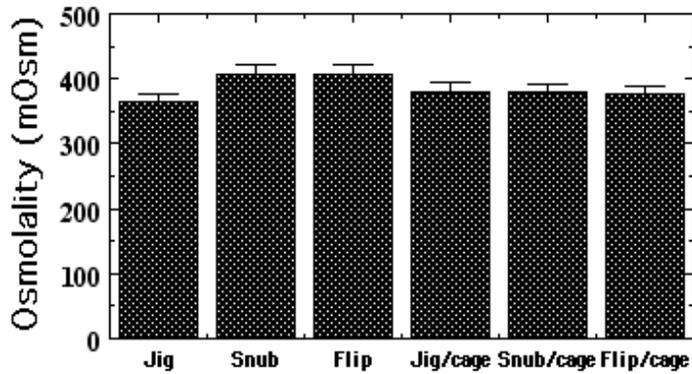
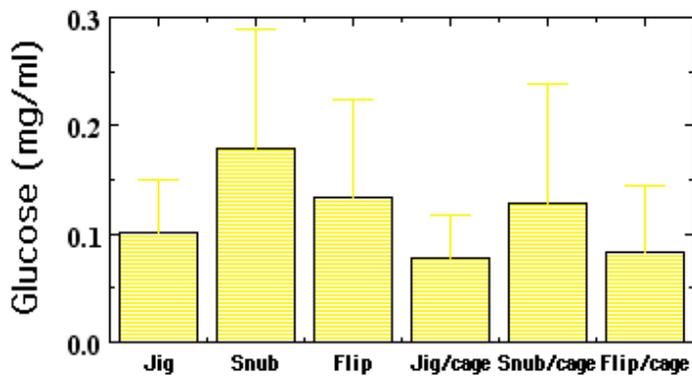
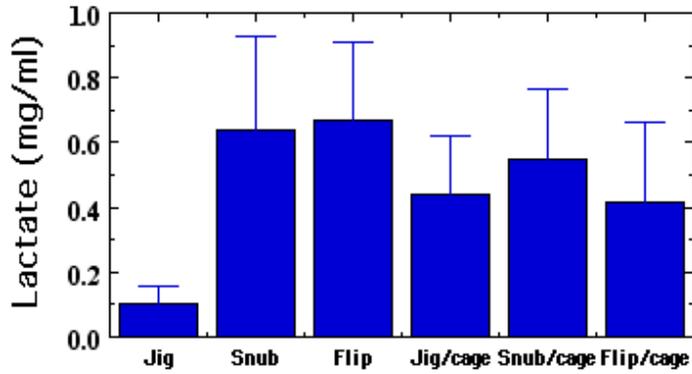


Figure 7. Comparison of lactate concentration, glucose concentration and osmolality in blood of sublegal-sized cod. Snub and flip samples are taken from cod immediately after removal from the longline as it is hauled. Flip/cage and Snub/cage represent samples taken from cod that have survived 72 hours after longline capture and have not been previously bled. Jig fish have been caught individually and bled within one minute of hooking. These most likely represent Normal values of blood components in cod. Jig/cage represent samples from fish that have survived 72 hours after capture and are used as the Control for these experiments. Sublegal-sized cod were obtained in the NW Atlantic in June 2001. Error bars represent one standard deviation of the means.

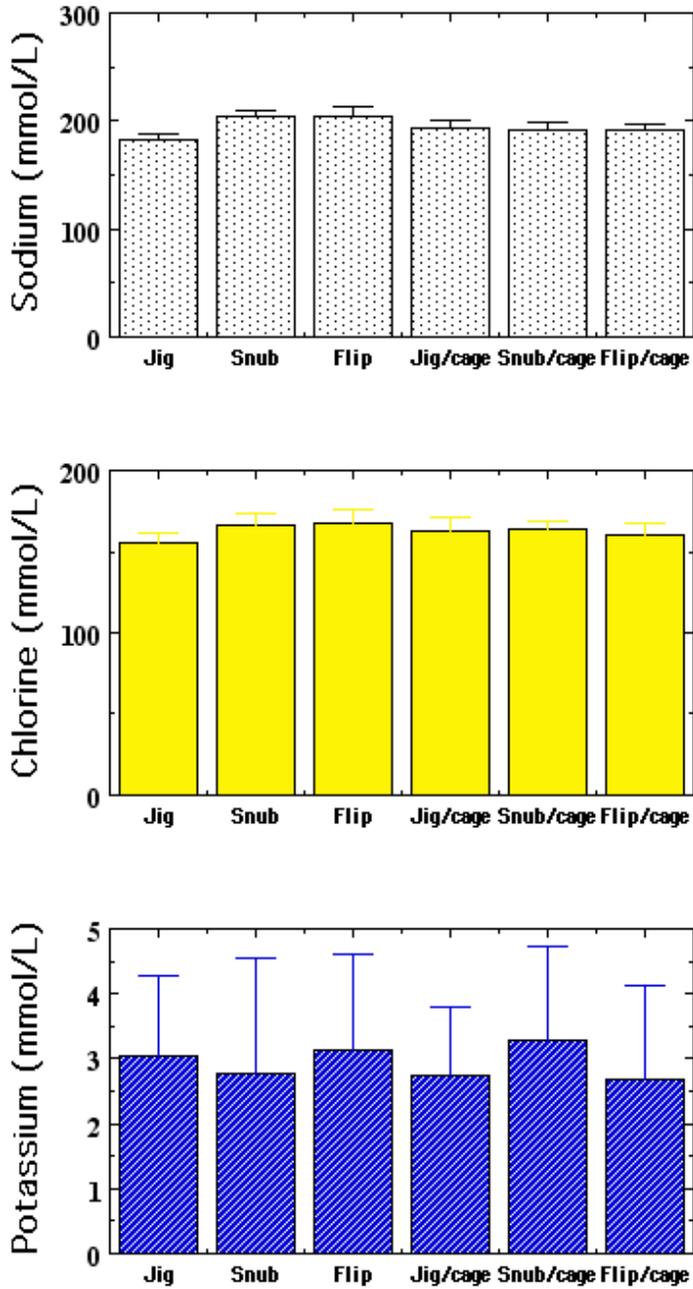


Figure 8. Comparison of ion concentration levels in the blood of sublegal-sized cod. Snub and flip samples are taken from cod immediately after removal from the longline as it is hauled. Flip/cage and Snub/cage represent samples taken from cod that have survived 72 hours after longline capture and have not been previously bled. Jig fish have been caught individually and bled within one minute of hooking. These most likely represent Normal values of blood components in cod. Jig/cage represent samples from fish that have survived 72 hours after capture and are used as the Control for these experiments. Sublegal-sized cod were obtained in the NW Atlantic in June 2001. Error bars represent one standard deviation of the means.

Although serum potassium ion levels exhibited a great deal of deviation in all fish, there were no measurable differences seen between any treatment groups (Figure 8).

Previous work on Atlantic cod indicated that changes in one blood parameter correlated to an effect on other blood parameters (Robinson et al., 1993). Spearman's rank-Order Correlation Analysis revealed that sodium ion co-varied with osmolality and chloride ion.

E. Conclusions

Since no benefit was ascribed to the survival of sublegal-sized fish by using an alternate dehooking protocol, it is difficult to use the biochemical data as it was originally intended. However, the modifications found in cod blood profiles have proved interesting.

The low levels of blood parameters in jig fish established that jigging was an effective means of producing "control" fish, and that the longline capture process induced biochemical reactions in cod regardless of dehooking technique. Also, elevated levels of stress factors measured in jigged fish after caging illustrated that the experimental holding technique may be inappropriate for survival assessment.

The use of blood chemistry to assess stress has been questioned by some researchers. Results show large differences between cod caught with a minimum of intervention (jigging) and longline-caught, as well as the effect of caging, suggest that blood chemistry is an accurate measure of stress in cod. Further, our approach establishes that the blood profiles of jigged cod represent a close approximation of "typical" sublegal cod. This information may be useful in other survival studies as biochemical reference points for cod.

Blood profiles in sublegal-sized cod resulting from longline capture and subsequent survival were remarkably similar in magnitude and response to handling as in previous studies except for the potassium ion results. Contrary to Milliken et al. (1999), potassium ion concentrations did not decrease with severity of wound. Instead there was no significant difference between either approach used to take fish off longline gear. Decreased potassium levels were attributed to blood loss in the first study (SK 95-NER-141). The absence of a potassium ion collapse in this study supports the idea that using the upright rollers instead of the block and tackle may have led to less severe injuries or injuries emphasizing more rapid blood coagulation and less blood loss.

The biochemical data also support the previous conjecture that some aspect of the caging and survival protocol may have skewed survival analyses. By comparing the blood profiles obtained from jig cod to jig/cage cod, it is apparent that the three days spent in the cage were very different physiologically than swimming in the ocean.

Elevated cortisol levels is one of the first responses to stress seen in fish (Mazeaud et al., 1977; Hazen and Balment, 1997, Wendelaar Bonga, 1997). Cortisol values obtained from

jig/cage cod were significantly elevated over initial values. Jig fishing and on-deck handling no doubt initiated this cortisol response but three days in a relatively unmolested environment did not alleviate it. Recovery in cod is either much longer than three days or the environment was not as innocuous as was assumed.

In addition, osmolality, sodium ion and chloride ion in jig/cage cod were significantly elevated ($p < 0.001$) over initial values. This is not surprising since adjustments in the electrolyte balance are an interrelated secondary response to corticosteroid induction. Also, hematocrit values in jig/cage cod were elevated over initial values, a further response tied to cortisol secretion (Wendelaar Bonga, 1997; Hazen and Balment, 1997).

Whole blood lactate levels were considerably elevated in fish just off the longline suggesting recent strenuous activity, no doubt related to physical restraint and struggling against the haul. In fish, lactate sequestered in muscle cells can discharge to the blood for up to 24 hours (Hoag, 1975; Wood et al., 1990; Gustavson et al., 1991). Nevertheless after 72 hours, no residual lactate should remain in the tissues. Significantly elevated lactate values in all caged cod including jigged cod suggests a response to some sort of physical activity while captive such as swimming against a current. This obliged activity added to managing post-capture handling could conceivably skew significance in the data that would otherwise prove a remedial protocol valid.

One final observation that should be mentioned is the categorically low serum cortisol values obtained from fish that were caught individually and immediately bled. Cortisol results reported here are consistent with previous reports (SK 95-NER-141) where all cortisol values obtained from jig fish were below the detection limit of the assay ($< 1 \mu\text{g/dL}$). Cortisol values for this study hovered near the lower detection limit of $< 0.2 \mu\text{g/dL}$ with 50% of the values at 0.2 or 0.3 and 50% being undetectable. The rapid methodology used to obtain these blood samples from wild stock argues that cortisol values in normal free swimming cod are very low. This result has important ramifications for all captive studies. Cortisol is the major corticosteroid secreted by saltwater teleosts. Cortisol concentrations are responsible for eliciting changes in energy metabolism, ion regulation and enzyme activity for intermediary metabolism in the liver. Regarding the stress response, cortisol is hyperglycemic through stimulation of glycolysis and gluconeogenesis. It will also stimulate the enzyme activity of Na^+/K^+ ATPase, the driving force in ion transport in brachial chloride cells. Long term consequences of elevated cortisol levels result in higher mortality rates due to increased susceptibility to disease driven by immunosuppression, decreased growth rates, and lower reproductive success. Accordingly, field research becomes extremely important in physiological studies especially for fisheries management purposes.

VII. General Conclusions

Despite our search for confounding factors, it is possible that careful handling or potassium supplementation may not yield higher survival rates. We caution, along with Neilson et al. (1989) and Farrell et al. (2001a) that assessment of the relative mortality of fishing types, handling techniques or post-capture treatments is at best difficult. Multiple iterations of experimental design are often required to determine actual rates of mortality. Unfortunately the expense of field research rarely offers this opportunity.

It is precisely this expense that drove a reexamination of the data gathered in the June 2001 field season. It has already been noted that the number of fish obtained for the protocol study may have limited the significance found between groups. Coincidentally, additional snub data were compiled that tested the effectiveness of potassium treatment on survival. The survival of snub fish treated with potassium ion was no different when compared to the snub figures used above (For complete information see SK ID#NA06FD0177; Appendix). In addition, biochemical analysis did not reveal any statistical differences in the mean potassium blood chemistries (SK ID#NA06FD0177; Appendix). In other words, these fish were no different in their survival or biochemistry than the snub fish previously used to determine significance. Accordingly, the potassium-treated snub cod survival figures were added to the seawater snub data (Table 3) and reevaluated for significance.

Table 3. Survival data that include snub figures from potassium treated fish.

Treatment	Alive	Dead	Total
Snubbed:	33	85	118
Flipped:	32	49	81
Total	65	134	199

Using these data, thirty-three or 28% of the snubbed fish were alive upon cage retrieval; thirty-two or 39.5% of the flipped fish were alive. Using the G-test for independence ($\alpha=0.1$), the survival of cod in these data were found to be dependent on the dehooking protocol ($G_{\text{adjusted}} = 3.20$, $df = 1$, $p = 0.074$). Fish removed from hooks by the Leach flip protocol demonstrated survival significantly greater than snubbing the fish from the hook.

It may seem here that the alpha level was being manipulated in order to obtain significance. However, relaxing the confidence interval was justified considering the data represent physiological distributions. The higher p-value is defensible considering the large biochemical differences seen among individuals within treatment categories. We have included the exact p-value for Table 3 to allow readers to judge for themselves. Nonetheless, significance at the $\alpha=0.1$ level is not meaningless and advocates further empirical investigation.

VIII. Significant Problems

A. 1999

Both PIs underwent personal family crises that required their attention and shifted work to the next field season.

B. 2000 Cruise

Serious absence of cod in fishing grounds that were historically productive precluded the acquisition of enough data for analysis.

Reproducing the “flip” technique over a higher water line than the usual in the longline fleet proved difficult to execute and recover the fish. In addition, the gunwale height is important for the technique because the angle of hauled gear to the gunwale is important in order to execute the movements cleanly.

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Appendix

Sample	MDM F #	Notes	Injury Type	Treatment/handling	length	Cl mmol /L	hematocrit	K mmol /L	Na mmol/ L	Osmolality mOsm	cortisol ug/dl * = Undetectable (< .2 ug/dl)	glucose mg/ml	lactate mg/mL	protein mg/mL
204				TLC	48	156.6	32	2.65	190.3	392.0	10.5	0.08	0.48818 14	43.68
205		No OS, small clot		TLC	45	160.9	25.5	1.14	191		10.2	0.09	0.38918 329	34.96
206				TLC	46	167.5	33	2.45	201.6	409.0	28.8	0.10	0.68074 2335	34.79
207		Slightly hemolized		SNUB	48	160.8	39.5	0.96	199.8	402.0	24.3	0.11	0.78842 6025	51.64
208		Slightly hemolized		SNUB	48	160.7	43	0.92	193.6	380.5	4.7	0.11	0.28596 1005	48.54
209		Small clot		SNUB	45	164.4	38.5	1.60	200.8	404.5	37.6	0.09	0.73256 063	39.15
210		No Cortisol (*air in sample- electrolyte assay)		SNUB	48	148	40	1.76	208.4	384.5		0.09	0.55225 0587	67.13
211		Medium clot (*air in sample- electrolyte assay)		SNUB	44	166	36	1.63	200.1	390.5	8.7	0.02	1.07631 6865	33.08
212				TLC	45	165.5	38.5	3.81	199.5	407.0	7.4	0.07	0.85372 7717	31.71
213		Medium clot, only 1 Hct		TLC	47	168.8	38	1.88	198.8	400.0	11.6	0.15		49.16
214		Medium clot (*air in sample- electrolyte assay)		TLC	44	160.5	39	2.69	203.1	384.5	25.3	0.06	0.44603 6367	46.17
215		Large clot,no CL, no OS		TLC	46		44					0.13	0.63589 4115	87.20
216				TLC	43	177.4	41.5	3.34	214.4	411.5	37.3	0.03	0.88080 7035	44.35
217		Large clot,no CL		TLC	48		39.5			401.0	37.2	0.37	1.07230 71	47.83
218		(*air in sample- electrolyte assay)		SNUB	40.5	163.7	40	5.24	198.7	415.0	2.9	0.07	0.69552 7925	69.26
219		Slightly hemolized		TLC	43	169.1	40.5	3.47	203.1	427.5	4.3	0.05	0.56518 0515	49.93
220		Medium clot (*air in sample- electrolyte assay)		TLC	46	147.7	43	1.30	222.8	452.5	8.6	0.07	0.61380 15	45.28
221		(*air in sample- electrolyte assay)		SNUB	41	150.8	43	2.10	221	410.0	19.4	0.12	0.61371 0262	78.64
222		Very small clot		SNUB	44	175.2	33	6.40	202.9	416.0	10.5	0.11	0.55783 5823	47.66
223		Red blood cell mixed in		SNUB	44	167.7	31.5	2.66	209.3	412.0	7	0.10	0.50313 5208	24.69
224				SNUB	44	168.6	36	1.14	200.8	409.5	12.3	0.08	0.57482 9978	33.25
225				SNUB	38	165.8	41	2.30	207.3	423.0	5.9	0.09	0.43763 8717	61.27
226		Hemolized		TLC	40	170.3	36.5	2.92	204.5	433.0	6.5	0.07	0.58315 1417	42.39
227		Medium clot (*air in sample- electrolyte assay)		TLC	44	173	36	2.23	203.7	416.0	17.5	0.06	0.56899 351	61.51
228		Lactate 430 ul		SNUB	46	170.4	35.5	4.86	202.3	447.0	8.6	0.09	0.47979 0833	65.37

229	Protein sample in #229 glucose with green cap			TLC	42	171.5	30.5	6.99	200.6	414.0	8.2	0.07	0.61602		
													381	43.70	
230				TLC	47	170.9	37.5	2.70	206.1	406.5	19.4	0.09	0.79140		
													3913		
231	No OS (*air in sample- electrolyte assay)			TLC	43	151.2	35	4.22	223		8	0.08	1.01527		
													408	50.02	
232	Slight clot (malfunction, No electrolytes)			TLC	46		37	*		398.5	3	0.05	0.87109		
													2677	54.75	
233				TLC	45	164.5	33.5	3.76	194.3	403.0	11.9	0.18	0.82757		
													5957	37.07	
234				TLC	50	170.9	41	1.17	205.6	406.0	10.7	0.20	1.01201		
													2907	41.46	
235				SNUB	44	170	37.5	0.84	206.4	398.0	1.5	0.15	0.70028		
													7053	147.33	
236	No red tops, total serum clot			SNUB	42		32.5						1.12251		
													0983	50.53	
237				TLC	42	170.9	35.5	1.06	205.8	403.0	11.1	0.18	1.06979		
													4373	116.60	
238				SNUB	43	173.8	37	0.88	216.4	400.0	9.8	0.17	1.04433		
													2103	42.22	
239	No cortisol (*air in sample- electrolyte assay)			TLC	46	164.4	37	2.28	198.7	412.0		0.27	1.29069		
													7787	45.28	
240				TLC	47	173.3	39.5	0.79	211.2	420.5	13.3	0.16	0.50754		
													3947	58.68	
241				SNUB	45	173	36.5	1.03	205.2	405.0	3.2	0.15	0.83223		
													4187	46.13	
242				SNUB	43	172.5	38	0.70	208.1	414.5	12.4	0.17	0.58541		
													8273	41.62	
243	Small to medium clot (*air in sample- electrolyte assay)			SNUB	46	162.1	38.5	1.15	199.4	407.0	10.7	0.3	1.02216		
													63	91.87	
244	No red tops, total serum clot			SNUB	48		39.5						0.61525		
													8545	98.72	
245				SNUB	47		37			393.0	20.5	0.33	1.81758		
													4317	68.61	
246	Slightly hemolized			SNUB	44	174.1	40.5	1.42	207.5	426.5	12.8		0.59348		
													81	94.39	
247	Small clot (*air in sample- electrolyte assay)			SNUB	41	157.4	35.5	1.25	209.4	421.0	13.1	0.27	0.40501		
													1727	38.50	
248	(*air in sample- electrolyte assay)			SNUB	38	156.4	42.5	1.72	202.6	424.0	13.4	0.25	0.33980		
													2653	68.09	
249				SNUB	48	170.7	35	1.04	206.6	414.0	23.4	0.26	0.58522		
													4983	51.46	
250				SNUB	47	165.5	27.5	2.69	198.2	391.0	34.9	0.52	0.38112		
													8967	70.20	
251	Slightly hemolized (*air in sample- electrolyte assay)			TLC	44	170.1	37	2.35	207.8		16	0.24	0.39284		
													144	47.42	
252				SNUB	47	174.9	30.5	5.65	200.7	407.0	19.1	0.24			
														41.16	
253	Large clot, no cortisol			SNUB	47	170.2	38.5	1.83	208	402.5		0.20	0.53662		
													898	38.06	
254	MDM F # 27	No Hct #2 reading		lower jaw broken	C/SNUB	48	155.7	25	2.18	181.2	362.5	2.3	0.13	1.03408	
													5233	28.23	
255	MDM	Medium clot (*air in sample- electrolyte		lower jaw	C/TLC	48	134.6	29	2.83	192.5	346	0.2	0.07	0.27143	28.86

F # 31	assay)	puncture											1343	
MDM	Medium clot (*air in sample- electrolyte	mid-low jaw											0.24436	
256	F # 20	assay)	puncture	C/TLC	48	148.4	29.5	2.58	195.3	375.0	1.6	0.11	1737	36.86
MDM			left lower jaw										1.43393	
257	F # 18	Medium clot	puncture	C/TLC	49	161.1	26	1.82	187.8	362.5	0.2	0.10	06	24.85
MDM	Large clot, no cortisol (*air in sample-	right upper jaw											0.22371	
258	F # 13	electrolyte assay)	torn	C/TLC	44	159.8	27.5	2.56	194.3	374.5		0.18	1017	20.22
MDM			right jaw										0.33746	
259	F # 30	No red top sample, Large clot	puncture	C/TLC	47		35						9617	44.37
MDM													0.23997	
260	F # 12	Large clot, cortisol sample only	left jaw puncture	C/TLC	50		28				1.3		45	34.97
MDM	Large clot (*air in sample- electrolyte	left upper jaw											0.44002	
261	F # 14	assay)	puncture	C/TLC	50	151	32	2.71	187.5	392.0	2.5		0553	38.88
													0.60893	
262				TLC	46	172.3	36	2.63	205.6	409.0	6.8	0.11	7543	46.83
													0.38292	
263				SNUB	47	166.3	33	1.50	198.2	399.5	8.3	0.16	925	34.78
													0.56463	
264				SNUB	47	171.1	32	2.25	204.1	407.5	15.5	0.23	2285	57.96
													0.51444	
265				TLC	44	163.2	36.5	1.48	199.3	388.5	5.8	0.11	1337	47.74
266		(*air in sample- electrolyte assay)		SNUB	43	160.9	31	3.02	199.9	387.0	0.9			61.14
													0.10395	
267				TLC	45	171.6	32.5	2.18	204.9	408.0	10.9	0.13	956	48.21
													0.54791	
268				SNUB	46	169.1	30.5	1.05	204.1	399.5	11.2	0.21	6697	60.57
													0.69093	
269				TLC	46	166.5	34	2.93	201.6	406.5	8.7	0.21	7343	44.53
													0.85588	
270				TLC	46	196.3	31.5	2.02	234.9	418.0	10.8	0.15	4677	75.83
													0.44567	
271		Was snubbed prior to catch		TLC	42	167.7	23.5	1.74	200.7	394.5	5.3	0.22	589	35.98
		serum cloudy (*air in sample- electrolyte												
272		assay)		SNUB	38	151.7	25.5	4.60	198.9	404.5	17.2	0.30		63.85
													0.65712	
273		(*air in sample- electrolyte assay)		SNUB	44	162.9	31	2.52	204	422.0	13	0.12	2897	44.59
													0.41683	
274				TLC	41	168.8	30	5.41	200	401.0	7	0.06	3443	50.76
													0.71003	
275				SNUB	46	172.9	31.5	2.27	210.2	407.0	12.7	0.18	003	58.43
													0.51483	
276				SNUB	42	165.5	33	3.94	197.9	394.5	10.2	0.25	0503	45.92
													0.52261	
277		(*air in sample- electrolyte assay)		TLC	42	160.8	32.5	4.26	204.1	391.5	9	0.17	4347	50.28
													0.09457	
278				JIG	42	155.1	27	4.69	181.7	361.0	0.4	0.11	8305	42.06
													0.07382	
279		Estimated length = 46, No cortisol		JIG		159.8	27.5	5.44	182.6			0.16	4903	35.19
													0.11203	
280				JIG	47	157.4	29.5	3.01	181.9	365.0	0.2	0.15	5232	38.75
281		NO SAMPLE		JIG										

282	Large clot, cortisol only	JIG	49	18.5					0.5		0.10214				
											137	42.75			
283	(*air in sample- electrolyte assay)	JIG	40	158.3	29.5	2.49	186.2	375.0	1	0.14	0.10246	2583	24.35		
284	Medium clot (*air in sample- electrolyte assay)	JIG	50	156.8	23.5	2.85	185	375.0	0.2	0.16	0.08367	0043	36.95		
285	MDM F# 45	right lower jaw broken	C/SNUB	48	160.8	28	2.48	185.7	363.0	7.1	0.09		30.72		
286	MDM F# 92	right jaw puncture	C/SNUB/K	48	159.4	27.5	4.26	184.9	376.5	6.2	0.06	0.21223	603	38.09	
287	MDM F# 42	? (control-jigged)	C/TLC	46	156.6	27	1.65	186.1	371.0	23.8	0.11	0.15790	5363	29.02	
288	NO TAG/ 80	lower jaw puncture		39	162	25	3.48	188.1	378.0	1	0.10	0.45729	2955	27.60	
289	MDM F# 37	? (control-jigged)	C/JIG	40	163.4	32	2.07	190.7	395.5	11.9	0.15	0.30454	677	31.00	
290	MDM F# 77	Large clot, no cor, no OS (*air in sample- electrolyte assay)	lower jaw broken	C/TLC	45	155.5	23	2.19	193		0.16	0.50039	096	29.90	
291	MDM F# 43		C/TLC	48	164	24.5	2.23	193.6	380.0	1.5	0.16	0.64317	586	40.64	
292	NO TAG	Large clot, cortisol sample only					37.5			1.6		0.55148	8827	36.20	
293	MDM F# 54	Medium clot, not enough sample for OS assay	lower left jaw torn	C/TLC	41	168.4	32	1.63	198.5	1.2	0.06	0.73736	532	30.62	
294	MDM F# 32	Large clot, not enough sample for OS assay (*air in sample- electrolyte assay)	right side (control- jigged)	C/JIG	49	143.9	29.5	2.14	205.7	36.6	0.11	0.51587	9243	23.40	
295	MDM F# 87		lower right jaw torn	C/SNUB	45	164.7	28.5	5.17	191.1	385.0	2	0.04	0.46702	5473	30.27
296	MDM F# 89	Medium clot (*air in sample- electrolyte assay)	lower left jaw broken	C/SNUB/K	41	165.5	30.5	2.90	194.6	391.5	14.2	0.11	0.63148	772	28.25
297	MDM F# 50		right jaw puncture	C/TLC	47	157.5	28.5	2.54	185.7	367.0	0.9	0.12	0.17690	9487	40.36
298	MDM F# 65	Small clot	lower right jaw broken	C/SNUB/K	44	157.9	29	2.82	185.9	372.0	0.2		0.28653	9617	27.84
299	MDM F# 84		puncture under eye	C/SNUB/K	41	157.8	29.5	4.10	183.4	374.0	10.4	0.10	0.15098	341	22.96
300	MDM F# 78		left lower jaw torn	C/SNUB/K	46	160.8	29.5	3.68	188.7	371.0	0.7	0.09	0.37945	368	27.71
301	MDM F# 33		right side dorsal (control- jigged)	C/JIG	47	161.1	25.5	3.43	188.5	360.0	9.5	0.14	0.19780	2617	23.42
302	MDM F# 39	Large clot, no NaK	? (control- jigged)	C/JIG	44		27.5		372.0	0.2	0.13	0.46124	3457	46.03	
303	MDM F# 53	Large clot, no Cl, Glu, OS	left upper jaw torn	C/SNUB	48		31.5			1.9		0.63679	8277	35.01	
304	MDM F# 38	(*air in sample- electrolyte assay)	? (control- jigged)	C/JIG	49	146.1	30	4.48	191.4	382.0	3.5	0.79	0.59973	7487	31.58
305	MDM F# 41	Small clot	? (control- jigged)	C/JIG	44	170	22.5	2.64	196.5	380.5	0.9	1.32	0.43069	8327	28.58
306	MDM F# 56	Small clot (*air in sample- electrolyte assay)	lower jaw broken	C/SNUB	47	164.1	28	4.69	191.7	367.0	11.7	0.97	0.70921	201	37.69
307	MDM F# 40	Small clot	? (control- jigged)	C/JIG	47	170.7	32.5	3.71	198.1	380.5	6.3	0.74	0.54176	087	31.80

308	NO TAG				173.7	32	6.01	202.4	399.0	25.1	0.02	0.10326	
	MDM											8123	27.84
309	F# 51	Green top hemolized	left lower jaw torn	C/SNUB	45	168.1	32.5	5.90	195.5	401.0	1.4	0.68088	
	MDM			C/SNUB/								1367	29.67
310	F# 88	Medium clot	puncture	K	43	166.4	27.5	3.30	194.9	413.0	6.3	0.82626	
	NO TAG											511	26.75
311	MDM					166.7	27	4.31	193.4	389.0	5.9	0.54735	
	F# 34	Small clot	? (control-jiggged)	C/JIG	50	165.7	35.5	2.89	196.5	369.0	1.2	0.61713	
312	MDM											4333	32.92
313	F# 48	Green top hemolized	puncture	C/TLC	41	164.5	30	7.34	195.9	383.0	1.7	0.33692	
												69	29.38
314				TLC	47	165.9	35.5	4.21	203.2	411.0	1.3	0.57652	
												8477	42.08
315				SNUB	47	169.7	29.5	4.15	203.4	413.5	*	0.44439	
												8633	37.36
316				SNUB	44	167.9	36.5	4.96	202.5	437.0	0.8	0.58167	
												2587	63.02
317		(*air in sample- electrolyte assay)		TLC	46	161.4	31.5	3.26	213	418.5	2.4	1.04785	
												477	49.62
318		Medium clot		SNUB	45	168.3	30	5.33	196	398.0	0.2	0.17176	
												892	41.21
319		(*air in sample- electrolyte assay)		SNUB	47	164.6	30	3.62	198.7	383.5	0.3	0.25754	
												7697	36.46
320		(*air in sample- electrolyte assay)		TLC	42	169.1	24.5	5.65	195.8	403.5	0.3	0.58802	
												4633	35.88
321				TLC	36	175.3	27	5.38	208.4	419.5	3.4	0.72022	
												5263	28.92
322				TLC	46	173	29.5	4.84	206.5	419.0	1.2	0.67320	
												1583	42.14
323				TLC	40	167	37	3.99	197.5	385.0	0.8	0.38921	
												888	39.10
324				SNUB	46	176.8	30	3.89	208.8	432.0	7	0.74619	
												093	44.66
325				SNUB	UNK NOW N	171.2	31.5	6.40	203.4	394.5	5.4	0.42908	
												7533	48.81
326		No red top, 450 ul lactate		TLC	31		33					0.68771	
												8427	42.09
327				TLC	49	164.2	39	3.77	202	404.0	12.9	0.53901	
												8027	36.22
328				TLC	47	166.8	31	5.49	201.7	397.0	6.9	0.46992	
												45	38.43
329				SNUB	43	174	32.5	6.35	208	420.5	16.8	0.87524	
												6483	47.06
330				SNUB	41	155.2	27	3.67	188.4	397.0	14.1	0.74153	
												8197	40.17
331	MDM F# 107		puncture gullar region	C/SNUB/ K	44		38			369.0	0.3	0.13046	
												7497	31.27
332	MDM F# 95	Medium clot, no chlorides	lower jaw torn	C/JIG	50	162.8	36.5	3.46	187	372.5	2.8	0.13324	
333	MDM		lower right jaw	C/SNUB/	48		27.5					8653	37.96
												0.25619	25.51

	F # 155 MDM		broken	K								6267		
334	F # 117 MDM		lower left jaw torn	C/SNUB	47	161.2	32	1.57	191.8	384.0	1.9	0.13	0.36414 4377	38.25
335	F # 153 MDM		puncture	C/SNUB	44	159.6	31	3.27	189	374.0	2.5	0.16	0.37223 9273	33.28
336	F # 163 MDM		puncture gullar region	C/TLC	41	161	31	1.51	186.9	375.0	27.1	0.29	0.11636 0753	24.35
337	F # 100 MDM		puncture (control- jigged)	C/JIG	46	173.2	25	3.11	201.7		9.1	0.13	0.49849 5963	29.88
338	F # 143 MDM		puncture	C/SNUB/ K	46	161.7	35	2.01	192.5	369.0	1.9	0.08	0.39248 7337	25.60
339	F # 167 MDM	Large clot, Cor and NaK samples only	puncture gullar region	C/SNUB/ K	44		27.5				2.7		0.33649 1463	27.84
340	F # 156 MDM		puncture gullar region	C/TLC	49	164.3	33.5	1.28	193.2	406.0	1.5	0.12	0.36638 503	34.94
341	F # 97 MDM	No red tops	puncture	C/JIG	48		37.5						0.30136 7537	29.53
342	F # 99 MDM		? (control- jigged)	C/JIG	47	166.7	34.5	1.02	195.7	363.5	1.7	0.11	0.33936 5947	32.04
343				JIG	49	158.4	29.5	3.48	184.7	368.0	0.2	0.09	0.09284 1863	41.22
344				JIG	49	160.4	24.5	5.50	182.9	379.0	0.2	0.13	0.06226 4737	35.75
345				JIG	47	158.9	33.5	5.09	185.1	382.5	0.7	0.17	0.07495 893	36.76
346		Na, K and OS samples only, small sample (*air in sample- electrolyte assay)		JIG	48	154	21	11.00	171.4	363.0			0.02542 4913	35.46
347		No Red tops		JIG	42		27						0.10808 1977	36.92
348		No Purple tops		JIG	47	160.3	22	5.04	184.1	364.0	0.2	0.09		31.00
349		No Red tops		JIG			22						0.04868 464	33.23
350		No Red tops, No Purple tops/Green top sample suspect		JIG	32		13.5							28.41
351		No Cortisol, large clot		JIG	40	154	28.5	3.66	178.7	352.0		0.11	0.10804 815	27.96
352		Lactate 350 ul, Jigged by Henry		JIG	47	155.1	26.5	3.71	184.4	359.0	*	0.07	0.04293 2813	32.36
353		Medium clot		JIG	47	154.3	26	3.38	182.2	367.0		0.13	0.13268 022	28.96
354		Not enough sample to run OS assay		JIG	49	154.9	24	3.25	183.2	374.5	0.3	0.19	0.11811 128	37.21
355		Suspect blood, lactate maybe OK, No green or Red tops		JIG	49								0.07720 3333	
356		Large clot, No cl or cor		JIG	49	154.5	27	2.95	182.7			0.13	0.21959 0527	35.98

	188												
	MDM												
384	F#		left upper jaw									0.45761	
	182		torn	C/JIG	48	157.1	26	2.32	183.1	364.5	7.9	1347	30.05
	MDM												
385	F#		puncture right									0.48906	
	232		side	C/TLC	48	161	32	2.65	189	377.0	1.3	407	29.27
	MDM												
386	F#		lower right jaw									0.52864	
	230		broken	C/SNUB/ K	47	164.3	34	2.15	193.7	375.0	9.1	95	29.39
	MDM												
387	F#		lower left jaw									0.50249	
	204		torn	C/TLC	46	162.4	34	1.84	190.6	389.0	2.1	449	29.81
	MDM												
388	F#		? (control-										
	172		jiggged)	C/JIG	39	163.6	32.5	3.73	192	376.0	2.9		33.03
	MDM												
389	F#	Small clot	? (control-									0.65920	
	174		jiggged)	C/JIG	44	171.1	30	1.66	203.3	424.5	4.6	4307	28.92
	MDM												
390	F#	Small clot, No OS or cl	lower right jaw									0.69550	
	225		broken	C/SNUB/ K	49		39				0.4	809	37.58
	MDM												
391	F#	fish caught in output pipe	small tear right									0.98914	
	180		underside	C/JIG	50	170.8	31.5	2.59	201	393.0	2.1	083	30.97
	MDM												
392	F#		? (control-									0.63275	
	193		jiggged)	C/JIG	47	163	35	2.04	192.4	400.5	0.2	7073	32.22
	MDM												
393	F#		puncture left side									0.52923	
	185			C/JIG	49	165.3	38	1.54	195.7	380.0	2.3	3977	30.46
	MDM												
394	F#		puncture left side									0.11037	
	197			C/TLC	48	158.2	28	6.18	182.2	369.0	0.2	407	29.53
	MDM												
395	F#		puncture left side									0.13057	
	203			C/TLC	44	161.1	32	5.83	185.9	376.0	0.5	96	27.58
	MDM												
396	F#	(*air in sample- electrolyte assay)	? (control-									0.28875	
	189		jiggged)	C/JIG	46	155.9	34	5.14	184	378.0	10.9	8643	37.80
	MDM												
397	F#		lower left jaw									0.20222	
	226		broken	C/SNUB/ K	44	166.1	32	5.13	190.2	393.5	6.4	358	28.32
	MDM												
398	F#		lower jaw broken									0.50640	
	214		gullar region	C/SNUB/ K	46	163.1	28	3.34	189.4	360.0	7.3	518	21.54
	MDM												
399	F#		? (control-									0.55239	
	190		jiggged)	C/JIG	47	162.1	30	3.60	190.1	365.0	*	1827	31.45
	MDM												
400	F#	NOT SURE ABOUT HANDLING!!!!	puncture left side									0.47849	
	213			C/SNUB/ K	47	157.7	31	3.18	187.7	360.0	0.2	6533	25.88
	MDM												
401	F#	Very small clot (*air in sample-	? (control-									0.43101	
	173	electrolyte assay)	jiggged)	C/JIG	48	161.4	29.5	2.90	188.9	370.0	0.3	2593	25.50
402	MDM		? (control-									0.35752	25.91
				C/JIG	48	166.4	32	3.65	195.1	395.5	1.8		

	F# 192 MDM		jigged)									8193		
403	F# 171 MDM		left upper jaw (control jigged)	C/JIG	46	162.4	28	4.11	187.6	370.0	3.4	0.03	0.51024 6557	23.15
404	F# 194 MDM		? (control- jigged)	C/JIG	45	164.8	32.5	3.11	192.9	381.0	8.2	0.05	0.26303 6233	31.95
405	F# 175 MDM		? (control- jigged)	C/JIG	45	160.5	33	4.66	190	380.0	9.9	0.07	0.37523 449	30.74
406	F# 231 MDM	Small clot	puncture right side	C/TLC	49	163.8	30	3.28	192.3	367.0	0.8	0.07	0.33949 467	35.85
407	F# 201 MDM		lower right jaw broken	C/SNUB	48	165.1	25.5	3.22	193	369.0	5.9	0.41	0.22872 905	23.49
408	F# 200	(malfunction, no electrolytes)	lower jaw broken	C/SNUB	44		30			402.0	8.9	0.04	0.54739 5317	31.85
409	336	(malfunction, no electrolytes)	puncture left side	C/TLC	48		30.5			369.0	0.4	0.05	0.46920 9583	29.56
410	265		puncture left side	C/TLC	46	167.7	36	2.38	198.5	357.0	49.1	0.01	0.16154 7607	31.54
411	293		puncture left side	C/TLC	49	161.8	33.5	2.02	194.5	374.0		0.04	0.43777 8943	34.92
412	333	Not enough sample to run OS assay	lower left jaw torn	C/TLC	44	163.9	31.5	2.73	192.1		3.7	0.11	0.40213 7703	31.74
413	291		puncture right eye	C/TLC	50	160.5	34	2.16	194	379.5	4	0	0.62647 9147	36.31
414	251		puncture	C/JIG	43	163.1	24	2.35	194.3	375.5	7	0.08	0.59401 308	24.79
415	276		puncture left side	C/TLC	48	163.9	30.5	4.38	195.4	372.0	3.7	0.04	0.45931 6013	31.64
416	243		puncture gullar region	C/JIG	46	187	27.5	4.27	215.6	422.5	41.1	0.06	0.16556 9693	80.04
417	337		puncture gullar region	C/TLC	48	158.8	33	2.65	188.5	373.0	1.7	0.04	0.42272 5673	26.08
418	269		lower left jaw broken	C/SNUB/ K	46	174.9	30.5	3.66	203.3	407.0	20.9	0.03	0.12229 3193	28.17
419	322		lower jaw torn	C/SNUB/ K	47	178.7	35	2.93	209.4	395.0	17	0.01	0.32648 4687	33.63
420	245		puncture gullar region	C/JIG	46	160.3	29.5	2.02	192.2	384.0	1.6	0.06	0.27596 8277	24.10
421	252		eye puncture	C/JIG	49	156.9	29	2.86	185.6	360.0	7.6	0.07	0.18318 3357	25.92
422	321	(malfunction, no electrolytes)	lower right jaw torn	C/SNUB	50		29.5			367.0	11.4	0.09	0.27005 5287	30.15
423	240		eye puncture	C/JIG	48	156.5	29	7.82	179.5	365.5	1.6		0.27060 175	26.68
424	244		dorsal foul hooked	C/JIG	47	160.8	28.5	1.49	190.5	364.0	9	0.05	0.56105 441	27.77
425	248		lower jaw	C/JIG	50	164.1	27	2.32	192.1	370.0	21.7	0.03	0.15916 7163	23.34

426	283		puncture gullar region	C/TLC	48	162.2	26.5	1.30	192	360.5	*	0.06	0.41134 6547	31.66
427	281		lower left jaw broken	C/SNUB	47	161.4	22.5	2.25	191.5	375.0	6.2	0.08	0.54681 4803	23.00
428	253	Glu error- no assay	puncture	C/JIG	48	158.7	35	2.62	190.5	364.5	0.4		0.85705 223	34.72
429	271		lower jaw broken	C/SNUB	47	170.7	22.5	3.76	202.4	387.0	10.8	0.10	0.54176 9213	25.91
430	255		lower jaw broken gullar region	C/SNUB/ K	48	167.7	30	1.99	196.5	381.0	29.8	0.09	0.32406 2027	24.88
431	328		lower left jaw broken	C/SNUB/ K	47	158.3	34.5	2.41	186.7	382.5			0.58877 5393	31.47
432	237	two vials labeled '432' for cortisol (see values)	hook in lower jaw	C/JIG	41	170.5	24.5	2.93	200.3	380.5	12.3 & 7.4	0.21	0.48658 9097	19.24
433	319	(malfunction, no electrolytes)	lower left jaw torn	C/SNUB/ K	47		32.5			391.0	4.6	0.04	0.40225 292	34.63
434	234		upper left jaw torn	C/JIG	50	159.2	30	3.45	185.9	388.0	1.3	0.04	0.52248 6857	31.04
435	335		puncture gullar region	C/TLC	43	165.4	35	2.14	197.6	404.5	4.4	0.04	0.65491 517	29.27
436	250	Glu error- no assay	lower left jaw torn	C/JIG	48	168.4	28.5	1.20	198.5	395.5	11.4		0.43222 7033	25.96
437	280		puncture left side	C/TLC	48	163.9	31.5	1.55	193.4	371.5	2	0.08	0.41438 6633	28.59
438	254	Glu error- no assay	puncture snout region	C/JIG	49	153.7	32.5	1.07	184.9	378.0	22.4		0.12978 6553	27.50
439	277		puncture left side	C/TLC	48	164.2	32	1.18	197.7	395.0	6.9	0.07	0.58521 8117	30.98
440	235		? (control-jigged)	C/JIG	45	162.8	35.5	1.56	197.8	381.5	4.6	0.07	0.49096 1723	30.18
441	241		punctures gullar and snout region	C/JIG	47	167.7	31	2.00	199.1	409.0	4.1	0.05	0.54496 6863	25.74
442	247		puncture right side	C/JIG	45	172.1	30.5	1.33	205.1	388.0	2.2	0.07	0.48687 3187	33.82
443	308		lower right jaw broken	C/SNUB	46	171.4	32.5	1.54	203.7	388.5	15.9	0.11	0.72121 8807	36.29
444	311		puncture left side	C/TLC	42	165.7	33.5	1.90	195.4	380.0	20.9	0.09	0.43834 323	29.07
445	249	(*air in sample- electrolyte assay)	puncture operculum	C/JIG	50	152.3	39	1.56	188	385.5	5.4	0.13	0.45946 5143	28.58